



**HAL**  
open science

## Non-contiguous finished genome sequence and description of *Bartonella saheliensis* sp. nov. from the blood of *Gerbilliscus gambianus* from Senegal

H. Dahmana, H. Medkour, H. Anani, Laurent Granjon, G. Diatta, F. Fenollar, O. Mediannikov

### ► To cite this version:

H. Dahmana, H. Medkour, H. Anani, Laurent Granjon, G. Diatta, et al.. Non-contiguous finished genome sequence and description of *Bartonella saheliensis* sp. nov. from the blood of *Gerbilliscus gambianus* from Senegal. *New Microbes and New Infections*, 2020, 35, pp.100667. 10.1016/j.nmni.2020.100667 . hal-02547902

**HAL Id: hal-02547902**

**<https://amu.hal.science/hal-02547902>**

Submitted on 22 Aug 2022

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons Attribution - NonCommercial - NoDerivatives 4.0 International License

**Noncontiguous finished genome sequence and description of *Bartonella sahelensis* sp.  
nov. from the blood of *Gerbilliscus gambianus* from Senegal**

Handi DAHMANA<sup>1,2</sup>, Hacène MEDKOUR<sup>1,2</sup>, Hussein ANANI<sup>2,3</sup>, Laurent Granjon<sup>4</sup>,  
Georges Diatta<sup>5</sup>, Florence FENOLLAR<sup>2,3</sup>, Oleg MEDIANNIKOV<sup>1,2\*</sup>

<sup>1</sup> Aix Marseille Univ, IRD, AP-HM, MEPHI, Marseille, France

<sup>2</sup> IHU-Méditerranée Infection, Marseille, France

<sup>3</sup> Aix Marseille Univ, IRD, AP-HM, SSA, VITROME, Marseille, France

<sup>4</sup> CBGP, IRD, CIRAD, INRA, Montpellier SupAgro, Univ Montpellier, Montpellier, France

<sup>5</sup> Campus Commun UCAD-IRD of Hann, Dakar, Senegal

**Corresponding author:** Oleg MEDIANNIKOV

**Address:** MEPHI, IRD, APHM, IHU-Méditerranée Infection, 19-21 Boulevard Jean Moulin,  
13385 Marseille Cedex 05

Tel: +33 (0)4 13 73 24 01, Fax: +33 (0)4 13 73 24 02, E-mail: olegusss1@gmail.com

**Word abstract count:** 75

**Word text count:** 2262

**Keywords:** *Bartonella*; *Bartonella sahelensis* sp. nov.; genome; *Gerbilliscus gambianus*;  
rodents; Senegal

1 **Abstract**

2 *Bartonella sahelensis* strain 077 (= CSUR B644<sup>T</sup>; = DSM 28003<sup>T</sup>) is a new bacterial species  
3 isolated from the rodent *Gerbilliscus gambianus* blood captured in the Sine-Saloum region of  
4 Senegal. In this work we describe both the characteristics of this microorganism, as well as  
5 the complete sequence of the genome and simultaneously its annotation. Its genome has  
6 2,327,299 bp, which includes 38.4% of G + C content and code for 2,015 proteins and 53  
7 RNA genes.

## 8 Introduction

9 *Bartonella* species are Gram-negative, small (0.5–0.6 × 1.0 µm) slightly curved rod-  
10 shaped fastidious bacteria. They may be seen in stained blood films appearing as rounded or  
11 ellipsoidal forms or as slender, straight, curved, or bent rods, occurring singly or in groups. It  
12 is the monotypic genus of the family *Bartonellaceae* among *Alphaproteobacteria*, and was  
13 described by Alberto L. Barton in 1909, after studying the agent of Carrion's disease [1].  
14 They are facultatively intracellular and use hemotrophy (infection of erythrocytes) as a  
15 parasitic strategy [2]. To date, just over thirty species have been described and officially  
16 validated and many others have not yet been described [3]. The species of *Bartonella* genus  
17 infects a wide range of animal species, including domestic animals, such as cats, dogs,  
18 rodents, rabbits and cattle as well as a diverse group of wild animals including wildcats,  
19 coyotes, deer, elks, foxes, insectivores and bats [3].

20 New species are always isolated and then characterized from rodents or their  
21 ectoparasites [4][5][6][7][8]. Interestingly, more than half of the species characterized are  
22 harboured by rodents and lagomorphs, such as *B. tribocorum*, *B. grahamii*, *B. elizabethae*, *B.*  
23 *vinsonii* subsp. *arupensis*, *B. washoensis* and *B. alsatica* which are known to be potentially  
24 zoonotic [9]. Although, in rodents ectoparasites, high prevalence's of zoonotic bartonellosis  
25 agents are found (43.75% of *B. elizabethae* in *Stenoponia tripectinata tripectinata*) [10].

26 *Bartonella* species are transmitted by different insects like lice, dipterans and fleas that are  
27 respectively the main vectors of *B. quintana*, *B. bacilliformis* and *B. henselae*, while the role  
28 of ticks in the transmission of bartonellosis remains uncertain [1][11][12][13]. The presence  
29 of *Bartonella* DNA does not necessarily mean that they transmit it to mammals [12].

30 Commensal rodents and associated soft ticks are vectors of relapsing fever caused by  
31 *Borrelia crocidurae* in Senegal [14]. To investigate the presence of *Bartonella* spp. in Sine-  
32 Saloum region, rodents and insectivores were captured alive in February 2013; 30 isolates of

33 *Bartonella* spp. were recovered from their blood. None of the isolates belonged to already  
34 described *Bartonella* species. The phylogenetic analysis showed that they belonged to three  
35 separate genetic clusters within the genus *Bartonella*. Comparison of *gltA* genes of recovered  
36 isolates with those of officially recognized species allowed to conclude that three clusters may  
37 present three separate new species of *Bartonella* [4].

38 In the present paper, we describe one of these *Bartonella* species, *Bartonella sahelensis*  
39 strain 077, isolated from the blood of *Gerbilliscus gambianus* in Senegal [4]. The bacterium  
40 strain was cultured and isolated. A taxono-genomics approach including matrix-assisted laser  
41 desorption-ionization time-of-flight mass spectrometry (MALDI-TOF MS) coupled with  
42 phylogenetic analysis was used, as well as main phenotypic description and genome  
43 sequencing, in order to fully describe it [15][16]. Here, we present a summary classification  
44 and a set of features for *B. sahelensis* sp. nov. strain 077 together with the description of the  
45 complete genomic sequence and annotation. All these characteristics support the definition of  
46 the species *B. sahelensis*.

#### 47 **Samples and bacterial culture**

48 In February 2013, as part of a 6-day prospective study on tick-borne relapsing fever in  
49 West Africa, 119 small mammals were captured alive in two sites (Dielmo and Ndiop) using  
50 wire mesh traps baited with peanut butter or onions: 116 rodents and 3 shrews (*Crocidura* cf.  
51 *olivieri*). The rodents were morphologically identified: 5 *Arvicantis niloticus*, 56 *Gerbilliscus*  
52 *gambianus*, 49 *Mastomys erythroleucus*, 5 *Mus musculus*, and 1 *Praomys daltoni*. They were  
53 anaesthetized and opened under sterile conditions. The isolation of the *Bartonella* strains was  
54 performed as described previously [6]. Briefly, blood was inoculated on Columbia agar plates  
55 supplemented with 5% sheep blood (bioMérieux, Marcy l'Etoile, France) and incubated at  
56 37°C in a 5% CO<sub>2</sub>-enriched atmosphere. Thus, 30 isolates of *Bartonella* spp. were recovered  
57 from the blood of rodents.

## 58 **Classification and features**

59       None of the isolates belonged to the *Bartonella* species that have already been  
60 described. The *gltA*, *rpoB*, 16S rRNA, *ftsZ* genes, and ITS were amplified and sequenced  
61 from the recovered *Bartonella* isolates. Phylogenetic analysis based on the *gltA* genes showed  
62 that the recovered isolates form three distinct groups compared to those of officially  
63 recognized species. This has led to the conclusion that these three groups may present three  
64 distinct new *Bartonella* species. Candidatus “*Bartonella raoultii*” (1 isolate) has not yet been  
65 described, *Bartonella mastomydis* (21 isolates) has been previously reported [17]. Both were  
66 recovered from *Mastomys erythroleucus*. However, the species we describe here, *Bartonella*  
67 *saheliensis* (8 isolates) has been recovered from *Gerbilliscus gambianus* only.

68       The closest validated *Bartonella* species to isolate 077 when compared to the 16S rRNA  
69 gene is *B. queenslandensis* (NR116176) with 99.5%. when other genes (*ftsZ*, *rpoB*, and *gltA*)  
70 and for ITS, were compared, the closest identity (95.6%, 94.8%, 95.6% and 86.8%,  
71 respectively) was found with *B. elizabethae* (LR134527). We used validated 16S rRNA  
72 sequences of *Bartonella* species to highlight the phylogenetic position of the isolate 077  
73 relative to the others (**Figure 1**).

74       Strain 077 (**Table 1**) was isolated from the *G. gambianus* blood after 10 days of culture.  
75 Subsequently, MALDI-TOF MS analysis has been performed on a Microflex LT spectrometer  
76 (Bruker Daltonics, Bremen, Germany), as previously described [18]. The spectra obtained  
77 (**Figure 2**) were imported into MALDI Biotyper 3.0 software (Bruker Daltonics) and analysed  
78 against the main spectra of the bacteria included in two databases (Bruker and constantly  
79 updated MEPHI databases).  
80 (<http://www.mediterraneeinfection.com/article.php?larub=280&titre=urms-database>). Briefly,  
81 the identification method included the m/z from 3000 to 15,000 Da. For each spectrum, a

82 maximum of 100 peaks was considered and compared with the spectra in the database and a  
83 score below 1.7 meant that identification was not possible.

84 The obtained scores of *B. sahelensis* strain 077 were always below 1.7, which  
85 confirmed that it was not a member of a known species. Thus, its spectrum was added to the  
86 database. A dendrogram comparing the spectrum of *B. sahelensis* strain 077 with those of  
87 other *Bartonella* species is shown in **Figure 3**.

### 88 **Biochemical characterization and images acquisition**

89 For its growth, different temperatures were tested (32, 37, and 42°C). The optimal  
90 growth was obtained at 32°C in 5% CO<sub>2</sub> atmosphere. The colonies had 0.3 mm to 1 mm in  
91 diameter; they were grey and opaque on blood enriched Columbia agar. Cells grown on agar  
92 are Gram negative and have a mean length and width of 1.05± 0.08 µm and 0.6±0.05 µm,  
93 respectively.

94 All specimens or samples or conditioning tested tubes were cyto-centrifuged on  
95 cytospin slides. Slides were then processed to images acquisition and were stained with PTA  
96 (phosphotungstic acid 1 %) in order to check any differences or morphological changes.  
97 We used a tabletop scanning electron microscope SEM (Hitachi TM4000) with approximately  
98 60 centimeters in height and 33 cm in width to evaluate bacterial structures. The SEM has a  
99 capability of observing specimen under low vacuum pressure (10<sup>0</sup> Pa to 10<sup>1</sup> Pa) to reduce  
100 charge-up on specimen surface by the irradiated electrons. Evacuation time after loading of  
101 specimen into the SEM Chamber is shorter than 2 minutes, which is much quicker than  
102 conventional SEMs with high vacuum condition. All samples were acquired at the same  
103 acquisition settings regarding magnification, intensity and voltage mode. All settings are  
104 displayed on micrographs.

105 Neither flagella, nor pili were observed using electron microscopy. *B. sahelensis* strain  
106 077 showed also did not show catalase or oxidase activity (**Figure 4**). Biochemical

107 characteristics were assessed using API 50 CH (bioMérieux, Marcy l'Etoile, France), API  
108 ZYM (bioMérieux) and API Coryne (bioMérieux); none of the available biochemical tests  
109 were positive. Similar patterns have previously been observed for *B. senegalensis* [19], *B.*  
110 *mastomydis* [17] and *B. massiliensis* [8].

## 111 **Genome sequencing information**

### 112 ***Genome project history***

113 On the basis of La Scola's criteria that include the similarity of 16S rRNA, ITS, *ftsZ*,  
114 *gltA*, and *rpoB* genes to classify the members of the family Bartonellaceae, strain 077 was  
115 considered as a new species within the genus *Bartonella* and selected for genome sequencing.  
116 This genome is the twenty-first of *Bartonella* species and the first of *Bartonella saheliensis*  
117 sp. nov. It was assembled and deposited under the following GenBank accession number:  
118 CABGUM010000001-CABGUM010000132. A summary of project information is presented  
119 in **Table 2**.

120 *Bartonella saheliensis* strain 077 (= CSUR B644<sup>T</sup>; = DSM 28003<sup>T</sup>) was cultured on  
121 Columbia agar enriched with sheep blood (bioMérieux) with 5% CO<sub>2</sub> at 32°C. Bacteria  
122 growing on two Petri dishes were recovered and then resuspended in 6x100µl of G2 buffer. A  
123 first mechanical lysis was performed with glass powder using the Fastprep-24 device (MP  
124 Biomedicals, Graffenstaden, France) during 2x20 seconds. Then, after 30 minutes' lysozyme  
125 incubation at 37°C, DNA was extracted on the EZ1 biorobot (Qiagen, Hilden, Germany) with  
126 the EZ1 DNA tissue kit. DNA was quantified by Quant-iT™ PicoGreen™ dsDNA Assay Kit  
127 (Invitrogen, Waltham, Massachusetts, USA) to 98.2 ng/µl.

### 128 ***Genome sequencing and assembly***

129 Genomic DNA (5 µg) was mechanically fragmented on a Hydroshear device (Digilab,  
130 Holliston, MA, USA) with an enrichment size of 3-4 kb. After that, the visualization was  
131 performed using the Agilent 2100 BioAnalyzer on a DNA labchip 7500 with an optimal size



132 of 3.475 kb. The library was constructed according to the 454 GS FLX Titanium paired-end  
133 protocol. Circularization and nebulization were performed and generated a pattern with an  
134 optimum at 641 bp. After PCR amplification over 17 cycles followed by double size  
135 selection, the single-stranded paired-end library was quantified on the Quant-it Ribogreen  
136 (Invitrogen) on the Genios\_Tecan fluorometer at 7,360 pg/ $\mu$ L. The library concentration  
137 equivalence was calculated as 9.24E+08 molecules/ $\mu$ L. The library was stored at - 20°C until  
138 further use.

139 The library was clonally amplified with 1 cpb and 1.5 cpb in 4 and 3 emPCR reactions,  
140 respectively, with the GS Titanium SV emPCR Kit (Lib-L) v2(Roche, Basel, Switzerland).  
141 The yields of the 1 cpb and 1.5 cpb emPCR were determined to be 3.08% and 8%,  
142 respectively. After amplification, 790,000 beads from the 2 emPCR conditions were loaded  
143 on a  $\frac{1}{4}$  region on the GS Titanium PicoTiterPlate PTP Kit 70 $\times$ 75 and sequenced with the GS  
144 FLX Titanium Sequencing Kit XLR70 (Roche). The run was analysed on the cluster using the  
145 gsRunBrowser and Newbler assembler (Roche). A total of 200,243 passed filter wells were  
146 obtained and generated 57.62 Mb of DNA sequence with an average length of 287 bp. The  
147 passed filter sequences were assembled using gsAssembler with 90% identity and 40 bp for  
148 overlap requirements. The final assembly identified 132 scaffolds and 173 large contigs  
149 ( $\geq$ 1,500 bp), generating a genome size of 2,327,299-bp, which corresponds to 28 $\times$  equivalent  
150 genome.

### 151 ***Genome annotation***

152 Open reading frames (ORFs) were predicted using PRODIGAL [20] using default  
153 parameters, but predicted ORFs were excluded if they spanned a sequencing gap region. The  
154 predicted bacterial protein sequences were searched against the GenBank database [21] using  
155 BLASTP and the Clusters of Orthologous Groups (COG) database using COGNITOR [22].  
156 The prediction of RNA genes, i.e., rRNAs, tRNAs and other RNAs, was performed using the

157 RNAmmer [23] and ARAGORN [24] algorithms. The transmembrane helices and signal  
158 peptides were identified using TMHMM v.2.0 [25] and SignalP [26], respectively.

### 159 **Genome properties**

160 The genome is 2,327,299-bpbp long with 38.4 mol% GC content (**Figure 5**). It is  
161 composed of 173 contigs. Of the 2,015 predicted genes, 1,925 were protein-coding genes and  
162 53 were RNAs (including one tmRNA, 6 rRNA, and 46 tRNA genes). A total of 949 genes  
163 (47%) were assigned a putative function (by COG or NR blast). The distribution of genes into  
164 COGs functional categories is presented in **Table 3**.

165 The properties and the statistics of the genome are summarized in **Tables 3** and **4**. The  
166 degree of genomic similarity of *B. saheliensis* strain 077 closely related species was estimated  
167 using the OrthoANI software [27]. Values among closely related species (**Figure 6**) ranged  
168 from 81.45%, between *B. massiliensis* strain OS09T and *B. rattaaustraliani* AUST NH4, to  
169 95.23%, between *B. elizabethae* strain NCTC12898 and *B. mastomydis*. When the isolate was  
170 compared to these closely species, values ranged from 81.47% with *B. rattaaustraliani* AUST  
171 NH4 to 91.13% with *B. elizabethae* strain NCTC12898.

### 172 **Conclusion**

173 Based on what was described in this paper, including unique phenotypic and genotypic  
174 characteristics using MALDI-TOF spectrum with sequencing of the 16S rRNA, ITS, *ftsZ*,  
175 *rpoB*, and *gltA* genes (sequence divergences >99.5%, >86.8.5%, >95.6%, >94.8%, and  
176 >95.6%, respectively), and an OrthoANI value of about only 95% with the phylogenetically  
177 closest species with standing in nomenclature.

178 Consequently, we propose *B. saheliensis* strain 077 as the type strain of *Bartonella*  
179 *saheliensis* sp. nov., a new bacterial species within the family Bartonellaceae. The strain was  
180 isolated from the blood of *Gerbilliscus gambianus* captured in the Sine-Saloum region of  
181 Senegal.

182

183 **Description of *Bartonella sahelensis* sp. nov.**

184 *Bartonella sahelensis* sp. nov., (sah.el.li.en'sis. L. masc. adj. sahelensis of Sahel, the  
185 ecoclimatic and biogeographic zone of transition in Africa between the Sahara in the north  
186 and the Sudanian Savanna in the south, common living region of the *Gerbilliscus gambianus*  
187 from which the type strain has been isolated) is a nonmotile, Gram-negative rod. Colonies are  
188 opaque, grey with a diameter of 0.3 to 1 mm on Columbia blood-enriched agar. Optimal  
189 growth is observed at 32°C in an aerobic atmosphere. Length and width are  $1.05\pm 0.08$   $\mu\text{m}$  and  
190  $0.6\pm 0.05$   $\mu\text{m}$ , respectively. Cells are rod shaped without flagella or pili. *Bartonella sahelensis*  
191 sp. nov., strain 077 exhibits low biochemical and enzymatic activities. The genome size and  
192 GC content are 2.23 Mb and 38.4 mol%, respectively. The type strain 077 (= CSUR B644<sup>T</sup>; =  
193 DSM 28003<sup>T</sup>) was isolated from the blood of *Gerbilliscus gambianus* captured in the Sine-  
194 Saloum region of Senegal.

195 **Nucleotide sequence accession number**

196 The complete annotation, as well as genome sequences of *Bartonella sahelensis* sp.  
197 nov., strain 077 are deposited in GenBank under accession numbers: NZ\_LR607204 and  
198 CABGUM010000001-CABGUM010000132, respectively.

199 **Deposit in culture collections**

200 Strain 077 was deposited in two different strain collections under numbers: CSUR  
201 B644<sup>T</sup> and DSM 28003<sup>T</sup>.

202

203 **Conflict of interest**

204 None to declare.

205 **Acknowledgements**

206 The authors thank Cheikh Sokhna for the assistance and organisation of field missions,  
207 Maxence Aubadie-Ladrix† and Masse Sambou for the field work, Catherine Robert for  
208 sequencing the genome, Aurelia Caputo for submitting the genomic sequence to GenBank,  
209 Robinne Simon and Carine Couderc for performing the MALDI-TOF reference spectrum.

210 We also thank Takashi Irie, Kyoko Imai, Shigeki Matsubara, Taku Sakazume, Yusuke  
211 Ominami, Hisada Akiko and the Hitachi team of Japan (Hitachi High-Technologies  
212 Corporation, Science & Medical Systems Business Group 24-14, Nishi-shimbashi 1-chome,  
213 Minato-ku, Tokyo 105-8717 Japan) for the collaborative study conducted together with the  
214 IHU Méditerranée Infection, and for the installation of a TM4000 microscope at the IHU  
215 Méditerranée Infection.

216

217 **Funding sources**

218 This study was supported by the Institut Hospitalo-Universitaire (IHU) Méditerranée  
219 Infection, the National Research Agency under the program « Investissements d’avenir »,  
220 reference ANR-10-IAHU-03.

221

## References

- 222 1. Welch DF. *Bartonella*. Bergey's Manual of Systematics of Archaea and Bacteria.  
223 Chichester, UK: John Wiley & Sons, Ltd; 2015. pp. 1–15.  
224 doi:10.1002/9781118960608.gbm00794
- 225 2. Birtles RJ. Bartonellae as elegant hemotropic parasites. Annals of the New York  
226 Academy of Sciences. 2005. pp. 270–279. doi:10.1196/annals.1355.044
- 227 3. Mediannikov O, Karkouri K El, Robert C, Fournier PE, Raoult D. Non-contiguous  
228 finished genome sequence and description of *Bartonella florenciae* sp. nov. Stand  
229 Genomic Sci. 2013;9: 185–196. doi:10.4056/sigs.4358060
- 230 4. Mediannikov O, Aubadie M, Bassene H, Diatta G, Granjon L, Fenollar F. Three new  
231 *Bartonella* species from rodents in Senegal. Int J Infect Dis. 2014;21: 335.  
232 doi:10.1016/j.ijid.2014.03.1112
- 233 5. Gundi VAKB, Davoust B, Khamis A, Boni M, Raoult D, La Scola B. Isolation of  
234 *Bartonella rattimassiliensis* sp. nov. and *Bartonella phoceensis* sp. nov. from European  
235 *Rattus norvegicus*. J Clin Microbiol. 2004;42: 3816–3818.  
236 doi:10.1128/JCM.42.8.3816-3818.2004
- 237 6. Heller R, Riegel P, Hansmann Y, Delacour G, Bermond D, Dehio C, et al. *Bartonella*  
238 *tribocorum* sp. nov., a new *Bartonella* species isolated from the blood of wild rats. Int J  
239 Syst Bacteriol. 1998;48: 1333–1339. doi:10.1099/00207713-48-4-1333
- 240 7. Gundi VAKB, Taylor C, Raoult D, La Scola B. *Bartonella rattaaustraliani* sp. nov.,  
241 *Bartonella queenslandensis* sp. nov. and *Bartonella coopersplainsensis* sp. nov.,  
242 identified in Australian rats. Int J Syst Evol Microbiol. 2009;59: 2956–2961.  
243 doi:10.1099/ijs.0.002865-0
- 244 8. MEDKOUR H, Ibrahima LO C, ANANI H, FENOLLAR F, MEDIANNIKOV O.  
245 *Bartonella massiliensis* sp. nov., a new bacterial species isolated from an *Ornithodoros*  
246 *sonrai* tick from Senegal. New Microbes New Infect. 2019; 100596.  
247 doi:10.1016/j.nmni.2019.100596
- 248 9. Kraljik J, Paziewska-Harris A, Miklisová D, Blaňarová L, Mošanský L, Bona M, et al.  
249 Genetic diversity of *Bartonella* genotypes found in the striped field mouse (*Apodemus*  
250 *agrarius*) in Central Europe. Parasitology. 2016;143: 1437–1442.  
251 doi:10.1017/S0031182016000962
- 252 10. Abreu-yanes E, Martin-alonso A, Martin-carrillo N, Livia KG, Marrero-gagliardi A,  
253 Valladares B, et al. *Bartonella* in Rodents and Ectoparasites in the Canary Islands ,  
254 Spain : New Insights into Host – Vector – Pathogen Relationships. 2018; 264–273.  
255 doi:10.1007/s00248-017-1022-y
- 256 11. Angelakis E, Billeter SA, Breitschwerdt EB, Chomel BB, Raoult D. Potential for Tick-  
257 borne Bartonellosis. Emerg Infect Dis. 2010;16: 385–391.  
258 doi:10.3201/eid1603.091685
- 259 12. Angelakis E, Pulcini C, Waton J, Imbert P, Socolovschi C, Edouard S, et al. Scalp  
260 Eschar and Neck Lymphadenopathy Caused by *Bartonella henselae* after Tick Bite .  
261 Clin Infect Dis. 2010;50: 549–551. doi:10.1086/650172
- 262 13. Telford SR, Wormser GP. *Bartonella* spp. transmission by ticks not established.

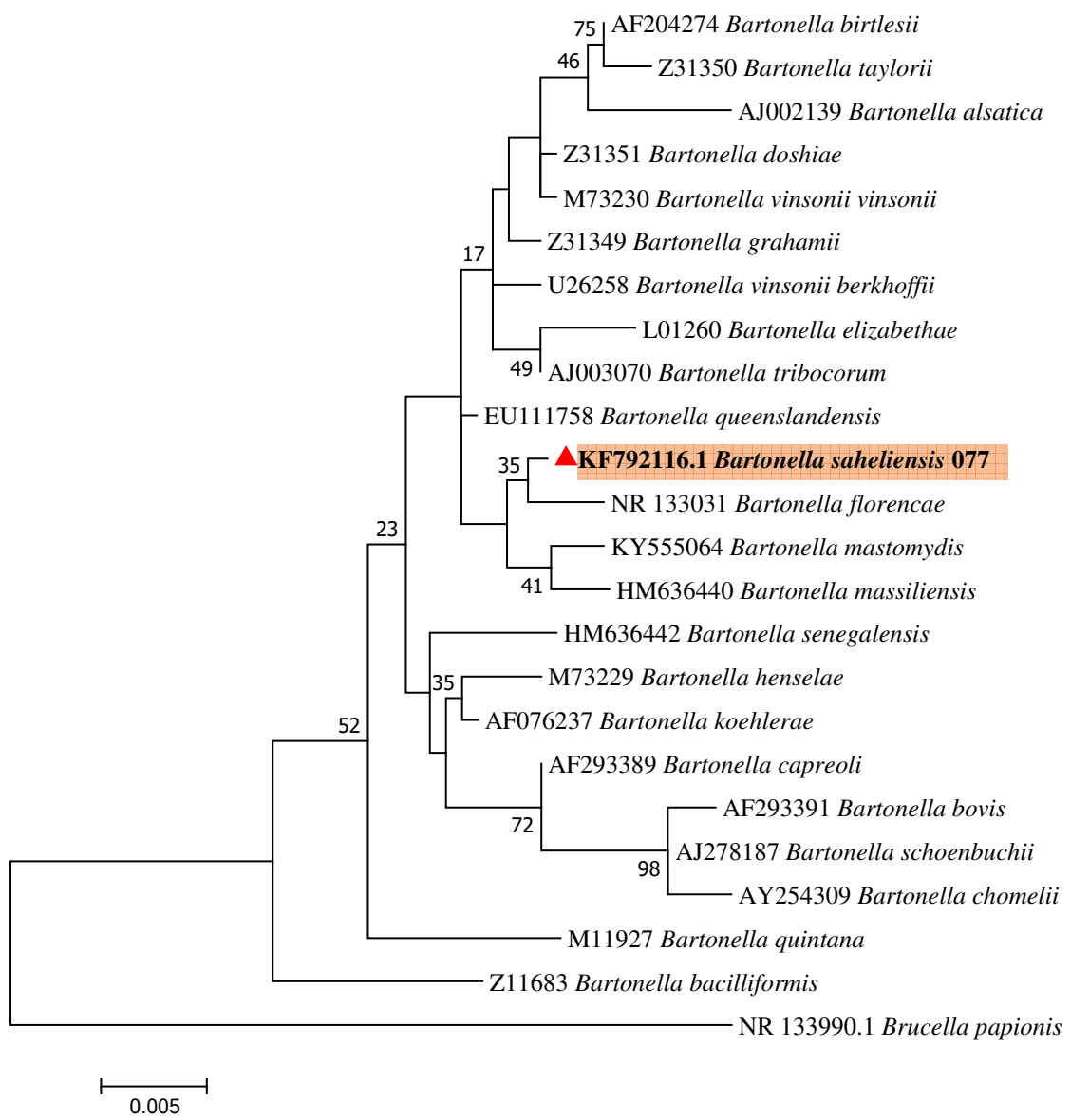
- 263 Emerging Infectious Diseases. 2010. pp. 379–384. doi:10.3201/eid1603.090443
- 264 14. Mediannikov O, Socolovschi C, Bassene H, Diatta G, Ratmanov P, Fenollar F, et al.  
265 *Borrelia crociduræ* infection in acutely febrile patients, Senegal. *Emerg Infect Dis*.  
266 2014;20: 1335–1338. doi:10.3201/eid2008.130550
- 267 15. Ramasamy D, Mishra AK, Lagier J-C, Padhmanabhan R, Rossi M, Sentausa E, et al. A  
268 polyphasic strategy incorporating genomic data for the taxonomic description of novel  
269 bacterial species. *Int J Syst Evol Microbiol*. 2014;64: 384–91.  
270 doi:10.1099/ijs.0.057091-0
- 271 16. Fournier P-E, Lagier J-C, Dubourg G, Raoult D. From culturomics to  
272 taxonomogenomics: A need to change the taxonomy of prokaryotes in clinical  
273 microbiology. *Anaerobe*. 2015;36: 73–8. doi:10.1016/j.anaerobe.2015.10.011
- 274 17. Dahmani M, Diatta G, Labas N, Diop A, Bassene H, Raoult D, et al. Noncontiguous  
275 finished genome sequence and description of *Bartonella mastomydis* sp. nov. *New*  
276 *Microbes New Infect*. 2018;25: 60–70. doi:10.1016/j.nmni.2018.03.005
- 277 18. Seng P, Drancourt M, Gouriet F, La Scola B, Fournier P-E, Rolain JM, et al. Ongoing  
278 revolution in bacteriology: routine identification of bacteria by matrix-assisted laser  
279 desorption ionization time-of-flight mass spectrometry. *Clin Infect Dis*. 2009;49: 543–  
280 51. doi:10.1086/600885
- 281 19. Mediannikov O, El Karkouri K, Diatta G, Robert C, Fournier P-E, Raoult D. Non-  
282 contiguous finished genome sequence and description of *Bartonella senegalensis* sp.  
283 nov. *Stand Genomic Sci*. 2013;8: 279–289. doi:10.4056/sigs.3807472
- 284 20. Hyatt D, Chen G-L, Locascio PF, Land ML, Larimer FW, Hauser LJ. Prodigal:  
285 prokaryotic gene recognition and translation initiation site identification. *BMC*  
286 *Bioinformatics*. 2010;11: 119. doi:10.1186/1471-2105-11-119
- 287 21. BLAST: Basic Local Alignment Search Tool [Internet]. [cited 16 Sep 2019]. Available:  
288 <https://blast.ncbi.nlm.nih.gov/Blast.cgi>
- 289 22. Tatusov RL, Galperin MY, Natale DA, Koonin E V. The COG database: a tool for  
290 genome-scale analysis of protein functions and evolution. *Nucleic Acids Res*. 2000;28:  
291 33–6. doi:10.1093/nar/28.1.33
- 292 23. Lagesen K, Hallin P, Rødland EA, Stærfeldt HH, Rognes T, Ussery DW. RNAmmer:  
293 Consistent and rapid annotation of ribosomal RNA genes. *Nucleic Acids Res*. 2007;35:  
294 3100–3108. doi:10.1093/nar/gkm160
- 295 24. Laslett D, Canback B. ARAGORN, a program to detect tRNA genes and tmRNA genes  
296 in nucleotide sequences. *Nucleic Acids Res*. 2004;32: 11–6. doi:10.1093/nar/gkh152
- 297 25. TMHMM Server, v. 2.0 [Internet]. [cited 16 Sep 2019]. Available:  
298 <http://www.cbs.dtu.dk/services/TMHMM/>
- 299 26. Petersen TN, Brunak S, Von Heijne G, Nielsen H. SignalP 4.0: Discriminating signal  
300 peptides from transmembrane regions. *Nature Methods*. 2011. pp. 785–786.  
301 doi:10.1038/nmeth.1701
- 302 27. Lee I, Ouk Kim Y, Park S-C, Chun J. OrthoANI: An improved algorithm and software  
303 for calculating average nucleotide identity. *Int J Syst Evol Microbiol*. 2016;66: 1100–  
304 1103. doi:10.1099/ijsem.0.000760

- 305 28. Woese CR, Kandler O, Wheelis ML. Towards a natural system of organisms: proposal  
306 for the domains Archaea, Bacteria, and Eucarya. Proc Natl Acad Sci U S A. 1990;87:  
307 4576–9. doi:10.1073/pnas.87.12.4576
- 308 29. Manual D, Manual S, Manual S. Preface to Volume Two of the Second Edition of  
309 Bergey's Manual of Systematic Bacteriology.
- 310 30. Staley JT, Boone DR, Brenner DJ, De Vos P, Garrity GM, Goodfellow M, et al.  
311 Volume two: The Proteobacteria, Part B: The Gammaproteobacteria. Bergey's Manual  
312 of Systematic Bacteriology. Springer; 2005. p. 1106. doi:10.1007/0-387-28022-7
- 313 31. Garrity G, Lilburn T, Cole J, Harrison S, ... JE-TTO of, 2007 U. Part 3-The Bacteria:  
314 Phylum Proteobacteria, Class Alphaproteobacteria. Bergey's Man Syst Bacteriol. 2005;  
315 1–574.
- 316 32. Ashburner M, Ball CA, Blake JA, Botstein D, Butler H, Cherry JM, et al. Gene  
317 ontology: Tool for the unification of biology. Nature Genetics. 2000. pp. 25–29.  
318 doi:10.1038/75556
- 319 33. David Kuykendall L. Rhizobiales ord. nov. Bergey's Manual of Systematics of  
320 Archaea and Bacteria. John Wiley & Sons, Ltd; 2015. pp. 1–1.  
321 doi:10.1002/9781118960608.obm00071
- 322 34. Skerman VBD, McGowan V, Sneath PHA. Approved list of bacterial names, amended  
323 edition. [Internet]. American Society for Microbiology. American Society for  
324 Microbiology; 1989. doi:10.1099/00207713-30-1-225
- 325 35. Brenner DJ, O'Connor SP, Winkler HH, Steigerwalt AG. Proposals to unify the genera  
326 bartonella and Rochalimaea, with descriptions of Bartonella quintana comb. nov.,  
327 Bartonella vinsonii comb. nov., Bartonella henselae comb. nov., and Bartonella  
328 elizabethae comb. nov., and to remove the family Bartonellaceae from the order  
329 Rickettsiales. Int J Syst Bacteriol. 1993;43: 777–786. doi:10.1099/00207713-43-4-777
- 330 36. Birtles RJ, Harrison TG, Saunders NA, Molyneux DH. Proposals to unify the genera  
331 Grahamella and Bartonella, with descriptions of Bartonella talpae comb. nov.,  
332 Bartonella peromysci comb. nov., and three new species, Bartonella grahamii sp. nov.,  
333 Bartonella taylorii sp. nov., and Bartonella doshiae sp. nov. Int J Syst Bacteriol.  
334 1995;45: 1–8. doi:10.1099/00207713-45-1-1
- 335 37. Weinman D GI. Bartonella Strong & al., 1915. In: Tyzzer and Sellards; Buchanan R,  
336 Gibbons, editors. Bergey's Manual of Determinative Bacteriology. 8th ed. Baltimore:  
337 Williams and Wilkins Co [Internet]. 1974 [cited 16 Sep 2019] pp. 904–05. Available:  
338 <https://www.gbif.org/pt/species/108277807/verbatim>

339

340

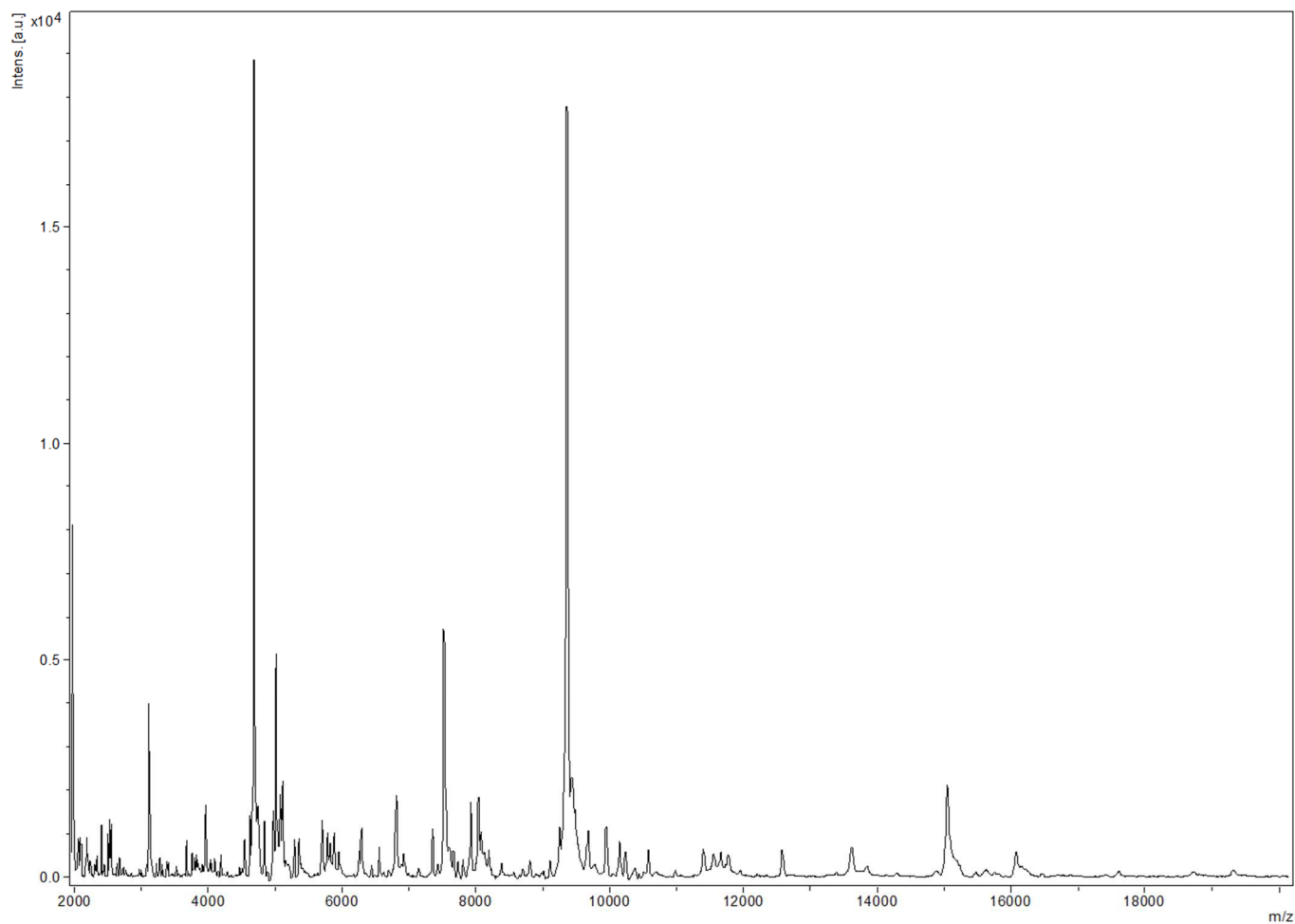
341  
342  
343  
344  
345  
346  
347  
348  
349  
350  
351  
352  
353  
354  
355  
356  
357  
358  
359  
360  
361  
362  
363  
364  
365  
366  
367  
368  
369



**Figure 1.** Phylogenetic tree showing the position of *Bartonella sahelensis* sp. nov., strain 077 relative to other phylogenetically-close neighbors. Sequences were aligned using ClustalW parameters within MEGA 7 software. The evolutionary history was inferred using the Minimum Evolution method. The respective Genbank accession numbers for 16S rRNA genes are indicated before each species. Numbers at the nodes are percentages of bootstrap values obtained by repeating the analysis 1000 times to generate a majority consensus tree. The scale bar indicates a 5% nucleotide sequence divergence.



370 **Figure 2.** MALDI-TOF MS Reference mass spectrum *Bartonella saheliensis* sp. nov. spectra  
371 from 12 individual colonies were compared and a reference spectrum was generated.



372

373

374

375

376

377

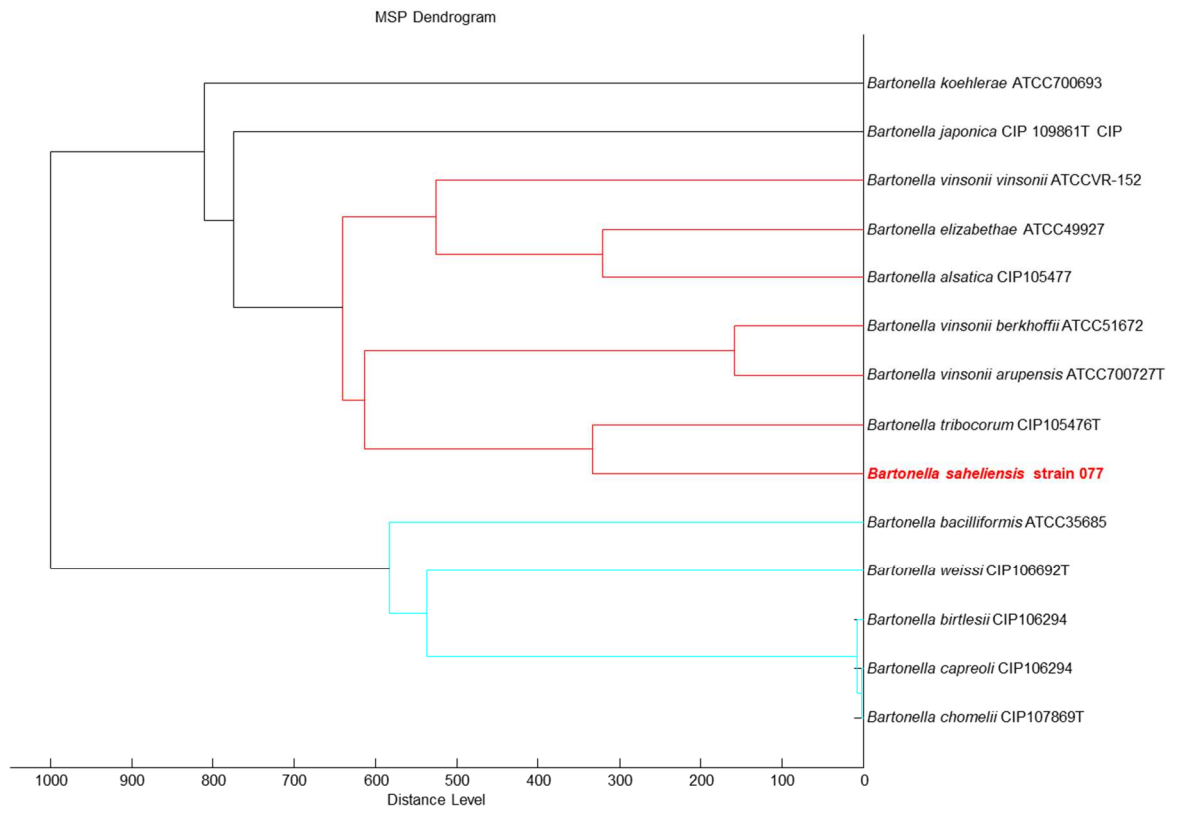
378

379

380

381

382 **Figure 3.** Dendrogram comparing the MALDI-TOF spectra of *Bartonella sahelensis* sp.  
383 nov., strain 077 with those of other members of *Bartonella* genus.



384

385

386 **Figure 4.** Transmission electron micrograph of *Bartonella saheliensis* strain 077, using a  
387 Morgagni 268D (Philips) transmission electron microscope at an operating voltage of 60 kV.  
388 The scale bar represents 500 nm.

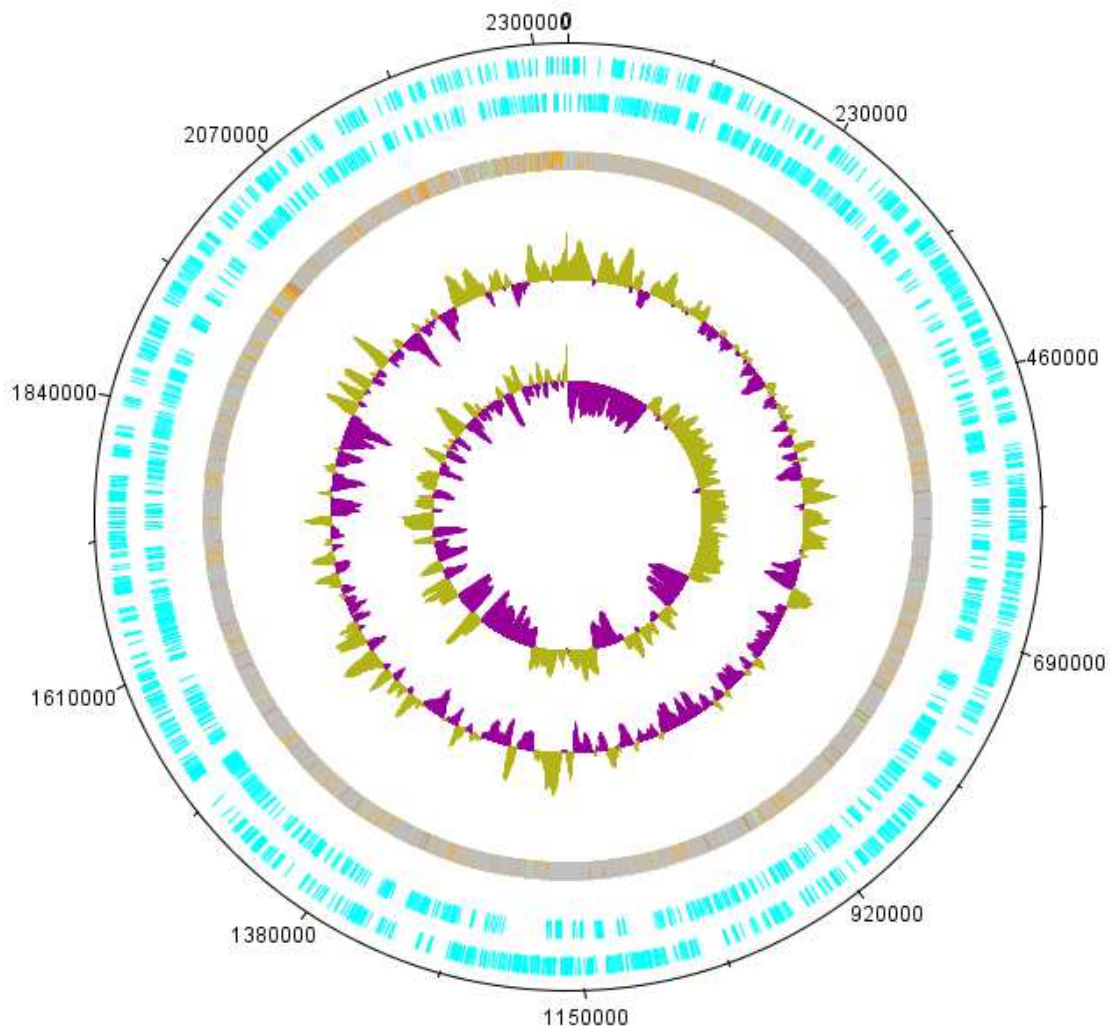


389

390

391

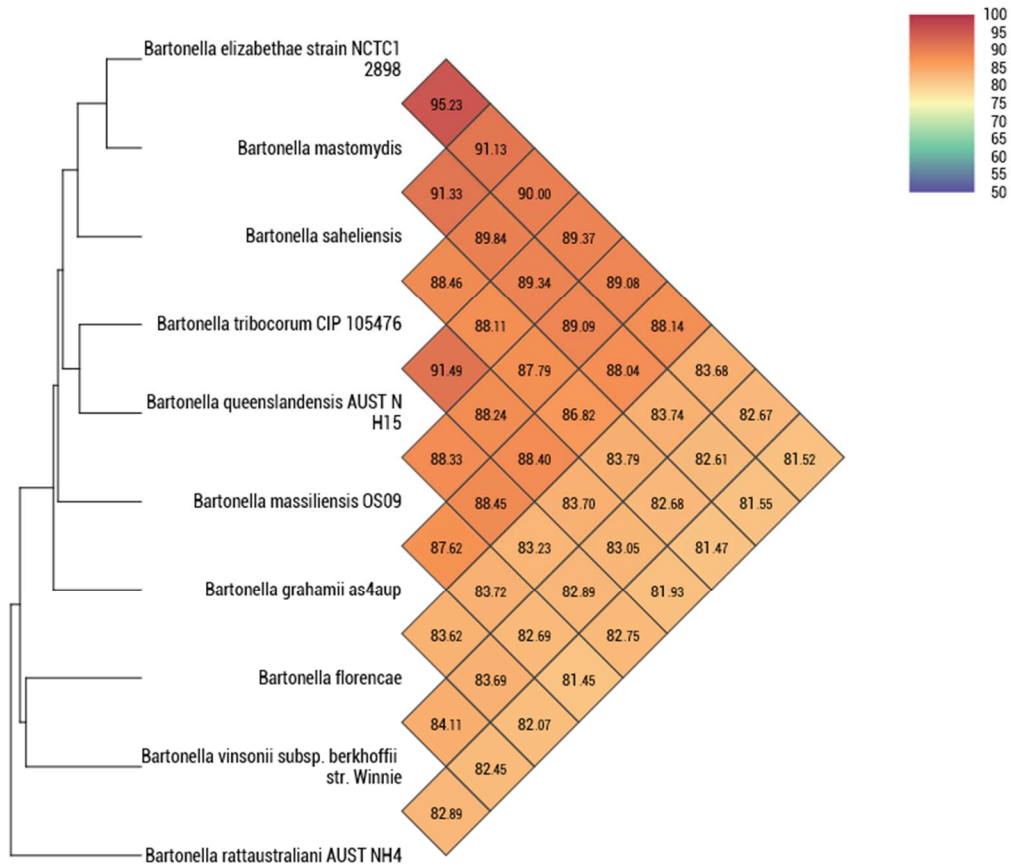
392 **Figure 5.** Graphical circular map of the genome. From outside to the center: genes on the  
393 forward strand, genes on the reverse strand colored in red, all contigs, G + C content and G +  
394 C skew.  
395  
396



397  
398  
399  
400  
401

402 **Figure 6.** Heatmap generated with OrthoANI values calculated using the OAT software  
 403 between *Bartonella sahelensis* sp. nov., strain 077 and other closely related species with  
 404 standing in nomenclature.

405



406

407

408 **Table 1.** Classification and general features of *Bartonella sahelensis* sp. nov., strain 077.

<b>MIGS ID</b>	<b>Property</b>	<b>Term</b>	<b>Evidence code a</b>
	Current classification	Domain <i>Bacteria</i>	TAS[28]
		Phylum <i>Proteobacteria</i>	TAS [29][30]
		Class <i>Alphaproteobacteria</i>	TAS[31]
		Order <i>Rhizobiales</i>	TAS[32][33]
		Family <i>Bartonellaceae</i>	TAS[34][35]
		Genus <i>Bartonella</i>	TAS[36][35][37]
		Species <i>Bartonella sahelensis</i>	IDA
		Type strain 077	IDA
	Gram stain	Negative	IDA
	Cell shape	Rod	IDA
	Motility	Nonmotile	IDA
	Sporulation	Nonsporulating	IDA
	Temperature range	Mesophilic	IDA
	Optimum temperature	32°C	IDA
<b>MIGS-22</b>	Oxygen requirement	Aerobic	IDA
	Carbon source	Unknown	IDA
	Energy source	Unknown	IDA
<b>MIGS-6</b>	Habitat	<i>Gerbilliscus gambianus</i> blood	IDA
<b>MIGS-15</b>	Biotic relationship	Facultative intracellular	IDA
	Pathogenicity	Unknown	IDA
	Biosafety level	3	IDA
<b>MIGS-14</b>	Isolation	<i>Gerbilliscus gambianus</i> blood	IDA
<b>MIGS-4</b>	Geographic location	Senegal	IDA
<b>MIGS-5</b>	Sample collection	February 2013	IDA
<b>MIGS-4.1</b>	Latitude	14°030N°	IDA
<b>MIGS-4.2</b>	Longitude	15°310W°	IDA
<b>MIGS-4.3</b>	Depth	Surface of the earth	IDA
<b>MIGS-4.4</b>	Altitude	5 m above sea level	IDA

409

410 **Table 2.** Project information.

<b>MISG ID</b>	<b>Property</b>	<b>Term</b>
<b>MIGS-31</b>	Finishing quality	High-quality draft
<b>MIGS-28</b>	Libraries used	One paired-end 3 kb library
<b>MIGS-29</b>	Sequencing platforms	454 GS FLX Titanium
<b>MIGS-31.2</b>	Fold coverage	28x
<b>MIGS-30</b>	Assemblers	gsAssembler from Roche
<b>MIGS-12</b>	Gene calling method	Prodigal
	GenBank ID	CABGUM010000001-CABGUM010000132
<b>MIGS-13</b>	Project relevance	Investigate the presence of <i>Bartonella</i> spp. in commensal rodents in Sine-Saloum region of Senegal.

411

412

413 **Table 3.** Number of genes associated with the 25 general COG functional categories.

<b>Code</b>	<b>Value</b>	<b>% of total<sup>a</sup></b>	<b>Description</b>
<b>J</b>	89	4.41	Translation
<b>A</b>	0	0	RNA processing and modification
<b>K</b>	41	2.03	Transcription
<b>L</b>	76	3.77	Replication, recombination and repair
<b>B</b>	0	0	Chromatin structure and dynamic
<b>D</b>	22	1.09	Cell cycle control, mitosis and meiosis
<b>Y</b>	0	0	Nuclear structure
<b>V</b>	10	0.49	Defense mechanisms
<b>T</b>	17	0.84	Signal transduction mechanisms
<b>M</b>	65	3.22	Cell wall/membrane biogenesis
<b>N</b>	2	0.09	Cell motility
<b>Z</b>	0	0	Cytoskeleton
<b>W</b>	0	0	Extracellular structures
<b>U</b>	40	1.98	Intracellular trafficking and secretion
<b>O</b>		2.63	Post tanslational modification, protein turnover, chaperones
	53		
<b>C</b>	0	0	Energy production and conversion
<b>G</b>	59	2.92	Carbohydrate transport and metabolism
<b>E</b>	38	1.88	Amino acid transport and metabolism
<b>F</b>	68	3.37	Nucleotide transport and metabolism
<b>H</b>	34	1.68	Coenzyme transport and metabolism
<b>I</b>	55	2.72	Lipid transport and metabolism
<b>P</b>	42	2.08	Inorganic ion transport and metabolism
<b>Q</b>		1.63	Secondary metabolites biosynthesis, transport and catabolism
	33		
<b>R</b>	3	0.14	General function prediction only
<b>S</b>	96	4.76	Function unknown
<b>-</b>	106	5.26	Not in COGs

414