Detection of a Potential New *Bartonella* Species “Candidatus *Bartonella rondoniensis*” in Human Biting Kissing Bugs (Reduviidae; Triatominae)

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Abstract

**Background**

Among the Reduviidae family, triatomines are giant blood-sucking bugs. They are well known in Central and South America where they transmit *Trypanosoma cruzi* to mammals, including humans, through their feces. This parasitic protozoan is the causative agent of Chagas disease, a major public health issue in endemic areas. Because of the medical and economic impact of Chagas disease, the presence of other arthropod-borne pathogens in triatomines was rarely investigated.

**Methodology/Principal findings**

In this study, seven triatomines species involved in the transmission of *T. cruzi* were molecularly screened for the presence of known pathogens generally associated with arthropods, such as *Rickettsia*, *Bartonella*, *Anaplasmataceae*, *Borrelia* species and *Coxiella burnetii*. Of all included triatomine species, only *Eratyrus mucronatus* specimens tested positive for *Bartonella* species for 56% of tested samples. A new genotype of *Bartonella* spp. was detected in 13/23 *Eratyrus mucronatus* specimens, an important vector of *T. cruzi* to humans. This bacterium was further characterized by sequencing fragments of the *ftsZ*, *gltA* and *rpoB* genes. Depending on the targeted gene, this agent shares 84% to 91% of identity with *B. bacilliformis*, the agent of Carrion’s disease, a deadly sandfly-borne infectious disease endemic in South America. It is also closely related to animal pathogens such as *B. bovis* and *B. chomelii*.

**Conclusions**

As *E. mucronatus* is an invasive species that occasionally feeds on humans, the presence of potentially pathogenic *Bartonella*-infected bugs could present another risk for human health, along with the *T. cruzi* issue.
Author Summary

Triatomines are hematophagous insects including vectors of *T. cruzi*, the agent of Chagas disease, a huge public health issue, especially in South America. Whether these arthropods carry other pathogenic microorganisms is currently unknown. We investigated the presence of different arthropod-borne pathogens, including *Bartonella* spp., by quantitative PCR. *Bartonella* species were identified using *ftsZ*, *gltA* and *rpoB* gene sequencing and a new genotype of *Bartonella* spp. was detected in *Eratyrus mucronatus* specimens, an important vector of *T. cruzi* to humans. This agent is closely related to several human and animal pathogens. Depending on the gene fragment used, this agent shares 84% to 91% of identity with *B. bacilliformis*, the agent of the deadly Carrion’s disease. The possibility of transmission of potentially pathogenic bacteria could be an additional threat to human health since *E. mucronatus* bugs are more and more anthropophilic.

Introduction

Triatomine bugs (order Hemiptera, family Reduviidae, subfamily Triatominae) are blood-sucking arthropods ("kissing bugs"), most of which can feed both on animals and humans. All stages from first instar to male and female adults are strictly hematophagous and responsible for a relatively large blood intake due to their large size. They are mainly sylvatic and feed on small wild mammals but can also feed on birds and bats [1]. Triatomines occupy diverse natural ecotopes, such as mammal and bird nests, hollow trees, caves and rock fissures [2], but also rural environments, as they can prosper in crevices in houses [1]. These arthropods are distributed world-wide but the vast majority of the 140 recognized species is found in the Americas [3]. They are particularly well studied in South America, where they transmit an endemic flagellate pathogen, *T. cruzi*, the etiological agent of Chagas disease [1]. Also known as the American trypanosomiasis, Chagas disease is a neglected tropical disease, the first human parasitic disease in the endemic areas. *T. cruzi* is transmitted through the feces of infected kissing bugs, causing heart failure 10 to 30 years post-infection for almost 30% of individuals [4].

Because of the public health impact of Chagas disease, studies on kissing bugs are mainly focused on this theme. As a matter of fact, the presence of other human pathogens was never described in the hundred years that it has been known that kissing bugs could transmit pathogens. Only the presence of *Wolbachia* and *Arsenophonus* species was investigated based on the fact that these obligate intracellular bacteria are known to be endosymbionts of many arthropods [5,6]. The presence of zoopathogenic arthropod-borne viruses was also investigated. To our knowledge, there is no report of pathogen detection in dejections or in triatomines themselves, except for *T. cruzi*, although *Arsenophonus nasoniae* was once reported to be detected in an eschar of a human [7]. Regarding viruses, two have been described in these bugs. *Triatoma virus* is reported as strictly entomopathogenic, particularly for its principal host, *Triatoma infestans* [8], while *African swine fever virus* was detected in *Triatoma gerstaeckeri* but not transmitted to pigs [9].

French Guiana is an 84,000 km² overseas department and region of France bordered by Brazil and Suriname. Because of its many different ecosystems, particularly a dense rainforest, French Guiana is a biodiversity hotspot and one of the 21 areas where Chagas disease is endemic [10]. Among the 27 described species of triatomines in this area, many are invasive species. That is to say that many of them temporarily leave their sylvatic or peridomestic dwellings in order to invade houses. The main vector community of French Guiana comprises...
highly anthropophilic bugs belonging to the *Panstrongylus*, *Rhodnius* and *Eratyrus* genera [10]. They accidentally feed on humans [11] and also on potentially infected animals since they easily feed on domestic animals or wild mammals.

Aiming to add to knowledge regarding bacteria and triatomine association, we screened seven species of triatomines bugs from French Guiana by molecular biology for the presence of arthropod-borne bacteria such as *Rickettsia*, *Bartonella*, *Borrelia*, *Anaplasma*, *Wolbachia*, *Ehrlichia* species and *Coxiella burnetii*.
Methods

Triatomine collection, identification and selection

Triatomine specimens were collected in French Guiana from 1991 to 2013 using light traps or interception traps by one of the authors (JMB) and by the Société Entomologique Antillades-Guyane (SEAG) as part of an inventory of French Guiana’s insects. Triatomines were caught in forests (Horses Mountains, Kaw Mountains), peri domiciliary areas (Degrad Kwata, Kaw Mountains, Nancibo) or urban areas (Sinnamary, Kourou savannah) as displayed in Fig 1.

All triatomine specimens were morphological identified with the Bérenger et al. morphological key [12] and kept dried as insect collections. Seven T.cruzi vectors were included in this study: Rhodnius prolixus (n = 10), Rh. pictipes (n = 10), Rh. robustus (n = 10), Triatoma infestans (n = 10), Panstrongylus geniculatus (n = 10), P. rufotuberculatus (n = 4), Eratyrus mucronatus (n = 23).

DNA extraction

Dried triatomines were rinsed in sterile water and air-dried on filter paper before cutting lengthwise in two equal halves, using a sterile surgical blade for each specimen. One half and the legs were stored at -20˚C as a backup sample and the other legless half was selected for molecular analyses. Each half triatomine was crushed with a sterile pestle in 400 μL of a G2 buffer solution containing 40 μM of proteinase K (Qiagen) and incubated at 56˚C overnight. After 1 minute of centrifugation at 7000 x g, 200 μL of the supernatant was then collected prior to DNA extraction. Triatominae genomic DNA was individually extracted using the EZ1 DNA tissue extraction kit (Qiagen, Hilden, Germany) according to the manufacturer’s instructions. Triatominae DNAs were then eluted in 100 μL of Tris EDTA (TE) buffer using the DNA extracting EZ1 Advanced XL Robot (Qiagen) as previously described [13]. DNAs were either immediately used or stored at -20˚C until used for molecular analysis. The DNA extracting EZ1 Advanced XL Robot was disinfected after each batch of extraction as per the manufacturer recommendations to avoid cross-contamination.

Table 1. Sequences of qPCR primers used to investigate the presence of pathogens’ DNA in the E. mucronatus samples. F: forward primer, R: reverse primer, P: qPCR probe.

<table>
<thead>
<tr>
<th>Target organism</th>
<th>Target gene</th>
<th>Primer’s name</th>
<th>Sequence (5’-3’)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bartonella spp.</td>
<td>Intergenic spacer</td>
<td>IT2_F</td>
<td>GGGGCCGTAGCTAGCTG</td>
</tr>
<tr>
<td></td>
<td></td>
<td>IT2_R</td>
<td>TGAATATCCTTCTTACCAATTTC</td>
</tr>
<tr>
<td></td>
<td></td>
<td>IT2_P</td>
<td>6FAM-GATCCGGTCCGGCTCCACCA</td>
</tr>
<tr>
<td>Rickettsia spp.</td>
<td>gltA</td>
<td>gltA_F</td>
<td>GTGGAATTATGACTAATTATAT</td>
</tr>
<tr>
<td></td>
<td></td>
<td>gltA_R</td>
<td>GTATCTTACCAATCATCTTATGC</td>
</tr>
<tr>
<td></td>
<td></td>
<td>gltA_P</td>
<td>6FAM-CAAATATACACATTACATTTAT</td>
</tr>
<tr>
<td>Coxiella burnetii</td>
<td>IS30A</td>
<td>ITS30A_F</td>
<td>CGCTGACCTACAGAATAATGTC</td>
</tr>
<tr>
<td></td>
<td></td>
<td>ITS30A_R</td>
<td>GGGGTAGTTAATAATACCTTCTGG</td>
</tr>
<tr>
<td></td>
<td></td>
<td>ITS30A_P</td>
<td>6FAM-CATGAAACGTAATTTATCAGTGTGATGC</td>
</tr>
<tr>
<td>Borrelia spp.</td>
<td>16S</td>
<td>16S_F</td>
<td>AGCCCTTTAAGCTCGCTTTGAG</td>
</tr>
<tr>
<td></td>
<td></td>
<td>16S_R</td>
<td>GCCCTTGCAGGTACGTG</td>
</tr>
<tr>
<td></td>
<td></td>
<td>16S_P</td>
<td>6FAM-CGGGCCTAGAGGCTGAGG</td>
</tr>
<tr>
<td>Anaplasmataceae</td>
<td>23S</td>
<td>23S_F</td>
<td>TGACAGCGGTACCTTTTGATC</td>
</tr>
<tr>
<td></td>
<td></td>
<td>23S_R</td>
<td>GTAACAGGTCGTCCTTCCA</td>
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<tr>
<td></td>
<td></td>
<td>23S_P</td>
<td>6FAM-GGATTAGACCGGAACCAAG</td>
</tr>
</tbody>
</table>

doi:10.1371/journal.pntd.0005297.t001
Molecular analysis

DNA samples were individually tested by genus-specific PCR using primers and probes targeting specific sequences of Bartonella spp., but also Rickettsia spp., Coxiella burnetii, Borrelia spp., and all Anaplasmataceae species [14] as previously described [15] (Table 1). Real-time quantitative PCR (qPCR) was carried out according to the manufacturer’s protocol using a CFX Connect Real-Time PCR Detection System (Bio-rad, Hercules, CA, USA) with the Eurogentec Takyon qPCR kit (Eurogentec, Seraing, Belgium).

*Bartonella elizabethae, Rickettsia montanensis, Coxiella burnetii, Anaplasma phagocytophilum* and *Borrelia crocidurae* DNAs were used as positive qPCR controls for the primers and probe targeting respectively all Bartonella, Rickettsia, Coxiiella burnetii and Borrelia species.

DNAs were tested at different concentrations to avoid PCR inhibition. For each run, a PCR mix without DNA was used as negative control. Standard PCR targeting a 710 bp region of the invertebrate *cytochrome oxidase I* (COI) gene was performed on PCR negative triatomines to control DNA extraction.
Sequencing and GenBank accession numbers

DNA samples that were positive with Bartonella-qPCR were submitted to conventional PCR amplification using a Bio-Rad Thermocycler (Bio-Rad Laboratories, Hercules, CA) prior to sequencing. For Bartonella species identification, primers targeting Bartonella rpoB, gltA and ftsZ genes fragments were used as previously described [16]. DNA from Bartonella elizabethae served as PCR positive control and mixture without DNA as negative control. The cycling protocol consisted of 15 min at 95˚C followed by 35 cycles of denaturing at 95˚C for 30 s, annealing at 50˚C for 30 s (58˚C for rpoB gene), extension 1 min at 72˚C, followed by a final cycle of 1 min at 72˚C and sampling held at 4˚C. Amplification products were separated by electrophoresis through a 1.5% agarose-tris-borate-EDTA gel containing ethidium bromide. PCR products were sequenced using a Big Dye Terminator kit and an ABI PRISM 3130 Genetic Analyser (Applied BioSystems, Courtabeuf, France). The sequences were analyzed using the ABI PRISM DNA Sequencing Analysis software version 3.0 (Applied BioSystems) and compared to sequences available in the GenBank database using the BLAST algorithm (http://blast.ncbi.nlm.nih.gov/Blast.cgi). The partial sequences of ftsZ and rpoB genes of Bartonella amplified from the sample EmG01 are available in GenBank at #KX377404 and #KX377405.

Phylogenic analysis

Phylogeny of the detected Bartonella with other members of the Bartonella genus was established with TOPALi 2.5 software (Biomathematics and Statistics Scotland, Edinburgh, UK).
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Bartonella spp. Eratyrus mucronatus 1 French Guiana

AY562181 Brucella melitensis

KC886738 Bartonella ancashensis 20.60
KP720646 Bartonella ancashensis 41.60

Bartonella spp. Eratyrus mucronatus 1 French Guiana

CP000524 Bartonella bacilliformis KC583
HG328244 Bartonella bacilliformis USM-LMMB-007
KF218224 Bartonella bovis B32730
KF218218 Bartonella bovis B33664
KJ909808 Bartonella bovis 234
HM167505 Bartonella capreoli B28880
EF605288 Bartonella melophagi K-2C
gJN646666 Bartonella chomelii BNC03
HG977196 Bartonella schoenbuchensis MVT06
FJ147196 Bartonella rochalimae BR2
FN645454 Bartonella clarridgeiae 73
AB674242 Bartonella washoensis Sb1865nv
AF165994 Bartonella quintana
KF466253 Bartonella henselae SC443
AY166580 Bartonella koehlerae
FJ832089 Bartonella koehlerae CE3
AF165995 Bartonella taylorii
gAB196425 Bartonella birtlesii
AF165987 Bartonella alsatica
AY166582 Bartonella vinsonii arupensis
HQ444159 Bartonella cooperensis 63A
AB242288 Bartonella japonica
AB242292 Bartonella silvatica
AY515131 Bartonella rattimassiliensis 16115
JN647928 Bartonella grahamii KR32
CP001562 Bartonella grahamii as4aup
JX158367 Bartonella elizabethae THSKR-057
HG969192 Bartonella tribocorum BM1374166
JX158368 Bartonella queenslandensis SKR-002
Available sequences of ftsZ, gltA and rpoB genes of validated Bartonella species were retrieved from the National Center for Biotechnology Information (NCBI) based on the results of the BLAST program. Multiple sequence alignment was performed with the ClustalW multiple sequence alignment program, which is included in the BioEdit software.

Results

Triatominae collection

Triatomines were collected in eight different localities in French Guiana with no selection regarding species and sex (convenient sampling). Among the triatomines collected, Eratyrus mucronatus (Fig 2) accounted for 20% of catches and 29.8% of the specimens analyzed. Details related to collection, such as sampling area and triatomines’ sex, are indicated in Table 2. Of 23 E. mucronatus samples, 22 (95.6%) were male. Further details regarding other collected species have been listed elsewhere [12].

Molecular detection

DNAs extracted from all the triatomines were included to assess the presence of Bartonella species. Of 23 Eratyrus mucronatus samples, 13 (56.5%) were positive by Bartonella spp.-specific qPCR, with cycle threshold (Ct) values ranging from 13.23 to 25.91 (mean: 21.44) (Table 2). These specimens were from six distinct regions and collected between 1993 and 2003. All positive specimens were male, and a majority of them were collected in sylvatic and peridomestic areas: the Kaw Mountains (38.4%) and Horses Mountains (38.4%).

All samples tested negative for the presence of Rickettsia spp., Borrelia spp., Anaplasma spp., Ehrlichia spp., Wolbachia spp. and Coxiella burnetii. Bartonella spp. was only detected in Eratyrus mucronatus specimens.

Sequencing

A 787 bp fragment of the Bartonella spp. rpoB gene was amplified using conventional PCR primers prior to sequencing. Only three ITS2-qPCR positive samples were also positive for rpoB by standard PCR. Sequencing failed for two of them. Comparison of the one rpoB resulting sequence against the NCBI database using the BLASTN program indicated that it possessed 90% identity with the ATCC Bartonella bacilliformis 35685D-5 strain (#CP014012.1) and with the B. bacilliformis KC583 strain (#CP000524.1). The next closest cultivated strains were a B. bovis strain [17] and a B. chomelii strain [18], both with 89% identity. Our genotype also possesses 87% identity with a Bartonella ancashensis strain [19,20]. Available sequences of Bartonella rpoB gene were retrieved from NCBI and compared to the Bartonella sequence described hereby. This Bartonella genotype formed a distinct clade, with a strain of Bartonella bacilliformis as the closest clade based on rpoB gene analysis (Fig 3).

All samples allowed amplification of a single 333 bp fragment of the ftsZ gene by standard PCR. Blast analysis revealed 91% identity with the aforementioned 35685D-5 and KC583 B. bacilliformis strains (Fig 4).

Fig 3. A consensus phylogenetic tree showing the relationships of the studied species of Bartonella species based on a portion of rpoB gene sequence comparison. GenBank accession numbers (or the only genome accession number) are indicated when the sequences initially originated from Genbank. The sequences were aligned using ClustalW, and phylogenetic inferences were obtained using Bayesian phylogenetic analysis with TOPALi 2.5 software (Biomathematics and Statistics Scotland, Edinburgh, UK) within the integrated Maximum Likelihood application using the TrN + I + Γ model. Numbers at the nodes are percentages of bootstrap values obtained by repeating the analysis 100 times to generate a majority consensus tree. Bootstrap values below 80 were deleted from the final tree. The final set includes 756 base pairs. The new Bartonella sequence described in the present study is written in red.

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**Bartonella spp. Eratyrus mucronatus 1 French Guiana**

- AF360732 Brucella melitensis
- DQ179112 Bartonella bacilliformis EC01
- AY599560 Bartonella bacilliformis KC584
- CP014012 Bartonella bacilliformis ATCC35685D-5
- AB292602 Bartonella bacilliformis KC583
- HM363772 Bartonella E3-106 bat-Nigeria
- KJ99967 Bartonella B23797 bat-Nigeria
- KJ99967 Bartonella B23812 bat-Nigeria
- KJ99968 Bartonella B32395 bat-Nigeria
- KJ99967 Bartonella B23973 bat-Nigeria
- 319402157 Bartonella clarridgeiae 73
- EU571942 Bartonella clarridgeiae M9HN-SHQ
- DQ676490 Bartonella rochalimae SM318006
- DQ676486 Bartonella rochalimae Humboldt131
- KF193413 Bartonella bovis B32730
- KR733181 Bartonella bovis I724598
- KM215689 Bartonella chomelii Ru34
- AF467765 Bartonella schoenbuchensis
- EF505286 Bartonella melophagi K-2C
- KM215688 Bartonella chomelii A828
- HM167504 Bartonella capreol B28980
- AF467762 Bartonella birtlesii
- KP720647 Bartonella ancashensis 41.60
- CP010401 Bartonella ancashensis 20.00
- AY515134 Bartonella ratti massiliensis 16115
- AB426647 Bartonella grahamii PTZ30/3
- AB426640 Bartonella grahamii Aomori 23-1
- EU111780 Bartonella queenslandensis AUST/NH15
- AF467760 Bartonella elizabethae
- AF467759 Bartonella tribocorum
- AB519080 Bartonella washoensis ER14-3
- AB440637 Bartonella silvatica
- HQ444158 Bartonella coop ers plainsensis
- AB440633 Bartonella japonica
- HQ014623 Bartonella quintana S13
- HQ014630 Bartonella quintana M22
- KP822812 Bartonella henselae B40577
- KF246532 Bartonella koehlerae boulouuisi L-27-96
- KF246538 Bartonella koehlerae bothieri L-08-96
Detection of a New Bartonella Spp. in Eratyrus mucronatus Triatomines

Fig 4. A consensus phylogenetic tree showing the relationships of the Bartonella species studied based on a portion of ftsZ gene sequence comparison. GenBank accession numbers (or the only genome accession number) are indicated when the sequences originated from Genbank at the beginning. The sequences were aligned using ClustalW, and phylogenetic inferences were obtained using Bayesian phylogenetic analysis with TOPALi 2.5 software (Biomathematics and Statistics Scotland, Edinburgh, UK) within the integrated Maximum Likelihood application using the ML SYM+I+F model. Numbers at the nodes are percentages of bootstrap values obtained by repeating the analysis 100 times to generate a majority consensus tree. Bootstrap values below 80 were deleted from the final tree. The final set includes 292 base pairs. The new Bartonella sequence described in this study is written in red.

doi:10.1371/journal.pntd.0005297.g004

A total of 12 out of 13 samples were successfully amplified by standard PCR targeting a fragment of the gltA gene. BLAST analysis showed 88% identity with uncultured Bartonella species detected in bank voles [21], deer [22] and bats from Africa [23], but also with B. bovis and B. chomelii strains (Fig 5). Based on the gltA gene, our genotype is 84% similar to B. bacilliformis strains. Only a single rpoB sequence was obtained but all ftsZ and gltA sequences obtained were identical for all E. mucronatus specimens.

BLAST analysis of the concatenated sequence of the three genes revealed 99% of coverage and 90% similarity with the two aforementioned B. bacilliformis strains. Phylogenetic analysis based on the concatenated sequences revealed clustering of our Bartonella strain with two B. bacilliformis and B. ancashensis strains (Fig 6).

Discussion

Bartonella species are small fastidious gram-negative bacteria belonging to the Alphaproteobacteria class that are able to infect many mammals, including humans [24]. They are mostly transmitted by arthropod vectors such as sandflies (Lutzomyia verrucarum), human body lice (Pediculus humanus humanus), different fleas including cat fleas (Ctenocephalides felis), biting flies and ticks [25]. Among the several Bartonella species, some have been identified as human pathogens, causing well-known vector-borne diseases such as Carrion’s disease (B. bacilliformis), trench fever (B. quintana), cat scratch disease (B. henselae) as well as endocarditis [24].

We hereby describe a novel Bartonella genotype, phylogenetically related to several human and animal pathogens, as shown by the phylogenetic analyses. B. bacilliformis, a closely related species, is the causative agent of the first and well-described human bartonellosis called Carrion’s disease [26]. Transmitted through the bite of an infected phlebotomine sand fly, L. verrucarum, this South American endemic bacterium can induce a biphasic illness with two distinct syndromes that can be concomitant or independent. An acute phase known as Oroya fever manifests as a hemolytic fever linked to bacteremia that can range from 10 to 210 days and can be fatal in 40–88% of individuals without treatment. The second syndrome called verruga peruana manifests as blood-filled hemangiomas due to infection of the endothelium [26]. No human cases of B. bacilliformis infection have been reported in French Guiana to date [27]. Our genotype is also closely related to a strain of B. ancashensis, a recently described Bartonella species closely related to B. bacilliformis that was isolated from the blood of two patients diagnosed with a chronic stage of verruga peruana in Peru [20]. All data suggest that B. ancashensis could be a second agent. Our new agent is also closely related to B. bovis strains. Isolated from cats, which are only accidental hosts, this endocarditis [28].

E. mucronatus is a sylvatic triatominae bug involved in the transmission of T. cruzi [11]. It is recognized now as an invasive species as it has been described around and inside houses since 1959 [29] because of its attraction to artificial light sources [30]. They are known to feed on bats, but also on small mammals such as xenarhtrans and opossums [11]. Bats are widely reported to be sources of many viral and bacterial pathogens [31], including Bartonella spp. worldwide, including in French Guiana [32], Nigeria [33], Guatemala [34] and Vietnam, for
Detection of a New Bartonella Spp. in Eratyrus mucronatus Triatomines

Bartonella spp. Eratyrus mucronatus 1 French Guiana

- KC886736 Bartonella ancashensis 20.60
- AF293394 Bartonella bovis
  - KJ909850 Bartonella bovis 1539
- AF293392 Bartonella capreoli
- AY724768 Bartonella melophagi K-2C
  - KM215690 Bartonella chomelii A828
    - KM215693 Bartonella chomelii Ru56
- AF204272 Bartonella birtlesii
- U28075 Bartonella vinsonii berkholzii
- AB242287 Bartonella silvatica
- JQ694021 Bartonella sp A582RCG bankvole
  - JX158353 Bartonella elizabethae THSKR-057
    - FJ655405 Bartonella sp Rn1914001 rodent Thailand
  - KT324581 Bartonella tribocorum F2540
    - KT327031 Bartonella tribocorum B29906
      - AY515125 Bartonella rattimassiliensis 16115
        - CP001562 Bartonella grahamii as4aup
          - EU111802 Bartonella queenslandensis AUST/NH15
        - LC031777 Bartonella quintana MF1-1
          - HQ014627 Bartonella quintana M22
            - KF246523 Bartonella koehlerae boulouisi L-27-96
              - KF246529 Bartonella koehlerae bothieri L-08-96
                - KF133830 Bartonella henselae M6ND
                  - EF451789 Bartonella henselae Berlin-2
                    - KT324574 Bartonella cooperiplainsensis T1521
                      - AB242289 Bartonella japonica
                        - DQ897367 Bartonella washoensis cynomys CL8606cc
                          - AF440276 Bartonella washoensis cynomys CL1281cc
                            - EU770616 Bartonella claridgeiae M9HN-SHQ
                              - DQ676488 Bartonella rochalimae SM318006
                                - DQ676484 Bartonella rochalimae Humboldt131

225851546 Brucella melitensis ATCC 23457

- DQ200879 Bartonella bacilliformis Vega
- DQ200881 Bartonella bacilliformis Vero75
- DQ452937 Bartonella bacilliformis Choo Col-01
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Fig 5. A consensus phylogenetic tree showing the relationships of the Bartonella species studied based on a portion of gltA gene sequence comparison. GenBank accession numbers (or the only genome accession number) are indicated when the sequences originated from Genbank at the beginning. The sequences were aligned using ClustalW, and phylogenetic inferences were obtained using Bayesian phylogenetic analysis with TOPALi2.5 software (Biomathematics and Statistics Scotland, Edinburgh, UK) within the integrated Maximum Likelihood application using the K81uf + I + F model. Numbers at the nodes are percentages of bootstrap values obtained by repeating the analysis 100 times to generate a majority consensus tree. Bootstrap values below 80 were deleted from the final tree. The final set includes 200 base pairs. The new Bartonella sequence described in this study is written in red.

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Fig 6. A consensus concatenated phylogenetic tree showing the relationships of the Bartonella species studied based on a concatenated sequence of Bartonella rpoB, ftsZ and gltA gene fragment. Concatenated rpoB, ftsZ and gltA sequences were aligned using CLUSTALW and phylogenetic inferences obtained using Bayesian phylogenetic analysis [Ronquist F, Huelsenbeck JP. MrBayes 3: Bayesian phylogenetic inference under mixed models. Bio-informatics 2003; 19:1572–1574] with the TOPALi2.5 software (Biomathematics and Statistics Scotland, Edinburgh, UK) with the integrated MrBayes application [ftp://mrbayes.csit.fsu.edu] with the HKY+I+F substitution model. GenBank accession numbers are indicated at the beginning. Numbers at the nodes are bootstrap values obtained by repeating the analysis 100 times to generate a majority consensus tree. There were a total of 1245 positions in the final dataset. The scale bar indicates a 10% nucleotide sequence divergence.

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example [35]. Therefore, *Bartonella* spp. were frequently detected in hematophagous arthropods feeding on bats such as bat flies (*Hippoboscidae, Streblidae, Nycteribiidae*) [36] or *Cimex adjunctus* [37]. Triatome vectors belonging to genera *Triatoma, Rhodnius, Panstrongylus* and *Eratyrus* can be totally domiciliated or invasive, since they occasionally visit houses as described in Bolivia [38], Brazil [39], Argentina [40] and Venezuela [11]. The presence of these bugs around houses has long been known and has justified the establishment of chemical control campaigns, which after 5 years remain a failure in Bolivia [38]. The invasive behavior of *E. mucronatus* has not yet been described in French Guiana but data from Bolivia suggest that eliminating it once it is settled is challenging [38]. Living in various ecotopes and not host-specific [41], these bugs can easily feed both on humans and animals [38], both of them potentially bacteremic, parasitemic or viremic at the blood meal time point.

Triatominae species are well-studied bugs, however, this work provides the first evidence to our knowledge of infection with a bacterium that is not *a priori* endosymbiotic. The specimens we analyzed were dry, with no information regarding their engorgement status at the time of collection. However, as they were collected using light traps or interception traps, we can assume that they were seeking hosts and therefore probably non-engorged. Thus, we can suppose that we did not detect DNA of a bacterium present in recently ingested blood but a genuine infection. To support this hypothesis, the infection rate was considerable (56%) among triatomines collected in very distant sampling periods, geographically and over time. Ct values were also very low, increasing the possibilities that this bacterium multiplies within the bug’s body. However, to evaluate the possibility of transmission of these *Bartonella* spp., an experimental model of infection, or at least information regarding the location of the bacteria in the bug, would be necessary. Such information could not be obtained from our samples as they were dry and old. Cultivation of any bacteria or any attempt to localize with immunofluorescence, for example, was not possible.

In continuing this work, it would be interesting to collect wild *E. mucronatus* specimens in order to isolate the bacterium and establish an experimental model of infection with this arthropod/pathogen pair. This would reveal whether the bug is a simple carrier or an efficient vector of this *Bartonella*. The possible interaction between *T. cruzi* and this *Bartonella* spp. in this insect is also unknown and could be investigated by monitoring the trypanosome’s cycle and transmission in co-infected *E. mucronatus*. Being phylogenetically closely related to two severe human pathogens (*B. bacilliformis* and *B. ancashensis*), it would also be important to evaluate its pathogenicity. Because of the huge public health impact of Chagas disease in South America, investigations on *Triatominae* were limited to the study of their interactions with *T. cruzi*. In fact, Triatominae bugs may host such bacteria as *Bartonella* species and, probably, may be its vector.

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**Author Contributions**

**Conceptualization:** ML, JMB, PP.

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**Investigation:** ML, JMB.

**Methodology:** ML, JMB.
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Supervision: PP.
Validation: ML.
Writing – original draft: ML JMB.
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