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# Molecular investigation of tick-borne pathogens in ixodid ticks infesting domestic animals (cattle and sheep) and small rodents (black rats) of Corsica, France

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ABSTRACT

Although livestock farming (sheep, goats, pigs, and cattle) is an important economic activity in Corsica, a French Mediterranean island, knowledge about the tick fauna and microorganisms carried by them remains scarce. This study aimed to investigate the presence and perform molecular characterization of Anaplasmataceae, *Rickettsia* spp., and *Borrelia burgdorferi* sensu lato (sl) in tick species collected in Corsica. Ticks from cattle (*Bos taurus*), sheep (*Ovis aries*), and rodents (*Rattus rattus*) were collected from May to September 2016. DNA was purified from ticks, submitted to quantitative real-time polymerase chain reaction (qPCR) and sequenced for phylogenetic analysis. In total, 660 ticks were collected from 111 animals during the study. The most abundant collected tick species from cattle was *Rhipicephalus bursa* (n = 495; 84.5%), followed by *Hyalomma marginatum* (n = 91; 15.5%). *Rhipicephalus bursa* and *Ixodes ricinus* were the only tick species collected from sheep and rodents, respectively. Overall, *Rickettsia* was the most common pathogen group (n = 48; 24%) detected in ticks. Sequence analysis of partial *gltA* and *ompA* genes revealed the presence of *Ri. aeschlimannii* and *Candidatus* Ri. barbariae. Anaplasmataceae DNA was detected in eight (6%) of the 127 cattle pools and in one (2%) of the 61 *R. bursa* specimens collected from sheep. Sequence analysis of the *rpoB* gene revealed the presence of one *Anaplasma* species, *A. marginale*.

*Borrelia burgdorferi* sl DNA was detected in one pool of *H. marginatum* collected from cattle and in two (15%) of the 13 *I. ricinus* pools collected from nine black rats. To our knowledge, this is the first report of the occurrence and molecular characterization of *Candidatus* Ri. barbariae, an emerging member of the *Rickettsia* group causing spotted fever, in Corsica. The detection of *B. burgdorferi* sl DNA, which was previously believed to be rare in Corsica, confirms the presence of this agent on the island.

## 1. Introduction

Ticks, ubiquitous ectoparasitic arthropods that belong to the subclass Acari, order Ixodida, are considered to be the most common vectors of disease-causing pathogens in animals (domestic and wild), and are the second most common vector of human pathogens worldwide after mosquitoes (Colwell et al., 2011; Rizzoli et al., 2014). Arthropod-borne microorganisms, such as Toscana virus in dogs (Dahmani et al., 2016) and West Nile virus in domestic animals have been reported in Corsica (Maquart et al., 2017). Corsica, a French Mediterranean island, is characterized by a mild Mediterranean climate and a high variability of microclimates because of its specific

*Abbreviations:* qPCR, quantitative real-time polymerase chain reaction; CCHFV, Crimean-Congo hemorrhagic fever virus

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geographical situation (Grech-Angelini et al., 2016). Corsican livestock farming (sheep, goats, pigs, and cattle) is extensive, so important interactions between livestock, wildlife, and human populations could favor the circulation of tick-borne diseases. A systematic survey of tick fauna on Corsican livestock reported the dominance of the typical Mediterranean species *Rhipicephalus bursa* and *Hyalomma marginatum* (Grech-Angelini et al., 2016). *Hyalomma marginatum* is one of the main vectors of the zoonotic Crimean–Congo hemorrhagic fever virus (CCHFV) in the Mediterranean Basin and the Afrotropical region (Estrada-Pena et al., 2012). *Rhipicephalus* bursa is recognized as a primary vector of *Babesia ovis* (Moltmann et al., 1982), but it transmits other pathogens such as *Rickettsia* spp. and *Anaplasma* spp. (Dahmani et al., 2017; Raele et al., 2015). An epidemiological survey on Anaplasmataceae species infecting domestic animals and ticks in Corsica described the presence of six species belonging to the genus *Anaplasma* from blood samples taken from ruminants and from ticks infecting cattle (Dahmani et al., 2017). *Ehrlichia canis* was detected once in Corsica in a non-engorged *R. bursa* tick collected from a cow (Dahmani et al., 2017). *Anaplasma* spp. and *Ehrlichia* spp. are transmitted by ticks, and both genera contain obligate intracellular Gram-negative bacterial parasites. In the vertebrate host, these bacteria infect hematopoietic cells. There are four pathogens of ruminants in the genus *Anaplasma*: *A. marginale*, *A. centrale*, *A. bovis*, and *A. ovis*; in addition, there is *A. phagocytophilum*, which infects a variety of hosts, including humans and other animals, and *A. platys*, which infects dogs (Ndip et al., 2010). *Rickettsia* aeschlimannii was detected and isolated in *H. marginatum* in Corsica (Matsumoto et al., 2004).

This study aimed to investigate the presence of Anaplasmataceae, *Rickettsia* spp. and *B. burgdorferi* sensu lato (sl) in tick species collected from cattle, sheep, and black rats in Corsica, and to characterize them using molecular approaches.

## 2. Materials and methods

### 2.1. Tick collection and morphological identification

Ticks from cattle (*Bos taurus*), sheep (*Ovis aries*), and rodents (*Rattus rattus*) were collected from May to September 2016 in northern Corsica, France (Fig. 1). Ticks were collected from June to July 2016 in the Ponte-Leccia slaughterhouse in Haute Corse. During each visit, the whole skin of slaughtered animals was inspected, and ticks were collected manually. The national cattle identification system, which uses ear tags, allowed the origin of animals to be tracked and identified the farm owners. Cattle sampled in the Ponte-Leccia slaughterhouse come from Haute Corse municipalities. Ticks were also collected from sheep (n = 60) belonging to one farm in Corte on May 2016 and from rodents (n = 9) trapped on September 2016 in Lozari, Urbino, and Terrenzana (Fig. 1). Ticks were identified at species level using a pictorial guide (Estrada-Pena et al., 2004). *Ixodes* ticks were checked for being *I. inopinatus* (Estrada-Pena et al., 2014).

### 2.2. DNA extraction

Ticks were washed once in 70% ethanol for 5 min and then twice in distilled water for 5 min. Those collected from sheep and black rats were analyzed individually, whereas those collected from cattle were analyzed after monospecific pools consisting of 2–6 ticks were collected from the same animal.

Individual ticks or pools of ticks were crushed using the TissueLyser II (Qiagen, Hilden, Germany) in phosphate-buffered saline at 5500 rpm for 20 s. DNA extraction was performed on a QIAcube HT (Qiagen) using a QIAamp cador Pathogen Mini kit according to the manufacturer's instructions. DNA was eluted in 150 µl of buffer and stored at −20 °C. For each PCR reaction, the template DNA had a final concentration < 200 ng.

### 2.3. Molecular analysis of ticks and bacteria

Morphological identification of ticks was confirmed by polymerase chain reaction (PCR) amplification and sequencing mitochondrial 16S rDNA (Table 1) (Black and Piesman, 1994). At least two specimens from each species were randomly selected and subjected to molecular identification. The PCR assays for Anaplasmataceae (Dahmani et al., 2017), *Rickettsia* spp. (Labruna et al., 2004), *B. burgdorferi* sl (Courtney et al., 2004) and *B. miyamotoi* (Diaz et al., 2012) were performed using the primers and probes listed in Table 1. Reactions were performed on a 96-well Applied Biosystems™ QuantStudio™ 3 Real-Time PCR System using QuantiFast Pathogen + Internal Control Kits (Qiagen). Internal and negative controls were included in each run. Each run was repeated three times. Samples that were positive for Anaplasmataceae were tested by conventional PCR using *Anaplasma* genus-specific primers targeting the 525-bp fragment of the RNA polymerase subunit beta (*rpoB*) gene (Dahmani et al., 2017) (Table 1). Positive samples for *Rickettsia* spp. were analyzed using primers that amplified the 850-bp fragment of the *gltA* gene encoding citrate synthase (Table 1) (Mediannikov et al., 2004; Roux et al., 1997) and primers to amplify the 532-bp fragment of the 190-kDa outer membrane protein (*ompA*) gene (Regnery et al., 1991). The reactions were carried out using Applied Biosystems GeneAmp PCR System 9700 (Courtabouef, France). Negative and positive controls were included. The PCR products were visualized in 2% agarose gels in Tris-Acetate-EDTA (TAE Buffer) and were visualized under ultraviolet light after staining with ethidium bromide. A 100-bp DNA ladder was used as a standard marker.

The pathogens detected in pools were expressed as the percentage and minimum infection rate based on the assumption that each PCR-positive pool contained at least one positive tick (Sosa-Gutierrez et al., 2016). Detection rate of DNA bacteria were compared by using Fisher exact test ($p < 0.05$). The analysis was conducted using the R statistical platform (version 3.1.2) (R Development Core Team, 2015).

### 2.4. Sequencing and phylogenetic analysis

A selected number of positive samples for *Rickettsia* and Anaplasmataceae were purified and directly sequenced using an Applied Biosystems model 3730XL (Fisher Scientific S. A. S., Illkirch-Graffenstaden, France). Sequences of ticks, Anaplamataceae, or *Rickettsia* spp. were aligned using MEGA X (Kumar et al., 2018). All sequences were assembled and compared with similar sequences retrieved from the GenBank nucleotide database using BLASTn (Altschul et al., 1997). Phylogenetic analyses were inferred using the maximum-likelihood method implemented on Mega X.

## 3. Results

### 3.1. Tick identification

In total, 660 ticks were collected during the study (Table 2). Forty-two cattle were inspected in the Ponte-Leccia slaughterhouse from June to July 2016 and 586 ticks were collected. Among the 42 infested cows, 57% were infested with less than 10 ticks, 28% with 10–30 ticks, and 15% with more of 30 ticks. Two tick species were identified morphologically on cattle (Table 2). The most abundant species was *R.* bursa (n = 495; 84.5%), followed by *H. marginatum* (n = 91; 15.5%). Among the 586 ticks collected from cattle, 541 (94.1%) were adults (38.6% females and 55.5% males) and 34 (5.9%) were nymphs. Sex and life stage were not determined for 11 ticks. Sixty-one ticks were collected from 60 sheep in one farm on May 2016 (Corte, Haute-Corse) (Table 2). All these were R. bursa; 51 (83.6%) were adults (33% males and 51% females). Thirteen *I. ricinus* ticks (all adult females) were collected from nine rodents (black rats) captured in Lozari, Urbino, and Terrenzana (Haute-Corse) in September 2016. Among them, six were infested by one tick, two by two ticks, and one by three ticks. The sequences of

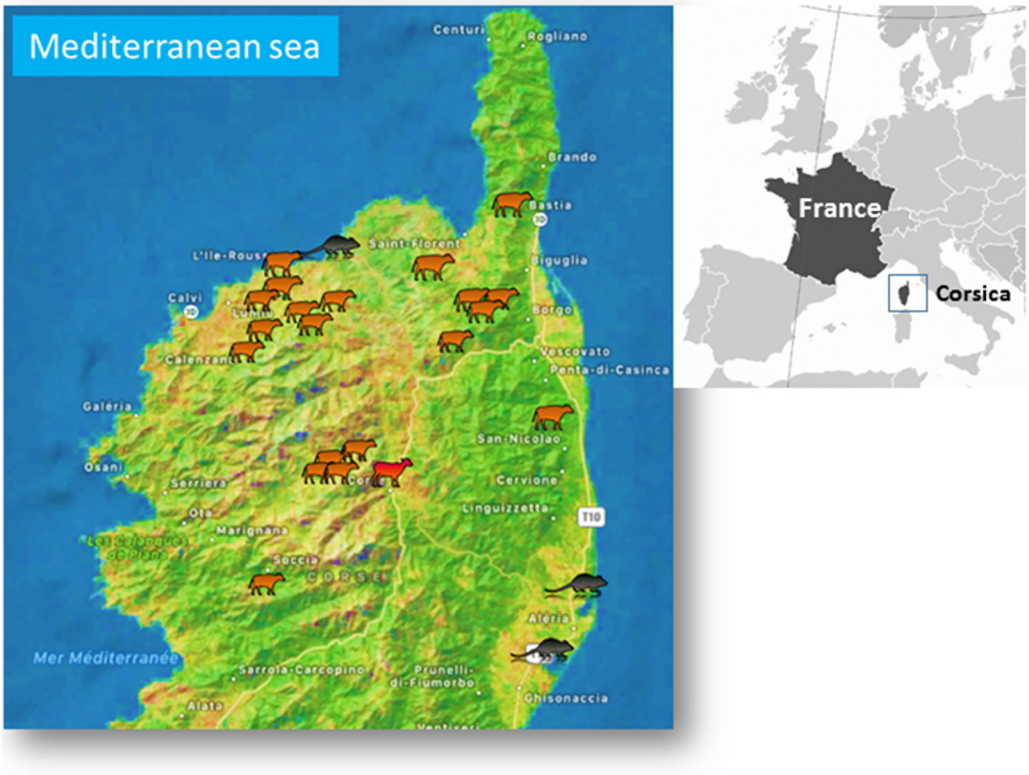


**Fig. 1.** Map of Corsica showing the study area where the animals lived and the GPS coordinates.
For cattle: Occhiatana (42° 34′ 30″ N, 9° 00′ 33″ E), Pighjolu (42° 10′ 40″ N, 8° 54′ 37″ E), Calacuccia (42° 20′ 12″N, 9° 01′ 05″ E), Casamaccioli (42° 19′ 06″N, 9° 00′ 07″ E), Olmi-Capella (42° 31′ 38″ N, 9° 01′ 09″ E), Lozzi (42° 20′ 45″N, 9° 00′ 14″ E), Speluncatu (42° 33′ 46″ N, 8° 58′ 54″ E), Muratu (42° 34′ 41″ N, 9° 19′ 36″ E), Soriu (42° 35′ 02″N, 9° 16′ 28″ E) Lentu (42° 31′ 22″N, 9° 16′ 57″ E), Albertacce (42° 19′ 41″ N, 8° 59′ 04″ E), Zilia (42° 31′ 52″N, 8° 54′ 06″ E), Barbaghju (42° 41′ 26″N, 9° 22′ 42″ E), Calinzana (42° 30′ 31″ N, 8° 51′ 21″ E), Valone-Orneto (42° 24′ 06″ N, 9° 28′ 18″ E), Pieve (42° 34′ 51″N, 9° 17′ 18″ E), Santu Petru di Tenda (42° 36′ 22″ N, 9° 15′ 30″ E), Lavatoghju (42° 34′ 29″ N, 8° 52′ 42″ E), Lisula (42° 38′ 08″ N, 8° 56′ 17″ E) and Santa Reperata Di Balagna (42° 36′ 16″N, 8° 55′ 45″ E). For sheep: Corti (42° 20′ 16″N, 9° 15′ 45″ E) and for rodents: Lozari (42°38′28 N, 9°00′56E), Urbinu (42° 02′ 53″ N, 9° 28′ 22″ E), and Terrenzana (42° 9′ 28″N, 9° 32′ 58″E).

**Table 1**
Primers and probes used in this study and PCR conditions (annealing temperature).

| Species | Target | Name | Sequence | Annealing temperature (°C) | References |
|---|---|---|---|---|---|
| | **qPCR** | | | | |
| *B. burgdorferi* sl | *23S RNA* | *Bb23Sf* | CGAGTCTTAAAAGGGCGATTTAGT | 60 | Courtney et al. (2004) |
| | | *Bb23Sfr* | GCTTCAGCCTGGCCATAAATAG | | |
| | | *Bb23Sp* | AGATGTGGTAGACCCGAAGCCGAGTG | | |
| *B. miyamotoi* | *glpQ* | *Bmi-F* | CACGACCCAGAAATTGACACA | 60 | Diaz et al. (2012) |
| | | *Bmi-R* | GTGTGAAGTCAGTGGCGTAAT | | |
| | | *Bmi-P* | TCGTCCGTTTTCTCTAGCTCGATTGGG | | |
| *Rickettsia* spp. | *gltA* | *Rspp-F* | GAGAGAAAATTATATCCAAATGTTGAT | 60 | Labruna et al. (2004) |
| | | *Rspp-R* | AGGGTCTTCGTGCATTTCTT | | |
| | | *Rspp-P* | CATTGTGCCATCCAGCCTACGGT | | |
| Anaplasmataceae | *23S rRNA* | *Tt-Ana-F* | TGACAGCGTACCTTTTGCAT | 60 | Dahmani et al. (2017) |
| | | *Tt-Ana-R* | GTAACAGGTTCGGTCCTCCA | | |
| | | *Tt-Ana-P* | GGATTAGACCCGAAACCAAG | | |
| | **Conventional PCR (sequencing)** | | | | |
| *Rickettsia* spp. | *gltA* | *CS2D* | ATGACCAATGAAAATAATAAT | 54 | Roux et al. (1997), Mediannikov et al. (2004) |
| | | *CSEndR* | CTTATACTCTCTATGTACA | | |
| | | *409D* | CCTATGGCTATTATGCTTGC | 54 | |
| | | *1258R* | ATTGCAAAAAGTACAGTGAACA | | |
| | ompA | *Rr190.70p* | ATGGCGAATATTTCTCCAAAA | 48 | Regnery et al. (1991) |
| | | *Rr190.602n* | AGTGCAGCATTCGCTCCCCCT | | |
| *Anaplasma* spp. | *rpoB* | *Ana-rpoBF* | GCTGTTCCTAGGCTYTCTTACGCGA | 55 | Dahmani et al. (2017) |
| | | *Ana-rpoBR* | AATCRAGCCAVGAGCCCCTRTAWGG | | |
| Ticks | 16S rDNA | *16S + 1* | CTGCTCAATGATTTTTTAAATTGCTGTGG | 48 and 54 | Black and Piesman (1994) |
| | | *16S-1* | CCGGTCTGAACTCAGATCAAGT | | |

mitochondrial 16S rDNA fragments of 16 ticks selected in this study after blast analysis were confirmed to be *R. bursa* (n = 7), *H. marginatum* (n = 7), and *I. ricinus* (n = 2) (GenBank accession numbers MH663984-90 for *R. bursa*, MH663977-83 for *H. marginatum and* MH663991-92 for *I. ricinus*). The seven 16S rDNA sequences of *H marginatum* had 98–100% identity with each other and 99–100% identity with *H. marginatum* from Sardinia (KT931964), Israel (KT391060), Turkey (KR870973), and Algeria (KP776645) (Fig. 2).

The seven (MH663984-90) 16S rDNA sequences of *R. bursa* were identical to each other and showed 100% identity with *R. bursa* from the Iberian Peninsula (AJ002956), Italy (KJ814007-KJ814010), and Turkey (KR870983.1-KU664350.1) (Fig. 2). The two (MH663991-92) 16S rDNA sequences of *I. ricinus* were identical to each other and showed 100% identity with *I. ricinus* from Germany (JF928526), Italy (KF197116), Sweden (KX384811), Belgium (KJ414457), and Turkey (KR870982). They had 98% identity with 16S rDNA sequences from Morocco (GU074602) and France (GU074612) (Fig. 2). Seventy-four individual ticks (61 collected from sheep and 13 from rodents) and 127

**Table 2**
Total tick species collected from hosts.

| Host (n) | Tick species | n ticks (%) | Male n (%) | Female n (%) | Nymph n (%) |
|---|---|---|---|---|---|
| Cattle (n = 42) | *R. bursa* | 495 (84.5) | 251 (51.1) | 207 (42.2) | 33 (6.7) |
| | *H. marginatum* | 91 (15.5) | 68 (81.0) | 15 (18.0) | 1 (1.0) |
| | Total[a] | 586 | 319 (55.5) | 222 (38.6) | 34 (5.9) |
| Sheep (n = 60) | *R. bursa* | 61 (100) | 20 (33.0) | 31 (51.0) | 10 (16.0) |
| Rodents (n = 9) | *I. ricinus* | 13 (100) | 0 (0) | 13 (100) | 0 (0) |
| Total (n = 111) | Total tick species | 660 | 339 (52.2) | 266 (41.0) | 34 (6.8) |

[a] Sex and life were not determined for 11 ticks.

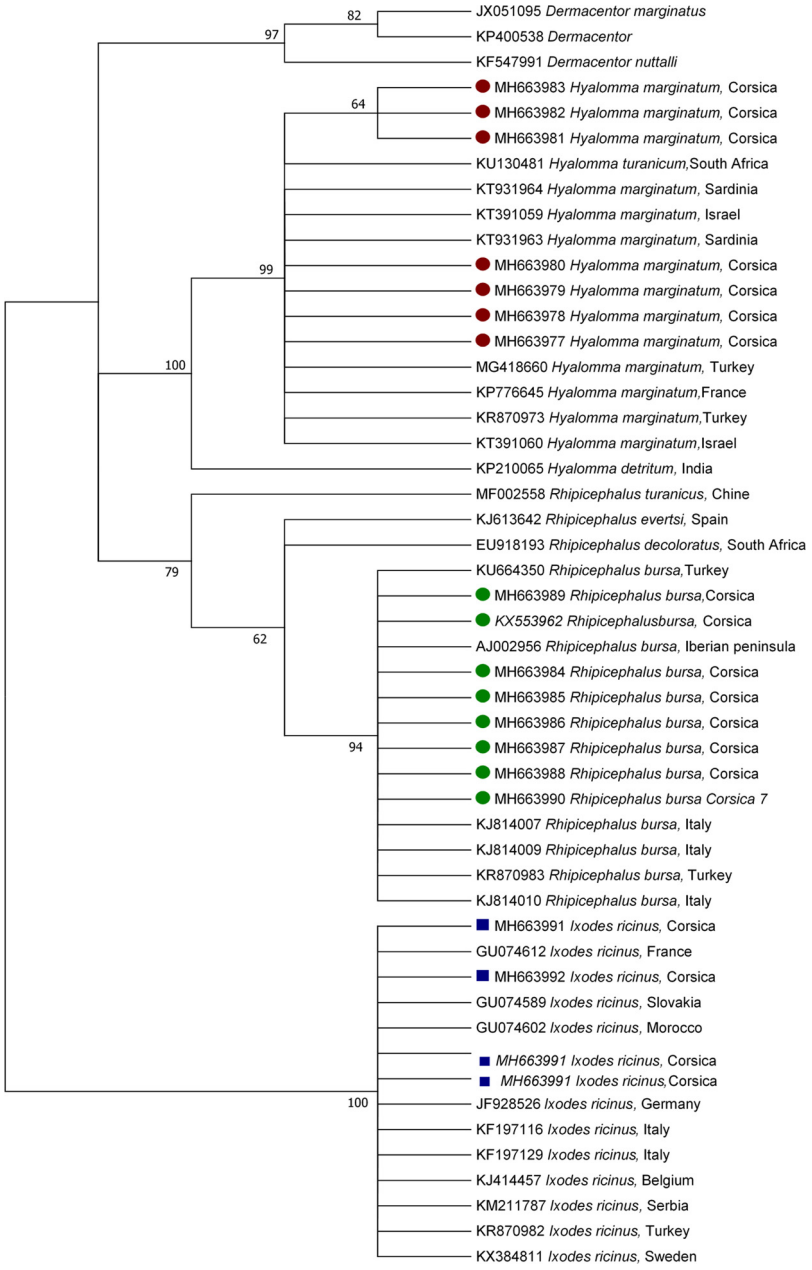


**Fig. 2.** Phylogenetic tree showing the position of Rhipicephalus bursa (green circles), Hyalomma marginatum (red circles) and Ixodes ricinus (blue squares) compared to other tick species based on 16S rDNA sequences. The evolutionary history was inferred by using the Maximum Likelihood method. The analysis involved 50 nucleotide sequences. There were a total of 291 positions in the final dataset. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

**Table 3**
Infection rate of bacteria in ticks collected from cattle.

| Number of ticks per pool (n) | Number of pools with n ticks *H. marginatum* | Number of pools with n ticks *R. bursa* | Positive *Rickettsia* spp. n (%) | | | Positive Anaplasmataceae n (%) | | | Positive *Borrelia burgdorferi* (sl) n (%) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | *H. marginatum* | *R.bursa* | Total | *H. marginatum* | *R. bursa* | Total | *H. marginatum* | *R. bursa* | Total |
| 1 | 8 | 1 | 2 (25) | 0 (0) | 2 (22) | 1(12) | 0 (0) | 1 (11) | 0(0) | 0 (0) | 0 (0) |
| 2 | 5 | 7 | 4 (80) | 1 (14) | 5 (42) | 0 (0) | 0 (0) | 0 (0) | 1 (20) | 0 (0) | 1 (8) |
| 3 | 2 | 9 | 1 (50) | 1 (11) | 2 (18) | 2 (100) | 0 (0) | 2 (18) | 0 (0) | 0 (0) | 0 (0) |
| 4 | 3 | 15 | 2 (66) | 3 (20) | 5(28) | 0 (0) | 1 (33) | 1 (5) | 0 (0) | 0 (0) | 0 (0) |
| 5 | 3 | 12 | 2 (66) | 2 (17) | 4 (27) | 1 (33) | 0 (0) | 1 (7) | 0 (0) | 0 (0) | 0 (0) |
| 6 | 6 | 16 | 5 (83) | 14 (87) | 19 (86) | 1 (17) | 2 (12) | 3 (14) | 0 (0) | 0 (0) | 0 (0) |
| Total pools | 27 | 100 | 16 (59) | 21 (21) | 37 (29) | 5 (18) | 3 (3) | 8 (6) | 1 (4) | 0 (0) | 1 (1) |

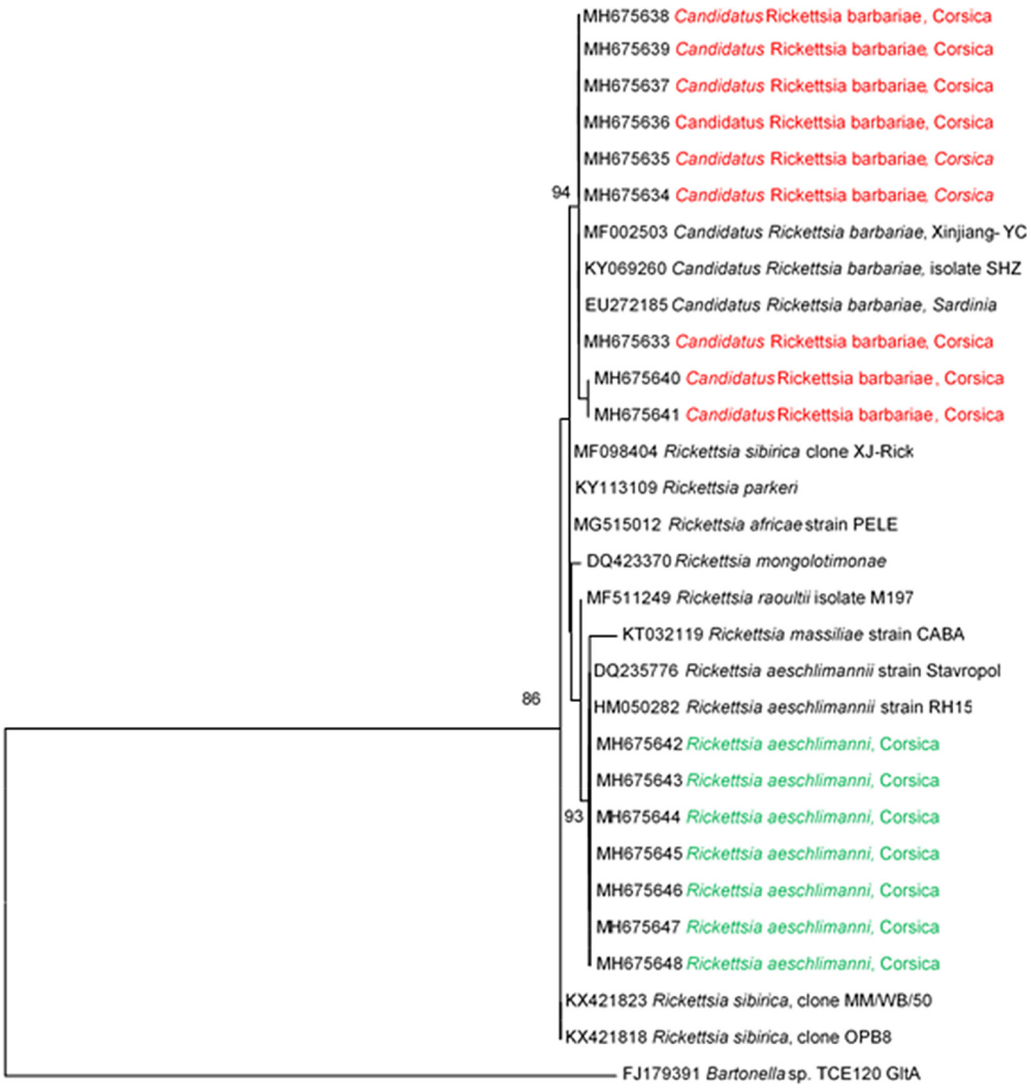


**Fig. 3.** Phylogenetic analysis of *Rickettsia* spp. identified in Corsica. The evolutionary history was inferred by using the Maximum Likelihood method based on Rickettsia *gltA* sequences. The analysis involved 30 nucleotide sequences. All positions containing gaps and missing data were eliminated. There were a total of 331 positions in the final dataset.

pools prepared with cattle ticks (100 pools of *R. bursa* and 27 pools of *H. marginatum* in cattle) were analyzed (Tables 2 and 3).

### 3.2. Detection of Rickettsia spp

In ticks collected from cattle, *Rickettsia* spp. DNA was detected in 37 pools (29%), with the highest detection rate in *H. marginatum* (59%) compared with *R. bursa* (21%) ($p = 0.00$; Table 3). Similar *Rickettsia* spp. detection rates were observed in *R. bursa* collected from cattle and sheep (21% and 18%, respectively; $p = 0.68$). The 13 *I. ricinus* specimens collected from rodents were all negative for *Rickettsia* spp. Nine pools of ticks (three *R. bursa* and six *H. marginatum*) and seven individual ticks (*R. bursa*) collected from cattle and sheep, respectively, were screened for the presence of the *gltA* gene (GenBank accession numbers MH675633-MH675648). Sequence analysis of the *gltA* sequences revealed the presence of two *Rickettsia* species: *Ri. aeschlimannii* and *Candidatus* Ri. barbariae. The seven *gltA* sequences derived from five pools of *H. marginatum* and two pools of *R. bursa* collected from

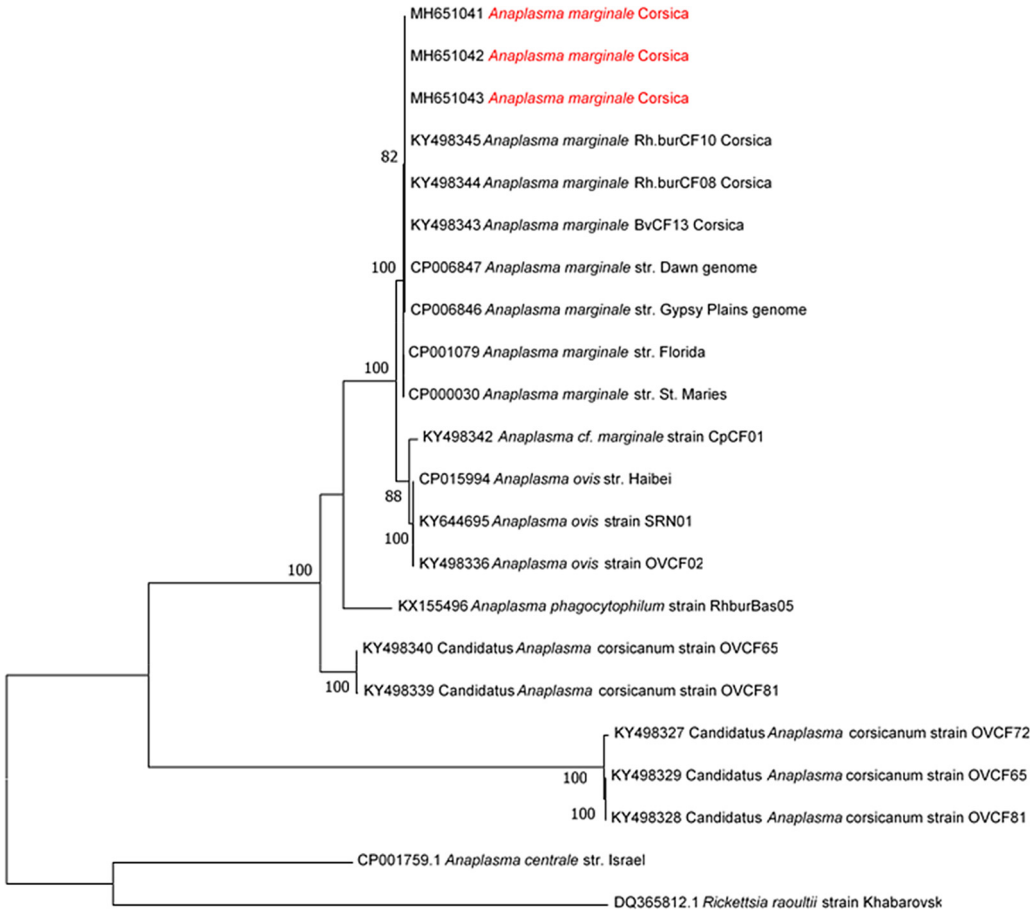


**Fig. 4.** Phylogenetic analysis of Anaplasmataceae identified in Corsica. The evolutionary history was inferred by using the Maximum Likelihood method based on Anaplasmataceae *rpob* gene sequences. The analysis involved 30 nucleotide sequences. There were a total of 331 positions in the final dataset.

cattle were 100% identical to each other and clustered together with *Ri. aeschlimannii* sequences (KU961540, DQ235776, and HM50282) (Fig. 3). Nine *gltA* sequences derived from eight *R. bursa* specimens collected from sheep and one pool of *H. marginatum* ticks collected from cattle showed 99–100% identity with each other and 99% identity with *Candidatus* Ri. barbariae collected in Sardinia (EU272185) (Fig. 3). The analyses of the *ompA* gene (GenBank accession numbers MH797764–MH79777) confirmed these results (99% of identity with *Candidatus* Ri. barbariae with KY233248-49 from Lebanon).

### 3.3. Detection of Anaplasmataceae

Anaplasmataceae DNA was detected in eight (6%) of the 127 tick cattle pools. Among them, DNA was detected in five (18%) of the 27 *H. marginatum* pools and in three (3%) of the 100 *R. bursa* pools (Table 3). Anaplamataceae DNA was detected in one (2%) of the 61 *R. bursa* ticks collected from sheep and analyzed individually. Anaplamataceae DNA was not detected in *I. ricinus*. Sequence analysis of the *rpoB* gene sequencing of three pools (one *R. bursa* and two *H. marginatum*) revealed the presence of one *Anaplasma* species, *A. marginale* (GenBank accession numbers MH651041-MH651043). The three sequences of *A. marginale* had 99–100% identity with each other and showed 99–100% identity with an *A. marginale* strain reported in Corsica (KY498343–KY498345) (Fig. 4).

### 3.4. Detection of B. burgdorferi (sl)

*Borrelia burgdorferi* sl DNA was detected in two (15%) out of the 13 *I. ricinus* ticks collected from nine rodents and in *H. marginatum* one (4%) cattle pool. *B. miyamotoi* DNA was not detected in any of the 13 *I. ricinus* analyzed.

## 4. Discussion

Here we report the detection rate of *Rickettsia* spp., Anaplasmataceae, and *B. burgdorferi* sl in ticks collected from cattle, sheep, and black rats in Corsica, France. Although *R. bursa* was the most-represented tick species, *H. marginatum* ticks showed the highest detection rate for *Rickettsia* spp. and Anaplasmataceae. *Rickettsia* spp. DNA was present in almost 20% of the ticks collected from sheep and cattle examined. To our knowledge, we report here for the first time the detection of *Candidatus* Ri. barbariae and *B. burgdorferi* sl DNA in ticks collected from ruminants and black rats in Corsica, respectively.

*Rhipicephalus bursa* was the most abundant tick species collected from cattle (> 80%) and the only tick species collected from small ruminants. This was in line with previous findings from Corsica (Dahmani et al., 2017; Grech-Angelini et al., 2016). In the Mediterranean basin, this species is considered as the major ectoparasite of sheep (Yeruham et al., 2000). The majority of *R. bursa* specimens collected in this study were adults, as they were collected during the summer period (Ferrolho et al., 2016). Majority of R. bursa ticks analyzed were positive for *Rickettsia* spp., with a similar detection rate between cattle and sheep. Sequence analysis revealed the detection of *Ri. aeschlimannii* and *Candidatus* Ri. barbariae in *R. bursa*. *Rickettsia* spp., such as *Ri. aeschlimannii* and *Ri. massiliae*, had previously been detected in *R. bursa* in several Mediterranean countries in a wide range of animal hosts (Parola et al., 2013); however, to the best of our knowledge, this is the first detection report of *Candidatus* Ri. barbariae in *R. bursa* ticks collected

from domestic animals in Corsica. These species were described previously in several tick species in Portugal (*R. bursa*), Cyprus (*R. turanicus*), Sardinia (*R. turanicus*), and China in the flea (Vermipsylla alakurt) (Zhao et al., 2016). Here, 3% of R. bursa tick cattle pools were positive for Anaplasmataceae DNA. We found a detection rate of Anaplamataceae DNA of 2% among the 61 R. bursa collected from sheep, which is similar to the detection rate reported in other Mediterranean regions (Satta et al., 2011; Torina et al., 2010). We also detected the DNA of *A. marginale*. This is consistent with a previous study reporting that the DNA of *A. marginale* was detected in two engorged female ticks removed from cattle (2%) and from all (12/12) cattle blood samples in Corsica (Dahmani et al., 2017).

*Hyalomma marginatum* was the second most abundant tick species collected from cattle (15.5%), as described previously (Grech-Angelini et al., 2016). *Hyalomma marginatum*, which is the vector of CCHFV, is present in southeastern continental France (Vial et al., 2016), but also in Mediterranean islands such as Sicily (Italy) and Minorca (Spain) (Castella et al., 2001). More than 50% of *H. marginatum* pools were positive for DNA of Ri. aeschlimannii and *Candidatus* Ri. barbariae. Such a high infection rate is in agreement with Ri. aeschlimannii infection rate of *Hyalomma* (> 70%) previously reported in Corsica (Matsumoto et al., 2004) and Croatia (64%) (Punda-Polic et al., 2002). Lower rates of *Rickettsia* spp. were observed in Sicily (4%) (Torina et al., 2010) and Turkey (6.5%) (Keskin et al., 2016). These differences in results could be related to differences in environmental factors, animal species, the numbers of ticks collected and the analysis methods. In our study, the detection of *Candidatus* Ri. barbariae in a pool of *H. marginatum,* not a recognized vector for this *Rickettsia* species (Parola et al., 2013), could also be explained by the presence of this bacterium in the blood meal of the ticks.

*Borrelia burgdorferi* sl is the causative agent of Lyme borreliosis, which is the most prevalent tick-borne disease in Europe and North America (Raileanu et al., 2017). The French *Sentinelles* General Practitioner Network observed that the incidence of Lyme borreliosis is much lower in Corsica (incidence rate reported, 20–50 cases per 100,000 inhabitants) compared with endemic regions of France (> 150 per 100,000 inhabitants) (Vandenesch et al., 2014). Here we report for the first time the detection of *B. burgdorferi* sl DNA in two *I. ricinus* ticks collected from rodents (15%). The detection rate of *B. burgdorferi* sl reported here was similar to that reported for *I. ricinus* collected from rodents in Italy (14.7%) (Pascucci et al., 2015) and was in the range of previous studies from France, Ireland, and Austria, which found a detection rate of *B. burgdorferi* sl in small mammal species ranging from 2.3% to 24% (Gray et al., 1999; Khanakah et al., 2006; Marsot et al., 2011). Although cattle are not a reservoir host, we detected *B. burgdorferi* sl DNA in one *H. marginatum* cattle pool. This detection could be explained by the presence of reservoir hosts of *B. burgdorferi* in the same location (Gern, 2009).

The limitations of our study were that first, the ticks were collected mostly in a municipal slaughterhouse in northern Corsica. We have no data about the southern part of the island. Second, ticks were analyzed mostly in pooled samples and not individually. The screening of pooled samples is complicated because it is impossible to determine whether a positive result is caused by one or more infected ticks. Third, the ticks investigated here were removed from hosts. The presence of bacterial DNA in an engorged tick could be caused by its presence in the blood meal.

These results contribute to the knowledge of tick-borne disease in Corsica and provide a useful contribution to understanding the epidemiology. Further research should be carried out to investigate the eco-epidemiological cycle of the pathogenic agents detected here.

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## Competing interests

The authors declare that they have no competing interests.

## Ethics approval and consent to participate

The inspected cattle were slaughtered for human consumption. Living sheep were examined with the assistance of their owner. Trapping of black rats was organized near to livestock facilities or fence of dwellings and provided according to Regulation No. 20 (order of 29 january 2007 and of 29 june 2011, L. 427-8 of the Environment Code) of the French Ministry of Ecology, Energy and Sustainable Development.

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