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1 ***Anaplasma capra* in sheep and goats on Corsica Island, France: a European lineage within**
2 ***A. capra* clade II?**

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Abstract: Anaplasmosis is a tick-transmitted disease due to several species of the genus *Anaplasma*. In 2019, we demonstrated the presence of *Anaplasma capra* in two deer species at a zoological park in mainland France. As we suspected its presence in Corsica, we surveyed 11 geographically distant sheep or goat farms. Using molecular tools such as nested PCR targeting 16S ribosomal RNA (rRNA), citrate synthase (*gltA*) and heat-shock protein (*groEL*) genes, we detected the presence of *A. capra* on 5/11 farms, in 26/108 blood samples (24%), in sheep as well as in goats. Genotyping and phylogenetic analysis of *A. capra* revealed that isolates from Corsica island grouped closely with *A. capra* isolates reported in red deer and swamp deer from a zoological reserve in mainland France, as well as in roe deer from Spain, in a separate and well supported clade within *A. capra* clade II. This third report of the tick-borne bacterium *A. capra* in Europe suggests a potentially larger presence of this pathogen on the European continent, on domestic, native as well as wild ruminants, a broad host range already described in Asian countries for this species.

Keywords: *Anaplasma capra*, sheep, goat, phylogeny, Corsica, Europe

34

35 **1. Introduction**

36 The order Rickettsiales was reorganized in 2001 based on 16S rRNA and *groEL* sequence
37 analyses and six *Anaplasma* species have been recognized in this genus: *Anaplasma bovis*,
38 *Anaplasma centrale*, *Anaplasma marginale*, *Anaplasma ovis*, *Anaplasma phagocytophilum* and
39 *Anaplasma platys* (Dumler et al., 2001). Since this last reclassification of Anaplasmataceae
40 twenty years ago, two new *Anaplasma* species have been identified, *Anaplasma odocoilei* and
41 *Anaplasma capra*, as well as a large number of unclassified genovariants that cannot be
42 assigned to known species (Rar et al., 2021). *Anaplasma* spp. are Gram-negative obligate
43 intracellular bacteria transmitted by ticks to vertebrate hosts, which may include humans. In
44 wild and domestic animals, bacteria infect hematopoietic cells and can cause a persistent
45 infection, which means that these hosts represent important long-term reservoirs (Rar et al.,
46 2021).

47 *Anaplasma capra* has been recently described in several Asian countries (China, Japan,
48 Malaysia and Korea), in domestic as well as wild animals. *Anaplasma capra* was named for the
49 first time in northern China following the isolation and characterization of this pathogen in 120
50 samples from asymptomatic goats in 2012 (Li et al., 2015). It was then reported in sheep from
51 12 provinces in China (Yang et al., 2017), in sheep and goats in China at 52 sites from 8
52 provinces (Peng et al., 2018) as well as in bovines from south Korea (Seo et al., 2018). In Japan,
53 *A. capra*-related infection of wild ruminants was detected a few years earlier (Kawahara et al.,
54 2006; Sato et al., 2009), and more recently also in Malaysia (Koh et al., 2018), China (Yang et
55 al., 2018) and South Korea (Amer et al., 2019). The host range of this species is not limited to
56 ruminants, as *A. capra* also infects dogs (Shi et al., 2019) and humans (Li et al., 2015),
57 suggesting a wide host range panel for this species. *Anaplasma capra* was indeed detected in
58 28 patients in northern China, in a survey of 477 patients suffering from non-specific febrile

59 manifestations, including fever, headache, malaise, myalgia, chills and report of a tick bite
60 within past 2 months (Li et al., 2015).

61 Based on *gltA* or *GroEL* phylogenies, two separate clades, I and II (thus named in Miranda et
62 al., 2021, also previously referred to as genotypes 1 and 2) are consistently delineated within *A.*
63 *capra* (Yang et al., 2018; Peng et al., 2018; Jouglin et al., 2019; Miranda et al., 2021). Clade I
64 gathers *A. capra* sequences from sheep, goats, cattle, dogs, humans and ticks. Clade II also
65 contains *A. capra* sequences from sheep and goats, and also from a variety of wild ruminants.

66 The geographical distribution of *A. capra* is not restricted to Asian countries, as our recent study
67 demonstrated its presence in red deer and swamp deer at a zoological park in France, the first
68 report of *A. capra* in Europe (Jouglin et al., 2019). The occurrence of *A. capra* on roe deer was
69 later reported in Spain, from several and geographically distant areas (Remesar et al., 2021).

70 This recent finding confirms the presence of *A. capra* on deer species in non-captive conditions,
71 and raises the question of its geographical distribution in Europe.

72 In this previous study (Jouglin et al., 2019), we discovered that the 23S rRNA deer sequences
73 of *A. capra* aligned closely with a sequence from an unknown *Anaplasma* species provisionally
74 named “Candidatus *Anaplasma mediterraneum*”, detected from sheep in Corsica (Dahmani et
75 al., 2017). Corsica is a French island located in the Mediterranean Sea, south east of the French
76 mainland (Figure 1). Corsica consists of two administrative departments (Corse - du - Sud and
77 Haute - Corse) and 360 communes (the smallest administrative unit in France) (Figure 1).

78 Animal husbandry represents an important economic activity (sheep, goats, cattle and pigs) on
79 the island. Various species of *Anaplasma* have been detected in Corsica: *A. ovis*, *A. bovis*, *A.*
80 *phagocytophilum* and *A. marginale* (Grech-Angelini et al, 2019; Dahmani et al., 2017) but the
81 occurrence of *A. capra* was never reported. We decided to conduct the present survey to

82 specifically search for *A. capra* in sheep and goats on this island, and thus to eventually confirm
83 autochthonous presence of *A. capra* in Europe.

84 **2. Material and methods**

85 *2.1. Animal sampling*

86 Between February and August 2020, we monitored infections on sheep and goat farms by
87 *Anaplasma* spp. in collaboration with the Corsican Livestock Health Defense Group (GDS). As
88 part of national surveillance for animal diseases, veterinarians collected goat and sheep blood
89 samples for this study. The manipulation and blood sampling of animals was approved by the
90 local animal ethical committee (CERVO request CERVO-2019-7-V). A total of 108 heparin
91 blood samples were obtained from domestic animals. On each farm, up to ten animals were
92 selected. In order to ensure that the animals had been in contact with ticks previously, the
93 sampled goats and sheep were at least 2 years old. Eleven farms were included in this study, 7
94 sheep farms and 4 goat farms, 7 from Corse-du-Sud and 4 from Haute-Corse (Figure 1). They
95 were localized in 9 geographically distant municipalities.

96 *2.2. Molecular detection and characterization of Anaplasma spp.*

97 Genomic DNA was extracted from blood following previously described protocols (Jouglin et
98 al., 2017). DNA was stored at -20°C until further use. Firstly, all samples were tested by a PCR
99 targeting the 16S RNA ribosomal gene: infection by *Anaplasma* spp. was screened using the
100 Ana16Sup1/Ana16Sdo3 primer pair which produces an amplicon of 1089 bp. Then a nested
101 PCR using the Ana16SIntup1/Ana16sdo1 primer pair was performed which produced a 581 bp
102 amplicon (Jouglin et al., 2019). All positive amplicons were sequenced to identify the detected
103 *Anaplasma* species. In order to further characterize the *Anaplasma* species, all samples were
104 also subjected to the amplification of heat-shock protein (*groEL*) and citrate synthase (*gltA*)
105 gene fragments, by nested PCR using *A. capra* specific primer sets (Li et al., 2015; Jouglin et
106 al., 2019).

107 To prevent cross contamination, DNA extraction, amplification and detection of PCR products
108 were done in separate rooms and even different buildings. A negative control (extraction and
109 amplification) was included to control potential contamination in each of these two procedures.
110 PCR products were analyzed on 1% agarose gels supplemented with ethidium bromide, and
111 visualized under UV light. A 100-bp DNA ladder (Solis BioDyne, Estonia) was used. The
112 amplicons were purified using ExoSAP-IT (Ozyme, France) and sequenced bi-directionally
113 (Sanger conventional sequencing, GATC, Germany).

114 2.3. *Sequence alignment and phylogenetic analysis*

115 After the sequencing, chromatograms were analyzed with 4peaks
116 (<https://nucleobytes.com/4peaks>) and cleaned sequences were identified using the BLASTn
117 program (<https://www.ncbi.nlm.nih.gov/Blast>). All sequences were aligned together to
118 identify potential nucleotide polymorphisms.

119 The sequences obtained in our study were first aligned with Muscle ([Edgar, 2004](#)) to determine
120 redundancy and to keep only unique sequences in the phylogenetic analyses. For phylogenies,
121 we built sequence collections of *Anaplasma* spp., choosing, when possible, several
122 representative sequences from each species of *Anaplasma* described so far. This collection was
123 completed with a BLASTn search against the nucleotide non-redundant (nr) database from
124 Genbank (<http://www.ncbi.nlm.nih.gov/genbank>). Then the unique sequences obtained from
125 this study were included. All sequences were aligned with Muscle (with the “codon” option for
126 *groEL* and *gltA*) and the resulting alignments were trimmed manually to contain only the region
127 corresponding to the longest unique sequences produced in our study. Then a phylogenetic tree
128 was obtained with the maximum likelihood method (ML), with the partial deletion option,
129 choosing the Tamura-Nei model of substitution with uniform rates. Phylogenies were tested by
130 bootstrap (n=100). All analyses (alignments and phylogenies) were performed using MegaX
131 ([Kumar et al., 2018](#)). Phylogenetic trees were edited graphically with Iroki ([Moore et al., 2020](#)).

132 3. Results

133 Out of 108 heparinized blood samples from sheep and goats, 26 (24%) were positive for *A.*
134 *capra* on the basis of cumulative 16S rRNA PCR amplification, sequencing and Blastn results.
135 The positivity rate was 30% (21/70) and 13% (5/38) in sheep and goats respectively. *A. capra*
136 was detected on four sheep farms and one goat farm, in both Haute-Corse and Corse du Sud
137 (Figure 1). The number of infected animals in each positive herd varied between 1 and 10, on
138 10 sampled animals. *Anaplasma capra* was not detected in 6 farms (3 sheep farms and 3 goat
139 farms), five in Corse du Sud and one in Haute-Corse.

140 Further analyses of the 108 blood samples yielded 20 partial *groEL* gene sequences and 18
141 partial *gltA* sequences from blood samples already found positive using 16S rRNA nPCR.
142 Sequence lengths ranged between 263-526 bp for the 16S rRNA gene, 420-735 bp for *gltA*, and
143 418-967 bp for *groEL*. All 16S rRNA (n=26) and *gltA* (n=18) sequences obtained in this study,
144 from either goat or sheep, were identical, and showed 100% identity with *A. capra* sequences
145 reported in red deer and swamp deer from the Haute Touche zoological park in France
146 (Genbank accession numbers MH084719-22). In Corsica, two different partial *A. capra groEL*
147 sequences were obtained, differing by a single SNP (G/A at position 623 when compared to
148 MH084717 from previous study), a substitution found in *A. capra* from only two sheep from
149 the same farm (OVI20-01-E10 and OVI20-05-E10). Otherwise, all Corsican sheep and goat *A.*
150 *capra groEL* partial sequences differed from continental deer sequences by another SNP (C/A
151 at position 425 when compared to MH084717). Identities between the *A. capra groEL*
152 sequences from Corsican sheep and goats and the *A. capra groEL* sequences from deer in
153 France ranged between 99.78 and 99.89%.

154 The *A. capra* partial and representative sequences obtained in this study were deposited in the
155 Genbank database under the following accession numbers: *groEL* (MW930530-32), *gltA*
156 (MW930533-35), and 16S rRNA (MW930536-38).

157 The phylogenetic study of 16S rRNA sequences showed that isolates from Corsica (this study)
158 grouped with other sequences assigned to *A. capra*, from different geographic origins (mostly
159 Asian countries), supported by a high bootstrap value (Figure 2). Of note, within this group, the
160 Corsican isolates grouped more closely with the two isolates from mainland France (Jouglin et
161 al., 2019) and with three isolates from Spain. However, as the 16S rRNA sequence is partial,
162 short, and highly conserved, this grouping of the isolates from mainland France, Corsica and
163 Spain was not strongly supported. The phylogenies of two coding genes, *gltA* and *groEL*, also
164 confirmed that the Corsican isolates belong to *A. capra* (Figures 3 and 4). Besides, sequences
165 from these two genes, more variable than the 16S rRNA, allowed to distinguish two well
166 differentiated clades within the *A. capra* species, also referred to as clades I and II in several
167 previous studies (Yang et al., 2017, 2018; Peng et al., 2018; Miranda et al., 2021). For both *gltA*
168 and *groEL*, we found that the Corsican isolates grouped in one of these clusters (clade II), and
169 that within this clade, they grouped with strong bootstrap support with the mainland France
170 isolates discovered in our previous study. The longest *A. capra groEL* sequence from roe deer
171 in Spain was included and also grouped with the sequences from the French isolates, forming
172 a potential European lineage.

173 **4. Discussion**

174 In this study we confirm the presence of *A. capra* in Corsica, both in goats and sheep, in about
175 half of the tested flocks, spatially well distributed on the island. The number of infected animals
176 was also quite high on some farms, with overall 24% of the small ruminants tested being
177 infected, indicating an important circulation of the bacteria and/or its maintenance in the host
178 once transmitted by ticks. This is the third report of *A. capra* in Europe, the second in France,
179 but this time on two different domestic animal species, and on several different locations in
180 Corsica, indicating the endemicity of this bacteria in this island.

181 The impact of *A. capra* on animal health has not been evaluated yet, but it was suspected to be
182 responsible of non-specific febrile manifestations (fever, headache, malaise, myalgia, chills) in
183 several tick-bitten patients in China (Li et al., 2015). In a future study, we will be evaluate a
184 potential role of *A. capra* on abortion by comparing *A. capra* prevalences in herds with history
185 of abortions and herds without.

186 *Anaplasma capra* exhibits at least two markedly divergent clades (named clades I and II or
187 genotypes 1 and 2 in Yang et al., 2017, 2018; Peng et al., 2018; Miranda et al., 2021).
188 Phylogenetic analysis of Corsican *A. capra*, especially thanks to the use of resolute markers
189 like the partial sequences of two coding genes, *gltA* and *groEL*, shows that all isolates from
190 sheep and goats in Corsica belong to clade II, which is relatively divergent from clade I. Human
191 isolates of *A. capra* belong to clade I, sheep and goat isolates belong to either clade I or II, while
192 most genotypes identified in wild ruminants (Asian or European) cluster within clade II (Yang
193 et al. 2018; Jouglin et al., 2019). Finally, we stress that the sequences described in this study
194 from Corsica showed a low level of genetic variation (all sequences for the three genes were
195 identical, with the exception of a single substitution for *groEL*) suggesting a possible single and
196 recent event of introduction of *A. capra*. Furthermore, the sequences from Corsica, mainland
197 France and Spain are genetically very close, although not identical. They indeed form a well-
198 supported clade (bootstrap value > 90 for *groEL*) within clade II. For *gltA*, we also found a
199 robust grouping (bootstrap value > 90) of the sequences from Corsica and mainland France (no
200 Spanish sequences were available for this gene). Overall, this suggests a common ancestry and
201 a relatively recent divergence on the European continent. New samples that could be discovered
202 elsewhere in Europe would greatly help to clarify the history of this pathogen on this continent.
203 *Anaplasma capra* was detected in several tick species in China (Li et al., 2015; Guo et al.,
204 2019), but the vectorial competence of these vectors for *A. capra* transmission has not yet been
205 evaluated. Nine different tick species have been identified on Corsican animals: *Rhipicephalus*

206 *bursa*, *Rhipicephalus sanguineus sensu lato*, *Rhipicephalus (Boophilus) annulatus*, *Hyalomma*
207 *marginatum*, *Hyalomma scupense*, *Dermacentor marginatus*, *Ixodes ricinus*, *Haemaphysalis*
208 *punctata* and *Haemaphysalis sulcata* (Grech-Angelini et al., 2016; 2020), but the almost sole
209 tick species found on sheep and goats in Corsica was *R. bursa* (Grech-Angelini et al., 2016;
210 Cabezas-Cruz et al., 2019), therefore a good candidate to be an *A. capra* vector.

211 **5. Conclusion**

212 The presence of *A. capra* was confirmed in 26 blood samples from five different farms from
213 Corsica, both in goats and sheep. Furthermore, our phylogenetic analyses indicate that the
214 newly identified *A. capra* isolates from Corsica are genetically very close to the *A. capra*
215 recently identified on the French mainland and to isolates from Spain. Corsica, mainland France
216 and Spanish isolates, despite host and geographical origin differences, cluster together in one
217 clade within clade II of *A. capra*, suggesting a relatively recent common ancestor of European
218 isolates.

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228

229 **Conflict of interest:** none.

230

231 **Authors' contribution**

232 **Maggy Jouglin:** Investigation, Formal analysis, Methodology, Visualization, Writing-

233 Original draft. **Claude Rispe:** Formal analysis, Visualization, Reviewing and Editing.

234 **Sébastien Grech-Angelini:** Resources, Reviewing and Editing. **Mélanie Gallois:** Resources,

235 Conceptualization, Supervision. **Laurence Malandrin:** Funding acquisition,

236 Conceptualization, Supervision, Writing-Original draft, Reviewing and Editing.

237

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354

355 **Legends of figures**

356

357 **Figure 1:** Map of Corsica (France) showing the location of goat and sheep farms and the number
358 of *A. capra* positive animals when detected in a farm.

359

360 **Figure 2:** Maximum likelihood phylogeny of partial 16S rRNA sequences of *Anaplasma*
361 isolates. Alignment length was 582 base pairs. Sequences of *Ehrlichia* and *Rickettsia* were
362 used as outgroups. Sequence labels are composed of the species names (between inverted
363 commas if needing re-assignment), accession number, origin (vertebrate host, or other),
364 country. Label of sequences obtained in this study is in blue. Representative sequences
365 from both goat and sheep samples obtained in this study were used in the phylogeny.
366 Branches corresponding to *A. capra* are in purple. High bootstrap support for nodes is shown
367 by filled circles (bootstrap > 90) or open circles (bootstrap > 80).

368

369

370 **Figure 3:** Maximum likelihood phylogeny of partial *gltA* sequences of *Anaplasma* isolates.
371 Alignment length was 762 base pairs. Sequences of *Ehrlichia* and *Rickettsia* were used as
372 outgroups. Sequence labels are composed of the species names (between inverted commas if
373 needing reassignment), accession number, origin (vertebrate host, or other), country. Label of
374 sequences obtained in this study is in blue. Representative sequences from both goat and sheep

375 samples obtained in this study were used in the phylogeny. Branches corresponding to *A. capra*
376 are in purple. High bootstrap support for nodes is shown by filled circles (bootstrap > 90) or
377 open circles (bootstrap > 80). Clades I and II are indicated as well as the European lineage
378 (green).

379

380 **Figure 4:** Maximum likelihood phylogeny of partial *groEL* sequences of *Anaplasma* isolates.
381 Alignment length was 987 base pairs. Sequences of *Ehrlichia* and *Rickettsia* were used as
382 outgroups. Sequence labels are composed of the species names (between inverted commas if
383 needing re-assignment), accession number, origin (vertebrate host, or other), country. Labels of
384 sequences obtained in this study are in blue. Representative sequences from both goat and sheep
385 samples obtained in this study were used in the phylogeny. Branches corresponding to *A. capra*
386 are in purple. High bootstrap support for nodes is shown by filled circles (bootstrap > 90) or
387 open circles (bootstrap > 80). Clades I and II are indicated as well as the European lineage
388 (green).



1/10 sheep

10/10 sheep

Departement

Corse-du-Sud

Haute-Corse

Goats farms sampled

▲ No positive animal

▲ At least one positive animal

Sheep farms sampled

● No positive animal

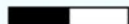
● At least one positive animal

2/10 sheep

5/10 goat

9/10 sheep

0 10 20 km



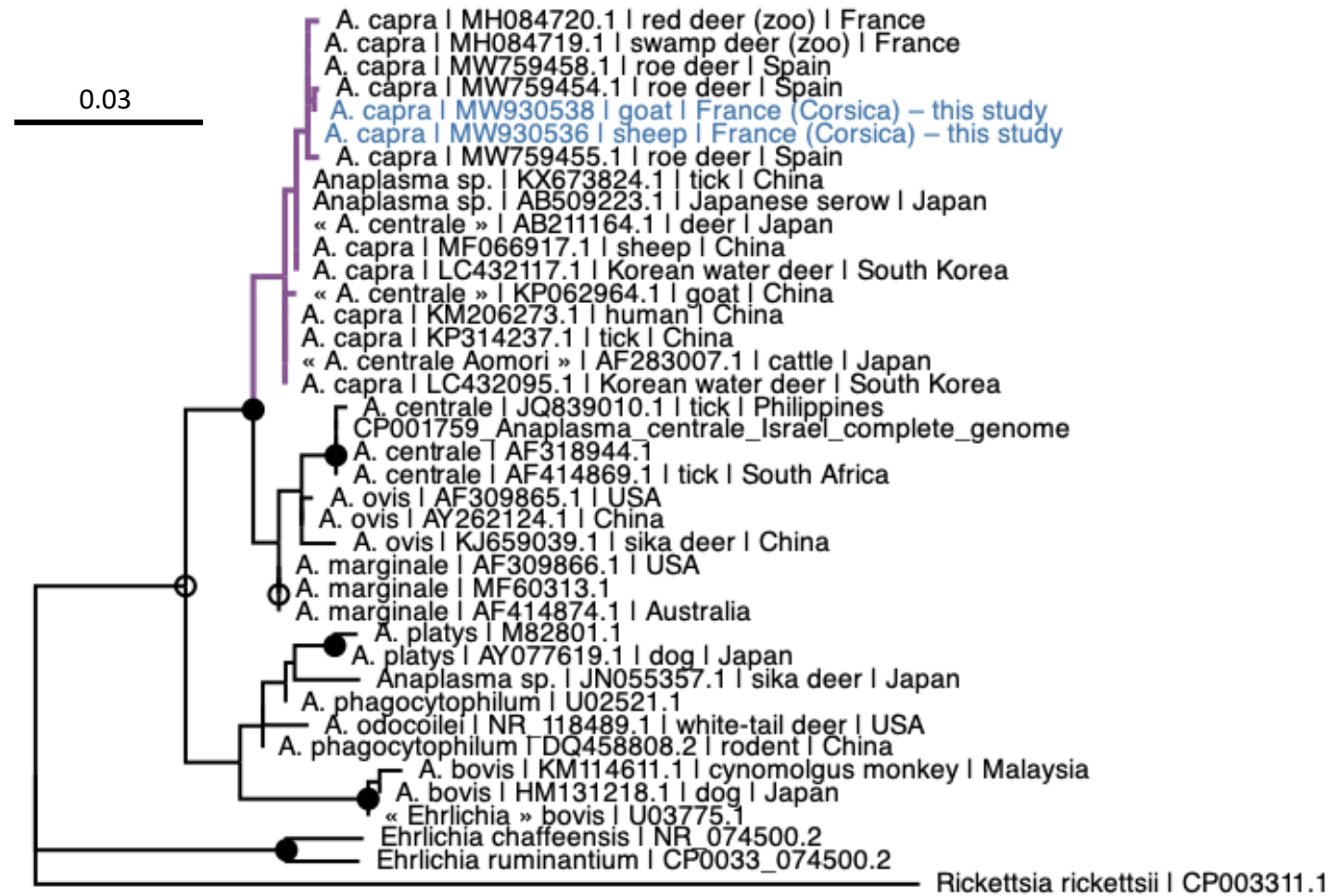


Figure 2: Maximum likelihood phylogeny of partial 16S rRNA sequences of *Anaplasma* isolates. Alignment length was 582 base pairs. Sequences of *Ehrlichia* and *Rickettsia* were used as outgroups. Sequence labels are composed of the species names (between inverted commas if needing re-assignment), accession number, origin (vertebrate host, or other), country. Label of sequences obtained in this study is in blue. Representative sequences from both goat and sheep samples obtained in this study were used in the phylogeny. Branches corresponding to *A. capra* are in purple. High bootstrap support for nodes is shown by filled circles (bootstrap > 90) or open circles (bootstrap > 80).

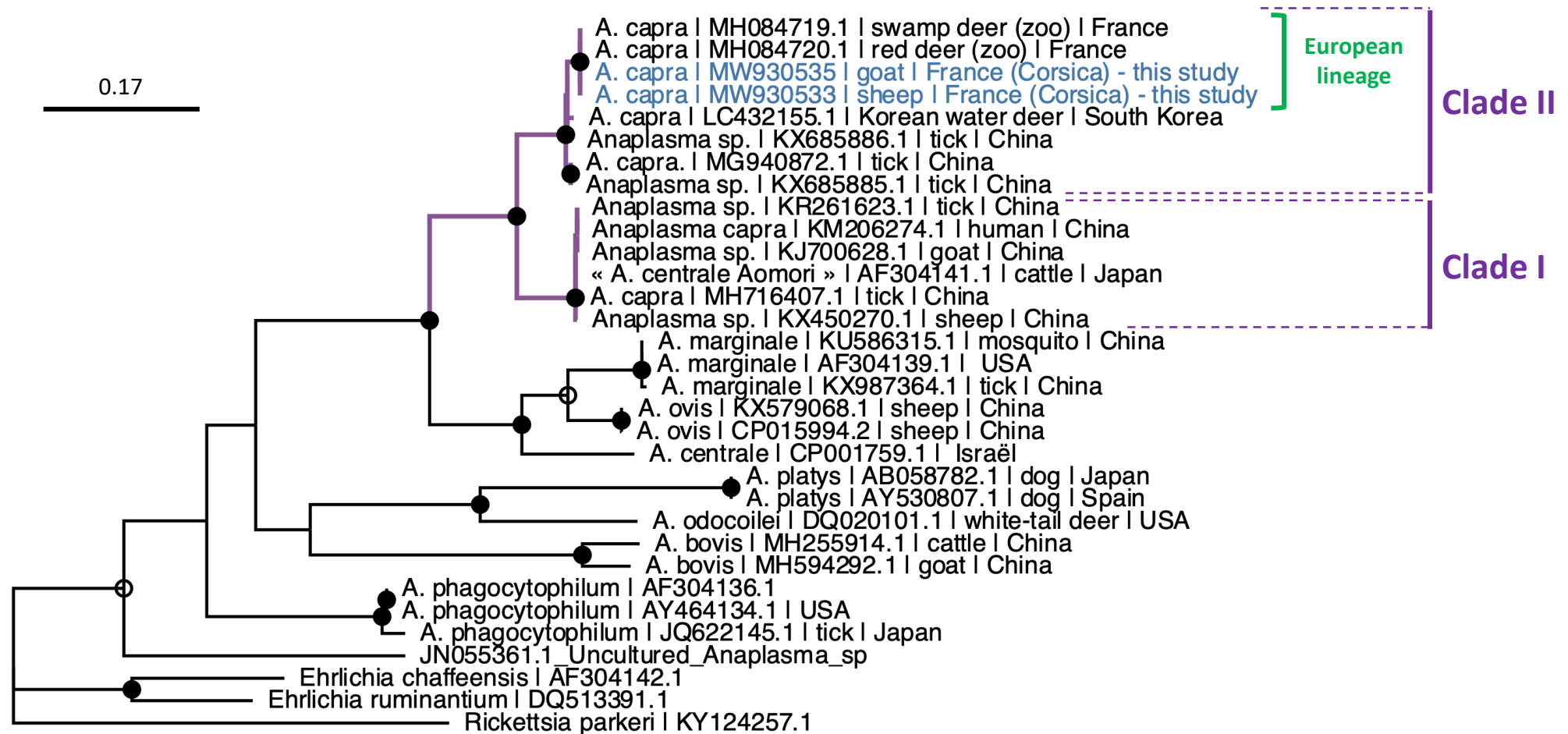


Figure 3: Maximum likelihood phylogeny of partial *gltA* sequences of *Anaplasma* isolates. Alignment length was 762 base pairs. Sequences of *Ehrlichia* and *Rickettsia* were used as outgroups. Sequence labels are composed of the species names (between inverted commas if needing reassignment), accession number, origin (vertebrate host, or other), country. Label of sequences obtained in this study is in blue. Representative sequences from both goat and sheep samples obtained in this study were used in the phylogeny. Branches corresponding to *A. capra* are in purple. High bootstrap support for nodes is shown by filled circles (bootstrap > 90) or open circles (bootstrap > 80). Clades I and II are indicated as well as the European lineage (green).

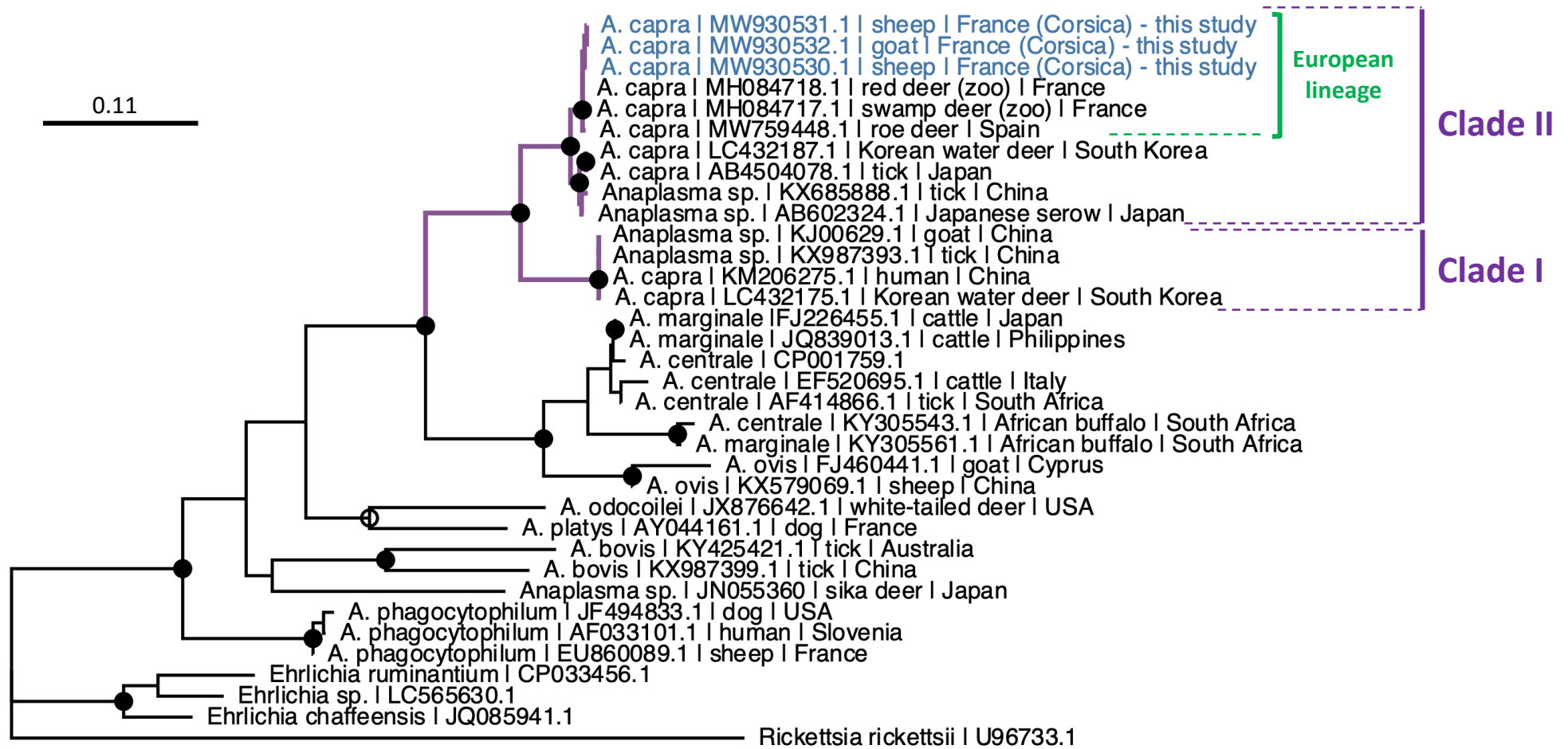


Figure 4: Maximum likelihood phylogeny of partial *groEL* sequences of *Anaplasma* isolates. Alignment length was 987 base pairs. Sequences of *Ehrlichia* and *Rickettsia* were used as outgroups. Sequence labels are composed of the species names (between inverted commas if needing re-assignment), accession number, origin (vertebrate host, or other), country. Labels of sequences obtained in this study are in blue. Representative sequences from both goat and sheep samples obtained in this study were used in the phylogeny. Branches corresponding to *A. capra* are in purple. High bootstrap support for nodes is shown by filled circles (bootstrap > 90) or open circles (bootstrap > 80). Clades I and II are indicated as well as the European lineage (green).