Veterinary Parasitology: Regional Studies and Reports 61 (2025) 101272

Contents lists available at ScienceDirect

Veterinary Parasitology: Regional Studies and Reports

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

Short Communication

Two *Cytauxzoon* species in Brazil? Ocelots (*Leopardus pardalis*) and pumas (*Puma concolor*) as reservoir for *Cytauxzoon* spp.: Molecular characterization in three Brazilian biomes

Joares A. May Jr [a,b,c,d], Vinícius Baggio-Souza [a], Laura Berger [a], Rafaela Mallmann-Bohn [a], Adeyldes Oliveira Reis [a], Aline Girotto-Soares [a], Raíssa Sepulvida [b], Jorge Salomão Junior [d], Leonardo Sartorello [e], Fabio Souza da Silva [e], Hugo Borghezan Mozerle [f], Marcos Adriano Tortato [f], Adriano Rodrigues Lagos [g], Felipe Viana Manzano [g], Fernando Vieira Machado [g], Jorge José Cherem [h], Renata Fagundes-Moreira [i], João F. Soares [a,*]

[a] *Laboratório de Protozoologia e Rickettsioses Vetoriais, Universidade Federal do Rio Grande do Sul, RS, Brazil*
[b] *Panthera Brasil, Poconé, MT, Brazil*
[c] *Centro Universitário Univinte, Capivari de Baixo, SC, Brazil*
[d] *Ampara Silvestres, Poconé, MT, Brazil*
[e] *Associação Onçafari, São Paulo, SP, Brazil*
[f] *Tapir Consultoria Ambiental, Gravatal, SC, Brazil*
[g] *Eletrobras, Rio de Janeiro, RJ, Brazil*
[h] *Instituto Tabuleiro, Trindade, Florianopolis, SC, Brazil*
[i] *University of Bari Aldo Moro, Bari, Italy*

ARTICLE INFO

Keywords:
Wild cats
Piroplasmids
Tick-borne agents

ABSTRACT

Cytauxzoonosis. is tick-borne disease that can cause subclinical to fatal manifestations in felids worldwide. Our study aimed to molecularly characterize *Cytauxzoon* spp. in two felid species in three different Brazilian biomes. Blood samples were collected from 27 ocelots and 10 pumas during health monitoring in the Pantanal, Cerrado, and Amazon biomes. Conventional PCR and sequencing of the 18S rRNA and *CytB* genes were performed, followed by a Bayesian phylogenetic analysis. Of the 37 samples, 73.52 % of ocelots and 60 % of pumas tested positive for *Cytauxzoon* spp. Phylogenetic analysis confirmed the presence of Cytauxzoon brasiliensis and a strain geentically close to Cytauxzoon felis in pumas and ocelots. Different tick species, such as *Amblyomma sculptum* were found on infected animals, suggesting a potential vector role for the species. Results support that ocelots and pumas are possible natural reservoirs for *Cytauxzoon* spp. in different Brazilian biomes. Even so, we highlight that the ecological implications of habitat loss and anthropogenic pressures, may exacerbate the *Cytauxzoon* pathogenicity for wild felids. Further studies are needed to elucidate the epidemiology and impact of *Cytauxzoon* spp. on wildlife health.

1. Introduction

Cytauxzoon spp. are a tick-borne parasite classified as a small piroplasmid from the order Piroplasmida and Theileriidae family. In North America, these hemoparasites are reported to cause an acute disease in domestic cats, however, in wild felines it has a chronic manifestation, which can be fatal (André et al., 2015). Natural, subclinical, or asymptomatic infections can also occur (Meinkoth et al., 2000; Lewis et al., 2012). Apart from a few clinical reports, wild felids are generally reservoirs to some species: *Cytauxzoon manul* in Asia (Ketz-Riley et al., 2003), *Cytauxzoon felis* in America (US), also the three recently described in Europe, mainly through phylogenetic analysis of the *CytB* gene, *Cytauxzoon europaeus*, *Cytauxzoon otrantorum,* and *Cytauxzoon banethi* (Panait et al., *2021*), and most recently, *Cytauxzoon brasiliensis* in

* Corresponding author at: Laboratory of Protozoology and Vector-Borne Rickettsiosis, Federal University of Rio Grande do Sul, Avenida Bento Gonçalves 9090, Porto Alegre 91540-000, Brazil.
E-mail address: joao.soares@ufrgs.br (J.F. Soares).

https://doi.org/10.1016/j.vprsr.2025.101272
Received 19 December 2024; Received in revised form 28 April 2025; Accepted 29 April 2025
Available online 30 April 2025

Brazil (Duarte et al., 2024).

Previously, as only fragments of the 18S rRNA were used for *Cytauxzoon* diagnosis, only *C. felis* was reported as the occurring species in Brazil. Afterwards, as larger fragments were required, studies started to report as *Cytauxzoon* spp., as differences were observed in phylogenetic analysis. For example, captive felids from São Paulo and Distrito Federal (André et al., 2009), and free ranging jaguars, pumas and ocelots were positive to *Cytauxzoon* sp. (Furtado et al., 2017; Moreira et al., 2022). Only recently, *C. brasiliensis* description brought to discussion that earlier studies and reports only used a short and partial region of a conserved gene, 18S rRNA, that in BLAST had high query coverage and similarity to *C. felis (*Duarte et al., *2024)*.

The puma (*Puma concolor*) and the ocelot (*Leopardus pardalis*) are native wild felids present in every Brazilian biome. Both species constantly suffer great risks towards its extinction, such as habitat loss due to deforestation and urbanization, poaching, and agriculture (Nielsen et al., 2015; Paviolo et al., 2015). The habitat loss pose a risk to public safety, as it elevates the chance of contact between humans and domestic animals with wild species, which can facilitate the spillover of agents that these felids serve as reservoirs, but could be pathogenic to domestic animals and humans.

This study molecularly characterizes through 18S rRNA along with *CytB* genes, the presence of *C. brasiliensis* and *C. felis* in free-ranging pumas and ocelots from different Brazilian biomes, furthermore, confirms its roles as reservoirs for this hemoparasites.

2. Materials and methods

2.1. Ethical and regulatory approvals

All animal capture, handling, and sedation procedures were approved by the "Instituto Brasileiro do Meio Ambiente e dos Recursos Naturais Renováveis" (IBAMA) under authorization numbers ABIO No. 92/2021 and SISBio 43980, 75397, 78476, 61844, and 75457. The subsequent reception, storage, and use of biological samples were reviewed and approved by the Ethics Committee on Animal Use of the Federal University of Rio Grande do Sul (CEUA/UFRGS) under protocol number 44246, issued on 23 May 2023.

2.2. Capture sites

The study areas cover three distinct Brazilian biomes: Cerrado, Amazon, and Pantanal, each with unique environmental characteristics which can influence feline capture. The captures occurred in the municipalities of Paracatu, Minas Gerais (MG) (Cerrado biome) (17°13′41.092"S 46°52′25.226"W), Poconé, Mato Grosso (MT) (Northern Pantanal biome) (16°15′50.58"S 56°37′55.556"W), Miranda, Mato Grosso do Sul (MS) (Southern Pantanal biome) (19°57′9.565"S 56°18′29.545"W), and Jacareacanga, Pará (PA) (Amazon biome) (9°03′11.2"S 56°35′08.5"W).

The Cerrado site, located in the northwest of Minas Gerais, comprises diverse vegetation types, including Cerrado sensu stricto, rupestrian grasslands at higher altitudes, and riparian forests along watercourses. Ocelots were captured on private land used for cattle ranching and agriculture, near a hydroelectric power plant and a state park—all areas close to a metropolitan region. In the Amazon, the study area lies within the deforestation arc in southern Pará, where logging and cattle ranching have significantly altered the landscape. However, conservation-focused areas, such as those near the Serra do Cachimbo, provide connectivity for large carnivores like jaguars and pumas, as well as smaller species such as ocelots and bush dogs. The Pantanal site was divided into two regions: the northern area (MT), part of the Jofre Velho Ranch managed by Panthera Brasil, where ocelots were captured, and the southern area (MS), part of Caiman Ranch managed by Onçafari, where pumas were captured. This region features a mosaic of forests, open savannas, and wetlands, where wild felid habitats overlap with cattle and buffalo farming, as well as domestic dogs and cats (camera trap monitoring, unpublished data).

2.3. Handling maneuvers and sample storage

Camera traps were used to monitor feline movement, and physical restraints were set up to capture individuals (Frank et al., 2003). Pumas were captured between 2019 and 2023, and ocelots between 2021 and 2024. Pumas were captured using foot snares, while ocelots were caught in box traps (Tomahawk-style). Once secured, the animals were sedated with an intramuscular injection of 5 mg/kg tiletamine–zolazepam and 0.04 mg/kg dexmedetomidine (Poole et al., 1993; Onuma et al., 2015)—administered via CO_2 rifle dart for pumas and by hand syringe for ocelots.

During the clinical examination, each animal was weighed at least three times using a scale (Cabelas, USA) to confirm its weight, and a licensed veterinarian assessed for hydration status (skin turgor), mucous membrane color, coat condition, capillary refill time, presence of lesions and ectoparasites, dentition, and reproductive traits (mammary glands, vulva, and scrotal sac). Throughout the procedure, the animals were monitored by a veterinarian with stethoscope (Littmann, USA), thermometer, direct counting of thoracic and abdominal movements for respiratory rate, a pulse oximeter (NT1-V, Newtech, Inc., China) to measure heart rate and oxygen saturation, a blood pressure monitor (More Fitness, China) attached to a limb, and a glucometer (Accu-Chek Active, Mannheim, Germany). A full-body examination was performed to identify and collect ectoparasites, which were preserved in ethanol tubes. Blood samples were obtained through venipuncture and stored in EDTA-treated and plain tubes at −20 °C until molecular analysis. Captured individuals were fitted with radio collars and microchipped for identification in case of recapture, enabling continuous monitoring alongside camera trap data. After completing the procedures (which took 40 min to one hour), ocelots were placed back into their box traps and released once sedation wore off. Pumas were returned to their original capture location and observed until they could walk unassisted.

2.4. Ectoparasites identification

Ectoparasites were sent to the Laboratório de Protozoologia e Rickettsioses Vetoriais from the Universidade Federal do Rio Grande do Sul – RS – Brazil, where the specimens were identified through stereomicroscopy and confirmed by dichotomous keys (Linardi, 2000; Barros-Battesti et al., 2006).

2.5. DNA extraction and PCR assays

DNA was extracted from 200 µl of whole blood using a DNA Extraction Kit (Invitrogen®), according to manufacturer's instructions. Samples underwent a conventional PCR (cPCR) targeting the *gapdh* (glyceraldehyde-3-phosphate dehydrogenase) gene, to confirm the presence of mammal DNA (Birkenheuer et al., 2003). After that, all samples were tested for a partial 18S rRNA for the presence of piroplasmid agents using the protocol suggested by Soares et al. (Soares et al., 2011), and positive samples were then submitted for a *Cytauxzoon* spp. specific primer (Panait et al., 2021), in order to amplify a partial region of the 18S rRNA gene. If positive for *Cytauxzoon* spp., one sample from each geographic region went for a final amplification for the *cytochrome b* gene to amplify a fragment of nearly 1300pb (Panait et al., 2021; Schreeg et al., 2013). Ultrapure MiliQ water was used a negative control, and DNA from a naturally infected jaguar (Moreira et al., 2022) was used as positive control. PCR results were checked through agarose gel at 1.5 % in a LED transilluminator.

Samples that amplified in the 18S rRNA and *CytB* genes were purified using the Invitrogen® Purification Kit and sequenced in both senses using Sanger method. Consensus sequences were made with BioEdit v3.1. Sequences were submitted to BLASTn Tool analysis to check for

sequence similarity, query coverage, and *E*-value.

2.6. Phylogenetic trees

A Bayesian phylogenetic tree was constructed for the 18S rRNA and *CytB* genes, with homolog sequences and samples from this study. Sequences were aligned with MEGA 11, using the ClustalW algorithm. The best evolutionary model was selected using the IQTree online server (http://iqtree.cibiv.univie.ac.at/). Two simultaneous runs with 1×10^7 iterations and a 25 % burn-in rate was performed to retrieve the consensus tree.

3. Results

Blood samples were collected from 27 individuals of *L. pardalis* from two different regions (16 in Paracatu-MG and 11 in Poconé-MT). Three individuals were recaptured in different campaigns in Paracatu; one of these was captured three times with a time span of three months between first and second capture, and six months between second and third capture. A total of 21 (73.52 %) blood samples from L. *pardalis*

Fig. 1. Bayesian phylogenetic tree from the 18S rRNA gene consisting of 60 homolog sequences from the Piroplasmida order, and 7 sequences from this study (in bold); Two simultaneous runs of 100,000,000 replicates and a 25 % burn-in rate were used to generate the best tree; Cardiosporidium cionae was used as outgroup.

tested positive in the cPCR. One of the recaptured animals was negative at first sampling, and positive three months later. The other recaptured animals were positive and maintained it in the posterior samples.

Regarding *P. concolor*, nine blood samples were collected from Miranda-MS and one from Jacareacanga-PA, considering two recaptures from different individuals. Six (60 %) blood samples were positive and the two recaptured animals were negative in both cPCR assays, with a time between capture of 4 and 7 months. All individuals from both species were clinically healthy.

Seven sequences were retrieved for the 18S rRNA gene. Sequences were aligned with 60 homolog sequences to generate a tree through Bayesian inference (Fig. 1). A sequence from *Cardiosporidium cionae* (EU052685) was used as outgroup. Five L. *pardalis* samples, three from MG and two from MT regions, clustered with *C. brasiliensis*, while two puma samples from MS region positioned closer to *C. felis*. Four samples were amplified for the *CytB* gene with nearly 1000 base pairs and aligned with 7 homolog sequences. A sample from *B. microti* (MT114077) was used as outgroup (Fig. 3). Two L. *pardalis* from the Pantanal biome (MT and MS regions) and one *P. concolor* from the Amazon biome (PA) were more similar to *C. brasiliensis* in the Blast analysis. One L. *pardalis* from the Pantanal biome (MG) had 100 % cover and 99 % similarity to *C. felis*. Fig. 2 shows the geographic distribution of the sampled animals, with collection sites detailed across different regions and biomes.

Also, ectoparasites were collected and identified from positive animals. One *L. pardalis* (Code 289) positive for a genetically similar *C. brasiliensis,* was coparasitized by *Amblyomma sculptum* and *Amblyomma ovale*. Two *P. concolor* (Codes 357 and 353), positive for genetically similar *C. brasiliensis* and *C. felis* respectively, presented to be simultaneously parasitized by *A. sculptum*, *A. ovale* and *Amblyomma triste*, as well as *Rhipicephalus microplus* (puma 357). The L. *pardalis* (Code 1117), positive for genetically similar *C. felis*, was only parasitized by *A. sculptum*.

Information for each individual is shown in Supplementary Table 1.

4. Discussion

This study confirms the presence of two *Cytauxzoon* species in Brazil, *C. brasiliensis* and one genetically close to *C. felis*. Moreira et al., (2022) suspected that jaguars and other wild felids could act as a natural reservoir for *Cytauxzoon* spp. in Brazil. The results of this study confirm this suspicion in three different biomes: the Pantanal (Two L. *pardalis* positive to *C. brasiliensis* and two *P. concolor* positive to *C. felis*), the Cerrado (One L. *pardalis* positive to *C. felis* and another positive to *C. brasiliensis*), and the Amazonic (One *P. concolor* positive to *C. brasiliensis*). These findings were further supported in the present study through phylogenetic analysis of the 18S rRNA and *CytB* genes. Only longer sequences of the 18S rRNA and *CytB* genes were able to phylogenetic separate these species. Our captures confirms an infection or reinfection for a period of eight months, which is normal for the Theileriidae family, as these can have a long period of infection (up to four years) (Barnett, 1968). No clinical signs related to Cytauxzoonosis were observed in any of the captured animals.

The *Cytauxzoon* spp. infection was reported in captive ocelots, pumas, and jaguars (André et al., 2009), with a prevalence of 20.7 % (6/29), 22.2 % (2/9), and 11.1 % (1/9), respectively. The low prevalence in

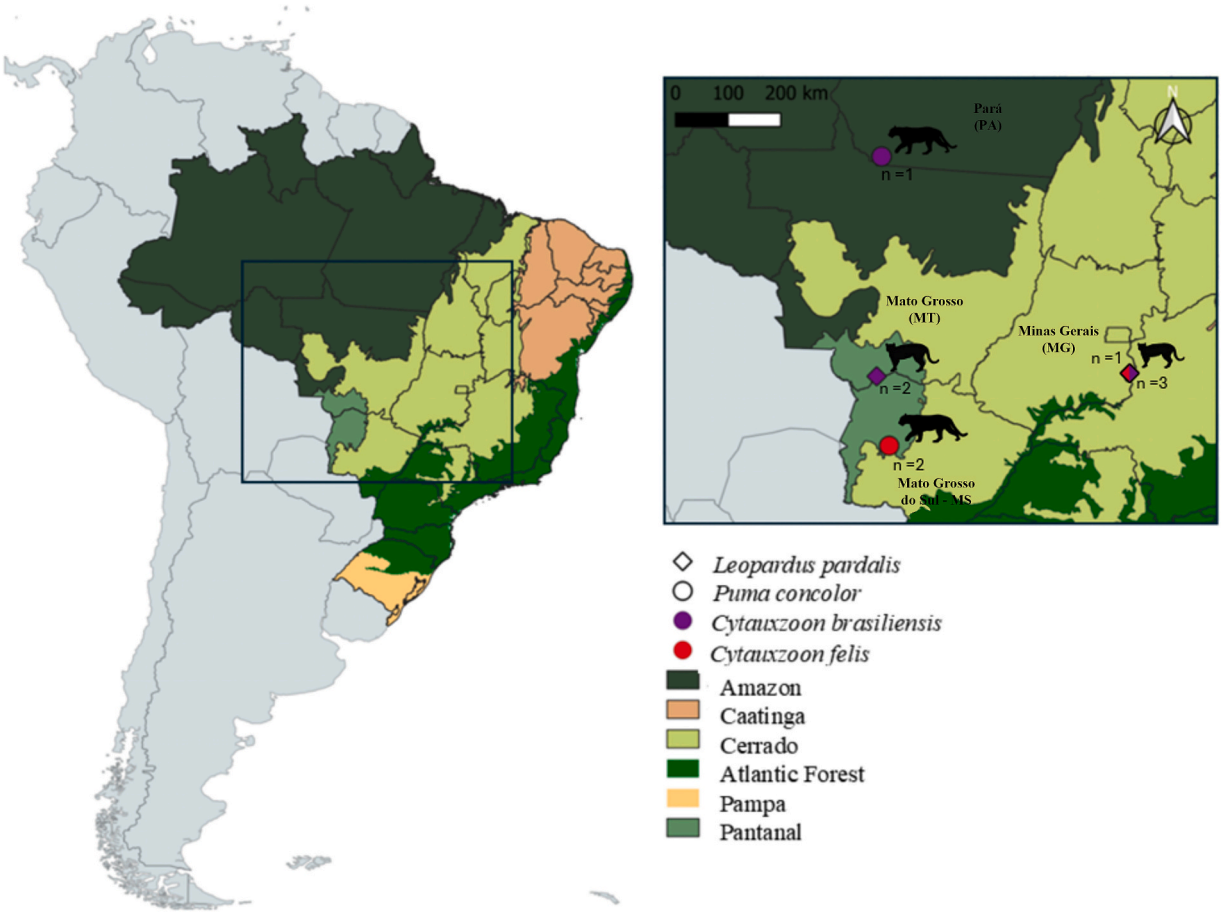

Fig. 2. Location of sequenced samples of *Cytauxzoon brasiliensis* in purple and *Cytauxzoon felis* in red, from pumas (*Puma concolor*) (circles) and ocelots (*Leopardus pardalis*) (diamond shape) in the three different biomes (Pantanal, Cerrado and Amazon). Numbers show the quantity of sequences obtained for *Cytauxzoon* species in each place. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

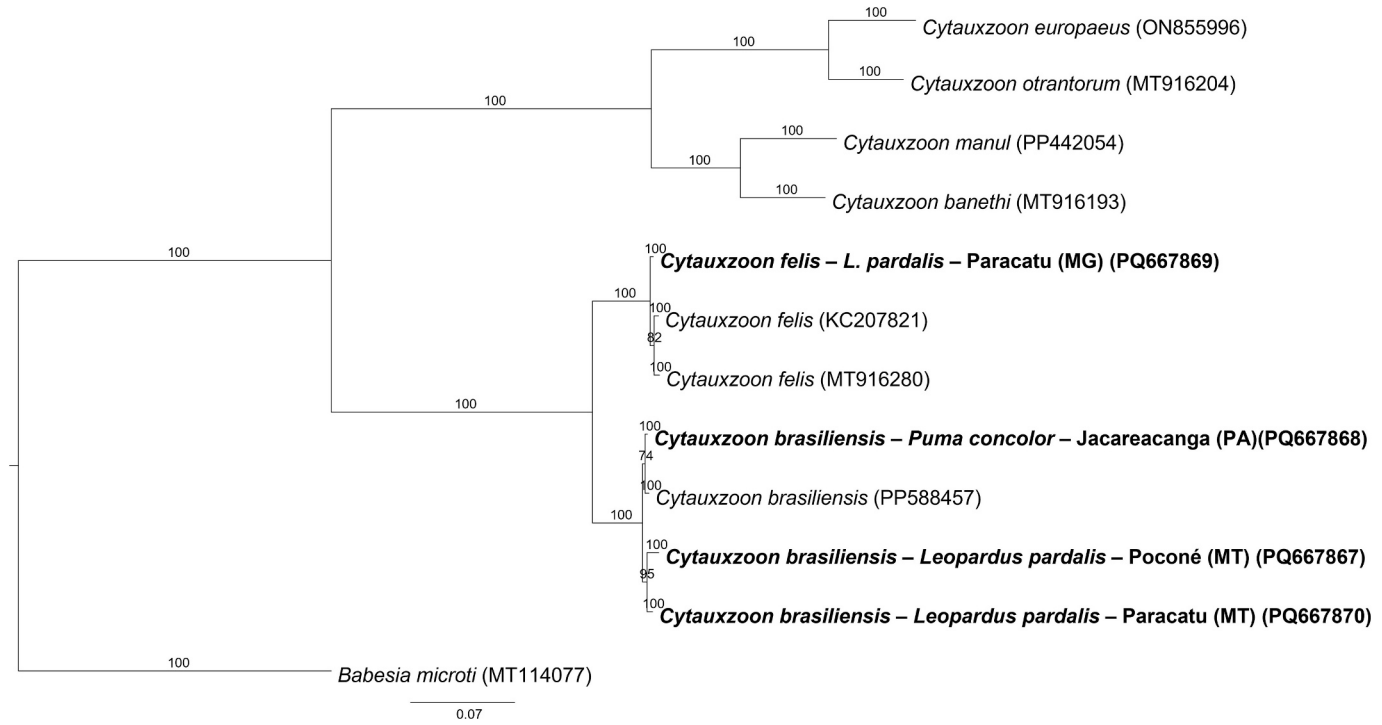

Fig. 3. Bayesian phylogenetic tree from the *CytB* gene consisting of 7 homolog sequences from the Piroplasmida order, and 4 sequences from this study (in bold); Two simultaneous runs of 100,000,000 replicates and a 25 % burn-in rate were used to generate the best tree. Babesia microti (MT114077) was used as outgroup.

captive animals may be due to the absence of the suspected vector (*A. sculptum*) (Moreira et al., 2022). A US study found a low prevalence in general (20 % 138/696), but it varied from state to state as, for example, the prevalence ranged from 79 % in Missouri, 65 % in Oklahoma, 63 % in North Carolina, 57 % in South Carolina, down to 9 % in Georgia, and 2 % in North Dakota (Shock et al., 2011). The prevalence for *C. brasiliensis* in Brazilian wild felids seems to differ from *C. felis* in the US, as almost 100 % of studied felids were positive in two studies (Furtado et al., 2017; Moreira et al., 2022). A *post-mortem* study also evaluated the presence of protozoan hemoparasites infection in wild felids and found only 25 % (2/8) of the pumas positive to *Cytauxzoon* sp. (De Barros Silva et al., 2021).

A. sculptum was already studied and suspected to be the main vector of *Cytauxzoon* sp. to jaguars in the MS region (Moreira et al., 2022). Two pumas from MS and two ocelots from MG were parasitized by *A. sculptum*, but one of each was infected by *C. felis* and *C. brasiliensis*. The aforementioned article already treated the sequences obtained as *Cytauxzoon* sp., as these weren't large enough and the authors observed dissimilarities. Our findings increases the number of *Cytauxzoon* positive felines from areas with occurrence of *A. sculptum*, and, apart from the hypothesis that it is probably the vector to *C. felis*, *A. sculptum* can also be the vector to *C. brasiliensis* to these felids, reinforcing the results from Fagundes-Moreira et al. (2022). Unfortunately, we were unable to perform any statistical analysis correlating the ectoparasites as vectors for these *Cytauxzoon* species, as only a few ectoparasites were collected and even fewer sequences were retrieved.

As suggested for jaguars (Furtado et al., 2017; Moreira et al., 2022), pumas and ocelots seems to be reservoirs to *Cytauxzoon* spp., as the prevalence in wild individuals are high. In a complete and healthy environment, these hemoparasites may not be pathogenic to wild felids. However, for constantly suffer from anthropic factors, such as deforestation, the stress caused by habitat loss associated with other parasites synergism, *Cytauxozoon* sp. infection can pose a risk to their safety[.

5. Conclusion

This study confirms the presence of two *Cytauxzoon* species in three different Brazilian regions, and portrays L. *pardalis* and *P. concolor* as reservoirs for both parasites. The presence of *C. felis* and *C. brasiliensis* in Brazil brings novel data on its reservoirs and ecology, although future studies seems necessary to better molecularly characterize and understand these species geographic range and clinical impact on wildlife.

Ethical statement for solid state ionics

Hereby, I Joares Adenilson May Jr consciously assure that for the manuscript "Two *Cytauxzoon* species in Brazil? Ocelots (*Leopardus pardalis*) and pumas (*Puma concolor*) as reservoir for *Cytauxzoon* spp.: Molecular characterization in three Brazilian biomes" the.

following is fulfilled:

1) This material is the authors' own original work, which has not been previously published elsewhere.
2) The paper is not currently being considered for publication elsewhere.
3) The paper reflects the authors' own research and analysis in a truthful and complete manner.
4) The paper properly credits the meaningful contributions of co-authors and co-researchers.
5) The results are appropriately placed in the context of prior and existing research.
6) All sources used are properly disclosed (correct citation). Literally copying of text must be indicated as such by using quotation marks and giving proper reference.
7) All authors have been personally and actively involved in substantial work leading to the paper, and will take public responsibility for its content.

The violation of the Ethical Statement rules may result in severe consequences.

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I agree with the above statements and declare that this submission follows the policies of Solid State Ionics as outlined in the Guide for Authors and in the Ethical Statement.

CRediT authorship contribution statement

Joares A. May: Writing – review & editing, Writing – original draft, Resources, Methodology, Investigation, Funding acquisition, Formal analysis, Conceptualization. **Vinícius Baggio-Souza:** Writing – review & editing, Writing – original draft, Methodology, Investigation. **Laura Berger:** Writing – review & editing, Methodology, Investigation. **Rafaela Mallmann-Bohn:** Writing – review & editing, Methodology. **Adeyldes Oliveira Reis:** Writing – review & editing, Methodology. **Aline Girotto-Soares:** Methodology. **Raíssa Sepulvida:** Methodology, Funding acquisition. **Jorge Salomão Junior:** Resources, Investigation. **Leonardo Sartorello:** Investigation. **Fabio Souza da Silva:** Investigation. **Hugo Borghezan Mozerle:** Investigation. **Marcos Adriano Tortato:** Resources, Investigation. **Adriano Rodrigues Lagos:** Resources, Investigation, Funding acquisition. **Felipe Viana Manzano:** Resources, Investigation, Funding acquisition. **Fernando Vieira Machado:** Resources, Investigation, Funding acquisition. **Jorge José Cherem:** Investigation. **Renata Fagundes-Moreira:** Writing – review & editing, Methodology, Investigation, Conceptualization. **João F. Soares:** Writing – review & editing, Writing – original draft, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

The authors would like to express their gratitude to the projects and organizations that contribute significantly to wildlife conservation and partially finance studies such as this one: the NGO Panthera, the Onçafari Association, and the Geoffroy's Cat Working Group (GCWG). This study was also partially financed by Eletrobras Furnas (IBAMA ABIO No. 92/2021). The author JFS was supported by CNPq (National Council for Scientific and Technological Development - Productivity Grant (#312576/2021–8) and Fundação de Amparo à Pesquisa do Rio Grande do Sul (FAPERGS) (finance codes: 19/2551–0001842–8; 24/2551–0001157-9). Coordination for the Improvement of Higher Education Personnel (CAPES) #001.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.vprsr.2025.101272.

References

André, M.R., Adania, C.H., Machado, R.Z., Allegrettl, S.M., Fellppe, P.A.N., Silva, K.F., Nakaghi, A.C.H., Dagnone, A.S., 2009. Molecular detection of cytauxzoon spp. in asymptomatic brazilian wild captive felids. J. Wildl. Dis. 45, 234–237. https://doi.org/10.7589/0090-3558-45.1.234.

André, M.R., Herrera, H.M., de Jesus Fernandes, S., de Sousa, K.C.M., Gonçalves, L.R., Domingos, I.H., de Macedo, G.C., Machado, R.Z., 2015. Tick-borne agents in domesticated and stray cats from the city of Campo Grande, state of Mato Grosso do Sul, midwestern Brazil, ticks tick. Borne. Dis. 6, 779–786. https://doi.org/10.1016/j.ttbdis.2015.07.004.

S. F. Barnett, Theileriasis, in: M.R.M.R. D. Weinman D. Weinman (Ed.), Infect. Blood Dis. Man Anim., New York & London: Academic Press, 1968: pp. 269–328.

Barros-Battesti, D., Arzua, M., Bechara, G., 2006. Carrapatos de importância médico-veterinária da região neotropical: um guia ilustrado para identificação de espécies, 1st ed. Butantan, São Paulo.

Birkenheuer, A.J., Levy, M.G., Breitschwerdt, E.B., 2003. Development and evaluation of a Seminested PCR for detection and differentiation of Babesia gibsoni (Asian genotype) and B. Canis DNA in canine blood samples. J. Clin. Microbiol. 41. https://doi.org/10.1128/jcm.41.9.4172-4177.2003.

De Barros Silva, V.L., De Almeida, S.L.H., Maia, M.O., Santos, T.Á., Pavelegini, L.A.D., Zaffalon, G.B., Marcili, A., Morgado, T.O., Dutra, V., Nakazato, L., De Campos Pacheco, R., 2021. Post mortem protozoan hemoparasites detection in wild mammals from Mato Grosso state, midwestern Brazil. Rev. Bras. Parasitol. Vet. 30, 1–9. https://doi.org/10.1590/S1984-29612021083.

Duarte, M.A., de Oliveira, C.M., Honorato, S.M., Batista, L.M. Rosario, Mendonça, J.T., de Sousa, D.E.R., Hirano, L.Q.L., André, M.R., Castro, M.B., Paludo, G.R., 2024. Cytauxzoon brasiliensis sp. nov. (Apicomplexa: Theileriidae), a new species infecting a little-spotted-cat (*Leopardus tigrinus*) (Carnivora: Felidae) from Brazil. Syst. Parasitol. 101. https://doi.org/10.1007/s11230-024-10178-9.

Furtado, M.M., Taniwaki, S.A., Metzger, B., dos Santos Paduan, K., O'Dwyer, H.L., de Almeida Jácomo, A.T., Porfírio, G.E.O., Silveira, L., Sollmann, R., Tôrres, N.M., Ferreira Neto, J.S., 2017. Is the free-ranging jaguar (Panthera onca) a reservoir for Cytauxzoon felis in Brazil?, ticks tick. Borne. Dis. 8, 470–476. https://doi.org/10.1016/j.ttbdis.2017.02.005.

Ketz-Riley, C.J., Reichard, M.V., Van Den Bussche, R.A., Hoover, J.P., Meinkoth, J., Kocan, A.A., 2003. An intraerythrocytic small piroplasm in wild-caught Pallas's cats (Otocolobus manul) from Mongolia. J. Wildl. Dis. 39, 424–430. https://doi.org/10.7589/0090-3558-39.2.424.

Lewis, K.M., Cohn, L.A., Downey, M.E., Whitney, M.S., Birkenheuer, A.J., 2012. Evaluation of Cytauxzoon felis infection status in captive-born wild felids housed in an area endemic for the pathogen. J. Am. Vet. Med. Assoc. 241, 1088–1092. https://doi.org/10.2460/javma.241.8.1088.

Linardi, L.R., Guimarães, 2000. Sifonápteros do Brasil, Museu de Zoologia USP/Fapesp, São Paulo.

Meinkoth, J., Kocan, A.A., Whitworth, L., Murphy, G., Fox, J.C., Woods, J.P., 2000. Cats surviving natural infection with Cytauxzoon felis: 18 cases (1997-1998). J. Vet. Intern. Med. 14, 521–525. https://doi.org/10.1111/j.1939-1676.2000.tb02270.x.

Moreira, R., Souza, U.A., May-Junior, J.A., Baggio-Souza, V., Berger, L., Wagner, P.G.C., Mazim, F.D., Peters, F.B., Favarini, M.O., Tortato, M.A., Albano, A.P.N., Fagundes, D. D., Haberfeld, M.B., Sartorelo, L.R., Ranpim, L.E., Fragoso, C.E., Girotto-Soares, A., Martins, T.F., Valle, S.F., Soares, J.F., 2022. Epidemiological compatibility of Amblyomma sculptum as possible vector and Panthera onca as reservoir of Cytauxzoon spp. in Midwestern Brazil, ticks tick. Borne. Dis. 13, 102021. https://doi.org/10.1016/j.ttbdis.2022.102021.

Nielsen, C., Thompson, D., Kelly, M., Lopez Gonzalez, C., 2015. *Puma concolor*, Puma Errata version THE IUCN RED LIST OF THREATENED SPECIES™, IUCN Red List Threat. Species, 8235, pp. 1–12. https://www.iucnredlist.org/species/18868/97216466.

Panait, L.C., Mihalca, A.D., Modrý, D., Juránková, J., Ionică, A.M., Deak, G., Gherman, C. M., Heddergott, M., Hodžić, A., Veronesi, F., Reichard, M., Zieman, E.A., Nielsen, C. K., Jiménez-Ruiz, F.A., Hrazdilová, K., 2021. Three new species of Cytauxzoon in European wild felids. Vet. Parasitol. 290. https://doi.org/10.1016/j.vetpar.2021.109344.

Paviolo, A., Crawshaw, P., Caso, A., Oliveira, T.G., Lopez-Gonzalez, C., Kelly, M., De Angelo, C., Payan, E., Leopardus pardalis., 2015. The IUCN red List of Threatened species 2015: e.T11509A97212355. IUCN Red List Threat. Species 8235. https://doi.org/10.2305/IUCN.UK.2015-4.RLTS.T11509A50653476.en e.T11509A97212355. https://www.iucnredlist.org/species/11509/97212355.

Schreeg, M.E., Marr, H.S., Tarigo, J., Cohn, L.A., Levy, M.G., Birkenheuer, A.J., 2013. Pharmacogenomics of Cytauxzoon felis cytochrome b : implications for Atovaquone and azithromycin therapy in domestic cats with Cytauxzoonosis. J. Clin. Microbiol. 51, 3066–3069. https://doi.org/10.1128/JCM.01407-13.

Shock, B.C., Murphy, S.M., Patton, L.L., Shock, P.M., Olfenbuttel, C., Beringer, J., Prange, S., Grove, D.M., Peek, M., Butfiloski, J.W., Hughes, D.W., Lockhart, J.M., Bevins, S.N., VandeWoude, S., Crooks, K.R., Nettles, V.F., Brown, H.M., Peterson, D. S., Yabsley, M.J., 2011. Distribution and prevalence of Cytauxzoon felis in bobcats (Lynx Rufus), the natural reservoir, and other wild felids in thirteen states. Vet. Parasitol. 175, 325–330. https://doi.org/10.1016/j.vetpar.2010.10.009.

Soares, J.F., Girotto, A., Brandão, P.E., Da Silva, A.S., França, R.T., Lopes, S.T.A., Labruna, M.B., 2011. Detection and molecular characterization of a canine piroplasm from Brazil. Vet. Parasitol. 180, 203–208. https://doi.org/10.1016/j.vetpar.2011.03.024.