ISABELLA PEREIRA PESENATO

ECTOPARASITOFAUNA (ACARI: IXODIDA AND GAMASIDA; INSECTA: SIPHONAPTERA) FROM TERRESTRIAL MAMMALS: DIVERSITY, TAXONOMIC ASPECTS AND ASSOCIATED ZOONOTIC RICKETTSIALES IN LEGADO DAS ÁGUAS - RESERVA VOTORANTIM, IN TAPIRAÍ AND MIRACATU, SÃO PAULO

São Paulo

2023

ISABELLA PEREIRA PESENATO

Ectoparasitofauna (Acari: Ixodida and Gamasida; Insecta:Siphonaptera) from terrestrial mammals: diversity, taxonomic aspects and associated zoonotic rickettsiales in Legado Das Águas - Reserva Votorantim, in Tapiraí and Miracatu, São Paulo

corrected version

Thesis submitted to the Postgraduate Program in Experimental Epidemiology applied to Zoonosis of the School of Veterinary Medicine and Animal Science of the University of São Paulo to obtain the Doctor's degree in Sciences.

Department:

Preventive Veterinary Medicine and Animal Health

Area:

Experimental Epidemiology Applied to Zoonoses

Advisor:

Prof. Arlei Marcili, Ph.D.

Co-Advisor:

Prof. Fernanda Aparecida Nieri Bastos, Ph.D.

São Paulo

2023

DADOS INTERNACIONAIS DE CATALOGAÇÃO NA PUBLICAÇÃO

(Biblioteca Virginie Buff D'Ápice da Faculdade de Medicina Veterinária e Zootecnia da Universidade de São Paulo)

Pesenato, Isabella Pereira
Ectoparasitofauna (Acari: Ixodida and Gamasida; Insecta: Siphonaptera) from terrestrial mammals: diversity, taxonomic aspects and associated zoonotic rickettsiales in Legado das Águas - Reserva Votorantim, in Tapiraí and Miracatu, São Paulo / Isabella Pereira Pesenato ; orientador Arlei Marcili ; coorientadora Fernanda Aparecida Nieri Bastos. – São Paulo, 2023.
109 f. : il.

Título traduzido: Ectoparasitofauna (Acari: Ixodida e Gamasida; Insecta: Siphonaptera) de mamíferos terrestres: diversidade, aspectos taxonômicos e rickettsiales zoonóticos associados na reserva particular Legado das Águas – Reserva Votorantim, nos municípios de Tapiraí e Miracatu, São Paulo.

Tese (Doutorado – Programa de Pós-Graduação em Epidemiologia Experimental Aplicada às Zoonoses – Departamento de Medicina Veterinária Preventiva e Saúde Animal) – Faculdade de Medicina Veterinária e Zootecnia, Universidade de São Paulo, 2023.

1. Rickettsia. 2. Carrapatos. 3. Ácaros. 4. Pulgas. 5. Mata Atlântica. I. Título.

Ficha catalográfica elaborada pela bibliotecária Maria Aparecida Laet, CRB 5673-8, da FMVZ/USP.

Faculdade de Medicina Veterinária e Zootecnia Universidade de São Paulo

Comissão de Ética no Uso de Animais

São Paulo, 5th October 2023

CERTIFIED

We certify that the proposal entitled: "*ECTOPARASITOFAUNA (ACARI: IXODIDA AND GAMASIDA; INSECTA: SIPHONAPTERA) FROM TERRESTRIAL MAMMALS: DIVERSITY, TAXONOMIC ASPECTS AND ASSOCIATED IN LEGADO DAS ÁGUAS - RESERVA VOTORANTIM, IN TAPIRAÍ AND MIRACATU, OF SÃO PAULO*", protocol number CEUAx 6509131119 (ID 001278), under the responsibility Arlei Marcili, agree with Ethical Principles in Animal Research adopted by Ethic Committee in the Use of Animals of School of Veterinary Medicine and Animal Science (University of São Paulo), and was approved in the meeting of day December 17, 2019.

Certificamos que a proposta intitulada: "*ECTOPARASITOFAUNA (ACARI: IXODIDA E GAMASIDA; INSECTA: SIPHONAPTERA) DE MAMÍFEROS TERRESTRES: DIVERSIDADE, ASPECTOS TAXONÔMICOS E RICKETTSIALES ZOONÓTICOS ASSOCIADOS NA RESERVA PARTICULAR LEGADO DAS ÁGUAS RESERVA VOTORANTIM, NOS MUNICÍPIOS DE TAPIRAÍ E MIRACATU, SÃO PAULO.*", protocolado sob o CEUAx nº 6509131119, sob a responsabilidade de Arlei Marcili, está de acordo com os princípios éticos de experimentação animal da Comissão de Ética no Uso de Animais da Faculdade de Medicina Veterinária e Zootecnia Universidade de São Paulo, e foi aprovado na reunião de 17 de dezembro de 2019.

Prof. Dr. Marcelo Bahia Labruna
Coordenador da Comissão de Ética no Uso de Animais
Faculdade de Medicina Veterinária e Zootecnia
Universidade de São Paulo

Profa. Dra. Camilla Mota Mendes
Vice-Coordenadora da Comissão de Ética no Uso de Animais
Faculdade de Medicina Veterinária e Zootecnia
Universidade de São Paulo

UNiSA
Universidade Santo Amaro

COMISSÃO DE ÉTICA NO USO DE ANIMAIS

PARECER N.46.1/2017/2018

Projeto de Pesquisa: "Estudo de agentes zoonóticos e seus vetores em animais domésticos e silvestres na reserva particular legado das águas – Reserva Votorantim, nos municípios de Tapiraí e Miracatu São Paulo.".

Pesquisadores Responsáveis: Prof. Arlei Marcili

Curso: Medicina Veterinária

Prezado Pesquisador:

Ao se proceder à análise do processo em questão, coube a seguinte deliberação:

O Comitê de Ética em Pesquisa no Uso de Animais **(CEUA-UNISA),** seguindo as diretrizes e normas regulamentadoras de pesquisa envolvendo animais, conforme a Lei federal nº 11.794 (Lei Arouca), as resoluções do CONCEA, que estabelecem os procedimentos para o uso científico de animais no país e a Lei Estadual nº 11.977/05 que institui o Código de Proteção aos Animais do Estado de São Paulo, deliberando pela **Aprovação** do Projeto **"Estudo de agentes zoonóticos e seus vetores em animais domésticos e silvestres na reserva particular legado das águas – Reserva Votorantim, nos municípios de Tapiraí e Miracatu São Paulo.".**

São Paulo, 16 de março de 2018.

PROFA. DRA. VALERIA CASTILHO ONOFRIO
Coordenadora do Comitê de Ética no Uso de Animais – CEUA
UNISA - Universidade Santo Amaro

FOLHA DE AVALIAÇÃO

Autor: PESENATO, Isabella Pereira

Título: Ectoparasitofauna (Acari: Ixodida and Gamasida; Insecta:Siphonaptera) from terrestrial mammals: diversity, taxonomic aspects and associated zoonotic rickettsiales in Legado das Águas - Reserva Votorantim, in Tapiraí and Miracatu, São Paulo

Thesis submitted to the Postgraduate Program in Experimental Epidemiology applied to Zoonosis of the School of Veterinary Medicine and Animal Science of the University of São Paulo to obtain the Doctor's degree in Sciences.

Data: _____/_____/_____

Banca Examinadora

Prof. Dr._____

Instituição:_____ Julgamento:_____

Prof. Dr._____

Instituição:_____ Julgamento:_____

Prof. Dr._____

Instituição:_____ Julgamento:_____

Prof. Dr._____

Instituição:_____ Julgamento:_____

Prof. Dr._____

Instituição:_____ Julgamento:_____

This thesis is dedicated to my mother, Maria Inês Pereira (in memoriam), who always supported me and proudly told everyone her daughter studied at USP.

ACKNOWLEDGEMENTS

First of all, I'd like to thank God for giving me the opportunity to continually evolve and for my family, who have been with me from the moment I started my journey on this planet, especially my father Sergio, my mother Inês, my brother Felipe and my grandparents Sergio and Janete, who have always been very proud of me. To my boyfriend Giovanni, who before being my boyfriend is my Friend, for always being by my side, supporting me and always being so kind and loving to me.

To Dr. Arlei Marcili and Dr. Fernanda Nieri, who gave me the opportunity to learn immensely as a professional and as a person, both in the laboratory and in the field, and for having believed in my potential even before they met me. To MSc. Luís Flávio Costa, MSc. Renato Alvisi and Dr. Ana Rita Nardi for believing that I had the academic profile and for encouraging me to pursue this area since I was in college.

To my field trip friends, Herbert Soares, Jaciara Costa, Thiago Fakelmann, Giovanna Nosberto and Mayara Bispo, for always being there to share laughs and sweat during the days and nights of collecting. For all the Legado das Águas team who accompanied our group on the crazy field trips, having fun with us, especially Miguel and José for their help with trails and traps. To the members of the Ribeirão da Anta Community who opened their homes and hearts to welcome us and taught us so much with their years of experience in the forest, especially Valter for accompanying us on the trails and Val with her delicacies from the gods.

To my lab friends who, as well as teaching me so many techniques and tips, also supported me in the endless samples and tests I needed to carry out. To Ryan Silva and Jaciara Costa for welcoming me so well into the 'Tryp/Leish' group and accompanying me during my trial period, to Carolina Serpa for her endless experience with serology, molecular and cell culture and to my 'Ticks' friends for the breakfast conversations and discussions of articles: Igor Silito, Barbara Weck, Lina Binder, Glauber Meneses, Felipe Jorge, Matheus Pasini and Talita Beck, and also Mara Gravinatti and Bruna Alves.

To Professor Dr. Marcelo Labruna for inviting me to the weekly meetings of his group, which helped me so much to form my critical reading, and for always being available to clarify any doubts I might have. Dr. Hilda Pena for teaching us so many laboratory techniques and for providing such an organized and well-equipped environment for our daily work. Dr. Thiago Martins for always being willing to identify my ticks, regardless of the time or day, and for teaching me so much about taxonomy during our meetings. To Dr. Marcos Amaku for the help with the statistical analysis. To the technicians Renato, Marcio and Pedro for all their help

during my doctoral studies and for being so welcoming to everyone who came into our laboratories. To Danival Lopes, for valuable administrative assistance.

To the friends I made during my analyses at the Butantan Institute and the LCZ team. Dr. Valéria Onofrio for opening the doors of the institute to me, trusting my work. Dr. Fernando Jacinavicius and Dr. Ricardo Bassini for always being there when it came to mites and for giving up so much of their time so that I could finish my analyses. Gabrielle Andrade, Cristina do Rosário and Lívia Correa for being incredible people and so careful with everything they do. Matheus Huang and Gledson Bandeira for giving me the opportunity to share my knowledge with you.

To my long-time friends, that accompanied me throughout my doctorate years, who are now considered family because we know that our journey together is still a long one and our friendship goes back many lifetimes. For this reason, my deepest gratitude goes to our greatest friend, Dr. Celso Charuri, who brought us together and gave us something so important to work on.

I would like to thank São Paulo Research Foundation (FAPESP) for financial support of this work (FAPESP no. 2018/18992-3). This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) - Finance Code 001.

Finally, I thank all those who contributed directly to this work, and those who indirectly, knowing it or not, were present in my life.

“Start by doing what’s necessary; then do what’s possible; and suddenly you are doing the impossible”.

Saint Francis of Assisi

RESUMO

PESENATO, IP. **Ectoparasitofauna (Acari: Ixodida e Gamasida; Insecta: Siphonaptera) de Mamíferos Terrestres:** Diversidade, Aspectos Taxonômicos e Rickettsiales Zoonóticos associados na reserva particular Legado das Águas – Reserva Votorantim, nos municípios de Tapiraí e Miracatu, São Paulo. 2023. 109f. Tese (Doutorado em Ciências) – Faculdade de Medicina Veterinária e Zootecnia, Universidade de São Paulo, São Paulo, 2023.

As bactérias do gênero *Rickettsia* são responsáveis por causar diversas doenças ao redor do mundo e por terem causado grandes epidemias no passado. No Brasil o Grupo Febre Maculosa é responsável por grande preocupação em questão de saúde pública, envolvendo questões ambientais e animais. A transmissão das riquétsias está associada primariamente a carrapatos, mas piolhos, ácaros e pulgas podem estar envolvidos na disseminação. O presente estudo teve como objetivo coletar a ectoparasitofauna presente em um fragmento de Mata Atlântica primária localizado dentro de uma reserva particular (Legado das Águas – Reserva Votorantim) no estado de São Paulo, Brasil. Os carrapatos, ácaros e pulgas foram coletados a partir de pequenos mamíferos terrestres, cães, seres humanos e também através da observação da vegetação nos estágios de vida livre, além disso foram obtidas amostras de sangue dos roedores, marsupiais e cães. Após a coleta, estas amostras foram identificadas e testadas para bactérias do gênero *Rickettsia*, através de ferramentas moleculares, sorológicas e cultivo em células Vero. No total foram capturados 476 pequenos mamíferos terrestres, 189 morcegos e 12 cães, a partir destes e do ambiente foram coletados 6.947 ácaros, 1.458 pulgas e 2.714 carrapatos. Foram observadas novas relações de parasitismo relacionados ao hospedeiro e também novos registros de localidade de espécies. Após os testes moleculares foram evidenciadas três espécies de riquétsias circulando na população de carrapatos: *Rickettsia bellii, Rickettsia parkeri* cepa Mata Atlântica e *Rickettsia rhipicephali*, e destas foram obtidos dois isolados de *R. bellii* a partir de carrapatos da espécie *Amblyomma ovale* através da técnica de *shell vial*. Os testes sorológicos foram compatíveis com os resultados dos testes de carrapatos, com títulos altos para as três bactérias em questão e apenas um espécime de *Didelphis aurita* apresentou títulos compatíveis com *Rickettsia amblyommatis*. Os espécimes de pulgas e ácaros foram testados utilizando os mesmos marcadores, porém nenhum amplificou DNA riquetsial. Os resultados deste trabalho servem para uma maior compreensão da ecologia de hospedeiros, ectoparasitos e bactérias do gênero *Rickettsia* em condições ambientais sem ou com pouca ação humana e como as ações antrópicas afetam a distribuição destes patógenos.

Palavras-chave: *Rickettsia*. Carrapatos. Ácaros. Pulgas. Mata Atlântica.

ABSTRACT

PESENATO, IP. **Ectoparasitofauna (Acari: Ixodida and Gamasida; Insecta:Siphonaptera) from terrestrial mammals:** diversity, taxonomic aspects and associated zoonotic rickettsiales in Legado das Águas - Reserva Votorantim, in Tapiraí and Miracatu, São Paulo. 2023. 109f. Tese (Doutorado em Ciências) – Faculdade de Medicina Veterinária e Zootecnia, Universidade de São Paulo, São Paulo, 2023.

Bacteria of the *Rickettsia* are responsible for causing various diseases around the world and have caused major epidemics in the past. In Brazil, the spotted fever group is a serious public health concern, involving environmental and animal issues. The transmission of rickettsiae is primarily associated with ticks, but lice, mites and fleas can also be involved in the dissemination. The aim of this study was to collect the ectoparasitofauna present in a fragment of primary Atlantic Rainforest located within a private reserve (Legado das Águas - Reserva Votorantim) in the state of São Paulo, Brazil. Ticks, mites and fleas were collected from small terrestrial mammals, dogs, humans and also by observing the vegetation in its free-living stages, and blood samples were obtained from rodents, marsupials and dogs. After collection, these samples were identified and tested for bacteria of the *Rickettsia* genus using molecular and serological tools and culture on Vero cells. A total of 476 small terrestrial mammals, 189 bats, and 12 dogs were captured, from which 6,947 mites, 1,458 fleas and, 2,714 ticks were collected. New host-related parasitism relationships were observed, as well as new species locality records. Molecular tests revealed three species of rickettsia circulating in the tick population: *Rickettsia bellii*, *Rickettsia parkeri* strain Atlantic Rainforest and *Rickettsia rhipicephali*, and two isolates of *R. bellii* were obtained from *Amblyomma ovale* ticks using the viral shell technique. The serological tests were compatible with the results of the tick tests, with high titres for the three bacteria in question and only one specimen from *Didelphis aurita* showed titres compatible with *Rickettsia amblyommatis*. Flea and mite specimens were tested using the same targets, but none amplified rickettsial DNA. The results of this study provide a better understanding of the ecology of hosts, ectoparasites and bacteria of the *Rickettsia* genus in environmental conditions without or with little human action and how anthropogenic actions affect the distribution of these pathogens.

Keywords: *Rickettsia*. Ticks. Mites. Fleas. Atlantic Rainforest.

LIST OF FIGURES

LIST OF TABLES

LIST OF CHARTS

SUMMARY

1 INTRODUCTION

Ectoparasites are arthropods that do at least one part of their life cycle on the skin of their hosts and are those that cause some kind of damage to another individual by removing nutrients or basic components from it for their own food or benefit (Otranto, 2018). These individuals present a continuous subject of studies for many years since their discoveries, mainly addressing its role as pathogen vectors, local or systemic reactions, ecological aspects and host-parasite interactions (Szabó et al., 2002; Webster et al., 2014). Within the ecosystem, species specific parasites can also impact on biodiversity, reproduction and survival of their host, acting as an ambient indicator in terms of biodiversity loss (Santos et al., 2018).

The ectoparasites interacting with wildlife are extremely relevant when it comes to zoonosis and public health, since these animals are reportedly recognized as reservoirs of vector-borne pathogens (Bezerra-Santos et al., 2020; Estevam et al., 2020). With the growth of human activities many biomes are being destroyed and these animals are being forced to adapt to human settlements (Bellard et al., 2014). When it comes to natural environments this host-parasite relationship is a result of coevolution, a complex but also balanced interaction, which is extremely important to study for a better understanding of the patterns observed in anthropized localities (Bush; Reed; Maher, 2013). Several ectoparasites show great relevance for medical and veterinary health and among them hematophagous parasites are particularly relevant for transmitting pathogens, such as rickettsiae bacteria.

Rickettsiae are gram-negative bacteria, represented by the genera *Rickettsia* and *Orientia*, the latter is the causative agent of "rural typhus" or scrub typhus. All the species included in the genus *Rickettsia* are obligate intracellular bacteria and can be present in plants, amoebas, arthropods, annelids and vertebrates (DAVIS et al., 1998; KIKUCHI et al., 2002; DYKOVÁ et al., 2003). There is a division within this genus comprising five main groups: bellii group, canadensis group, typhus group, transitional group and spotted fever group (GILLESPIE et al., 2008; WEINERT et al., 2009). In Brazil the most important group for epidemiological studies is the Spotted Fever Group (SFG) that contains both *Rickettsia rickettsia* and *Rickettsia parkeri* strain Atlantic Rainforest, causatives of mild to severe diseases in humans (de Oliveira et al., 2016).

The area addressed in our study is located in the Brazilian southeastern called Legado das Águas – Reserva Votorantim, being the largest private Atlantic Rainforest reserve in the country, located between the municipalities of Taipiraí and Miracatu in the state of São Paulo, in the Ribeira Valley, covering an area of 31,000 hectares. About 50 years ago, Votorantim

(private institution) acquired several properties with preserved forested areas in the Ribeira Valley and built seven hydroelectric plants at various points along the Juquiá River. In 2012, the site was transformed into Legado das Águas, as it is known today, with a focus on ecotourism in this area. Around 75% of the total area of the reserve is made up of primary dense ombrophilous forest with an average temperature of 25ºC and high rainfall rates, with great preservation of the original flora and fauna with a focus on protecting these species, which are often at risk of extinction. Inside the reserve were selected four areas for the collections to be conducted (figure 1).

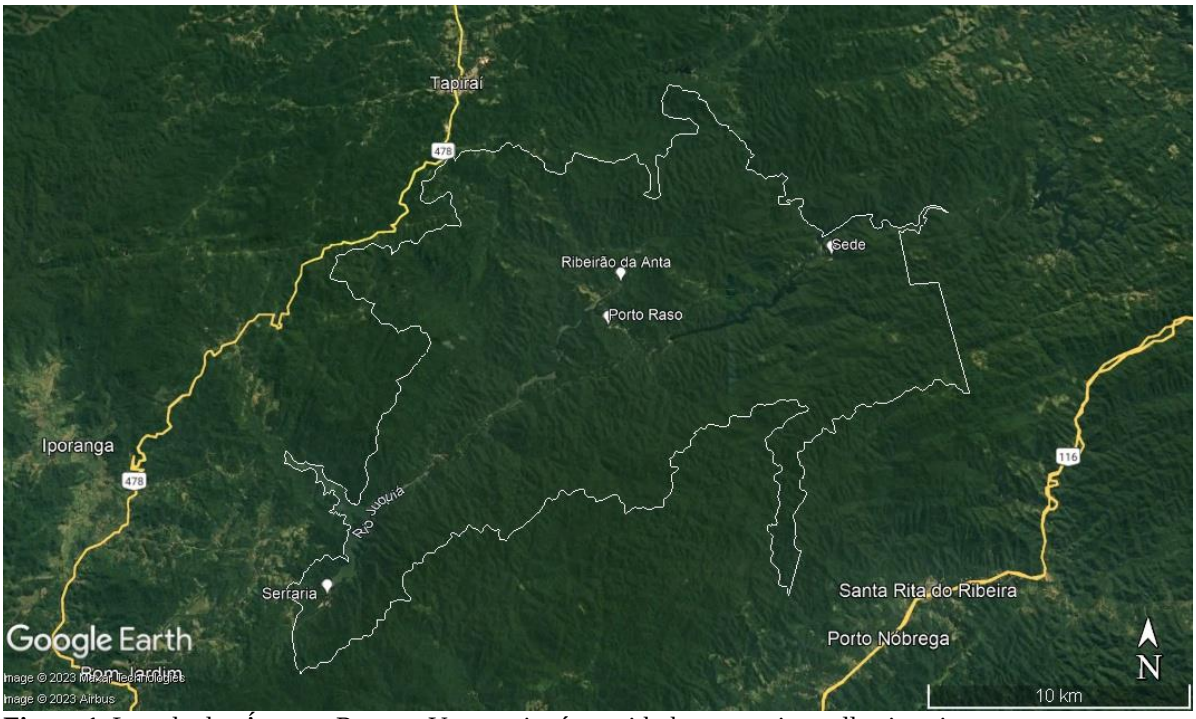

Figure 1. Legado das Águas – Reserva Votorantim área with the respective collection sites**.**

1.1 CHAPTER PRESENTATION

The results obtained in this study are organized in chapters. The chapter 1 is a result about the mites examined form the families Trombiculidae s.s., Macronyssidae, and Laelapidae collected on small terrestrial mammals, including some new locality and host association records for Brazil and rickettsial monitoring; the chapter 2 is an updated checklist of Brazilian Siphonaptera, comprising rickettsial monitoring from samples collected in Legado das Águas-Reserva Votorantim preserved area; the chapter 3 comprises a study of ticks collected from the vegetation and on small terrestrial mammals, dogs and humans, and rickettsial characterization by three techniques: isolation in Vero cells, molecular and serological testing. All the chapters are adapted in manuscript format, as follows:

- Chapter 1 - Mites present in small terrestrial mammals in a preserved ecosystem inside the Atlantic Rainforest including pathogen monitoring. [unpublished yet].
- Chapter 2 - Brazilian fleas (Hexapoda: Siphonaptera): diversity, host associations and new records on small mammals from the Atlantic Rainforest [unpublished yet].
- Chapter 3 - Epidemiological aspects of *Rickettsia* in mammals and ticks in a preserved Atlantic Rainforest fragment in southeastern, Brazil. [unpublished yet].

1.2 GENERAL OBJECTIVE

The general objective of this work was to carry out a prospective study by collecting small mammals and dogs and investigating the ectoparasitofauna present in the hosts and vegetation in a primary Atlantic Rainforest reserve (Legado das Águas – Reserva Votorantim), as well as testing for bacteria of the genus Rickettsia in the arthropods and hosts.

1.2.1 Specific objectives

- Taxonomic identification of ectoparasites collected from small mammals, dogs, humans and free-living ticks.
- To search bacteria of the genus *Rickettsia* using molecular tools and in vitro isolation from ectoparasites collected from small mammals and also dogs and free-living ticks.
- Search for antibodies in serological samples collected from small mammals using the indirect immunofluorescence assay (IFA).
- Perform phylogenetic positioning of the detected and isolated rickettsiae.

1.3 REFERENCES

BELLARD, C. *et al*. Vulnerability of biodiversity hotspots to global change. **Global Ecology and Biogeography**, v. 23, n. 12, p. 1376–1386, 1 dez. 2014.

BEZERRA-SANTOS, M. A. *et al*. Ticks, fleas and endosymbionts in the ectoparasite fauna of the black-eared opossum *Dipelphis aurita* in Brazil. **Experimental and Applied Acarology**, v. 80, n. 3, p. 329–338, 2020.

BUSH, S. E.; REED, M.; MAHER, S. Impact of forest size on parasite biodiversity: Implications for conservation of hosts and parasites. **Biodiversity and Conservation**, v. 22, n. 6–7, p. 1391–1404, 2013.

DAVIS, M. J.*et al*. Rickettsial Relative Associated with Papaya Bunchy Top Disease. **Current Microbiology**, v. 36, n. 2, p. 80–84, 1998.

DE OLIVEIRA, S. V. *et al*. An update on the epidemiological situation of spotted fever in Brazil. **Journal of Venomous Animals and Toxins including Tropical Diseases**, v. 22, n. 1, p. 22, 2016.

DYKOVÁ, I. *et al*. *Nuclearia pattersoni sp. n*. (Filosea), a new species of amphizoic amoeba isolated from gills of roach (*Rutilus rutilus*), and its rickettsial endosymbiont. **Folia Parasitologica**, v. 50, n. 3, p. 161–170, 2003.

ESTEVAM, L. G. T. M. *et al*. Seven years of evaluation of ectoparasites and vector-borne pathogens among ring-tailed coatis in an urban park in southeastern Brazil. **Veterinary Parasitology: Regional Studies and Reports**, v. 21, n. February, p. 100442, 2020.

GILLESPIE, J. J. *et al. Rickettsia* Phylogenomics: Unwinding the Intricacies of Obligate Intracellular Life. **PLoS ONE**, v. 3, n. 4, p. e2018, 2008.

KIKUCHI, Y. *et al.* Novel Clade of *Rickettsia* spp. from Leeches. **Applied and Environmental Microbiology**, v. 68, n. 2, p. 999–1004, 2002.

OTRANTO, D. Arthropod-borne pathogens of dogs and cats: From pathways and times of transmission to disease control. **Veterinary Parasitology**, v. 251, n. November 2017, p. 68–77, 2018.

SANTOS, N. de J. *et al*. Effect of forest cover on ectoparasites of small mammals in the Brazilian Atlantic forest. **Oecologia Australis**, v. 22, n. 3, p. 279–290, 2018.

SZABÓ, K. *et al*. Effects of haematophagous mites on nestling house sparrows (*Passer domesticus*). **Acta Parasitologica**, v. 47, n. 4, p. 318–322, 2002.

WEBSTER, K. N. *et al*. Ectoparasite infestation patterns, haematology and serum biochemistry of urban-dwelling common brushtail possums. **Wildlife Biology**, v. 20, n. 4, p. 206–216, 2014.

WEINERT, L. A. *et al.* Evolution and diversity of *Rickettsia* bacteria. **BMC Biology**, v. 7, n. 6, p. 1-15, 2009.

2 CHAPTER 1 - MITES PRESENT IN SMALL TERRESTRIAL MAMMALS IN A PRESERVED ECOSYSTEM INSIDE THE ATLANTIC RAINFOREST INCLUDING PATHOGEN MONITORING

2.1 INTRODUCTION

Ectoparasites are commonly found in natural environments without anthropic actions, living along with small terrestrial mammals, such as rodents and marsupials, causing little or no harm, being a result of many years of coevolution (Cançado et al., 2017). These parasites are part of a prominent role concerning zoonotic diseases, acting as vectors to humans or other groups of animals. Among them, ticks are the most studied regarding the presence of *Rickettsia* bacteria, leaving mites in a neglected landscape (Souza et al., 2021).

Mites are chelicerate arthropods with a wide variety of feeding habits, and some families can present parasitic habits, primarily interacting with small mammals (Walter; Proctor, 2013). Association patterns of these ectoparasites with specific hosts have been studied in the Neotropical region, with differences between degraded and preserved areas (Jacinavicius et al., 2018a; Bassini-Silva et al., 2021).

The most common group of mites found parasitizing rodents and marsupials are mesostigmatids (Mesostigmata), followed by chiggers (Trombidiformes: Trombiculidae *sensu lato*) with already described roles in some zoonotic diseases such as rickettsialpox (caused by *Rickettsia akari* vectored by mesostigmatids) and scrub typhus (caused by *Orientia tsutsugamushi* transmitted by chiggers) (Herrera-Mares et al., 2022). Within the Mesostigmata two families have high relevancy specially when it comes to small mammals, the family Macronyssidae has specific and generalist species with hematophagous habits (Bassini-Silva et al., 2021) and the family Laelapidae, is mainly associated with rodents and the most diverse family in this order, found on their body surface or nests, but not demonstrating hematophagous habits (Masan; Fenda, 2010). Although, some studies are being conducted to investigate pathogens in these two groups of mites (Reeves et al., 2007; Jacinavicius et al., 2019; Bassini-Silva et al., 2023), the number is minimal when compared to other ectoparasites, such as ticks.

The Brazilian Atlantic Rainforest is one of the 35 biodiversity hotspots on the planet, sheltering regions with high levels of endemism but heavily threatened by human activities, replaced by pastures and monocultures, resulting in a loss of habitat for animals and increasing human-animal interaction with parasites exchange (Linhares de Rezende et al., 2015; Vidal-Martínez; Wunderlich, 2017). Most of what remains of the Atlantic Forest is under the

protection of laws and forest reserves, which encourage the maintenance of these areas with the introduction of scientific research, partnerships with conservationists and tourism to sustain local biodiversity (Mittermeier et al., 2004; Legado das Águas, 2018).

The objective of this study was to identify the species of mites that were collected from rodents and marsupials in three areas of the Atlantic Rainforest reserve Legado das Águas - Reserva Votorantim, São Paulo State, Brazil, and detection of *Rickettsia* spp. in these mites.

2.2 MATERIALS AND METHODS

2.2.1 Specimens collection

The mites examined were collected in the private reserve Legado das Águas - Reserva Votorantim, Miracatu, São Paulo, Brazil, located in a fragment of Atlantic Forest, with approximately 75% of the total area composed of dense primary ombrophilous forest. Within the perimeter of the reserve, three areas were chosen for capture: Sede (24° 1' 49.51" S, 47° 21' 8.36" W), Porto Raso (24° 3' 25.90" S, 47° 26' 30.07" W) and Serraria (24° 9' 9.63" S, 47° 32' 53.49" W), the locality and the areas are better shown in the Figure 1.

Between January 2018 and December 2021, eight campaigns were conducted, lasting an average of seven to twelve days, with three campaigns in the Sede area (January, July and December of 2018 with six days each), three in Porto Raso (July of 2019, February of 2020 and October of 2021 with six days each), and two longer ones in Serraria (September and December of 2022 with nine days each) due to the COVID-19 pandemic resulting in a total of 18 days in each sampled area.

The trails chosen were based on the vegetation and tracks of wild animals. In total, 240 traps (Sherman and Tomahawk traps) were used in each campaign, and the small mammals were captured using bait made with a mixture of sardines, cornmeal, coconut oil, vanilla, and peanut paste. After capture, the animals were anesthetized with ketamine hydrochloride (15-30mg/kg), and following sample collection and recovery, the rodents and marsupials were identified using taxonomic keys (Bonvicino; Oliveira; D'Andrea, 2008; Faria, 2019) then returned to the wild at the same site as captured. All mites were collected using tweezers and stored in a microtube containing absolute ethanol.

2.2.2 Mite morphological identification

After collection, the samples were sent to the Laboratório de Coleções Zoológicas of the Instituto Butantan (LCZ-IB) for identification.

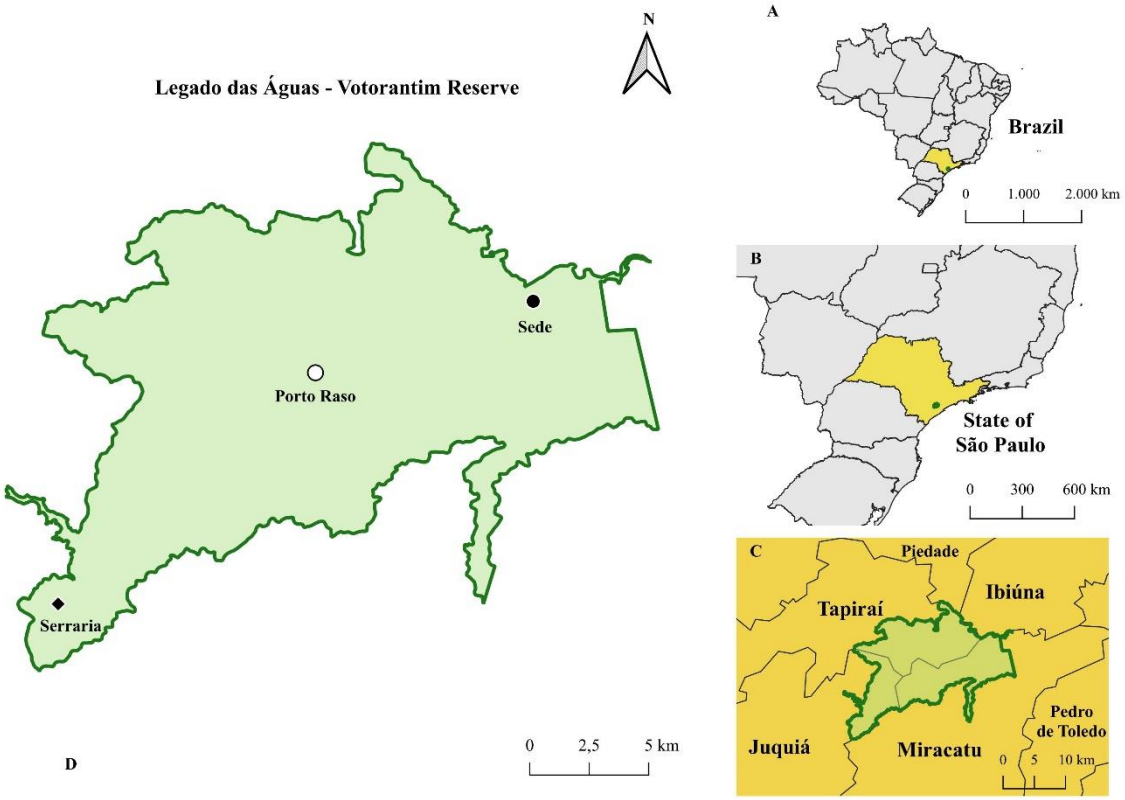

Figure 2: Map showing the collection area. **A.** Brazil and State of São Paulo highlighted; **B.** Position of the reserve inside São Paulo's state; **C.** municipalities surrounding the reserve area; **D.** Collection sites within the Legado das Águas limit.

The mites were slide-mounted using Hoyer's medium following the protocols described by Walter and Krantz (2009). For the identification of genera, we used Radovsky (2010), Brennan and Goff (1977), and Fonseca (1936). We compared our material for species level with the original descriptions of each species within the genus identified.

Images and measurements were taken using a Leica DM4000B microscope and compiled with Leica Application Suite version 2.5.0. All slide-mounted specimens were deposited in the Acarological Collection at the Laboratory of Zoological Collections of the Butantan Institute (LCZ-IB) under the accession numbers IBSP18723 - IBSP18862.

2.2.3 Molecular analysis

Part of the collected mites were stored for molecular analysis. For this, each mite was individualized in a microtube and submitted to DNA extraction using the commercial DNeasy

Blood and Tissue Kit (Qiagen®), protocol suggested by the manufacturer was followed. After DNA extraction, each exoskeleton was recovered from the columns and slide-mounted for identification following the steps previously described.

From the extracted DNA, conventional PCRs were performed using primers that amplify endogenous genes of the mite, the small subunit (SSU) 18S rRNA gene following the thermal cycler conditions proposed by Otto and Wilson (2001), the 16S mitochondrial rRNA gene, following the thermal cycler conditions proposed by Mangold; Bargues and Mas-coma (1998) and the Cytochrome c oxidase subunit 1 gene following the thermal cycler conditions proposed by Folmer et al. (1994). All reactions included controls: positive, DNA extracted from *Blankartia sinammaryi* (Floch & Fauran, 1956) and negative, ultrapure water type I.

Samples positive for one or two of the endogenous genes were considered viable, thus screened for bacteria of the genus *Rickettsia*. First, was performed a real-time polymerase chain reaction (qPCR) using the primers CS-5 and CS-6 and including an internal probe to amplify a 147 bp fragment of the *gltA* gene, present in all bacteria of this genus. This reaction was performed following the protocols described by Labruna et al. (2004) and Guedes et al. (2005). All reactions included positive (DNA extracted from cell culture infected with *Rickettsia vini*) and negative (ultrapure water type I) controls. All the primers used in the molecular tests are described in the Table 1.

Table 1. Primer pairs used for the amplification of rickettsial and mite's genes.

Organism	Gene	Sequence 5'3'	Size (pb)	Reference
Rickettsia	gltA	CS-5: GAGAGAAAATTATATCCAAATGTTGAT CS-6: AGGGTCTTCGTGCATTTCTT 6-FAM d(CATTGTCGGATCCAGCCTACGGT) BHQ-1	147	Guedes et al. (2005) and Labruna et al. (2004)
Mite	18S rRNA	18S-1F: ATATTGGAGGGCAAGTCTGG 18S-1R: TGGCATCGTTTATGGTTAG	500	Otto and Wilson (2001)
Mite	16S rRNA	16S+1: CCGGTCTGAACTCAGATCAAGT 16S-1: GCTCAATGATTTTTTAAATTGCTGT	460	Mangold et al. (1998)
Mite	COI	LCO1490: GGTCAACAAATCATAAAGATATTGG HCO2198: TAAACTTCAGGGTGACCAAAAAATCA	710	Folmer et al., 1994

The reactions that generated amplicons were purified with ExoSAP-IT (USB Corporation®, OH), using the manufacturer's instructions, and subsequently sequenced through Sanger sequencing performed at the "Human Genome and Stem Cell Research Center of the Institute of Biosciences of the USP". The sequences obtained were edited using the program SeqMan (Lasergene, DNAstar, Madison, Wis.) and then submitted to analysis using the program BLASTn to infer similarity with other homologous sequences already deposited in the GenBank (Altschul et al., 1990).

2.2.4 Ethical statement

The Ethics Committee of the Faculty of Veterinary Medicine and Zootechny of the University of São Paulo (FMVZ-USP) under the number 6509131119 approved the present study.

2.3 RESULTS

2.3.1 Host-associations

During the sampling years, 478 mammals were collected (368 rodents and 110 marsupials). Of these, 283 rodents and 42 marsupials were infested with mites from three families: Trombiculidae, Macronyssidae, and Laelapidae, resulting in a total of 6988 specimens.

As for the family Trombiculidae, six different rodent species from the families Cricetidae and Sciuridae and two species of marsupials were found parasitized with 34 batches of mites, ranging from 1 to 50 specimens per batch. The ectoparasites were collected, parasitizing mainly the ear canal of these vertebrates, and in a few cases, were found on the animal's body (Figure 2a). Co-parasitism among species was also observed, including two specimens attached to the same skin fragment, *Quadraseta pazca* (Brennan & Jones, 1964) and *Quadraseta brasiliensis* Goff & Gettinger, 1989 collected from *Euryoryzomys russatus* (Wagner, 1848) (Figure 2b).

The chiggers collected were identified in three genera and four species. The genera *Quadraseta* Brennan, 1970 is the most abundant in this study, represented by two species, *Q. pazca* and *Q. brasiliensis*, collected from rodents belonging to the following species: *E. russatus* (33 individuals), *Delomys* sp. Thomas, 1917 (2 individuals), *Oligoryzomys* sp. Bangs, 1900 (5 individuals), *Rhipidomys* sp. Tschudi, 1845 (1 individual) and *Thaptomys nigrita* (Lichtenstein, 1829) (1 individual). The species *Eutrombicula tinami* Oudemans, 1910 was collected from two species of rodents, *E. russatus* (1 individual) and *Guerlinguetus brasiliensis* (Gmelin, 1788) (1 individual), and one marsupial, *Didelphis aurita* (Wied-Neuwied, 1826) (3 individuals). Lastly, *Colicus spinosus* Goff & Gettinger, 1989 parasitized only a marsupial, *Monodelphis* sp. Burnett, 1830 (1 individual).

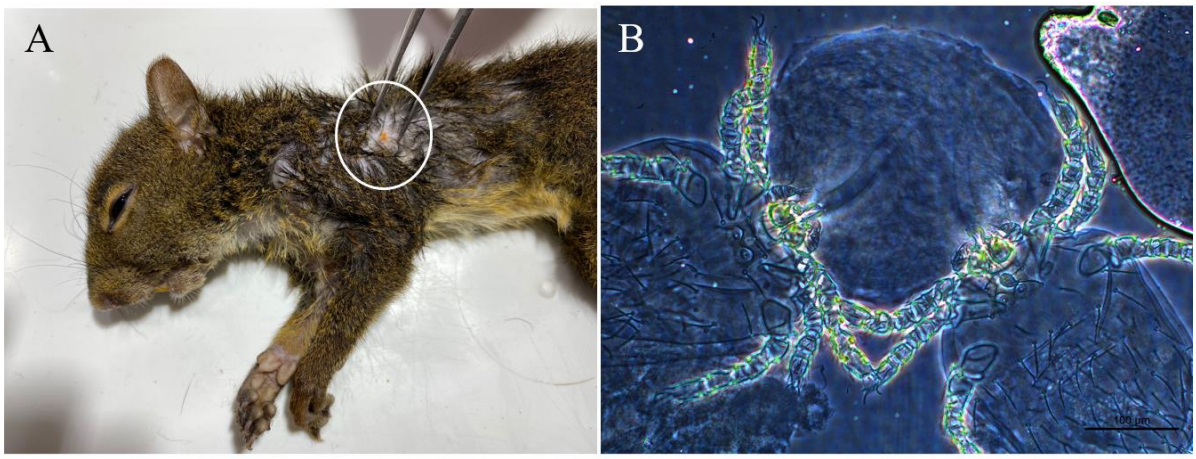

Figure 3: A. parasitism of chigger mites over the body of *Guerlinguetus brasiliensis*. **B.** Co-parasitism between *Quadraseta pazca* and *Quadraseta brasiliensis* attached to the same skin fragment collected from a *Euryoryzomys russatus*.

The mites belonging to the order Mesostigmata are divided in two families Laelapidae and Macronyssidae. The family Laelapidae was the most diverse (four genera and eleven species), collected and associated with rodents and marsupials in the study area. Also, there were several life stages: protonymphs, deutonymphs, males, and females. Twenty-nine lots were collected in marsupials and 285 in rodents, ranging from 1 to 105 specimens each.

The species in this family belong to four different genera and were associated with many hosts species. *Androlaelaps fahrenholzi* (Berlese, 1911) was found to be associated with eleven rodent species and three marsupial species. In contrast, another species belonging to the same genera, *Androlaelaps ilhacardosoi* Gettinger and Martins-Hatano (2003) was found associated only with a particular species of marsupial, *Monodelphis* sp. The same specificity was observed in the species *Androlaelaps rotundus* (Fonseca, 1935) collected associated only with the rodent *Akodon* sp. Meyen, 1833. The genus *Gigantolaelaps* Fonseca, 1939 is represented by three species: *Gigantolaelaps gilmorei* Fonseca, 1939, *Gigantolaelaps oudemansi* Fonseca, 1939, and *Gigantolaelaps wolffsohni* (Oudemans, 1910) found associated with rodents and marsupials, including a wide variety of hosts. Five species were found associated with rodents and identified as belonging to the genera *Laelaps* Koch, 1836, as follows: *Laelaps castroi* Fonseca, 1957, *Laelaps differens* Fonseca, 1935, *Laelaps manguinhosi* Fonseca, 1935, *Laelaps paulistanensis* Fonseca, 1935, and *Laelaps thori* Fonseca, 1939 (figure 3). Some immature stages specimens were identified to the genus level of *Laelaps* sp. and found to be associated with rodents and marsupials collected in this study.

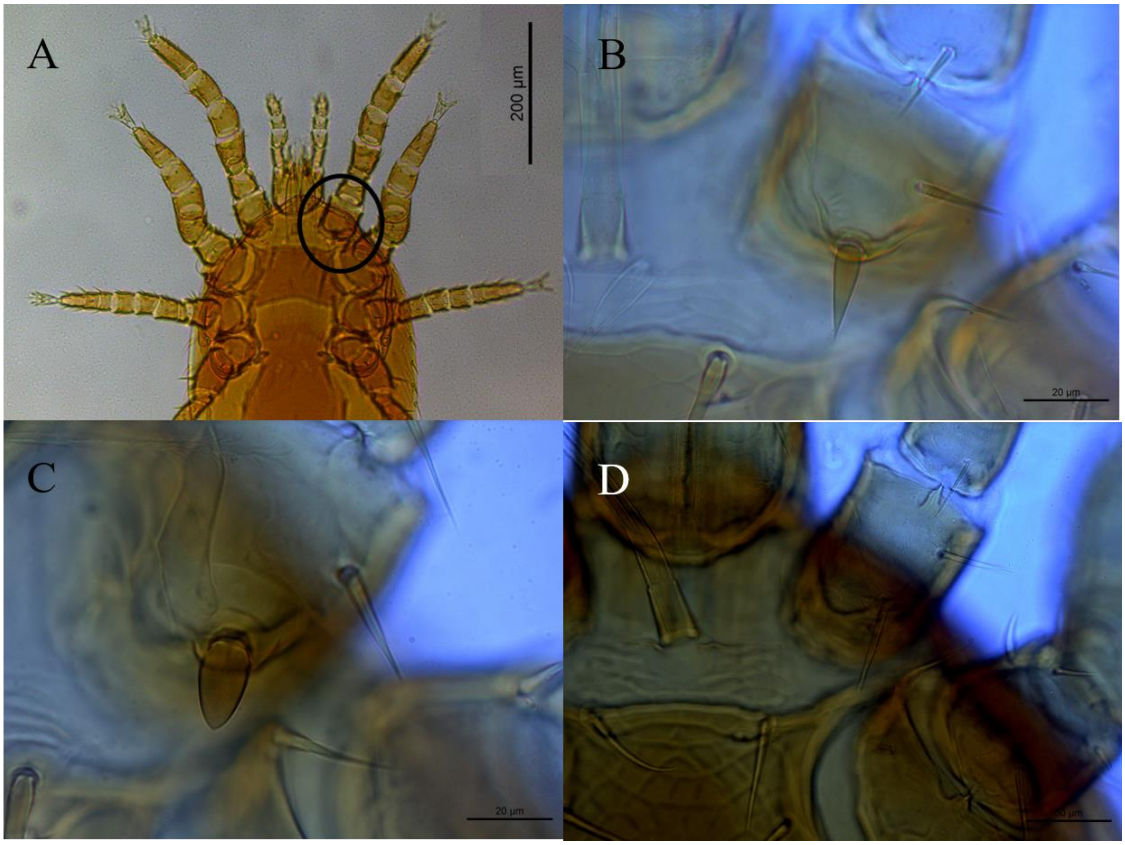

Figure 4: Differences observed between the spine-like setae present on the coxae I among laelapids**. A.** Locality of the spine; **B.** spine of *Laelaps differens*; **C**. spine of *Laelaps castroi*; **D.** spine of *Laelaps thori*.

The other family, Macronyssidae, had the lowest diversity of species. Some specimens from this family were not identified to species level because they were protonymphs or males since most taxonomic keys for this group are based on females, as the other stages are rare. Thirty lots were collected from rodents ranging from 1 to 102 specimens per lot. No macronyssid specimens were collected associated with marsupials. The genus collected was *Ornithonyssus* sp. Sambon, 1928 and associated with four species of rodents, *Brucepattersonius* sp. Hershkovitz, 1998, *Nectomys* sp. Peters, 1861, *Oxymycterus* sp. Waterhouse, 1837 and *Holochilus* sp Brandt, 1835. The information on mites collected on rodents is compiled in Table 2 and the mites collected on marsupials is on Table 3.

Table 2: Occurence of mites collected on rodents in a preserved ecological area (Legado das Águas – Reserva Votorantim) inside the Atlantic Rainforest Biome, during the years of 2018 to 2021.

Mite species	Hosts (infested/captured)													
	Akodon sp. (n=2) SR	*Brucepattersonius* sp. (n=5) PR	*Delomys* sp. (n=1) SE	*Euryoryzomys russatus* (n=225) SE, PR, SR	*Guerlinguetus* sp. (n=1) SR	*Holochilus* sp. (n=58) PR	*Hylaeamys* sp. (n=8) SE, PR	*Nectomys squamipes* (n=15) PR	*Oligoryzomys* sp. (n=15) SE, PR, SR	*Oxymycterus* sp. (n=26) SE, PR, SR	*Rhipidomys* sp. (n=2) PR	*Sooretamys* sp. (n=2) SE	*Thaptomys nigrita* (n=3) SR	TOTAL
	Infestation prevalence % (infested hosts/total hosts) number of mite specimens n													
Androlaelaps fahrenholzi	50 (1/2) 9	20 (1/5) 1	100 (1/1) 1	38.67 (87/225) 472	-	20.69 (12/58) 79	25 (2/8) 12	33.33 (5/15) 31	40 (6/15) 17	15.38 (4/26) 18	100 (2/2) 147	-	33.33 (1/3) 1	788
A. rotundus	50 (1/2) 29	-	-	-	-	-	-	-	-	-	-	-	-	29
Eutrombicola tinami	-	-	-	0.44 (1/225) 4	100 (1/1) 128	-	-	-	-	-	-	-	-	132
Gigantolaelaps gilmorei	-	-	100 (1/1) 1	52 (117/225) 573	-	-	25 (2/8) 6	6.67 (1/15) 4	40 (6/15) 20	-	100 (2/2) 22	50 (1/2) 5	-	631
G. oudemansi	-	-	100 (1/1) 1	83.56 (188/225) 3027	-	3.45 (2/58) 10	75 (6/8) 77	6.67 (1/15) 17	60 (9/15) 138	-	100 (2/2) 69	50 (1/2) 20	-	3359
G. wolffsoni	-	-	-	1.78 (4/225) 30	-	44.83 (26/58) 210	12.50 (1/8) 2	60 (9/15) 92	20 (3/15) 32	-	50 (1/2) 29	-	-	395
Laelaps castroi	-	-	-	9.78 (22/225) 52	-	-	12.50 (1/8) 1	-	6.67 (1/15) 4	-	50 (1/2) 2	50 (1/2) 9	-	68
L. differens	-	-	-	9.33 (21/225) 80	-	-	-	-	13.33 (2/15) 6	3.85 (1/26) 1	50 (1/2) 3	-	-	90
L. manguinhosi	-	-	-	-	-	12.07 (7/58) 49	-	13.33 (2/15) 29	-	-	-	-	-	78
L. paulistanensis	-	-	-	-	-	-	-	-	6.67 (1/15) 2	-	-	-	-	2
L. thori	-	20 (1/5) 2	-	0.44 (1/225) 1	-	1.72 (1/58) 3	-	-	-	19.23 (5/26) 97	-	-	-	103
Laelaps sp.	-	-	-	1.78 (4/225) 15	-	3.45 (2/58) 5	-	-	-	15.38 (4/26) 6	-	-	-	26
Ornithonyssus sp.	50 (1/2) 2	60 (3/5) 36	-	-	-	3.45 (2/58) 4	-	6.67 (1/15) 1	-	80.77 (21/26) 278	-	-	-	321
Quadraseta brasiliensis	-	-	100 (1/1) 8	8.44 (19/225) 119	-	-	-	-	13.33 (2/15) 13	-	50 (1/2) 1	-	-	141
Q. pazca	-	-	100 (1/1) 12	6.22 (14/225) 234	-	-	-	-	13.33 (2/15) 18	-	-	-	33.33 (1/3) 2	266
TOTAL	40	39	23	4607	128	360	98	174	250	400	273	34	3	6429

SE: Sede; PR: Porto Raso; SR: Serraria

Table 3: Occurence of mites collected on marsupials in a preserved ecological area (Legado das Águas – Reserva Votorantim) inside the Atlantic Rainforest Biome, during the years of 2018 to 2021.

Mite species	Hosts (infested/captured)						
	Didelphis aurita (n=28) SE, PR, SR	*Gracilinanus sp.* (n=1) SE	*Marmosa (Micoureus) demerarae* (n=3) SE	*Marmosops sp.* (n=2) SE	*Metachirus nudicaudatus* (n=72) SE, PR	*Monodelphis sp.* (n=1) SR	TOTAL
	Infestation prevalence % (infested hosts/total hosts) number of mite specimens n						
Androlaelaps fahrenholzi	3.57 (1/28) 11	-	-	100 (2/2) 132	47.22 (34/72) 314	-	457
A. ilhacardosoi	-	-	-	-	-	100 (1/1) 1	1
Colicus spinosus	-	-	-	-	-	100 (1/1) 2	2
Eutrombicola tinami	10.71 (3/28) 11	-	-	-	-	-	11
Gigantolaelaps gilmorei	3.57 (1/28) 7	-	-	-	-	-	7
G. oudemansi	-	100 (1/1) 6	-	-	1.39 (1/72) 30	-	36
Laelaps sp.	-	-	33.33 (1/3) 3	-	1.39 (1/72) 1	-	4
TOTAL	29	6	3	132	345	3	518

SE: Sede; PR: Porto Raso; SR: Serraria

2.3.2 Molecular analysis

One hundred fifteen specimens of chigger mites were processed individually for the 18S rRNA gene, and 99 samples generated expected amplicons with the expected size, these 14 samples generated viable sequences for the species *Q. pazca* (OQ026942 to OQ0269550). The other species of chiggers did not generate viable sequences. All the 99 successful samples for the endogenous control were tested for the *gltA* gene present in all *Rickettsia* bacteria, but none showed positivity for the pathogen. Attempts to amplify fragments of the COI gene were unsuccessful in chigger mites.

From the macronyssid mites collected, 71 were processed individually for the 16S rRNA and the 18S rRNA genes; of these 55 samples amplified expected amplicons for both genes and were subsequently tested for the *gltA* gene. However, none showed positivity for the genus *Rickettsia*.

2.4 DISCUSSION

The study showed the presence of a vast acarofauna in a preserved fragment inside the Atlantic Rainforest, interacting with rodents and marsupials in three areas of the private reserve Legado das Águas – Reserva Votorantim. In addition, there was no evidence of bacteria of the genus *Rickettsia* circulating in this group of ectoparasites that can also interact with humans.

The number of chigger species found was relatively low when compared to other studies conducted in preserved areas. Here, we identified three genera and four species in four years of collection, with no new species found. Jacinavicius et al. (2020) studied a conserved unit in the Pará state (Brazil) within the caatinga biome and described four new species of chigger mites. In another study conducted with chigger mites collected in the São Paulo state, among 317 specimens examined, Jacinavicius et al. (2019) identified six genera and twelve species also retrieved from small terrestrial mammals (rodents and marsupials).

Here, we report a new locality record for the species *Colicus spinosus*. The previous records were in the checklist for chigger mites from Brazil (Jacinavicius et al., 2018a), which stated that this species was recorded in Brasilia (Federal District) and Itapevi (São Paulo) associated with Didelphimorphia. Our record was collected in the same host genera reported from Itapevi, *Monodelphis*.

The species *Eutrombicula tinami* has been described as a neglected ectoparasite already found parasitizing humans (Bassini-Silva et al., 2019) in the same biome as collected here, with reports of trombiculiasis symptoms, such as pruritus, dermatitis, and inflammatory reactions. The researchers that conducted the field experiment had similar symptoms during the collections, but these mites have reduced sizes, making it hard to find. This mite has no preferential host association and has been described as parasitizing several bird species, rodents, humans (Bassini-Silva et al., 2019), marsupials (Jacinavicius et al., 2018a), and even domestic animals that have free accession to forested areas (Cousandier et al., 2021). Here, we report the first time this mite is described as parasitizing a rodent of the genus *Guerlinguetus*.

The Neotropical genus *Quadraseta* presents a wide variety of species and is mainly associated with small terrestrial mammals (Jacinavicius et al., 2018a) and has been associated with the presence of *Orientia* bacteria in specimens collected from rodents in Chile (Silva De La Fuente et al., 2023). This research found two species belonging to this genus, *Q. pazca* and *Q. brasiliensis* parasitizing five different species of rodents. Jacinavicius et al. (2018b) contributed with the knowledge of the post-larval stages of the *Q. brasiliensis* , reporting new hosts and localities from Brazil, and Arbex et al. (2021) reported this species in Suriname in an echimyid rodent. Here, we report a new locality for this species in the Miracatu and Tapiraí municipalities (São Paulo) and also a new host interaction with *Rhipidomys* sp.

The co-parasitism among chiggers has been highlighted by many authors (Bassini-Silva; Welbourn; Ochoa, 2021; Jacinavicius et al., 2021; Stekolnikov et al., 2022) since this group of mites is not very selective in terms of hosts. Jacinavicius et al. (2023) reported co-infestation of three different species of trombiculids in the same cluster collected from a cat. Also, Jacinavicius et al. (2021) reported the same occurrence in different sites along the host body. Here, we report a case of co-parasitism of *Q. pazca* and *Q. brasiliensis* collected from an *E. russatus*, inside the ear canal of this host.

Another important observed feature is the attachment placed on the host's body. A particular preference is observed because the genus *Quadraseta* was only found inside the ear canal of the hosts. The same pattern was observed by Jacinavicius et al. (2021). The authors suggested that is no observed competition regarding the genera collected. However, it is possible that the *Quadraseta* species compete with each other, which this study corroborates. Meanwhile, the genera *Eutrombicula* and *Colicus* have been found in the torso and abdomen of the hosts.

The mites of the family Laelapidae are often associated with rodents and, more commonly, the females belonging to this family; the other stages can be found in the nest of these hosts feeding on organic matter (Netušil et al., 2013). In the present study, we collected in the hosts, besides females, males, deutonymphs, and protonymphs. The genus *Gigantolaelaps* comprises twenty species (Furman, 1972; Gettinger; Martins-Hatano; Gardner, 2011), and their main character is their large size. The most collected species was *G. oudemansi*, found in rodents and marsupials, but most of the species was collected from the rodent species *E. russatus*. Our observation corroborates the study by Gettinger (1987); he observed this same species mainly associated with the oryzomyine group (Cricetidae: Sigmodontinae). A strong association was also observed between this mite species and *G. gilmorei*; meanwhile, the species *G. wolffsohni* was not associated with other mites.

The species group complex *A. rotundus* was only found interacting with rodents of the genus *Akodon*. In Paraguay, Sánchez-Martínez and Owen (2021) observed that this rodent rathers a more preserved environment. However, we only collected this host in our most degraded area (Serraria), so the number of mite specimens belonging to this species complex was reduced. Lareschi (2020) proposed that this species-group complex probably constitutes a new genus, but since it remains a species-group, those specimens were identified as that. The same situation occurs with *A. fahrenholzi*, since it has a morphometric difference between the localities of the collection (Silva-De La Fuente et al., 2020). The species *A. ilhacardosoi* was reported only once at Ilha do Cardoso – SP by Gettinger and Martins-Hatano (2003), found in a marsupial of the genus *Monodelphis*. Here, we report this species collected from the same host genus but in a different locality, Vale do Ribeira region.

Regarding the genus *Laelaps*, we report five species mainly related to rodents and rarely to marsupials. Gettinger (1992) observed that some species of this genus are host specific such as *L. castroi*, *L. differens*, *L. manguinhosi*, and *L. thori*. However, for the *L. castroi* species, we observed a different pattern, we collected this mite in five different rodent species: *E. russatus*, *Oligoryzomys* sp., *Hylaeamys* sp. Weksler, Percequillo & Voss, 2006, *Sooretamys* sp. Weksler, Percequillo & Voss, 2006, and *Rhipidomys* sp, as opposed to Nieri-Bastos et al. (2004) that collected this species from only one host (*Oligoryzomys* sp.). The same happened with *L. differens* and *L. thori*, collected from four different rodent genera. *L. manguinhosi* was only retrieved from *Nectomys squamipes* (Brants, 1827) and *Holochilus* sp., which can be explained because both rodents shared

the same collection spots and agreed with Lareschi et al. (2006) results. These different patterns can be explained by the different biomes collected once Gettinger (1992) researched Brazil's Cerrado, and this study was conducted in the Atlantic Rainforest biome with a wide variety of hosts inhabiting the same spot. The only species we observed as a host specificity was *L. paulistanensis*, associated only with rodents from the *Oligoryzomys* sp. species, the same pattern was observed by Lareschi et al. (2006) in Uruguay and Savchenko et al. (2021) in Argentina, but not by Barros et al. (1993) who retrieved this mite from two rodent species: *Akodon montensis* Thomas, 1913, and *Oligoryzomys nigripes* (Olfers, 1818).

In this study, we report the collection of three different genera of laelapid mites comprehending eleven species in a total of 6,074 specimens, having a lower diversity when compared with the data by Gettinger et al. (2005) conducted in the Amazonian biome that presented five genera and twenty-one species with a significantly smaller number of specimens (1,014), but when compared to another study conducted in the same biome as ours (Nieri-Bastos et al., 2004), the diversity is similar, reporting four genera and ten species among 729 specimens collected. The biome difference and the abundance of preferential hosts can explain this event.

The genus *Ornithonyssus* was the only representative collected belonging to the Macronyssidae family. The species belonging to this genus have hematophagous habits. Fonseca (1948) described eleven species in Brazil, with some species already synonymized over the following years. In our study, we observed a host preference regarding this mite. The mites belonging to this family were collected from the following rodents: *Oxymycterus* sp. and *Brucepattersonius* sp, *Nectomys squamipes*, and no specimens were retrieved from marsupials. Meanwhile, Nieri-Bastos et al. (2011) reported collecting the same genera from eleven hosts in three Brazilian states. Sponchiado et al. (2015) studied the Cerrado biome and retrieved this mite only from one species of marsupial, *Gracilinanus agilis* (Burmeister, 1854). Complying with the checklist by Bassini-Silva et al. (2021), we recorded this genus in a preserved area inside the Atlantic Rainforest.

Many authors have reported the presence of *Rickettsia* DNA in mites collected from wild and synanthropic hosts belonging to the families reported in this study: Trombiculidae (Choi et al., 2007; Jacinavicius et al., 2019; Ponnusamy et al., 2022), Laelapidae (Miťková et al., 2015; Kuo; Lee; Wang, 2020) and Macronyssidae (Reeves et al., 2007). On the other hand, there was no amplification for the bacteria *Rickettsia* in our

study. One hypothesis for this result is that the rickettsiae circulating in the collected area are not able to generate infection in the sampled mite species and can also be explained by the low amount of target DNA of this bacteria, a common fact when it comes to its main vector (Serpa et al., 2021).

2.5 CONCLUSION

This study contributes to a better understanding on the relationships between ectoparasitic mites and small terrestrial mammals in the Vale do Ribeira region, inside a conservational area in the Atlantic Rainforest biome. We report new records of locality, host-parasite interactions, and the ectoparasitic fauna inside a never studied area. This study's results highlight the importance of natural areas, their inner balance, and the harmful actions that urbanization may bring. Further research on other mites-associated pathogens is essential for epidemiological public health.

2.6 REFERENCES

ARBEX, R. *et al.* New species records of *Quadraseta* Brennan, 1970 (Trombidiformes: Trombiculidae) in South America. **Entomological Communications**, v. 3, p. ec03042, 2021.

BARROS, D. M.; LINARDI, P. M.; BOTELHO, J. R. Ectoparasittes of some wild rodents from Paraná State, Brazil. **Journal of Medical Entomology**, v. 30, n. 6, p. 1068-1070, 1993.

BASSINI-SILVA, R. *et al. Eutrombicula tinami* (Oudemans, 1910) (Trombidiformes: Trombiculidae) in Brazil: a neglected ectoparasite of several animals including humans. **Acarologia**, v. 59, n. 4, p. 412–423, 2019.

BASSINI-SILVA, R. *et al.* A Checklist of Macronyssid Species (Mesostigmata: Macronyssidae) From Brazil. **Journal of Medical Entomology**, v. 58, n. 2, p. 625–633, 2021.

BASSINI-SILVA, R. *et al.* Bacterial pathogens' screening in Brazilian chigger mites (Trombidiformes: Trombiculidae), with the first report of *'Candidatus* Rickettsia colombianensi'-like in avian-associated chiggers. **Archives of Microbiology**, v. 205, n. 1, p. 1–8, 2023.

BASSINI-SILVA, R.; WELBOURN, C.; OCHOA, R. Two New Species of Chiggers (Trombidiformes: Trombiculidae) From Brazil. **Journal of Medical Entomology**, v. 58, n. 4, p. 1725–1732, 2021.

BONVICINO, C.; OLIVEIRA, J.; D'ANDREA, P. **Guia dos Roedores do Brasil:** Chaves para gêneros baseadas em caracteres externos. 1. ed. Rio de Janeiro: Centro Pan-Americano de febre Aftosa - OPAS/OMS, 2008.

BRENNAN, J. M.; GOFF, M. L. Keys to the Genera of Chiggers of the Western Hemisphere (Acarina: Trombiculidae). **The Journal of Parasitology**, v. 63, n. 3, p. 554–566, 1977.

CANÇADO, P. H. D. *et al*. Estado atual dos carrapatos em relação a seus animais-hospedeiros domésticos e selvagens do Pantanal no Estado do Mato Grosso do Sul, Brasil. **Iheringia - Serie Zoologia**, v. 107, p. 1–8, 2017.

CHOI, Y.-J. *et al*. Molecular Detection of Various Rickettsiae in Mites (Acari: Trombiculidae) in Southern Jeolla Province, Korea. Microbiology and Immunology, v. 51, n. 3, p. 307–312, 2007.

COUSANDIER, G. *et al.* First record of *Eutrombicula tinami* (Oudemans, 1910) (Trombidiformes: Trombiculidae) parasitizing a cat in Brazil. **Parasitology Research**, v. 120, n. 1, p. 337–339, 2021.

FARIA, M. B. **Os marsupiais do Brasil:** guia de identificação com base em caracteres morfológicos externos e cranianos. 1. ed. São Caetano do Sul: Amélie Editorial, 2019.

FOLMER, O. *et al*. DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. **Molecular Marine Biology and Biotechnology,** v. 3, n. 5, p. 294–299, 1994.

FONSECA, F. da. A Monograph of the Genera and Species of Macronyssidae Oudemans, 1936 (synom: Liponissidae Vitzthum, 1931) (Acari). **Proceedings of the Zoological Society of London**, v. 118, n. 2, p. 249–334, 30 ago. 1948.

FONSECA, F. Notas de Acarologia XX. Espécies novas de acarianos do gênero Laelaps, parasitas de ratos do Brasil (Acari: Laelaptidae). **Memórias do Instituto Butantan**, v. 10, p. 33–37, 1936.

FURMAN, D. P. Mites of the family Laelapidae in Venezuela (Acarina: Laelapidae). **Brigham Young University Science Bulletin, Biological Series**, v. 17, n. 3, p. 1–58, 1972.

GETTINGER, D. Host Associations of *Gigantolaelaps* (Acari: Laelapidae) in the Cerrado Province of Central Brazil. Journal of Medical Entomology, v. 24, n. 5, p. 559–565, 1987.

GETTINGER, D. Host Specificity of *Laelaps* (Acari: Laelapidae) in Central Brazil. Journal of Medical Entomology, v. 29, n. 1, p. 71–77, 1992.

GETTINGER, D. *et al.* Laelapine Mites (Acari: Laelapidae) associated with small mammals from Amazonas, Brazil, including a new species from marsupials. Journal of Parasitology, v. 91, n. 1, p. 45–48, 2005.

GETTINGER, D.; MARTINS-HATANO, F. A new species of Neotropical Laelapine Mite (Acari: Parasitiformes, Laelapidae) from the marsupial *Monodelphis americana.* **Journal of Parasitology,** v. 89, n. 5, p. 909–912, 2003.

GETTINGER, D.; MARTINS-HATANO, F.; GARDNER, S. L. Some Laelapine Mites (Acari: Laelapidae) Ectoparasitic on Small Mammals in the Galapagos Islands, Including a New Species of *Gigantolaelaps* from *Aegialomys galapagoensis*. Journal of Parasitology, v. 97, n. 4, p. 574–576, 2011.

GUEDES, E. *et al*. Detection of *Rickettsia rickettsii* in the tick *Amblyomma cajennense* in a new Brazilian spotted fever-endemic area in the state of Minas Gerais. **Memórias do Instituto Oswaldo Cruz**, v. 100, n. 8, p. 841–845, 2005.

HERRERA-MARES, A. *et al*. Mites, rodents, and pathogens: A global review for a multi-species interaction in disease ecology. **Acta Tropica**, v. 232, n. May, p. 106509, 2022.

JACINAVICIUS, F. C. *et al*. Three chigger species (Trombidiformes: Trombiculidae) co-parasitizing a domestic cat in Brazil, including pathogen monitoring. International **Journal of Acarology**, v. 49, n. 2, p. 120–127, 2023.

JACINAVICIUS, F. C. *et al*. Evidence of co-parasitism between different species of chiggers (Trombidiformes: Trombiculidae) parasitizing cricetid rodents in Morro Grande Forest Reserve, São Paulo State, Brazil. **Entomological Communications**, v. 3, n. 3, p. ec03043, 2021.

JACINAVICIUS, F. C. *et al*. New Species of Chiggers (Trombidiformes: Trombiculidae and Leeuwenhoekiidae) From the Conservation Unit Parque Nacional da Serra das Confusões, Brazil. **Journal of Medical Entomology**, v. 58, n. 1, p. 286–297, 2020.

JACINAVICIUS, F. C. *et al.* Molecular detection of *Rickettsia* genus in chigger mites (Trombidiformes: Trombiculidae) collected on small mammals in southeastern brazilian. **Revista Brasileira de Parasitologia Veterinária**, v. 28, n. 4, p. 563–568, 2019.

JACINAVICIUS, F. C. *et al*. A contribution to the knowledge of *Quadraseta brasiliensis* Goff and Gettinger, 1989 (Trombidiformes: Trombiculidae), with description of the deutonymph instar. **Acarologia**, v. 58, n. 2, p. 442–456, 2018a.

JACINAVICIUS, F. C. *et al*. A checklist of chiggers from Brazil, including new records (Acari: Trombidiformes: Trombiculidae and Leeuwenhoekiidae). **ZooKeys**, v. 743, n. 743, p. 1–41, 2018b.

KUO, C. C.; LEE, P. L.; WANG, H. C. Molecular detection of *Rickettsia* species and host associations of *Laelaps* mites (Acari: Laelapidae) in Taiwan. **Experimental and Applied Acarology,** v. 81, n. 4, p. 547–559, 2020.

LABRUNA, M. B. *et al*. *Rickettsia* Species Infecting *Amblyomma cooperi* Ticks from an Area in the State of São Paulo, Brazil, Where Brazilian Spotted Fever Is Endemic. **Journal of Clinical Microbiology**, v. 42, n. 1, p. 90–98, 2004.

LARESCHI, M. *et al*. First report of mites (Gamasida: Laelapidae) parasitic on wild rodents in Uruguay, with new host records. **Neotropical Entomology**, v. 35, n. 5, p. 596–601, 2006.

LARESCHI, M. Three new species of Laelapidae mites (Mesostigmata) parasitic of species of *Akodon* (Rodentia: Cricetidae: Sigmodontinae) on the basis of female, male and deutonymph specimens. Veterinary Parasitology: Regional Studies and Reports, v. 22, p. 100500, 2020.

LEGADO DAS ÁGUAS. **A sustentabilidade de uma reserva.** 1. ed. São Paulo: Ipsis Gráfica e Editora, 2018.

LINHARES DE REZENDE, C. *et al.* Atlantic Forest spontaneous regeneration at landscape scale. **Biodiversity and Conservation**, v. 24, p. 2255–2272, 2015.

MANGOLD, A. J.; BARGUES, M. D.; MAS-COMA, S. Mitochondrial 16S rDNA sequences and phylogenetic relationships of species of Rhipicephalus and other tick genera among Metastriata (Acari: Ixodidae). **Parasitology Research**, v. 84, n. 6, p. 478–484, 1998.

MAŠÁN, P.; FENDA, P. **A Review of the Laelapid Mites Associated with Terrestrial Mammals in Slovakia, with a Key to the European Species**. Institute of Zoology, Slovak Academy of Sciences: Bratislava, Slovakia, 2010.

MIŤKOVÁ, K. *et al*. First detections of *Rickettsia helvetica* and *R. monacensis* in ectoparasitic mites (Laelapidae and Trombiculidae) infesting rodents in south-western Slovakia. **Parasitology Research**, v. 114, n. 7, p. 2465–2472, 2015.

MITTERMEIER, R. A. *et al*. Atlantic Forest. In: **Hotspots:** Earth's biologically richest and most endangered terrestrial ecoregions. Mexico City: Cemex, 2004. p. 84–92.

NETUŠIL, J. *et al.* The occurrence of *Borrelia burgdorferi sensu lato* in certain ectoparasites (Mesostigmata, Siphonaptera) of *Apodemus flavicollis* and *Myodes glareolus* in chosen localities in the Czech Republic. Acta Parasitologica, v. 58, n. 3, p. 337–341, 2013.

NIERI-BASTOS, F. A. *et al.* Ectoparasites of Wild Rodents From Parque Estadual Da Cantareira (Pedra Grande Nuclei), São Paulo, Brazil. **Revista Brasileira de Parasitologia Veterinária**, v. 13, p. 29–35, 2004.

NIERI-BASTOS, F. A. *et al.* Morphological and molecular analysis of *Ornithonyssus* spp. (Acari: Macronyssidae) from small terrestrial mammals in Brazil. **Experimental and Applied Acarology**, v. 55, n. 4, p. 305–327, 2011.

OTTO, J. C.; WILSON, K. J. Assessment of the usefulness of ribosomal 18S and mitochondrial COI sequences in Prostigmata phylogeny. (H. C. Proctor, R. A. Norton, M. J. Colloff, Eds.) In: **Acarology**: Proceedings of the 10th International Congress, Melbourne: CSIRO Publisher, 2001.

PONNUSAMY, L. *et al. Rickettsia felis* and Other *Rickettsia* Species in Chigger Mites Collected from Wild Rodents in North Carolina, USA. **Microorganisms**, v. 10, n. 7, p. 1342, 2022.

RADOVSKY, F. J. **Revision of genera of the parasitic mite family Macronyssidae (Mesostigmata: Dermanyssoidea) of the world.** West Bloomfield: Indira Publishing House, 2010.

REEVES, W. K. *et al.* Rickettsial pathogens in the tropical rat mite *Ornithonyssus bacoti* (Acari: Macronyssidae) from Egyptian rats (*Rattus* spp.). **Experimental and Applied Acarology**, v. 41, n. 1–2, p. 101–107, 2007.

SÁNCHEZ-MARTÍNEZ, J. P.; OWEN, R. D. Population characteristics of *Akodon montensis* (Sigmodontinae) in response to habitat degradation and food availability. Therya, v. 12, n. 1, p. 5–13, 2021.

SAVCHENKO, E.; MELIS, M.; LARESCHI, M. Laelapid mites (Mesostigmata) ectoparasites of *Oligoryzomys* (Rodentia: Cricetidae) in north-eastern and central Argentina. **Mastozoología Neotropical**, v. 28, n. 1, p. 001–012, 2021.

SERPA, M. C. de A. *et al.* Small mammals, ticks and rickettsiae in natural and human-modified landscapes: Diversity and occurrence of Brazilian spotted fever in Brazil. **Ticks and Tick-borne Diseases**, v. 12, n. 6, p. 101805, 2021.

SILVA-DE LA FUENTE, M. C. *et al*. Morphometric variation of *Androlaelaps fahrenholzi* (Mesostigmata: Laelapidae) associated with three Sigmodontinae (Rodentia: Cricetidae) from the north of Chile. **Experimental and Applied Acarology**, v. 81, n. 1, p. 135–148, 2020.

SILVA DE LA FUENTE, M. C. *et al*. Eco-epidemiology of rodent-associated trombiculid mites and infection with *Orientia* spp. in Southern Chile. **PLOS Neglected Tropical Diseases**, v. 17, n. 1, p. e0011051, 12 jan. 2023.

SOUZA, U. A. *et al.* Ticks, mites, fleas, and vector-borne pathogens in free-ranging neotropical wild felids from southern Brazil. **Ticks and Tick-borne Diseases**, v. 12, n. 4, p. 101706, 2021.

SPONCHIADO, J. *et al.* Interaction of ectoparasites (Mesostigmata, Phthiraptera and Siphonaptera) with small mammals in Cerrado fragments, western Brazil. **Experimental and Applied Acarology,** v. 66, n. 3, p. 369–381, 2015.

STEKOLNIKOV, A. A. *et al.* Distribution of chigger mites (Acari: Trombiculidae) over hosts, parasitopes, collection localities, and seasons in northern Iran. **Experimental and Applied Acarology,** v. 86, n. 1, p. 21–47, 2022.

VIDAL-MARTÍNEZ, V. M.; WUNDERLICH, A. C. Parasites as bioindicators of environmental degradation in Latin America: A meta-analysis. **Journal of Helminthology,** v. 91, n. 2, p. 165–173, 27 mar. 2017.

WALTER, D. E.; PROCTOR, H. C. What Good are Mites? In: **Mites:** Ecology, Evolution & Behaviour: Life at a Microscale. Dordrecht: Springer Netherlands, 2013. p. 1–11.

3 CHAPTER 2 – BRAZILIAN FLEAS (HEXAPODA: SIPHONAPTERA): DIVERSITY, HOST ASSOCIATIONS AND NEW RECORDS ON SMALL MAMMALS FROM THE ATLANTIC RAINFOREST

3.1 INTRODUCTION

The order Siphonaptera Latreille 1825 belongs to the class Insecta. It presents a similar morphology to other arthropods of this group, with three pairs of legs and a body divided into a head, thorax (with three segments), and abdomen. This order's main differences are that they have flattened dorsolaterally, hind legs adapted for jumping, and the absence of wings. They are tiny insects with a brownish color and may or may not have ctenids, which are structures used for attachment to the host (Medvedev; Krasnov, 2006).

The fleas are holometabolous, and under ideal conditions, the cycle is completed in approximately 30 days. The stages are egg, larva (with three instars), pupa, and adults. Sexual dimorphism between males and females is present, with females larger than males. In the adult stage, the mouthparts are sucking-pungitive, performing a solenophagous blood meal on their hosts (Linardi, 2011). Adults are usually found on the hosts or in their nests, especially after they have fed, as the female needs this factor for ovarian maturation and oviposition (Korine et al., 2012). While the larvae are legless and vermiform, they have well-developed chewing-type mouthparts because the basis of their diet is organic matter, including adult feces. (Almeida et al., 2020).

Fleas mainly parasitize mammals mostly associated with rodents and marsupials, with a minority associated with birds (Lareschi et al., 2018). The parasite-host relationship can be classified as specific (monoxene and oligoxene) or eclectic (polyxene), and the fleas are well adapted to the microclimate and characteristics of the host's body and coat. The fleas considered eclectic are in the minority (Medvedev; Krasnov, 2002). However, they are the ones that constitute the biggest problem in public health because they exchange hosts and may be present in populations of wild and synanthropic rodents that live close to urban areas and accidentally can bite humans (Lewis, 1998).

The fleas have a worldwide distribution, including Antarctica, where *Glaciopsyllus antarticus* Smit and Dunnet 1962 (Ceratophyllidae) was described as parasitizing birds (Murray; Orton; Cameron, 2013). The greater diversity of species is

observed in the temperate region (Whiting et al., 2008), and it is believed that this group has existed since the Eocene period after some researchers found evidence in fossils of mammals from this period (De Lima; Porpino, 2018).

Regarding their permanence on the host, fleas can be subdivided into three categories. (i) Penetrating fleas introduce the head, thorax, and part of its abdomen into the epidermis of its host, forming a neosome around it as it feeds and is filled with blood. After entirely feeding, it lays eggs in the environment and finally dies inside the host. An example of this habit is the species *Tunga penetrans* Linnaeus 1758 (Tungidae) (Nagy et al., 2007). (ii) The fleas of the genus *Ctenocephalides* Stiles and Collins 1930 (Pulicidae: Archaeopsyllinae) live most of the time on their hosts, even while not feeding (Dryden; Gaafar, 1991). (iii) The last observed behavior is fleas that climb on their hosts only to feed but spend most of their adult stage living in mammalian nests, as observed in the genus *Pulex* Linnaeus 1758 (Pulicidae: Pulicinae) (Zurita et al., 2019).

These ectoparasites can cause several disorders in their hosts, causing itching, discomfort, and dermatitis, spoliative actions that are observed in large infestations that can lead the host to profound anemia and death, in addition to inflammatory actions observed in infestations by penetrating fleas or semi-penetrating that act as a gateway for opportunistic pathogens, such as bacteria present on the surface of the skin (Durden; Hinkle, 2019). In addition, fleas are of great medical importance because they act as vectors or intermediate hosts of a wide range of pathogens such as viruses, bacteria, protozoa, and helminths that can cause death in animals and humans (Bitam et al., 2010; Lappin; Tasker; Roura, 2020).

Currently, more than 3000 species are known and grouped into 240 genera distributed throughout the world (Lewis, 1998). In Brazil, there are about 223 species, of which 115 are mainly found interacting with rodents and marsupials, regarding the bats, besides the generalist fleas, there is also a family that interacts specifically with this host group. We reviewed the eight leading families, including the endemic species, through a detailed bibliographic revision, and additionally contributed with new records of fleas collected on small mammals from a private reserve located within the Atlantic Rainforest in the Brazilian southeastern.

3.2 MATERIALS AND METHODS

3.2.1 Review

This review was carried out from April 2022 to October 2023 and based on the search for articles available in the PubMed and Google Scholar databases, using keywords in Portuguese and English and often using combined words such as “flea AND Brazil,” “Brazil AND Siphonaptera” and “pulgas AND Brasil.” Some works were found through the diseases that these arthropods can transmit (bubonic plague and murine typhus). Most of the references used were based on the work of two great Brazilian researchers (Dr. Pedro Marcos Linardi and Dr. Lindolpho Rocha Guimaraes), whose work was mainly focused on the Order Siphonaptera. This study's nomenclature and taxonomic division follow the study made by Linardi and Guimarães (2000).

3.2.2 Specimens’ collection

The fleas examined were collected in the private reserve Legado das Águas - Reserva Votorantim, Miracatu, São Paulo, Brazil, located in a fragment of Atlantic Forest, with approximately 75% of the total area composed of dense primary ombrophilous forest. Within the perimeter of the reserve, three areas were chosen for capture: Sede (24° 1' 49.51" S, 47° 21' 8.36" W), Porto Raso (24° 3' 25.90" S, 47° 26' 30.07" W) and Serraria (24° 9' 9.63" S, 47° 32' 53.49" W).

Between January 2018 and December 2021, eight campaigns were conducted, lasting an average of seven to twelve days, with three campaigns in the Sede area (January, July and December of 2018 with six days each), three in Porto Raso (July of 2019, February of 2020 and October of 2021 with six days each), and two longer ones in Serraria (September and December of 2022 with nine days each) due to the COVID-19 pandemic resulting in a total of 18 days in each sampled area.

The trails chosen were based on the vegetation and tracks of wild animals. In total, 240 traps (Sherman and Tomahawk traps) were used in each campaign, and the small terrestrial mammals were captured using bait made with a mixture of sardines, cornmeal, coconut oil, vanilla, and peanut paste.

For the bat captures, were used mist nets with 3,0 X 6,0 meters long, placed at the sunset and kept open for four hours during the sampling nights. For some bat species, an active search was performed at the shelters and the animals were carried inside black fabric bags to the field laboratory.

After capture, the animals were anesthetized with ketamine hydrochloride (15-30mg/kg), and following sample collection and recovery, the rodents, marsupials, and bats were identified using taxonomic keys (Gregorin; Taddei, 2002; Bonvicino; Oliveira; D'Andrea, 2008; Faria, 2019) then returned to the wild at the same site of capture. All fleas were collected using tweezers and stored in a microtube containing absolute ethanol.

3.2.3 Flea Morphological identification

After collection, the samples were sent to the Laboratório de Coleções Zoológicas of the Instituto Butantan (LCZ-IB) for identification. The fleas were clarified using a potassium hydroxide solution of 10% and slide-mounted using Hoyer's medium. Only one or two fleas were chosen from each batch collected from the hosts as a method of sampling. We used Linardi and Guimarães (2000) for the identification of genera.

Images and measurements were taken using a Leica DM4000B microscope and compiled with Leica Application Suite version 2.5.0. All slide-mounted specimens were deposited in the Entomological Collection at the Laboratory of Zoological Collections of the Butantan Institute (LCZ-IB) under the accession numbers IBSP-Ent 14641 - IBSP-Ent 14965.

3.2.4 Molecular analysis

Part of the collected fleas were stored for molecular analysis. Each flea was individualized in a microtube and submitted to DNA extraction using the commercial DNeasy Blood and Tissue Kit (Qiagen®). The protocol suggested by the manufacturer was followed. After DNA extraction, each exoskeleton was recovered from the columns and slide-mounted for identification following the steps previously described.

From the extracted DNA, conventional PCRs were performed using primers that amplify an endogenous gene of the flea, mitochondrial cytochrome oxidase II (COII), using the primers F-Leu and R-Lys that amplify a fragment of 612bp, as described by Whiting (2002).

Samples positive for the endogenous gene were considered viable and thus screened for bacteria of the genus *Rickettsia*. It was performed a real-time polymerase chain reaction (qPCR) using the primers CS-5 and CS-6 and including an internal probe, to amplify a 147 bp fragment of the *gltA* gene, present in all bacteria of this genus. This

reaction was performed following Labruna et al. (2004) and Guedes et al. (2005) protocols. All reactions included positive (DNA extracted from cell culture infected with *Rickettsia vini*) and negative (ultrapure water type I) controls.

3.2.5 Ethical statement

The Ethics Committee of the Faculty of Veterinary Medicine and Zootechny of the University of São Paulo (FMVZ-USP) under the number 6509131119 approved the present study.

3.3 RESULTS

3.3.1 Literature review

The data of this study were extracted from about 200 relevant articles in English and Portuguese with the publishing dates ranging from 1915 to 2023. Only articles available on the Internet were included in the study, older articles available only in printed form were disregarded. Some species are vastly described in literature, for this reason the studies were limited by host or locality descriptions. The reports showed the presence of fleas through the 26 states of Brazil, including the Federal District (DF). The figure 4 represents the localities of families and subfamilies of fleas in the Brazilian states with the literature records and the new records of this study.

Family Ceratophyllidae Dampf, 1908

This family has 44 genera worldwide, but only the species *Nosopsyllus fasciatus* (Bosc, 1800), occurs in Brazil and has been associated with wild and synanthropic rodents (Lewis, 1993). It can also erratically parasitize dogs and humans, assuming a potential role as a vector of bacteria pathogens (Špitalská et al., 2015; Durden; Hinkle, 2019), as well as protozoa pathogens (Molyneux, 1969). Nowadays, this genus is considered cosmopolitan due to human locomotion and consequent accidental transport of rodents (Lewis, 1993).

Only the species *Oropsylla montana* (Baker, 1895) (Ceratophyllinae) stands out regarding health importance. This species has been proven to be able to maintain the

bacteria *Yersinia pestis* at low temperatures in North America, correlated with a possible role in the epidemiology of plague and the Tropical regions (Williams et al., 2013).

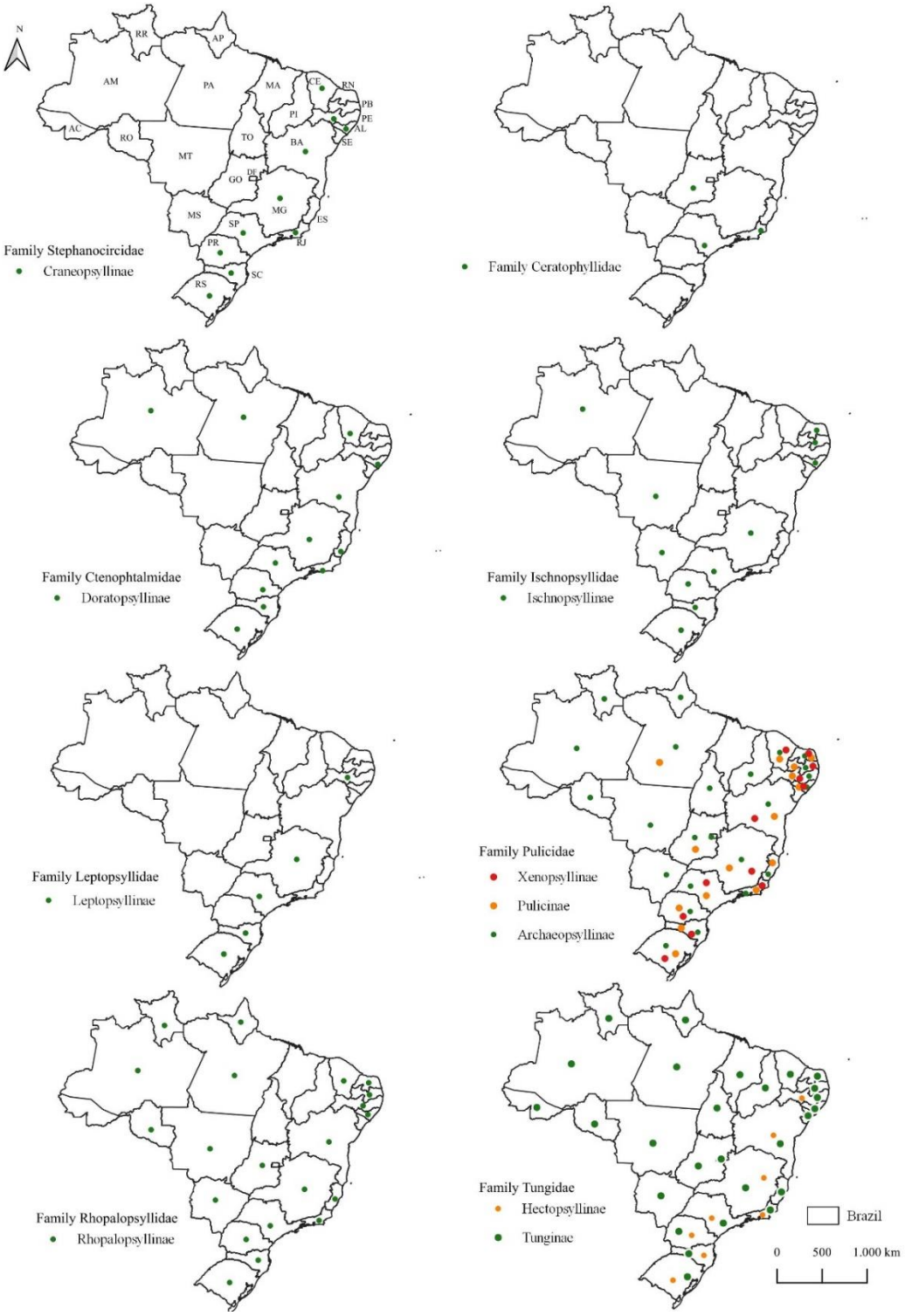

Figure 5: Map showing the distribution of families and subfamilies of fleas in Brazil. States as represented in the map. AC: Acre, AL: Alagoas, AM: Amazonas, AP: Amapá, BA: Bahia, CE: Ceará, DF: Distrito Federal, ES: Espírito Santo, GO: Goiás, MA: Maranhão, MG: Minas Gerais, MS: Mato Grosso do Sul, MT: Mato Grosso, PA: Pará, PB: Paraíba, PE: Pernambuco, PI: Piauí, PR: Paraná, RJ: Rio de Janeiro, RN: Rio Grande do Norte, RO: Rondônia, RS: Rio Grande do Sul, RR: Roraima, SC: Santa Catarina, SE: Sergipe, SP: São Paulo, TO: Tocantins.

Family Ctenophtalmidae Rothschild, 1915

The Ctenophtalmidae is composed of nine subfamilies, 17 tribes, and 42 genera that contain about 600 species. About 25% of the flea species described are included in this family. However, new species described that are difficult to classify are also grouped in this family, and because of this, this family may be considered a paraphyletic taxon (Whiting et al., 2008).

The Neotropical genus *Adoratopsylla* Ewing 1925 (Doratopsyllinae: Tritopsyllini) is the only one of this family that occurs in Brazil, and parasite preferably marsupials of the subfamily Didelphinae Gray 1821, but it has already been found in cricetid and sigmodontine rodents (Lareschi et al. al., 2010; Oliveira et al., 2010; Urdapilleta; Linardi; Lareschi, 2019; Durden et al., 2021). This genus is divided into the subgenera *Tritopsylla* Cunha 1914 and *Adoratopsylla*, and five species have been recorded in Brazil: *A. (A.) antiquorum antiquorum* (Rothschild 1904), *A. (A.) antiquorum ronnai* Guimarães 1954, *A. (A.) bisetosa* Ewing 1925, *A. (T.) intermedia intermedia* (Wagner 1901), and *A. (T.) sinuata* Guimarães 1945 (Barros-Battesti; Arzua, 1997; Beaucournu; Reynes; Vié, 1998; Pinto et al., 2009). Until now, there are no reports in the literature of fleas of this genus acting as vectors of pathogens of importance in public health or veterinary medicine.

Family Ischnopsyllidae Tiraboschi, 1904

The Ischnopsyllidae are bat-ectoparasites, associated mainly with the families Vespertilionidae Gray 1821, Molossidae Gervais 1856, and Rhinolophidae Gray 1825, presenting a very high specificity concerning their host, and due to this association, these fleas have a wide geographic distribution (Lewis, 1993; 1994a). There is a hypothesis that insectivorous bats would be more susceptible to infestation because they use caves and tree hollows as shelter, which would be more suitable developing the immature stages of these fleas (Johnson, 1957).

The knowledge about the life cycle of the species belonging to this family is still limited, knowing that the larva has three larval instars and the adults are not known for their ability to jump long distances but for their high climbing ability, mainly because its initial stages develop in the accumulation of bat feces, and when they leave the pupa, the adults have to climb the walls of the shelters to find the hosts (Smith; Clay, 1988; Liang; Houyong, 2003; Hastriter et al., 2017).

There are 20 genera, five tribes, and two subfamilies, and these representatives can be found on all continents except Antarctica (Lewis, 1993). Of the 20 known genera, only five are described in Brazilian territory, distributed among bat species with different eating habits and habitats, spread across all five regions of the country. Supplementary Chart 1 demonstrates these genera, highlighting the species of fleas and hosts and the record status of occurrences, including our new record towards this family.

Family Leptopsyllidae Baker, 1904

Fleas of the Leptopsyllidae are mainly found parasitizing rodents and sometimes associated synanthropic rodents, such as *Rattus rattus* Linnaeus 1758, *R. norvegicus* (Berkenhout 1769), and *Mus musculus* Linnaeus 1758, as well as wild rodents, but also can be found parasitizing lagomorphs and carnivores (Maleki-Ravasan et al., 2017; Keskin; Hastriter; Beaucournu, 2018). In Brazil, this family is represented by a single genus called *Leptopsylla* Jordan & Rothschild 1911, within nine valid species, and just one is cosmopolitan and already recorded in Brazil - *Leptopsylla segnis* (Schönherr, 1811). The distribution of this species is related to rodents present on ships, similar to what happened with the genus *Xenopsylla* Glinkiewicz 1907.

Some studies have reported that the species *L. segnis* naturally infected with *Rickettsia typhi* parasitizing *R. rattus.* Experimental studies corroborated the vectorial potential of *L. segnis* for this pathogen, which is sometimes more effective than *X. cheopis* (Rothschild, 1903), as it settles for a long period in its host, favoring a high concentration of rickettsiae (Azad; Traub, 1987; Christou et al., 2010). However, this species has little importance because they hardly parasite humans (Bacellar et al., 2006). Besides that, *Bartonella* bacteria was also detected in this species (Loftis et al., 2006).

Studies that aim at infectious agents of public health importance in members of this family are needed to elucidate these insects' participation as vectors better, map the ecosystem where it is found, and avoid possible outbreaks.

Family Pulicidae Billberg, 1820

Pulicidae is one of the most studied flea families in the world because it is considered a family of high relevance for medicine and veterinary medicine, harboring generalist species that were able to infest humans and companion animals, being able to act as vectors of pathogens (Linardi; Guimarães, 2000). This family includes around 21 genera divided into four subfamilies: Pulicinae Billberg, 1820, Xenopsyllinae

Glienkiewicz, 1907, Archaeopsyllinae Oudemans, 1909, and Spylopsyllinae, the first three subfamilies can be found in Brazil (Lewis, 1998; Linardi; Guimarães, 2000).

Nonetheless, Pulicidae and Tungidae were treated as a single group (Lewis, 1998). However, based on phylogenetic analyses, it is possible to confirm Pulicidae as a monophyletic group distinct from Tungidae (Whiting et al., 2008). On the other hand, Krasnov, et al. (2015) suggested that the Pulicidae and Leptopsyllidae families should be grouped based on phylogenetic analyses, their origin, and migration in the Nearctic region. However, this new classification is still being studied.

The subfamily Pulicidae is represented by the tribes Pulicini and Echidnophagini, the latter being represented only by the genus *Echidnophaga* Olliff, 1886. In contrast, the first tribe has four genera: *Pulex* Linnaeus, 1758, *Delopsylla* Jordan, 1926, *Juxtapulex* Wagner, 1933, and *Moeopsylla* Rothschild, 1908 (Zurita et al., 2019). Of these, only the genus *Pulex* is cosmopolitan and, consequently, also found in Brazil.

The genus *Echidnophaga* has been reported in several countries in South America, including Brazil, Chile, and Peru, and the only species found in these places is *Echidnophaga gallinacea* (Westwood, 1875). The adult female is known to have semi-penetrating habits, attached to the host through its mouthparts during feeding (Aboulaila; Menshawy, 2020). This species infests birds of the orders Galliformes (Koehler; Pereira; Kaufman, 1991), Anseriformes (Waruiru et al., 2017), and Columbiformes (Rezaei et al., 2016). However, they can also be present in mammals such as rabbits (Shepherd; Edmonds, 1978), dogs (Changbunjong et al., 2009), cats (Changbunjong et al., 2009), rats (Guernier et al., 2014), and wild carnivores (López-Pérez et al., 2018).

These species can cause anemia, skin ulcers, and itching, and they have been found harboring several pathogens, such as *Rickettsia felis* and *R. asembonensis* (López-Pérez et al., 2021), *Bartonella rochalimae* (López-Pérez et al., 2017), fowl pox (Gustafson et al., 1997) and *Yersinia pestis* (Wheeler; Douglas; Evans, 1941).

The genus *Pulex* is also commonly found in the Neotropics, but the only species reported in Brazil is *Pulex irritans* Linnaeus, 1758. This species can be associated with rodents, bats, and birds but has a preference for large wild and domestic mammals, such as dogs and cattle, and sometimes bite humans (Trembley; Bishopp, 1940; Buckland; Sadler, 1989; Moemenbellah-Fard et al., 2016).

Pulex irritans is one of the first to be identified and one of the most studied species in terms of public health because they harbor several pathogens, such as *Bartonella* spp. (Fontalvo et al., 2017) and *R. felis* (SAckal et al., 2008). As much as this

species is susceptible to infection by *Y. pestis*, its vectorial competence is low, already proven through experimental infection, and it is unlikely that this species has any epidemiological relevance in the Black Plague cycle (Miarinjara et al., 2021). In addition, other pathogens have already been detected in this species, such as *Hymenolepis microstoma* Dujardin, 1845 and *Dipylidium caninum* Linnaeus, 1758, but the epidemiological importance of this species in the cycle of these endoparasites is not known (Hu et al., 2021).

The subfamily Xenopsyllinae is composed of seven genera that are distributed in the African and Asian continents, and the genus *Xenopsylla* Glienkiewicz, 1907 is the one of greatest concern in human medicine and in Brazil there are two species already described: *X. cheopis* (Rothschild, 1903) and *X. brasiliensis* (Baker, 1904). It is believed that both species originate from the African continent and after this became cosmopolitan through rodents sheltered on ships during the colonization period (Faccini-Martínez; Sotomayor, 2014; Dean et al., 2018).

The species *X. cheopis*, considered the most efficient vector for *Y. pestis*, is frequently found in several regions of Brazil, parasitizing wild animals and synanthropic rodents (Salvador et al., 2007; Souza et al., 2021). In addition, this bacterium can happen in two types of ecosystems: 1) in anthropized environments, with rodents of the genus *Rattus* and *Mus* that harboring the vector and disseminating the pathogen to companion animals and humans, this cycle is called urban plague; 2) in rural or wild environments, far from large cities, where the vector is maintained in small wild mammals (mainly rodents and marsupials), called wild plague (Durden; Hinkle, 2019).

Inside the flea, the bacteria *Y. pestis* causes a proventricular block that prevents the feeding of the arthropod, with a regurgitation of a blood in the place of blood meal that carries the bacteria that will infect the host (Bacot; Martin, 1915; Eisen et al., 2006). The absence of feeding by the vector generates an aggressive behavior with repeated attempts to feed on different hosts, favoring pathogen's spread. This fact makes *Y. pestis* pathogenic for both, the vertebrate and invertebrate hosts, as the lack of food will lead to flea dehydration, anorexia and death (Hinnebusch, 2005; Hinnebusch; Erickson, 2008; Silva-Rohwer et al., 2021).

In addition to *Y. pestis*, the flea *X. cheopis* also participates as a vector in the transmission cycle of murine typhus or endemic typhus, caused by the bacterium *Rickettsia typhi*. This *Rickettsia* needs mammals and the arthropod vector to maintain itself in the environment, and unlike the *Y. pestis* cycle, this bacterium is transmitted

through flea feces that are deposited on the host's skin (Azad, 1990; Civen; Ngo, 2008; Dumler; Walker, 2017). Once the flea acquires the bacteria through feeding on infected mammals, *R. typhi* penetrates the intestinal epithelium and after ten days of infection the insect is already capable of transmitting. In addition to infecting this system, it also infects the muscle layer and reproductive organs, enabling transovarian perpetuation, not being lethal to the invertebrate host or its progeny (Azad et al., 1997; Blanton; Walker, 2017).

The subfamily Archaepsyllinae contains five genera, but the only one that occurs in Brazil, the genus *Ctenocephalides* Stiles & Collins, 1930, that have two species recorded nationwide: *C. canis* (Curtis, 1826) and *C. felis felis* (Bouché, 1835), both of which are highly relevant in veterinary medicine. The species *C. canis* has a more specific character in relation to the hosts, being of rare occurrence and found parasitizing only carnivores and its distribution is more related to regions of temperate climate (Linardi; Nagem, 1973; De Castro; Rafael, 2006; Stalliviere et al., 2009). On the other hand, the species *C. felis felis* is highly generalist, being able to parasitize a wide range of hosts, such as carnivores, xenarthrans, rodents, marsupials, primates and lagomorphs (Linardi; Guimarães, 2000; Clark et al., 2018).

The allergic dermatitis syndrome to ectoparasite stings is a set of clinical signs commonly observed in dogs and cats, with alopecia in the hip region being the most common symptomatology caused by the species of *Ctenocephalides*. This syndrome was related only to flea bites, but has recently been described in the parasitism of several ectoparasites and triggered by a type I (immediate) and IV (late) hypersensitivity reaction to proteins present in the saliva of these arthropods (Briand et al., 2019; Forster; Wiseman; Snyder, 2021).

The flea *C. felis felis* has been found infected with the bacterium *Rickettsia felis*. This bacterium belonged to the spotted fever group, but in recent studies it was reclassified to the transitional group through molecular data and phylogenetic analysis (Ogata et al., 2005; Gillespie et al., 2007). Also, *Rickettsia felis* has already been evidenced in other species of fleas collected throughout Brazil (Horta et al., 2014), and its symptomatology in humans is still debatable. However, there are some reports of febrile episodes along with the development of a lesion at the site of the bite of the ectoparasite, and in more severe cases, it can cause neurological injuries such as encephalopathy, cerebral edema, and meningoencephalitis (Zavala-Velázquez et al., 2000; Ye et al., 2021; Teng et al., 2022). Other infectious agents that are related to the cat

flea are *Bartonella* spp. (Schott et al., 2019), *D. caninum* (DE AVELAR et al., 2007) and *Dipetalonema reconditum* (Grassi, 1890) (Linardi, 2002).

Family Rhopalopsyllidae Oudemans, 1909

Among the eight families of the Order Siphonaptera that occur in Brazil, this is the one with the highest endemicity of species and can be considered the most important for the Brazilian territory. It is divided into two subfamilies, Rhopalopsyllinae Oudemans, 1909 and Parapsyllinae Enderlein, 1903, concentrated in the Neotropical regions. Furthermore, only the first is recorded in Brazil (Lewis; Lewis, 1994b; Beaucournu et al., 2013).

The subfamily Rhopalopsyllinae comprises eight genera, five of which occur in Brazil: *Gephyropsylla* Barrera, 1952, *Hechtiella* Barrera, 1952, *Neotropsylla* Linardi & Guimarães, 1993, *Polygenis* Jordan, 1939, and *Rhopalopsyllus* Baker, 1905. The other three species are: (1) *Scolopsyllus*, which presents a single species, *S. columbianus* Mendez, 1968, described in Colombia parasitizing rodents of the genus *Euryoryzomys* Weksler, Percequillo & Voss, 2006 and appears to be restricted to this locality (Méndez, 1968); (2) *Ayeshaepsylla* Smit, 1987, formerly considered as *Polygenis*, and is also monotypic having only *A. thurmani* Traub, 1972 described (Linardi; Guimarães, 1993); (3) *Tiamastus* Jordan, 1939, that contains seven species distributed in several countries in South America, except Brazil (Linardi; Guimarães, 1993).

The genus *Gephyropsylla* is rarely described in the literature, but it is related to parasitism in small rodents hystricomorphs when mentioned. This genus has just one species, *G. klagesi* (Rothschild, 1904), divided into three subspecies, all recorded in the Neotropical Regions. According to the literature, the center of dispersion of this species is in the territory of Venezuela, as it has the occurrence of all described subspecies (Pucu; Lareschi; Gardner, 2014). The most generalist subspecies is *G. klagesi samuelis* (Jordan & Rothschild, 1923) that have been recorded parasitizing species of the following orders: Rodentia, Didelphimorphia, Carnivora, Edentata, Chiroptera, Artiodactyla and Sciuromorpha (Linardi; Guimarães, 2000).

Mainly parasitizing rodents of the family Echimyidae Gray, 1825, the genus *Hechtiella* has three species described to the Brazilian Atlantic Forest - *H. lakoi* (Guimarães, 1948), *H. lopesi* Guimarães & Linardi, 1993, *H. nitidus* (Johnson, 1957) (Guimaräes; Linardi, 1993; Bittencourt; Rocha, 2003). All parasitizing species of the genus *Proechimys* Allen, 1899 (Guimaräes; Linardi, 1993; Bittencourt; Rocha, 2003).

The monotypic genus *Neotropsylla* (*Neotropsylla guimaraesi* (Linardi, 1978)) is considered endemic to the state of São Paulo, Brazil, associated with cricetid rodents (Linardi et al., 1991; Linardi; Guimarães, 1993; Linardi, 2011).

The genus *Polygenis* has the largest number of species within the Family Rhopalopsyllidae and its distribution ranges from the southern tip of South America to the United States, being divided into two subgenera: *Polygenis* and *Neopolygenis*. Most species belonging to this genus are rodent parasites, but there are specimens collected parasitizing large mammals, demonstrating a low specificity of this genus in relation to its hosts (Lareschi; Sanchez; Autino, 2016; Linardi, 2017; Brum, 2018; De Oliveira et al., 2020).

It is believed that the origin of the genus *Rhopalopsyllus* was within the Brazilian territory, as most species belonging to this genus occur here (Barros; Linardi; Botelho, 1993), as well as their main hosts - rodents, marsupials and xenarthrans. However, they can also occur in other wild mammals, such as coatis (*Nasua nasua* (Linnaeus, 1766)) and crab-eating foxes (*Cerdocyon thous* (Linnaeus, 1766)) and also in domestic dogs (Wells et al., 1981; Cerqueira et al., 2000; Scofield et al., 2005; Horta et al., 2007; Rodrigues; Daemon; Rodrigues, 2008; Silveira, 2012; Estevam et al., 2020). The genus contains seven species and two subspecies, being better elucidated in Supplementary Chart 1, as well as for all the other genera listed above.

Unlike the other genera found in this family, the genus *Polygenis* stands out. This genus can cause discomfort and itching, as well as, is also associated with several pathogens, being responsible for maintaining the wild plague (*Yersinia pestis*) circulating in forest environments using wild rodents, as amplifying hosts. So, species of this genus are being considered highly efficient vector for this disease, proving to be even more efficient than the main vector - *Xenopsylla cheopis* (Holdenried, 1952; Macchiavello, 1957; Carvalho et al., 2001). Another pathogen that is recently associated with this genus is *Rickettsia felis*, initially detected in *Ctenocephalides felis felis*, Melis et al. (2020) suggesting that this flea is capable of maintaining and transmitting *Rickettsia* in forest environments. Besides that, Schott et al. (2020) identified *Bartonella* sp. and *Rickettsia* sp. strain Taim that showed phylogenetic proximity to *Rickettsia parkeri* in *Polygenis* spp.

Other infectious agents associated with fleas of the genus *Polygenis* are *Ehrlichia* sp. detected from *P.* (*P.*) *bohlsi bohlsi* (Wagner, 1901) in the Brazilian Pantanal region collected parasitizing *Trichomys* sp. Trouessart, 1880 (De Sousa et al., 2017). On

the other hand, the nematode commonly found in rodents belonging to the genus *Hymenolepis* was also detected in fleas of the species *P.* (*P.*) *tripus* (Jordan, 1933), being able to act as an intermediate host in its cycle (Botelho; Linardi, 1992).

Family Stephanocircidae Wagner, 1928

This family is divided into two subfamilies – (1) Stephanocircinae, which comprises ectoparasite species of Australian marsupials; and (2) Craneopsyllinae Wagner, 1939, which is described as parasitizing marsupials and rodents in South America (Linardi and Guimarães, 2000).

The tribe Craneopsyllini (Craneopsyllinae) includes the genus *Craneopsylla* Rothschild, 1911, that are represented by a single species divided into two subspecies, namely *C. minerva minerva* (Rothschild, 1903) and *C. minerva wolffhuegeli* (Rothschild, 1909) These species have been reported in several countries in South America, parasitizing rodents, marsupials and bats, and only the subspecies *C. minerva minerva* was found in Brazil (Lewis, 1974; Nava; Lareschi; Voglino, 2003; Urdapilleta; Linardi; Lareschi, 2019). Besides that, these fleas were also reported to be naturally infected with strains of the bacteria genera *Bartonella*, *Rickettsia,* and *Yersinia* (De La Barrera, 1942; Schott et al., 2020).

In Brazil, *C. minerva minerva* was recorded in the South, Southeast, and Northeast Regions, parasitizing species of bats and a wide variety of wild rodents, including in regions that recorded plague endemism, albeit at low prevalence rates (Guimaraes, 1972; Linardi e Guimarães, 2000).

Family Tungidae Taschenberg, 1880

The Family Tungidae is formed by two subfamilies, Hectopsyllinae Baker, 1904 and Tunginae Taschenberg, 1880, distributed in four genera and 23 species. The larvae of these fleas are easily found in the organic matter of sandy soils, commonly present in coastal regions or residential areas where houses are built with dirt floors (Linardi et al., 2010).

The subfamily Hectopsyllinae is composed of two genera, *Rhynchopsyllus* Haller, 1880 and *Hectopsylla* Frauenfeld, 1860. The genus *Hectopsylla* can be found parasitizing birds and mammals (mainly rodents and bats), the specimens of this genus only insert their mouthparts into the skin of their hosts, considered semi-penetrating, and they are already reported in Brazil and several other countries in South America (Luz et

al., 2009; Hastriter et al., 2014). This genus is represented by the species *Hectopsylla psittaci* Frauenfeld, 1860, found in birds in South and Southeast Brazil (Linardi and Guimaraes, 2000).

In the same way as the previous genus, the genus *Rhynchopsyllus* also has a semi-penetrating habit and is found parasitizing several species of bats in South America, occurring exclusively in the Neotropical region, and only the species *R. pulex* Haller, 1880 have been recorded in Brazil (Hastriter; Méndez, 2000; Esbérard, 2001). Although there are records of this genus parasitizing birds and rodents, these records are considered accidental (Tipton and Machado-Allison 1972).

There are two genera in the subfamily Tunginae: (1) *Neotunga* Smit, 1962, with the type-species *Neotunga euloidea* Smit, 1962 recorded parasitizing placental mammals of the order Pholidota on the African continent (Lewis, 1993); and (2) the important genus *Tunga* Jarocki, 1838, that is represented by species with penetrating habits when in contact with the host, the female flea will insert part of its body into its epidermis, leaving the last two abdominal segments in contact with the external environment, making it possible to visualize only the genital pore and its respiratory stigma. After carrying out this process, the flea begins to feed and it is possible to verify a hypertrophy of the body as a whole, forming a neosome of 5-13mm and after the peak of engorgement, the ectoparasite begins oviposition, releasing the eggs directly into the environment (Nagy et al. al., 2007).

This genus occurs mainly in Neotropical regions, due the fact that nine of the 13 species occur distributed in South America and one species, *Tunga penetrans* (Linnaeus, 1758*)*, also in this portion of the African continent (Barnes, Radovsky, 1969, De Avelar et al., 2013). Also, this genus can be found parasitizing humans, dogs, cats, cattle, pigs, goats, sheep, xenarthrans and rodents, and accidentally in elephants and primates (De Avelar; Linhares; Linardi, 2012; De Avelar; Facury Filho; Linardi, 2013; Linardi; Di Nucci; Ezquiaga; Abba, 2017).

Currently, the species of the genus *Tunga* are divided into "Group *penetrans*" (*T. penetrans*, *T. trimamillata* Pampiglione, Trentini, Fioravanti, Onore & Rivasi, 2002, *T. hexalobulata* Avelar, Facury Filho & Linardi, 2013, *T. travassosi* Pinto & Dreyfus, 1927, *T. bondari* Wagner, 1932, and *T. terasma* Jordan, 1937) that are associated mainly parasitizing xenarthrans, and six of them are recorded in Brazil; and "Grupo *caecata*" (*T. caecata* (Enderlein, 1901), *T. caecigena* Jordan & Rothschild, 1921, *T. callida Li & Chin, 1957*, *T. libis* Smit, 1962, *T. monositus* Barnes & Radovsky, 1969, *T. bossii* Avelar,

Linhares & Linardi, 2012, and *T. bonneti* Beaucournu & González-Acuña, 2012) with species that parasitize only rodents, and two occurring in the Brazilian territory (Beaucournu et al., 2012; De Avelar et al., 2012). This division into two groups is based on morphological differences, geographical distribution, and differences in hosts (Wagner, 1932; Pinto and Dreyfus, 1927; Jordan and Rothschild, 1921; Li and Chin 1957; Smit, 1962). Supplementary Chart 1 shows the eight species that occur in Brazil, with their respective locality records.

The species *Tunga penetrans* has the widest distribution within the genus, occurring throughout the Neotropical region through sub-Saharan Africa, with greater relevance in populations in a situation of socioeconomic vulnerability (Lewis, 1998; Macias and Sachida, 2000; Heukelbach et al., 2001; Wilcke et al., 2002; Carvalho et al., 2003, Muehlen et al., 2003).

Tungiasis is a condition caused by females of *T. penetrans* and *T. trimamillata*. The female of this species inserted into the host's skin causes discomfort, itching, and even local ulcerations, depending on the level of infestation (Pampiglione et al., 2003). In addition, these injuries caused by female engorgement can cause nail and tegument deformities, difficulty in locomotion, or even trigger secondary infections that can culminate in the death of the host due to complications (Feldmeier et al., 2004; Veraldi et al., 2007; Criado et al., 2013).

3.3.2 New records

From the field collection, 1,564 fleas were recovered from rodents, marsupials, and bats from Legado das Águas Reserve; of these specimens, 208 fleas were submitted to the clarifying and slide-mounting method, being separated in three genera and seven species were identified (table 4). Within the Rhopalopsyllidae family, the genera *Polygenis* was collected, interacting with rodents and marsupials. Three species from this genus were documented: *Polygenis (Polygenis) bohlsi jordani* (Lima, 1937), *Polygenis (Polygenis) rimatus* (Jordan, 1932), and *Polygenis (Polygenis) roberti roberti* (Rothschild, 1905). Here we register the first encounter of *P. (P.) bohlsi jordani* in the state of São Paulo and also present new host associations highlighted in the Supplementary Chart 1. The other two species had already been recorded at the collection state, but new host interactions are also observed here.

The other specimens belong to the Ctenophtalmidae family with also three representatives: *Adoratopsylla (Adoratopsylla) antiquorum antiquorum* (Rothschild, 1904), *Adoratopsylla (Tritopsylla) intermedia intermedia* (Wagner, 1901) and *Adoratopsylla (Tritopsylla) sinuata* (Guimarães, 1945). All the species identified present new host associations and *A. (T.) sinuata* that had been previously described only in the state of Paraná, was also found in this study, then being a new locality record. All the new records are pointed out in Supplementary Chart 1. The images taken from the collected fleas are presented in figures 5, 6, and 7.

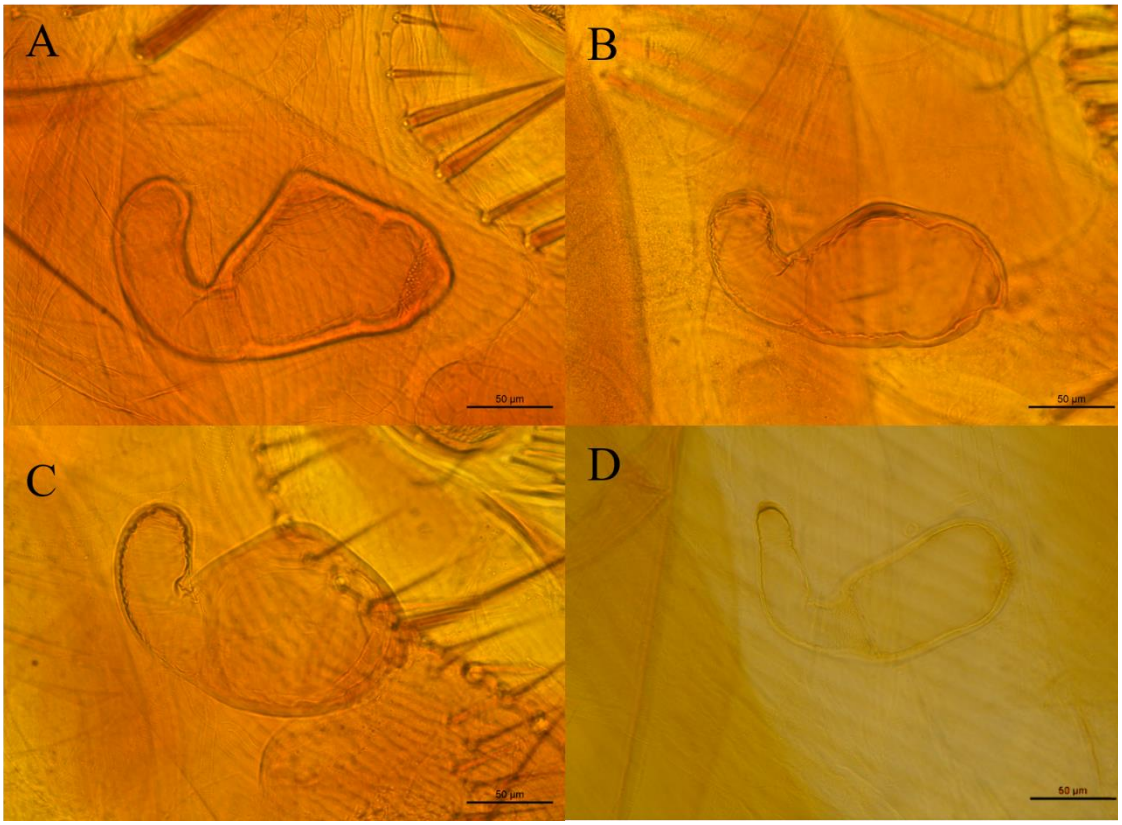

Figure 6: Spermathecae of the collected female fleas; **A.** *Polygenis* (*Polygenis*) *roberti roberti*; **B.** *Polygenis* (*Polygenis*) *rimatus*; **C.** *Polygenis* (*Polygenis*) *bohlsi jordani*; **D.** *Adoratopsylla* (*Tritopsylla*) *intermedia intermedia*.

Two species were recovered from the sampled bats, one female of *P. (P.) roberti roberti* from a *Chrotopterus auritus* (Peters, 1856) and five specimens of *Hormopsylla fosteri* (Rothschild, 1903) from three *Nyctinomops* sp. Miller, 1902. This is the first time the species *P. (P.) roberti roberti* is found interacting with bats, making this a new record. The genus *Hormopsylla* belongs to the Ischnopsyllidae family, known for having a

specific association with bats (figure 8). All the identified fleas, submitted to the clarifying and slide-mounting method cited previously are shown in Table 1.

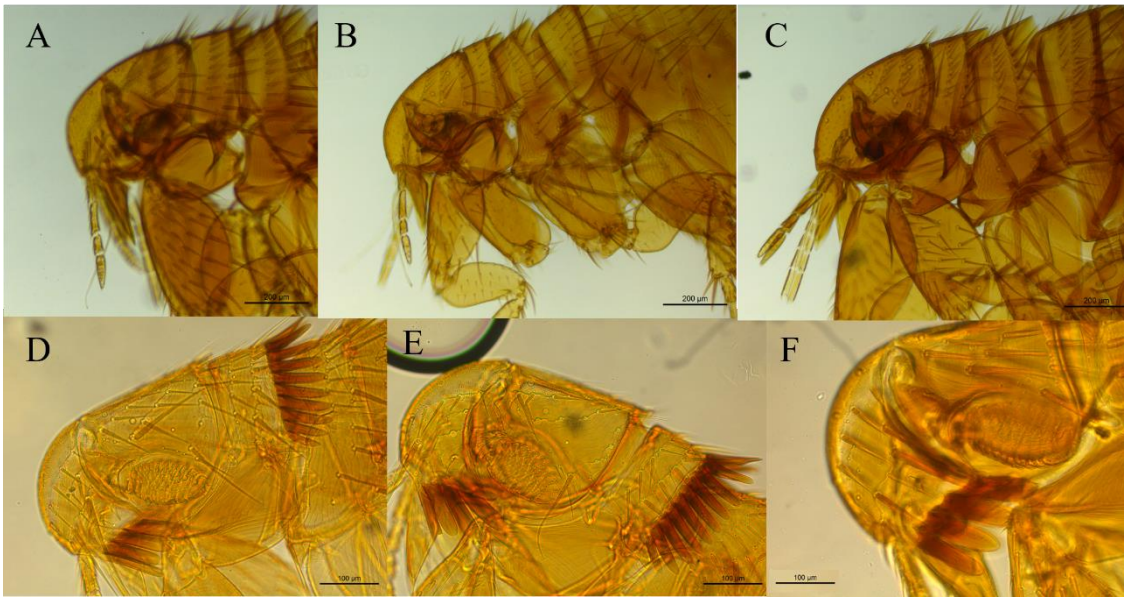

Figure 7: Morphology of the head of the collected fleas. **A.** *Polygenis* (*Polygenis*) *roberti roberti*; **B.** *Polygenis* (*Polygenis*) *rimatus*; **C.** *Polygenis* (*Polygenis*) *bohlsi jordani*; **D.** *Adoratopsylla* (*Adoratopsylla*) *antiquorum antiquorum*; **E.** *Adoratopsylla* (*Tritopsylla*) *intermedia intermedia*; **F.** *Adoratopsylla* (*Tritopsylla*) *sinuata*.

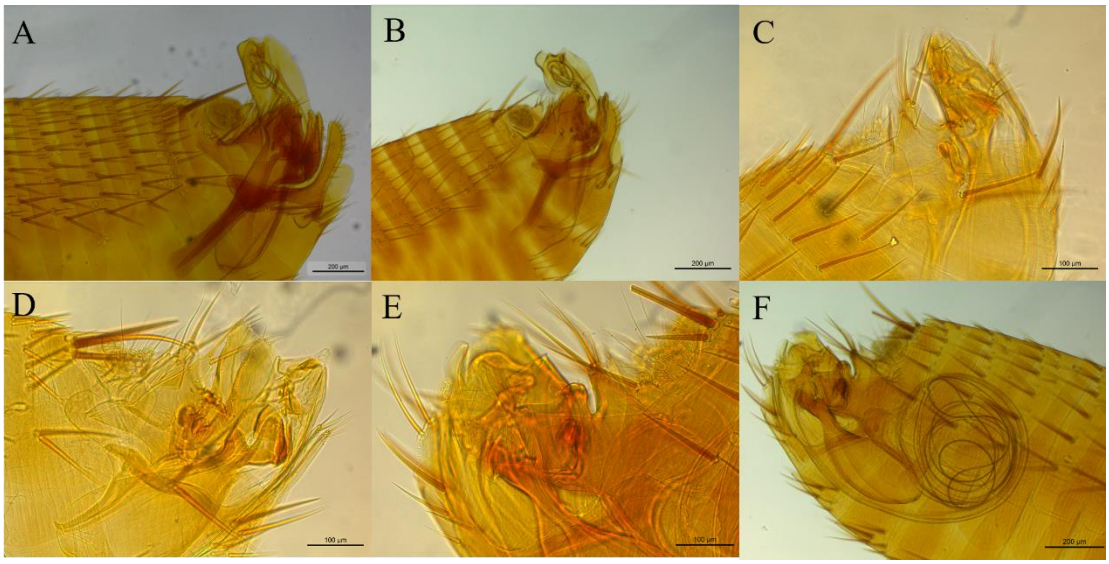

Figure 8: Morphology of aedagus and claspers of collected male fleas. **A.** *Polygenis* (*Polygenis*) *rimatus*; **B.** *Polygenis* (*Polygenis*) *roberti roberti*; **C.** *Adoratopsylla* (*Adoratopsylla*) *antiquorum antiquorum*; **D.** *Adoratopsylla* (*Tritopsylla*) *intermedia intermedia*; **E/F.** *Adoratopsylla* (*Tritopsylla*) *sinuata*.

3.3.3 Molecular analysis

One hundred and five flea specimens were processed individually through molecular

tools. All the samples produced expected amplicons when submitted to the COXII analysis, validating the extraction procedures. After that, all samples were screened for rickettsial DNA as previously described, but none amplified the pathogen gene.

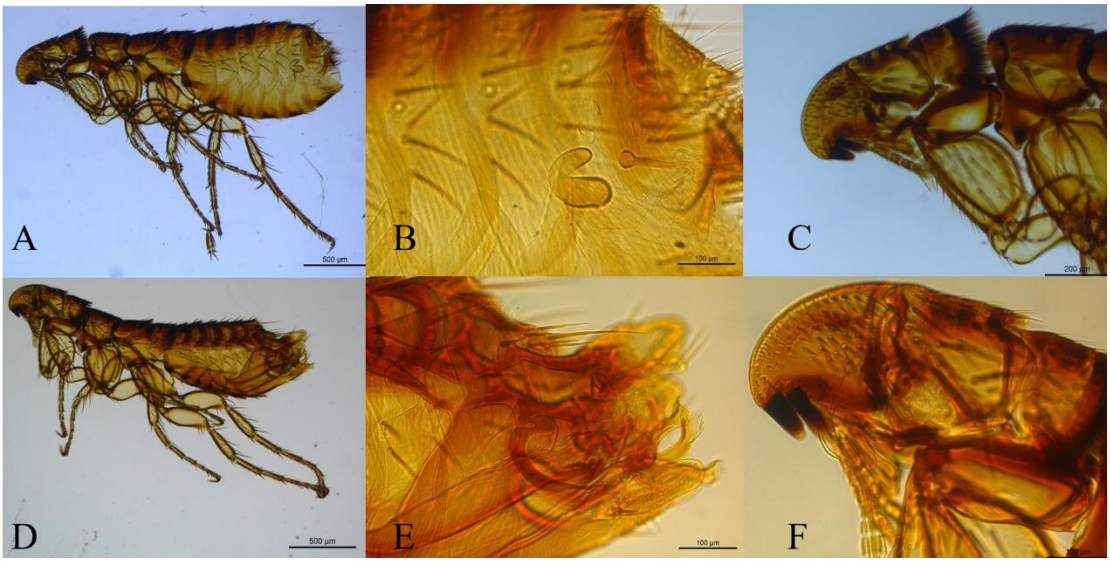

Figure 9: Morphology of *Hormopsylla fosteri*. **A.** Female; **B.** Spermathecae; **C.** Head of the female; **D.** Male; **E.** Clasper and aedagus; **F.** Head of the male.

3.4 CONCLUSION

Fleas are of great importance in human health and veterinary medicine, as in addition to causing physical discomfort due to their blood meal, they are also capable of transmitting various pathogens that can cause great damage to the vertebrate host.

Studies involving fleas are limited and focused on a few leading families already known, disregarding the lesser-known families that are incredibly scarce, demonstrating the need for further analyses evaluating not only the epidemiology of fleas as parasites and detection of zoonotic pathogens but also expanding the knowledge about the classifications and subclassifications of these invertebrates, reducing the gaps in knowledge about the characteristics of the species and subgroups, thus making possible a greater association with the epidemiology of infestations and related comorbidities, through a more accurate knowledge of the real diversity of the order Siphonaptera.

Table 4: Slide-mounted flea specimens collected on small mammals in a preserved ecological area (Legado das Águas – Reserva Votorantim) inside the Atlantic Rainforest Biome, during the years of 2018 to 2021.

	Flea species							
Host	*Adoratopsylla (A.) antiquorum antiquorum*	*Adoratopsylla (T.) intermedia intermedia*	*Adoratopsylla (T.) sinuata*	*Hormopsylla fosteri*	*Polygenis (P.) bohlsi jordani*	*Polygenis (P.) rimatus*	*Polygenis (P.) roberti roberti*	**TOTAL**
Chiroptera								
Chrotopterus auritus	-	-	-	-	-	-	1F	1
Nyctinomops sp.	-	-	-	1M, 4F	-	-	-	5
Marsupials								
Didelphis aurita	-	1F	-	-	-	-	4M, 3F	8
Gracilinanus sp.	-	-	-	-	-	-	1M, 1F	2
Metachirus nudicaudatus	2M, 2F	1F	6M, 2F	-	2F	2F	10M, 10F	37
Monodelphis sp.	-	-	1M	-	-	-	1M, 1F	3
Rodents								
Akodon sp.	-	-	-	-	-	1F	2M, 1F	4
Brucepattersonius sp.	-	-	-	-	1F	-	4M	5
Euryoryzomys russatus	1M, 1F	1M, 1F	-	-	12F	2M, 2F	34M, 36F	90
Guerlinguetus sp.	-	-	-	-	-	-	1M, 1F	2
Holochilus sp.	-	-	-	-	-	-	4M, 2F	6
Hylaeamys sp.	-	-	-	-	-	-	5M, 2F	7
Nectomys squamipes	-	-	-	-	-	-	3M, 7F	10
Oligoryzomys sp.	-	-	-	-	1F	-	1M, 4F	6
Oxymycterus sp.	1F	-	-	-	2F	1M, 1F	4F	9
Phyllomys sp.	-	-	-	-	-	-	2M, 1F	3
Rhipidomys sp.	-	-	-	-	-	-	5M, 1F	6
Sooretamys sp.	-	-	-	-	-	-	1M, 2F	3
Thaptomys nigrita	1F	-	-	-	-	-	-	1
TOTAL	8	4	9	5	18	9	155	**208**

3.5 REFERENCES

ABOULAILA, M.; MENSHAWY, S. Infection rate and molecular characterization of *Echidnophaga gallinacea* in chickens from Matrouh Governorate, Egypt. **Veterinary Parasitology: Regional Studies and Reports**, v. 22, n. August, p. 100457, 2020.

ALMEIDA, C. R. *et al*. Estudo do roedor *Akodon arviculoides*, Wagner, 1842 (Cricetidae) - Importância nos focos pestosos do Brasil. **Memórias do Instituto Oswaldo Cruz**, v. 81, n. 4, p. 409-416, 1986.

ALMEIDA, G. P. S. *et al*. Development of *Ctenocephalides felis felis* (Siphonaptera: Pulicidae) in different substrates for maintenance under laboratory conditions. **Revista Brasileira de Parasitologia Veterinária**, v. 29, n. 2, 2020.

ANTUNES, J. M. A. P. *et al.* Registro de *Dasypus novemcinctus* (Mammalia: Xenarthra) parasitado por *Tunga terasma* (Siphonaptera: Tungidae) em Alegre, estado do Espírito Santo, Brasil. **Revista Brasileira de Parasitologia**, v. 15, n. 4, p. 206-207, 2006.

ARZUA, M.; LINARDI, P. M.; BARROS-BATTESTI, D. M. *Myodopsylla wolffsohni wolffsohni* (Rothschild, 1903) (Siphonaptera, Ischnopsyllidae) on *Myotis nigricans* Schinz, 1821 (Chiroptera, Vespertilionidae), from the State of Paraná, Southern Brazil. **Revista Brasileira de Entomologia**, v. 46, n. 2, p. 255-256, 2002.

AZAD, A. F. Epidemiology of Murine Typhus. **Annual Review of Entomology**, v. 35, n. 1, p. 553–570, 1990.

AZAD, A. F.; TRAUB, R. Transmission of Murine Typhus Rickettsiae by *Leptopsylla segnis* (Siphonaptera: Leptopsyllidae). **Journal of Medical Entomology**, v. 24, n. 6, p. 689–693, 1987.

AZAD, A. F.; *et al*. Flea-borne Rickettsioses: Ecologic Considerations. **Emerging Infectious Diseases**, v. 3, n. 3, p. 319-327, 1997.

BACELLAR, F. *et al*. Molecular detection of *Rickettsia felis*, *Rickettsia typhi* and two genotypes closely related to *Bartonella elizabethae*. **The American Journal of Tropical Medicine and Hygiene**, v. 75, n. 4, p. 727–731, 2006.

BACOT, A. W.; MARTIN, C. J. LXVII. Observations on the mechanism of the transmission of plague by fleas. **J. Hyg.**, v. 14, n. 774, p. 423-438, 1915.

BARNES, A.M. & RADOVSKY, F.J. A new *Tunga* (Siphonaptera) from the Neartic region with description of all stages. **J. Med. Entomol**., v. 6, n.1, p.19-36, 1969.

BARROS-BATTESTI, D. M.; ARZUA, M. Geographical Distribution by Biomes of some Marsupial Siphonaptera from the State of Paraná, Brazil. **Memórias do Instituto Oswaldo Cruz**, v. 92, n. 4, p. 485–486, 1997.

BARROS, D. M.; LINARDI, P. M.; BOTELHO, J. R. Ectoparasites of Some Wild Rodents from Paraná State, Brazil. **Journal of Medical Entomology**, v. 30, n. 6, p. 1068–1070, 1993.

BEAUCOURNU, J. C. *et al.* Description de *Tunga bonneti* n. sp. du Chili (Siphonaptera: Tungidae) et notes sur sa spécificité, sa chorologie, son dermecos et sa phénologie [Description of *Tunga bonneti* n. sp. from Chile (Siphonaptera: Tungidae) with notes on specificity, chorology, dermecos and phenology]. **Parasite**, v. 19, n.3, p. 207-216, 2012.

BEAUCOURNU, J. C. *et al*. A new flea, *Ectinorus (Ectinorus) insignis* n. sp. (Siphonaptera, Rhopalopsyllidae, Parapsyllinae), with notes on the subgenus *Ectinorus* in Chile and comments on unciform sclerotization in the superfamily Malacopsylloidea. **Parasite**, v. 20, n. 1, p. 35, 2013.

BEAUCOURNU, J. C.; REYNES, J. M.; VIÉ, J. C. Fleas in French Guiana (Insecta:

Siphonaptera). **Journal of Medical Entomology**, v. 35, n. 1, p. 3–10, 1998.

BEZERRA-SANTOS, M. A.; *et al.* Ticks, fleas and endosymbionts in the ectoparasite fauna of the black-eared opossum *Dipelphis aurita* in Brazil. **Experimental and Applied Acarology**, v. 80, p. 329-338. Disponível em:

BITAM, I. *et al.* Fleas and flea-borne diseases. **International Journal of Infectious Diseases**, v. 14, n. 8, p. e667–e676, ago. 2010.

BITTENCOURT, E. B.; ROCHA, C. F. D. Host-ectoparasite Specificity in a Small Mammal Community in an Area of Atlantic Rain Forest (Ilha Grande, State of Rio de Janeiro), Southeastern Brazil. **Memorias do Instituto Oswaldo Cruz**, v. 98, n. 6, p. 793–798, 2003.

BLANTON, L. S.; WALKER, D. H. Flea-Borne Rickettsioses and Rickettsiae. **Am. J. Trop. Med. Hyg,** v. 96, n. 1, p. 53-56, 2017.

BONVICINO, C.; OLIVEIRA, J.; D'ANDREA, P. **Guia dos Roedores do Brasil: Chaves para gêneros baseadas em caracteres externos.** 1. ed. Rio de Janeiro: Centro Pan-Americano de febre Aftosa - OPAS/OMS, 2008.

BOTELHO, J. R. et al. Alguns hospedeiros reais de ectoparasitos do município de Caratinga, Minas Gerais, Brasil. **Memórias do Instituto Oswaldo Cruz**, v. 76, n. 1, p. 57-59, 1981.

BOTELHO, J. R.; LINARDI, P. M. Endoparasites of *Polygenis tripus* (Siphonaptera:Rhopalopsyllidae) of wild rodents from Belo Horizonte, Minas Gerais, Brazil. **Memórias do Instituto Oswaldo Cruz**, v. 87, n. 3, p. 453–455, 1992.

BRIAND, A. *et al.* Open field study on the efficacy of fluralaner topical solution for long-term control of flea bite allergy dermatitis in client owned cats in Ile-de-France region. **BMC Veterinary Research**, v. 15, n. 1, p. 337, 2019.

BRUM, J. G. W. Diversidade e potencial zoonótico de parasitos de *Didelphis albiventris* Lund, 1841 (Marsupialia:Didelphidae). **Acta Scientiae Veterinariae**, v. 33, n. 3, p. 335, 2018.

BUCKLAND, P. C.; SADLER, J. P. A Biogeography of the Human Flea, *Pulex irritans* L. (Siphonaptera: Pulicidae). **Journal of Biogeography**, v. 16, n. 2, p. 115, 1989.

CARVALHO, R. W. *et al*. Small rodents fleas from the bubonic plague focus located in the Serra dos Órgãos Mountain Range, State of Rio de Janeiro, Brazil. **Memórias do Instituto Oswaldo Cruz**, v. 96, n. 5, p. 603–609, 2001.

CARVALHO, R. W. *et al.* The patterns of tungiasis in Araruama township, state of Rio de Janeiro, Brazil. **Mem Inst Oswaldo** Cruz, v. 98, p.31–36, 2003.

CERQUEIRA, E. J. L. *et al*. Considerações sobre pulgas (Siphonaptera) da raposa *Cerdocyon thous* (Canidae) da área endêmica de leishmaniose visceral de Jacobina, Bahia, Brasil. **Revista da Sociedade Brasileira de Medicina Tropical**, v. 33, n. 1, p. 91–93, 2000.

CHANGBUNJONG, T. *et al.* A survey of ectoparasitic arthropods on domestic animals In Tak Province, Thailand. **Southeast Asian Journal of Tropical Medicine and Public Health**, v. 40, n. 3, p. 435–442, 2009.

CHRISTOU, C. *et al. Rickettsia typhi* and *Rickettsia felis* in *Xenopsylla cheopis* and *Leptopsylla segnis* Parasitizing Rats in Cyprus. **The American Journal of Tropical Medicine and Hygiene**, v. 83, n. 6, p. 1301–1304, 2010.

CIVEN, R.; NGO, V. Murine Typhus: An Unrecognized Suburban Vectorborne Disease. **Clinical Infectious Diseases**, v. 46, n. 6, p. 913–918, 2008.

CLARK, N. J. *et al*. Parasite spread at the domestic animal - wildlife interface: anthropogenic habitat use, phylogeny and body mass drive risk of cat and dog

flea (*Ctenocephalides* spp.) infestation in wild mammals. **Parasites and Vectors**, v. 11, n. 1, p. 8, 2018.

CRIADO P. R. *et al.* Tungiasis under dermoscopy: in vivo and ex vivo examination of the cutaneous infestation due to *Tunga penetrans*. **An. Bras. Dermatol.**, v.88, n. 4, p.649-51, 2013.

CURI, N. H. A.; *et al*. Wild canids, domestic dogs and their pathogens in Southeast Brazil: disease threats for canid conservation. **Biodiversity Conservation**, v. 19, p. 3513-3524, 2010.

DE AVELAR, D. M. *et al.* Endosymbionts of *Ctenocephalides felis felis* (Siphonaptera: Pulicidae) obtained from dogs captured in Belo Horizonte, Minas Gerais, Brazil. **Journal of Invertebrate Pathology**, v. 94, n. 2, p. 149–152, 2007.

DE AVELAR, D. M.; LINHARES, A. X.; LINARDI, P. M. A New Species of *Tunga* (Siphonaptera: Tungidae) From Brazil With a Key to the Adult Species and Neosomes. **Journal of Medical Entomology**, v. 49, p. 23-28, 2012.

DE AVELAR, D.M., FACURY FILHO, E.J.; LINARDI, P.M. A new species of *Tunga* (Siphonaptera: Tungidae) parasitizing cattle from Brazil. **Journal of Medical Entomology**, 50, 679–684, 2013.

DE CASTRO, M. C. M.; RAFAEL, J. A. Ectoparasitos de cães e gatos da cidade de Manaus, Amazonas, Brasil. **Acta Amazonica**, v. 36, n. 4, p. 535–538, 2006.

DE LA BARRERA, J.M. La peste rural en la Argentina. **Primer Congr. Nac. Enferm. Endemoepidémicas**, B. Aires, p. 431-2, 1942.

DE LIMA, F. C. G.; PORPINO, K. de O. Ectoparasitism and infections in the exoskeletons of large fossil cingulates. **Plos One**, v. 13, n. 10, p. e0205656, 2018.

DE MORAES, L. B. *et al.* Siphonaptera Parasites of Wild Rodents and Marsupials Trapped in Three Mountain Ranges of the Atlantic Forest in Southeastern Brazil. **Memórias do Instituto Oswaldo Cruz**, v. 98, n. 8, p. 1071-1076, 2003.

DE OLIVEIRA, G. M. B. *et al.* Tick-borne pathogens in dogs, wild small mammals and their ectoparasites in the semi-arid Caatinga biome, northeastern Brazil. **Ticks and Tick-borne Diseases**, v. 11, n. 4, p. 101409, 2020.

DE SOUSA, K. C. M. *et al*. Anaplasmataceae agents among wild mammals and ectoparasites in Brazil. **Epidemiology and Infection**, v. 145, n. 16, p. 3424–3437, 2017.

DE SOUSA, K. C. M. *et al.* Genetic Diversity of *Bartonella* spp. in Wild Mammals and Ectoparasites in Brazilian Pantanal. **Microbial Ecology**, v. 76, p. 544-554, 2018.

DEAN, K. R. *et al.* Human ectoparasites and the spread of plague in Europe during the Second Pandemic. **Proceedings of the National Academy of Sciences**, v. 115, n. 6, p. 1304–1309, 2018.

DI NUCCI, D. L.; EZQUIAGA, M. C.; ABBA, A. M. *Tunga penetrans* in Giant anteater (*Myrmecophaga tridactyla*) from Argentina. **Veterinary Parasitology: Regional Studies and Reports**, v. 10, p. 82–84, 2017.

DRYDEN, M. W.; GAAFAR, S. M. Blood Consumption by the Cat Flea, *Ctenocephalides felis* (Siphonaptera: Pulicidae). **Journal of Medical Entomology**, v. 28, n. 3, p. 394–400, 1991.

DUMLER, J.; WALKER, D. *Rickettsia typhi* (murine typhus). In: MANDELL, G.; BENNETT, J.; DOLIN, R. (Ed.). **Principles and practices of infectious diseases**. 8. ed. Philadelphia: Elsevier Churchill Livingstone, 2017. p. 264.

DURDEN, L. A. *et al.* Fleas (Siphonaptera) Parasitizing Peridomestic and Indigenous Mammals in Panamá and Screening of Selected Fleas for Vector-Borne Bacterial Pathogens. **Journal of Medical Entomology**, v. 58, n. 3, p. 1316–

1321, 2021.

DURDEN, L. A.; HINKLE, N. C. Fleas (Siphonaptera). In: DURDEN, L. A.; MULLEN, G. R. (Ed.). **Medical and Veterinary Entomology**. 3. ed. Londres: Academic Press, 2019. p. 145–170.

EISEN, R. J.; *et al.* Early-phase transmission of *Yersinia pestis* by unblocked fleas as a mechanism explaining rapidly spreading plague epizootics. **Proceedings of the National Academy of Sciences of the United States of America**, v. 103, n. 42, p. 15380 –15385, 2006.

ESBÉRARD, C. Infestation of *Rhynchopsyllus pulex* (Siphonaptera: Tungidae) on *Molossus molossus* (Chiroptera) in Southestern Brazil. **Memórias do Instituto Oswaldo Cruz**, v. 96, n. 8, p. 1169–1170, 2001.

ESTEVAM, L. G. T. M. *et al.* Seven years of evaluation of ectoparasites and vector-borne pathogens among ring-tailed coatis in an urban park in southeastern Brazil. **Veterinary Parasitology: Regional Studies and Reports**, v. 21, p. 100442, 2020.

FACCINI-MARTÍNEZ, Á. A.; SOTOMAYOR, H. A. Historical review of the plague in South America: a little-known disease in Colombia. **Biomedica: revista del Instituto Nacional de Salud**, v. 33, n. 1, p. 8–27, 2014.

FARIA, M. B. **Os marsupiais do Brasil:** guia de edentificação com base em caracteres morfológicos externos e cranianos. 1. ed. São Caetano do Sul: Amélie Editorial, 2019.

FELDMEIER, H., EISELE, M., VAN MARCK, E. et al. Investigations on the biology, epidemiology, pathology and control of *Tunga penetrans* in Brazil: IV. Clinical and histopathology. **Parasitology Research**, v. 94, p. 275–282, 2004.

FIORAVANTI, M. L.; PAMPIGLIONE, S.; TRENTINI, M. A. second species of *Tunga* (Insecta, Siphonaptera) infecting man: *Tunga trimamillata*. **Parasite**, v. 10, n. 3, p. 282-283.

FONTALVO, M. C. *et al*. *Bartonella* species pathogenic for humans infect pets, free-ranging wild mammals and their ectoparasites in the Caatinga biome, Northeastern Brazil: a serological and molecular study. **The Brazilian Journal of Infectious Diseases**, v. 21, n. 3, p. 290–296, 2017.

FORSTER, S.; WISEMAN, S.; SNYDER, D. E. Field study to investigate the effectiveness and safety of a novel orally administered combination drug product containing milbemycin oxime and lotilaner (Credelio® Plus) against natural flea and tick infestations on dogs presented as veterinary patients. **Parasites and Vectors**, v. 14, n. 1, p. 299, 2021.

FRANK, R.; *et al. Tunga penetrans* and further parasites in the giant anteater (*Myrmecophaga tridactyla*) from Minas Gerais, Brazil. **Parasitology Research**, v. 111, p. 1907-1912, 2012.

GILLESPIE, J. J. et al. Plasmids and Rickettsial Evolution: Insight from *Rickettsia felis*. **PloS One**, v. 2, n. 3, p. 1–17, 2007. Disponível em: <www.plosone.org>. Acesso em: 9 ago. 2021.

GONÇALVES, T. S. *et al.* Tick and flea infestations in captive *Tapirus terrestris* and *Tapirus kabomani* (Perissodactyla: Tapiridae) in the Brazilian Amazon. **Ticks and Tick-borne Diseases**, v. 14, 102234, 2023.

GREGORIN, R.; TADDEI, V. A. Chave artificial para a identificação de molossídeos brasileiros (Mammalia, Chiroptera). **Mastozoologia Neotropical**, v. 9, n. 1. P. 13-32, 2002.

GUEDES, E. *et al.* Detection of *Rickettsia rickettsii* in the tick *Amblyomma cajennense* in a new Brazilian spotted fever-endemic area in the state of Minas Gerais.

Memórias do Instituto Oswaldo Cruz, v. 100, n. 8, p. 841–845, 2005.

GUERNIER, V. *et al.* Fleas of Small Mammals on Reunion Island: Diversity, Distribution and Epidemiological Consequences. **PLoS Neglected Tropical Diseases**, v. 8, n. 9, p. e3129, 2014.

GUIMARAES, L.R. Contribuição à epidemiologia da peste endêmica no nordeste do Brasil e estado da Bahia. Estudo das pulgas encontradas nessa região. **Rev. Brasil. Malariol. D. Trop,** v. 24, n. ¼, p. 95-163, 1972.

GUIMARÄES, L. R.; LINARDI, P. M. *Hechtiella lopesi* sp. n. from São Paulo state, Brazil (Siphonaptera: Rhopalopsyllidae). **Memórias do Instituto Oswaldo Cruz**, v. 88, n. 4, p. 547–550, 1993.

GUIMARÃES, A. M.; LIMA, B. S.; ROCHA, C. M. B. M. Ectofauna Parasitária de Cães Urbanos Domiciliados Atendidos em Clínicas Veterinárias Particulares na cidade de Lavras, MG. **Ciência Animal Brasileira**, v. 12, n. 1, p. 172-177, 2011.

GUSTAFSON, C. R. *et al.* Sticktight Fleas Associated with Fowl Pox in a Backyard Chicken Flock in California. **Avian Diseases**, v. 41, n. 4, p. 1006, 1997.

HARVEY, T. V. *et al.* Tungiasis in domestic, wild, and synanthropic animals in Brazil. **Acta Tropica**, v. 222, p. 106068, 2021.

HASTRITER, M. W.; PETERSON, N. E. Notes on some fleas (Siphonaptera) from Amazonas and Bahia states, Brazil. **Entomological News**, v. 108, n. 4, p. 290-296, set/out 1997.

HASTRITER, M. W.; MÉNDEZ, E. A review of the flea genera *Hectopsylla frauenfeld* and *Rhynchopsyllus haller* (Siphonaptera: Pulicidae). **Proceedings of the Entomological Society of Washington**, v. 102, p. 613–624, 2000.

HASTRITER, M. *et al.* New distribution and host records for *Hectopsylla pulex* Haller (Siphonaptera, Tungidae) with notes on biology and morphology. **ZooKeys**, v. 389, p. 1–7, 2014.

HASTRITER, M. W. *et al.* New record of a phoretic flea associated with earwigs (Dermaptera, Arixeniidae) and a redescription of the bat flea *Lagaropsylla signata* (Siphonaptera, Ischnopsyllidae). **ZooKeys**, v. 657, p. 67–79, 2017.

HEUKELBACH J, DE OLIVEIRA FA, HESSE G, FELDMEIER H. Tungiasis: a neglected health problem of poor communities. **Trop Med Int Health**, v. 6, p. 267–272, 2001.

HEUKELBACH, J. *et al.* High prevalence of intestinal infections and ectoparasites in dogs, Minas Gerais State (southeast Brazil). **Parasitology Research**, v. 111, p. 1913-1921, 2012.

HINNEBUSCH, B. J. The Evolution of Flea-borne Transmission in *Yersinia pestis*. **Current Issues in Molecular Biology**, v. 7, n. 2, p. 197–212, 2005.

HINNEBUSCH, B. J.; ERICKSON, D. L. *Yersinia pestis* Biofilm in the Flea Vector and Its Role in the Transmission of Plage. **Current Topics in Microbiology and Immunology**, v. 322, p. 229–248, 2008.

HOLDENRIED, R. Sylvatic Plague Studies: VII. Plague Transmission Potentials of the Fleas *Diamanus montanus* and *Polygenis gwyni* Compared with *Xenopsylla cheopis*. **Journal of Infectious Diseases**, v. 90, n. 2, p. 131–140, 1952.

HOPKINS, G. H. E., ROTHSCHILD, M. **An illustrated catalogue of the Rothschild collection of fleas (Siphonaptera) in the British Museum (Natural History).** Vol. 2 Coptopsyllidae, Vermipsyllidae, Stephanocircidae, Ischnopsyllidae, Hypsophthalmidae and Xiphiopsyllidae. British Museum (Natural History), 445 p. 1956.

HORTA, M. C. *et al*. Prevalence of *Rickettsia felis* in the Fleas *Ctenocephalides felis*

felis and *Ctenocephalides canis* from Two Indian Villages in São Paulo Municipality, Brazil. **Annals New York Academy of Sciences**, v. 1078, p. 361-363, 2006.

HORTA, M. C. *et al. Rickettsia* infection in five areas of the state of São Paulo, Brazil. **Memórias do Instituto Oswaldo Cruz**, v. 102, n. 7, p. 793–801, nov. 2007.

HORTA, M. C. *et al*. *Rickettsia felis* in *Ctenocephalides felis felis* from five geographic regions of Brazil. **American Journal of Tropical Medicine and Hygiene**, v. 91, n. 1, p. 96–100, 2014.

HU, L. *et al.* Molecular Identification, Transcriptome Sequencing and Functional Annotation of *Pulex irritans*. **Acta Parasitologica**, v. 66, n. 2, p. 605–614, 3 jun. 2021.

JESUS, A. V. *et al*. Spatial Distribution of Off-Host Stages of *Tunga penetrans* in the Soil within the Home Range of Nine Infected Dogs in An Endemic Tourist Area in Brazil. **Tropical Medicine and Infectious Diseases**, v. 8, n. 98, p. 1-13, 2023.

JOHNSON, P.T. A classification of the Siphonaptera of South America with descriptions of new species. **Mem. Ent. Soc. Wash**, v. 5, p. 1-298, 1957.

JORDAN, K.; ROTHSCHILD, M. A new species of Sarcopsyllidae. **Ectoparasites,** v.1, n.3, p.131-132, 1921.

KESKIN, A.; HASTRITER, M. W.; BEAUCOURNU, J. C. Fleas (Siphonaptera) of Turkey: species composition, geographical distribution and host associations. **Zootaxa**, v. 4420, n. 2, p. 211, 2018.

KOEHLER, P. G.; PEREIRA, R. M.; KAUFMAN, P. E. Sticktight Flea, *Echidnophaga gallinacea*. **University of Florida Cooperative Extension Service, Institute of Food and Agriculture Sciences, EDIS**, 1991.

KORINE, C. *et al.* Effects of host diet and thermal state on feeding performance of the flea *Xenopsylla ramesis*. **Journal of Experimental Biology**, v. 215, n. 9, p. 1435–1441, 2012.

KRASNOV, B. R.; SHENBROT, G. I.; KHOKHLOVA, I. S. Historical biogeography of fleas: the former Bering Land Bridge and phylogenetic dissimilarity between the Nearctic and Palearctic assemblages. **Parasitology Research**, v. 114, n. 5, p. 1677–1686, 2015.

LABRUNA, M. B. *et al. Rickettsia* Species Infecting *Amblyomma cooperi* Ticks from an Area in the State of São Paulo, Brazil, Where Brazilian Spotted Fever Is Endemic. **Journal of Clinical Microbiology,** v. 42, n. 1, p. 90–98, 2004.

LAPPIN, M. R.; TASKER, S.; ROURA, X. Role of vector-borne pathogens in the development of fever in cats: 1. Flea-associated diseases. **Journal of Feline Medicine and Surgery**, v. 22, n. 1, p. 31–39, 2020.

LARESCHI, M. *et al.* Fleas Associated with Mammals from Northwestern Argentina, with New Distributional Reports. **Comparative Parasitology**, v. 77, n. 2, p. 207–213, 2010.

LARESCHI, M.; SANCHEZ, J.; AUTINO, A. A review of the fleas (Insecta: Siphonaptera) from Argentina. **Zootaxa**, v. 4103, n. 3, p. 239, 2016.

LARESCHI, M. *et al.* The human flea *Pulex irritans* (Siphonaptera: Pulicidae) in northwestern Argentina, with and investigation of *Bartonella* and *Rickettsia* spp. **Revista Mexicana de Biodiversidad**, v. 89, p. 375-381, 2018.

LEWIS, R. E. Notes on the Geographical Distribution and Host Preferences in the Order Siphonaptera Part 4. Coptopsyllidae, Pygiopsyllidae, Stephanocircidae and Xiphiopsyllidae. **Journal of Medical Entomology**, v. 11, n. 4, p. 403–413, 1974.

LEWIS, R. E. Notes on the Geographical Distribution and Host Preferences in the Order Siphonaptera. Part 8. New Taxa Described Between 1984 and 1990, with a Current Classification of the Order. **Journal of Medical Entomology**, v. 30, n. 1, p. 239–256, 1993.

LEWIS, R. E.; LEWIS, J. H. Siphonaptera of North America North of Mexico: Ischnopsyllidae. **Journal of Medical Entomology**, v. 31, n. 3, p. 348–368, 1 nov. 1994a.

LEWIS, R. E.; LEWIS, J. H. Siphonaptera of North America North of Mexico: Vermipsyllidae and Rhopalopsyllidae. **Journal of Medical Entomology**, v. 31, n. 1, p. 82–98, 1994b.

LEWIS, R. E. Résumé of the Siphonaptera (Insecta) of the World. **Journal of Medical Entomology**, v. 35, n. 4, p. 377–389, 1998.

LI, K.C.; CHIN, T.H. *Tunga callida* sp. nov., a new species of sandflea from Yunnan. **Acta Entomol. Sinica,** v.7, n.1, p.113-120, 1957.

LIANG, L.; HOUYONG, W. A new species and a new record of *Nycteridopsylla* Oudemans, 1906 (Siphonaptera: Ischnopsyllidae) from China. **Systematic Parasitology**, v. 56, p. 57–61, 2003.

LINARDI, P. M. *et al.* Ectoparasitos de roedores em ambientes silvestres do município de Juiz de Fora, Minas Gerais. **Memórias do Instituto Oswaldo Cruz**, v. 82, n. 1, p. 137-139, 1987.

LINARDI, P. M. *et al.* Ectoparasitos de pequenos mamíferos da Ilha de Maracá, Roraima, Brasil. I. Ectoparasitofauna, registros geográficos e de hospedeiros. **Acta Amazonica**, v. 21, n. 0, p. 131–140, 1991.

LINARDI, P. M. Endoparasitos de *Ctenocephalides felis* (Siphonaptera: Pulicidae). **O Biológico**, v. 64, n. 1, p. 65, 2002.

LINARDI, P. M. *et al. Polygenis (Polygenis) platensis* (Jordan & Rothschild) (Siphonaptera:Rhopalopsyllinae), a New Record in Brazil. **Neotropical Entomology**, v. 34, n. 5, p. 837-841, 2005.

LINARDI, P. M. *et al.* Occurrence of the off-host life stages of *Tunga penetrans* (Siphonaptera) in various environments in Brazil. **Annals of Tropical Medicine & Parasitology**, v. 104, n. 4, p. 337–345, 2010.

LINARDI, P. M. Checklist de Siphonaptera (Insecta) do Estado de São Paulo. **Biota Neotropica**, v. 11, n. 1a, p. 607–617, 2011. Disponível em:

LINARDI, P. M.; DE AVELAR, D. M. Neosomes of tungid fleas on wild and domestic animals. **Parasitology Research**, v. 113, p. 3517-3533, 2014.

LINARDI, P. M. Checklist dos Siphonaptera do Estado do Mato Grosso do Sul. **Iheringia. Série Zoologia**, v. 107, n. suppl, p. 1–6, 2017.

LINARDI, P. M.; GUIMARÃES, L. R. Systematic review of genera and subgenera of Rhopalopsyllinae (Siphonaptera: Rhopalopsyllidae) by phenetic and cladistic methods. **Journal of medical entomology**, v. 30, n. 1, p. 161–170, 1993.

LINARDI, P. M.; GUIMARÃES, L. R. **Sifonápteros do Brasil**. São Paulo: Museu de Zoologia da USP, Fapesp, 2000.

LINARDI, P. M.; NAGEM, R. L. Pulicídeos e outros ectoparasitos de cães de Belo Horizonte e municípios vizinhos. **Revista brasileira de biologia**, v. 33, p. 529–538, 1973.

LOFTIS, A. D. *et al*. Surveillance of egyptian fleas for agents of public health significance: *Anaplasma, Bartonella, Coxiella, Ehrlichia, Rickettsia*, and *Yersinia pestis*. **The American Journal of Tropical Medicine and Hygiene**, v. 75, n. 1, p. 41–48, 2006.

LÓPEZ-PÉREZ, A. M. *et al.* Prevalence and Phylogenetic Analysis of *Bartonella*

Species of Wild Carnivores and Their Fleas in Northwestern Mexico. **EcoHealth**, v. 14, n. 1, p. 116–129, 2017.

LÓPEZ-PÉREZ, A. M. *et al.* Drivers of flea (Siphonaptera) community structure in sympatric wild carnivores in northwestern Mexico. **Journal of Vector Ecology**, v. 43, n. 1, p. 15–25, 2018.

LÓPEZ-PÉREZ, A. M. *et al.* Diversity of rickettsiae in domestic, synanthropic, and sylvatic mammals and their ectoparasites in a spotted fever-epidemic region at the western US-Mexico border. **Transboundary and Emerging Diseases**, n. January, p. 1–14, 2021.

LUZ, J. L. *et al.* The chiggerflea *Hectopsylla pulex* (Siphonaptera: Tungidae) as an ectoparasite of free-tailed bats (Chiroptera: Molossidae). **Memórias do Instituto Oswaldo Cruz**, v. 104, n. 4, p. 567–569, 2009.

MACCHIAVELLO, A. Estudios sobre Peste Selvatica en America del Sur IV. Transmision Experimental de la Peste por *Polygenis litargus*. **Boletín de la Oficina Sanitaria Panamericana**, v. 43, n. 3, p. 122–131, 1957.

MACIAS P. C., SASHIDA P. M. Cutaneous infestation by *Tunga penetrans*. **Int J Dermatol** 39:296–298, 2000.

MALEKI-RAVASAN, N. *et al.* The Fleas (Siphonaptera) in Iran: Diversity, Host Range, and Medical Importance. **PLOS Neglected Tropical Diseases**, v. 11, n. 1, p. e0005260, 2017.

MEDVEDEV, S. G. Specific Features of the Distribution and Host Associations of Fleas (Siphonaptera). **Entomological Review**, v. 82, n. 9, p. 1165–1177, 2002.

MEDVEDEV, S. G.; KRASNOV, B. R. Fleas: Permanent satellites of small mammals. In: MORAND, S.; KRASNOV, B. R.; POULIN, R. (Ed.). **Micromammals and Macroparasites: From Evolutionary Ecology to Management**. Springer, 2006. p. 161–177.

MELIS, M. et al. Molecular detection and identification of *Rickettsia felis* in *Polygenis* (Siphonaptera, Rhopalopsyllidae, Rhopalopsyllinae) associated with cricetid rodents in a rural area from central Argentina. **Veterinary Parasitology: Regional Studies and Reports**, v. 21, p. 100445, 2020.

MENDES-DE-ALMEIDA, F. *et al.* Characterization of ectoparasites in an urban cat (*Felis catus* Linnaeus, 1758) population of Rio de Janeiro, Brazil. **Parasitology Research**, v. 108, p. 1431-1435, 2011.

MÉNDEZ, E. *Scolopsyllus colombianus*, New Genus and Species of the Family Rhopalopsyllidae (Siphonaptera) from Colombia. **Journal of Medical Entomology**, v. 5, n. 3, p. 405–410, 1968.

MENDONÇA, R. F. B. *et al.* Ectoparasites of small mammals in a fragmented area of the southern Amazonia: interaction networks and correlations with seasonality and host sext. **Experimental and Applied Acarology**, v. 81, p. 117-134, 2020.

MIARINJARA, A. *et al*. Poor vector competence of the human flea, *Pulex irritans*, to transmit *Yersinia pestis*. **Parasites & Vectors**, v. 14, n. 1, p. 317, 2021.

MOEMENBELLAH-FARD, M. D. *et al*. Faunal distribution of fleas and their blood-feeding preferences using enzyme-linked immunosorbent assays from farm animals and human shelters in a new rural region of southern Iran. **Journal of Parasitic Diseases**, v. 40, n. 1, p. 169–175, 2016.

MOLYNEUX, D. The fine-structure of the epimastigote forms of *Trypanosoma lewisi* in the rectum of the flea, *Nosopsyllus fasciatus*. **Parasitology**, v. 59, n. 1, p. 55-66, 1969.

MUEHLEN M. *et al.* Investigations on the biology, epidemiology, pathology and control of *Tunga penetrans* in Brazil II. Prevalence, parasite load and

topographic distribution of lesions in the population of a traditional fishing village. **Parasitology Research**, v. 90, p. 449–455, 2003.

MULLER, G. *et al*. Parasitos de animais silvestres com potencial zoonótico no Rio Grande do Sul. **Veterinária em Foco**, v. 6, n. 2, p. 185-190, 2009.

MURRAY, M. D.; ORTON, M. N.; CAMERON, A. S. The Antarctic Flea *Glaciopsyllus antarcticus.* Smit and Dunnet. In: **Entomology of Antarctica**. p. 393–395.

NAGY, N. *et al.* Investigations on the life cycle and morphology of *Tunga penetrans* in Brazil. **Parasitology Research**, v. 101, n. S2, p. 233–242, 2007.

NAVA, S.; LARESCHI, M.; VOGLINO, D. Interrelationship between ectoparasites and wild rodents from northeastern Buenos Aires province, Argentina. **Memórias do Instituto Oswaldo Cruz**, v. 98, n. 1, p. 45–49, jan. 2003.

OGATA, H. *et al*. The genome sequence of *Rickettsia felis* identifies the first putative conjugative plasmid in an obligate intracellular parasite. **PLoS Biology**, v. 3, n. 8, 2005.

OLIVEIRA, G. M. *et al.* Sifonápteros de roedores no foco de peste da Chapada do Araripe, Pernambuco, Brasil, 2002-2008. **Revista de Patologia Tropical**, v. 38, n. 3, p. 213-219.

OLIVEIRA, H. H. *et al.* Siphonaptera of small rodents and marsupials in the Pedra Branca State Park, State of Rio de Janeiro, Brazil. **Revista Brasileira de Parasitologia Veterinária**, v. 19, n. 1, p. 49–54, 2010.

OLIVEIRA, J. C. P. *et al*. Ectoparasites infesting animals living in close contact with human beings: a real trouble for One Health perspective? **Arquivo Brasileiro de Medicina Veterinária e Zootecnia**, v. 73, n. 1, p. 55-61, 2021.

PAMPIGLIONE, S. *et al*. On a new species of sand flea from Ecuador and tungiasis, a problem of public health in many developing countries. **Ann. Igiene**, 15: 747-752, 2003.

PAZ, G. F. *et al*. Dynamics of *Ctenocephalides felis felis* (Siphonaptera: Pulicidae) Infestations on Urban Dogs in Southeastern Brazil. **Journal of Medical Entomology**, v. 52, n. 2, p. 1159-1164, 2015.

PAZ, K. C. R. C. et al. Parasitism of Nelore (*Bos indicus*) calf from northern Brazil by *Ctenocephalides felis*. **Veterinary Parasitology: Regional Studies and Reports**, v. 34, p. 100770, 2022.

PINTO, C.; DREYFUS, A. *Tunga travassosi* n. sp., parasita de *Tatusia novemcinctus* do Brasil. **Bol. Biol**. 9:174- 1927.

PEREIRA, J. S. *et al*. Ocorrência de *Pulex irritans* Linnaeus,1758 em *Tamandua tetradactyla* no município de serra do mel, Rio Grande do Norte, Brasil. **Acta Veterinaria Brasilica**, v. 8, n. 2, p. 121-123, 2014.

PINTO, I. D. S. *et al.* Siphonaptera Associated With Wild Mammals From the Central Atlantic Forest Biodiversity Corridor in Southeastern Brazil. **Journal of Medical Entomology**, v. 46, n. 5, p. 1146–1151, 2009.

PORTA, D. *et al.* Parasites in synanthropic rodents in municipality of the Northwest region of the State of Paraná, Brazil. **African Journal of Microbiology Research**, v. 8, n. 16, p. 1684-1689, 2014.

PUCU, E.; LARESCHI, M.; GARDNER, S. L. Bolivian ectoparasites: A survey of the fleas of *Ctenomys* (Rodentia: Ctenomyidae). **Comparative Parasitology**, v. 81, n. 1, p. 114–118, 2014.

RAFAEL, J. A. Ocorrência de *Polygenis klagesi samuelis* na Amazônia Brasileira (Siphonaptera: Rhopalopsyllidae). **Acta Amazonica**, v. 12, n. 1, p. 231-232, 1982.

REGOLIN, A. L. *et al.* Ectoparasites of the critically endangered insular cavy, *Cavia intermedia* (Rodentia: Caviidae), southern Brazil. **International Journal for Parasitology: Parasites and Wildlife**, v. 4, p. 37-42, 2015.

REZAEI, F. *et al*. Prevalence of ectoparasites in free-range backyard chickens, domestic pigeons (*Columba livia domestica*) and turkeys of Kermanshah province, west of Iran. **Journal of Parasitic Diseases**, v. 40, n. 2, p. 448–453, 2016.

RODRIGUES, A. F. S. F.; DAEMON, E. MASSARD, C. L. Ectoparasites of *Nasua nasua* (Carnivora, Procyonidae) from an urban forest in Southeastern Brazil. **Arquivo Brasileiro de Medicina Veterinária e Zootecnia**, v. 58, n. 5, p. 969-971, 2006.

RODRIGUES, D. F.; DAEMON, E.; RODRIGUES, A. F. S. F. Caracterização da população de ectoparasitos em cães de núcleos de expansão urbana de Juiz de Fora, Minas Gerais, Brasil. **Revista Brasileira de Parasitologia Veterinária**, v. 17, n. 4, p. 185–188, 2008.

RODRIGUÉZ, Z.; MOREIRA, E. C.; LINARDI, P. M.; SANTOS, H. A. Notes on the Bat Flea *Hormopsylla fosteri* (Siphonaptera: Ischnopsyllidae) Infesting *Molossops abrasus* (Chiroptera). **Memórias do Instituto Oswaldo Cruz**, v. 94, n.6, p. 727-728, 1999.

SACKAL, C. *et al. Bartonella* spp. and *Rickettsia felis* in Fleas, Democratic Republic of Congo. **Emerging Infectious Diseases**, v. 14, n. 12, p. 1972–1974, 2008.

SALVADOR, C. H. *et al.* Interação parasito-hospedeiro entre ectoparasitos (Ixodida & Siphonaptera) e gambás *Didelphis aurita* Wied-Neuwied, 1826 (Mammalia: Didelphimorphia), no continente e em ilhas do litoral de Santa Catarina, Sul do Brasil Carlos. **Biotemas**, v. 20, n. 4, p. 81–90, 2007.

SANTOS, E. M. S. *et al.* Ticks and fleas in crab-eating fox (*Cerdocyon thous*) of Pernambuco state, Brazil. **Brazilian Journal of Veterinary Research and Animal Science**, v. 53, n. 3, p. 295-299, 2016.

SANTOS, K. C. *et al*. Efficacy of oral fluralaner (Bravecto) against *Tunga penetrans* in dogs: A negative control, randomized field study in an endemic community in Brazil. **Tropical Diseases**, v. 16, n. 3, p. e0010251, 2022.

SCHOTT, D. *et al.* Detection of *Rickettsia* spp. and *Bartonella* spp. in *Ctenocephalides felis* fleas from free-ranging crab-eating foxes (*Cerdocyon thous*). **Medical and Veterinary Entomology**, v. 33, n. 4, p. 536–540, 2019.

SCHOTT, D. *et al.* Detection of *Bartonella* sp. and a novel spotted fever group *Rickettsia* sp. in Neotropical fleas of wild rodents (Cricetidae) from Southern Brazil. **Comparative Immunology, Microbiology and Infectious Diseases**, v. 73, p. 101568, 2020.

SCOFIELD, A. *et al*. Ocorrência de *Rhopalopsyllus lutzi lutzi* (Baker) (Siphonaptera, Rhopalopsyllidae) em *Canis familiaris* (Linnaeus) de zona rural do município de Piraí, Rio de Janeiro, Brasil. **Revista Brasileira de Entomologia**, v. 49, n. 1, p. 159–161, 2005.

SHEPHERD, R. C. H.; EDMONDS, J. W. The occurrence of stickfast fleas *Echidnophaga* spp. on wild rabbits *Oryctolagus cuniculus* (L.) in Victoria. **Austral Ecology**, v. 3, n. 3, p. 287–295, set. 1978.

SILVA, T. K. S. *et al.* Investigation of *Ehrlichia* spp., *Anaplasma* spp. and *Rickettsia* in ectoparasites collected from domestic animals, Rio de Janeiro State, Brazil. **Virus Review and Research**, v. 22, 2017.

SILVA, V. P. R. *et al.* Multiparasitism in *Sapajus nigritus* (Goldfuss 1809) (Primates: Cebidae) kept illegally in home captivity in the state of Santa Catarina, Brazil – Case report. **Brazilian Journal of Development**, v. 9, n. 1, p. 232-243, 2023.

SILVA-ROHWER, A. R. *et al*. *CsrA* Enhances Cyclic-di-GMP Biosynthesis and *Yersinia pestis* Biofilm Blockage of the Flea Foregut by Alleviating *Hfq*-Dependent Repression of the *hmsT* mRNA. **mBio**, v. 12, n. 4, p. 1–16, 2021.

SILVEIRA, J. A. G. **Ocorrência de hemoparasitos e ectoparasitos em veado-catingueiro (*Mazama gouazoubira* Fischer, 1814), veado-campeiro (*Ozotocerus bezoarticus* Linnaeus, 1758) e cervo-do-pantanal (*Blastocerus dichotomus* Illiger, 1815): utilização de métodos parasitológicos e moleculares**. 2012. Tese. Universidade Federal de Minas Gerais, 2012.

SMIT, F.G.A.M. A new sand-flea from Ecuador. **Entomologist**, v. 95, n. 1187, p. 89-93, 1962.

SMITH, S. A.; CLAY, M. E. Biological and Morphological Studies on the Bat Flea, *Myodopsylla insignis* (Siphonaptera: Ischnopsyllidae). **Journal of Medical Entomology**, v. 25, n. 5, p. 413–424, 1988.

SOUZA, U. A. *et al.* Ticks and Tick-borne Diseases Ticks, mites, fleas, and vector-borne pathogens in free-ranging neotropical wild felids from southern Brazil. **Ticks and Tick-borne Diseases**, v. 12, n. February, p. 1–8, 2021.

ŠPITALSKÁ, E. *et al. Rickettsia* species in fleas collected from small mammals in Slovakia. **Parasitology Research**, v. 114, n. 11, p. 4333–4339, 2015.

SPONCHIADO, J. *et al.* Interaction of ectoparasites (Mesostigmata, Phthiraptera and Siphonaptera) with small mammals in Cerrado fragments, western Brazil. **Experimental and Applied Acarology**, v. 66, p. 369-381, 2015.

STALLIVIERE, F. M. *et al.* Ectoparasitos e helmintos intestinais em *Felis catus domesticus*, da cidade de Lages, SC, Brasil e aspectos socioeconômicos e culturais das famílias dos proprietários dos animais. **Revista Brasileira de Parasitologia Veterinária**, v. 18, n. 04, p. 26–31, 2009.

SZABÓ, M. P. J. *et al.* Cat Flea (*Ctenocephalides felis*) Infestation in Quarantined Marsh Deer (*Blastocerus dichotomus*) Populations. **Journal of Zoo and Wildlife Medicine**, v. 31, n. 4, p. 576-577, 2000.

TANCREDI I. P. *et al. Tunga penetrans* occurrence in *Panthera onca* crashed in the Mato Grosso northern region. **Scientific Electronic Archives**, v.13, n.6, p. 49-54, 2021.

TENG, Z. *et al*. Human *Rickettsia felis* infections in Mainland China. **Front. Cell. Infect. Microbiol.** v. 12, p. 997315, 2022.

TIPTON V. J., MACHADO-ALLISON C. E. Fleas of Venezuela. Brigham Young Univer **Sci Bul**, v. 17, p. 1-115, 1972.

TREMBLEY, H. L.; BISHOPP, F. C. Distribution and Hosts of Some Fleas of Economic Importance. **Journal of Economic Entomology**, v. 33, n. 4, p. 701–703, 1940.

URDAPILLETA, M.; LINARDI, P. M.; LARESCHI, M. Fleas associated with sigmodontine rodents and marsupials from the Paranaense Forest in Northeastern Argentina. **Acta Tropica**, v. 193, p. 71–77, 2019.

VERALDI S, VALSECCHI M. Imported tungiasis: a report of 19 cases and review of the literature. **Int J Dermatol**, v. 46, n. 10, p. 1061-6, 2007.

WAGNER, J. *Tunga bondari*, eine neue Art der Sandflohe. **Novit. Zool**., v.38, p.248-249, 1932.

WARUIRU, M. R. *et al.* Prevalence of ecto- and haemo-parasites of free-range local ducks in Kenya. **Livestock Research for Rural Development**, v. 29, n. 126, 2017.

WELLS, E. A. *et al.* Mammalian wildlife diseases as hazards to man and livestock in an area of the Llanos Orientales of Colombia. **Journal of Wildlife Diseases**, v. 17,

n. 1, p. 153–162, 1981.
WHEELER, C. M.; DOUGLAS, J. R.; EVANS, F. C. The role of the burrowing owl and the sticktight flea in the spread of the plage. **Science**, v. 94, n. 2450, p. 560–561, 1941.
WHITING, M. F. *et al.* Mecoptera is paraphyletic: multiple genes and phylogeny of Mecoptera and Siphonaptera. **Zoologica Scripta**, v. 31, n. 1, p. 93-104, 2002.
WHITING, M. F. *et al.* A molecular phylogeny of fleas (Insecta: Siphonaptera): origins and host associations. **Cladistics**, v. 24, n. 5, p. 677–707, 2008.
WILCKE T. *et al*. High prevalence of tungiasis in a poor neighbourhood in Fortaleza, Northeast Brazil. **Acta Tropica**, v. 83, p. 255–258, 2002.
WILLIAMS, S. K. *et al*. Effects of Low-Temperature Flea Maintenance on the Transmission of *Yersinia pestis* by *Oropsylla montana*. **Vector-Borne and Zoonotic Diseases**, v. 13, n. 7, p. 468–478, 2013.
WINKEL, K. T. *et al*. Ectoparasitos em Murídeos Sinantrópicos (Rodentia) em Pelotas, Sul do Rio Grande Do Sul, Brasil. **XVIII Congresso Brasileiro de Parasitologia Veterinária**, Gramado, Rio Grande do Sul, 2014.
YE, G. *et al.* Neurological presentations caused by *Rickettsia felis* infection. **British Journal of Hospital Medicine**, v. 82, n. 6, p. 1-2, 2021.
ZAVALA-VELÁZQUEZ, J. E. *et al*. *Rickettsia felis* rickettsiosis in Yucatán. **The Lancet**, v. 356, n. 9235, p. 1079–1080, 2000.
ZURITA, A. *et al*. Origin, evolution, phylogeny and taxonomy of *Pulex irritans*. **Medical and Veterinary Entomology**, v. 33, n. 2, p. 296–311, 2019.

Supplementary chart 1: Flea species with state locality and hosts described in Brazil, including new records of fleas collected from a preserved Atlantic Rainforest reserve (Legado das Águas – Reserva Votorantim) from 2018 to 2021.

Taxon	Host	Brazilian State	Reference
Family Ceratophyllidae Dampf, 1908			
Genus Nosopsyllus Jordan, 1933			
N. fasciatus (Bosc, 1800)	**Carnivora**: *Canis lupus familiaris* Linnaeus, 1758. **Rodentia**: *Mus musculus* brevirostris (Waterhouse, 1837), *Rattus norvegicus* (Berkenhout, 1769), *Rattus rattus alexandrinus* (Geoffroy, 1803), *Rattus rattus rattus* (Linnaeus, 1758).	GO, RJ, SP	Linardi and Guimaraes, 2000
Family Ctenophthalmidae Rothschild, 1915			
Subfamily Doratopsyllinae Wagner, 1939			
Tribe Tritopsyllini Cunha, 1914			
Genus *Adoratopsylla* Ewing, 1925			
Subgenus *Adoratopsylla* Ewing, 1925			
A. (A.) antiquorum antiquorum (Rothschild, 1904)	**Carnivora:** *Puma yagouaroundi* (Geoffroy, 1803). **Didelphimorphia:** *Didelphis albiventris* Lund, 1840, *Didelphis aurita* (Wied-Neuwied, 1826), *Didelphis marsupialis* Linnaeus, 1758, *Marmosa (Micoureus) paraguayanus* (Tate, 1931), *Marmosa murina* (Linnaeus, 1758), *Marmosops incanus* (Lund, 1840), *Marmosops parvidens* (Tate, 1931), *Metachirus nudicaudatus* (Desmarest, 1817)*, *Monodelphis americana* (Müller, 1776), *Monodelphis dimidiata* (Wagner, 1847), *Monodelphis domestica* (Wagner, 1842), *Monodelphis (Microdelphys) iheringi (*Thomas, 1888), *Philander opossum* (Linnaeus, 1758). **Rodentia:** *Akodon cursor* (Winge, 1887), *Akodon montensis* Thomas, 1913, *Akodon serrensis* Thomas, 1902, *Cerradomys subflavus* (Wagner, 1842), *Delomys dorsalis* (Hensel, 1873), *Delomys sublineatus* (Thomas, 1903), *Euryoryzomys russatus* (Wagner, 1848)*, *Galea spixii* (Wagler, 1831), *Necromys lasiurus* (Lund, 1841), *Nectomys squamipes* (Brants, 1827), *Oligoryzomys moojeni* Weksler & Bonvicino, 2005, *Oligoryzomys nigripes*	AL, BA, CE, ES, MG, PR, RJ, SP*	Barros et al., 1993; Barros-Battesti and Arzua, 1997; Linardi and Guimaraes, 2000; Horta et al., 2007; Pinto et al., 2009; Oliveira et al., 2010; **this study***

Taxon	Host	Brazilian State	Reference
	(Olfers, 1818), *Oxymycterus* sp. Waterhouse, 1837*, *Rhipidomys mastacalis* (Lund, 1840), *Thaptomys nigrita* (Lichtenstein, 1829)*, *Trinomys setosus* (Desmarest, 1817).		
A. (A.) antiquorum ronnai Guimarães, 1954	**Didelphimorphia:** *Marmosa (Micoureus) paraguayanus*, *Philander opossum.*	RS, SC, SP	Linardi and Guimaraes, 2000
A. (A.) bisetosa Ewing, 1925	**Didelphimorphia:** *Monodelphis brevicaudata* (Erxleben, 1777).	AM	Linardi and Guimaraes, 2000
Subgenus *Tritopsylla* Cunha, 1914			
A. (T.) intermedia intermedia (Wagner, 1901)	**Carnivora:** *Cerdocyon thous* (Linnaeus, 1766), *Procyon cancrivorus* Cuvier, 1798. **Didelphimorphia:** Chironectes minimus (Zimmermann, 1780), *Didelphis albiventris*, *Didelphis aurita**, *Lutreolina crassicaudata* (Desmarest, 1804), *Marmosa (Marmosa) murina* (Linnaeus, 1758), *Marmosa (Micoureus) paraguayanus*, *Marmosops incanus*, *Metachirus nudicaudatus**, *Monodelphis americana*, *Monodelphis (Microdelphys) iheringi*, *Philander opossum.* **Rodentia:** *Cavia aperea* Erxleben, 1777, *Euryoryzomys russatus**, *Guerlinguetus* sp. Gray, 1821, *Nectomys squamipes*, *Trinomys paratus* (Moojen, 1948).	BA, ES, MG, PA, PR, RJ, SC, SP*	Barros-Battesti and Arzua, 1997; Linardi and Guimarães, 2000; Salvador et al., 2007; Pinto et al., 2009; Oliveira et al., 2010; **this study***
A. (T.) sinuata Guimarães, 1945	**Didelphimorphia:** *Metachirus nudicaudatus**, *Monodelphis* sp. Burnett, 1830*, *Philander opossum*. **Rodentia:** ***Euryoryzomys russatus*.**	PR, SP*	Linardi and Guimarães, 2000; **this study***
Family Ischnopsyllidae Tiraboschi, 1904			
Subfamily Ischnopsyllinae Wahlgren, 1907			
Tribe Ischnopsyllini Wahlgren, 1907			
Genus *Myodopsylla* Jordan & Rothschild, 1911			
M. wolffsohni wolffsohni (Rothschild, 1903)	**Chiroptera:** *Eptesicus* sp. Rafinesque, 1820, *Molossus currentium currentium* Thomas, 1901, *Myotis levis* (Geoffroy, 1824), *Myotis nigricans nigricans* (Schinz, 1821), *Noctilio leporinus* Linneus, 1758.	AL, AM, MT, PR, SC	Linardi and Guimaraes, 2000; Arzua et al., 2002

Taxon	Host	Brazilian State	Reference
Tribe Sternopsyllini Medvedev, 1985			
Genus *Hormopsylla* Jordan & Rothschild, 1921			
H. fosteri (Rothschild, 1903)	**Chiroptera:** *Cynomops abrasus abrasus* (Temminck, 1827), *Desmodus rotundus* (Geoffroy, 1810), *Lasiurus (Lasiurus) blossevilli* blossevilli (Lesson, 1826), *Nyctinomops* sp. Miller, 1902*, *Nyctinomops laticaudatus* (Geoffroy, 1805), *Phyllostomus hastatus* Pallas, 1767.	MG, PB, RN, SP	Rodriguez et al., 1999; Linardi and Guimaraes, 2000; **this study***
Genus *Ptilopsylla* Jordan & Rothschild, 1921			
P. leptina Jordan & Rothschild, 1921	**Chiroptera:** *Noctilio albiventris* Desmarest, 1818, *Nyctinomops laticaudatus europs* (Allen, 1889).	MS	Linardi and Guimaraes, 2000
Genus *Rothschildopsylla* Guimarães, 1953			
R. noctilionis (Costa Lima, 1920)	**Chiroptera:** *Noctilio albiventris.*	MS	Linardi and Guimaraes, 2000; Linardi, 2017
Genus *Sternopsylla* Jordan & Rothschild, 1921			
S. distincta distincta (Jordan & Rothschild, 1921)	**Chiroptera:** *Molossus currentium currentium*, *Nyctinomops laticaudatus*, *Tadarida brasiliensis* (Geoffroy, 1824)	MG, PR, RS	Linardi and Guimaraes, 2000
Family Leptopsyllidae Baker, 1904			
Subfamily Leptopsyllinae Baker, 1904			
Genus *Leptopsylla* Jordan & Rothschild, 1911			
L. segnis (Schönherr, 1811)	**Didelphimorphia:** *Didelphis aurita*. **Rodentia:** *Cerradomys subflavus*, *Mus musculus* Linnaeus, 1758, *Oxymycterus delator* Thomas, 1903, *Rattus norvegicus*, *Rattus rattus alexandrinus*, *Rattus* rattus *frugivorus* (Rafinesque, 1814), *Rattus rattus rattus*.	MG, PE, RS, SC, SP	Linardi and Guimaraes, 2000; Salvador et al., 2007; Winkel et al., 2014

Taxon	Host	Brazilian State	Reference
Family Pulicidae Billberg, 1820			
Tribe Archaeopsyllini Oudemans, 1909			
Genus *Ctenocephalides* Stiles & Collins, 1930			
C. canis (Curtis, 1826)	**Carnivora**: *Canis lupus familiaris*, *Cerdocyon thous*, *Felis catus* Linnaeus, 1758.	AM, BA, MG, PE, PR, RJ, RS, SC, SP	Cerqueira et al., 2000; Linardi and Guimaraes, 2000; Horta et al., 2006; Guimarães et al., 2011; Silva et al., 2017; Oliveira et al., 2021
C. felis felis (Bouché, 1835)	**Artiodactyla:** *Blastocerus dichotomus* Illiger, 1815, *Bos taurus indicus* (Linnaeus, 1758). **Carnivora**: *Canis lúpus familiaris* Linnaeus, 1758, *Cerdocyon thous*, *Chrysocyon brachyurus* (Illiger, 1815), *Eira barbara* (Linnaeus, 1758), *Felis catus*, *Leopardus pardalis* (Linnaeus, 1758), *Leopardus tigrinus* (Schreber, 1775), *Lycalopex vetulus* (Lund, 1842), *Nasua nasua* (Linnaeus, 1766), *Panthera onca* (Linnaeus, 1758), *Procyon cancrivorus*, *Puma yagouaroundi*. **Cingulata:** *Dasypus novemcinctus* Linnaeus, 1758. **Didelphimorphia:** *Didelphis albiventris*, *Didelphis aurita*, *Didelphis marsupialis*, *Lutreolina crassicaudata*, *Marmosa* (*Micoureus*) *paraguayanus*, *Monodelphis domestica*. **Lagomorpha:** *Sylvilagus brasiliensis* (Linnaeus, 1758). **Pilosa:** *Tamandua tetradactyla* (Linnaeus, 1758). **Perissodactyla:** *Tapirus terrestris* (Linnaeus, 1758). **Primata:** *Homo sapiens* (Linnaeus, 1758), *Sapajus nigritus* (Goldfuss 1809). **Rodentia:** *Akodon serrensis*, *Cavia porcellus* (Linnaeus, 1758), *Cerradomys subflavus*, *Euryzygomatomys spinosus* (G. Fischer, 1814), *Galea spixii*, *Guerlinguetus aestuans* (Linnaeus, 1766), *Hydrochoerus hydrochaeris* (Linnaeus, 1766), *Necromys* lasiurus, *Oligoryzomys nigripes*, *Oxymycterus dasytrichus* (Schinz, 1821), *Oxymycterus delator*, *Thrichomys laurentius* (Thomas, 1904), *Trinomys albispinus* (Geoffroy, 1838).	AL, AM, AP, BA, CE, ES, DF, GO, MG, MS, MT, PA, PB, PE, PI, PR, RJ, RN, RS, RO, RR, SC, SP, TO	Barros-Battesti and Arzua, 1997; Linardi and Guimarães, 2000; Szabó et al., 2000; Pinto et al., 2009; Mendes-de-Almeida et al., 2011; Heukelbach et al., 2012; Horta et al., 2014; Paz et al., 2015; Regolin et al., 2015; Schott et al., 2019; Paz et al., 2022; Gonçalves et al., 2023; Silva et al., 2023
Tribe Pulicini Billberg, 1820			

Taxon	Host	Brazilian State	Reference
Genus *Pulex* Linnaeus, 1758			
P. irritans Linnaeus, 1758	**Carnivora**: *Canis lupus familiaris*, *Cerdocyon thous*, *Conepatus chinga* (Molina, 1782), *Chrysocyon brachyurus*, *Galictis vittata* (Schreber, 1776), *Leopardus geoffroyi* (d'Orbigny & Gervais, 1844), *Leopardus pardalis*, *Panthera onca*, *Procyon cancrivorus*. **Chiroptera:** *Nyctinomops laticaudatus*. **Pilosa:** *Tamandua tetradactyla*. **Primata:** *Homo sapiens*. **Didelphimorphia:** *Monodelphis domestica*, *Philander opossum*. **Rodentia:** *Cuniculus paca* (Linnaeus, 1766), *Galea spixii*, *Guerlinguetus aestuans*, *Holochilus brasiliensis* (Desmarest, 1819), *Kerodon rupestris* (Wied-Neuwied, 1820), *Oligoryzomys nigripes*, *Thrichomys inermis* (Pictet, 1841), *Thrichomys laurentius*, *Trinomys dimidiatus* (Günther, 1877), *Trinomys setosus*, *Wiedomys pyrrhorhinos* (Wied-Neuwied, 1821).	AL, BA, CE, ES, GO, MG, PA, PB, PE, PI, PR, RJ, RN, RS, SC, SP	Linardi and Guimarães, 2000; Carvalho et al., 2001; Curi et al., 2010; Pereira et al., 2014; Santos et al., 2016; Fontalvo et al., 2017; Souza et al., 2021;
Tribe Xenopsyllini Glienkiewicz, 1907			
Genus *Xenopsylla* Glienkiewicz, 1907			
X. brasiliensis (Baker, 1904)	**Carnivora**: *Canis lupus familiaris*. **Rodentia:** *Necromys lasiurus*, *Oligoryzomys nigripes*, *Mus musculus*, *Rattus norvegicus*, *Rattus rattus alexandrinus*, *Rattus* rattus *frugivorus*, *Rattus rattus rattus*.	CE, PB, PE, RN, RJ, RS, SP	Linardi and Guimarães, 2000
X. cheopis (Rothschild, 1903)	**Carnivora**: *Canis lupus familiaris*, *Cerdocyon thous*. **Didelphimorphia:** *Didelphis aurita*, *Didelphis marsupialis*, *Monodelphis domestica.* **Rodentia:** *Akodon cursor*, *Akodon montensis*, *Cavia aperea*, *Cerradomys subflavus*, *Galea spixii*, *Holochilus brasiliensis*, *Holochilus sciureus* Wagner, 1842, *Necromys lasiurus*, *Nectomys squamipes*, *Rattus rattus rattus*, *Thrichomys apereoides* (Lund, 1839), *Thrichomys inermis*, *Thrichomys laurentius*, *Trinomys elegans* (Lund, 1841), *Trinomys setosus.*	AL, BA, CE, MG, PE, PR, RJ, RN, RS, SC, SP	Cerqueira et al., 2000; Linardi and Guimarães, 2000; Carvalho et al., 2001; Ribeiro et al., 2003; Oliveira et al., 2009; Porta et al., 2014; Bezerra-Santos et al., 2020
Family Rhopalopsyllidae Oudemans, 1909			

Taxon	Host	Brazilian State	Reference
Subfamily Rhopalopsyllinae Oudemans, 1909			
Tribe Polygenini Linardi & Guimarães, 1993			
Genus *Neotropsylla* Linardi & Guimarães, 1993			
N. guimaraesi (Linardi, 1978)	**Rodentia:** *Calomys* sp. Waterhouse, 1837	SP	Linardi and Guimarães, 2000; Linardi, 2011
Genus Polygenis Jordan, 1939			
Subgenus Polygenis (Neopolygenis) Linardi & Guimarães, 1993			
P. N. atopus (Jordan & Rothschild, 1922)	**Carnivora**: *Eira barbara*, *Felis catus*, *Procyon cancrivorus.* **Didelphimorphia:** *Didelphis albiventris*, *Didelphis aurita*, *Didelphis marsupialis*, *Philander opossum*. **Passeriformes:** *Haplospiza unicolor* Linnaeus, 1766. **Rodentia:** *Akodon cursor*, *Akodon montensis*, *Caluromys* philander (Linnaeus, 1758), *Cerradomys subflavus*, *Delomys dorsalis*, *Euryoryzomys russatus*, *Guerlinguetus* sp., *Holochilus brasiliensis*, *Nectomys squamipes*, *Oligoryzomys flavescens* (Waterhouse, 1837), *Oligoryzomys nigripes*, *Oxymycterus dasytrichus*, *Rhipidomys mastacalis*, *Sooretamys angouya* (Fischer, 1814).	MG, PR, RS, RJ, SC, SP	Barros-Battesti and Arzua, 1997; Linardi and Guimarães, 2000; Horta et al., 2007; Muller et al., 2009; Oliveira et al., 2010; Brum, 2018
P. N. dentei Guimarães, 1947	**Rodentia:** *Akodon cursor*, *Akodon montensis*, *Delomys dorsalis*, *Oxymycterus quaestor* Thomas, 1903, *Thaptomys nigrita*	SP, RJ	Linardi and Guimarães, 2000; De Moraes et al., 2003; Linardi, 2011
P. N. frustratus Johnson, 1957	**Didelphimorphia:** *Didelphis marsupialis*, *Philander opossum*. **Rodentia:** *Akodon montensis*, *Cavia aperea*, *Delomys dorsalis*, *Oxymycterus dasytrichus*, *Oxymycterus quaestor* Thomas, 1903, *Thaptomys nigrita*.	SP, RJ, SC, PR	Linardi and Guimarães, 2000; de Moraes et al., 2003; Linardi, 2011
P. N. pradoi (Wagner, 1937)	**Carnivora:** *Nasua nasua*. **Didelphimorphia:** *Didelphis albiventris*, *Didelphis marsupialis*, *Philander opossum*.	BA, ES, PR, RJ, RS, SC, SP	Barros et al., 1993; Hastriter and Peterson, 1997; Linardi and

Taxon	Host	Brazilian State	Reference
	Rodentia: *Akodon cursor*, *Akodon montensis*, *Akodon reigi* González, Langguth & Oliveira, 1998, *Akodon serrensis*, *Euryoryzomys russatus*, *Euryzygomatomys spinosus*, *Necromys lasiurus*, *Nectomys squamipes*, *Oligoryzomys nigripes*, *Oxymycterus quaestor*, *Rattus rattus rattus*, *Thaptomys nigrita*, *Trinomys iheringi* (Thomas, 1911).		Guimarães, 2000; Carvalho et al., 2001; Linardi, 2011; Schott et al., 2020
P. N. pygaerus (Wagner, 1937)	**Didelphimorphia:** *Didelphis aurita* **Rodentia:** *Akodon cursor*, *Akodon montensis*, *Akodon serrensis*, *Euryoryzomys russatus*, *Necromys lasiurus*, *Nectomys squamipes*, *Oxymycterus quaestor*, *Rattus rattus rattus*, *Thaptomys nigrita*.	MG, PR, RJ, SC	Linardi and Guimarães, 2000; Linardi et al., 1987; Carvalho et al., 2001
Subgenus Polygenis (Polygenis) Jordan, 1939			
P. P. acodontis (Jordan & Rothschild, 1923)	**Rodentia:** *Guerlinguetus aestuans*.	SC	Linardi and Guimarães, 2000
P. P. adelus (Jordan & Rothschild, 1923)	**Didelphimorphia:** *Monodelphis domestica*. **Rodentia:** *Akodon montensis*, *Calomys tener* (Winge, 1887), *Cerradomys subflavus*, *Euryoryzomys russatus*, *Necromys lasiurus*, *Rhipidomys mastacalis*, *Trinomys albispinus*, *Trinomys setosus*, *Wiedomys pyrrhorhinos*.	BA, MG, PE, SP	Linardi and Guimarães, 2000; Linardi, 2011
P. P. axius axius (Jordan & Rothschild, 1923)	**Didelphimorphia:** *Didelphis albiventris*, *Lutreolina crassicaudata*. **Rodentia:** *Akodon cursor*, *Necromys lasiurus*, *Nectomys squamipes*, *Oxymycterus dasytrichus*.	MG, PR, RS, SP	Barros-Battesti and Arzua, 1997; Linardi and Guimarães, 2000; Linardi, 2011
P. P. axius pessoai Guimarães, 1956	**Rodentia:** *Cerradomys subflavus*, *Oligoryzomys nigripes*, *Oxymycterus dasytrichus*.	AL, PE	Linardi and Guimarães, 2000
P. P. axius proxima Guimarães, 1948	**Didelphimorphia:** *Lutreolina crassicaudata.* **Rodentia:** *Akodon montensis*, *Necromys lasiurus*.	MG, MS, RS, SP	Linardi and Guimarães, 2000; Muller et al., 2009; Linardi, 2011; 2017
P. P. bohlsi bohlsi (Wagner, 1901)	**Didelphimorphia:** *Didelphis* sp. Linnaeus, 1758. **Rodentia:** *Calomys callosus* (Rengger, 1830), *Calomys tener*, *Cerradomys subflavus*, *Cuniculus paca*, *Necromys lasiurus*, *Nectomys squamipes*, *Oligoryzomys nigripes*, *Oxymycterus dasytrichus*, *Rattus rattus frugivorus*, *Thrichomys apereoides*.	ES, GO, MG, MS	Linardi and Guimarães, 2000; Pinto et al., 2009; de Sousa et al., 2017; 2018

Taxon	Host	Brazilian State	Reference
P. P. bohlsi jordani (Lima, 1937)	**Carnivora:** *Cerdocyon thous*, *Galictis vittata*. **Didelphimorphia:** *Didelphis albiventris*, *Metachirus nudicaudatus**, *Monodelphis domestica*. **Lagomorpha:** *Sylvilagus brasiliensis*. **Primata:** *Callithrix jacchus* (Linnaeus, 1758). **Rodentia:** *Akodon montensis*, *Calomys expulsus* (Lund, 1840), *Calomys tener*, *Cavia aperea*, *Cerradomys subflavus*, *Echimys chrysurus* (Zimmermann, 1780), *Euryoryzomys lamia* (Thomas, 1901), *Euryoryzomys russatus**, *Galea spixii*, *Holochilus brasiliensis*, *Holochilus sciureus*, *Kerodon rupestris*, *Mus musculus brevirostris*, *Necromys lasiurus*, *Nectomys squamipes*, *Oligoryzomys flavescens*, *Oligoryzomys* sp.*, *Oxymycterus* sp.*, *Rattus norvegicus*, *Rattus rattus alexandrinus*, *Rattus rattus frugivorus*, *Rhipidomys mastacalis*, *Thrichomys inermis*, *Thrichomys laurentius*, *Trinomys albispinus*, *Trinomys setosus*, *Wiedomys pyrrhorhinos*.	AL, BA, CE, PE, PB, RN, SP*	Almeida et al., 1986; Hastriter and Peterson, 1997; Linardi and Guimarães, 2000; Oliveira et al., 2009; de Oliveira et al., 2021; **this study***
P. P. occidentalis occidentalis (Jordan & Rothschild, 1923)	**Carnivora:** *Cerdocyon thous*. **Cingulata:** *Dasypus novemcinctus*. **Didelphimorphia:** *Didelphis aurita*, *Didelphis marsupialis*. **Rodentia:** *Akodon* spp. Meyen, 1833, *Delomys dorsalis*, *Guerlinguetus aestuans*, *Guerlinguetus brasiliensis ingrami* (Thomas, 1901), *Necromys lasiurus*, *Oligoryzomys nigripes*, *Oxymycterus nasutus* (Waterhouse, 1837), *Rattus norvegicus*, *Rhipidomys mastacalis*, *Scapteromys tumidus* (Waterhouse, 1837), *Thrichomys inermis*. **Tinamiformes:** *Crypturellus obsoletus obsoletus* (Sclater, 1865).	AL, CE, ES, PR, RJ, RS, SC, SP	Linardi and Guimarães, 2000; Pinto et al., 2009; Oliveira et al., 2010; Linardi, 2011; Schott et al., 2020
P. P. occidentalis steganus (Jordan & Rothschild, 1923)	**Rodentia:** *Rhipidomys mastacalis*.	CE, GO, PA, RR	Linardi and Guimarães, 2000
P. P. platensis platensis (Jordan & Rothschild, 1908)	**Rodentia:** *Akodon azarae* (Fischer, 1829), *Ctenomys flamarioni* Travi, 1981, *Ctenomys minutus* Nehring, 1887, *Delomys dorsalis*, *Oligoryzomys nigripes*, *Scapteromys tumidus*.	RS	Linardi and Guimarães, 2000; Linardi et al., 2005; Schott et al., 2020
P. P. rimatus (Jordan, 1932)	**Didelphimorphia:** *Didelphis albiventris*, *Didelphis marsupialis*, *Monodelphis brevicaudata*, *Philander* sp. Brisson, 1762, *Metachirus nudicaudatus**. **Rodentia:** *Akodon* sp.*, *Akodon cursor*, *Akodon montensis*, *Akodon serrensis*, *Calomys expulsus*, *Cerradomys subflavus*, *Delomys dorsalis*, *Euryoryzomys russatus**, *Euryzygomatomys spinosus*, *Guerlinguetus brasiliensis ingrami*, *Necromys lasiurus*, *Nectomys squamipes*, *Oligoryzomys mattogrossae* Allen, 1916, *Oligoryzomys nigripes*,	BA, ES, GO, MG, PA, PR, RJ, RS, SC, SP	Barros et al., 1993; Barros-Battesti and Arzua, 1997; Hastriter and Peterson, 1997; Linardi and Guimarães, 2000; Carvalho et al.,

Taxon	Host	Brazilian State	Reference
	Oxymycterus sp.*, *Oxymycterus quaestor*, *Rattus norvegicus*, *Rattus rattus alexandrinus*, *Sooretamys angouya*, *Thaptomys nigrita*, *Trinomys dimidiatus*.		2001; Horta et al., 2007; Muller et al., 2009; Oliveira et al., 2010; Brum, 2018; Schott et al., 2020; **this study***
P. P. roberti beebei (Fox, 1947)	**Rodentia:** *Euryoryzomys* spp.	AP	Linardi and Guimarães, 2000
P. P. roberti roberti (Rothschild, 1905)	**Carnivora:** *Leopardus pardalis*. **Chiroptera:** *Chrotopterus auritus* (Peters, 1856)* **Cingulata:** *Dasypus novemcinctus*. **Didelphimorphia:** *Didelphis albiventris*, *Didelphis aurita**, *Didelphis marsupialis*, *Gracilinanus* sp. Gardner & Creighton, 1989*, *Marmosa* (*Micoureus*) *paraguayanus*, *Metachirus nudicaudatus**, *Monodelphis* sp.* **Pilosa:** *Tamandua tetradactyla*. **Rodentia:** *Akodon* sp.*, *Akodon montensis*, *Brucepattersonius* sp. Hershkovitz, 1998*, *Cerradomys subflavus*, *Dasyprocta azarae* Lichtenstein, 1823, *Dasyprocta* (Linnaeus, 1758), *Delomys dorsalis*, *Euryoryzomys lamia*, *Euryoryzomys russatus**, *Guerlinguetus brasiliensis ingrami**, *Holochilus brasiliensis**, *Hylaeamys megacephalus* (Fischer, 1814)*, *Hylaeamys oniscus* (Thomas, 1904), *Nectomys squamipes**, *Oligoryzomys nigripes**, *Oxymycterus* sp.*, *Oxymycterus quaestor*, *Phyllomys* sp. Lund, 1839*, *Proechimys guyannensis* (Geoffroy, 1803), *Rattus norvegicus*, *Rhipidomys mastacalis**, *Sooretamys angouya**, *Thaptomys nigrita*, *Trinomys dimidiatus*, *Trinomys setosus*.	BA, ES, GO, MG, MS, PE, PR, RJ, RS, SC, SP	Barros et al., 1993; Hastriter and Peterson, 1997; Carvalho et al., 2001; Horta et al., 2007; Muller et al., 2009; Brum, 2018; Schott et al., 2020; **this study***
P. P. tripopsis Guimarães, 1948	**Carnivora:** *Leopardus pardalis*. **Cingulata:** *Dasypus novemcinctus*. **Rodentia:** *Cerradomys subflavus*, *Echimys chrysurus*, *Euryoryzomys lamia*, *Holochilus brasiliensis*, *Hylaeamys megacephalus*, *Necromys lasiurus*, *Oligoryzomys nigripes*, *Oxymycterus dasytrichus*, *Rhipidomys mastacalis*.	BA, CE, GO, MS, PE	Linardi and Guimarães, 2000; Sponchiado et al., 2015; Linardi, 2017
P. P. tripus (Jordan, 1933)	**Didelphimorphia:** *Didelphis albiventris*, *Didelphis aurita*, *Didelphis marsupialis*, *Lutreolina crassicaudata*, *Monodelphis domestica*. **Rodentia:** *Akodon cursor*, *Akodon montensis*, *Calomys expulsus*, *Calomys tener*, *Cavia aperea*, *Cerradomys subflavus*, *Euryzygomatomys spinosus*, Galea *spixii*, *Holochilus brasiliensis*, *Holochilus sciureus*, *Mus musculus brevirostris*, *Necromys lasiurus*, *Nectomys*	AL, BA, CE, ES, GO, MG, PE, PR, RJ, RN, SP	Botelho et al.,1981; Almeida et al., 1986; Botelho; Linardi, 1992; Barros-Battesti and Arzua, 1997; Hastriter

Taxon	Host	Brazilian State	Reference
	squamipes, *Oligoryzomys nigripes*, *Oxymycterus dasytrichus*, *Rattus norvegicus*, *Rattus rattus alexandrinus*, *Rattus rattus frugivorus*, *Rhipidomys mastacalis*, *Thrichomys apereoides*, *Thrichomys inermis*, *Thrichomys laurentius*, *Trinomys albispinus*, *Trinomys setosus*, *Wiedomys pyrrhorhinos*.		and Peterson, 1997; Linardi and Guimarães, 2000; Carvalho et al., 2001; Horta et al., 2007; Oliveira et al., 2009; Pinto et al., 2009
Tribe Rhopalopsyllini Oudemans, 1909			
Genus *Gephyropsylla Barrera, 1952*			
G. klagesi klagesi (Rothschild, 1904)	**Cingulata:** *Dasypus novemcinctus*. **Didelphimorphia:** *Didelphis* spp., *Philander opossum*. **Rodentia:** *Cerradomys subflavus*, *Proechimys guyannensis*, *Rhipidomys mastacalis*.	AM, CE, GO, PA, RR	Rafael, 1982; Linardi et al., 1991; Linardi and Guimarães, 2000
G. klagesi samuelis (Jordan & Rothschild, 1923)	**Didelphimorphia:** *Didelphis marsupialis*. **Rodentia:** *Holochilus brasiliensis*, *Proechimys guyannensis*, *Proechimys longicaudatus* (Rengger, 1830).	AM, GO, RO, RR	Linardi et al., 1991; Hastriter and Peterson, 1997; Linardi and Guimarães, 2000
Genus *Hechtiella* Barrera, 1952			
H. lakoi (Guimarães, 1948)	**Didelphimorphia:** *Philander opossum*. **Rodentia:** *Euryoryzomys lamia*, *Oligoryzomys nigripes*, *Philander*, *Trinomys dimidiatus*, *Trinomys iheringi*.	ES, MG, RJ, SP	Linardi and Guimarães, 2000; Bittencourt and Rocha, 2003; de Moraes et al., 2003; Linardi, 2011
H. lopesi Guimarães & Linardi, 1993	**Rodentia:** *Proechymis* spp., *Trinomys iheringi*.	SP	Guimarães and Linardi, 1993, Linardi and Guimarães, 2000; Linardi, 2011
H. nitidus (Johnson, 1957)	**Cingulata:** *Dasypus novemcinctus*. **Didelphimorphia:** *Didelphis marsupialis*, *Marmosops incanus*, *Metachirus nudicaudatus*. **Rodentia:** *Necromys lasiurus*, *Nectomys squamipes*, *Trinomys dimidiatus*, *Trinomys iheringi*, *Trinomys paratus*.	BA, ES, MG, RJ	Botelho et al.,1981; Hastriter and Peterson, 1997; Linardi and

Taxon	Host	Brazilian State	Reference
			Guimarães, 2000; Pinto et al., 2009
Genus *Rhopalopsyllus* Baker, 1905			
R. australis australis Rothschild, 1904	**Perissodactyla:** *Tapirus terrestris*. **Rodentia:** *Dasyprocta fuliginosa* Wagler, 1832, *Proechimys guyannensis*.	AP, PA, RO, RR	Linardi et al., 1991; Linardi and Guimarães, 2000; Gonçalves et al; 2023
R. australis tamoyus Jordan & Rothschild, 1923	**Artiodactyla:** *Mazama rufa* Illiger, 1815. **Carnivora:** *Eira barbara*, *Nasua nasua*, *Procyon cancrivorus*. **Cingulata:** *Dasypus novemcinctus*. **Pilosa:** *Tamandua* spp. Gray, 1825. **Rodentia:** *Cuniculus paca*, *Dasyprocta azarae*, *Dasyprocta fuliginosa*.	GO, MG, MS, MT, RO, SP	Linardi and Guimarães, 2000; Linardi, 2011; 2017; Mendonça et al., 2020
R. australis tupiniquinus Guimarães, 1940	**Carnivora:** *Eira barbara*, *Leopardus pardalis*.	SP	Linardi and Guimarães, 2000; Linardi, 2011
R. australis tupinus Jordan & Rothschild, 1923	**Rodentia:** *Myoprocta acouchy* (Erxleben, 1777).	PA	Linardi and Guimarães, 2000
R. crypturi Wagner, 1939	**Tinamiformes:** *Crypturellus obsoletus obsoletus.*	SC	Linardi and Guimarães, 2000
R. garbei Guimarães, 1940	**Rodentia:** *Myoprocta acouchy.*	PA	Linardi and Guimarães, 2000
R. lugubris lugubris Jordan & Rothschild, 1908	**Artiodactyla:** *Mazama americana* (Erxleben, 1777). **Cingulata:** *Dasypus novemcinctus.* **Didelphimorphia:** *Didelphis marsupialis.* **Rodentia:** *Akodon montensis*, *Cuniculus paca*, *Dasyprocta leporina*, *Oxymycterus quaestor*, *Trinomys dimidiatus*, *Trinomys iheringi.*	ES, GO, MG, MS, MT, PA, RJ, RO, SC, SP	Linardi and Guimarães, 2000; Horta et al., 2007; Silveira, 2012; Linardi, 2017, Mendonça et al., 2020
R. lutzi lutzi (Baker, 1904)	**Carnivora:** *Canis lupus familiaris*, *Cerdocyon thous*, *Leopardus pardalis*, *Nasua nasua*, *Puma yagouaroundi*, *Galictis vittata*. **Cingulata:** *Dasypus novemcinctus.*	BA, ES, GO, MG, MS, PR, RJ, RO, SP	Barros et al., 1993; Barros-Battesti and Arzua; 1997 Cerqueira

Taxon	Host	Brazilian State	Reference
	Didelphimorphia: *Didelphis albiventris*, *Didelphis aurita*, *Didelphis marsupialis*, *Philander opossum*. **Pilosa:** *Tamandua tetradactyla*. **Rodentia:** *Akodon serrensis*, *Dasyprocta azarae*, *Dasyprocta leporina*.		et al., 2000; Linardi and Guimarães, 2000; Scofield et al., 2005; Rodrigues et al., 2006; Horta et al., 2007; Rodrigues et al., 2008; Pinto et al., 2009; Linardi, 2017; Estevam et al., 2020
R. saevus Jordan & Rothschild, 1923	**Cingulata:** *Dasypus novemcinctus.* **Didelphimorphia:** *Didelphis marsupialis.* **Rodentia:** *Dasyprocta fuliginosa*.	MT, RO	Linardi; Guimarães, 2000, Mendonça et al., 2020
Family Stephanocircidae Wagner, 1928			
Subfamily Craneopsyllinae Wagner, 1939			
Tribe Craneopsyllini Wagner, 1939			
Genus *Craneopsylla* Rothschild, 1911			
C. minerva minerva (Rothschild, 1903)	**Chiroptera:** *Anoura geoffroyi geoffroyi* Gray, 1838, *Sturnira* (*Sturnira*) *lilium* (Geoffroy, 1810). **Didelphimorphia:** *Didelphis albiventris*, *Lutreolina crassicaudata*, *Marmosops incanus*, *Monodelphis domestica*, *Philander opossum*. **Rodentia:** *Akodon cursor*, *Akodon montensis*, *Akodon reigi*, *Akodon serrensis*, *Brucepattersonius iheringi*, *Calomys tener*, *Cerradomys subflavus*, *Delomys dorsalis*, *Euryoryzomys lamia*, *Euryoryzomys russatus*, *Guerlinguetus aestuans*, *Holochilus sciureus*, *Necromys lasiurus*, *Nectomys squamipes*, *Oligoryzomys flavescens*, *Oligoryzomys nigripes*, *Oxymycterus dasytrichus*, *Oxymycterus quaestor*, *Proechimys guyannensis*, *Rattus rattus rattus*, *Rhipidomys mastacalis*, *Sooretamys angouya*, *Thaptomys nigrita*, *Trinomys dimidiatus*, *Wiedomys pyrrhorhinos*.	AL, BA, CE, MG, PE, PR, RJ, RS, SC, SP	Linardi; Guimarães, 2000; Carvalho et al., 2001; Bittencourt and Rocha, 2003; Schott et al., 2020

Taxon	Host	Brazilian State	Reference
Family Tungidae Taschenberg, 1880			
Subfamily Hectopsyllinae Baker, 1904			
Genus *Hectopsylla* Frauenfeld, 1860			
H. psittaci Frauenfeld, 1860	**Columbiformes:** *Columba livia* Gmelin, 1789. **Passeriformes:** *Progne chalybea* Gmelin, 1789, *Turdus leucomelas* Vieillot, 1818.	RJ, RS, SP	Linardi and Guimarães, 2000
Hectopsylla pulex (Haller, 1880)	**Chiroptera:** *Histiotus velatus* (Geoffroy, 1824), *Molossus molossus* Pallas, 1766, *Molossus rufus* Geoffroy, 1805, *Peropteryx macrotis* (Wagner, 1843), *Phyllostomus hastatus*.	BA, MG, PE, PR, RJ, RS, SC, SP	Linardi and Guimarães, 2000; Esbérard, 2001; Luz et al., 2009
Subfamily Tunginae Taschenberg, 1880			
Genus *Tunga* Jarocki, 1838			
T. bondari Wagner, 1932	**Cariamiformes:** *Cariama cristata* (Temminck, 1823). **Pilosa:** *Tamandua tetradactyla*.	BA, MG, SP	Hopkins and Rothschild, 1956; Linardi and Guimarães, 2000
T. bossii Avelar, Linhares & Linardi, 2012	**Rodentia:** *Delomys dorsalis.*	RJ	Linardi and Guimarães, 2000; de Avelar et al., 2012
T. caecata (Enderlein, 1901)	**Rodentia:** *Mus musculus, Rattus rattus rattus, Rattus norvegicus, Akodon cursor, Necromys pixuna, Nectomys squamipes, Oligoryzomys nigripes, Oxymycterus sp., Rhypidomys mastacalis.*	MG, PR, SP, RJ	Linardi and Guimarães, 2000; Linardi and Avelar, 2014
T. hexalobulata Avelar, Facury Filho & Linardi, 2013	**Artiodactyla:** *Bos taurus indicus.*	MG	de Avelar et al., 2013
T. penetrans (Linnaeus, 1758)	**Artiodactyla:** *Bos taurus indicus, Sus scrofa* Linnaeus, 1758, *Capra hircus* Linnaeus, 1758, *Ovis aries* Linnaeus, 1758, *Pecari tajacu* Linnaeus, 1758. **Carnivora:** *Canis lupus familiaris, Felis cattus* Linnaeus, 1758, *Panthera onca*. **Cingulata:** *Dasypus novemcinctus*. **Passeriformes:** *Volatinia jacarina* (Linnaeus, 1766).	AC, AL, AM, AP, BA, CE, DF, ES, GO, MA, MG, MS, MT, PA,	Linardi, 1998; Linardi and Guimarães, 2000; Frank et al., 2012; de Avelar et al., 2013; Linardi and de Avelar,

Taxon	Host	Brazilian State	Reference
	Perissodactyla: *Equus caballus* Linnaeus, 1758, *Tapirus terrestris*. **Pilosa:** *Tamandua tetradactyla, Myrmecophaga tridactyla* (Linnaeus, 1758). **Primata**: *Alouatta guariba clamitans* Cabrera, 1940, *Homo sapiens*. **Rodentia:** *Cuniculus paca, Mus musculus, Rattus rattus rattus, Rattus norvegicus.*	PB, PE, PI, PR, RJ, RN, RO, RS, RR, SC, SE, SP, TO	2014; Schott et al., 2020; Tancredi et al., 2021; Santos et al., 2022; Jesus et al., 2023
T. terasma Jordan, 1937	**Cingulata:** *Cabassous unicinctus* (Linnaeus, 1758), *Dasypus novemcinctus, Euphractus sexcinctus* (Linnaeus, 1758), *Priodontes maximus* (Kerr, 1792).	ES, GO, MA, MG, MS, SP	Linardi and Guimarães, 2000; Antunes et al., 2006; Linardi and de Avelar, 2014
T. travassosi Pinto & Dreyfus, 1927	**Cingulata:** *Dasypus novemcinctus*.	MG, SP	Pinto and Dreyfus; 1927; Linardi and Guimarães, 2000; Linardi and de Avelar, 2014
T. trimamillata Pampiglione, Trentini, Fioravanti, Onore & Rivasi, 2002	**Artiodactyla:** *Bos taurus indicus, Sus scrofa, Capra hircus, Ovis aries.* **Primata:** *Homo sapiens.* **Rodentia:** *Hydrochoerus hydrochaeris*.	MG, SP	Fioravanti et al., 2003; Linardi and de Avelar, 2014; Harvey et al., 2021

Legend: *new hosts or locations provided in this study, AC: Acre, AL: Alagoas, AM: Amazonas, AP: Amapá, BA: Bahia, CE: Ceará, DF: Distrito Federal, ES: Espírito Santo, GO: Goiás, MA: Maranhão, MG: Minas Gerais, MS: Mato Grosso do Sul, MT: Mato Grosso, PA: Pará, PB: Paraíba, PE: Pernambuco, PI: Piauí, PR: Paraná, RJ: Rio de Janeiro, RN: Rio Grande do Norte, RO: Rondônia, RS: Rio Grande do Sul, RR: Roraima, SC: Santa Catarina, SE: Sergipe, SP: São Paulo, TO: Tocantins.

4 CHAPTER 3 – EPIDEMIOLOGICAL ASPECTS OF RICKETTSIA IN MAMMALS AND TICKS IN A PRESERVED ATLANTIC RAINFOREST FRAGMENT IN SOUTHEASTERN BRAZIL

4.1 INTRODUCTION

Studies show that high levels of anthropization can impact the parasites and the pathogen life cycles, mainly because there are host loss and environmental fragmentation, and in these scenarios, parasites can act as bioindicators (Lafferty, 1997; Sures et all., 2004). With the loss of forested areas, human populations are exposed to more significant interaction with parasites and pathogens that once lived in a balanced ecosystem with their main hosts (Vidal-Martinéz et al., 2009). The diversity of ectoparasites is closely related to the host populations at that site, meaning if species richness declines over time, it indicates the fauna of that ambient is also suffering with human growth (Bush; Reed; Maher, 2013).

The Brazilian Atlantic Rainforest is one of the 35 biodiversity hotspots of the planet, harbouring areas with high levels of endemic species and highly threatened by human activities (Colombo; Joly, 2010) that have been reduced to 7.5% of its original area (Scarano, 2002). The mammal orders with the higher number of species in this biome are Chiroptera, Rodentia and Didelphimorphia (Paglia et al., 2012), known for acting as reservoirs to pathogens and hosts to ectoparasites (Blanco et al., 2017; Rozental et al., 2017) maintaining the balance and not showing distress signals towards these interactions. Hence, studying pathogen-host relationships in the primary environment is essential to understanding the evolution of these microorganisms in anthropized conditions.

Ticks are obligate hematophagous ectoparasites, and they can cause skin discomfort to the host and act as important vectors of numerous agents (Sonenshine; Roe, 2014). Several species of ticks found in Brazil have been reported to parasitize humans or live in close contact within natural ecosystems or anthropized environments, and some of these ticks have been proven or are considered to be vectors of rickettsial pathogens, being a relevant public health issue in Latin America (Dantas-Torres et al., 2019). Among the rickettsiae circulating in Brazil, the Spotted Fever Group (SFG) comprises pathogenic and non-pathogenic species and is considered the most important health concern (Szabó; Pinter; Labruna, 2013). Even though the Bellii group presents a non-pathogenic nature

(Parola et al., 2013; Krawczak et al., 2018), it is also relevant for being capable of infecting many tick species and possibly acting as an inhibitor of SFG rickettsiae to the progeny of infected ticks (Sakai et al., 2014).

The objective of this study was to identify the species of ticks collected parasitizing rodents, marsupials, dogs, humans, and free-living on the vegetation in four areas of the Atlantic Rainforest, São Paulo State, Brazil, and detect the circulating rickettsiae in the vectors and hosts.

4.2 MATERIALS AND METHODS

4.2.1 Specimens collection

The ticks examined were collected in the private reserve Legado das Águas - Reserva Votorantim, Miracatu, São Paulo, Brazil, located in a fragment of Atlantic Forest, with approximately 75% of the total area composed of dense primary ombrophilous forest. Nowadays, the main activity performed there is the ecotourism. Within the perimeter of the reserve, four areas were chosen: Sede (24° 1' 49.51" S, 47° 21' 8.36" W; 499m), Porto Raso (24° 3' 25.90" S, 47° 26' 30.07" W; 274m), Serraria (24° 9' 9.63" S, 47° 32' 53.49" W; 117m) and Ribeirão da Anta Cabocla Community (24° 4’ 8.58” S, 47° 43’ 75.52” W; 277m), All the areas described above are better shown in Figure 9.

Each area selected has its characteristics. The first area (Sede) is highly anthropized, where most of the tourism happens and where the visitants stay at night. The vegetation in the trails from this area is slightly modified with the constant flow of visitors. The second area (Porto Raso) is the most preserved area within our study, this collection point is located in the center of the reserve, and the chosen trails rarely have a tourist in them. The Serraria area has highly degraded vegetation as it is located on the reserve’s border and close to a highway, thus keeping all the large mammals away. Lastly, the Ribeirão da Anta area is a Cabocla Community where the villagers interact with the preserved Atlantic Forest and at this last collection point, ticks were only collected on leaf tips and from the dogs of residents. This area was chosen because the residents keep domestic dogs with free access to the forest, acting as possible tick carriers.

Between January 2018 and December 2021, eight campaigns were conducted, lasting an average of seven to twelve days, with three campaigns in the Sede area

(January, July and December of 2018 with six days each), three in Porto Raso (July of 2019, February of 2020 and October of 2021 with six days each), and two longer ones in Serraria and Ribeirão da Anta Cabocla Community (September and December of 2022 with nine days each) due to the COVID-19 pandemic resulting in a total of 18 days in each sampled area.

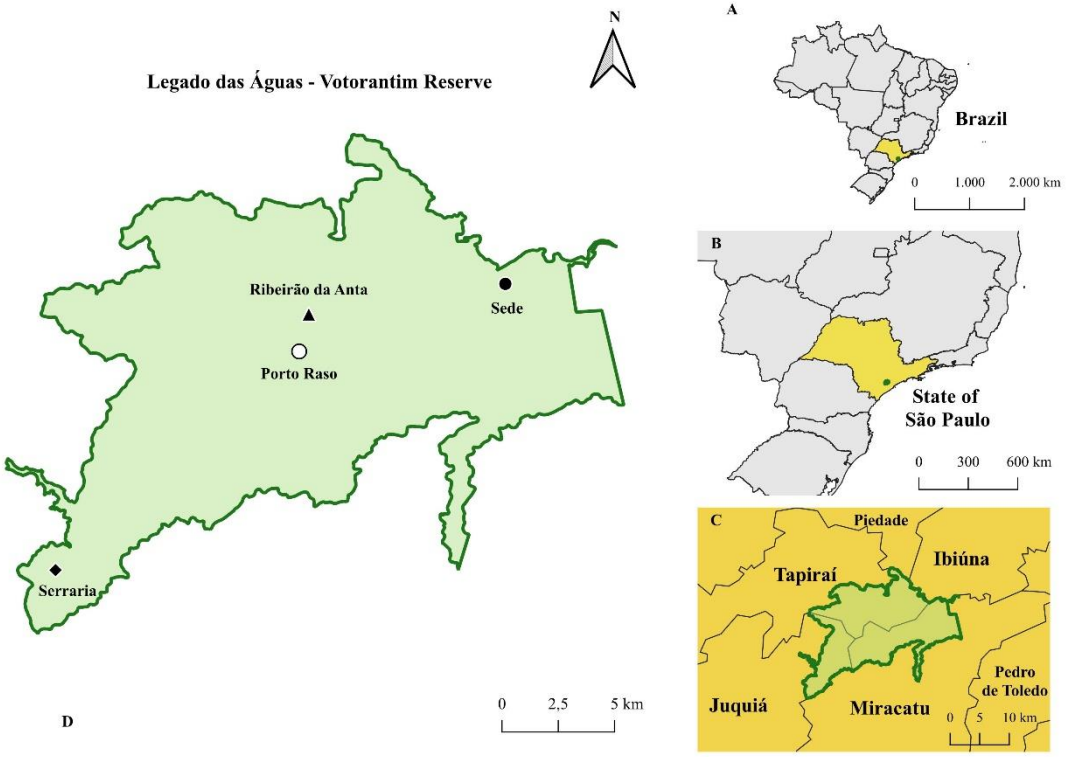

Figure 10: Map showing the collection area. **A.** Brazil and State of São Paulo highlighted; **B.** Position of the reserve inside São Paulo's state; **C.** municipalities surrounding the reserve area; **D.** Collection sites within the Legado das Águas limit.

The trails chosen were based on the vegetation and tracks of wild animals. In total, 240 traps (Sherman and Tomahawk traps) were used in each campaign in the Sede, Porto Raso, and Serraria areas. The small mammals were captured using bait made with a mixture of sardines, cornmeal, coconut oil, vanilla, and peanut paste. After capture, the animals were taken to the field laboratory and anesthetized with ketamine hydrochloride (15-30mg/kg). The animals were inspected for attached ticks, and the blood was collected through cardiac or tail vein puncture.

The rodents and marsupials were identified using taxonomic keys (Bonvicino; Oliveira; D'Andrea, 2008; Faria, 2019) and returned to the wild. The new host reports or cryptic species were euthanized with xylazine and ketamine sedation, followed by inhalation of isoflurane. After death confirmation, the specimens were fixed in 10%

formaldehyde and transported to the Zoology Museum of the University of São Paulo for storage.

The ticks collected from the dogs were recovered using tweezers, and the free-living ticks were found on leaf tips every day during the trail inspection. After returning from the trails, the collection team would scrutinize their bodies carefully to search for any attached ticks, which were also removed with a tweezer. The fed immatures and adult ticks were stored in dry plastic containers, and the unfed specimens were stored in microtubes containing absolute ethanol.

4.2.2 Tick Morphological identification

After collection, the samples were sent to the Laboratório de Doenças Parasitárias of the Faculty of Veterinary Medicine and Zootechny of the University of São Paulo (VPS-FMVZ) for identification. The fed immatures and adult ticks were kept in a biochemical oxygen demand (BOD) incubator until molting. After that, the hemolymph test was performed, and the specimen is stored in a dry microtube inside a -80°C freezer. The ticks stored in ethanol absolute were kept in identified boxes until molecular analysis.

The taxonomic identification followed the dichotomous keys proposed by Barros-Battesti et al. (2006) and Martins et al. (2010).

The specimens that were not submitted to any molecular or isolation testing were deposited in the National Tick Collection of the Faculty of Veterinary Medicine and Zootechny of the University of São Paulo (CNC-FMVZ) under the accession numbers CNC4665 – CNC4680.

4.2.3 Hemolymph test

The hemolymph test is performed by sectioning the first pair of legs of the adult ticks with sterilized scissors after the specimens had been left in the BOD incubator at 36°C. The fluid is deposited onto a slide with eighteen wells each and then fixed at room temperature. The slides are stained using the Gimenez (1964) method and examined under an optical microscope at 1000x magnification with immersion oil to seek *Rickettsia*-like organisms. Immediately after the slides are prepared, the specimens are individually stored in microtubes and frozen in a freezer at -80ºC to isolate *Rickettsia* in cell culture using the Shell Vial technique.

4.2.4 Isolation of Rickettsia from the tick

The positive ticks on the hemolymph test were submitted to the isolation procedure throughout the shell vial technique, according to Labruna et al. (2004a). Briefly, after the ticks were sterilized, macerated, and resuspended in 1.8ml of brain heart infusion (BHI), the sample was deposited onto a monolayer of Vero cells and centrifuged for 1h at 700g and 22°C. The exoskeleton of each inoculated tick was separated and then tested by PCR to validate the presence of Rickettsia. Each tick originated two vials. After that, the BHI medium was removed and replaced with RPMI (Gibco) supplemented with 10% of bovine calf serum (Hyclone) and antibiotic (streptomycin) and antimycotic (amphotericin B), then the vials were incubated at 28°C. Every 3 days, the medium was replaced by one without antibiotics and checked by Gimenez staining. When the infection of the cells reached 100%, the passage was made to 25cm² bottles. Then the rickettsia isolate was subjected to serial passages in 75cm² bottles containing a monolayer of Vero cells.

The bottles with monolayers showing a level of infection close to 100%, were scraped, and the suspension was centrifuged. The cell sediment was resuspended in sterile sucrose-phosphate-glutamate (SPG) buffer, pH 7.0, then frozen at -80ºC, composing reference samples for the isolate obtained.

For the molecular characterization and taxonomic positioning of the isolates, the DNA of the cells was extracted using the Purelink Genomic DNA commercial kit (Thermofisher) following the manufacturer recommendations and later processed targeting the citrate synthase gene (*gltA*) common to bacteria of the *Rickettsia* genus (Labruna et al., 2004a).

4.2.5 Molecular analysis

Each tick was individualized in a microtube and submitted to DNA extraction. The adult ticks were extracted by the guanidine isothiocyanate-phenol protocol (Sangioni et al., 2005) with a final elution of 60ul. The nymphs were processed by the boiling method described by Dupont et al. (1994) and eluted in 20ul of Tris-EDTA buffer solution.

All the extracted samples were screened initially by a real-time PCR (qPCR) using the primers CS-5 (Guedes et al., 2005) and CS-6 (Labruna et al., 2004a) and an internal probe that amplify a 147-bp fragment of the *gltA* gene, standard in all *Rickettsia* bacteria, following cycling conditions of Soares et al. (2012). All reactions included positive (DNA of *Rickettsia vini* cultivated in Vero cells) and negative controls (ultrapure water). This assay was performed in a 7500 real-time PCR system (Applied BioSystems).

The samples considered positive in the qPCR were further tested by a conventional PCR (cPCR) on two more genes. Initially the samples were submitted to a cPCR using the primers CS-239 and CS-1069 (Labruna et al., 2004b), which amplifies a fragment of 834-bp of the *gltA* gene and the samples that yielded expected amplicons were further tested to the cPCR targeting a 632bp fragment of the 190-kDa outer membrane protein gene (*ompA*), using the primers Rr190.70 (Regnery et al., 1991) and Rr190.701 (Roux et al., 1997), present only in rickettsiae from the Spotted Fever Group (SFG). Samples that were positive only in the qPCR but not on the cPCR assays were considered negative. The same controls described in the qPCR were used in the mentioned above assays.

The samples negative in both techniques were tested by a cPCR targeting a 460-bp fragment of the tick mitochondrial 16S rRNA gene (Mangold et al., 1998) to validate the extraction methods. All the primers are shown in the Table 5.

Table 5: Primer pairs used for the amplification of rickettsial and tick's genes.

Organism	Gene	Sequence 5'3'	Size (pb)	Reference
Rickettsia	gltA	CS-5: GAGAGAAAATTATATCCAAATGTTGAT CS-6: AGGGTCTTCGTGCATTTCTT 6-FAM d(CATTGTCGGATCCAGCCTACGGT) BHQ-1	147	Guedes et al. (2005) and Labruna et al. (2004)
Rickettsia	gltA	CS-239: GCTCTTCTCATCCTATGGCTATTAT CS-1069: CAGGGTCTTCGTGCATTTCTT	834	Labruna et al. (2004)
Rickettsia	ompA	Rr190.70: ATGGCGAATATTTCTCCAAAA Rr190.701: GTTCCGTTAATGGCAGCATCT	632	Regnery et al. (1991) and Roux et al. (1997)
Tick	16S rRNA	16S+1: CCGGTCTGAACTCAGATCAAGT 16S-1: GCTCAATGATTTTTTAAATTGCTGT	460	Mangold et al. (1998)

The reactions that generated amplicons were purified with ExoSAP-IT (USB Corporation®, OH), using the manufacturer's instructions, and subsequently sequenced by using the BigDye Terminator Kit (Perkin Elmer Applied Biosystems) as recommended by the manufacturer and processed by Sanger sequencing in an ABI automated sequencer (Applied BioSystems/ThermoFisher Scientific, model ABI 3500 Genetic Analyzer) The sequences obtained were edited using the program SeqMan (Lasergene, DNAstar) and then submitted to phylogenetic analysis.

4.2.6 Phylogenetic analysis

DNA sequences were edited using the SeqMan software (DNAStar, Inc., Madison, WI), and submitted to multiple alignments by using the program Clustal X (Thompson et al., 1997) and manually adjusted by using GeneDoc v. 2.6.01 (Nicholas et al., 1997). Phylogenetic trees were inferred by Bayesian (B), and maximum parsimony (MP) methods. MP trees were constructed using the PAUP * v program. 4.0b10 (Swofford, 1998), via heuristic search with 100 replicates of random addition of the terminals followed by branching (RAS-TBR Branch-breaking). Bootstrap support analyzes were performed on 100 replicates. Bayesian analyzes were performed in the MrBayes v.3.1.2 program (Ronquist and Huelsenbeck, 2003); 1,000,000 generations were employed using GTR as a substitution model and four range categories plus invariant proportion of sites. For the verification of support of branches in the Bayesian analyzes, the "posteriori" probability values obtained using the MrBayes program were used. Similarity matrices (based on uncorrected p-distance) were constructed using the Poit Replacer v.2.0 program provided by the author (Alves, J. M.) at http://www.geocities.com/alvesjmp/software.html.

4.2.7 Serological analysis

The blood collected from rodents, marsupials, and dogs was centrifuged to separate only the serum and kept inside 1,5ml microtubes at -20°C until further testing. The serum samples were tested by an immunofluorescent assay (IFA) following the protocols described by Horta et al. (2004), using five antigens from Brazilian territory: *R. rickettsii* strain Taiaçu (Pinter and Labruna, 2006), *R. parkeri* strain AT24 (Silveira et al., 2007), *R. rhipicephali* strain HJ5 (Labruna et al., 2005), *R. amblyommatis* strain AC37 (Labruna et al., 2004b) and *R. bellii* strain Mogi (Pinter and Labruna, 2006).

The reactions were performed on the host group, since the conjugate is specific. The sera were initially tested at the 1:64 titer as a cut-off point. The positive samples were further tested in a two-fold dilution to determine the endpoint titer. The sample that shows positivity to a determined species 4 times higher than the other species is considered homologous. All the reactions included positive and negative controls with previously tested sera.

4.2.8 Statistical analysis

Comparisons were made between the proportions of Rickettsia-positive ticks between the areas (Porto Raso, Ribeirão da Anta, Sede and Serraria) and also between the tick species that were found parasitizing humans (A. brasiliense, A. incisum and A. ovale), using the chi-squared test. Comparisons were made using a 5% significance level. In the analysis of serology data on hosts, comparisons were made between the proportions of positives for Rickettsia in relation to negatives and non-homologous rodents and marsupials and also between areas (Porto Raso, Sede and Serraria), using the chi-squared test. Ribeirão da Anta was excluded from this analysis because only 5 dogs were sampled in this area, an insufficient number for this area to be included in the analysis.

Comparisons were made using a 5% significance level. The 95% confidence intervals (95%CI) were estimated using the exact binomial distribution.

4.2.9 Ethical statement

This study is part of the project entitled "Study of zoonotic agents in wild and domestic animals and their associated vectors in the private reserve Legado das Águas - Reserva Votorantim, in the municipalities of Tapiraí and Miracatu, São Paulo" and funded by the São Paulo Research Foundation (FAPESP Process 2018/19882-3).

The Ethics Committee of the Faculty of Veterinary Medicine and Zootechny of the University of São Paulo (FMVZ-USP) under the number 6509131119 approved the present study.

4.3 RESULTS

4.3.1 Host-associations and tick identification

The ticks were recovered from eleven rodents and four marsupial species, besides the specimens collected from domestic dogs (10 individuals), humans, and vegetation. Throughout the fieldwork, 476 small terrestrial mammals were captured, comprehending 15 rodent species and six marsupial species. In the Sede area, the most abundant rodent was *Euryoryzomys russatus* (Wagner, 1848), and *Metachirus nudicaudatus* (Desmarest,

1817) was the most captured marsupial, which was the same pattern observed at the Porto Raso area, but with a different rodent species that was *Holochilus* sp. Brandt, 1835, a common animal in damp areas, the main characteristic of this second area. Lastly, at Serraria, the most common marsupial was *Didelphis aurita* (Wied-Neuwied, 1826), and *E. russatus* was again the most prevalent rodent. The other species, and the locality are listed in Table 6.

After all the collections were done, 2,714 tick specimens were collected. Among these, three genera and thirteen species were identified. Amidst the collected ticks, three genera were found: *Amblyomma* Koch, 1844 (n=2,662), *Ixodes* Latreille, 1795 (n=40), and *Haemaphysalis* Koch, 1844 (n=11) and the first genus is the most numerous in terms of species and specimens. From the *Amblyomma*, eight species were identified, and the larvae collected were identified only to the genus level. The most frequent species from this genus was *Amblyomma incisum* Neumann, 1906 (n=1,230), followed by *Amblyomma ovale* Koch, 1844 (n=574) and *Amblyomma brasiliense* Aragão, 1908 (n=313). The species with the highest host diversity was *A. ovale*, parasitizing rodents, marsupials, and humans in its immature form and adult stages were found on the inspected dogs. In comparison, only two specimens of *Amblyomma aureolatum* (Pallas, 1878) were found on the tip of a leaf at the first area, making this species the lesser found in numbers.

Table 6: Small mammals captured at Legado das Águas – Reserva Votorantim in three areas within the reserve, from 2018 to 2021.

Species	Area			
	Sede	Porto Raso	Serraria	**TOTAL**
Rodents				
Akodon sp.			2	2
Brucepattersonius sp.	1	5		6
Delomys sp.	1			1
Euryoryzomys russatus	149	45	30	224
Guerlinguetus brasiliensis			1	1
Holochilus sp.		59	2	61
Hylaeamys sp.	4	4		8
Kannabateomys sp.			1	1
Nectomys squamipes		16		16
Oligoryzomys sp.	9	4	3	16
Oxymycterus sp.	2	18	4	24
Phyllomys sp.		1		1
Rhipidomys sp.		2		2
Sooretamys angouya	2			2
Thaptomys nigrita			3	3
Marsupials				
Didelphis aurita	2	18	8	28
Gracilinanus sp.	1			1
Marmosa (Micoureus) demerarae	3			3
Marmosops sp.	2	1	1	4
Metachirus nudicaudatus	27	43	1	71
Monodelphis sp.			1	1
TOTAL	203	216	57	476

The *Haemaphysalis* had one species representative in this study, adults of *Haemaphysalis juxtakochi* Cooley, 1946 found during the vegetation inspection; no immature stages were found for this species. From the Ixodes, two species were collected: *Ixodes loricatus* Neumann, 1899 (n=22), mainly parasitizing animals from the Didelphimorphia, and only one male was found on an *E. russatus*; the other species was *Ixodes schulzei* Aragão & Fonseca, 1951 (n=11) associated only with cricetid rodents. All orders of captured hosts presented tick parasitism, including humans and the inspected vegetation (figure 10). All the collected species and their host associations are shown in Table 7.

4.3.2 Hemolymph test

After each campaign, all the adult ticks and fed nymphs that arrived alive at the lab after molting were submitted to the hemolymph test, totaling 1,068 ticks belonging to two genera and eight species.

Figure 11: A. *Didelphis aurita* parasitized with a female of *Ixodes loricatus* on its ear; **B.** *Holochilus* sp. with several females of *Ixodes schulzei* attached to its head; **C.** *Amblyomma incisum* nymph on a human forearm; **D.** Female of an *Amblyomma ovale* on a leaf tip.

The most tested species was *A. incisum* (621 individuals), but the *Amblyomma* species with the higher percentage of positivity was *A. ovale* (14.89%, 42/282). The other genus submitted to this test was *Ixodes*, and the species with the highest positivity was *I. loricatus*, with two specimens positive out of five tested (40%). Other species that showed positivity to the test were *A. brasiliense*, *Amblyomma naponense* (Packard, 1869), also collected during the captures, and *I. schulzei* (table 8).

Table 7: Ticks collected on small terrestrial mammals, dogs, humans, and free-living in four different areas in a preserved ecological area (Legado das Águas – Reserva Votorantim) inside the Atlantic Rainforest Biome, from 2018 to 2021.

Areas	Host (infested/captured)	Tick species														
		AA [1]	*AB* [2]	*AD* [3]	*AF* [4]	*AI* [5]	*AN* [6]	*AO* [7]	*AP* [8]	*A.* sp.[9]	*HJ* [10]	*IL* [11]	*IS* [12]	*I.* sp.[13]	Total by host	Total by area
	Rodents															
	Euryoryzomys russatus (32/149)	-	-	-	3N	-	-	84N	-	4L	-	1M	-	-	92	
	Oligoryzomys sp. (1/9)	-	-	-	-	-	-	6N	-	-	-		-	-	6	
	Marsupials															
SE*	*Didelphis aurita* (2/2)	-	-	-	1N	-	-	1N	1N	2L	-	4F	-	-	9	520
	Others															(19.16%)
	Canis familiaris (1/1)	-	-	-	-	1N	-	-	-	-	-	-	-	-	1	
	Free-living	2F	12M, 14F	-	-	163M, 151F, 36N	1M	12M, 14F	-	-	1F	-	-	-	406	
	Human (3/5)	-	-	-	-	5N	-	-	-	1L	-	-	-	-	6	
	Rodents															
	E. russatus (31/45)	-	-		6N	-	-	70N	-	47L	-	-	1N	1N	125	
	Holochilus sp. (41/59)	-	-	4N	1F, 32N	-	-	48N	1N	23L	-	-	7F, 2N	1N	119	
	Hylaeamys sp. (2/4)	-	-	-	-	-	-	1N	-	1L	-	-	-	-	2	
	Nectomys squamipes (4/16)	-	-	-	-	-	-	10N	-	-	-	-	1F		11	
	Oligoryzomys sp. (3/4)	-	-	-	-	-	-	4N	-	2L	-	-	-	-	6	
	Oxymycterus sp. (4/18)	-	-	-	-	-	-	1N, 1L	-	3L	-	-	-	-	5	
	Rhipidomys sp. (2/2)	-	-	-	-	-	-	16N	-	35L	-	-	-	-	51	1501
PR**	**Marsupials**															(55.31%)
	D. aurita (15/18)	-	16N, 1L	1N	72N, 34L	2N	-	7N	1N	66L	-	1M, 4F	-	-	205	
	Metachirus nudicaudatus (1/43)	-	-	-	3N	-	-	-	-	-	-	-	-	-	3	
	Others															
	C. familiaris (1/1)	-	-	-	-	-	-	1M	-	-	-	-	-	-	1	
	Free-living	-	41M, 38F, 55N	-	-	254M, 263F, 223N	1M, 2N	16M, 36F	-	6L	2M, 5F	-	-	-	942	
	Human (5/5)	-	10N	-	-	11N	-	1N	-	9L	-	-	-	-	31	
	Tupinambis sp. (1/1)	-	-	-	1F, 8N	-	-	-	-	-	-	-	-	-	9	
	Rodents															
	Akodon sp. (1/2)	-	-	-	-	-	-		-	1L	-	-	-	-	1	
	E. russatus (20/30)	-	-	-	-	-	-	64N	-	6N, 26L	-	-	-	-	96	
	Guerlinguetus brasiliensis (1/1)	-	-	1N	1N	-	-	1N	-	-	-	-	-	-	3	
	Holochilus sp. (2/2)	-	-	-	1N	-	-	1N	-	17L	-	-	-	-	19	
	Kannabateomys sp. (1/1)	-	-	-	-	-	-	2N	-	-	-	-	-	-	2	
	Oligoryzomys sp. (2/3)	-	-	-	-	-	-	3N	-	-	-	-	-	-	3	
	Oxymycterus sp. (3/4)	-	-	-	1N	-	-	78N, 20L	-	66L	-	-	-	-	165	667
SR+	**Marsupials**															(24.58%)
	D. aurita (7/8)	-	1N	-	10N	-	-	-	-	32L	-	2M, 8F	-	1L	54	
	Marmosops sp. (1/1)	-	-	-	-	-	-	-	-	1L	-	-	-	-	1	
	M. nudicaudatus (1/1)	-	-	-	-	-	-	-	-	1L	-	-	-	-	1	
	Monodelphis sp. (1/1)	-	-	-	-	-	-	-	-	-	-	2N	-	4L	6	
	Others															
	Free-living	-	38M, 35F, 20N, 32L	-	-	30M, 27F, 54N	1M, 5N	14M, 21F	-	-	1M, 2F	-	-	-	280	
	Human (5/5)	-	-	-	-	1F, 9N	-	-	-	-	-	-	-	-	10	
	Others															41
RA++	*C. familiaris* (9/10)	-	-	-	-	-	-	16M, 25F	-	-	-	-	-	-	41	(1.51%)
	Total by tick species	2	313	6	174	1230	10	574	3	350	11	22	11	7		**2714**

[1]*Amblyomma aureolatum*; [2]*Amblyomma brasiliense*; [3]*Amblyomma dubitatum*; [4]*Amblyomma fuscum*; [5]*Amblyomma incisum*; [6]*Amblyomma naponense*; [7]*Amblyomma ovale*; [8]*Amblyomma pacae*; [9]*Amblyomma* sp.; [10]*Haemaphysalis juxtakochi*; [11]*Ixodes loricatus*; [12]*Ixodes schulzei*.; [13]*Ixodes* sp.; *Sede; **Porto Raso; +Serraria; ++Ribeirão da Anta.; L: larvae; N: nymph; F: female; M: male.

Table 8: Tick species submitted to the hemolymph test for rickettsial checking; the table below shows the site of collection, sex, and positivity.

Species	Sex	Sede	Porto Raso	Serraria	Ribeirão da Anta	Total Examined	Positives
Amblyomma incisum	Males	64	226	23	0	313	4.86% (16/313)
	Females	56	232	20	0	308	4.94% (16/308)
Amblyomma ovale	Males	17	40	30	16	103	14.56% (15/103)
	Females	25	109	33	12	179	15.08% (27/179)
Amblyomma brasiliense	Males	9	29	27	0	65	10.96% (8/65)
	Females	9	39	31	0	79	8.14% (7/79)
Amblyomma naponense	Males	1	1	1	0	3	33.33% (1/3)
	Females	0	0	0	0	0	0 (0/0)
Amblyomma fuscum	Males	0	1	0	0	1	0 (0/1)
	Females	0	4	1	0	5	0 (0/5)
Amblyomma dubitatum	Males	0	2	0	0	2	0 (0/2)
	Females	0	2	0	0	2	0 (0/2)
Ixodes schulzei	Males	0	0	0	0	0	0 (0/0)
	Females	0	3	0	0	3	33.33% (1/3)
Ixodes loricatus	Males	0	0	2	0	2	50% (1/2)
	Females	0	1	2	0	3	33.33% (1/3)
Total		181	689	170	28	**1068**	**8.71% (93/1068)**

4.3.3 Isolation of *Rickettsia* from the tick

Twenty-three ticks were selected for the isolation in Vero cells technique after showing positivity when submitted to the hemolymph test, belonging to the species *A. brasiliense* (2), *A. incisum* (3), *A. ovale* (18). All the ticks processed through the Shell Vial technique showed previous positivity to the hemolymph test, and two isolates were successfully obtained. 10 *R. bellii* and 3 *R. parkeri* organisms were isolated in Vero cells from *A. ovale*, but only two of the isolates, cultured from *A. ovale*, was established in continuous cell culture passage. All the macerated ticks were screened through PCR after inoculation resulting positive for rickettsiae bacteria.

The first originated from a male *A. ovale* collected from the vegetation, and the second was from a male *A. ovale* collected from a dog from Ribeirão da Anta. Both isolates showed 100% identity with the rickettsial genes obtained from the ticks after molecular tests. After establishing the isolate, attempts to amplify the *gltA* gene were successful. After the sequencing, the isolates were compatible with other *Rickettsia bellii* sequences already available on GenBank (figure 11).

4.3.4 Molecular analysis

Out of the 2,714 collected ticks, 803 specimens were submitted individually to the DNA analysis targeting *Rickettsia* fragments comprehending the following species: 262 *A. incisum*, 230 *A. ovale*, 156 *A. brasiliense*, 111 *Amblyomma fuscum* Neumann, 1907, 6 *Amblyomma naponense* (Packard, 1869), 6 *Amblyomma dubitatum* Neumann, 1899, 1 *Amblyomma pacae* Aragão, 1911, 8 *Ha. juxtakochi*, 12 *I. loricatus*, 10 *I. schulzei*, and 1 *Ixodes* sp.

The overall number of positives, regardless of the *Rickettsia* species, was 5.48% (44/803), and the bacterium DNA was detected in five of the ten tested species. The species *A. ovale* had the highest number of positive specimens (81.8%, 36/44), with 19 samples (52.8%, 19/36) that amplified only the *gltA* gene and 17 (47.2%, 17/36) that amplified both *gltA* and *ompA*. Within this group, eight samples were sequenced resulting in two different rickettsiae species: *R. bellii* (99.5% similarity) and *Rickettsia parkeri* strain Atlantic Rainforest (99.9% similarity) (figure 11).

The species *A. brasiliense* had one sample (2.3%, 1/44) that amplified expected amplicons to the *gltA* gene and, once sequenced, the sample was compatible with *R. bellii* (99.5% similarity). As for the *Ha. juxtakochi* species four samples (9.1%, 4/44) generated amplicons, one for *gltA* gene and three for both genes, and after sequencing, the sequences aligned with *R. bellii* (99.5% similarity) and *R. rhipicephali* (100% similarity) (figure 11). Lastly, *I. loricatus* was the only species belonging to this genus that showed positivity to rickettsiae, with three samples (6.8%, 3/44) amplifying the *gltA* gene. Once sequenced, the BLAST analysis showed similarity with *R. bellii* (99.8% similarity).

In terms of *A. ovale* positivity per area sampled, the area with the highest number of positives was Porto Raso with 17 positive ticks (10 *R. parkeri* and 7 *R. bellii*), followed by the Ribeirão da Anta area with 7 positives (1 *R. parkeri* and 6 *R. bellii*), the Sede area with 6 positives (4 *R. parkeri* and 2 *R. bellii*) and lastly the Serraria area with 4 positives (2 *R. parkeri* and 2 *R. bellii*). The only positive *A. brasiliense* was collected at the Serraria area as well as the positive *I. loricatus*. The positive *Ha. juxtakochi* were collected at the Porto Raso (1 *R. bellii* and 2 *R. rhipicephali*) and Serraria areas (1 *R. rhipicephali*).

4.3.5 Serological analysis

Two hundred and eight animal sera were submitted to the IFA. Of these 166 rodents, 37 marsupials and five dogs. Fifteen species of rodents were tested and 50 specimens showed positivity at the lower dilution (1:64). However, only 21 sera demonstrated homology to a

Rickettsia species, 2 for *R. bellii* (with titers of 1:512 and 1:8,192), 16 for *R. parkeri* (with titers ranging from 1:512 to 1:8,192) and 3 for *R. rhipicephali* (with titers ranging from 1:512 to 1:16,384). Five species of marsupials were tested, with seventeen specimens showing positivity on the initial testing and ten provided homologies, 6 for *R. bellii* (with titers ranging from 1:1,024 to 1:2,048), 2 for *R. parkeri* (with titers of 1:512 and 1:16,384), one for *R. rhipicephali* (1:4,096) and one for *Rickettsia amblyommatis* (1:4,096).

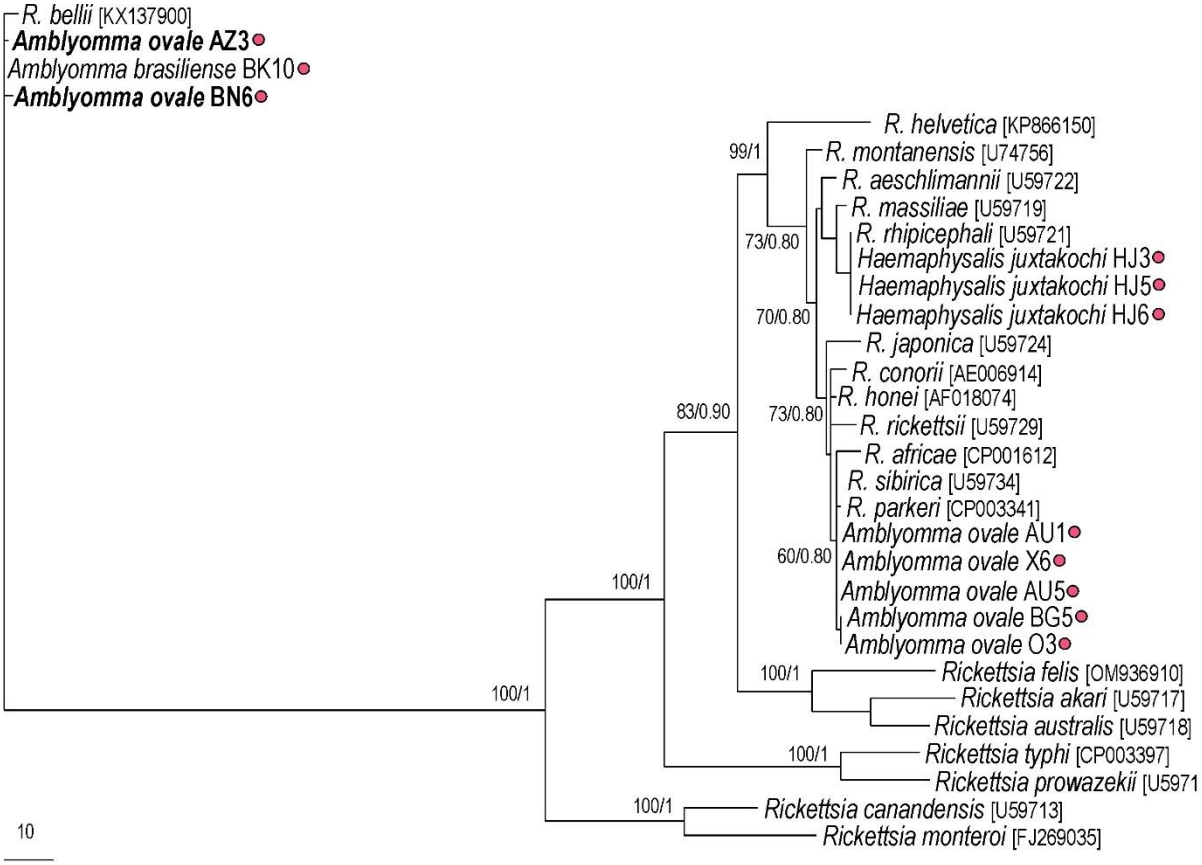

Figure 12: Phylogenetic tree based on the gltA gene sequences of *Rickettsia* species. *Rickettsia bellii* was used to outgroup. A total of 1039 characters (206 informative characters). Numbers at nodes are support values derived from bootstrap and posterior probability for MP and BA analyses (MP/BA).

Five domestic dogs from Ribeirão da Anta were tested for the presence of *Rickettsia* antibodies, and four of them showed positivity with high titers for *R. parkeri* (1:2,048 – 1:16,384). All tested dogs had *A. ovale* ticks attached to them at the collection moment and free access to the forest. All the results are shown in Table 9.

4.3.6 Statistical analysis

As for the results of the molecular analysis, there was a significant difference in the proportions of positives between the areas ($p < 0.001$). The proportions of positives in Porto

Table 9: Seroreactivity to rickettsiae bacteria in small terrestrial mammals and dogs sampled within four areas in an Atlantic Rainforest reserve (Legado das Águas), from 2018 to 2021.

Collection site	Host species (no. tested animals)	No. reactive animals for each rickettsiae species (% sororeactivity)					No. animals with PAIHR*
		Rickettsia rickettsii	*Rickettsia parkeri*	*Rickettsia rhipicephali*	*Rickettsia amblyommatis*	*Rickettsia bellii*	
SEDE	*Brucepattersonius* sp. (1)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	
	Euryoryzomys russatus (33)	3 (9)	4 (12)	3 (9)	3 (9)	3 (9)	
	Oligoryzomys sp. (2)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	
	Oxymycterus sp. (1)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	
	Sooretamys angouya (2)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	
	Didelphis aurita (1)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	
	Metachirus nudicaudatus (2)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	
	Marmosa (Micoureus) demerarae (2)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	
PORTO RASO	*Brucepattersonius* sp. (3)	1 (33)	1 (33)	1 (33)	1 (33)	1 (33)	1 (*R. rhipicephali*)
	E. russatus (16)	1 (6)	3 (19)	1 (6)	1 (6)	1 (6)	1 (*R. parkeri*)
	Holochilus sp. (50)	16 (32)	21 (42)	19 (38)	18 (36)	15 (30)	8 (*R. parkeri*), 2 (*R. rhipicephali*), 1 (*R. bellii*)
	Hylaeamys sp. (1)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	
	Nectomys sp. (5)	1 (20)	2 (40)	2 (40)	2 (40)	2 (40)	1 (*R. bellii*)
	Oligoryzomys sp. (2)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	
	Oxymycterus sp. (7)	1 (14)	1 (14)	1 (14)	1 (14)	1 (14)	
	Phyllomys sp. (1)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	
	Rhipidomys sp. (2)	2 (100)	2 (100)	2 (100)	2 (100)	2 (100)	
	D. aurita (8)	6 (75)	7 (88)	7 (88)	6 (75)	2 (25)	1 (*R. parkeri*), 1 (*R. amblyommatis*), 2 (*R. bellii*)
	M. nudicaudatus (13)	0 (0)	1 (8)	1 (8)	1 (8)	1 (8)	1 (*R. parkeri*), 1 (*R. bellii*)
SERRARIA	*Akodon* sp (2)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	
	E. russatus (19)	4 (21)	7 (37)	2 (11)	2 (11)	4 (21)	2 (*R. parkeri*)
	Guerlinguetus brasiliensis (1)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	
	Holochilus sp. (2)	2 (100)	2 (100)	2 (100)	2 (100)	2 (100)	2 (*R. parkeri*)
	Kannabateomys sp. (1)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	
	Oligoryzomys sp. (3)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	
	Oxymycterus sp. (4)	3 (75)	3 (75)	0 (0)	0 (0)	1 (25)	3 (*R. parkeri*)
	Thaptomys nigrita (3)	1 (33)	1 (33)	0 (0)	0 (0)	0 (0)	
	D. aurita (8)	6 (75)	6 (75)	7 (88)	6 (75)	6 (75)	1 (*R. rhipicephali*), 2 (*R. bellii*)
	M. nudicaudatus (1)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	
	Marmosops sp. (1)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	
	Monodelphis sp. (1)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	1 (*R. bellii*)
RIBEIRÃO DA ANTA	*C. lupus familiaris* (5)	5 (100)	5 (100)	5 (100)	5 (100)	5 (100)	4 (*R. parkeri*)

*PAIHR: possible antigen involved in a homologous reaction.

Raso, Ribeirão da Anta, Sede and Serraria were, respectively, 4.9% (95%CI: 3.0%; 7.5%), 25.9% (95%CI: 11.1%; 46.3%), 3.6% (95%CI: 1.3%; 7.7%) and 4.4% (95%CI: 2.0%; 8.2%). As for the ticks collected interacting with humans, there was a significant difference in the proportions of positives between the species *A. brasiliense, A. incisum* and *A. ovale* ($p < 0.001$). The proportions of positives for *A. brasiliense, A. incisum* and *A. ovale* were, respectively, 0.6% (95%CI: 0.02%; 3.5%), 0.0% (95%CI: 0.0%; 1.4%) and 14.8% (95%CI: 10.5%; 20.0%).

As for the results of the serological analysis, there was a significant difference in the proportions of positives for *Rickettsia* between rodents and marsupials ($p = 0.028$). The proportions of positives among marsupials and rodents were 27.0% (95%CI: 13.8%; 44.1%) and 12.7% (95%CI: 8.0%; 18.7%), respectively. There was a significant difference in the proportions of positives for *Rickettsia* between the areas ($p = 0.0038$). The proportion of positives observed in Porto Raso, Sede and Serraria were 18.0% (95%CI: 11.4%; 26.4%), 0.0% (95%CI: 0.0%; 7.9%) and 23.4% (95%CI: 12.3%; 38.0%), respectively.

4.4 DISCUSSION

The present study showed a high diversity of tick species, as well as demonstrating the active circulation of three species of rickettsiae based on molecular and serological analysis and in vitro growth, in a primary Atlantic Rainforest reserve located in the São Paulo state, Brazil, where there had been no previous surveys.

Our study collected eleven different species of ticks comprising three genera. The most collected species was *A. incisum*, with all its adult stages collected from the vegetation. The primary host for this tick's mature stages is the tapirs, animals highly present in our study area, making the reserve known for its specimens, agreeing with the data by Szabó et al. (2009a, b). As for the immature stages of this species, the specimens were collected from the vegetation and parasitized one dog and thirteen humans. These stages tend to be more generalist and described by Lamattina et al. (2018) and Suzin et al. (2022), infesting humans once we can act as accidental hosts for many tick species. It is important to state that the ticks found over the vegetation have no correlation with the specimens found infesting the hosts.

The species *A. ovale* was of great interest for acting as the main vector of *Rickettsia parkeri* strain Atlantic Rainforest, an etiological agent of mild spotted fever (Sabatini et al., 2010). Once the study was finished, this was the second most collected species, interacting with many rodent species, dogs, humans, and marsupials, and found free-living in the environment.

The rodent with the most records for this species was *E. russatus*, representing the most collected rodent species. Our data is exciting since this rodent is the primary amplifying host for the *Rickettsia* species mentioned above (Krawczak; Labruna, 2018).

Other species in our collections was *A. brasiliense*, retrieved from the vegetation and parasitizing humans, and *Didelphis aurita* (Wied-Neuwied, 1826). Only immature stages interacted with this mammal, recorded in our most preserved area. Szabó et al. (2013) also recorded immature stages interacting with this Didelphidae species. Luz et al. (2018) described a host preference of this species for peccaries during their adult stages. In our collections, we observed that the position between *A. incisum* and *A. brasiliense* on the leaves' tip was different, whether the first species was found higher and the second lower in terms of height (Suzin et al., 2020), being compatible with the expected host's sizes. Similarly, the findings of Szabó et al. (2006), the species *A. incisum*, *A. ovale*, and *A. brasiliense* were collected attached from our collection crew after the active surveillance for ticks and hosts inside the forest.

Here, we collected two specimens of *Amblyomma aureolatum* (Pallas, 1878), which were found over a leaf tip at our higher spot in altitude matters (Sede, 499m). This was expected since this tick species occurs mainly in higher altitudes at the Brazilian southeastern (Pinter et al., 2004; Barbieri et al., 2015), rarely occurring in sympatry with *A. ovale*, since this species rather lower altitudes. Szabó et al. (2009b) also collected this species in a fragment of Atlantic Rainforest in higher numbers than this study, but all the trails the authors sampled had higher altitude than our higher area. Only nymphs and females of *I. schulzei* were collected, corroborating the work done by Soares et al. (2021) that this species reproduces by parthenogenesis since the male remains unknown.

Regarding the *Rickettsia* species detected in ticks, *R. bellii* was the most prevalent species and with the most incredible range of tick species infection. Representing the only species found interacting with *I. loricatus* populations, mentioned in other studies from Brazil (Horta et al., 2007; Krawczak et al., 2022) and also being the first rickettsiae described infecting this tick species (Horta et al., 2006). One *A. brasiliense* was positive for this *Rickettsia* species, making this a new record since many other studies tested this tick molecularly, not being able to amplify any rickettsial DNA from this species (Sabatini et al., 2010; Acosta et al., 2016; Luz et al., 2018), the other species already found interacting with this tick is *R. amblyommatis* as shown by Krawczak et al., 2022.

The *Ha. juxtakochi* populations were detected infected by two rickettsiae species, *R. bellii* and *R. rhipicephali*, and these findings are corroborated by many studies that reported the same results from this tick species (Labruna et al., 2005; Labruna et al., 2007; Soares et al.,

2015). Although some specimens of *A. incisum* showed *Rickettsia*-like structures, no sample of this tick was positive for *Rickettsia* after molecular testing. This result is in contrast with the findings of Pacheco et al. (2011), that include the detection of a new rickettsia, '*Candidatus* Rickettsia monteiroi', in populations of *A. incisum* collected in an area near to the one in this study and after the study mentioned above, this rickettsia species was never detected again. Besides the rickettsiae species mentioned above, *A. incisum* was also detected harboring the DNA of *R. bellii* in two studies conducted in the Brazilian territory (Sabatini et al., 2010; Pacheco et al., 2011).

The most important finding of this study in terms of public health was the presence of *R. parkeri* strain Atlantic Rainforest infecting *A. ovale* ticks at a rate of 7.33% (17/232) in comparison to 8.19% (19/232) of *R. bellii* infection. Barbieri et al. (2023) observed a negative correlation between these rickettsiae species in *A. ovale* populations, and this pattern was not observed in the Porto Raso area, the most preserved area in our study and the specie *R. parkeri* was the most detected, so further studies are necessary to prove these authors' hypothesis. This tick species was collected on rodents, marsupials, dogs, and humans. When it comes to the sampled areas the one with the highest positivity was the one with also the one with higher level of preservation, this can be explained by the fact that a greater number of hosts were available to complete the tick's life cycle and amplify the detected rickettsiae thus infecting new specimens.

The presence of *R. parkeri* strain Atlantic Rainforest in this population could increase the risk of tourists or people living in close contact with the forest, and the infection could be enhanced by unrestrained domestic dogs with free access to the forest, which is the scenario observed at Ribeirão da Anta community, which can be compared with the scenario observed in areas of *R. rickettsii* circulation with transmission by the tick species *A. aureolatum* (Ogrzewalska et al., 2012).

Serological analysis showed five rodent species showing reactivity to three rickettsiae species that were also shown through molecular testing of the ticks, suggesting a previous exposure, and the species with a higher number of homologies was *R. parkeri*, agreeing with the fact that rodents act as its main amplifying host (Krawczak et al., 2016). Most of the tested marsupials reacted to the *R. bellii* antigen, which corroborates our molecular findings since most of the tick species collected from marsupials were also positive for this same rickettsia species, which was not observed by Milagres et al. (2010) at another southeastern region.

Serum samples were collected from five domestic dogs inhabiting the peripheries of the forest and having free access to it. Out of the five samples, four (80%) had endpoint titers ≥ 4-

fold to *R. parkeri* than to any other species, showing the dog importance in this bacterium life cycle when it comes to human infections (Kmetiuk et al., 2022). All the dogs tested had *A. ovale* attached to their bodies in the collection moment. These ticks were also tested, showing positivity to both *R. bellii* and *R. parkeri*. This fact led us to conclude that *R. parkeri* is more antigenic than *R. bellii*, a pattern also observed by Babieri et al. (2014) and Weck et al. (2020).

4.5 CONCLUSION

A high number of tick species and rickettsia-associated is presented in this study conducted in the largest private reserve of the Atlantic Rainforest of Brazil, a biome known for its endemicity of species and fragmentation caused by human activities. The rickettsiae found here can cause none or mild symptoms in humans, contrasting with other studies conducted in the same biome but with little preservation where *Rickettsia rickettsii* was able to establish, highlighting the importance of nature balance when it comes to emerging diseases. Future research must identify the critical factors that can lead to the spread of tick-borne spotted fever in specific regions of the Atlantic Forest biome.

4.6 REFERENCES

ACOSTA, I. C. L. *et al.* Ticks (Acari: Ixodidae, Argasidae) from humans, domestic and wild animals in the state of Espírito Santo, Brazil, with notes on rickettsial infection. **Veterinary Parasitology: Regional Studies and Reports**, v. 3–4, p. 66–69, 2016.

BARBIERI, A. R. M. *et al*. Epidemiology of *Rickettsia* sp. strain Atlantic rainforest in a spotted fever-endemic area of southern Brazil. **Ticks and Tick-borne Diseases**, v. 5, n. 6, p. 848–853, 2014.

BARBIERI, A. R. M. *et al. Rickettsia* communities and their relationship with tick species within and around the national park of Iguaçu, Brazil. **Experimental and Applied Acarology**, v. 91, n. 2, p. 339-358, 2023.

BARBIERI, J. M. *et al.* Altitudinal Assessment of *Amblyomma aureolatum* and *Amblyomma ovale* (Acari: Ixodidae), Vectors of Spotted Fever Group Rickettsiosis in the State of São Paulo, Brazil. **Journal of Medical Entomology**, v. 52, n. 5, p. 1170–1174, 2015.

BARROS-BATTESTI, D. M.; ARZUA, M.; BECHARA, G. H. **Carrapatos de Importância Médico-Veterinária da Região Neotropical:** Um guia ilustrado para identificação de espécies. São Paulo: Vox/ICTTD-3/Butantan, 2006.

BLANCO, C. M. *et al*. Microorganisms in ticks (Acari: Ixodidae) collected on marsupials and rodents from Santa Catarina, Paraná and Mato Grosso do Sul states, Brazil. **Ticks and Tick-borne Diseases,** v. 8, n. 1, p. 90–98, 2017.

BUSH, S. E.; REED, M.; MAHER, S. Impact of forest size on parasite biodiversity: Implications for conservation of hosts and parasites. **Biodiversity and Conservation**, v. 22, n. 6–7, p. 1391–1404, 2013.

COLOMBO, A. F.; JOLY, C. A. Brazilian Atlantic Forest lato sensu: The most ancient Brazilian forest, and a biodiversity hotspot, is highly threatened by climate change. Brazilian Journal of Biology, v. 70, n. 3 SUPPL., p. 697–708, 2010.

DANTAS-TORRES, F. *et al*. Ticks (Ixodida: Argasidae, Ixodidae) of Brazil: Updated species checklist and taxonomic keys. **Ticks and Tick-borne Diseases**, v. 10, n. 6, 2019.

DUPONT, H. T.; CORNET, J. P.; RAOULT, D. Identification of Rickettsiae from Ticks Collected in the Central African Republic using the Polymerase Chain Reaction. **American Society of Tropical Medicine and Hygiene**, v. 50, n. 3, p. 373–380, 1994.

GIMÉNEZ, D. F. Staining Rickettsiae in Yolk-Sac Cultures. **Stain Technology**, v. 39, n. 3, p. 135–140, 1964.

GUEDES, E. et al. Detection of *Rickettsia rickettsii* in the tick *Amblyomma cajennense* in a new Brazilian spotted fever-endemic area in the state of Minas Gerais. **Memórias do Instituto Oswaldo Cruz**, v. 100, n. 8, p. 841–845, 2005.

HORTA, M. C. *et al*. Prevalence of antibodies to spotted fever group rickettsiae in humans and domestic animals in a brazilian spotted fever-endemic area in the state of São Paulo, Brazil: Serologic evidence for infection by *Rickettsia rickettsii* and another spotted fever. **The American Journal of Tropical Medicine and Hygiene**, v. 71, n. 1, p. 93–97, 2004.

HORTA, M. C. *et al.* Natural infection, transovarial transmission, and transstadial survival of *Rickettsia bellii* in the tick *Ixodes loricatus* (Acari: Ixodidae) from Brazil. **Annals of the New York Academy of Sciences**, v. 1078, p. 285–290, 2006.

HORTA, M. C. *et al*. *Rickettsia* infection in five areas of the state of São Paulo, Brazil. **Memorias do Instituto Oswaldo Cruz**, v. 102, n. 7, p. 793–801, 2007.

KMETIUK, L. B. *et al*. Epidemiology of *Rickettsia* spp. in Atlantic rainforest areas of island and seashore mainland, southern Brazil. **Transboundary and Emerging Diseases**, v. 69, n. 6, p. 3597–3605, 2022.

KRAWCZAK, F. da S. *et al.* Rickettsial infection in ticks from a natural area of Atlantic Forest biome in southern Brazil. **Experimental and Applied Acarology**, v. 88, n. 3–4, p. 371–386, 2022.

KRAWCZAK, F. S. *et al*. Ecology of a tick-borne spotted fever in southern Brazil. **Experimental and Applied Acarology**, v. 70, n. 2, p. 219–229, 2016.

KRAWCZAK, F. S. *et al*. Genotypic Characterization of *Rickettsia bellii* Reveals Distinct Lineages in the United States and South America. **BioMed Research International**, v. 2018, 2018.

KRAWCZAK, F. S.; LABRUNA, M. B. The rice rat *Euryoryzomys russatus*, a competent amplifying host of *Rickettsia parkeri* strain Atlantic rainforest for the tick *Amblyomma ovale*. **Ticks and Tick-borne Diseases**, v. 9, n. 5, p. 1133–1136, 2018.

LABRUNA, M. B. *et al. Rickettsia* Species Infecting *Amblyomma cooperi* Ticks from an Area in the State of São Paulo, Brazil, Where Brazilian Spotted Fever Is Endemic. **Journal of Clinical Microbiology,** v. 42, n. 1, p. 90–98, 2004a.

LABRUNA, M. B. *et al*. *Rickettsia bellii* and *Rickettsia amblyommii* in *Amblyomma* ticks from the State of Rondônia, Western Amazon, Brazil. **Journal of Medical Entomology**, v. 41, n. 6, p. 1073–1081, 1 nov. 2004b.

LABRUNA, M. B. *et al*. Detection of a spotted fever group *Rickettsia* in the tick *Haemaphysalis juxtakochi* in Rondonia, Brazil. Veterinary Parasitology, v. 127, n. 2, p. 169–174, 2005.

LABRUNA, M. B. *et al*. Isolation of *Rickettsia rhipicephali* and *Rickettsia bellii* from Haemaphysalis juxtakochi ticks in the state of São Paulo, Brazil. **Applied and Environmental Microbiology,** v. 73, n. 3, p. 869–873, 2007.

LAFFERTY, K. D. Environmental parasitology: What can parasites tell us about human impacts on the environment? **Parasitology Today**, v. 13, n. 7, p. 251–255, 1997.

LAMATTINA, D. *et al.* Ecological characterization of a tick community across a landscape gradient exhibiting differential anthropogenic disturbance in the Atlantic Forest ecoregion in Argentina. **Medical and Veterinary Entomology**, v. 32, n. 3, p. 271–281, 2018.

LUZ, H. R. et al. A proposed description of the natural life cycle of *Amblyomma brasiliense* (Acari: Ixodidae) in a primary Atlantic rainforest environment in Brazil. **Systematic and Applied Acarology,** v. 23, n. 6, p. 1138–1147, 2018.

MANGOLD, A. J.; BARGUES, M. D.; MAS-COMA, S. Mitochondrial 16S rDNA sequences and phylogenetic relationships of species of *Rhipicephalus* and other tick genera among Metastriata (Acari: Ixodidae). **Parasitology Research**, v. 84, n. 6, p. 478–484, 1998.

MARTINS, T. F. *et al.* Nymphs of the genus *Amblyomma* (Acari: Ixodidae) of Brazil: descriptions, redescriptions, and identification key. **Ticks and Tick-borne Diseases**, v. 1, n. 2, p. 75–99, 2010.

MILAGRES, B. S. *et al. Rickettsia* in synanthropic and domestic animals and their hosts from two areas of low endemicity for Brazilian spotted fever in the Eastern Region of Minas Gerais, Brazil. **American Journal of Tropical Medicine and Hygiene**, v. 83, n. 6, p. 1305–1307, 2010.

OGRZEWALSKA, M. *et al.* Epidemiology of Brazilian spotted fever in the Atlantic Forest, state of São Paulo, Brazil. Parasitology, v. 139, n. 10, p. 1283–1300, 2012.

PAGLIA, A. P. *et al.* Lista Anotada dos Mamíferos do Brasil. 2. ed. [s.l.] Occasional Papers in Conservation Biology: Conservation International, 2012.

PAROLA, P. *et al.* Update on tick-borne rickettsioses around the world: a geographic approach. **Clinical Microbiology Reviews**, v. 26, n. 4, p. 657-702, 2013.

PINTER, A. et al. Study of the seasonal dynamics, life cycle, and host specificity of *Amblyomma aureolatum* (Acari: Ixodidae). **Journal of Medical Entomology**, v. 41, n. 3, p. 324–332, 2004.

PINTER, A.; LABRUNA, M. B. Isolation of *Rickettsia rickettsii* and *Rickettsia bellii* in Cell Culture from the Tick *Amblyomma aureolatum* in Brazil. **Annals of the New York Academy of Sciences,** v. 1078, n. 1, p. 523–529, 2006.

REGNERY, R. L.; SPRUILL, C. L.; PLIKAYTIS, B. D. Genotypic identification of rickettsiae and estimation of intraspecies sequence divergence for portions of two rickettsial genes. **Journal of Bacteriology**, v. 173, n. 5, p. 1576–1589, 1991.

ROUX, V. *et al.* Citrate Synthase Gene Comparison, a New Tool for Phylogenetic Analysis, and Its Application for the Rickettsiae. **International Journal of Systematic Bacteriology**, v. 47, n. 2, p. 252–261, 1997.

ROZENTAL, T. *et al.* Zoonotic pathogens in Atlantic Forest wild rodents in Brazil: Bartonella and Coxiella infections. **Acta Tropica**, v. 168, p. 64–73, 2017.

SABATINI, G. S. *et al.* Survey of ticks (Acari: Ixodidae) and their rickettsia in an Atlantic rain forest reserve in the state of São Paulo, Brazil. **Journal of Medical Entomology**, v. 47, n. 5, p. 913–916, 2010.

SAKAI, R. K. *et al*. Experimental infection with *Rickettsia rickettsii* in an *Amblyomma dubitatum* tick colony, naturally infected by *Rickettsia bellii*. **Ticks and Tick-borne Diseases**, v. 5, n. 6, p. 917–923, 2014.

SANGIONI, L. A. et al. Rickettsial Infection in Animals and Brazilian Spotted Fever Endemicity. **Emerging Infectious Diseases**, v. 11, n. 2, p. 265–270, 2005.

SCARANO, F. R. Structure, function and floristic relationships of plant communities in stressful habitats marginal to the Brazilian Atlantic rainforest. **Annals of Botany**, v. 90, n. 4, p. 517–524, 2002.

SILVEIRA, I. et al. *Rickettsia parkeri* in Brazil. **Emerging Infectious Diseases**, v. 13, n. 7, p. 1111–1113, 2007.

SOARES, H. S. *et al.* Ticks and rickettsial infection in the wildlife of two regions of the Brazilian Amazon. **Experimental and Applied Acarology**, v. 65, n. 1, p. 125–140, 2015.

SOARES, J. F. *et al*. Experimental infection of the tick *Amblyomma cajennense*, Cayenne tick, with *Rickettsia rickettsii*, the agent of Rocky Mountain spotted fever. **Medical and Veterinary Entomology**, v. 26, n. 2, p. 139–151, 2012.

SOARES, J. F. et al. Life cycle of *Ixodes schulzei* (Acari: Ixodidae) in the laboratory, and demonstration of reproduction by parthenogenesis. **Parasitology Research**, v. 120, n. 1, p. 9–13, 2021.

SONENSHINE, D.; ROE, R. **Overview:** Ticks, people and animals. In: Biology of Ticks. 2. ed. [s.l.] Oxford University Press, 2014. 1p. 3–16.

SURES, B. Environmental parasitology: Relevancy of parasites in monitoring environmental pollution. **Trends in Parasitology,** v. 20, n. 4, p. 170–177, 2004.

SUZIN, A. *et al.* Free-living ticks (Acari: Ixodidae) in the Iguaçu National Park, Brazil: Temporal dynamics and questing behavior on vegetation. **Ticks and Tick-borne Diseases**, v. 11, n. 5, p. 101471, 2020.

SUZIN, A. *et al*. Ticks on humans in an Atlantic rainforest preserved ecosystem in Brazil: Species, life stages, attachment sites, and temporal pattern of infestation. **Ticks and Tick-borne Diseases**, v. 13, n. 1, 2022.

SZABÓ, M. P. J. *et al.* Ticks (Acari: Ixodidae) parasitizing humans in an Atlantic rainforest reserve of Southeastern Brazil with notes on host suitability. **Experimental and Applied Acarology**, v. 39, n. 3–4, p. 339–346, 2006.

SZABÓ, M. P. J. *et al.* Biology and life cycle of *Amblyomma incisum* (Acari: Ixodidae). **Experimental and Applied Acarology**, v. 48, n. 3, p. 263–271, 2009a.

SZABÓ, M. P. J. *et al.* Ecological aspects of the free-living ticks (Acari: Ixodidae) on animal trails within Atlantic rainforest in south-eastern Brazil. **Annals of Tropical Medicine and Parasitology,** v. 103, n. 1, p. 57–72, 2009b.

SZABÓ, M. P. J. *et al.* In vitro isolation from *Amblyomma ovale* (Acari: Ixodidae) and ecological aspects of the Atlantic rainforest *Rickettsia*, the causative agent of a novel spotted fever rickettsiosis in Brazil. **Parasitology**, v. 140, n. 6, p. 719–728, 2013.

SZABÓ, M. P. J.; PINTER, A.; LABRUNA, M. B. Ecology, biology and distribution of spotted-fever tick vectors in Brazil. **Frontiers in Cellular and Infection Microbiology**, v. 4, n. JUL, p. 1–9, 2013.

VIDAL-MARTÍNEZ, V. M. *et al*. Can parasites really reveal environmental impact? **Trends in Parasitology**, v. 26, n. 1, p. 44–51, 2009.

WECK, B. et al. *Rickettsia parkeri* in the Pampa biome of southern Brazil: Isolation, molecular characterization, and serological evidence of canine infection. **Veterinary Parasitology: Regional Studies and Reports**, v. 22, n. June, p. 100448, 2020.

5 CONCLUSIONS

- In this study it was possible to closely observe the importance of conservation areas for maintaining local biodiversity. A wide variety of rodent and marsupial species were captured, not to mention the large mammals seen along the trails, such as non-human primates, canids, tapirids, deer and felids.
- In this study, three mite families were collected abundantly interacting with small terrestrial mammals. It is important to emphasize that within the Laelapidae and Macronyssidae families, the female stage is the most commonly collected in other works and here we collected nymphal stages as well as males, allowing for future molecular and taxonomic studies on these stages.
- This study contributes with new parasite-host interactions and new locality reports regarding mites and fleas, that also showed no infection to rickettsiae species.
- This is the first record of the detection of *Rickettsia bellii* DNA in the *Amblyomma brasiliensis* tick.
- This study showed the presence of *Amblyomma ovale* ticks infected with *Rickettsia parkeri* strain Atlantic Rainforest in all the areas sampled within the reserve, posing a potential risk to local residents and tourists.