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

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Abstract

Bartonella saheliensis strain 077 (= CSUR B644T; = DSM 28003T) is a new bacterial species isolated from blood of the rodent *Gerbilliscus gambianus* captured in the Sine-Saloum region of Senegal. In this work we describe the characteristics of this microorganism, as well as the complete sequence of the genome and its annotation. Its genome has 2 327 299 bp (G+C content 38.4%) and codes for 2015 proteins and 53 RNA genes.

Keywords: *Bartonella*, *Bartonella saheliensis*sp. nov., genome, *Gerbilliscus gambianus*, rodents, senegal
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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 of the Alphaproteobacteria, and was described by Alberto L. Barton in 1909 after he studied the agent of Carrion's disease [1]. These bacteria are facultatively intracellular and use haemotrophy (infection of erythrocytes) as a parasitic strategy [2]. To date, just over 30 species have been described and officially validated, and many others have not yet been described [3]. The species of *Bartonella* infect a wide range of animals, including domestic animals such as cats, dogs, rodents, rabbits and cattle, as well as a diverse group of wild animals, including wildcats, coyotes, deer, elk, foxes, insectivores and bats [3].

New species are always isolated and then characterized from rodents or their ectoparasites [4–8]. Interestingly, more than half of the species characterized are harboured by rodents and lagomorphs; these include *B. tribocorum*, *B. grahamii*, *B. elizabethae*, *B. vinsonii* subsp. *arupensis*, *B. washoensis* and *B. alsatica* which are known to be potentially zoonotic [9]. High prevalences of zoonotic bartonellosis agents are found in rodent ectoparasites (for example 43.75% of *B. elizabethae* strains are found in *Stenoponia tripectinata tripectinata*) [10]. *Bartonella* species are transmitted by different insects—lice, dipterans and fleas are the main vectors of *B. quintana, B. bacilliformis* and *B. henselae* respectively—while the role of ticks in the transmission of bartonellosis remains uncertain [1,11–13]. The presence of *Bartonella* DNA in the tick does not necessarily mean that the tick transmits *Bartonella* to mammals [12].

In Senegal commensal rodents and associated soft ticks are vectors of relapsing fever caused by *Borrelia crocidurae* [14]. To investigate the presence of *Bartonella* spp. in the 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. Phylogenetic analysis showed that they belonged to three separate genetic clusters within the genus *Bartonella*. Comparison between *gltA* genes of recovered isolates and those of officially recognized species allowed the

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conclusion that the three clusters may represent three distinct new species of *Bartonella* [4].

In this paper we describe one of these *Bartonella* species, *Bartonella saheliensis* strain 077, isolated from the blood of *Gerbilliscus gambianus* in Senegal [4]. The bacterial strain was cultured and isolated. A taxonogenomics 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; they included 116 rodents and three shrews (Crocidura cf. olivieri). The rodents were morphologically identified: five 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 onto Columbia agar plates supplemented with 5% sheep blood (bioMérieux, Marcy l'Etoile, France) and incubated at 37°C in a 5% CO2-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 previously 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 formed three distinct groups compared to those of officially recognized species. This led to the conclusion that these three groups may represent three distinct new Bartonella species. Candidatus "Bartonella raoultii" (one 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 (eight isolates) has been recovered from Gerbilliscus gambianus only.

Of the validated *Bartonella* species, the closest to isolate 077 on comparison of the 16S rRNA gene is *B. queenslandensis* (NR116176) at 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 (Fig. 1).

Strain 077 (Table 1) was isolated from G. gambianus blood after 10 days of culture. Subsequently, MALDI-TOF MS analysis was performed on a Microflex LT spectrometer (Bruker Daltonics, Bremen, Germany), as described previously [18]. The spectra obtained (Fig. 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 <1.7 meant that identification was not possible.

The obtained scores of *B. saheliensis* strain 077 were always <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 Fig. 3.

Biochemical characterization and image acquisition

For its growth, different temperatures were tested (32°C, 37°C, and 42°C). Optimal growth was obtained at 32°C in a 5% CO_2 atmosphere. The colonies were 0.3–1.0 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 image 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) approximately 60 cm in height and 33 cm in width to evaluate bacterial structures. The SEM can observe specimens under low pressure (10^0–10^1 Pa) to reduce charge on the specimen surface by the irradiating electrons. Evacuation time after loading of the specimen into the SEM chamber is <2 min, which is much quicker than conventional SEMs with high

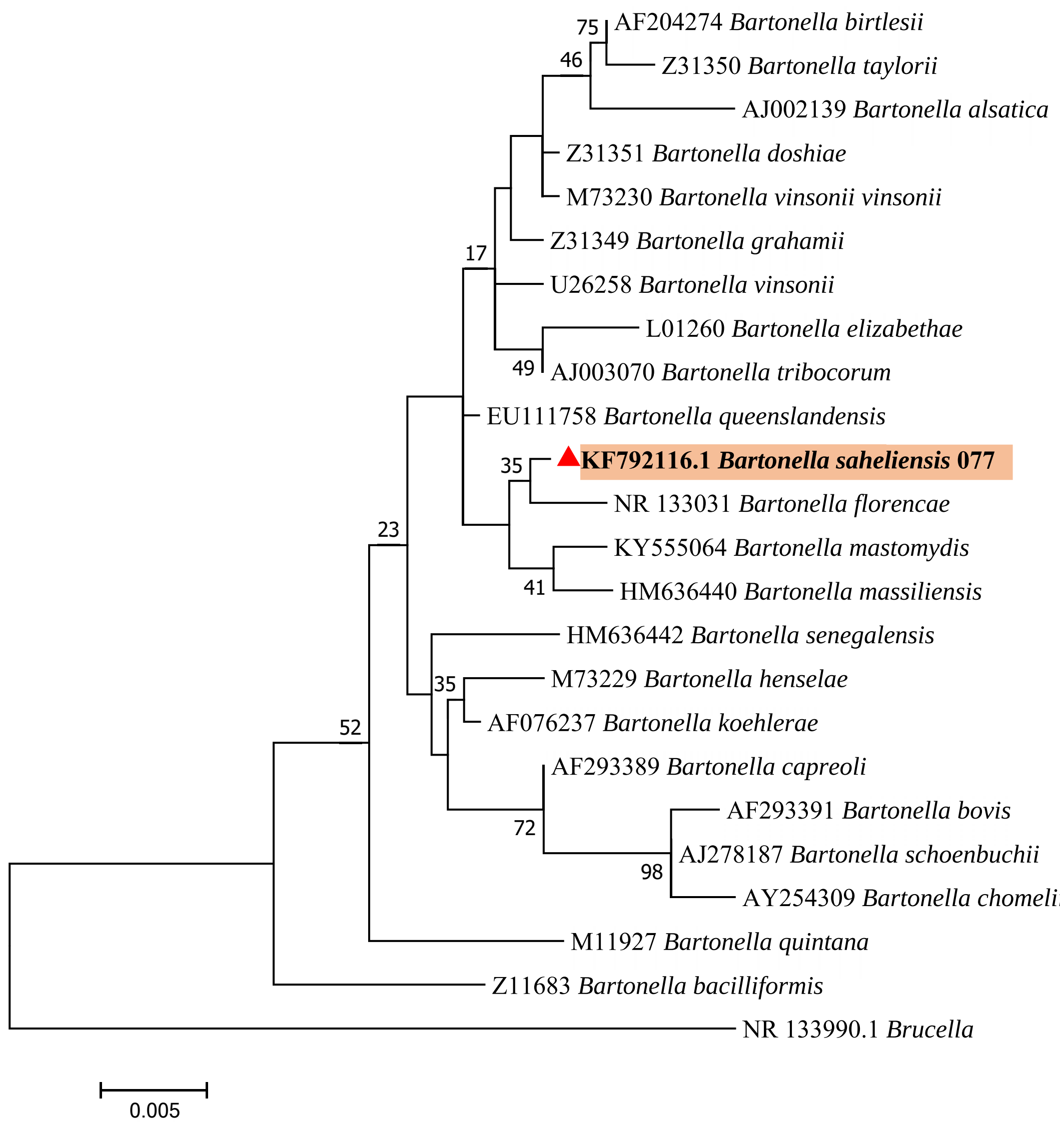

FIG. 1. Phylogenetic tree showing the position of *Bartonella saheliensis* sp. nov., strain 077 relative to other phylogenetically close neighbours. Sequences were aligned using ClustalW parameters within MEGA 7 software. The evolutionary history was inferred using the Minimum Evolution method. The respective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

vacuum conditions. 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 also did not show catalase or oxidase activity (Fig. 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 was 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 was selected for genome sequencing. This genome is the 21st from *Bartonella* species and the first of *Bartonella saheliensis* sp. nov. It was assembled and deposited under the following GenBank accession number:

TABLE 1. Classification and general features of *Bartonella saheliensis* sp. nov. strain 077

MIGS ID	Property	Term	Evidence code
	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 [35–37]
		Species *Bartonella saheliensis*	IDA
		Type strain 077	IDA
	Gram stain	Negative	IDA
	Cell shape	Rod	IDA
	Motility	Non-motile	IDA
	Sporulation	Non-sporulating	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

MIGS, Minimum Information About a Genome Sequence; IDA, Inferred from Direct Assay; TAS, Traceable Author Statement.

CABGUM010000001-CABGUM010000132. A summary of project information is presented in Table 2.

Bartonella saheliensis strain 077 (= CSUR B644T; = DSM 28003T) 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 6 × 100 μL of G2 buffer. A first mechanical lysis was performed with glass powder using the Fastprep-24 device (MP Biomedicals, Graffenstaden, France) during 2 × 20 s. Then, after 30 min of 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

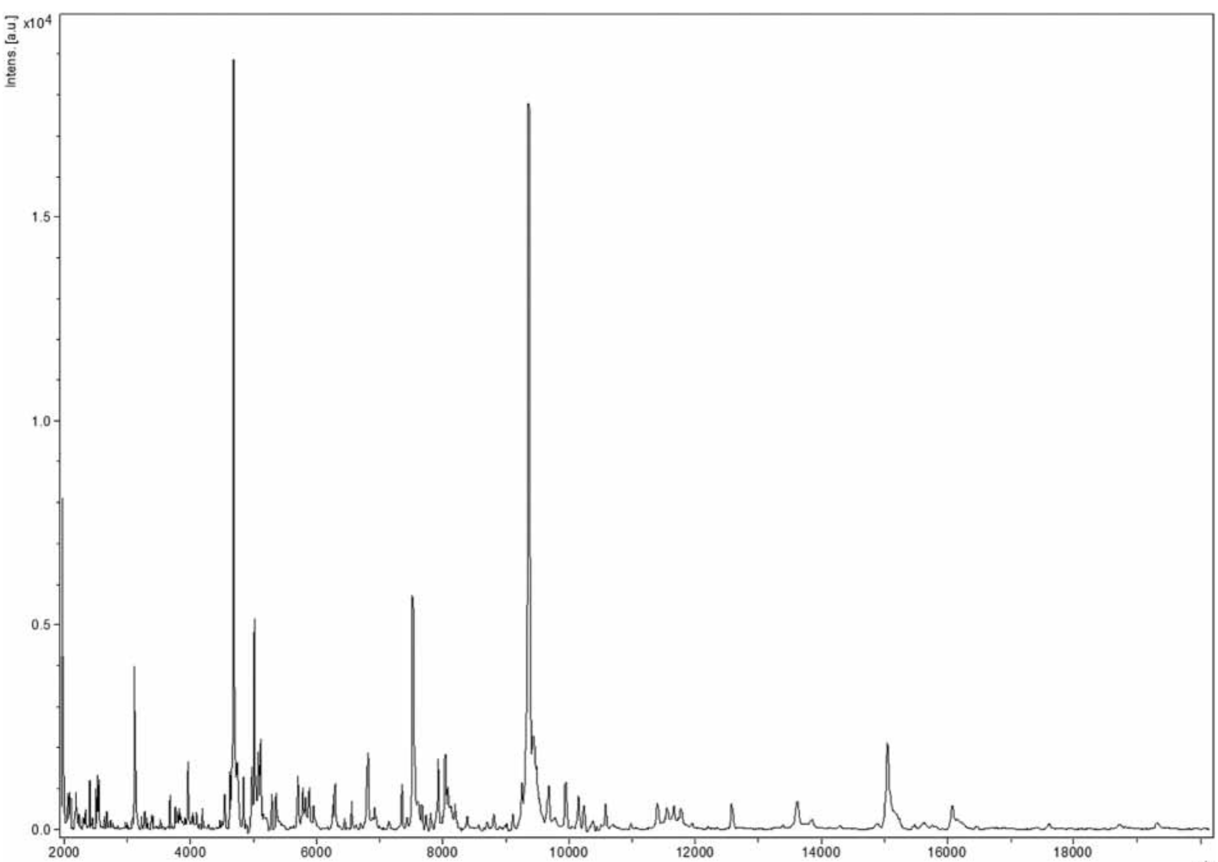

FIG. 2. MALDI-TOF MS reference mass spectrum. *Bartonella saheliensis* sp. nov. spectra from 12 individual colonies were compared and a reference spectrum was generated.

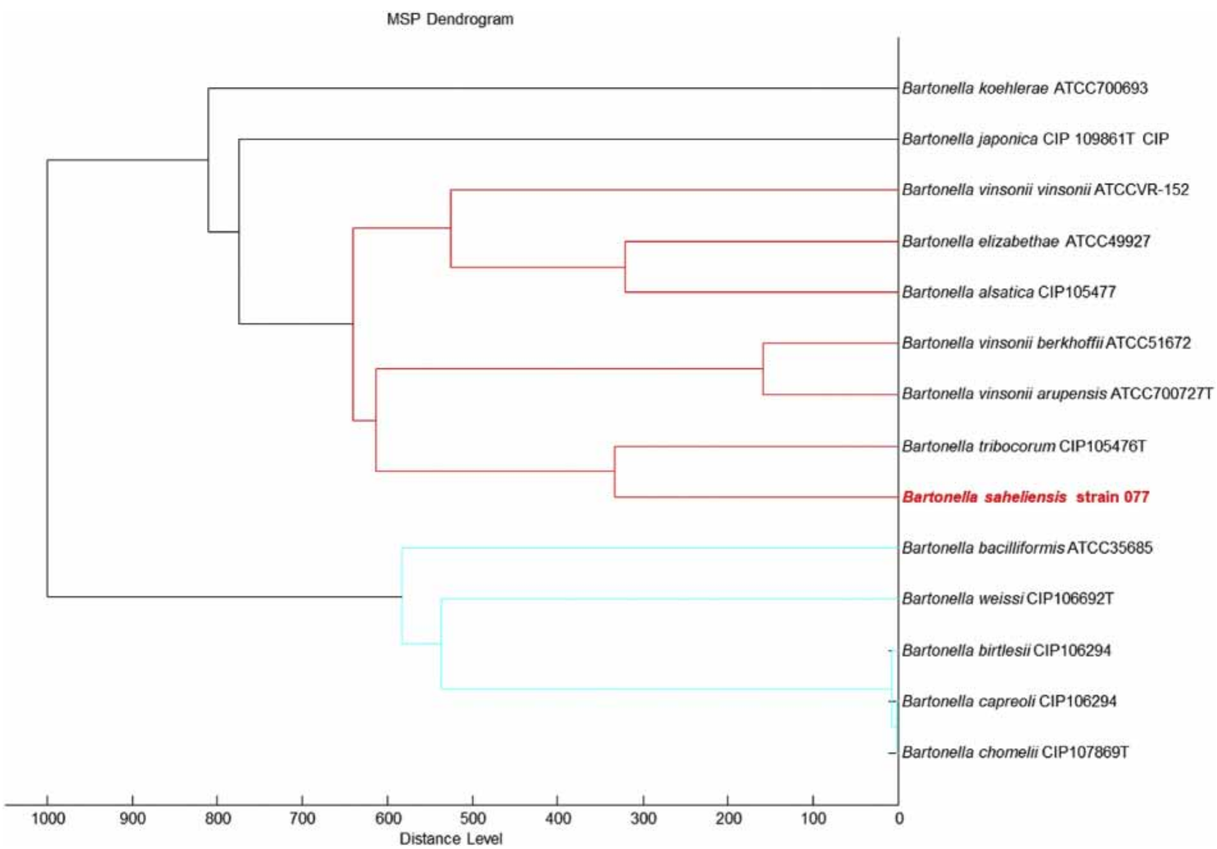

FIG. 3. Dendrogram comparing the MALDI-TOF spectra of *Bartonella saheliensis* sp. nov. strain 077 with those of other members of *Bartonella* genus.

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 7360 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 four and three 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 two emPCR conditions were loaded on a $^1/_4$ 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 (≥1500 bp), generating a genome size of 2 327 299 bp, which corresponds to 28 × equivalent genome.

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

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.

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 bp long with 38.4 mol% GC content (Fig. 5). It is composed of 173 contigs. Of the 2015 predicted genes, 1925 were protein-coding genes and 53 were RNAs (including one tmRNA, six 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. The degree of genomic similarity between *B. saheliensis* strain 077 and closely related species was estimated using the OrthoANI software [27]. Values among closely

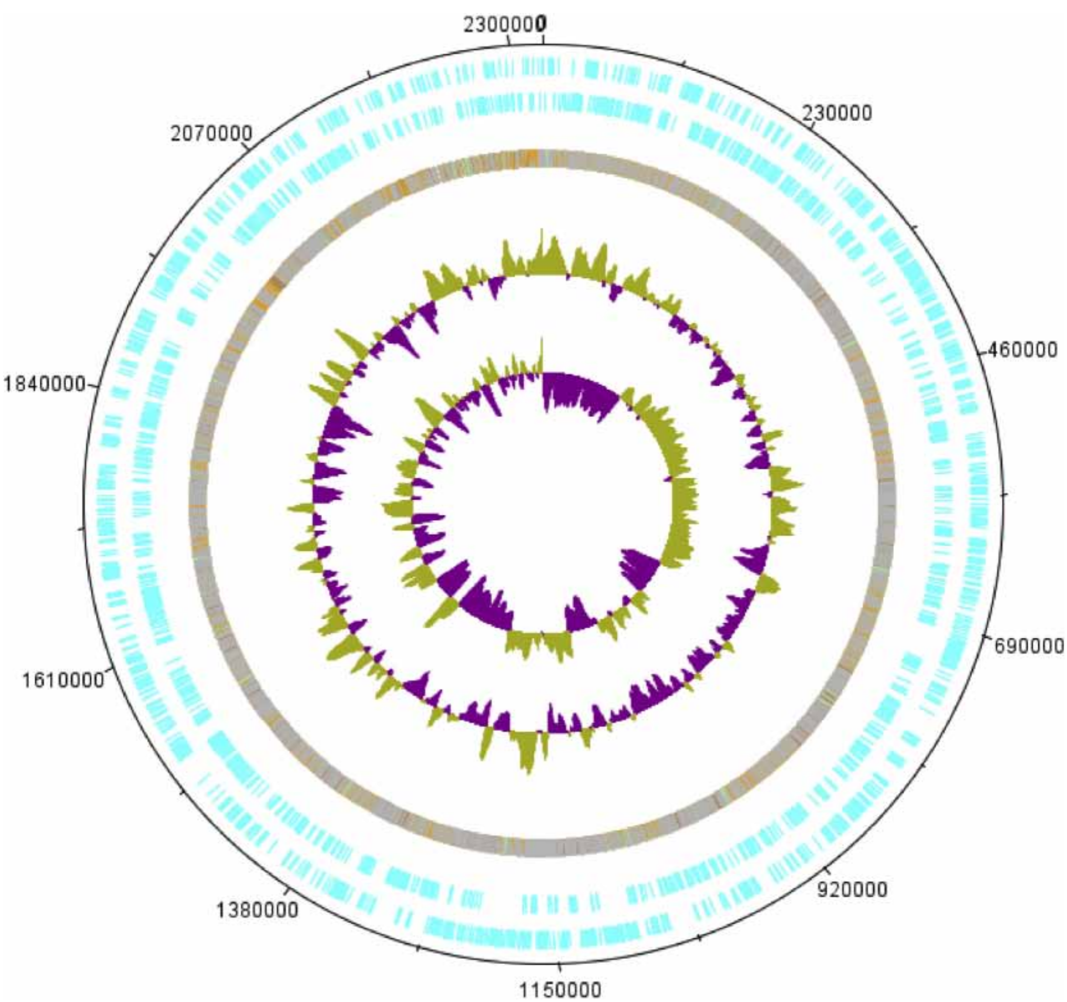

FIG. 5. Graphical circular map of the genome. From outside to the centre: genes on the forward strand, genes on the reverse strand coloured in red, all contigs, G + C content and G + C skew.

TABLE 3. Number of genes associated with the 25 general COG functional categories

Code	Value	% of tota[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	Defence 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

[a]The total is based on the total number of protein coding genes in the annotated genome.

related species (Fig. 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 related species, values ranged from 81.47% with B. rattaustraliani AUST NH4 to 91.13% with B. elizabethae strain NCTC12898.

Conclusion

Based on what has been 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—we propose *B. saheliensis* strain 077 as the type strain of Bartonella *saheliensis* sp. nov., a new bacterial species within the family Bartonellaceae. The strain

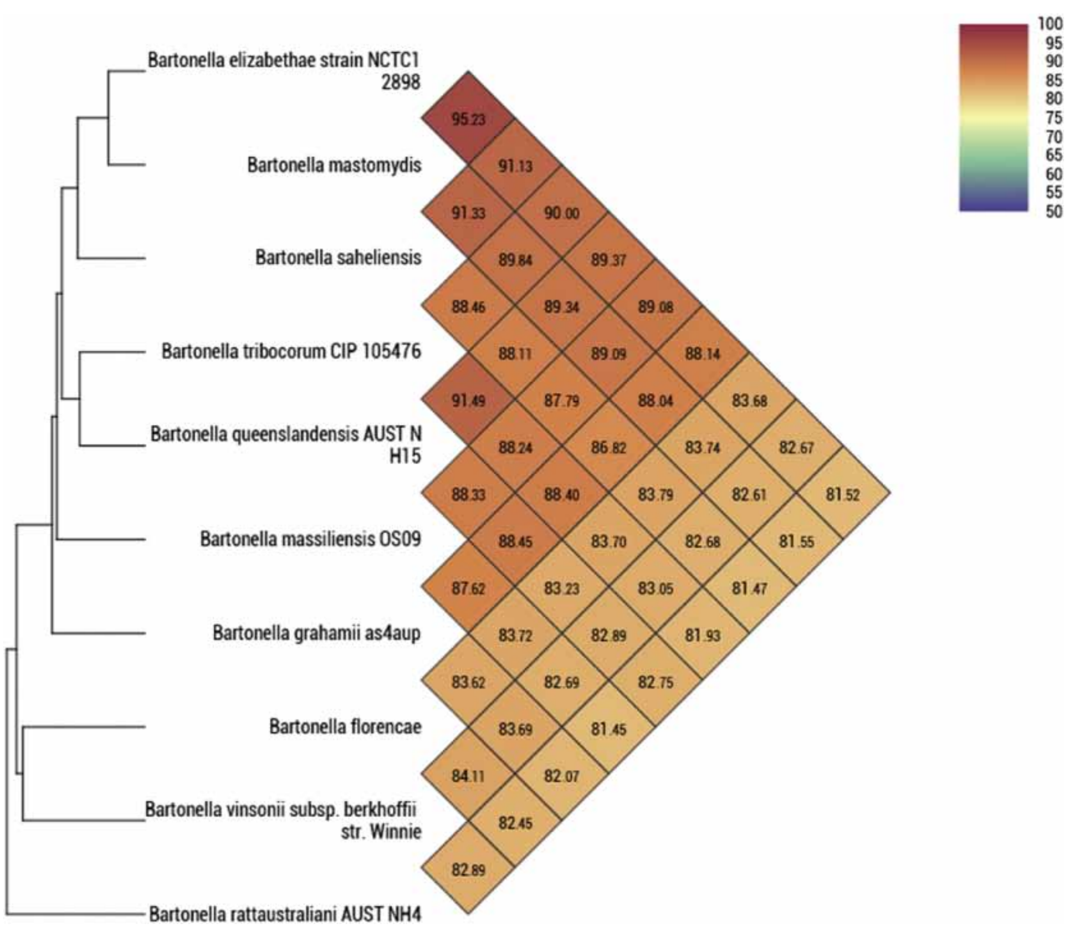

FIG. 6. Heatmap generated with OrthoANI values calculated using the OAT software between *Bartonella saheliensis* sp. nov. strain 077 and other closely related species with standing in nomenclature.

was isolated from the blood of *Gerbilliscus gambianus* captured in the Sine-Saloum region of Senegal.

Description of *Bartonella saheliensis* sp. nov.

Bartonella saheliensis sp. nov., (sah.el.li.en'sis. L. masc. adj. saheliensis of Sahel, the ecoclimatic and biogeographic zone of transition in Africa between the Sahara in the north and the Sudanian Savanna in the south, where *Gerbilliscus gambianus* from which the type strain was isolated is endemic) is a non-motile, gram-negative rod. Colonies are opaque, grey, with a diameter of 0.3–1 mm on blood-enriched Columbia agar. Optimal growth is observed at 32°C in an aerobic atmosphere. Length and width are 1.05 ± 0.08 μm and 0.6 ± 0.05 μm, respectively. Cells are rod-shaped without flagella or pili. Bartonella saheliensis sp. nov. strain 077 exhibits low biochemical and enzymatic activities. The genome size and GC content are 2.23 Mb and 38.4 mol%, respectively. The type strain 077 (= CSUR B644T; = DSM 28003T) was isolated from the blood of *Gerbilliscus gambianus* captured in the Sine-Saloum region of Senegal.

Nucleotide sequence accession number

The complete annotation as well as genome sequences of Bartonella saheliensis sp. nov. strain 077 are deposited in GenBank un 7204 and CABGUM010000001-CABGUM010000132, respectively.

Deposit in culture collections

Strain 077 was deposited in two different strain collections under numbers: CSUR B644T and DSM 28003T.

Transparency declaration

The authors declare no conflicts of interest.

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References

[1] Welch DF. *Bartonella*. Bergey's manual of systematics of archaea and bacteria. Chichester, UK: John Wiley & Sons, Ltd; 2015. p. 1–15. https://doi.org/10.1002/9781118960608.gbm00794.

[2] Birtles RJ. Bartonellae as elegant hemotropic parasites. Ann N Y Acad Sci 2005;1063:270–9. https://doi.org/10.1196/annals.1355.044.

[3] Mediannikov O, Karkouri K El, Robert C, Fournier PE, Raoult D. Non-contiguous finished genome sequence and description of *Bartonella florenciae* sp. nov. Stand Genomic Sci 2013;9:185–96. https://doi.org/10.4056/sigs.4358060.

[4] Mediannikov O, Aubadie M, Bassene H, Diatta G, Granjon L, Fenollar F. Three new *Bartonella* species from rodents in Senegal. Int J Infect Dis 2014;21:335. https://doi.org/10.1016/j.ijid.2014.03.1112.

[5] Gundi VAKB, Davoust B, Khamis A, Boni M, Raoult D, La Scola B. Isolation of *Bartonella rattimassiliensis* sp. nov. and *Bartonella phoceensis* sp. nov. from European *Rattus norvegicus*. J Clin Microbiol 2004;42:3816–8. https://doi.org/10.1128/JCM.42.8.3816-3818.2004.

[6] Heller R, Riegel P, Hansmann Y, Delacour G, Bermond D, Dehio C, et al. *Bartonella tribocorum* sp. nov., a new *Bartonella* species isolated from the blood of wild rats. Int J Syst Bacteriol 1998;48:1333–9. https://doi.org/10.1099/00207713-48-4-1333.

[7] Gundi VAKB, Taylor C, Raoult D, La Scola B. *Bartonella rattaustraliani* sp. nov., *Bartonella queenslandensis* sp. nov. and *Bartonella coopersplainsensis* sp. nov., identified in Australian rats. Int J Syst Evol Microbiol 2009;59:2956–61. https://doi.org/10.1099/ijs.0.002865-0.

[8] Medkour H, Ibrahima LO C, Anani H, Fenollar F, Mediannikov O. *Bartonella massiliensis* sp. nov., a new bacterial species isolated from an *Ornithodoros sonrai* tick from Senegal. New Microbe. New Infect 2019;100596. https://doi.org/10.1016/j.nmni.2019.100596.

[9] Kraljik J, Paziewska-Harris A, Miklisová D, Blaňarová L, Mošanský L, Bona M, et al. Genetic diversity of *Bartonella* genotypes found in the striped field mouse (*Apodemus agrarius*) in Central Europe. Parasitology 2016;143:1437–42. https://doi.org/10.1017/S0031182016000962.

[10] Abreu-yanes E, Martin-Alonso A, Martin-Carrillo N, Livia KG, Marrero-Gagliardi A, Valladares B, et al. *Bartonella* in rodents and ectoparasites in the Canary Islands, Spain: new insights into host–vector–pathogen relationships. Microb Ecol 2018;75:264–73. https://doi.org/10.1007/s00248-017-1022-y.

[11] Angelakis E, Billeter SA, Breitschwerdt EB, Chomel BB, Raoult D. Potential for tick-borne bartonelloses. Emerg Infect Dis 2010;16:385–91. https://doi.org/10.3201/eid1603.091685.

[12] Angelakis E, Pulcini C, Waton J, Imbert P, Socolovschi C, Edouard S, et al. Scalp eschar and neck lymphadenopathy caused by *Bartonella henselae* after tick bite. Clin Infect Dis 2010;50:549–51. https://doi.org/10.1086/650172.
[13] Telford SR, Wormser GP. *Bartonella* spp. transmission by ticks not established. Emerg Infect Dis 2010;16:379–84. https://doi.org/10.3201/eid1603.090443.
[14] Mediannikov O, Socolovschi C, Bassene H, Diatta G, Ratmanov P, Fenollar F, et al. *Borrelia crocidurae* infection in acutely febrile patients, Senegal. Emerg Infect Dis 2014;20:1335–8. https://doi.org/10.3201/eid2008.130550.
[15] Ramasamy D, Mishra AK, Lagier J-C, Padhmanabhan R, Rossi M, Sentausa E, et al. A polyphasic strategy incorporating genomic data for the taxonomic description of novel bacterial species. Int J Syst Evol Microbiol 2014;64:384–91. https://doi.org/10.1099/ijs.0.057091-0.
[16] Fournier P-E, Lagier J-C, Dubourg G, Raoult D. From culturomics to taxonomogenomics: a need to change the taxonomy of prokaryotes in clinical microbiology. Anaerobe 2015;36:73–8. https://doi.org/10.1016/j.anaerobe.2015.10.011.
[17] Dahmani M, Diatta G, Labas N, Diop A, Bassene H, Raoult D, et al. Noncontiguous finished genome sequence and description of *Bartonella mastomydis* sp. nov. New Microbe. New Infect 2018;25:60–70. https://doi.org/10.1016/j.nmni.2018.03.005.
[18] Seng P, Drancourt M, Gouriet F, La Scola B, Fournier P-E, Rolain JM, et al. Ongoing revolution in bacteriology: routine identification of bacteria by matrix-assisted laser desorption ionization time-of-flight mass spectrometry. Clin Infect Dis 2009;49:543–51. https://doi.org/10.1086/600885.
[19] Mediannikov O, El Karkouri K, Diatta G, Robert C, Fournier P-E, Raoult D. Non-contiguous finished genome sequence and description of *Bartonella senegalensis* sp. nov. Stand Genomic Sci 2013;8:279–89. https://doi.org/10.4056/sigs.3807472.
[20] Hyatt D, Chen G-L, Locascio PF, Land ML, Larimer FW, Hauser LJ. Prodigal: prokaryotic gene recognition and translation initiation site identification. BMC Bioinform 2010;11:119. https://doi.org/10.1186/1471-2105-11-119.
[21] BLAST. Basic local alignment search tool [Internet] [cited 16 Sep 2019]. Available: https://blast.ncbi.nlm.nih.gov/Blast.cgi.
[22] Tatusov RL, Galperin MY, Natale DA, Koonin EV. The COG database: a tool for genome-scale analysis of protein functions and evolution. Nucleic Acids Res 2000;28:33–6. https://doi.org/10.1093/nar/28.1.33.
[23] Lagesen K, Hallin P, Rødland EA, Stærfeldt HH, Rognes T, Ussery DW. RNAmmer: consistent and rapid annotation of ribosomal RNA genes. Nucleic Acids Res 2007;35:3100–8. https://doi.org/10.1093/nar/gkm160.
[24] Laslett D, Canback B. ARAGORN, a program to detect tRNA genes and tmRNA genes in nucleotide sequences. Nucleic Acids Res 2004;32:11–6. https://doi.org/10.1093/nar/gkh152.
[25] TMHMM Server, v. 2.0 [Internet] [cited 16 Sep 2019]. Available: http://www.cbs.dtu.dk/services/TMHMM/.
[26] Petersen TN, Brunak S, von Heijne G, Nielsen H. SignalP 4.0: discriminating signal peptides from transmembrane regions. Nat Methods 2011:785–6. https://doi.org/10.1038/nmeth.1701.
[27] Lee I, Ouk Kim Y, Park S-C, Chun J. OrthoANI: an improved algorithm and software for calculating average nucleotide identity. Int J Syst Evol Microbiol 2016;66:1100–3. https://doi.org/10.1099/ijsem.0.000760.
[28] Woese CR, Kandler O, Wheelis ML. Towards a natural system of organisms: proposal for the domains Archaea, Bacteria, and Eucarya. Proc Natl Acad Sci USA 1990;87:4576–9. https://doi.org/10.1073/pnas.87.12.4576.
[29] Lilburn T, Garrity GM, Bell JA. Phylum XIV. Proteobacteria phyl. nov. In: Garrity GM, editor. Bergey's Manual of Systematic Bacteriology. 2nd ed. New York: Springer; 2005. p. 1–574.
[30] Staley JT, Boone DR, Brenner DJ, De Vos P, Garrity GM, Goodfellow M, et al. The proteobacteria, Part B: the gammaproteobacteria. Bergey's manual of systematic bacteriology, ume two. Springer; 2005. p. 1106. https://doi.org/10.1007/0-387-28022-7.
[31] Garrity GM, Bell JA, Lilburn T. Class I: Alpha-proteobacteria. Phyl nov. In: Brenner DJ, Krieg NR, Staley JT, editors. Bergey's Manual of Systemmatic Bacteriology, The Proteobacteria, Part C: The Alpha, Beta, Delta, and Epsilon-proteobacteria, Vol. 2. U.S: Springer; 2005. p. 1–574.
[32] Ashburner M, Ball CA, Blake JA, Botstein D, Butler H, Cherry JM, et al. Gene ontology: tool for the unification of biology. Nat Genet 2000;25:25–9. https://doi.org/10.1038/75556.
[33] David Kuykendall L. Rhizobiales ord. nov . Bergey's manual of systematics of archaea and bacteria. John Wiley & Sons, Ltd; 2015. https://doi.org/10.1002/9781118960608.obm00071. 1–1.
[34] Skerman VBD, McGowan V, Sneath PHA. Approved list of bacterial names. amended edition. American Society for Microbiology; 1989. https://doi.org/10.1099/00207713-30-1-225 [Internet].
[35] Brenner DJ, O'Connor SP, Winkler HH, Steigerwalt AG. Proposals to unify the genera *Bartonella* and *Rochalimaea*, with descriptions of *Bartonella quintana* comb. nov., *Bartonella vinsonii* comb. nov., *Bartonella henselae* comb. nov., and *Bartonella elizabethae* comb. nov., and to remove the family Bartonellaceae from the order Rickettsiales. Int J Syst Bacteriol 1993;43:777–86. https://doi.org/10.1099/00207713-43-4-777.
[36] Birtles RJ, Harrison TG, Saunders NA, Molyneux DH. Proposals to unify the genera *Grahamella* and *Bartonella*, with descriptions of *Bartonella talpae* comb. nov., *Bartonella peromysci* comb. nov., and three new species, *Bartonella grahamii* sp. nov., *Bartonella taylorii* sp. nov., and *Bartonella doshiae* sp. nov. Int J Syst Bacteriol 1995;45:1–8. https://doi.org/10.1099/00207713-45-1-1.
[37] Weinman D. Genus I. Bartonella strong, tyzzer and sellards 1915, 808. In: Buchanan RE, Gibbons NE, editors. Bergey's manual of determinative bacteriology (8th ed.). Baltimore: The Williams and Wilkins Co.; 1974. p. 904–5.