Infection, Genetics and Evolution 22 (2014) 250–256

Contents lists available at SciVerse ScienceDirect

Infection, Genetics and Evolution

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

Trypanosome species in neo-tropical bats: Biological, evolutionary and epidemiological implications

Juan David Ramírez [a], Gabriela Tapia-Calle [a], Geissler Muñoz-Cruz [b], Cristina Poveda [a], Lina M. Rendón [a], Eduwin Hincapié [b], Felipe Guhl [a,*]

[a] Centro de Investigaciones en Microbiología y Parasitología Tropical, CIMPAT, Universidad de los Andes, Bogotá, Colombia
[b] Grupo de Investigaciones entomológicas de la Orinoquía Colombiana, GIENOC, Unitropico, Yopal, Colombia

ARTICLE INFO

Article history:
Available online 2 July 2013

Keywords:
Barcoding
Chiroptera
Ecology
Evolution
Trypanosomes

ABSTRACT

Bats (Chiroptera) are the only mammals naturally able to fly. Due to this characteristic they play a relevant ecological role in the niches they inhabit. These mammals spread infectious diseases from enzootic to domestic foci. Rabbies, SARS, fungi, ebola and trypanosomes are the most common pathogens these animals may host. We conducted intensive sampling of bats from the phyllostomidae, vespertilionidae and emballonuridae families in six localities from Casanare department in eastern Colombia. Blood-EDTA samples were obtained and subsequently submitted to analyses of mitochondrial and nuclear genetic markers in order to conduct barcoding analyses to discriminate trypanosome species. The findings according to the congruence of the three molecular markers suggest the occurrence of *Trypanosoma cruzi cruzi* (51%), *T. c. marinkellei* (9%), *T. dionisii* (13%), *T. rangeli* (21%), *T. evansi* (4%) and *T. theileri* (2%) among 107 positive bat specimens. Regarding the *T. cruzi* DTUs, we observed the presence of TcI (60%), TcII (15%), TcIII (7%), TcIV (7%) and TcBAT (11%) being the first evidence to our concern of the foreseen genotype TcBAT in Colombia. These results allowed us to propose reliable hypotheses regarding the ecology and biology of the bats circulating in the area including the enigmatic question whether TcBAT should be considered a novel DTU. The epidemiological and evolutionary implications of these findings are herein discussed.

1. Introduction

Bats are considered the only mammals naturally capable of maintaining a stable flight. These organisms correspond to approximately 20% of known mammal species classified to date and divided into two suborders (*megachiroptera* and *microchiroptera*) (Teeling et al., 2005). Most bats are considered insectivores, the rest are frugivores, fish-eaters and blood eaters (vampire bats). They play a relevant role in flower pollination and fruit seed dispersal (Hodgkison et al., 2003). Bats are important components of neo-tropical communities as they occupy a large variety of trophic niches and are often the most species-rich and abundant mammals in the area (Dietz and Kalko, 2006; Giannini and Kalko, 2004). Neo-tropical bats such as *Carollia*, *Myotis*, *Artibeus* and *Desmodus* play a relevant role in neo-tropical ecosystems as they disperse seeds, pollinate flowers and control insect populations (Bernard, 2001; Kunz et al., 2007). The ecology of these mammals highlight their relevance as reservoirs of infectious diseases and zoonotic pathogens due to their high ability of mobility, broad distribution and social behaviour (communal roosting and fission–fusion social structure). Among these pathogens emerge rabies, severe acute respiratory syndrome (SARS), henipavirus, possibly ebola and the trypanosomes (Guyatt et al., 2003; Li et al., 2005; Halpin et al., 2000; Hamilton et al., 2012a,b).

Trypanosomes (genus *Trypanosoma*) are usually transmitted by arthropods or leech vectors. Among the *Trypanosoma* genus exists four clear clades depending on the host that the parasite infects (aquatic clade, mammalian clade, terrestrial clade and avian clade); within the mammalian clade emerges the *Trypanosoma cruzi* clade containing *T. c. cruzi*, *T. c. marinkellei*, *T. dionisii*, *T. conorhinii*, *T. rangeli* and other mammalian trypanosomes. This clade is of extreme interest in terms of public health; Chagas disease caused by *T. c. cruzi* represents an important human pathology in the Americas and the heterogeneity displayed by this taxon is exhibited in at least six discrete typing units (DTUs) widely distributed in humans, insect vectors and reservoirs named from *T. c. cruzi* I to *T. c. cruzi* VI (TcI–TcVI) with the emergence of one new genotype strictly associated to bat species (*Myotis* and *Noctilio*) in Brazil and Panama (Zingales et al., 2009, 2012; Marcili et al., 2009a; Pinto et al., 2012). This might suggest that host-fitting is the main mechanism of trypanosomes evolution (Hamilton et al., 2012b). Nevertheless,

---

* Corresponding author.
E-mail address: fguhl@uniandes.edu.co (F. Guhl).

1567-1348/$ - see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.meegid.2013.06.022

many authors have suggested that the *T. cruzi* clade is the sister clade of a recently discovered bat-trypanosome species from Africa named *T. erneyi* supporting what is now named the bat-seeding hypothesis in the diversification of *T. cruzi* clade (Lima et al., 2012; Hamilton et al., 2012b).

Different studies have attempted to elucidate the prevalence of Trypanosomes in bats observing the occurrence of 3–9% in surveys conducted in South America (Marinkelle, 1976; Dias et al., 1942; Deane, 1961). In neo-tropical bats the most common trypanosome species detected are *T. cruzi cruzi*, *T. c. marinkellei*, *T. dionisii, T. rangeli* and *T. conorhinii* (Baker et al., 1978; García et al., 2012; Marcili et al., 2009a,b). In Colombia exists one single survey where *T. cruzi*-like infections were detected in a considerable number of bat specimens (Marinkelle, 1976). Despite this effort, no conclusive results associating the biology, ecology and evolution of trypanosomes and bats have emerged. In this context, we undertook intensive sampling of neo-tropical bats from an endemic area of Chagas disease in Colombia with a high rate of *T. cruzi* transmission in order to unravel the distribution of bat trypanosomes in the area with the aim of obtaining reliable information about the transmission dynamics of these protozoans with special focus on elucidating the relevance of this subpatent infections in the biology, evolution and ecology of neo-tropical bats.

2. Materials and methods

2.1. Study area and specimen identification

Bat specimens were captured in six localities across the Casanare department in Eastern Colombia (Fig. 1). A total of 175 specimens were captured in Nunchia (3 bats), San Luis de Palenque (45 bats), Yopal (13 bats), Agua Azul (22 bats), Maní (21 bats) and Tamara (71 bats) using nets and procedures permitted by the Ministerio del Medio Ambiente in Colombia obtaining appropiate geographical coordinates of capture (Table S1). Blood samples (200 μL) were collected in EDTA and transferred to Guanidine-EDTA buffer for complete lysis of the blood and preservation of DNA fragments. Bats were identified to species level using DNA barcoding previously developed for neo-tropical bats (Clare et al., 2011).

2.2. Molecular identification of trypanosomes species

The blood samples in guanidine buffer (GEB) were submitted for DNA extraction using the Qiamp miniprep kit following the manufactureŕs instructions (Qiagen, Barcelona, Spain). The DNA aliquots were submitted to amplification based on a barcoding approach using three genomic regions; we employed the partial region of cytochrome b gene (cytb), the region V7 of the SSU rDNA 18S gene (SSU rDNA) fragment and gGAPDH as previously established for the discrimination of Trypanosomes species (Marcili et al., 2009a; García et al., 2012). The amplification was accomplished in a final volume of 20 μL using 1× Buffer (Corpogen, COL), 5 mM $MgCl_2$, 1 μM of primers, 10 μM of dNTPs, 0.5U of Taq Tucan (Corpogen, COL) and 20 ng of DNA. The mix was submitted to 29 cycles of amplification, the amplicons were visualised in 2% agarose gels stained with red gel. The PCR products were cleaned up by isopropanol precipitation and sequenced by the dideoxy-terminal method in an automated capillary sequencer (AB3730, Applied Biosystems, UK). The resulting sequences were edited in MEGA 5.0 (Tamura et al., 2011) and aligned using ClustalW 1.8 (Thompson et al., 1994). All edited sequences were deposited in GenBank and assigned accession numbers (KC951574–KC951627). For the identification of Trypanosomes species, the sequences obtained were compared with reference sequences from *T. c. cruzi* (from the six DTUs and the TcBAT genotype), *T. c. marinkellei, T. dionisii, T. conorhinii, T. evansi, T. theileri* and *T. rangeli* retrieved from GenBank.

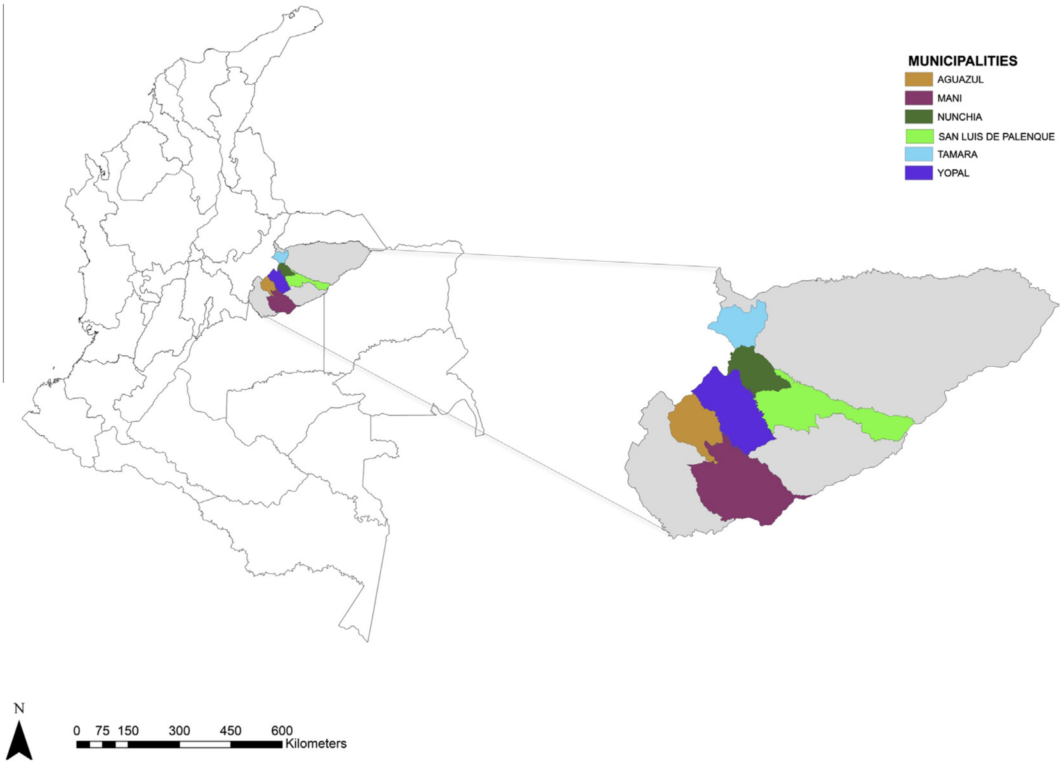

Fig. 1. Geographical distribution of the six provinces where bat species were sampled in Eastern Colombia (Nunchía, Yopal, Maní, San Luis de Palenque, Tamará and Agua Azul).