International Journal for Parasitology: Parasites and Wildlife 21 (2023) 83–88

Contents lists available at ScienceDirect

International Journal for Parasitology: Parasites and Wildlife

journal homepage: www.elsevier.com/locate/ijppaw

Urban biodiversity: Cuterebriasis in free-ranging Robinson's mouse opossum (*Marmosa robinsoni*) in the suburbs of Barranquilla, Colombia

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ARTICLE INFO

Keywords:
Cuterebra
Didelphidae
Marsupials
Bot fly
Barcode

ABSTRACT

The tropical dry forest is one of the world's most threatened ecosystems and is the habitat of the Robinson's Mouse Opossum (*Marmosa robinsoni*), a small marsupial within the Didelphidae family. This study aimed to describe cases of cuterebriases in free-ranging *M. robinsoni* by examining individuals caught in live animal traps. Sherman traps were deployed in four different sites over three different periods in five days. All animals passed through biometry, weighing, sampling parasites, and sampling feces. Only animals captured in the study site located close to the city were anesthetized and examined. The evaluation included blood samples and a clinical examination. Animals received anesthesia under physical restraint by intramuscular injections of ketamine and xylazine. For anesthetic reversion, the protocol was Yohimbine administered before release. In total, 8% (5/60) of all captured animals had fly larvae extracted from wounds. The molecular Barcode of the mitochondrial Cytochrome Oxidase I gene showed no match with any recognized species of *Cuterebra.* The animals weighed from 35 to 80 g and had lesions in the scapular region with parasites under their skin in sizes ranging from 1.3 to 2.2 cm. The animals with parasites were in good physical condition without evidence of disturbances in health conditions. This is compatible with literature, reporting little effect on population dynamics of other host species infected with *Cuterebra* larvae. The study included 24 animals captured in three areas far from any city, which showed no evidence of cuterebrid infection, suggesting that proximity to the city could increase exposure to cuterebriasis. There are reports of cuterebrids in *M. robinsoni* in Brazil; however, this is the first report of cuterebriasis in *M. robinsoni* in Colombia.

1. Introduction

The tropical dry forest is one of the most threatened ecosystems in the world and only 8% of its original distribution remains in Colombia, including continuous forest and fragments, mostly surrounded by areas dominated by agriculture or cattle farming (Pizano and García, 2014). This is the preferred habitat of the Robinson's Mouse Opossum (*Marmosa robinsoni* Bangs), a small marsupial of the Didelphidae family with five subspecies (Voss, 2013) including *M. r. robinsoni* in the Caribbean region, northeast of Colombia, and northern Venezuela (Gardner, 2008). Besides, the species occurs in several other countries of Central and South America as Belize, Ecuador, El Salvador, Grenada, Guatemala, and others (Pérez-Hernandez, 2016). Despite having a broad distribution, studies about the species' natural history are scarce, lacking information on nutrition, life expectancy and ecology. (Gutiérrez et al., 2010). This study aimed to describe and discuss cases of cuterebriasis in free-ranging Robinson's Mouse Opossums (*M. robinsoni*) captured in a small forest area within the metropolitan area of Barranquilla, Colombia (Fig. 1).

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https://doi.org/10.1016/j.ijppaw.2023.02.006
Received 23 November 2022; Received in revised form 24 February 2023; Accepted 25 February 2023

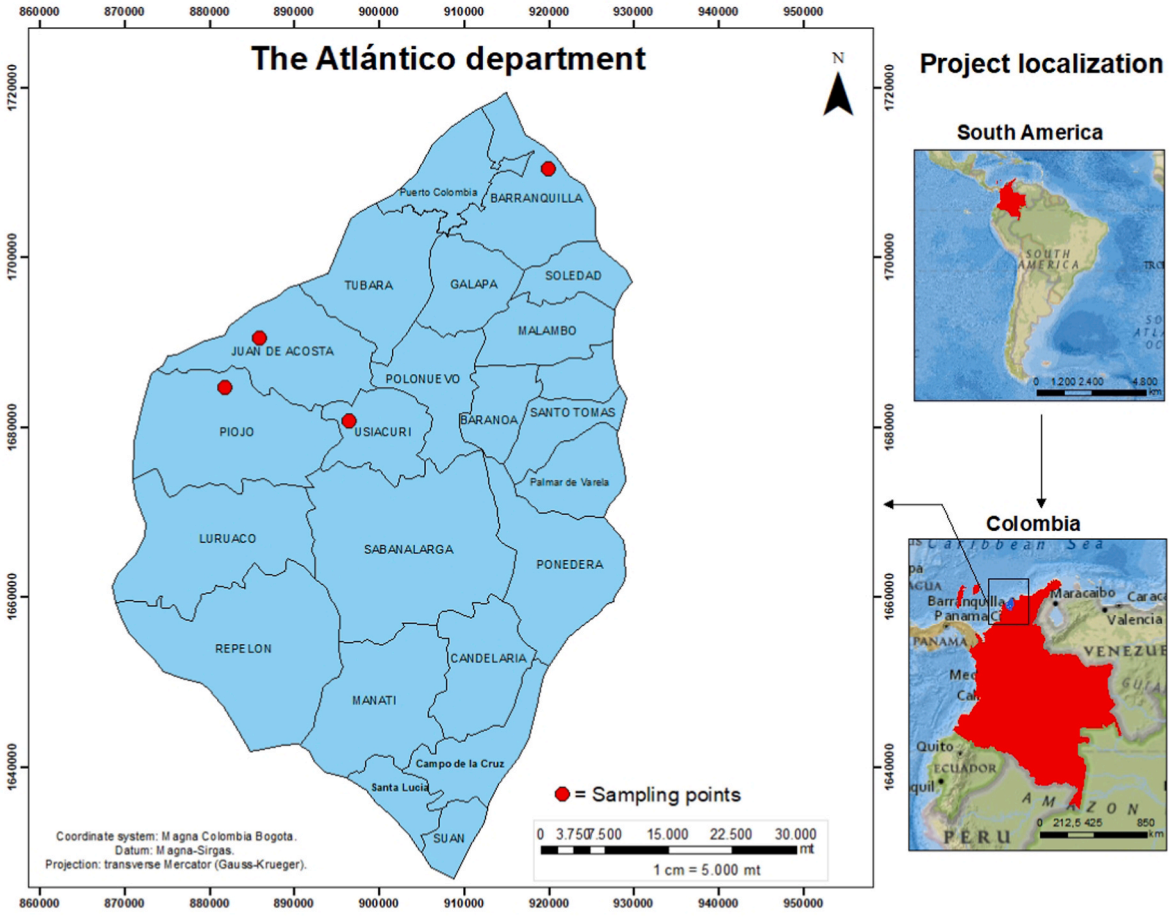

Fig. 1. Map of the Atlantic department of Colombia showing the four areas (red dots) of study of M. robinsoni: Palomar, Carreto, Luriza, and Zona Franca Celsia (visualized with Google Earth Pro). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Table 1
Description of study areas where M. robinsoni captures took place at the Atlántico department, Colombia.

Location	Municipality	Coordinates	Elevation (meters over sea level)	Human intervention status
Zona Franca Celsia	Barranquilla	11° 1′38.89"N 74°48′48.72"W	20	Intermediate. Peri-urban area with industrial activities nearby.
Carreto	Juan de Acosta	10°50′19.10"N 75° 7′5.66"W	20	Highly intervened with livestock and wood extraction.
Palomar	Piojó	10°47′37"N 75°09′31"W	200	Low intervention. Government protected areas without local communities.
Luriza	Usiacuri	10°45′10,84"N 75°01′12,64"W	100–200	Low intervention. Government protected areas with local communities.

2. Materials and methods

2.1. Study area

The study area included four sites of tropical dry forest ecosystem (Table 1): a forest fragment in the suburbs of Barranquilla near a residential and industrialized area (Zona Franca Celsia); a rural zone far from the city with livestock grazing and logging activity (Carreto); a protected area without human occupation (Palomar); and a protected area with human occupation (Luriza).

2.2. Trapping

At each of the four study sites, 10 trapping stations were distributed randomly on a grid with two Sherman traps (23 × 7.5 × 9 cm) deployed at each site, one at ground level and another attached to a tree at 1.8 m above ground level. Each trap was baited with oatmeal, peanut butter and vanilla extract. All traps were set in the evening and checked the next day in the early morning for five consecutive days, during three study periods between December 2016 and April 2017.

2.3. Anesthesia

Animals captured in the area close to the city (Zona Franca Celsia) passed through a medical evaluation while under anesthesia. Due to budget and logistical reasons, the animals captured at the other three locations were not anesthetized. After weighing, the individuals received anesthesia under physical restraint by intramuscular injections of 20 mg/kg of ketamine hydrochloride (HCl) and 3 mg/kg of xylazine hydrochloride with an additional 10 mg/kg of ketamine HCl when necessary. Anesthesia monitoring during the procedures included temperature, heart, and respiratory rates. Each animal also received Yohimbine HCL (0.125 mg/kg, IM) before release at the same spot where captured, after ensuring full recovery from anesthesia (Holz, 2014).

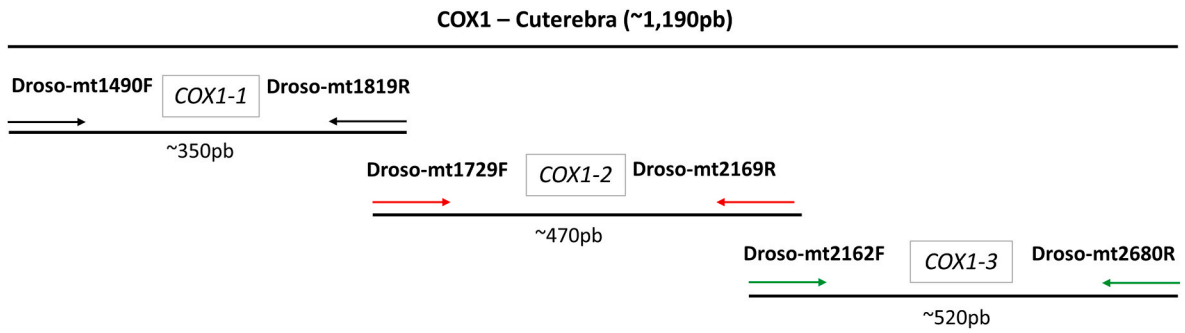

Fig. 2. Primers used to amplify and sequence the mitochondrial cytochrome Oxidase subunit I gene (COX1) of Cuterebra sp. List of primers: Droso-mt1490 5′-TTTCWACWAATCATAAAGATATYGG-3′, Droso-mt1729 5′-GGAGCYCCTGAYATRGCATTYCC-3′, Droso-mt1819 5′-GTRCCAGCYCCRTTTTCTAC-3′, Droso-mt2162 5′-CAACATTTATTYTGATTYTTTGG-3′, Droso-mt2169 5′-TAAACTTCAGGRTGWCCAAARAATCA-3′ y Droso-mt2680 5′-GYTAATCCWGTAAATAAWGG-3′.

2.4. Clinical exam and sample collecting

The procedure for all animals included biometry, weighing, sampling parasites, and sampling feces. Considering the transport distance to the laboratory, feces from Carreto, Palomar, and Luriza were stored in plastic containers with alcohol 70%. The animals were classified as adult or juvenile by weight (O'Connell, 1983).

The evaluation of animals captured at Zona Franca Celsia included blood samples and a clinical examination to search for abnormalities indicating health disorders such as parasites, skin lesions, swellings and secretion by natural cavities. A few drops of blood were collected from the lateral coccygeal vein, since this species is too small (ranging from 25 to 90 g of body weight) to perform a complete blood panel on. Parasite samples were collected in plastic containers and stored in 70% ethanol; a drop of blood was used for a blood smear, and feces were kept in plastic containers without a preservation medium.

2.5. Laboratory analysis

The Barranquilla Zoo Clinical Laboratory processed parasites, blood, and fecal samples. The fecal analysis included a direct coprological exam and a flotation technique using Sheather's solution (Bloor et al., 2015). Samples were evaluated with an optical microscope (Optika, B-382PL model) with 100x and 400x magnification. Processing of blood smears involved Wright's stain and evaluation using the same microscope (1000x magnification) to evaluate differential white cell count, cell morphology, hemoparasite search, and platelet count. The macroscopic identification of the parasites was carried out with a stereo microscope (Cambridge Instruments, Z30L model) and with published identification keys (Bowman, 2014; Colwell, 2001; Pape, 2001; Taylor et al., 2016).

2.6. DNA extraction and molecular barcode

Total DNA from three larvae of *Cuterebra* sp. was extracted using the Genomic DNA Purification Kit (Monarch®, Ipswich, CA, United Kingdom, Catalog no. NEB #T3010), following the protocol suggested by the manufacturer. Primers were designed against conserved regions of the mitochondrial Cytochrome Oxidase subunit I (COX1) gene in Diptera, using reference sequences publicly available through the Genbank. Initially, the primer pairs Droso-mt1490/Droso-mt2169 and Droso-mt2162/Droso-mt2680 were used to amplify two complementary sections of the COX1. However, the first pair of primers amplified a nuclear copy (numt) 242 bp shorter than the expected mitochondrial product. To avoid this problem, the region of interest was amplified in three complementary fragments following primer combinations: Droso-mt1490/Droso-mt1819, Droso-mt1729/Droso-mt2169 and Droso-mt2162/Droso-mt2680 amplified fragments approximately 350bp, 470bp and 520bp, respectively (Fig. 2).

PCR products were amplified in a final volume of 15 µl containing 1X KCl buffer, 1.5–2.5 mM $MgCl_2$, 0.2 mM dNTPs, 0.2 µM of each primer, 0.5 U Taq polymerase (Fermentas, Waltham, MA, USA), and 1 µL of genomic DNA. The reactions were carried out at an initial temperature of 94 °C - 5 min, followed by 35 amplification cycles of 94 °C - 30 s, 45 s of annealing temperature (52.4 °C, 56.4 °C, and 49.8 °C, for fragments 1, 2 and 3, respectively) and 72 °C–45 s, and a final step at 72 °C–7 min. The amplification of fragment 1 generated two amplicons (500 bp and 350 bp) and therefore the product of expected size (350 bp) was extracted from the agarose gel and purified using the DNA Gel Extraction Kit (Monarch®, Ipswich, MS, USA), following the protocol suggested by the manufacturer. This PCR product was re-amplified under the same initial conditions. PCR products were purified with a mix of 0.5 U Exonuclease I, and 0.25 U Alkaline Phosphatase (New England Biolabs) in a final volume of 30 µL incubated at 37 °C for 30 min. Both chains of each amplicon were sequenced by the Sanger method through a commercial service. Sequences were edited with Geneious v6.1.5 (Biomatters Ltd.) and aligned and concatenated using Mega11 (Kumar et al., 1993).

Species identification was made with the Identification Engine tool available in Barcode of Life Data Systems (BOLD), using the "Species Level Barcode Records" option (Ratnasingham and Hebert, 2007). For the species assignment, a similarity threshold of 99% was used, and the relationships with reference sequences were assessed through a Neighbor-Joining (NJ) tree of K2P distances.

2.7. Ethics statement

Three different permits of government authorities validated research activities through the following documents:

- Resolution 1498 of September 29th, 2016, from the DAMAB (Departamento Técnico Administrativo del Medio Ambiente de Barranquilla), the municipal governing authority.
- Resolution 0753 of 2016 from the CRA (Corporación Autónoma Regional del Atlántico), the state government authority.
- Resolution 0183 of March 13th, 2017, from the CRA.

2.6. Funding

This study was financed by the company Celsia S.A. E.S.P, part of the Argos Group. The sponsor did not participate in decisions of study design, analysis, or interpretation of the data.

3. Results and discussion

Traps in all four areas resulted in 60 *M. robinsoni* captured, 43 males and 17 females. Most captures occurred at the Zona Franca Celsia (60%, 36/60), followed by Carreto (22%, 13/60) and Luriza (18%, 11/60). No captures of *M. robinsoni* occurred at the Palomar location. All animals

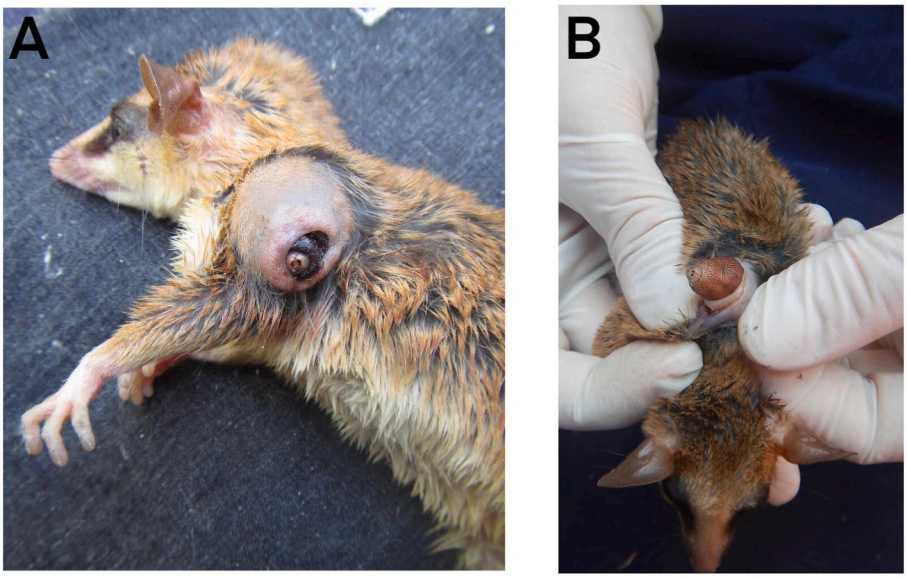

Fig. 3. Free range M. robinsoni with interscapular wounds. A. External appearance of bot fly larva wound. B. Extraction of Cuterebra sp. larvae.

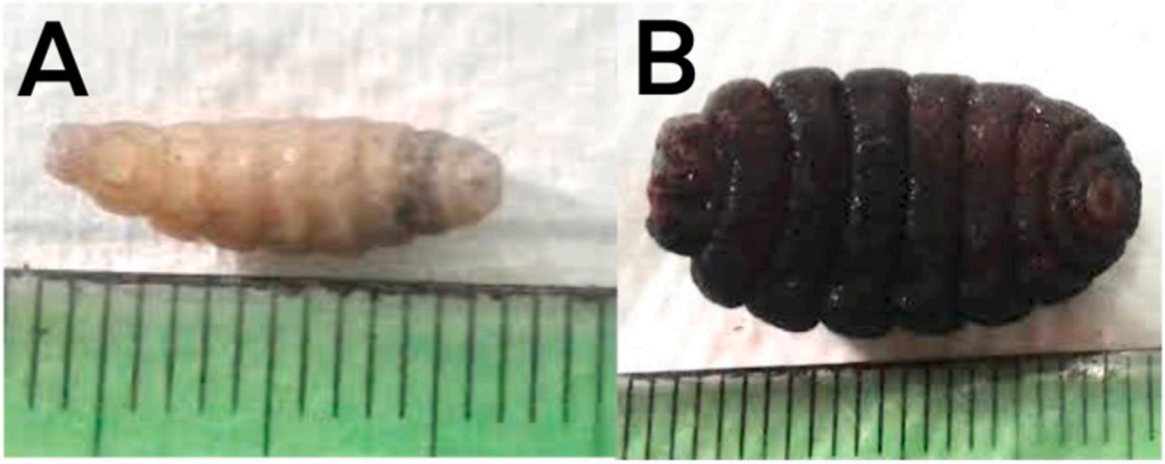

Fig. 4. Development stages of Cuterebra sp. found in different hosts of M. robinsoni. A. Second instar. B. Third instar.

were captured in the traps attached to trees.

In total, 8% (5/60) of all captured animals had fly larvae (Fig. 3A and B) under their skin identified as cuterebrid bot fly *Cuterebra* sp. (Fig. 4). Only animals captured in Zona Franca Celsia had bot fly larvae (14%, 5/36). *Cuterebra* larvae have strong dark spines all over their body (Fig. 5) (Bowman, 2014; Colwell, 2001; Taylor et al., 2016). The posterior spiracular plates are divided into three subunits and have the appearance tortuous and intertwining slit (Pape, 2001).

Additionally, the clinical examination showed areas with interscapular lesions in 28% (10/36) of the marsupials captured at Zona Franca Celsia. None of those lesions had fly larvae. Among those *M. robinsoni* found with interscapular lesions, 14% (5/36) had fresh wounds (two of them with purulent drainage) and 14% (5/36) had fibrosis in the region (scars) as observed in old lesions. The size and location of the lesions closely resemble interscapular wounds where *Cuterebra* affected other animals described in this study, possibly pointing out a higher prevalence. None of the 24 specimens captured at Palomar, Carreto, and Luriza had interscapular wounds.

Larvae of the fly genus Cuterebra have been reported in several species including dogs (Canis familiaris), cats (Felis catus), lions (Panthera leo), rabbits (Oryctolagus cuniculus) and rats (Rattus norvergicus) (cite literature). Although there is one report of infection in M. robinsoni in Brazil (citation), this is the first known report of Cuterebra in this host species documented in Colombia.

Larvae of the fly genus *Cuterebra* have been reported in several species including humans (Baird et al., 1989), rabbits (*Oryctolagus cuniculus*), cats (*Felis catus*) (Slansky, 2007), dogs (*Canis familiaris*) (Park et al., 2021) and rats (*Rattus norvergicus*) (Slansky, 2007). Although there is one report of infection in *M. robinsoni* in Brazil (Guimarães and Papavero, 1999), this is the first known register in Colombia of this parasite in this host species. Despite all five infected animals having large superficial wounds in the scapular region with large parasites (1.3–2.2 cm) relative to the size of the hosts (weighing 35–80 g), no significant disturbances were identified in body score or health condition.

The data found is compatible with literature reporting that these parasites have little effect on the fitness or population dynamics of their typical hosts (in general, at average intensities of one to three larvae per animal), despite high prevalence considering peak values commonly range from 30% to 70% (Slansky, 2007). Species of bot flies (Oestridae) typically have low pathogenicity and are host-specific, in contrast with parasitic species from other families such as Calliphoridae and

Fig. 5. Second instar of *Cuterebra* sp. from *M. robinsoni*. Note the body spines appear evenly distributed in the larva's body.

Sarcophagidae (Stevens et al., 2006).

An extent of 1,190bp of the COX1 gene was amplified and sequenced in three samples (GenBank accession numbers OQ301542, OQ301543 and OQ301544). They showed similarities between 94.8% and 94.9% and conformed to a monophyletic cluster with JQ246700 (*Cuterebra* sp.) in the NJ tree, nested within a cluster containing all the available sequences of *Cuterebra* in the database (Supplementary material). In these cases, "no match" was obtained in BOLD and taxonomic assignment could not be made to the species level.

Marmosa robinsoni is likely a typical host of *Cuterebra.* The adult form of the parasite oviposits around the entrance of burrows (Baird, 1997) and this species of opossum occupies burrows or builds nests in trees (O'Connell, 1983). All five animals infected were males and two of five were juveniles. That is also in line with the reports that infection levels of male hosts are higher than the levels of females, and young subadults have higher infection levels than other age groups (Catts, 1982).

Bot flies were absent in all 24 specimens captured at Palomar, Carreto, and Luriza. Zona Franca Celsia was the only study area close to a city and had several spots with trash and sightings of free domestic dogs. Species of the genus *Cuterebra* have been recorded from rodents, rabbits, and small marsupials, with cats and dogs being accidental hosts (Guimarães and Papavero, 1999). The other areas had different conservation status, but no urban area close by. This raises the hypothesis that the proximity to the city could increase or affect the prevalence of cuterebriasis.

4. Conclusions

The findings in this study point to *M. robinsoni* being a typical host for *Cuterebra* sp., considering different developing stages found and low pathogenicity. The samples in this study from *Cuterebra* infecting *M. robinsoni* did not match with sequences found in public databases. Further research is required to determine the precise species and to understand if the proximity to the city could affect cuterebriasis prevalence, as seen in this study.

Declaration of competing interest

None.

Acknowledgments

We thank the company CELSIA S.A. E.S.P for financing and supporting this research. We are grateful to Alise Rector for a valuable review and comments on English writing.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijppaw.2023.02.006.

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