Cryptosporidium spp. in wild murids (Rodentia) from Corsica, France

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Abstract

Cryptosporidium spp. are worldwide protozoan parasites that can affect a broad range of vertebrate hosts, including rodents. In the island of Corsica (France), there are no previous data about these protozoa infecting wild rodents. To estimate the distribution and occurrence, a total of 117 wild murine rodents of the species Rattus rattus (84), Mus musculus domesticus (21), Apodemus sylvaticus (11), and Rattus norvegicus (1) were captured in 24 different biotopes. Fecal samples were screened for Cryptosporidium spp. by nested PCR to amplify an 830 bp fragment of the 18S rRNA gene. As general occurrence, 15.4% of the rodents analyzed were positive for Cryptosporidium spp., being detected widely distributed along the island in R. rattus (17.6%) and M. m. domesticus (14.3%). Cryptosporidium viatorum, Cryptosporidium sp. rat genotype II, and Cryptosporidium sp. rat genotype III were successfully identified in R. rattus. The results herein reported provide the first data on Cryptosporidium spp. in wild murine species from a Mediterranean island and constitute the first report of the zoonotic species C. viatorum in R. rattus. Although a low occurrence of Cryptosporidium spp. in murids was obtained and only in one animal the zoonotic species C. viatorum was identified, our results highlight that wild murine rodents from Corsica could mediate in the maintenance and transmission of this protozoan to the environment and other hosts including humans and animals. Further studies are required to better understand the epidemiology of Cryptosporidium spp. in wild rodents from Corsica and their possible public health repercussions.

Keywords Rattus rattus · Rattus norvegicus · Mus musculus domesticus · Apodemus sylvaticus · Cryptosporidium viatorum · Corsica

Introduction

The protozoan Cryptosporidium spp. (phylum Apicomplexa) infects a broad range of vertebrate hosts that can play an important role in the maintenance and transmission of this pathogen (Kváč et al. 2014). It can be found in more than 150 mammalian species (Bauerfeind et al. 2016), being the order Rodentia, which represents at least 43% of the mammalian species (Wilson and Reeder 1993; Huchon et al. 2002), the most abundant and diversified order of mammals considered to be reservoirs of Cryptosporidium spp. (Feng 2010). The morphological and biological adaptations of rodents allow them to survive in any type of environments (Huchon et al. 2002), being significantly more abundant in anthropogenically modified habitats (e.g., agricultural lands, pasturelands, urban areas) than non-modified habitats (Mendoza et al. 2020). This ability facilitates rodents to spread and transfer their pathogens, such as Cryptosporidium spp., to humans and wild and domestic animals in rural and urban...
areas (Meerburg et al. 2009). *Cryptosporidium* spp. are the causal agent of cryptosporidiosis, a leading cause of diarrheal disease in both humans and animals worldwide (Innes et al. 2020). This protozoan can be transmitted by fecal–oral contamination, via ingestion of contaminated water or food, direct contact with infected persons or animals, indirect contact with contaminated fomites, and, in some cases, via inhalation (Sponseller et al. 2014; Bauerfeind et al. 2016).

The prevalence of *Cryptosporidium* spp. infection in rodents is highly variable worldwide (Feng 2010). A recent systematic review and meta-analysis estimated a 17% of pooled global prevalence of *Cryptosporidium* spp. infection in rodents, obtaining a 22% of prevalence in Europe (see Taghipour et al. 2020). In fact, previous studies reported prevalence rates of *Cryptosporidium* spp. infection in rodents ranging between 8.0 and 31.4% in mice and 2.1 and 63.0% in rats (Feng 2010; Koehler et al. 2018; Zhao et al. 2018, 2019). Currently, more than 40 *Cryptosporidium* species and similar number of genotypes have been recognized as valid worldwide (Zahedi and Ryan 2020; Ježková et al. 2020; Zahedi et al. 2021). At least 21 *Cryptosporidium* species and 21 genotypes have been cited in rodents: *Cryptosporidium muris*, *Cryptosporidium parvum*, *Cryptosporidium hominis*, *Cryptosporidium meleagridis*, *Cryptosporidium tyzzeri*, *Cryptosporidium ubiquitum*, *Cryptosporidium suis*, *Cryptosporidium scrofarum*, *Cryptosporidium erinacei*, *Cryptosporidium canis*, *Cryptosporidium wrairi*, *Cryptosporidium rubei*, *Cryptosporidium andersoni*, *Cryptosporidium proliferans*, *Cryptosporidium occultus*, *Cryptosporidium viatorum*, *Cryptosporidium ditrichi*, *Cryptosporidium apodemi*, *Cryptosporidium alticolis*, *Cryptosporidium microti*, *Cryptosporidium ratti*, rat genotypes II–IV, mouse genotypes II and III, naruko genotype, ferret genotype, chipmunk genotypes I and II, skunk genotype, hamster genotype, deer mouse genotypes I–IV, vole genotype, bear genotype, muskrat genotypes I and II, and ground squirrel genotypes I–III (see Lv et al. 2009; Paparini et al. 2012; Ng-Hublin et al. 2013; Kvač et al. 2014; Čondlová et al. 2018; Koehler et al. 2018; Zhang et al. 2018; Zhao et al. 2018; Horčičková et al. 2019; Zahedi and Ryan 2020; Ježková et al. 2020). Among them, *C. parvum* and *C. hominis* are the major species involved in human and waterborne outbreaks (Xiao 2010; Ryan and Xiao 2014). *Cryptosporidium parvum*, *C. muris*, and *Cryptosporidium* sp. rat genotype III are the species frequently detected in rats (Koehler et al. 2018), being *C. muris* and *Cryptosporidium* sp. mouse genotype I the most reported species in mice (Morgan et al. 1999, 2000; Foo et al. 2007).

Corsica (France), considered the fourth largest Mediterranean island and the most mountainous and forested one (Grecch-Angelini et al. 2016), is situated in the southeast of the French mainland and west of the Italian Peninsula (Fig. 1). This well-known tourist destination is characterized by a mild Mediterranean climate and a high variability of microclimates (Grecch-Angelini et al. 2016), which allows the development of a large diversity of natural environments where many animal species, including rodents, are well established. In terms of public health and veterinary, available data about the pathogens harboring wild murine rodents from Corsica are based on *Fasciola hepatica* in *Rattus rattus* and *Mus musculus* (Valero et al. 1998, 2002; Ménard et al. 2000; Magnanou et al. 2006), *Schistosoma* spp. in *R. rattus* (Oleaga et al. 2019), and *Borrelia burgdorferi* s.l. in *Ixodes ricinus* ticks of *R. rattus* (Cicculli et al. 2019). Within a multidisciplinary study, the species *Coxiella burnetii* and *Toxoplasma gondii* in *R. rattus* and *M. m. domesticus* (Izquierdo-Rodríguez et al. 2019) and pathogenic *Leptospira* species in *R. rattus*, *Mus musculus domesticus*, *Rattus norvegicus*, and *Apodemus sylvaticus* (Izquierdo-Rodríguez et al. 2020) were detected. As a part of this multidisciplinary study, and considering the lack of data on *Cryptosporidium* spp. in rodents from Corsica, we aimed to analyze its distribution, occurrence, and identity of the species and genotypes present in wild murids from Corsica.

### Materials and methods

#### Sample collection

From a multidisciplinary study carried out in Corsica (France) between February and June 2016, a total of 117 wild murine rodents of the species *R. rattus* (black rat) (*n* = 84), *M. m. domesticus* (domestic mouse) (*n* = 21), *A. sylvaticus* (wood mouse) (*n* = 11), and *R. norvegicus* (gray rat) (*n* = 1) were captured alive using live traps. With the aim of collecting animals from different biotopes, traps were set in 24 municipalities along Corsica considering North and South of the island following Izquierdo-Rodríguez and collaborators (Izquierdo-Rodríguez et al. 2020) (Table 1), mainly at suburban-rural sites near ponds, river mouths, and lakes (Fig. 1). Euthanasia was performed with CO₂ inhalation or by cervical dislocation, and, after dissection, a portion of fecal samples from the rectum of each animal were preserved in 2.5% potassium dichromate and conserved until examination. This study was carried out in strict accordance with the recommendations of the guidelines of animal welfare in experimental science and the European Union legislation (Directive 86/609/EEC). The protocol was approved by the Ethics Committee of Investigation and Animal Wellness of Universidad de La Laguna (register number CEIBA2018-0330).

#### DNA isolation

Before DNA extraction, 200 μL of the samples was washed with PBS-EDTA at room temperature to remove the
potassium dichromate. Next, total DNA was isolated directly by following the manufacturer’s instructions of the commercial FastDNA SPIN kit (Qbiogene, Illkirch Cedex, France) using the TissueLyser II (Qiagen, Hilden, Germany) as oocyst disruptor. DNA was stored at \(-20^\circ\text{C}\) until further processing.

### PCR amplification

*Cryptosporidium* spp. were detected by nested PCR targeting an 830 bp of the 18S ribosomal rRNA gene using the primer pair SSU-F1/SSU-R1 and SSU-F2/SSU-R2 for the primary and secondary PCR, respectively (Zhao et al. 2013). The set-up of the PCR reactions was carried out according to the method of Zhang and collaborators (Zhang et al. 2015). The reaction mixture for all pairs of primers contained 0.625 U Taq DNA polymerase, 0.4 µM of each primer, 200 µM each dNTPs, 2 mM MgCl\(_2\), 1× buffer (Mg\(_2+\) free), 2 µl of DNA template, and water to a total volume of 25 µl. The cycling conditions for both amplifications were initial denaturation of 95 °C for 5 min followed by 35 cycles 94 °C for 45 s, 45 s at suitable temperature (55 °C for primary PCR and 58 °C for secondary PCR), and 1 min at 72 °C, followed by a final extension step at 72 °C for 10 min. All PCR reactions were performed in a Labnet Thermocycler (Labnet International, Berkshire, UK). PCR products were resolved on 1.5% agarose gels, and PCR-positive products were purified using the UltraClean PCR Clean-up kit (Mo-Bio Laboratories, Inc., Carlsbad, CA, USA). In cases where bands of different sizes occurred, the desired size band was cut and purified with the QIAEX® II Gel Extraction kit (Qiagen, Hilden, Germany). In both cases, the manufacturer’s recommendations were followed.

### Sequencing and phylogenetic analyses

The purified PCR-positive products were sequenced at Macrogen Europe (Amsterdam, the Netherlands). Nucleotide sequences obtained were edited with the MEGA X program (Kumar et al. 2018) and subsequently aligned with the ClustalW program included in MEGA X. Minor corrections, to increase the aligned sequence similarity and improve the inferences on any positional homology, were then made by hand. A BLAST search was carried out in order to elucidate any homologies or similarities with the sequences previously published in the GenBank database. The molecular identification was achieved by phylogenetic analysis through the neighbor-joining distance method (Saitou and Nei 1987) with at least 1000 bootstrap replications. Nucleotide sequences obtained
in this work and reference sequences from other Cryptosporidium species and genotypes from the GenBank were aligned. Toxoplasma gondii was used as the outgroup. The obtained sequences generated in this study have been deposited in GenBank under the accession numbers MW590662–MW590669.

### Statistical analyses

A chi-square test, setting the P value in 0.05, was conducted for the comparison of Cryptosporidium spp. prevalence obtained between the rodent species and the capture area, North and South. Data analysis was carried out using SPSS v22.0 statistical software.

### Results

In this study, Cryptosporidium DNA was detected in wild murids fecal samples, and it was found widely distributed along Corsica, being detected both in the North and the South of the island homogeneously with 11.2% and 28.6% of occurrence, respectively, without statistical differences (Fig. 1, Table 1). Of the 117 wild murine rodents analyzed, 18 resulted positive for the amplification of the 18S rRNA gene fragment with a general occurrence of 15.4% (Table 1). Considering the four murine species analyzed, R. rattus and M. m. domesticus were the only species found infected by Cryptosporidium spp. Of the 18 PCR-positive samples, 15 correspond to rats, nine of them were captured in the North of Corsica, specifically in the regions of Diane (n = 2),

Table 1 Rodent species analyzed for the detection of Cryptosporidium spp., as well as the locations where they have been captured in Corsica. All species/genotypes identified were detected in R. rattus. Abbreviations: % occurrence of Cryptosporidium spp., +/n positive animals for Cryptosporidium spp./number of samples analyzed (* one Rattus norvegicus).

| Locations          | Rattus rattus (+/n) | Mus musculus domesticus (+/n) | Apodemus sylvaticus (+/n) | Total (+/n) | Species/genotype |
|--------------------|---------------------|-------------------------------|---------------------------|-------------|------------------|
| **North**          |                     |                               |                           |             |                  |
| Biguglia           | 2/3                 | 0/2                           | 0/0                       | 2/5         | Cryptosporidium sp. rat genotype II (n = 1) |
| Calacuccia         | 0/3                 | 0/1                           | 0/2                       | 0/6         |                  |
| Canale di Verde    | 0/5                 | 0/2                           | 0/0                       | 0/7         |                  |
| Chiatra            | 0/1                 | 0/0                           | 0/0                       | 0/1         |                  |
| Corte              | 2/5                 | 0/3                           | 0/3                       | 2/11        | Cryptosporidium sp. rat genotype II (n = 1) |
| Diane              | 2/5                 | 0/1                           | 0/0                       | 2/6         | Cryptosporidium sp. rat genotype II (n = 2) |
| Foce               | 2/7                 | 0/2                           | 0/1                       | 2/10        |                  |
| Golo               | 0/3*                | 1/3                           | 0/0                       | 1/6         |                  |
| Gradugine          | 1/3                 | 0/0                           | 0/0                       | 1/3         |                  |
| Matra              | 0/1                 | 0/0                           | 0/1                       | 0/2         |                  |
| Padula             | 0/2                 | 0/0                           | 0/0                       | 0/2         |                  |
| Palo               | 0/3                 | 0/1                           | 0/0                       | 0/4         |                  |
| Prunelli di-Fium’Orbu | 0/5              | 0/0                           | 0/0                       | 0/5         |                  |
| Saint Florent      | 0/5                 | 0/0                           | 0/2                       | 0/7         |                  |
| Terrenzana         | 0/6                 | 0/1                           | 0/0                       | 0/7         |                  |
| Tettola            | 0/0                 | 0/0                           | 0/2                       | 0/2         |                  |
| Urbino             | 0/5                 | 0/0                           | 0/0                       | 0/5         |                  |
| Total (+/n) (%)    | 9/62 (14.5)         | 1/16 (6.2)                    | 0/11 (0)                  | 10/89 (11.2)|                  |
| **South**          |                     |                               |                           |             |                  |
| Ajaccio            | 1/4                 | 0/0                           | 0/0                       | 1/4         |                  |
| Liamone            | 0/6                 | 0/1                           | 0/0                       | 0/7         |                  |
| Pinarellu          | 1/1                 | 2/2                           | 0/0                       | 3/3         | Cryptosporidium sp. rat genotype II (n = 1) |
| Porticcio          | 2/2                 | 0/0                           | 0/0                       | 2/2         | Cryptosporidium sp. rat genotype II (n = 1) |
| Rizzanese          | 2/5                 | 0/0                           | 0/0                       | 2/5         | Cryptosporidium viatorum (n = 1) |
| Saint Cyprien      | 0/2                 | 0/1                           | 0/0                       | 0/3         | Cryptosporidium sp. rat genotype III (n = 1) |
| Taravo             | 0/3                 | 0/1                           | 0/0                       | 0/4         |                  |
| Total (+/n) (%)    | 6/23 (26)           | 2/5 (40)                      | -                         | 8/28 (28.6) |                  |
| Total (+/n) (%)    | 15/85 (17.6)        | 3/21 (14.3)                   | 0/11 (0)                  | 18/117 (15.4)|                  |
Gradugine (n = 1), Foce (n = 2), Corte (n = 2), and Biguglia (n = 2). The other six rats were captured in the South regions of Porticcio (n = 2), Ajaccio (n = 1), Pinarellu (n = 1), and Rizzanese (n = 2). In the case of mice, three resulted infected by Cryptosporidium spp., two of them were captured in the South region of Pinarellu and the other one was captured in the North region of Golo (Fig. 1). No significant differences were found in the occurrence of murine species infected between North and South of Corsica. The general occurrences of infection in R. rattus and M. m. domesticus were 17.6% (15/85) and 14.3% (3/21), respectively (Table 1). In this study, only eight PCR-positive samples from R. rattus were successfully sequenced (Table 1). Phylogenetic analysis showed the presence of 3 Cryptosporidium species and genotypes (Fig. 2), including Cryptosporidium sp. rat genotype II. Cryptosporidium sp. rat genotype III, and the zoonotic species C. viatorum. Cryptosporidium sp. rat genotype II was the most detected genotype, which has been found widely distributed in North and South of Corsica, while Cryptosporidium sp. rat genotype III was only detected in one rat in the South, as was C. viatorum. A sequence obtained from R. rattus, captured in the South region of Rizzanese (isolate 111C), clustered together with a C. viatorum sequence obtained from Berylmys bowersi from China (GenBank: MK522270). Additionally, six sequences from R. rattus, captured in the North regions of Diane (isolates 7C and 10C), Corte (isolate 89C), and Biguglia (isolate 105C), and in the South regions of Ajaccio (isolate 77C) and Pinarellu (isolate 98C), clustered together with Cryptosporidium sp. rat genotype II from Rattus tanezumi from

![Fig. 2 Phyllogenetic analysis using the neighbor-joining method with p-distance and 1000 bootstrap replications based on the 18S ribosomal RNA fragment (830 bp). Toxoplasma gondii was used as the outgroup](image-url)
China (GenBank: GQ121025). One sequence from R. rattus, captured in the South region of Rizzanese (isolate 113C), clustered together with Cryptosporidium sp. rat genotype III from R. rattus from Australia (GenBank: JX294371).

Four different haplotypes of Cryptosporidium sp. rat genotype II were detected. The most prevalent, haplotype 1, was found in the North (isolates 10C and 89C) and South (isolate 77C) of Corsica. One second haplotype, haplotype 2, was identified in the North of the island (isolate 105C), whose nucleotide sequence presented two extra adenine bases in positions 172 and 173 with respect to haplotype 1. In the case of haplotype 3, detected in Southern Corsica (isolate 98C), it presented a base pair change of T→A and C→A at position 63 and 235 with respect to haplotype 1, respectively. In the haplotype 4, detected in Northern Corsica (isolate 7C), the nucleotide sequenced presented a base pair change of T→G in position 160, A→C in position 161, and C→G in positions 158 and 246.

Discussion

This is the first epidemiology survey of Cryptosporidium spp. in murine rodents from Corsica. A total of three Cryptosporidium species and genotypes, concretely, Cryptosporidium sp. rat genotype II, Cryptosporidium sp. rat genotype III, and C. viatorum, were successfully identified with a 15.4% of occurrence. The occurrences obtained for rats (17.6%) and mice (14.3%) are within the reported ranges for mice (8.0–31.4%) and rats (2.1–63.0%) (Feng 2010; Koehler et al. 2018; Zhao et al. 2018, 2019).

The most prevalent genotype was Cryptosporidium sp. rat genotype II, contrary to the genotype found in other studies where Cryptosporidium sp. rat genotype III is the most frequently reported (Koehler et al. 2018). Cryptosporidium sp. rat genotype II was found in six rats widely distributed both in the North and South of the island of Corsica. However, Cryptosporidium sp. rat genotype III and C. viatorum were only found in the South of the island, but as they have been detected in a single animal each, this is not a congruent result to assess if there are any difference between their distribution on the island. Therefore, more studies are required to search for these species and genotypes in the North of the island, considering the few animals from which we were able to successfully sequence Cryptosporidium spp.

Cryptosporidium sp. rat genotype II and Cryptosporidium sp. rat genotype III are host-adapted genotypes (Zahed et al. 2016) that have been detected in rats worldwide (Lv et al. 2009; Paparini et al. 2012; Ng-Hublin et al. 2013; Koehler et al. 2018; Tan et al. 2019; Zhao et al. 2019; García-Livia et al. 2020). Nevertheless, other hosts can be infected by Cryptosporidium sp. rat genotypes II and III, as has been reported in mice, cats, sheep, and goats (Ryan et al. 2005; Lv et al. 2009; Paparini et al. 2012; Ng-Hublin et al. 2013; Koinari et al. 2014; Yang et al. 2015; Hatam-Nahavandi et al. 2019). To date, the potential of the rat genotypes identified in this study to cause cryptosporidiosis in livestock or humans remains unclear.

Cryptosporidium viatorum, the only zoonotic species identified in this study, has been originally found exclusively in humans (Elwin et al. 2012; Insulander et al. 2013; Lebbad et al. 2013; Adamu et al. 2014; Ayinmode et al. 2014; Stensvold et al. 2015; De Lucio et al. 2016; Sánchez et al. 2017; Ukwah et al. 2017) and in urban wastewater and combined sewer overflows in China (Huang et al. 2017). Recently, in 2018, C. viatorum was reported in a non-human host, concretely in the wild rats Rattus lutreolus from Australia (Koehler et al. 2018), and in the species R. norvegicus, Leopoldamys edwardsi, and B. bowersi from China (Zhao et al. 2019; Chen et al. 2019). Therefore, our study constitutes the first report of C. viatorum in R. rattus and the fifth report in non-human hosts, expanding its host range. Our results support recent findings suggesting that wild murines could be hosts of the zoonotic species C. viatorum. In fact, C. viatorum XVa subtype family from wild rats has been recently reported to be genetically identical to those subtypes found in humans (Chen et al. 2019). Further molecular investigations are needed to better understand the epidemiology of C. viatorum and clarify its transmission routes.

Considering that the species C. viatorum was the only zoonotic species detected in a single animal, more analyses are required to determine the occurrence of this and other possible zoonotic Cryptosporidium species that could have not been detected in wild murids from Corsica. In this sense, it is important to note that a recent study, conducted between 2017 and 2019 with 750 online reports from the National Reference Center-Expert of cryptosporidiosis in France, showed that the 40% of the reported cases of human cryptosporidiosis in this country were mainly related to recreational water (48%) and animal contact (23%) (Costa et al. 2020). In Corsica, no cases of cryptosporidiosis in humans have been reported to date, but considering that murine rodents can maintain the transmission cycles of several Cryptosporidium zoonotic species, such as C. parvum, C. meleagris, C. scrofarum, C. viatorum, C. canis, C. tyzzeri, C. andersoni, C. hominis, C. suis, C. proliferans, C. occultus, C. wrairi, C. rubeyi, and C. ditrichi (Zhao et al. 2018; Koehler et al. 2018; Zhang et al. 2018; Beser et al. 2020), the detection of C. viatorum in this study should not be dismissed.

This study constitutes the first survey of Cryptosporidium spp. infection in wild murine species from a Mediterranean island. Some studies have previously confirmed the presence of this protozoan in murine rodent species from Atlantic islands (Feliu et al. 2012; García-Livia
et al. 2020), showing that Cryptosporidium spp. infection is common in R. rattus and M. m. domesticus from the Canary Islands (Spain), with a high diversity of Cryptosporidium species and genotypes. In Tenerife (Canary Islands), a total of seven Cryptosporidium species/genotypes were identified, while in Corsica, despite being an island much larger in size than Tenerife and both studies with similar sample size (n = 97 in Tenerife and n = 1117 in Corsica), a low occurrence was obtained and a total of three Cryptosporidium species/genotypes were identified. Further studies analyzing more samples are required in order to obtain additional data about the occurrence and biodiversity of Cryptosporidium spp. in wild murids from Corsica.

Factors such as host specificity, ecological interactions, geographic distribution, bioclimatic conditions, and sampled areas, among others, could influence in the occurrence and prevalence of Cryptosporidium spp. in wild rodents (Zhao et al. 2019). Considering that samples of our study were only obtained near ponds, river mouths, and lakes, together with the fact that only few samples were successfully sequenced, further investigations with an increased in the number of samples and in non-analyzed areas would improve the knowledge about the prevalence, distribution, and biodiversity of Cryptosporidium spp. in wild murine rodents from Corsica.

Conclusions

The present study constitutes the first report of Cryptosporidium spp. in wild murids from Corsica (France), thus creating an overview of the epidemiological situation of this parasite in this region. Cryptosporidium sp. rat genotype II, Cryptosporidium sp. rat genotype III, and C. viatorum were the Cryptosporidium species and genotypes successfully identified in fecal samples from wild murine rodents, being the first citation of C. viatorum in R. rattus.

Considering the interactions between wildlife, livestock, and humans, further investigations should be carried out in unsampled areas, in addition to other wild hosts, to better understand the epidemiology of Cryptosporidium spp. in Corsica and to determine the possible zoonotic risks of transmission.

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Author contribution KGL wrote the manuscript, designed the figures, and performed the phylogenetic analysis and the interpretation of the results. AFA has performed the experiments, formal analysis, and data curation and provided critical revision of the manuscript. CF and JM has contributed to the sampling of the rodents and provided critical revision of the manuscript. YQ has contributed with the funding acquisition and project administration and provided critical revision of the manuscript. PF has contributed with the conceptualization, funding acquisition, project administration, and interpretation of the results and drafted or provided critical revision of the manuscript. All authors read and approved the final manuscript.

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Availability of data and material The dataset used and analyzed by the authors during the present study are available from the corresponding author upon reasonable request.

Code availability Not applicable.

Declarations

Ethics approval and consent to participate This study was carried out in strict accordance with the recommendations of the guidelines of animal welfare in experimental science and the European Union legislation (Directive 86/609/EEC). The protocol was approved by “Comité de Ética de la Investigación y Bienestar Animal” of Universidad de La Laguna (Protocol Number: CEIBA2018-0330).

Consent for publication Not applicable.

Competing interests The authors declare no competing interests.

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