

“Mi Casa, Tu Casa”: the coati nest as a hub of *Trypanosoma cruzi* transmission in the southern Pantanal

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The study of the ecology of *Trypanosoma cruzi* is challenging due to its extreme adaptive plasticity, resulting in the parasitism of hundreds of mammal species and dozens of triatomine species. The genetic analysis of blood meal sources (BMS) from the triatomine vector is an accurate and practical approach for gathering information on which wild mammal species participate in a local transmission network. South American coatis, *Nasua nasua*, act as important reservoir host species of *T. cruzi* in the Pantanal biome because of their high rate of infection and elevated parasitemia, with the main discrete typing unit (DTU) lineages (TcI and TcII). Moreover, the carnivore coati is the only mammal species to build high arboreal nests for breeding and resting that can be shared by various vertebrate and invertebrate species. Herein, we applied the sensitive and specific methodology of DNA barcoding and molecular cloning to study triatomines found in a coati nest to access the diversity of mammal species that explore this structure, and therefore, may be involved in the parasite transmission network. Twenty-three *Triatoma sordida* were collected in one coati's nest in the subregion of Nhecolândia, Pantanal. The DNA isolated from the gut of insects was subjected to BMS detection by PCR using *cytb* and 12S rDNA targets from vertebrates. The *Trypanosoma* spp. diagnosis and DTU genotyping were based on 18S rDNA and *cytb* molecular markers. Phylogenetic analyses and chord diagrams were constructed to visualize BMS haplotypes, DTU lineages detected on vectors, and their interconnections. Twenty of 23 triatomines analyzed were PCR-positive (86.95%) showing lineages *T. cruzi* DTU TcI ($n = 2$), TcII ($n = 6$), and a predominance of TcI/TcII ($n = 12$) mixed infection. Intra-DTU diversity was observed mainly from different TcI haplotypes. Genetic analyses revealed that the southern anteater, *Tamandua tetradactyla*, was the unique species detected as the BMS of triatomines collected from the coati's nest. At least three different individuals of *T. tetradactyla* served as BMS of 21/23 bugs studied, as indicated by the *cytb* and 12S rDNA haplotypes identified. The identification of multiple BMS, and importantly, different individuals of the same species, was achieved by the methodology applied. The study demonstrated that the southern anteaters can occupy the South American coati's nest, serving as the BMS of *T. sordida* specimens. The TcI and TcII infection, and significantly, a predominance of TcI/TcII mixed infection profile with different TcI and TcII haplotypes were observed, due to the discriminatory capacity of the methodology applied. *Tamandua tetradactyla*, a host which has been little studied, may have an important role in the *T. cruzi* transmission in that Pantanal subregion. The data from the present study indicate the sharing of coatis' nests by other mammal species, expanding the possibilities for parasite transmission in the canopy strata. We propose that coatis' nests can act as the true hubs of the *T. cruzi* transmission web in Pantanal, instead of the coatis themselves, as previously suggested.