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

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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 sahelensis*  
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 sahelensis* 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-bp 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 tRNA, 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. sahelensis* 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. rattaustraliani* 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. rattaustraliani* 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. sahelensis* strain 077 as the type strain of *Bartonella*  
179 *sahelensis* 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.

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216

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221

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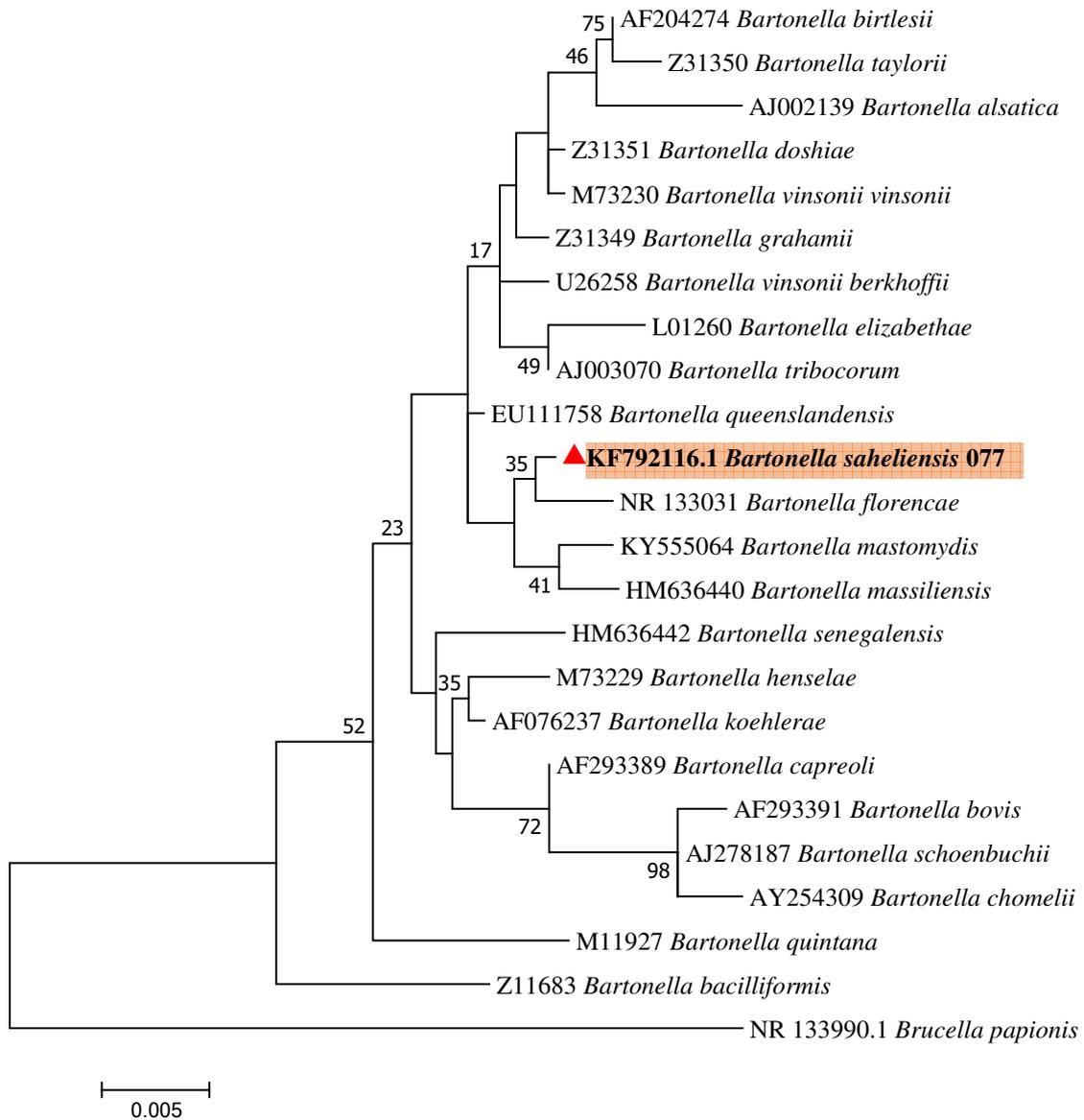
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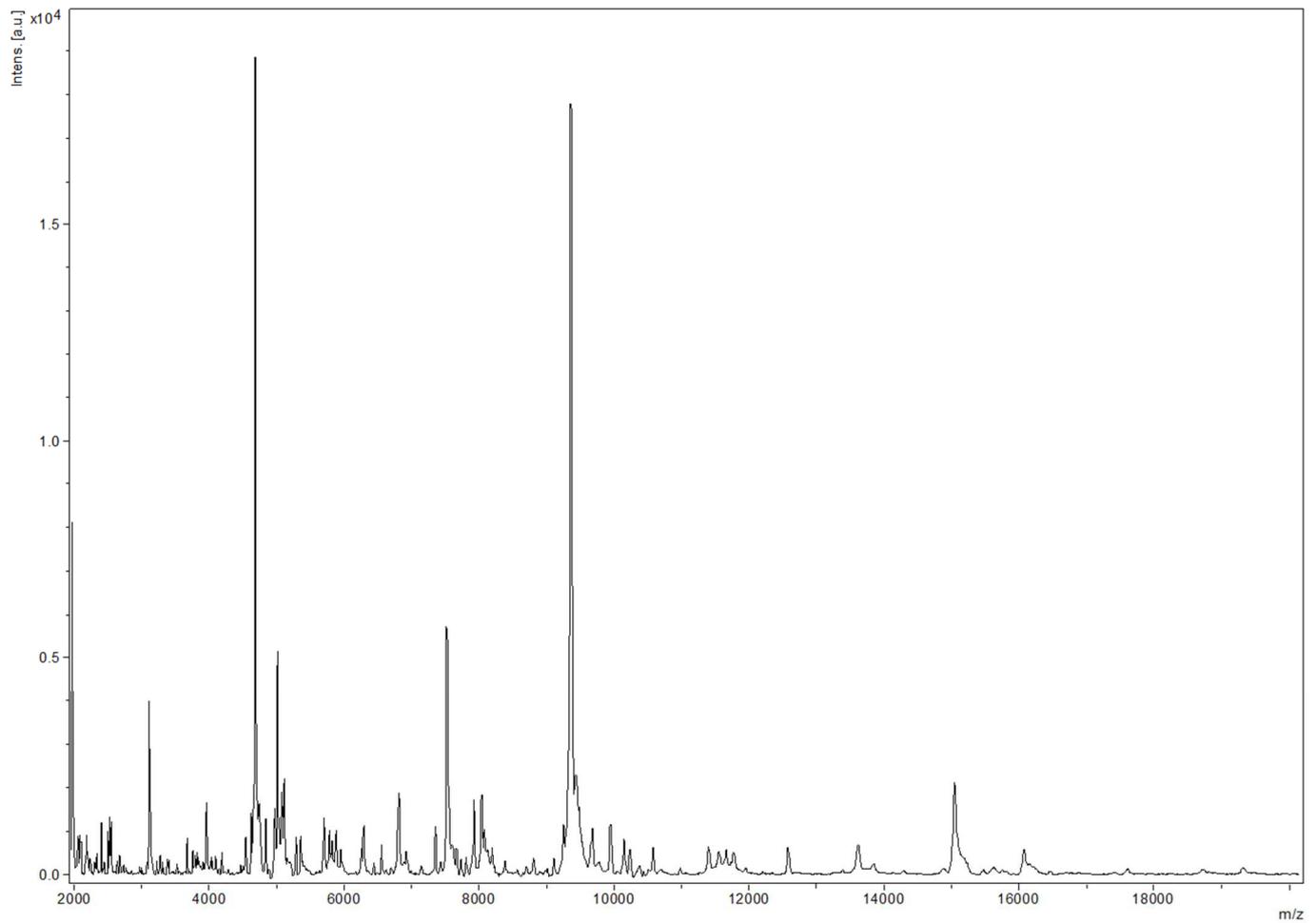
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362 **Figure 1.** Phylogenetic tree showing the position of *Bartonella sahelensis* sp. nov., strain 077  
363 relative to other phylogenetically-close neighbors. Sequences were aligned using ClustalW  
364 parameters within MEGA 7 software. The evolutionary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

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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.



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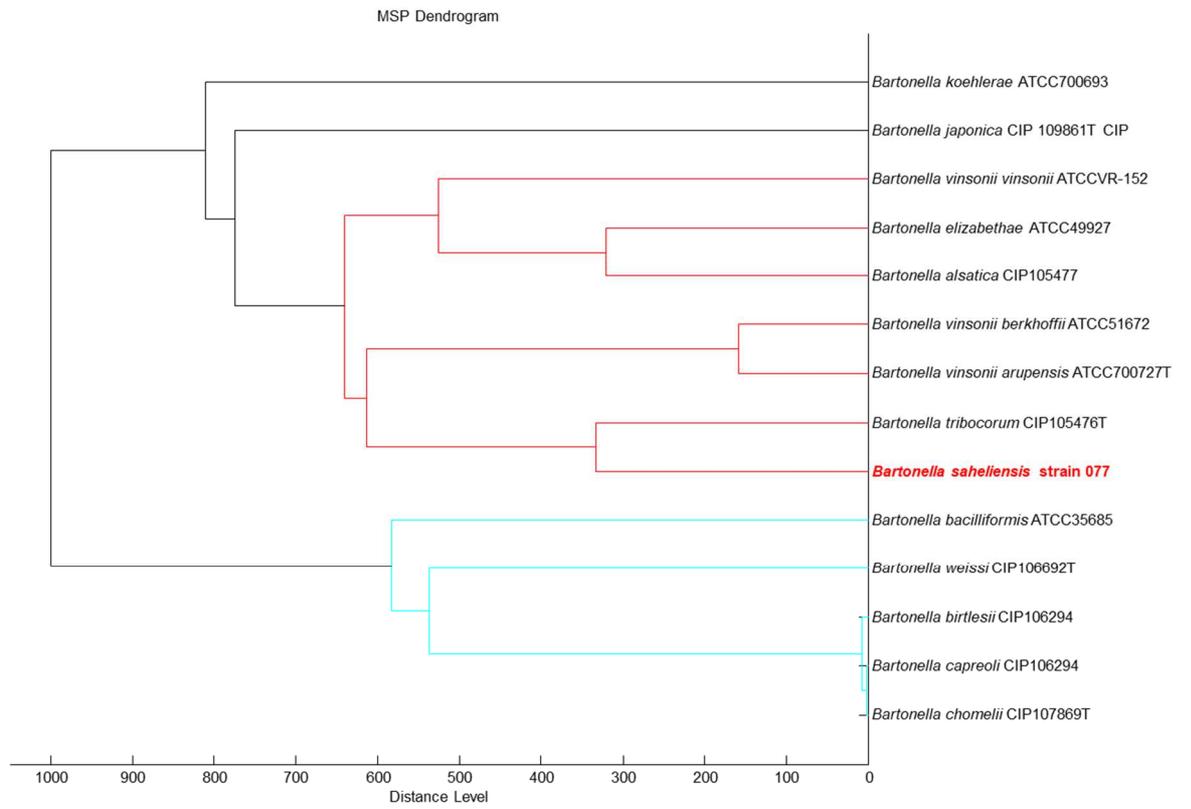
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382 **Figure 3.** Dendrogram comparing the MALDI-TOF spectra of *Bartonella sahelensis* sp.  
383 nov., strain 077 with those of other members of *Bartonella* genus.



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386 **Figure 4.** Transmission electron micrograph of *Bartonella sahelensis* 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.

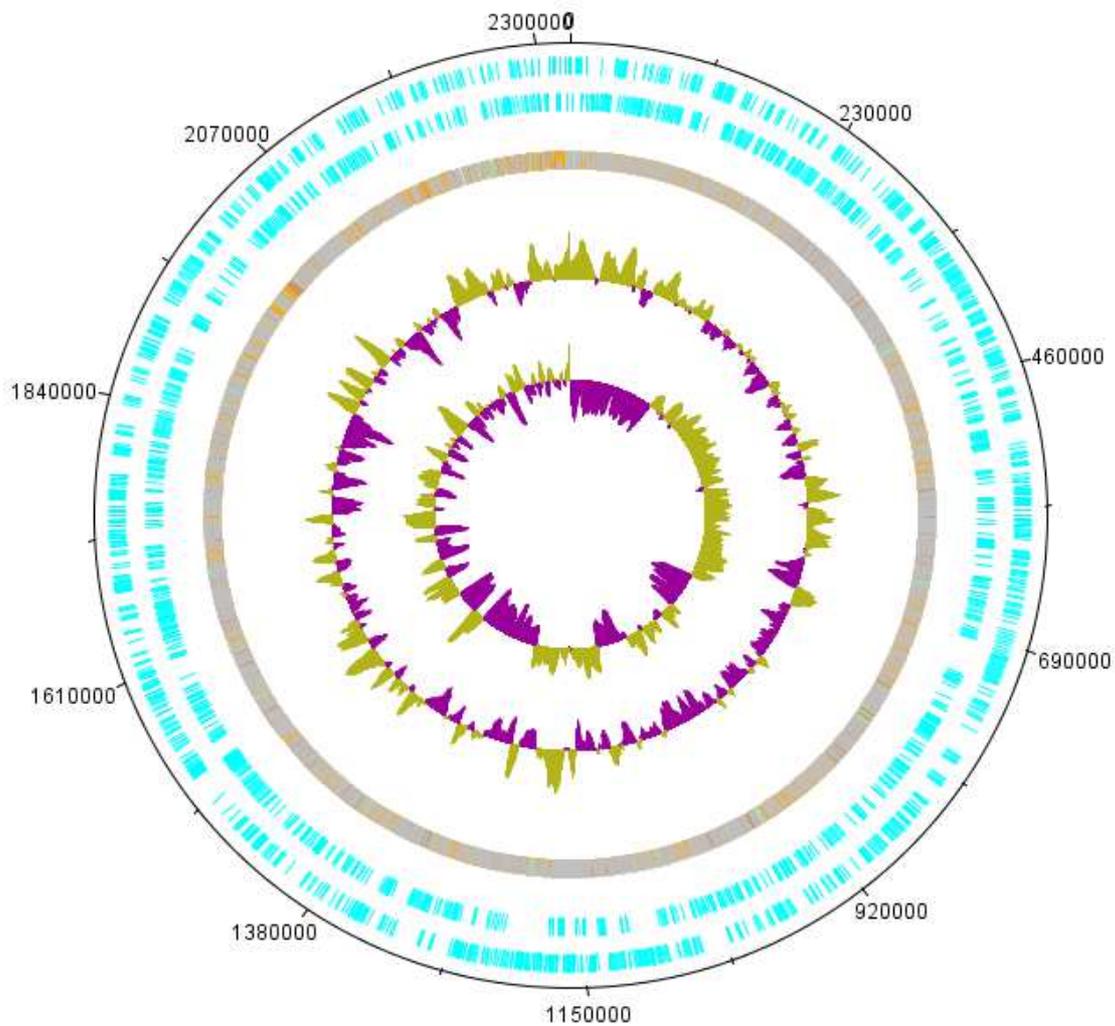


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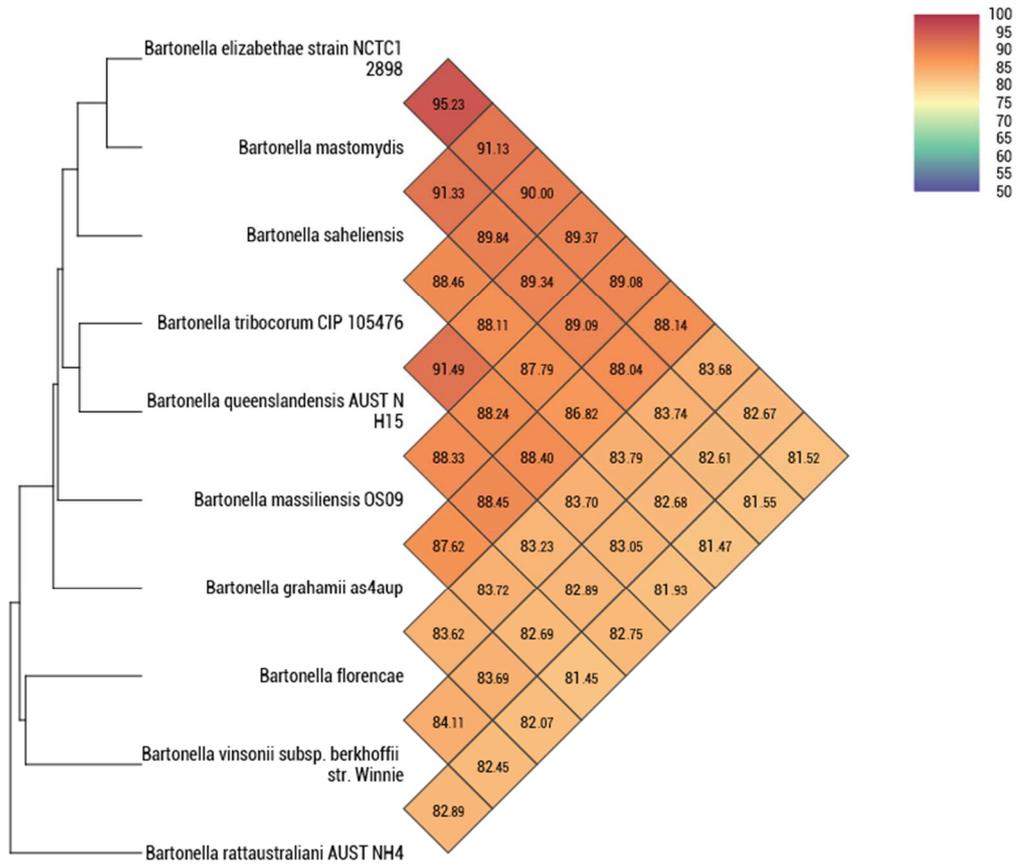
392 **Figure 5.** Graphical circular map of the genome. From outside to the center: genes on the  
393 forward strand, genes on the reverse strand colored in red, all contigs, G + C content and G +  
394 C skew.  
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402 **Figure 6.** Heatmap generated 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.

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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

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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.

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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

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