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Bartonella spp. in Small Mammals, Benin

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Abstract

This study aimed to investigate the prevalence and genetic diversity of *Bartonella* organisms in small mammals in Cotonou, Benin. We captured 163 rodents and 12 insectivores and successfully detected *Bartonella* DNA from 13 of the 175 small mammal individuals. *Bartonella* spp., identical or closely related to *Bartonella elizabethae*, *Bartonella tribocorum*, and *Bartonella rochalimae*, was detected. A potential new *Bartonella* species, proposed as Candidatus *Bartonella mastomydis*, was found in three *Mastomys* individuals and genetically characterized by targeting two housekeeping genes (*rpoB* and *gltA*) and the intergenic species region. However, 20.8% of gray rats were found to be infected with *Bartonella* spp., and none of the black rats analyzed was positive. This work may be important from a public health point of view due to the zoonotic nature of the *Bartonella* species detected and warrants further investigation on the unknown zoonotic potential of this newly proposed *Bartonella* species.

Key Words: Bartonella—PCR—Rodents—Vector-borne.

Introduction

The genus *Bartonella* includes more than 30 *Bartonella* spp., and several Candidatus spp. have been isolated from humans as well as from wild and domestic animals around the world (Angelakis and Raoult 2014). The transmission of bartonellae occurs through traumatic contact with infected animals (*e.g.*, *Bartonella henselae* by cat scratch) or through blood-sucking arthropods (Morick et al. 2013), the latter most likely through inoculation of host skin lesions by contaminated insect feces (Chomel et al. 2009). *Bartonella* infections in rodents have been reported worldwide, and close contacts between humans and rodents have been found to create excellent conditions for transmission of *Bartonella* spp. from animals to humans (Welc-Faleciak et al. 2008). In this sense, a few cases of human infections with *Bartonella* bacteria of rodent origin have been reported worldwide (Morick et al. 2010).

Although a recent study showed that the overall prevalence of *Bartonella* sp. in *Xenopsylla cheopis* fleas in Benin was 34.67% (Leulmi et al. 2014), no study was conducted in small mammals to identify *Bartonella* reservoirs. Consequently, our aim was to analyze the prevalence of *Bartonella* infections in small mammal populations of three wide areas in Cotonou, where half of the nation's population (over 3 million people) resides (Dossou and Glehouenou-Dossou 2007).

To estimate the zoonotic risk and evaluate the genetic diversity associated with these *Bartonella* organisms, genotypes of *Bartonella* spp. were determined using the citrate synthase gene (*gltA*), which is especially popular and a widely used target for distinguishing closely related *Bartonella* species and genotypes (Kosoy et al. 2012), RNA polymerase beta-subunit (*rpoB*), and 16S-23S rRNA intergenic species region (ITS). Evaluating the genetic heterogeneity of the *Bartonella* strains in small mammals in Benin will also serve to increase the knowledge about *Bartonella* host specificity.

Materials and Methods

A total of 163 rodents and 12 insectivores were captured in three zones of Cotonou, Republic of Benin (Fig. 1). Animals were trapped inside human residences and peridomestic areas between November 2009 and July 2010, as Houemenou et al. (2013) described previously. Rodent individuals belonged to the species *Rattus rattus* (black rats), *Rattus norvegicus* (gray rats), *Mastomys* sp., and *Mus* sp., whereas *Crocidura* sp. and *Crocidura olivieri* were the insectivore species included in this study (Table 1). The *Xenopsylla cheopis* fleas harbored by some of these small mammal individuals that had been

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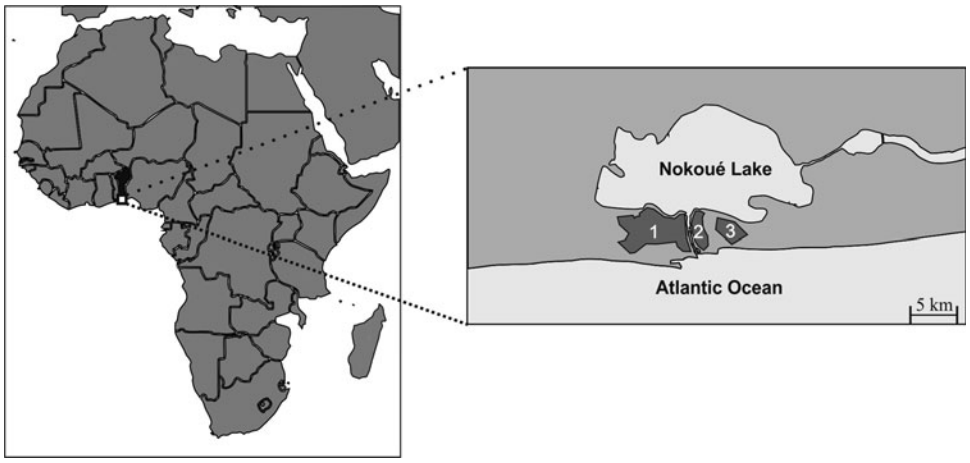

FIG. 1. Sampling areas in Cotonou, Benin.

already screened for the presence of *Bartonella* sp. (Leulmi et al. 2014).

Spleens of animals were obtained, and DNA was isolated by using the Illustra Tissue and Cells Genomic Prep Mini Spin Kit (GE Healthcare, Little Chalfont, UK). The presence of *Bartonella* was examined by PCR targeting the *gltA* gene as Billeter et al. (2011) described previously. This amplicon encompasses a 327-bp-specific zone that has been described to be useful for distinguishing *Bartonella* species (La Scola et al. 2003). In case of a positive PCR result, we performed a second PCR targeting the *rpoB* gene, as Renesto et al. (2001) described previously. The ITS was also sequenced if the homology of both *gltA* and *rpoB* genes with the rest of described *Bartonella* species suggested the existence of an undescribed *Bartonella* species. The ITS was amplified according to Roux and Raoult (1995).

Those positive rodent individuals identified morphologically only at the genus level were genotyped by amplification and sequencing the nuclear interphotoreceptor binding protein gene (IRBP), as Weksler (2003) described previously. This nuclear gene has been used extensively to address questions of mammalian interordinal phylogeny (Stanhope et al. 1996, Springer et al. 1999) and to discern relationships at lower taxonomic levels as well (Yoder and Irwin 1999, Jansa and Voss 2000).

Table 1. Prevalence of *Bartonella* spp. in Small Mammals in Cotonou, Benin

	Bartonella *spp.*	
Zone of study	*Host species* (N)	*+ (P%)*
Zone 1	*Rattus rattus* (61)	0 (0)
	Rattus norvegicus (19)	5 (26.3)
	Mastomys sp. (8)	1 (12.5)
	Crocidura olivieri (4)	1 (25)
	Crocidura sp. (2)	0 (0)
	Mus sp. (1)	0 (0)
Zone 2	*Rattus rattus* (21)	0 (0)
	Rattus norvegicus (5)	0 (0)
	Mastomys sp. (13)	4 (30.8)
	Crocidura olivieri (5)	0 (0)
Zone 3	*Rattus rattus* (28)	0 (0)
	Mastomys sp. (6)	2 (33.3)
	Crocidura olivieri (2)	0 (0)
Total	175	13 (7.4)

Spleen samples were collected from the small mammals *Rattus rattus*, *Rattus norvegicus*, *Mastomys* sp., *Crocidura olivieri*, and *Crocidura* sp. A total of 175 small mammals were examined for the presence of *Bartonella* species in three zones in Cotonou, Benin.

N, number of small mammals analyzed; +, number of positive samples; P, prevalence.

Sequencing reactions were performed for both strands at Macrogen, Inc. In the case of suspecting mixed infections due to the presence of clear double peaks in the chromatograms, the amplified products were cloned into the pGEM-T Easy cloning vector (Promega) for further sequencing using plasmid primers SP6 and T7. New and previously published *Bartonella* sequences, which were retrieved from GenBank, were aligned with the multiple alignment program ClustalW in MEGA 6.06 (Tamura et al. 2013). Sequences were compared by using the nucleotide–nucleotide BLAST (blastn) program.

Phylogenetic relationships of the *Bartonella* spp. genotypes based on partial sequences of the *gltA* and *rpoB* genes were assessed by the neighbor-joining method with Kimura's two-parameter distance model (Kimura 1980). Bootstrap analysis was performed with 1000 trials. In the case of suspecting the presence of an undescribed *Bartonella* species, a phylogenetic tree was constructed from concatenated sequences of *gltA* and *rpoB* genes and the ITS.

Chi-square contingency tables were used to compare the proportion of *Bartonella*-infected small mammals between the different host species and areas included in the study. Fisher's exact tests were used if expected cell counts were less than five. A probability value less than <0.05 was considered as statistically significant.

This study was approved by the Ethical Committee of l'Université d'Abomey-Calavi of Benin, reference no. 208/MCOT/SG/DRH/DDCPRS/SFERM.

Results

Thirteen out of the 175 small mammals analyzed were positive for *Bartonella* species. Therefore, the overall prevalence was 7.4%. *Bartonella* DNA was found in *R. norvegicus*, *Mastomys* sp., and *C. olivieri*. The highest *Bartonella* prevalences were found in *Mastomys* sp. (25.9%) and *R. norvegicus* (20.8%), whereas none of the black rats analyzed ($n=110$) were found to be harboring *Bartonella* organisms. As a result,

Bartonella prevalence was significantly higher in both *Mastomys* sp. and *R. norvegicus* than in *R. rattus* ($p < 0.001$ Fisher's exact test for both host comparisons). *Bartonella*-positive mammals belonged to the three areas studied; although the highest prevalence was observed in area 2 (9.1%), no significant differences of *Bartonella* prevalence were found among the three areas included in the study.

Seven *Mastomys* sp. individuals were positive for *Bartonella* spp., and five of them were identified as *Mastomys natalensis* on the basis of their IRBP gene sequences, which were submitted to the GenBank under the following Accession numbers: KP862858–KP862860 and 1863188.

Five *gltA* genotypes were detected and designated as genotypes g-A to g-E. The phylogenetic relationships between the isolates obtained in this study and previously described *Bartonella* species are shown in Figure 2, and additional information about these *gltA* genotypes is supplied in Table 2. The genotypes g-A and g-B showed 99% and 100% identity, respectively, with GenBank deposited *B. elizabethae* sequence (GenBank Accession number JX158351). The genotype g-C had 100% similarity to the GenBank deposited *B. tribocorum* sequence (GenBank Accession number JX158361), and the genotype g-D showed 100% identity with *B. rochalimae* (GenBank Accession number FN645459). Finally, the genotype g-E showed 94% BLAST similarity with the closest *gltA* sequence deposited in the GenBank database (GenBank Accession number AY584853), which corresponded to *Bartonella taylorii* isolated from *Apodemus agrarius* (Mediannikov et al. 2005).

During genetic analyses, clear double peaks (distinct from the baseline noise) were visible at species-specific positions on the sequencing chromatograms of the *gltA* products corresponding to three isolates, Ben165, Ben178, and Ben205, suggesting mixed infections. Among them, only the isolate Ben205 was cloned successfully, allowing us to distinguish two different *gltA* genotypes, g-A and g-E. Selected *Bartonella gltA* sequences were deposited in GenBank under the following accession numbers: KP857582–KP857587, KR107234, KT881099, and KT881100.

With regard to the *rpoB* gene, five partial *rpoB* genotypes were also obtained and designated as genotypes r-A to r-E. The phylogenetic relationships between the isolates obtained

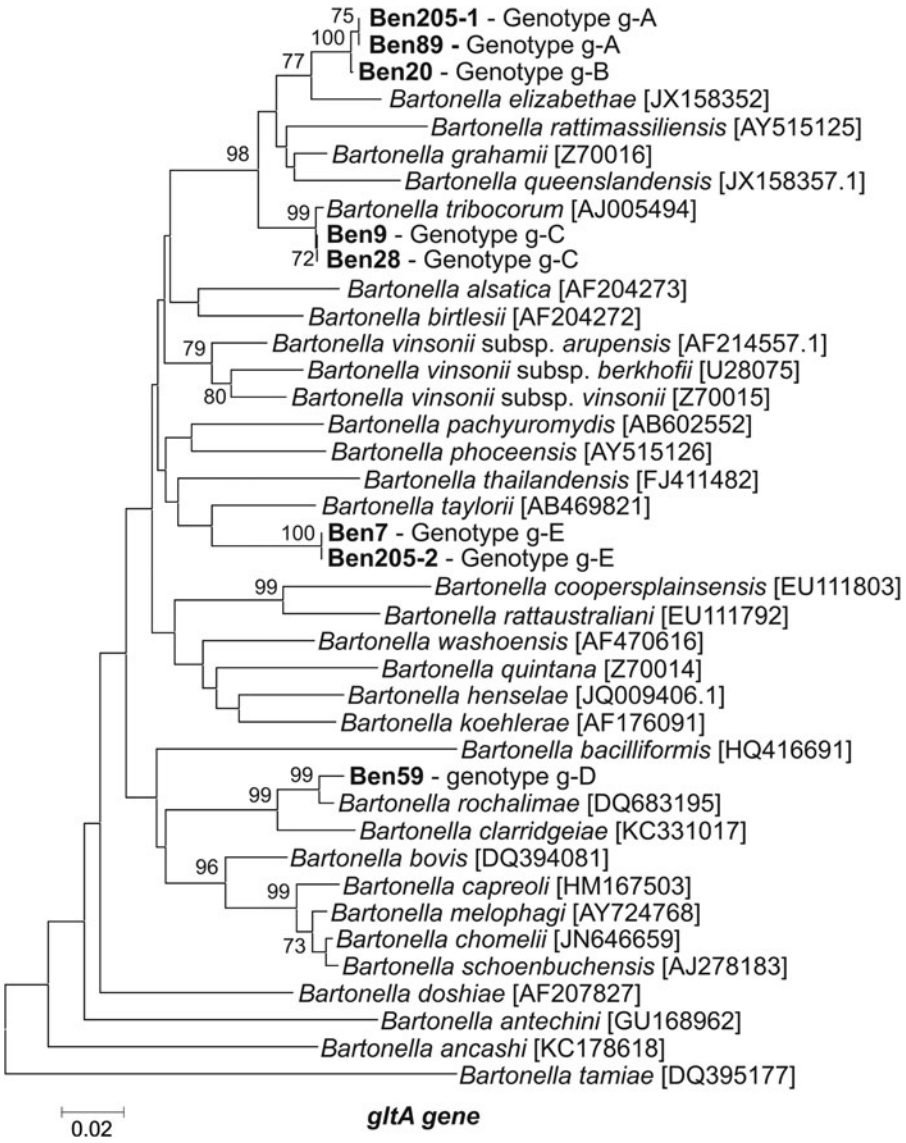

FIG. 2. Phylogenetic classification of *Bartonella* genotypes based on *gltA* gene sequences. Only the bootstrap values above 70% obtained are given. The GenBank Accession numbers for reference sequences are given between *brackets*. Isolates sequenced in this study are indicated in *bold*. Sequences obtained from coinfecting *Bartonella* organisms isolated from the same host individual were designated with a number after a dash. Scale bar indicates 20 substitutions per nucleotide position. The *gltA* sequence of *Bartonella tamiae* was included as the outgroup.

Table 2. Additional Information About *Bartonella* Genotypes Obtained from Small Mammals in Cotonou, Based on *gltA* and *rpoB* Sequence Analysis

			gltA *genotyping*			rpoB *genotyping*		
Isolate	*Mammal species*	*Area*	*Genotype*	*Closest* Bartonella *spp./ % similarity*	*GenBank Accession nos.*	*Genotype*	*Closest* Bartonella *spp./ % similarity*	*GenBank Accession nos.*
Ben7	*Mastomys* sp.	2	g-E	*B. taylorii*/94	KT881099	r-E	*B. birtlesii*/94	KP997022
Ben9	*Rattus norvegicus*	1	g-C	*B. tribocorum*/100	KP857582	r-B	*B. elizabethae*/99	KP997018
Ben20	*Mastomys natalensis*	2	g-B	*B. elizabethae*/100	KP857583	r-C	*B. elizabethae*/98	KT881101
Ben28	*Mastomys natalensis*	2	g-C	*B. tribocorum*/100	KP857584	r-A	*B. elizabethae*/98	KT881102
Ben59	*Rattus norvegicus*	1	g-D	*B. rochalimae*/100	KP857585	r-D	*B. rochalimae*/97	KP997019
Ben89	*Rattus norvegicus*	1	g-B	*B. elizabethae*/100	KP857586	r-A	*B. elizabethae*/98	KP997020
Ben143	*Mastomys natalensis*	1	—	—	—	—	—	—
Ben149	*Crocidura olivieri*	1	—	—	—	—	—	—
Ben165	*Rattus norvegicus*	1	—	—	—	r-B	*B. elizabethae*/99	KP997017
Ben178	*Mastomys* sp.	3	—	—	—	r-E	*B. birtlesii*/94	KT881103
Ben205-1	*Mastomys natalensis*	2	g-A	*B. elizabethae*/99	—	r-C	*B. elizabethae*/98	—
Ben205-2	*Mastomys natalensis*	2	g-E	*B. taylorii*/94	KR107234	r-E	*B. birtlesii*/94	KP997021
Ben211	*Mastomys natalensis*	3	—	—	—	—	—	—
Ben215	*Rattus norvegicus*	1	g-D	*B. rochalimae*/100	KT881100	—	—	—

A total of 175 small mammals were screened for the presence of *Bartonella* spp. by using citrate synthase gene (*gltA*) and RNA polymerase beta-subunit (*rpoB*) primers; 13 individuals harbored *Bartonella* organisms. Five *gltA* genotypes and five *rpoB* genotypes were observed. Similarities below 95% for both genes suggested the existence of a new *Bartonella* species. *Dashes* indicate that the corresponding isolate could not be sequenced. *Mastomys* individuals were genetically characterized by targeting the interphotoreceptor binding protein gene (IRBP) gene.

in this study and previously described *Bartonella* species according to *rpoB* sequences analysis are shown in Figure 3, and additional information is also depicted in Table 2. The genotypes r-A to r-C of *rpoB* gene were highly similar to *B. elizabethae* (98–99% BLAST similarity). The isolates Ben9 and Ben28, which had shown 100% similarity to *B. tribocorum* and had been consequently classified as genotype g-C when analyzing the *gltA* gene, were grouped together with *B. elizabethae* (98–99% BLAST similarity) on the basis of the *rpoB* genotype. On the contrary, the genotype r-D was concordant with the genotype g-D of the *gltA* gene and showed 97% similarity with *B. rochalimae*.

Finally, the genotype r-E of *rpoB*, which corresponds to the genotype g-E of the *gltA* gene, showed the highest similarity (99%) with an undescribed *Bartonella* organism that had been previously found in *Arvicanthis dembeensis* and *Mastomys awashensis* in Ethiopia (GenBank Accession number JQ425643 and JQ425641, respectively) (Meheretu et al. 2013). This genotype r-E showed 94% BLAST similarity with the closest, related described *Bartonella* species, *B. birtlesii*. Consequently, since partial sequences of both *gltA* and *rpoB* genes of isolates Ben7 and Ben205-2 (corresponding to genotypes g-E and r-E) were less than 95% similar to the rest of described *Bartonella* species, fulfilling the species description cutoff designated by La Scola et al. (2003), it is likely that this strain belongs to a new *Bartonella* species.

According to the isolate Ben205, double peaks were also found at species-specific positions, suggesting again the presence of two *Bartonella* strains. Cloning these *rpoB* PCR products allowed us to confirm the existence of two coinfecting *Bartonella* organisms, whose genotypes were classified as r-C and r-E. *Bartonella rpoB* sequences were deposited in GenBank under the following accession numbers: KP997017–KP997022 and KT881101–KT881103 (Table 2).

Those *Bartonella* organisms whose *gltA* and *rpoB* genotypes were less than 95% similar to the rest of described *Bartonella* species were further analyzed for the ITS, and the homology was 91% or less between our isolates and known *Bartonella* species (GenBank Accession number KT895985). The highest BLAST similarities for the ITS, along with *gltA* and *rpoB* genes, are given in Table 3. The phylogenetic tree of the concatenated sequences of *gltA* and *rpoB* genes and ITS showed that this undescribed *Bartonella* species aligned most closely to *B. birtlesii*, and both *Bartonella* organisms were grouped separately in a well-supported clade (99% bootstrap support; Fig. 4), as was observed in the *rpoB* phylogenetic tree (Fig. 3).

Discussion

The results of this study indicate that *Bartonella* organisms are widely distributed in Cotonou, as they were found in all the areas included in this study, which cover ~ 14 km in length.

To our knowledge, this work constitutes the first finding of *Bartonella* spp. in rodents in Benin. The overall prevalence observed in this study (7.4%) was clearly influenced by the absence of *Bartonella* spp. in black rats ($n = 110$).

The remarkable prevalence found in *R. norvegicus* and *Mastomys* sp. could indicate that *Bartonella* infections are common in these rodent species.

The discovery of five *gltA* and five *rpoB* genotypes in this restricted sampling area indicates a high level of *Bartonella* diversity among small mammals in Cotonou. The finding of *B. elizabethae* in small mammals in Cotonou increases the already widespread distribution of this zoonotic species throughout Africa, as it has been already found in countries as geographically distant as the Democratic Republic of Congo

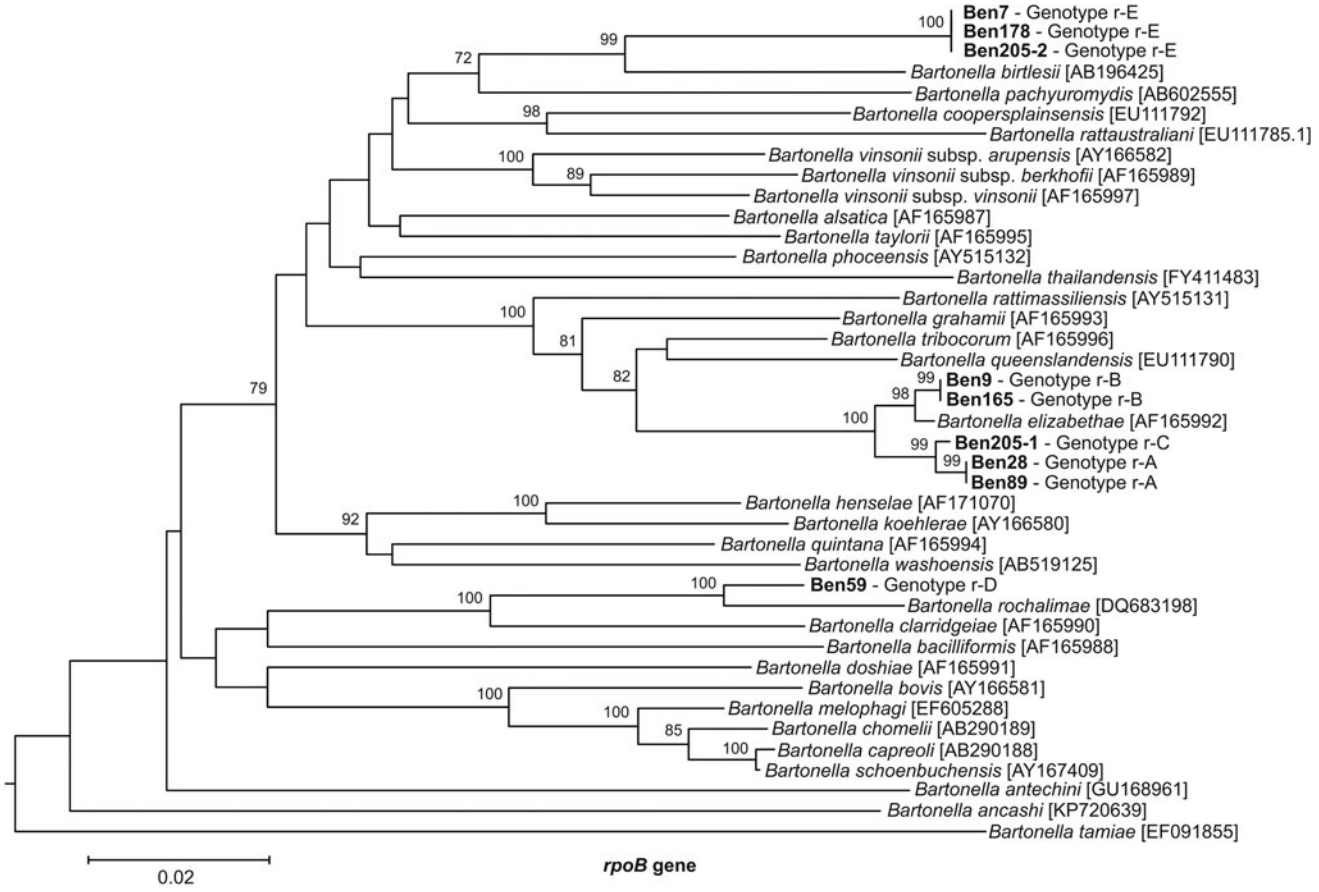

FIG. 3. Phylogenetic classification of *Bartonella* genotypes based on *rpoB* gene sequences. Only the bootstrap values above 70% obtained are given. The GenBank Accession numbers for reference sequences are given between *brackets*. Isolates sequenced in this study are indicated in *bold*. Sequences obtained from coinfecting *Bartonella* organisms isolated from the same host individual were designated with a number after a dash. Scale bar indicates 20 substitutions per nucleotide position. The *rpoB* sequence of *Bartonella tamiae* was included as the out-group.

(DRC), Tanzania (Gundi et al. 2012), Uganda (Billeter et al. 2014), South Africa (Brettschneider et al. 2012b), or Ethiopia (Meheretu et al. 2013). Almost the same could be said for *B. tribocorum*, as it has also been already isolated in many African countries, such as Nigeria (Kamani et al. 2013), South Africa (Pretorius et al. 2004), DRC, or Tanzania (Gundi et al. 2012).

La Scola et al. (2003) proposed that newly encountered *Bartonella* isolates should be considered a new species if the 327-bp *gltA* fragment shares <96.0% sequence similarity with those of the validated species and if an 825-bp *rpoB* fragment shares <95.4% sequence similarity with those of the validated species. Two isolates analyzed in this study satisfied this species description cutoff for both genes. Therefore, it is likely that this strain belongs to a new *Bartonella* species. This conclusion was supported by the fact that the analyzed portion of the ITS of this organism showed 91% similarity with the closest *Bartonella* species, whereas La Scola et al. (2003) demonstrated that the median value of interspecies

TABLE 3. SIMILARITY BETWEEN CANDIDATUS *BARTONELLA MASTOMYDIS* AND CLOSEST *BARTONELLA* SPECIES FOR *GLTA*, *RPOB*, AND ITS

	Base pair similarity (%) with gltA	*Base pair similarity (%) with* rpoB	*Base pair similarity (%) with ITS*
Bartonella taylorii	307/326 (94%) AY584853.1	739/825 (90%) AF165995	297/324 (92%) AJ269788
Bartonella birtlesii	293/320 (92%) AF204272.1	771/824 (94%) AB196425.1	282/330 (85%) AY116640.1
Bartonella pachyuromydis	290/321 (90%) AB444978	751/825 (91%) AB529949.1	329/361(91%) AB602561.1

Percentage and GenBank Accession numbers.
ITS, intergenic species region.

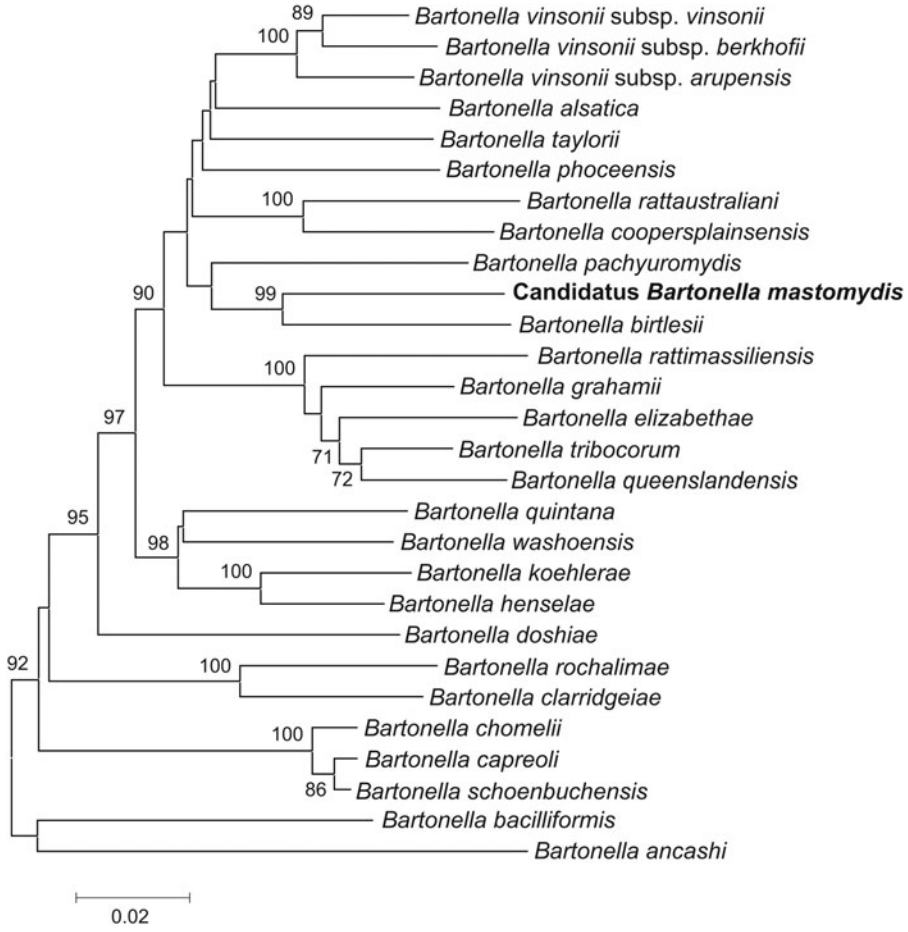

FIG. 4. Phylogenetic tree of Candidatus *Bartonella mastomydis* and other *Bartonella* species. The phylogenetic tree was based on concatenated sequences of *gltA* and *rpoB* genes and the 16S-23S rRNA intergenic spacer region and was constructed from 1378 base pair sequences, inferred using the Neighbor-Joining method, and 1000 replicates in the bootstrap test. Scale bar indicates 20 substitutions per nucleotide position. The newly proposed *Bartonella* species is indicated in *bold* letters. Only those *Bartonella* species for which *gltA*, *rpoB*, and intergenic species region sequences of the desired length were available at the GenBank database were included in this phylogeny.

similarity for the ITS, with regard to those *Bartonella* species already described at that time, was 93.9%.

Therefore, our study seems to ratify the finding carried out by Meheretu et al. (2013), who indicated that some of the *Bartonella* strains circulating among Murid rodents in Africa were likely to correspond to undescribed *Bartonella* species. In this sense, one of those undescribed *Bartonella* species, designated as clade 5 in their study, corresponds to our newly proposed species.

This *Bartonella* organism was found in *Mastomys* sp. individuals in our study, genetically identified as *M. natalensis* on the basis of their IRBP gene sequences, which were also found to carry *B. tribocorum* and *B. elizabethae* organisms in our study. This finding indicates the wide range of *Bartonella* species for which this rodent species may be acting as a reservoir in Africa. The zoonotic role of *M. natalensis* is highlighted by the fact that this rodent species is the only known reservoir for Lassa Fever (Lalis et al. 2012). On the contrary, taking into consideration that *272 species of Muridae exist in Africa (37% of the world total) (Musser and Carleton 2005), it seems obvious that many more *Bartonella* genotypes/species are going to be discovered in this continent.

Since it was not possible to obtain a pure microbiological culture, we propose this *Bartonella* organism to be named Candidatus *Bartonella mastomydis*, ''Mastomys'' being the name of the rodent genus from which the sequence was first obtained. However, to eventually confirm whether this is a new species, further genetic identification by genome sequencing will be required and is currently ongoing.

Some *Bartonella* species have been found to infect a limited number of rodent species (*i.e.*, *B. vinsonii* subsp. *arupensis* and *B. washoensis*; Kosoy et al. 2003; Inoue et al. 2011, Bai et al. 2011), whereas others such as *B. elizabethae* or *B. queenslandensis* have been found to infect many rodent species (Pangjai et al. 2014, Jiyipong et al. 2015). As has been previously observed (Gundi et al. 2010), we found a lack of host specificity for *B. elizabethae* and *B. tribocorum*, since similar or identical genotypes associated with these *Bartonella* organisms were isolated from different small mammal species in our study. This phenomenon could suggest that the transmission of both *Bartonella* species may occur by shared or common ectoparasites, such as one or more species of flea or tick, found distributed throughout the African continent. In this sense, and taking into consideration the results obtained by Leulmi et al. (2014), *X. cheopis* fleas are the most likely candidates playing this role in Cotonou.

Although intracellular bacteria have low rates of recombination and horizontal gene acquisition (Vos and Didelot 2009) probably because of their isolated growth niche, different *Bartonella* species may coexist during their life cycle, probably within their arthropod vectors, providing opportunities for recombination (Buffet et al. 2013). As a consequence, *Bartonella* strains circulating within rodents have showed more frequent recombination events compared to human and cat adapted species, which may have resulted in a broader host range for rodent adapted species (Berglund et al. 2010; Paziewska et al. 2011). With regard to *B. tribocorum*, a

recent study also demonstrated that its lack of host specificity may have been the result of the action of host adaptability genes that are packaged into bacteriophage particles, promoting rapid diversification and facilitating host shifting (Berglund et al. 2009).

With respect to two isolates (Ben9 and Ben28), they did not show the highest BLAST similarity to the same *Bartonella* species when comparing *gltA* and *rpoB* results. This finding may be due to the elevated number of recombination events that occurs within the *gltA* gene (Buffet et al. 2013). In this sense, this *gltA* sequence seems to suggest recombination between *B. tribocorum* and *B. elizabethae*, two *Bartonella* species that were found in other rodent individuals in this study. This recombination event may have probably occurred within the arthropod vector, as *X. cheopis* fleas have been found to be infected with both *Bartonella* species (Billeter et al. 2013). Therefore, only using this gene might lead to biases resulting in the false identification of genotypes (Paziewska et al. 2011).

Strikingly, none of the black rats analyzed in this study was found to harbor *Bartonella* organisms, whereas 20.8% of *R. norvegicus* individuals were PCR-positive. This result is similar to those obtained by many authors that have indicated that *Bartonella* prevalence differs markedly in *R. norvegicus* and *R. rattus*, being always higher in the former species (Ellis et al. 1999, Hsieh et al. 2010). These prevalence differences are of interest as one would anticipate that related species with similar biology that occurs in the same area would have comparable susceptibility to infection, suggesting that some factor is likely to be responsible for these differences (Brettschneider et al. 2012a).

In accordance with the study carried out by Leulmi et al. (2014), which analyzed the presence of *Bartonella* sp. in the ectoparasites carried by the same rodent individuals that were screened in this work, *Bartonella* prevalence was remarkably high in *X. cheopis* fleas obtained from both rat species (52.6% in gray rats and 34.3% in black rats). The high prevalence of *Bartonella* sp. in *X. cheopis* fleas obtained from black rats in that study, taken together with the absence of positive cases in this rodent species in our study, suggests that whatever is the exact nature of this (these) factor (s), it seems to be hampering the transmission of *Bartonella* spp. organisms from the vector to the host. Supporting this hypothesis, four of the *Bartonella*-negative black rats analyzed in this study were harboring infected fleas. In this sense, further studies are needed to ascertain the real zoonotic risk associated to black rats in Western Africa.

Our results could be important from a public health point of view due to the pathogenic potential of the species identified, which has been isolated from humans with severe clinical manifestations. In this sense, *B. elizabethae* has been reported to cause endocarditis (Daly et al. 1993), which is the most common clinical manifestation of *Bartonella* infection (Renesto et al. 2001), and *B. rochalimae* has been found in patients with bacteremia, fever, and splenomegaly (Tsai et al. 2010). Similarly, *B. tribocorum* has been found in a Thailand patient with fever (Kosoy et al. 2010). Therefore, the risk of human infection by Candidatus *Bartonella mastomydis* and the rest of *Bartonella* species found in this study should be studied in patients from Benin with fever of unknown origin. The risk of zoonotic transmission is underlined by the peridomestic and intradomestic environment of trapped animals, which suggests that they can come into close contact with humans with its consequent zoonotic risk.

At this point, it must be taken into consideration that *Mastomys* spp. and many other rodent species constitute the main source of animal food for rural populations in Benin (Assogbadjo et al. 2005). Consequently, transmission to humans is likely to be taking place in rural zones due to contacts with infected animals or through their blood-sucking arthropods. Therefore, this epidemiological study must be extended to rural areas to ascertain the incidence of *Bartonella*-related diseases in areas where potential *Bartonella* reservoirs constitute the main source of animal food.

Finally, this zoonotic risk could be exacerbated by the socioeconomic and political constraints affecting Cotonou, especially rapid demographic growth and inadequate resources for urban development, as previously described (Dossou and Glehouenou-Dossou 2007). In that sense, the inadequate waste management found in Cotonou may lead to an increase of rodent populations, a phenomenon that could imply an increase of human–rodent contacts and the number of *Bartonella*-infected rodents. In conclusion, our results demonstrate the importance of these mammal species as reservoirs for zoonotic *Bartonella* species and warrant increased awareness of physicians and healthcare workers for these pathogens, especially in unidentified febrile cases.

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No competing financial interests exist.

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