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

Handi DAHMANA[1,2], Hacène MEDKOUR[1,2], Hussein ANANI[2,3], Laurent Granjon[4], Georges Diatta[5], Florence FENOLLAR[2,3], Oleg MEDIANNIKOV[1,2*]

[1] Aix Marseille Univ, IRD, AP-HM, MEPHI, Marseille, France

[2] IHU-Méditerranée Infection, Marseille, France

[3] Aix Marseille Univ, IRD, AP-HM, SSA, VITROME, Marseille, France

[4]CBGP, IRD, CIRAD, INRA, Montpellier SupAgro, Univ Montpellier, Montpellier, France

[5]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

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Abstract

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

Introduction

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

New species are always isolated and then characterized from rodents or their ectoparasites [4][5][6][7][8]. Interestingly, more than half of the species characterized are harboured by rodents and lagomorphs, such as *B. tribocorum*, *B. grahamii*, *B. elizabethae*, *B. vinsonii* subsp. *arupensis*, *B. washoensis* and *B. alsatica* which are known to be potentially zoonotic [9]. Although, in rodents ectoparasites, high prevalence's of zoonotic bartonellosis agents are found (43.75% of *B. elizabethae* in *Stenoponia tripectinata tripectinata*) [10]. *Bartonella* species are transmitted by different insects like lice, dipterans and fleas that are respectively the main vectors of *B. quintana, B. bacilliformis* and *B. henselae*, while the role of ticks in the transmission of bartonellosis remains uncertain [1][11][12][13]. The presence of *Bartonella* DNA does not necessarily mean that they transmit it to mammals [12].

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

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

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

Samples and bacterial culture

In February 2013, as part of a 6-day prospective study on tick-borne relapsing fever in West Africa, 119 small mammals were captured alive in two sites (Dielmo and Ndiop) using wire mesh traps baited with peanut butter or onions: 116 rodents and 3 shrews (*Crocidura* cf. *olivieri*). The rodents were morphologically identified: 5 *Arvicantis niloticus*, 56 *Gerbilliscus gambianus*, 49 *Mastomys erythroleucus*, 5 *Mus musculus*, and 1 *Praomys daltoni*. They were anaesthetized and opened under sterile conditions. The isolation of the *Bartonella* strains was performed as described previously [6]. Briefly, blood was inoculated on Columbia agar plates supplemented with 5% sheep blood (bioMérieux, Marcy l'Etoile, France) and incubated at 37°C in a 5% CO_2-enriched atmosphere. Thus, 30 isolates of *Bartonella* spp. were recovered from the blood of rodents.

Classification and features

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

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

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

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

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

Biochemical characterization and images acquisition

For its growth, different temperatures were tested (32, 37, and 42°C). The optimal growth was obtained at 32°C in 5% CO_2 atmosphere. The colonies had 0.3 mm to 1 mm in diameter; they were grey and opaque on blood enriched Columbia agar. Cells grown on agar are Gram negative and have a mean length and width of 1.05± 0.08 µm and 0.6±0.05 µm, respectively.

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

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

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

Genome sequencing information

Genome project history

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

Bartonella saheliensis strain 077 (= CSUR B644^T; = DSM 28003^T) was cultured on Columbia agar enriched with sheep blood (bioMérieux) with 5% CO_2 at 32°C. Bacteria growing on two Petri dishes were recovered and then resuspended in 6x100µl of G2 buffer. A first mechanical lysis was performed with glass powder using the Fastprep-24 device (MP Biomedicals, Graffenstaden, France) during 2x20 seconds. Then, after 30 minutes' lysozyme incubation at 37°C, DNA was extracted on the EZ1 biorobot (Qiagen, Hilden, Germany) with the EZ1 DNA tissue kit. DNA was quantified by Quant-iT™ PicoGreen™ dsDNA Assay Kit (Invitrogen, Waltham, Massachusetts, USA) to 98.2 ng/µl.

Genome sequencing and assembly

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

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

The library was clonally amplified with 1 cpb and 1.5 cpb in 4 and 3 emPCR reactions, respectively, with the GS Titanium SV emPCR Kit (Lib-L) v2(Roche, Basel, Switzerland). The yields of the 1 cpb and 1.5 cpb emPCR were determined to be 3.08% and 8%, respectively. After amplification, 790,000 beads from the 2 emPCR conditions were loaded on a ¼ region on the GS Titanium PicoTiterPlate PTP Kit 70×75 and sequenced with the GS FLX Titanium Sequencing Kit XLR70 (Roche). The run was analysed on the cluster using the gsRunBrowser and Newbler assembler (Roche). A total of 200,243 passed filter wells were obtained and generated 57.62 Mb of DNA sequence with an average length of 287 bp. The passed filter sequences were assembled using gsAssembler with 90% identity and 40 bp for overlap requirements. The final assembly identified 132 scaffolds and 173 large contigs (≥1,500 bp), generating a genome size of 2,327,299-bp, which corresponds to 28× equivalent genome.

Genome annotation

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

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

Genome properties

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

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

Conclusion

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

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

182

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

184 *Bartonella saheliensis* sp. nov., (sah.el.li.en'sis. L. masc. adj. saheliensis 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±0.08 µm and
190 0.6±0.05 µm, respectively. Cells are rod shaped without flagella or pili. *Bartonella saheliensis*
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^T; =
193 DSM 28003^T) 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 saheliensis* 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^T and DSM 28003^T.

202

Conflict of interest

None to declare.

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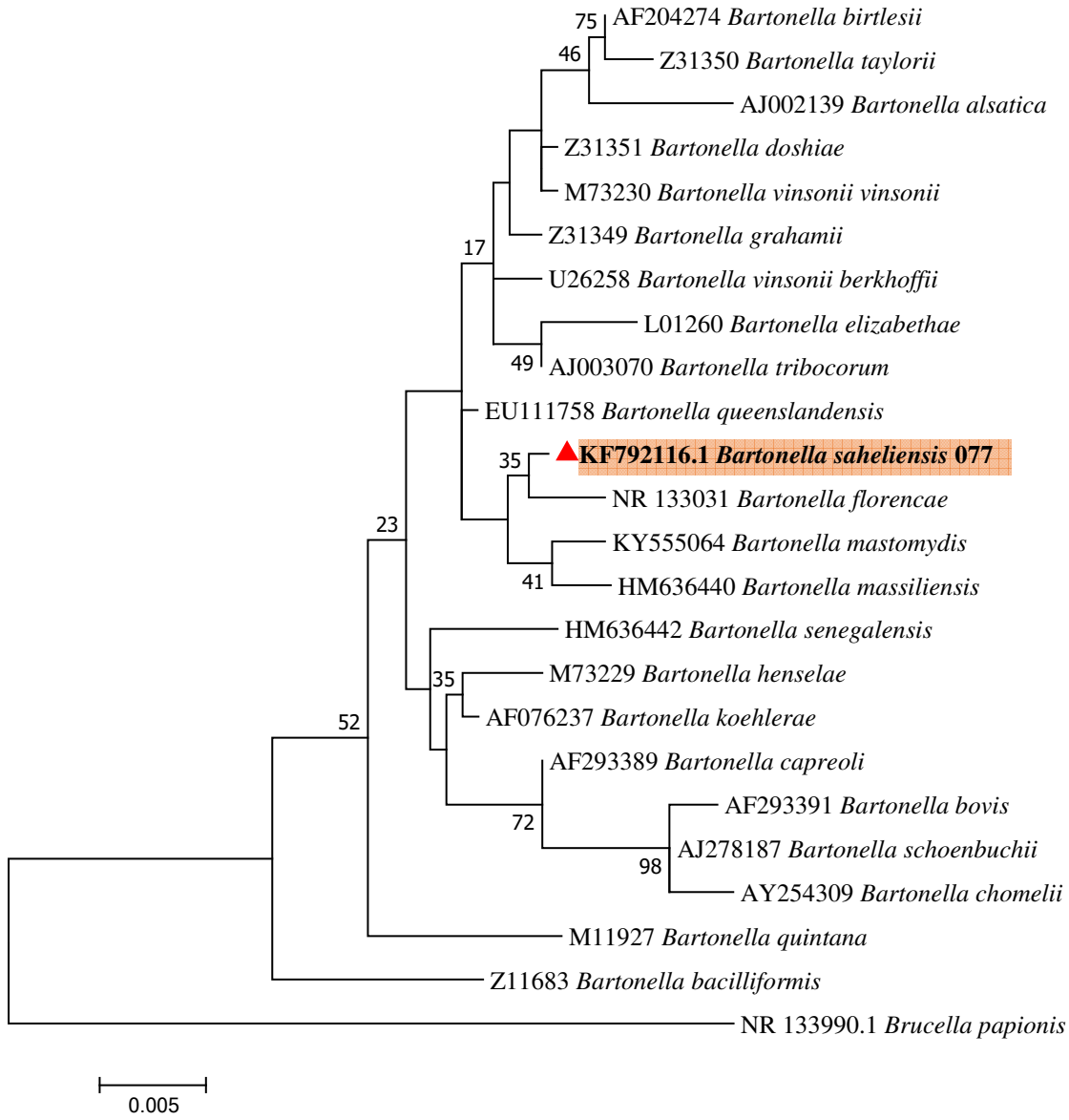


Figure 1. Phylogenetic tree showing the position of *Bartonella saheliensis* sp. nov., strain 077 relative to other phylogenetically-close neighbors. Sequences were aligned using ClustalW parameters within MEGA 7 software. The evolutionary history was inferred using the Minimum Evolution method. The 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.

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

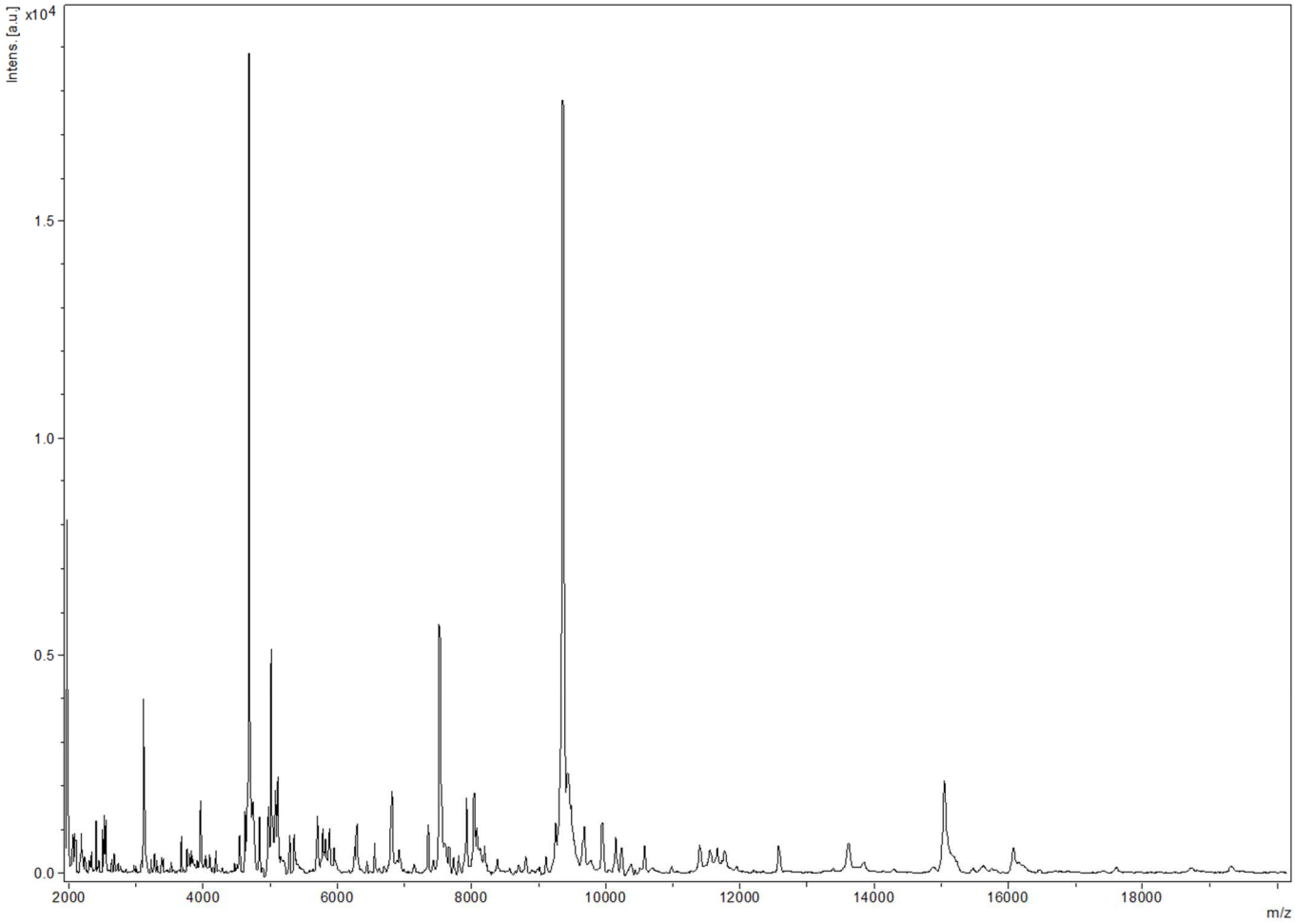
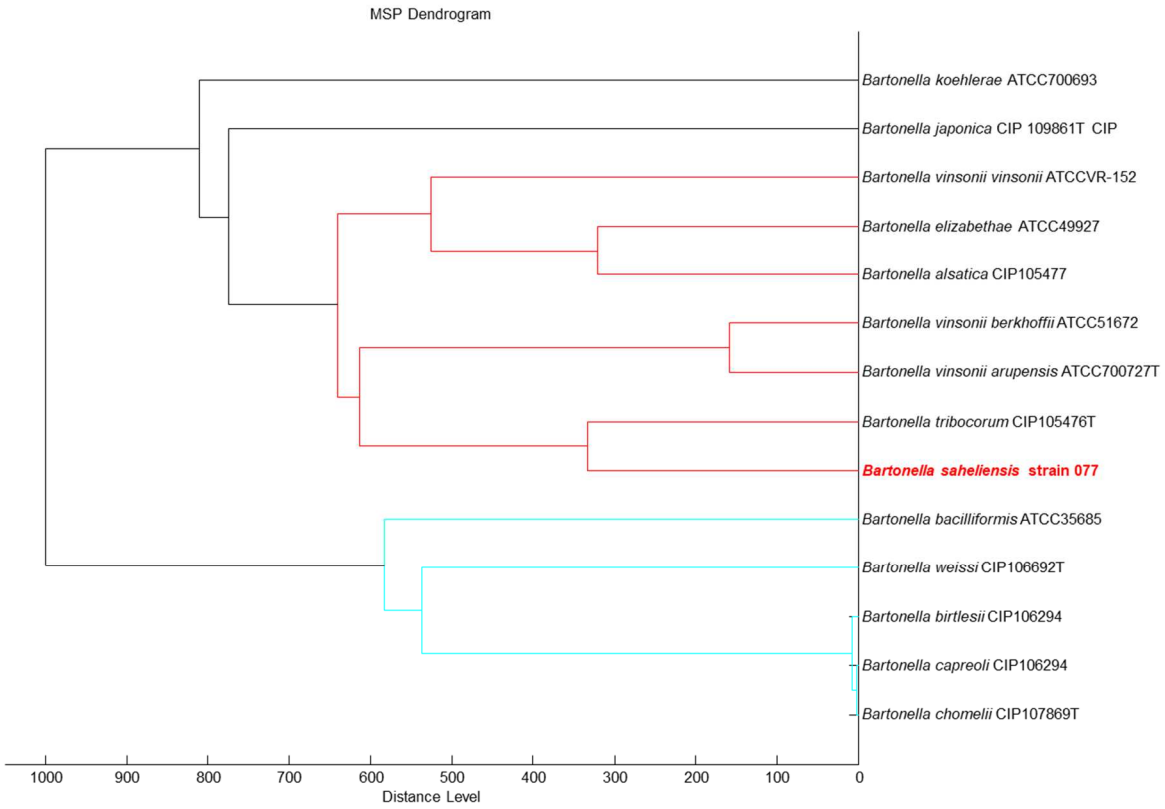


Figure 3. Dendrogram comparing the MALDI-TOF spectra of *Bartonella saheliensis* sp. nov., strain 077 with those of other members of *Bartonella* genus.



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.

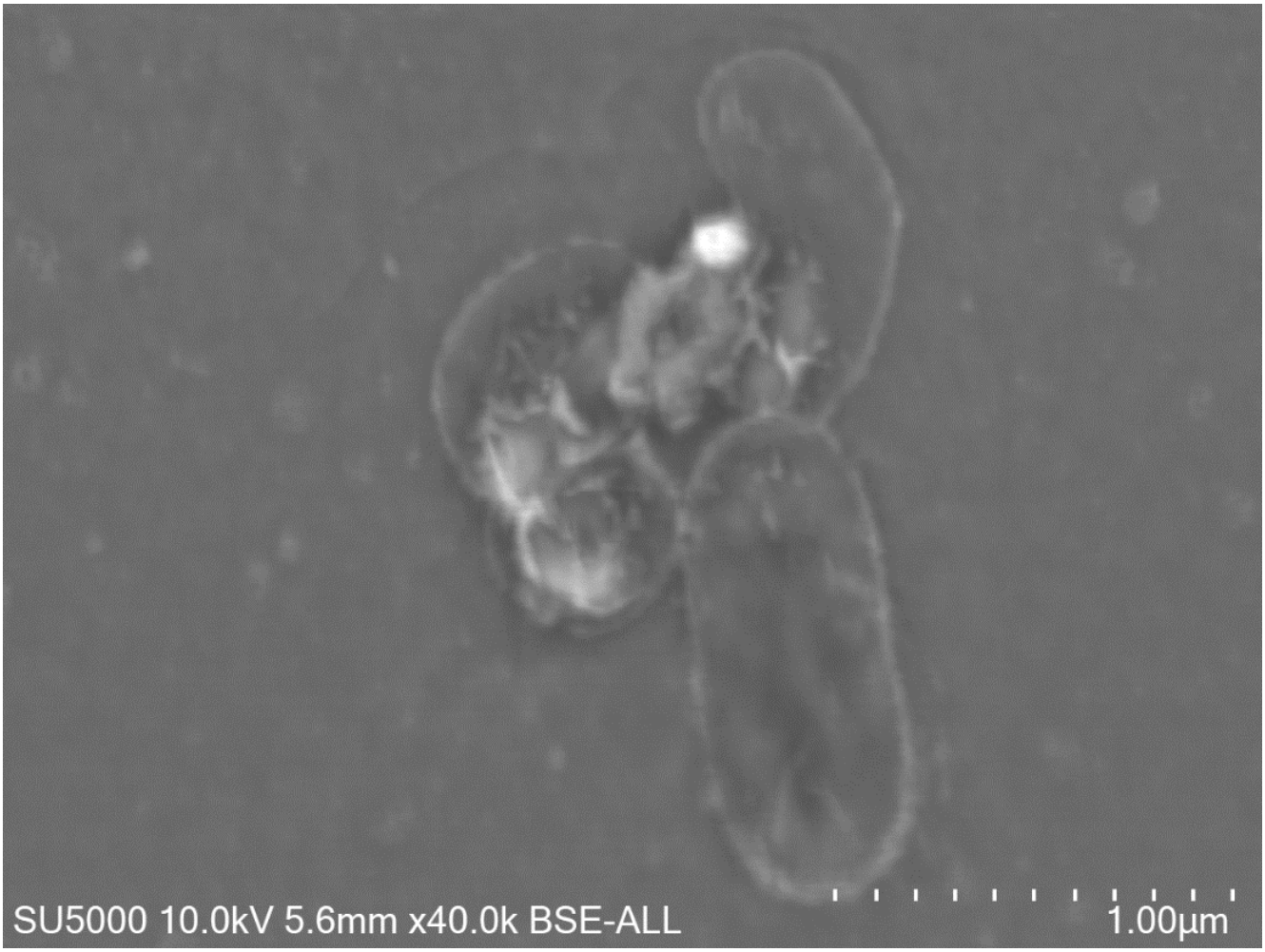


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Figure 5. Graphical circular map of the genome. From outside to the center: genes on the forward strand, genes on the reverse strand colored in red, all contigs, G + C content and G + C skew.

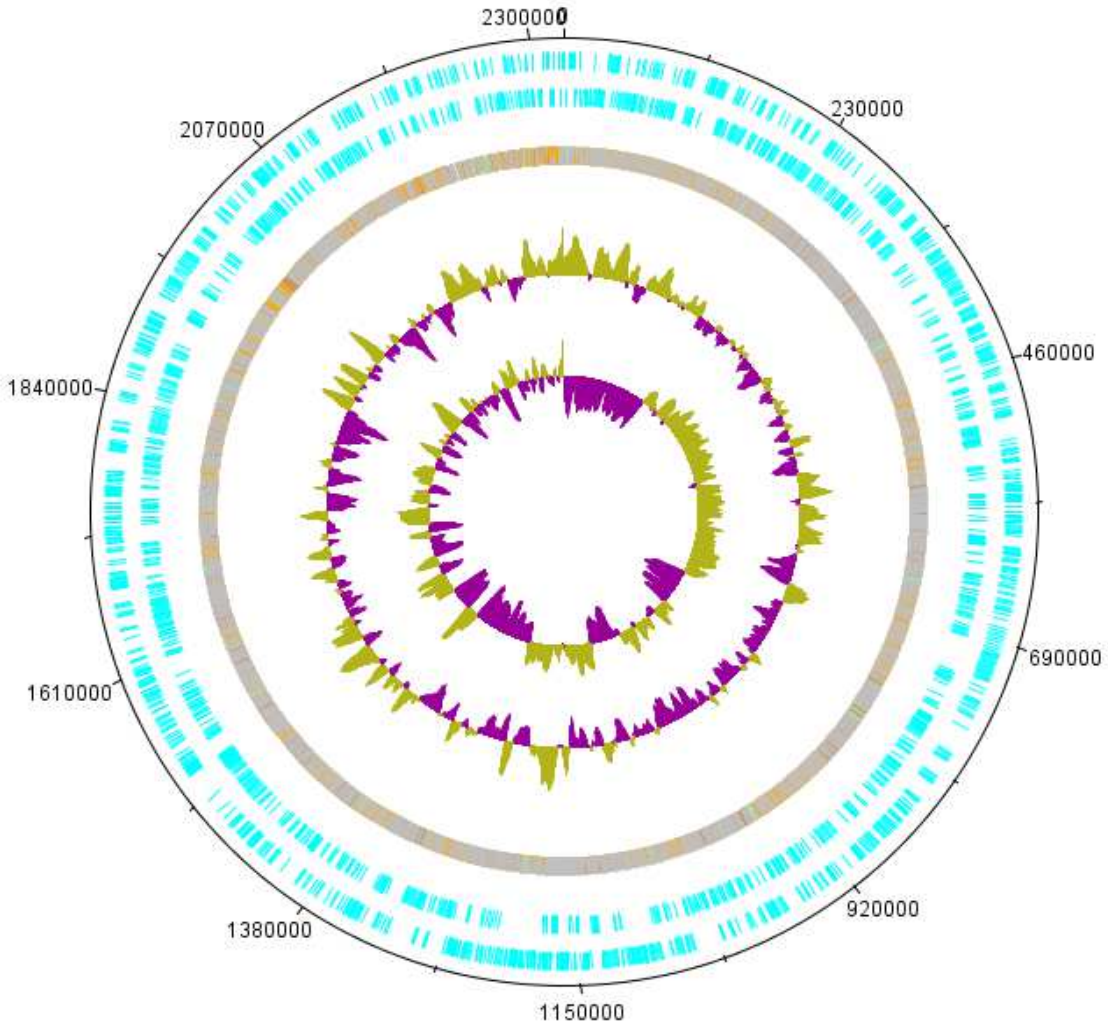
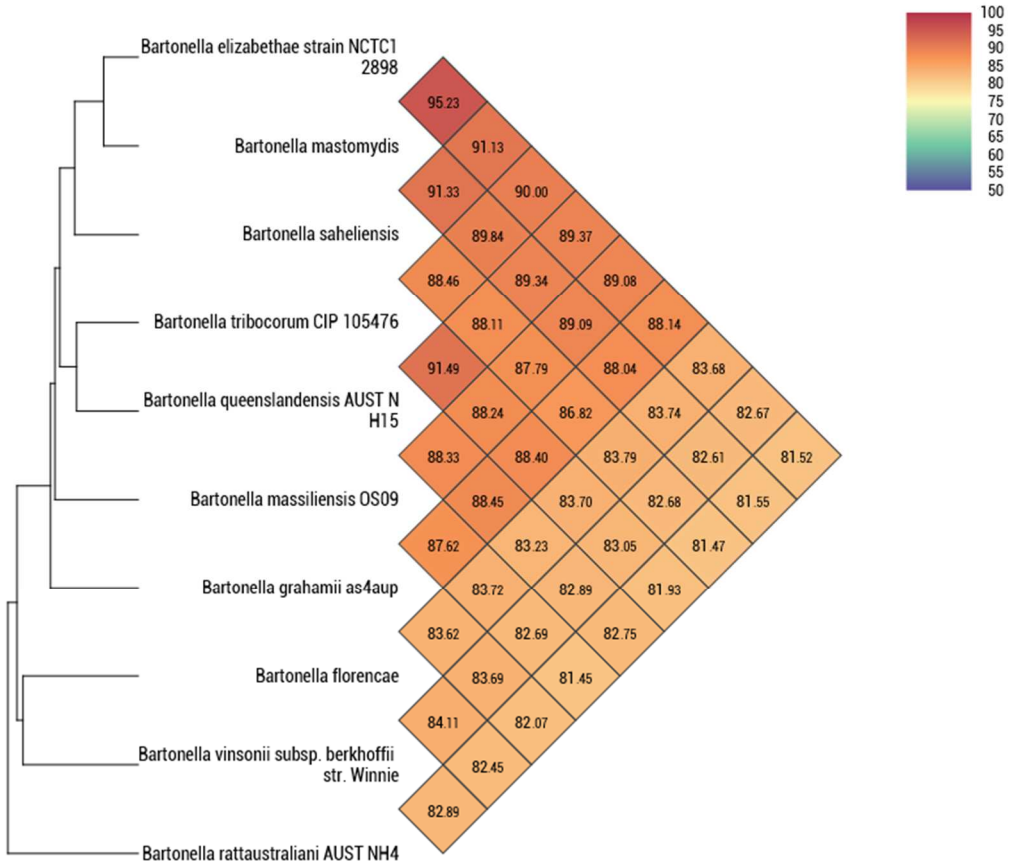


Figure 6. Heatmap generated with OrthoANI values calculated using the OAT software between *Bartonella saheliensis* sp. nov., strain 077 and other closely related species with standing in nomenclature.



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

MIGS ID	Property	Term	Evidence code a
	Current classification	Domain *Bacteria*	TAS[28]
		Phylum *Proteobacteria*	TAS [29][30]
		Class *Alphaproteobacteria*	TAS[31]
		Order *Rhizobiales*	TAS[32][33]
		Family *Bartonellaceae*	TAS[34][35]
		Genus *Bartonella*	TAS[36][35][37]
		Species *Bartonella saheliensis*	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
MIGS-22	Oxygen requirement	Aerobic	IDA
	Carbon source	Unknown	IDA
	Energy source	Unknown	IDA
MIGS-6	Habitat	*Gerbilliscus gambianus* blood	IDA
MIGS-15	Biotic relationship	Facultative intracellular	IDA
	Pathogenicity	Unknown	IDA
	Biosafety level	3	IDA
MIGS-14	Isolation	*Gerbilliscus gambianus* blood	IDA
MIGS-4	Geographic location	Senegal	IDA
MIGS-5	Sample collection	February 2013	IDA
MIGS-4.1	Latitude	14°030N°	IDA
MIGS-4.2	Longitude	15°310W°	IDA
MIGS-4.3	Depth	Surface of the earth	IDA
MIGS-4.4	Altitude	5 m above sea level	IDA

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410 **Table 2.** Project information.

MISG ID	Property	Term
MIGS-31	Finishing quality	High-quality draft
MIGS-28	Libraries used	One paired-end 3 kb library
MIGS-29	Sequencing platforms	454 GS FLX Titanium
MIGS-31.2	Fold coverage	28x
MIGS-30	Assemblers	gsAssembler from Roche
MIGS-12	Gene calling method	Prodigal
	GenBank ID	CABGUM010000001-CABGUM010000132
MIGS-13	Project relevance	Investigate the presence of *Bartonella* 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.

Code	Value	% of total[a]	Description
J	89	4.41	Translation
A	0	0	RNA processing and modification
K	41	2,03	Transcription
L	76	3.77	Replication, recombination and repair
B	0	0	Chromatin structure and dynamic
D	22	1.09	Cell cycle control, mitosis and meiosis
Y	0	0	Nuclear structure
V	10	0.49	Defense mechanisms
T	17	0.84	Signal transduction mechanisms
M	65	3.22	Cell wall/membrane biogenesis
N	2	0.09	Cell motility
Z	0	0	Cytoskeleton
W	0	0	Extracellular structures
U	40	1.98	Intracellular trafficking and secretion
O	53	2.63	Post tanslational modification, protein turnover, chaperones
C	0	0	Energy production and conversion
G	59	2.92	Carbohydrate transport and metabolism
E	38	1.88	Amino acid transport and metabolism
F	68	3.37	Nucleotide transport and metabolism
H	34	1.68	Coenzyme transport and metabolism
I	55	2.72	Lipid transport and metabolism
P	42	2.08	Inorganic ion transport and metabolism
Q	33	1.63	Secondary metabolites biosynthesis, transport and catabolism
R	3	0.14	General function prediction only
S	96	4.76	Function unknown
-	106	5.26	Not in COGs

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