Noncontiguous finished genome sequence and description of *Bartonella mastomydis* sp. nov.

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Abstract

Bartonella mastomydis sp. nov. strain 008 is the type strain of *B. mastomydis* sp. nov., a new species within the genus *Bartonella.* This strain was isolated from *Mastomys erythroleucus* rodents trapped in the Sine-Saloum region of Senegal. Here we describe the features of this organism, together with the complete genome sequence and its annotation. The 2 044 960 bp long genomes with 38.44% G + C content contains 1674 protein-coding and 42 RNA genes, including three rRNA genes.

Keywords: Bartonella mastomydis sp. nov., complete genome, *Mastomys erythroleucus*, *rodents*, *Senegal*
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Introduction

Just over a century ago, the first historical record of the emerging *Bartonella* genus was made during World War I, when a million frontline troops were shown to be plagued by a disease later known as trench fever. This was caused by the louse-borne bacterium now known as *Bartonella quintana* [1]. *Bartonella* are small facultative intracellular, vector-transmitted, Gram-negative, haemotropic bacilli, classified within the class of α-proteobacteria [2]. The genus was significantly expanded after Brenner et al. proposed the unification of genera *Bartonella* and *Rochalimaea* in 1993 and Birtles et al. unified *Bartonella* and *Grahamella* genera in 1995 [3]. The *Bartonellaceae* family (Gieszczykiewicz 1939) [4] contains 35 species and three subspecies (http://www.bacterio.net/) as of 1 August 2017 [5]. Bartonellae usually exist in two specific habitats: the gut of the obligately blood-sucking arthropod vector and the bloodstream of the mammalian host [1]. Among the 38 recognized *Bartonella* species, 17 have been described as pathogenic in humans [6]. In humans, *Bartonella* bacteria are among the most described as being associated with endocarditis or cardiopathy. In animal hosts, a wide array of clinical syndromes, as well as asymptomatic infection and endocarditis, have been described [6–8].

New species and subspecies are constantly being proposed. Candidate species belonging to the genus *Bartonella* from a wide range of animal reservoirs have been described but not yet assigned new species designations [1]. Parasitism by bartonellae is widespread among small mammals. Potentially new *Bartonella* species infecting bat communities were reported in Madagascar [9], Kenya [10], Puerto Rico [11] and French Guiana [12]. Rodents and insectivores were showed to maintain bartonellae infections. Additionally, a large number of partially characterized *Bartonella* have been isolated from rodents in Southeast Asia [13], South Africa [14,15], Europe, North and South America [16], Nigeria [17], the Republic of Congo and Tanzania [16]. In Senegal, West Africa, using the criteria proposed by La Scola et al. [18] based on the multilocus sequence analyses of four genes and the intergenic spacer (ITS) as a tool to the description of bartonellae, three new bartonellae were isolated and described: *Bartonella senegalensis, Bartonella massiliensis* from the soft tick *Ornithodoros sonrai* [13] and *Bartonella davoustii* from cattle [19].

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We sought to describe an additional *Bartonella* species isolated from small mammals in the region of Sine-Saloum, in western Senegal [20]. In this rural region, the biotype is favourable to the spread of commensal mammals harbouring pathogenic microorganisms and is often found in close contact with humans. This situation increases the risk of human and animal transmission of infectious disease from rodent-associated tick-borne pathogens. This work describes the genome sequence of the proposed candidate *Bartonella mastomydis* strain 008 isolated from *Mastomys erythroleucus* using a polyphasic approach combining matrix-assisted desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) and genomic properties, as well as next-generation sequencing technology to complete description of a potentially new species [21].

Here we present the summary classification and a set of features for *B. mastomydis* sp. nov. strain 008 together with the description of the complete genomic sequences and annotation. These characteristics support the definition of the species *B. mastomydis*.

Samples and bacterial culture

In February 2013, rodents and insectivores were captured alive at two sites (Dielmo and Ndiop) using wire mesh traps baited with peanut butter or onions. Our aim was to investigate the presence of *Bartonella* spp. in commensal rodents in Sine-Saloum, Senegal. In this region, rodents and rodent-associated soft ticks are respectively the reservoirs and vectors of relapsing fever caused by *Borrelia crocidurae*. Trapped rodents and insectivores were anesthetized and necropsied in sterile conditions. Sampled blood was inoculated on homemade Columbia agar plates supplemented with 5% sheep's blood. The results of this study have been reported elsewhere [20]. In total, within a 6-day period, 119 small mammals were captured: 116 rodents and three shrews (*Crocidura* cf. *olivieri*). Rodents were identified morphologically as follows: five *Arvicanthis niloticus,* 56 *Gerbillis-cus gambianus,* 49 *M. erythroleucus,* five *Mus musculus* and one *Praomys daltoni*. Thirty isolates of *Bartonella* spp. were recovered from the rodent bloodstreams. None of those isolated belonged to previously described *Bartonella* species (Table 1).

Classification and features

The *gltA, rpoB,* 16S rRNA and *ftsZ* genes as well as the ITS have been amplified and sequenced from recovered *Bartonella* isolates [18,22–25]. *Bartonella mastomydis* (21 isolates) recovered only from *M. erythroleucus* was obtained following the fifth to tenth incubations at 37°C in a 5% CO_2-enriched atmosphere on Columbia agar plates supplemented with 5% sheep's blood. Other morphologically and genetically indistinguishable strains were isolated from *M. erythroleucus*. The 21 isolates of *B. mastomydis* are almost genetically identical; however, strains type 008, 025, 086 and 202 showed different nucleotide identity. The identities between them are as follows: 100% for the *rrs* gene, 99% for the *rpoB* gene and 98% to 99% for the *ftsZ* and *gltA* genes. The sequence of the ITS of strain 008 present 94% to 99% identity with strains 025, 086 and 202, as presented by a 23 bp deletion and a 4 bp insertion compared to the other strains. This study focused on the taxonomic description and identification of strain 008.

Strain 008 exhibits the following nucleotide sequence similarities for the *rrs* gene (KY555064): 99% with *Bartonella tribocorum* str. BM1374166 (HG969192), *Bartonella grahamii* str. as4aup (CP001562), *Bartonella vinsonii* subsp. *arupensis* str. OK 94-513 (NR_104902) and subsp. *berkhoffii* (CP003124), *Bartonella elizabethae* str. F9251 (NR_025889), *Bartonella henselae* str. Houston-1 (NR_074335) and finally *Bartonella quintana* str. Toulouse (BX897700). For the ITS (KY555067), 95% similarity

TABLE 1. Classification and general features of *Bartonella massiliensis* strain 008.

MIGS ID	Property	Term	Evidence code[a]
	Current classification	Domain *Bacteria*	TAS [42]
		Phylum *Proteobacteria*	TAS [43]
		Class *Alphaproteobacteria*	TAS [44]
		Order *Rhizobiales*	TAS [45,46]
		Family *Bartonellaceae*	TAS [4,22]
		Genus *Bartonella*	TAS [3,4,22,47]
		Species *Bartonella mastomydis*	IDA
		Type strain 008	IDA
	Gram stain	Negative	IDA
	Cell shape	Rod	IDA
	Motility	Nonmotile	IDA
	Sporulation	Nonsporulating	IDA
	Temperature range	Mesophilic	IDA
	Optimum temperature	37°C	IDA
MIGS-22	Oxygen requirement	Aerobic	IDA
	Carbon source	Unknown	IDA
	Energy source	Unknown	IDA
MIGS-6	Habitat	*Mastomys erythroleucus* bloodstream	IDA
MIGS-15	Biotic relationship	Facultative intracellular	IDA
	Pathogenicity	Unknown	IDA
	Biosafety level	3	IDA
MIGS-14	Isolation	*M. erythroleucus*	IDA
MIGS-4	Geographic location	Senegal	IDA
MIGS-5	Sample collection	February 2013	IDA
MIGS-4.2	Latitude	14°03′N	IDA
MIGS-4.3	Longitude	15°31′W	IDA
MIGS-4.4	Altitude	8 m	IDA

MIGS, Minimum Information About a Genome Sequence.
[a]Evidence codes are as follows: IDA, inferred from direct assay; TAS, traceable author statement (i.e. a direct report exists in the literature); NAS, nontraceable author statement (i.e. not directly observed for the living, isolated sample, but based on a generally accepted property for the species or anecdotal evidence). These evidence codes are from the Gene Ontology project (http://www.geneontology.org/GO.evidence.shtml). If the evidence code is IDA, then the property should have been directly observed, for the purpose of this specific publication, for a live isolate by one of the authors, or by an expert or reputable institution mentioned in the acknowledgements.

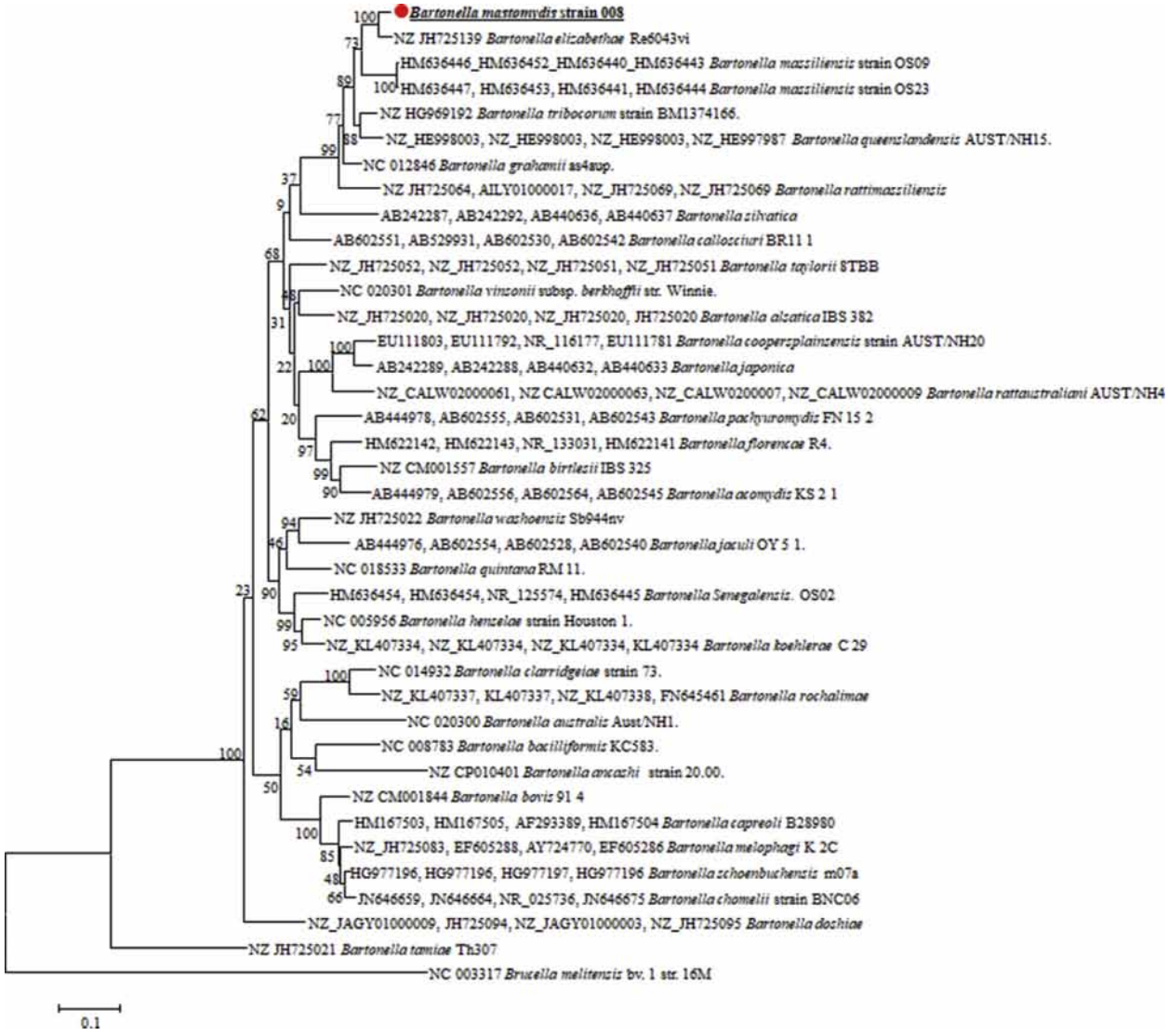

FIG. 1. Evolutionary history of sequenced samples was inferred using maximum likelihood method implemented in MEGA7 [40] and based on concatenated *gltA, rpoB,* 16S RNA and *ftsZ* (total length of 2731 bp) sequences. Sequences of *gltA, rpoB,*16S RNA and *ftsZ* genes used for comparison were obtained from the GenBank database (https://www.ncbi.nlm.nih.gov/genbank/). Sequences were aligned using BioEdit [41]. Firstly, for each gene individually, sequences we used for comparison were first aligned using CLUSTALW. All positions containing gaps and missing data were eliminated manually; then each alignment was concatenated and second alignment was performed. Evolutionary history was inferred by maximum likelihood method based on Hasegawa-Kishino-Yano model. Percentage of trees in which associated taxa clustered together is shown next to branches. Initial tree for heuristic search was obtained automatically by applying the Neighbor Joining and BioNJ algorithms to matrix of pairwise distances estimated using maximum composite likelihood (MCL) approach and then selecting topology with superior log likelihood value. Discrete gamma distribution was used to model evolutionary rate differences among sites (two categories (+G, parameter = 0.2144)). Tree is drawn to scale, with branch lengths measured in number of substitutions per site. Statistical support for internal branches of trees was evaluated by bootstrapping with 1000 iterations. Analysis involved 39 nucleotide sequences.

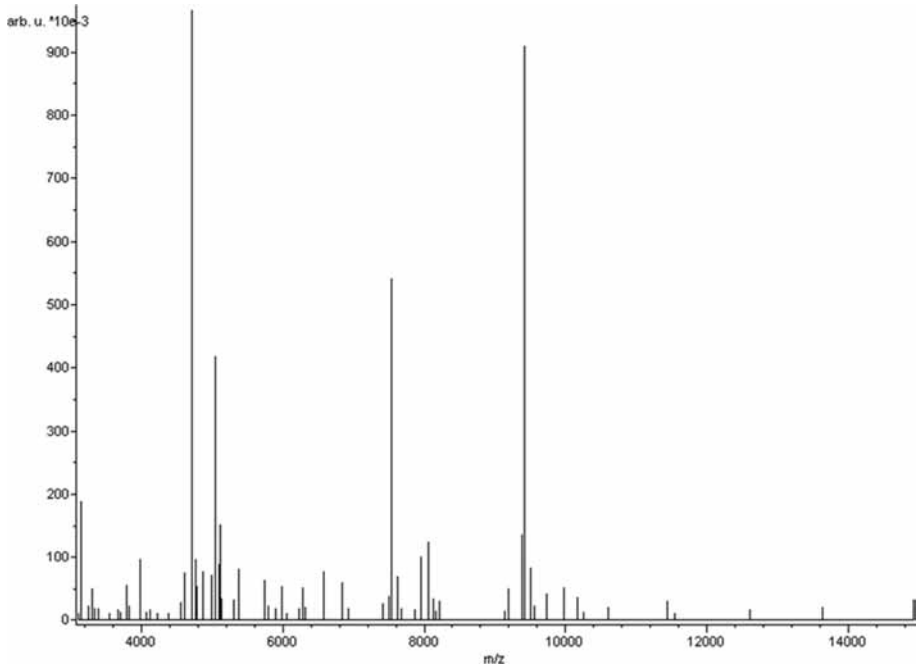

FIG. 2. Reference mass spectrum from *Bartonella mastomydis* strain 008. Spectra from 12 individual colonies were compared and reference spectrum was generated.

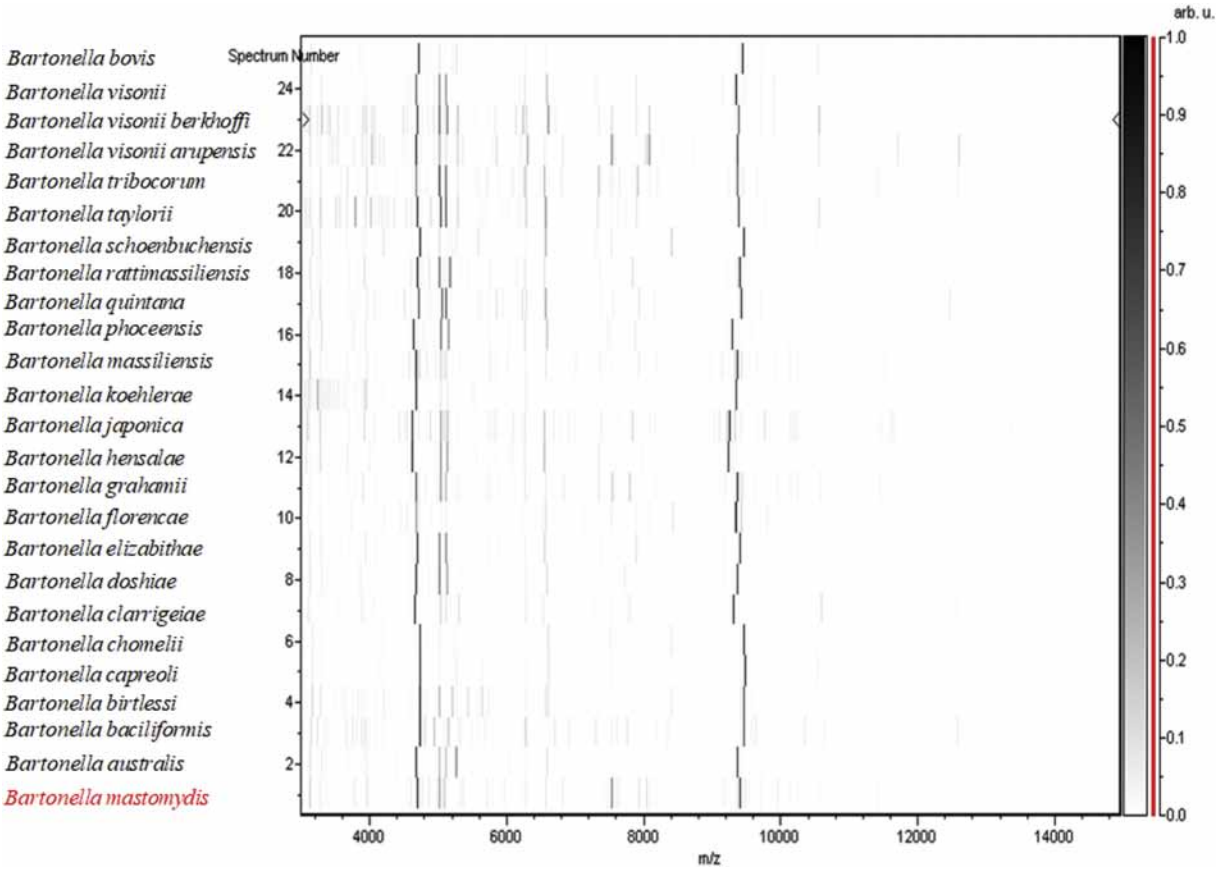

FIG. 3. Gel view comparing *Bartonella mastomydis* strain 008 spectra with other members of *Bartonella* genus. Gel view displays raw spectra of loaded spectrum files arranged in pseudo–gel-like look. *X*-axis records *m*/*z* value. Left *y*-axis displays running spectrum number originating from subsequent spectra loading. Peak intensity is expressed by greyscale scheme code. Colour bar and right *y*-axis indicate relation between colour in which peak is displayed and peak intensity in arbitrary units. Displayed species are indicated at left.

was observed with *B. elizabethae* (L35103). For the *gltA* gene (KY555066), 97% similarity was observed with *B. elizabethae* (Z70009), 94% with *B. tribocorum* str. BM1374166 (HG969192), *B. grahamii* str. as4aup (CP001562) and *Bartonella queenslandensis* str. AUST/NH12 (EU111798). For the *ftsZ* gene (KY555065), 98% of similarity was observed with *B. elizabethae* (AF467760), 96% with *B. tribocorum* str. BM1374166 (HG969192), *B. grahamii* str. as4aup (CP001562) and *B. queenslandensis* str. AUST/NH12 (EU111798). For the *rpoB* gene (KY555068), 99% similarity was observed with multiple uncultured *Bartonella* amplified from small mammals from Ethiopia [26], Benin [27], Congo and Tanzania [16] and Nepal [28]. The closest recognized species was *B. elizabethae* (AF165992) at 98% homology (Fig. 1).

MALDI-TOF MS protein analysis was carried out as previously described [21]. Five isolated colonies of strain 008 were deposited as individual spots on the MALDI target plate. Each smear was overlaid with 2 µL of matrix solution (a saturated solution of alpha-cyano-4-hydroxycinnamic acid) in 50% acetonitrile and 2.5% trifluoroacetic acid, and allowed to dry for 5 minutes. Measurements were performed with a Microflex spectrometer (Bruker Daltonics, Bremen, Germany). The five 008 spectra were imported into MALDI BioTyper software version 2.0 (Bruker) and were analysed by standard pattern matching (with default parameter settings) against the main spectra of 4613 bacteria in the BioTyper database and the 25 *Bartonella* species in our own database. The identification method included the *m/z* from 3000 to 15 000 Da. For every spectrum, a maximum of 100 peaks was considered and compared with the spectra in the database. A score of below 1.7 meant identification was not possible. For strain 008, the scores obtained were always below 1.5, suggesting that our isolate was not a member of a known species. We added the spectrum from strain 008 to the database (Fig. 2). A gel view comparing the spectrum of strain 008 with those of other *Bartonella* species is shown in Fig. 3.

Biochemical characterization and antibiotic susceptibility

Different growth temperatures (32, 37, 42°C) were tested. Growth occurred only at 37°C in 5% CO_2. Colonies were grey, opaque and 0.5 mm to 1 mm in diameter on blood-enriched Columbia agar. A motility test was negative. Cells grown on agar were Gram negative and had a mean length and width of 1369.8 ± 423.8 nm and 530.9 ± 105.8 nm respectively by electron microscopy (Fig. 4). No flagella or pili were observed. Strain 008 exhibited neither catalase nor oxidase activity. 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 profiles were previously observed for *B. senegalensis* [29]. *Bartonella mastomydis* is sensitive to amoxicillin, amoxicillin/clavulanic acid, oxacillin, imipenem, rifampicin, nitrofurantoin, doxycycline, linezolid, tobramycin, gentamycin, trimethoprim/sulfamethoxazole, fosfomycin and ciprofloxacin. *Bartonella mastomydis* is resistant to metronidazole and colistin.

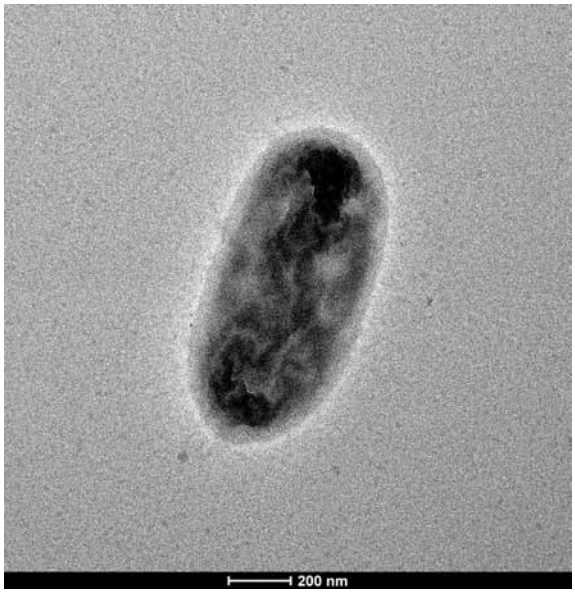

FIG. 4. Transmission electron microscopy of *Bartonella mastomydis* strain 008 using Tecnai G20 device (FEI Company, Limeil-Brévannes, France) at operating voltage of 200 keV. Scale bar = 200 nm.

Genome sequencing information

Genome project history

The organism was selected for sequencing on the basis of the similarity of its 16S rRNA, ITS, *ftsZ*, *gltA* and *rpoB* to other members of the genus *Bartonella*. Nucleotide sequence similarities for these genes suggested that strain 008 represents a

TABLE 2. **Project information**

MIGS 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	30×
MIGS-30	Assemblers	Newbler version 2.5.3
MIGS-12	Gene calling method	Prodigal
	GenBank ID	GCA_900185775
MIGS-13	Project relevance	Biodiversity of *Bartonella* spp. in rodents from Senegal

MIGS, Minimum Information About a Genome Sequence.

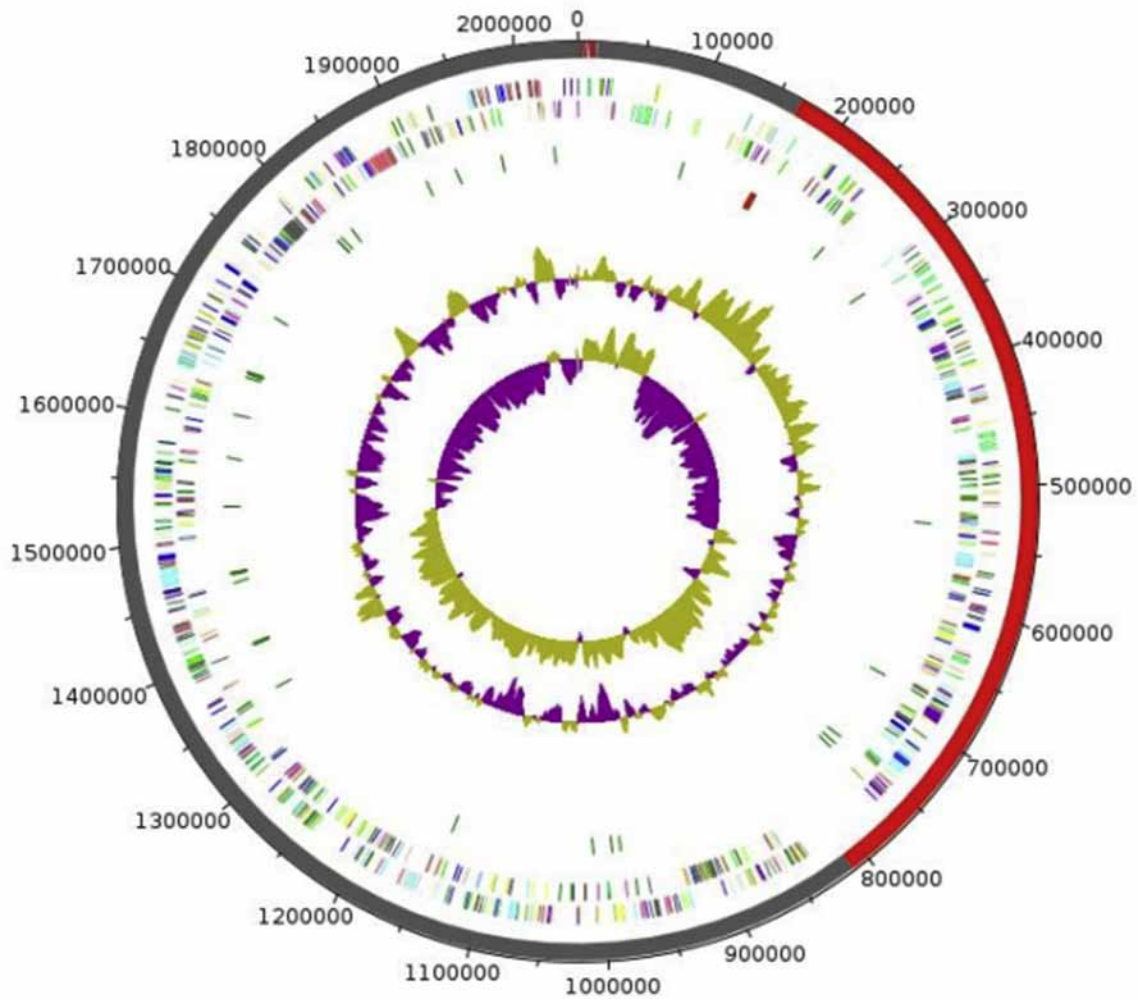

FIG. 5. Graphical circular map of chromosome. From outside to centre: genes on forward strand coloured by COGs categories (only genes assigned to COGs), genes on reverse strand coloured by COGs categories (only gene assigned to COGs), RNA genes (tRNAs green, rRNAs red), G + C content and G + C skew. COGs, Clusters of Orthologous Groups database.

new species in the genus *Bartonella*. A summary of the project information is shown in Table 2. The GenBank accession number is G + CA_900185775, and the entry consists of 12 scaffolds (>1500 bp). Table 2 shows the project information and its association with Minimum Information About a Genome Sequence (MIGS) version 2.0 compliance.

Genome sequencing and assembly

Bartonella mastomydis sp. nov. strain 008 (DSM 28002; CSUR B643) was grown on 5% sheep's blood–enriched Columbia agar at 37°C in a 5% CO_2 atmosphere. Genomic DNA of *B. mastomydis* sp. nov. strain 008 was extracted in two steps. A mechanical treatment was first performed by acid-washed glass beads (G4649-500g; MilliporeSigma, St Louis, MO, USA) using a FastPrep BIO 101 instrument (Qbiogene, Strasbourg, France) at maximum speed (6.5 m/s) for 90 s. Then after 2 hours' lysozyme incubation at 37°C, DNA was extracted on the EZ1 biorobot (Qiagen, Hilden, Germany) with the EZ1 DNA tissue kit. The elution volume was 50 µL. Genomic DNA was quantified by a Qubit assay with the High Sensitivity Kit (Life Technologies, Carlsbad, CA, USA) to 66 ng/µL. Genomic DNA was sequenced on the MiSeq Technology device (Illumina, San Diego, CA, USA) with the mate-pair strategy. The genomic DNA was barcoded to be mixed with 11 other projects with the Nextera Mate Pair sample prep kit (Illumina).

The mate-pair library was prepared with 1.5 µg of genomic DNA using the Nextera mate-pair Illumina guide. The genomic DNA sample was simultaneously fragmented and tagged with a mate-pair junction adapter. The profile of the fragmentation was validated on an Agilent 2100 BioAnalyzer (Agilent Technologies, Santa Clara, CA, USA) with a DNA 7500 labchip. The optimal size of obtained fragments was 7.77 kb. No size

selection was performed, and 600 ng of tagmented fragments were circularized. The circularized DNA was mechanically sheared to small fragments with Optima on a bimodal curve at 593 and 1377 bp on a Covaris (Woburn, MA, USA) S2 device in T6 tubes. The library profile was visualized on a High Sensitivity Bioanalyzer LabChip (Agilent), and the final concentration library was measured at 49.16 nmol/L. The libraries were normalized at 2 nM, pooled with 11 other projects, denatured and diluted at 15 pM. Automated cluster generation and 2 × 250 bp sequencing runs were performed in a 39-hour run.

Total information of 7.2 Gb was obtained from a 765K/mm^2 cluster density, with a cluster passing quality control filters of 94.7% (14 162 000 passed filter clusters). Within this run, the index representation for *B. mastomydis* was determined to 12.30%. The 1 742 441 paired end reads were filtered according to the read qualities.

Genome assembly

The genome's assembly was performed with a pipeline that enabled creation of an assembly with different software programs (Velvet [30], Spades [31] and Soap Denovo [32]), on trimmed (MiSeq and Trimmomatic [33]) or untrimmed data (only MiSeq). For each of the six assemblies performed, GapCloser [32] was used to reduce gaps. Then contamination with Phage Phix was identified (BLASTN against Phage Phix174 DNA sequence) and eliminated. Finally, scaffolds under 800 bp were removed, and scaffolds with a depth value lower than 25% of the mean depth were removed (identified as possible contaminants). The best assembly was selected by using different criteria (number of scaffolds, N50, number of N characters).

Genome annotation

Open reading frames (ORFs) were predicted using Prodigal [34] with default parameters, but the predicted ORFs were excluded if they spanned a sequencing gap region (contained N). The predicted bacterial protein sequences were searched against the Clusters of Orthologous Groups (COGs) database using BLASTP (*E* value of 1e-03, coverage 0.7 and identity percentage 30%). If no hit was found, it searches against the NR database using BLASTP (*E* value of 1e-03, coverage 0.7 and identity percentage of 30%). If the sequence length was <80 aa, we used an *E* value of 1e-05. The tRNAScanSE [35] tool was used to find transfer RNA genes, whereas ribosomal RNA genes were found by using RNAmmer [36]. Lipoprotein signal peptides and the number of transmembrane helices were predicted using Phobius [37]. ORFans were identified if not all of the BLASTP performed gave positive results (*E* value smaller than 1e-03 for ORFs with sequence size >80 aa or *E* value smaller than 1e-05 for ORFs with sequence <80 aa). Such parameter thresholds have already been used in previous work to define ORFans.

TABLE 3. Number of gene associated with 25 general COGs functional categories

Code	Value	% of total	Description
J	157	9.38	Translation
A	0	0	RNA processing and modification
K	58	3.46	Transcription
L	73	4.36	Replication, recombination and repair
B	0	0	Chromatin structure and dynamics
D	17	1.02	Cell cycle control, mitosis and meiosis
Y	0	0	Nuclear structure
V	21	1.25	Defense mechanisms
T	37	2.21	Signal transduction mechanisms
M	74	4.42	Cell wall/membrane biogenesis
N	4	0.24	Cell motility
Z	0	0	Cytoskeleton
W	0	0	Extracellular structures
U	42	2.51	Intracellular trafficking and secretion
O	74	4.42	Posttranslational modification, protein turnover, chaperones
X	25	1.49	Mobilome: prophages, transposons
C	74	4.42	Energy production and conversion
G	56	3.35	Carbohydrate transport and metabolism
E	106	6.33	Amino acid transport and metabolism
F	47	2.81	Nucleotide transport and metabolism
H	63	3.76	Coenzyme transport and metabolism
I	44	2.63	Lipid transport and metabolism
P	57	3.41	Inorganic ion transport and metabolism
Q	15	0.89	Secondary metabolites biosynthesis, transport and catabolism
R	74	4.42	General function prediction only
S	68	4.06	Function unknown
—	603	36.02	Not in COGs

COGs, Clusters of Orthologous Groups database.

Genome properties

The genome is 2 044 960 bp long with 38.44% G + C content. It is composed of 12 scaffolds (composed of 14 contigs) (Fig. 5). Of the 1716 predicted genes, 1674 were protein-coding genes and 42 were RNAs (one gene is 5S rRNA, one gene is 16S rRNA, one gene is 23S rRNA, 39 genes are tRNA genes). A total of 1212 genes (72.4%) were assigned as putative function (by COGs or by NR BLAST). Fifty-six genes were identified as

TABLE 4. Nucleotide content and gene count levels of genome

	Genome (total)	
Attribute	Value	% of Total[a]
Size (bp)	2 044 960	100
G + C content (bp)	785 960	38.44
Coding region	1 555 569	76.07
Total gene	1716	100
RNA genes	42	2.45
Protein-coding genes	1674	100
Protein assigned to COGs	1071	63.99
Protein with peptide signals	263	15.71
Genes with transmembrane helices	372	22.22

COGs, Clusters of Orthologous Groups database.
[a]Total is based on either the size of the genome in base pairs of the total of protein-coding genes in the annotated genome.

ORFans (3.35%). The remaining 338 genes were annotated as hypothetical proteins (20.19%). The distribution of genes into COGs functional categories is presented in Table 3. The propriety and statistics of the genome are summarized in Tables 3 and 4. The most predicted functional genes are associated with translation (9.38%), followed by those involved in the basic biological functions, such as amino acid transport and metabolism (6.33%), energy production and conversion (4.42%) and carbohydrate transport and metabolism (3.35%) (Table 4).

Insights from genome sequence

The draft genome sequence of *B. mastomydis* is smaller than those of *Bartonella rattaustraliani, Bartonella florencae, B. queenslandensis* and *B. tribocorum* (2045, 2158, 2054, 2378 and 2631 Mb respectively), but larger than those of *B. elizabethae* and *B. vinsonii* subsp. *berkhoffii* (1964 and 1803 Mb respectively). The G + C content of *B. mastomydis* is smaller than those of *B. rattaustraliani, B. vinsonii* subsp. *berkhoffii, B. florencae* and *B. tribocorum* (38.44, 38.8, 38.83, 38.45 and 38.81% respectively), but larger than those of *B. elizabethae* and *B. queenslandensis* (38.32 and 38.38% respectively). The protein-coding gene content of *B. mastomydis* is smaller than those of *B. rattaustraliani, B. florencae, B. queenslandensis* and *B. tribocorum* (1674, 1943, 1886, 2466 and 2295 respectively), but larger than those of *B. elizabethae* and *B. vinsonii* subsp. *berkhoffii* (1663 and 1434 respectively). Similarly, the gene content of *B. mastomydis* (1674) is smaller than those of *B. rattaustraliani, B. florencae, B. queenslandensis* and *B. tribocorum* (1943, 1886, 2466 and 2295 respectively), but larger than those of *B. elizabethae* and *B. vinsonii* subsp. *berkhoffii* (1663 and 1434 respectively). The COGs category gene distribution is not similar. *B. mastomydis* has fewer COGs category genes belonging to transcription (n = 58) than *B. tribocorum* (73). *Bartonella mastomydis* has also fewer genes belonging to the replication, recombination and repair COGs category (73) than *B. rattaustraliani* (108), *B. queenslandensis* (100) and *B. tribocorum* (95). Finally, *B. mastomydis* has also fewer genes belonging to mobilome: prophages, transposons COGs category (25) than *B. tribocorum, B. rattaustraliani, B. queenslandensis, B. vinsonii* subsp. *berkhoffii* and *B. florencae* (125, 56, 50, 45 and 43 respectively) (Fig. 6). Among species with standing in nomenclature, average genomic identity of orthologous gene sequences (AGIOS) values ranged from 0.96 between *B. mastomydis* and *B. elizabethae* to 0.66 between *B. vinsonii* subsp. *berkhoffii* and *B. rattaustraliani, B. queenslandensis, B. elizabethae, B. mastomydis, B. rattaustraliani, B. tribocorum, B. florencae* and *B. tribocorum* (Table 5). To evaluate the genomic similarity among the strains, we determined two parameters, digital DNA-DNA hybridization, which exhibits high correlation with DNA-DNA hybridization (DDH) [38] and AGIOS [39], which was designed to be independent of DDH (Table 6).

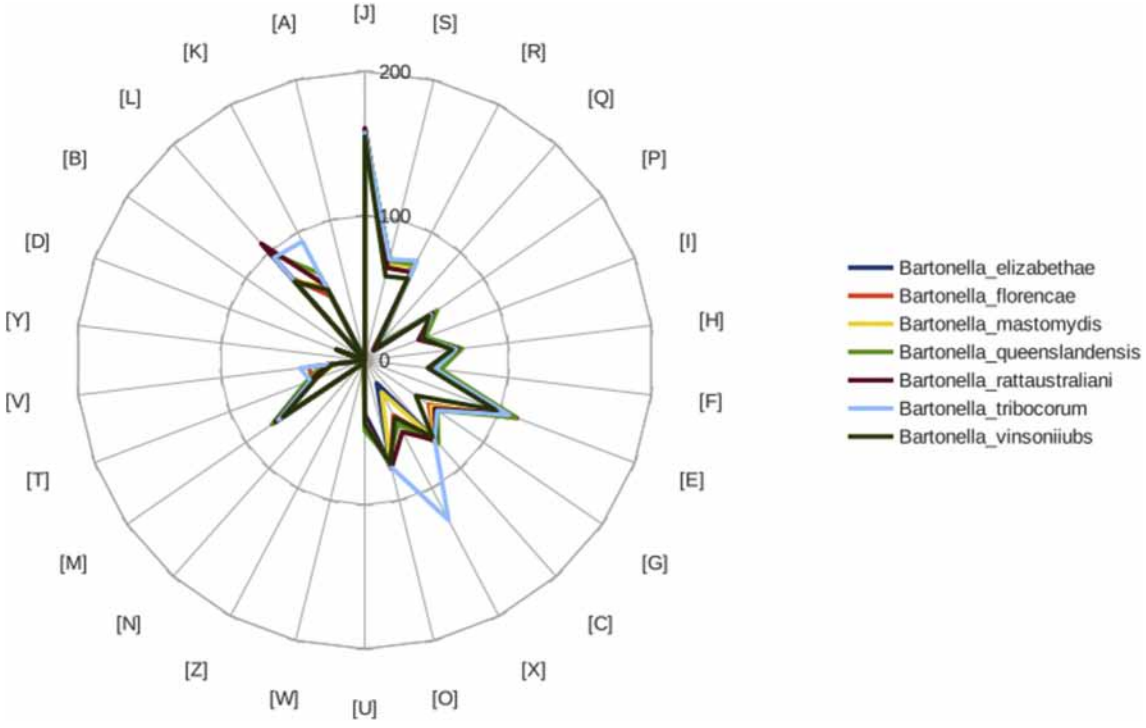

FIG. 6. Distribution of functional classes of predicted genes according to clusters of orthologous groups of proteins.

TABLE 5. **Numbers of orthologous protein shared between *Bartonella* genomes (upper right)**[a]

	B. vinsonii subsp. *berkhoffii*	*B. rattaustraliani*	*B. florencae*	*B. tribocorum*	*B. queenslandensis*	*B. elizabethae*	*B. mastomydis*
B. vinsonii subsp. *berkhoffii*	**1434**	1115	1121	1154	1043	1143	1144
B. rattaustraliani	0.66	**1943**	1134	1164	1057	1148	1154
B. florencae	0.67	0.83	**1886**	1210	1081	1201	1201
B. tribocorum	0.80	0.66	0.66	**2295**	1136	1257	1258
B. queenslandensis	0.66	0.82	0.83	0.70	**2466**	1114	1115
B. elizabethae	0.66	0.82	0.84	0.70	0.90	**1663**	1264
B. mastomydis	0.66	0.82	0.84	0.70	0.90	0.96	**1674**

[a]Average percentage similarity of nucleotides corresponding to orthologous protein shared between genomes (lower left) and numbers of proteins per genome (bold).

TABLE 6. **Pairwise comparison of *Bartonella mastomydis* with six other species using GGDC, formula 2 (DDH estimates based on identities/HSP length)**[a]

	B. vinsonii subsp. *berkhoffii*	*B. rattaustraliani*	*B. florencae*	*B. tribocorum*	*B. queenslandensis*	*B. elizabethae*	*B. mastomydis*
B. vinsonii subsp. *berkhoffii*	100% ± 00	25.8% ± 2.45	27.1% ± 2.45	25.8% ± 2.4	25.9% ± 2.4	25.6% ± 2.4	25.5% ± 2.4
B. rattaustraliani		100% ± 00	25.5% ± 2.4	25.1% ± 2.4	27.5% ± 2.45	24.4% ± 2.4	24.2% ± 2.4
B. florencae			100% ± 00	26.7% ± 2.4	26.3% ± 2.45	26.8% ± 2.4	26.7% ± 2.4
B. tribocorum				100% ± 00	42% ± 2.55	37.3% ± 2.45	36.8% ± 2.5
B. queenslandensis					100% ± 00	37.6% ± 2.45	37.3% ± 2.5
B. elizabethae						100% ± 00	60.3% ± 2.8
B. mastomydis							100% ± 00

DDH, DNA-DNA hybridization; GGDC, Genome-to-Genome Distance Calculator; HSP, high-scoring segment pairs.
[a]Confidence intervals indicate inherent uncertainty in estimating DDH values from intergenomic distances based on models derived from empirical test data sets (which are always limited in size). These results are in accordance with phylogenomic analyses as well as the GGDC results.

Conclusion

On the basis of phenotypic, phylogenetic and genomic analyses, we formally propose the creation of *Bartonella mastomydis* sp. nov. that contains the strain 008. This bacterial strain has been isolated from *M. erythroleucus* blood samples from animals trapped in the Sine-Saloum region of Senegal.

Description of *Bartonella mastomydis* sp. nov. strain 008

Bartonella mastomydis (mas.to'my.dis, N.L. gen. n., *mastomydis,* 'of *Mastomys,*' isolated from *Mastomys erythroleucus*) is a nonmotile, Gram-negative rod. Growth is only obtained at 37° C. Colonies are opaque, grey and 0.5 to 1 mm in diameter on blood-enriched Columbia agar. Cells are rod shaped without flagella or pili. Length and width are 1369.8 ± 423.8 nm and 530.9 ± 105.8 nm respectively. *Bartonella mastomydis* strain 008 exhibits neither biochemical nor enzymatic activities. The type strain 008 is sensitive to rifampicin, amoxicillin, amoxicillin/clavulanic acid, oxacillin, nitrofurantoin, doxycycline, linezolid, tobramycin, gentamycin, imipenem, trimethoprim/sulfamethoxazole, fosfomycin and ciprofloxacin and resistant to metronidazole and colistin. The G + C content of the genome is 38.44%. The 16S rRNA gene sequence and whole-genome shotgun sequence of strain 008 are deposited in GenBank under accession numbers KY555064 and GCA_900185775 respectively. The type strain 008 (CSUR B643, DSM2802) was isolated from the rodent *Mastomys erythroleucus* trapped in the region of Sine-Saloum, Senegal.

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Conflict of Interest

None declared.

References

[1] Okaro U, Addisu A, Casanas B, Anderson B. *Bartonella* species, an emerging cause of blood-culture–negative endocarditis. Clin Microbiol Rev 2017;30:709–46.
[2] Tsai YL, Chang CC, Chuang ST, Chomel BB. *Bartonella* species and their ectoparasites: selective host adaptation or strain selection

between the vector and the mammalian host? Comp Immunol Microbiol Infect Dis 2011;34:299–314.
[3] 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.
[4] Skerman VBD, McGowan V, Sneath PHA. Approved lists of bacterial names. Int J Syst Evol Microbiol 1980;30:225–420.
[5] Guptill L. Bartonellosis. Vet Microbiol 2010;140:347–59.
[6] Angelakis E, Raoult D. Pathogenicity and treatment of *Bartonella* infections. Int J Antimicrob Agents 2014;44:16–25.
[7] Brouqui P, Raoult D. New insight into the diagnosis of fastidious bacterial endocarditis. FEMS Immunol Med Microbiol 2006;47:1–13.
[8] Chomel BB, Kasten RW, Williams C, Wey AC, Henn JB, Maggi R, et al. *Bartonella* endocarditis: a pathology shared by animal reservoirs and patients. Ann N Y Acad Sci 2009;1166:120–6.
[9] Brook CE, Bai Y, Dobson AP, Osikowicz LM, Ranaivoson C, Zhu Q, et al. *Bartonella* spp. in fruit bats and blood-feeding ectoparasites in Madagascar. PLoS Negl Trop Dis 2015;9:e0003532.
[10] Kosoy M, Bai Y, Lynch T, Kuzmin IV, Niezgoda M, Franka R, et al. *Bartonella* spp. in bats. Kenya Emerg Infect Dis 2010;16:1875–81.
[11] Olival KJ, Dittmar K, Bai Y, Rostal MK, Lei BR, Daszak P. *Bartonella* spp. in a Puerto Rican bat community. J Wildl Dis 2015;51:274–8.
[12] Davoust B, Marié JL, Dahmani M, Berenger JM, Bompar JM, Blanchet D, et al. Evidence of *Bartonella* spp. in blood and ticks (*Ornithodoros hasei*) of bats, in French Guiana. Vector Borne Zoonotic Dis 2016;16:516–9.
[13] Jiyipong T, Jittapalapong S, Morand S, Raoult D, Rolain J. Prevalence and genetic diversity of *Bartonella* spp. in small mammals from southeastern Asia. Appl Environ Microbiol 2012;78:8463–6.
[14] Pretorius AM, Beati L, Birtles RJ. Diversity of bartonellae associated with small mammals inhabiting Free State province, South Africa. Int J Syst Evol Microbiol 2004;54:1959–67.
[15] Brettschneider H, Bennett NC, Chimimba CT, Bastos ADS. Bartonellae of the Namaqua rock mouse, *Micaelamys namaquensis* (Rodentia: Muridae) from South Africa. Vet Microbiol 2012;157:132–6.
[16] Gundi VA, Kosoy MY, Makundi RH, Laudisoit A. Identification of diverse *Bartonella* genotypes among small mammals from Democratic Republic of Congo and Tanzania. Am J Trop Med Hyg 2012;87:319–26.
[17] Kamani J, Morick D, Mumcuoglu KY, Harrus S. Prevalence and diversity of *Bartonella* species in commensal rodents and ectoparasites from Nigeria, West Africa. PLoS Negl Trop Dis 2013;7:e2246.
[18] La Scola B, Zeaiter Z, Khamis A, Raoult D. Gene-sequence–based criteria for species definition in bacteriology: the *Bartonella* paradigm. Trends Microbiol 2003;11:318–21.
[19] Dahmani M, Sambou M, Scandola P, Raoult D, Fenollar F, Mediannikov O. *Bartonella bovis* and *Candidatus Bartonella davousti* in cattle from Senegal. Comp Immunol Microbiol Infect Dis 2017;50:63–9.
[20] 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.
[21] Seng P, Drancourt M, Gouriet F, La Scola B, Fournier PE, 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.
[22] 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*. Int J Syst Bacteriol 1993;43:777–86.
[23] Birtles RJ, Raoult D. Comparison of partial citrate synthase gene (*gltA*) sequences for phylogenetic analysis of *Bartonella* species. Int J Syst Bacteriol 1996;46:891–7.
[24] Renesto P, Gouvernet J. Use of *rpoB* gene analysis for detection and identification of *Bartonella* species. J Clin Microbiol 2001;39:430–7.
[25] Zeaiter Z, Liang Z, Raoult D. Genetic classification and differentiation of *Bartonella* species based on comparison of partial *ftsZ* gene sequences. J Clin Microbiol 2002;40:3641–7.
[26] Meheretu Y, Leirs H, Welegerima K, Breno M, Tomas Z, Kidane D, et al. *Bartonella* prevalence and genetic diversity in small mammals from Ethiopia. Vector Borne Zoonotic Dis 2013;13:164–75.
[27] Martin-Alonso A, Houemenou G, Abreu-Yanes E, Valladares B, Feliu C, Foronda P. *Bartonella* spp. in small mammals. Benin Vector Borne Zoonotic Dis 2016;16:229–37.
[28] Gundi VA, Kosoy MY, Myint KS, Shrestha SK, Shrestha MP, Pavlin JA, et al. Prevalence and genetic diversity of *Bartonella* species detected in different tissues of small mammals in Nepal. Appl Environ Microbiol 2010;76:8247–54.
[29] Bakour S, Rathored J, Lo CI, Mediannikov O, Beye M, Ehounoud CB, et al. Non-contiguous finished genome sequence and description of *Bartonella senegalensis* sp. nov. New Microbe. New Infect 2016;11:93–102.
[30] Zerbino DR, Birney E. Velvet: algorithms for de novo short read assembly using de Bruijn graphs. Genome Res 2008;18:821–9.
[31] Bankevich A, Nurk S, Antipov D, Gurevich AA, Dvorkin M, Kulikov AS, et al. SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. J Comput Biol 2012;19:455–77.
[32] Luo R, Liu B, Xie Y, Li Z, Huang W, Yuan J, et al. SOAPdenovo2: an empirically improved memory-efficient short-read *de novo* assembler. Gigascience 2012;1:18.
[33] Bolger AM, Lohse M, Usadel B. Trimmomatic: a flexible trimmer for Illumina sequence data. Bioinformatics 2014;30:2114–20.
[34] Hyatt D, Chen GL, Locascio PF, Land ML, Larimer FW, Hauser LJ. Prodigal: prokaryotic gene recognition and translation initiation site identification. BMC Bioinform 2010;11:119.
[35] Lowe TM, Eddy SR. TRNAscan-SE: a program for improved detection of transfer RNA genes in genomic sequence. Nucleic Acids Res 1996;25:955–64.
[36] 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.
[37] Käll L, Krogh A, Sonnhammer ELL. A combined transmembrane topology and signal peptide prediction method. J Mol Biol 2004;338:1027–36.
[38] Auch AF, von Jan M, Klenk HP, Göker M. Digital DNA-DNA hybridization for microbial species delineation by means of genome-to-genome sequence comparison. Stand Genomic Sci 2010;2:117–34.
[39] Ramasamy D, Mishra AK, Lagier JC, 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.
[40] Kumar S, Stecher G, Tamura K. MEGA7: Molecular evolutionary Genetics analysis version 7.0 for bigger datasets. Mol Biol Evol 2016;33:msw054.
[41] Hall TA. BioEdit: a user-friendly biological sequences alignment editors and analysis program for Windows 95/98/NT. Nucl Acids Symp Ser 1999;41:95–8.
[42] Woese CR, Kandler O, Wheelis ML. Towards a natural system of organisms: proposal for the domains Archaea, Bacteria, and Eucarya. Proc Natl Acad Sci U S A 1990;87:4576–9.
[43] Lilburn T, Garrity GM, Bell JA. Phylum XIV. Proteobacteria phyl. nov. In: S.J, Garrity GM, Brenner DJ, Krieg NR, editors. Bergey's Manual of Systematic Bacteriology. 2nd ed. New York: Springer; 2005. p. 1–574.

[44] Lilburn T, Garrity GM, Bell JA. Class I. Alphaproteobacteria class. nov. In: S.J, Garrity GM, Brenner DJ, Krieg NR, editors. Bergey's Manual of Systematic Bacteriology. 2nd ed. New York: Springer; 2005. p. 1–574.

[45] Oren A, Garrity GM. List of new names and new combinations previously effectively, but not\rvalidly, published. List no. 132. Int J Syst Evol Microbiol 2010;10:469–72. https://doi.org/10.1099/ijsem.0.000737.

[46] Kuykendall L, Order VI. Rhizobiales ord. nov. In: S.J, Garrity GM, Brenner DJ, Krieg NR, editors. Bergey's Manual of Systematic Bacteriology. 2nd ed. New York: Springer; 2005. pp. 978-0-387-95040–2.

[47] Weinman D, Genus I. Bartonella Strong, Tyzzer and Sellards 1915. In: Buchanan R, Gibbons, editors. Bergey's Manual of Determinative Bacteriology. 8th ed. Baltimore: Williams and Wilkins Co; 1974. p. 904–5.