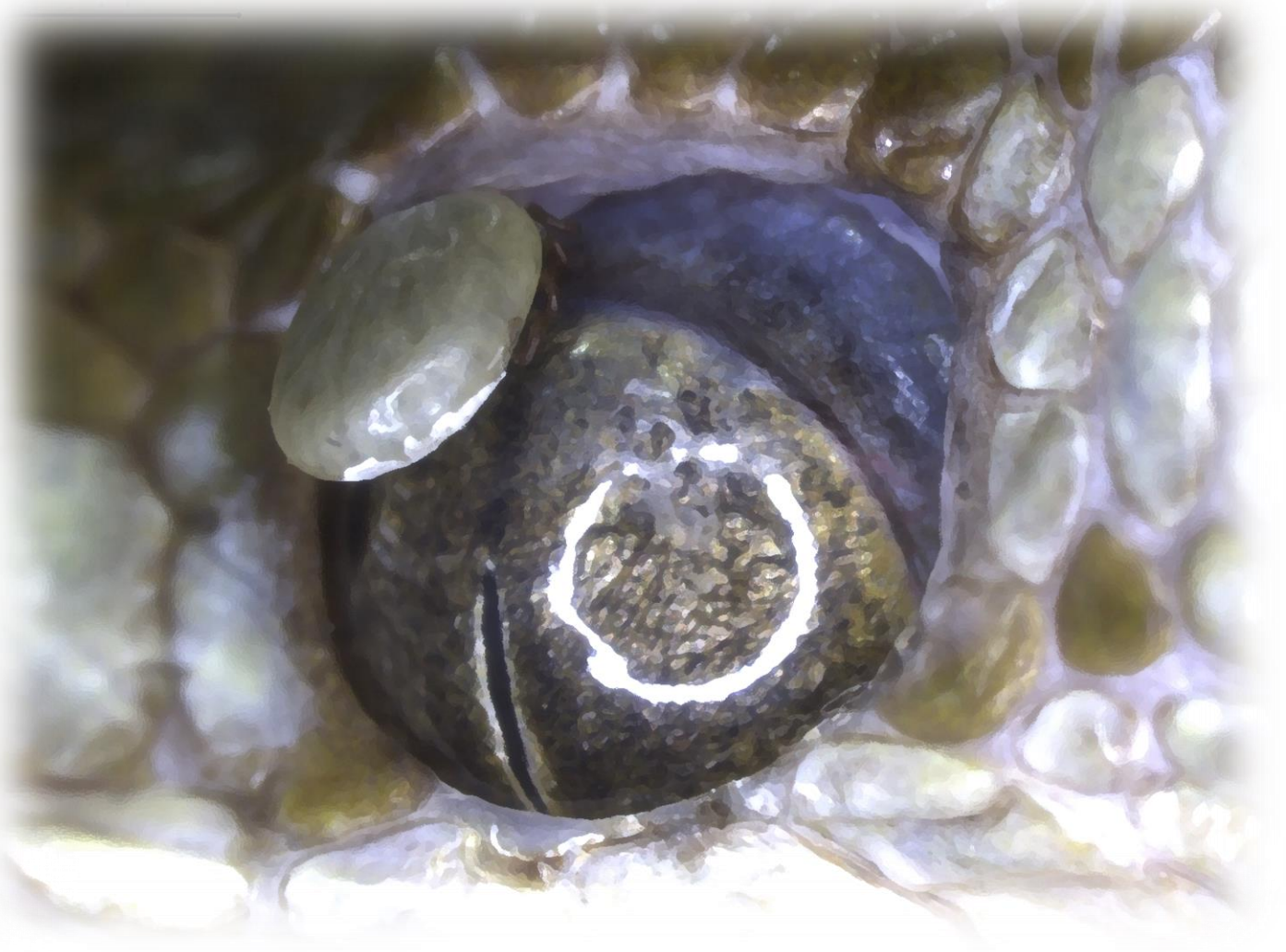
Acarofauna of reptiles and amphibians of Brazil:

Morphological and molecular studies and pathogens research



(Mendoza-Roldan, 2019)

Jairo Alfonso Mendoza Roldan

São Paulo

2019

JAIRO ALFONSO MENDOZA ROLDAN

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Morphological and molecular studies and pathogens research

Acarofauna de répteis e anfíbios do Brasil:

Estudos morfológicos, moleculares e investigação de patógenos

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São Paulo
2019

JAIRO ALFONSO MENDOZA ROLDAN

Acarofauna of reptiles and amphibians of Brazil: Morphological and molecular studies and pathogens research

Thesis presented to the Graduate Program in Experimental Epidemiology Applied to Zoonoses of the Faculty of Veterinary Medicine and Animal Science of the University of São Paulo, to obtain the title of Doctor in Science.

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São Paulo, 1st May 2019

CERTIFIED

We certify that the Research "Acari fauna of reptiles and amphibians of Brazil: Morphological and molecular studies and pathogens investigation", protocol number CEUAx 7491300715 (ID 000300), under the responsibility Darci Moraes Barros Battesti, 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 August 26, 2015.

Certificamos que o protocolo do Projeto de Pesquisa intitulado "Acarofauna de répteis e anfíbios do Brasil: Estudos morfológicos, moleculares e investigação de patógenos ", protocolado sob o CEUAx nº 7491300715, sob a responsabilidade de Darci Moraes Barros Battesti, 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 da Universidade de São Paulo, e foi aprovado na reunião de 26 de agosto de 2015.

Profa. Dra. Anneliese de Souza Traldi
Presidente da Comissão de Ética no Uso de Animais
Faculdade de Medicina Veterinária e Zootecnia da Universidade de São Paulo

Roseli da Costa Gomes
Secretária
Faculdade de Medicina Veterinária e Zootecnia da Universidade de São Paulo

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Prof Dr._____

Institution:_____ Judgment_____

Dedico esta tesis a la persona que me inspira a siempre ser mejor, a mi mejor amigo y confidente, a mi hermano gemelo Miguel Angel.

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“Reptiles and amphibians are sometimes thought of as primitive, dull and dimwitted. In fact, of course, they can be lethally fast, spectacularly beautiful, surprisingly affectionate and very sophisticated”

Sir David Frederick Attenborough

“Every great story seems to begin with a snake”

Nicolas Cage

RESUMO

MENDOZA-ROLDAN, J. A. **Acarofauna de répteis e anfíbios do Brasil: Estudos morfológicos, moleculares e investigação de patógenos.** [Acarofauna of reptiles and amphibians of Brazil: Morphological and molecular studies and pathogens research]. 2019. 461 f. Tese (Doutorado em Ciências) – Faculdade de Medicina Veterinária e Zootecnia, Universidade de São Paulo, São Paulo. 2019

O Brasil é um país megadiverso em herpetofauna, com 796 espécies de répteis e 1.080 de anfíbios. A grande urbanização e o desmatamento acentuado têm ocasionado o aumento de encontros entre a herpetofauna e a população. Esse fato faz com que algumas espécies, antes florestais, sejam atualmente consideradas sinantrópicas. Répteis e anfíbios são amplificadores e reservatórios conhecidos de vários patógenos, mas o papel destes animais no ciclo de doenças e o potencial vetorial dos ectoparasitas desses vertebrados são ainda pouco conhecidos. Répteis e anfíbios são parasitados por mais de 500 espécies de Acari, distribuídas em 61 gêneros de 13 famílias pertencentes às ordens Trombidiformes (Acariformes), Mesostigmata e Ixodida (Parasitiformes). No Brasil, a situação fragmentária dos registros de ácaros dessas ordens, principalmente nas regiões norte e nordeste, além da sua complexidade taxonômica e a escassês de informações sobre sua participação na epidemiologia de doenças, foram os principais motivos que levaram à proposição do presente estudo. Ácaros de répteis e anfíbios, que estão depositados na coleção acarológica de Instituto Butantan (IBSP) foram revisados e, em sua maioria, identificados. Também foram revisadas seis coleções em diferentes localidades (Argentina, Brasil, Estados Unidos, França e Bélgica). Igualmente, foram identificados os ácaros obtidos na recepção de animais do Instituto Butantan e nas coletas em campo de diferentes projetos. Parte do material foi preparada para estudos moleculares e inferência filogenética, usando genes ribossomais e mitrocondriais, e parte foi investigada para a presença de *Borrelia* spp., *Coxiella* spp., *Hepatozoon* spp. e *Rickettsia* sp. Da classe Acari, seis famílias, 12 gêneros e 32 espécies de ácaros Trombidiformes foram identificados, 23 delas ocorrendo no Brasil, incluindo seis novos registros de espécies para o país. O ácaro oribatídeo *Archegozetes longisetosus* Aoki, 1965 foi encontrado possivelmente parasitando um sapo, sendo esta uma nova associação parasito-hospedeiro. Foram identificadas seis famílias, 11 gêneros e 17 espécies de ácaros Mesostigmata, com 16 espécies ocorrendo no Brasil, sendo que uma nova espécie foi descrita (*Chironobius* n. sp.). Duas famílias, quatro gêneros e 19 espécies de carrapatos foram identificadas, 17 ocorrendo no Brasil, com uma

espécie de carrapato argasídeo pertencente ao genero *Ornithodoros* (*Alectorobius*) sp. O número de Acari da herpetofauna brasileira, após este estudo, é de atualmente em 56 espécies. Muitos hospedeiros são novos registros, bem como algumas localidades são novos registros de distribuição. Um total de 4,515 répteis e anfíbios foram examinados, dos quais 170 estavam infestados com ácaros e carrapatos. A avaliação de esfregaços de sangue permitiu correlacionar a presença de hemoparasitas com a prevalência ectoparasitária, e as lâminas histológicas de anfíbios ajudaram a caracterizar a lesão típica produzida pelos ácaros intradérmicos do gênero *Hannemania*. Foi proposta uma filogenia utilizando-se o gene 18S V4 rRNA para Acari, que inferiu a polifilia de Acari e a monofilia de Acariformes e Parasitiformes. Espécies do gênero *Hepatozoon* foram detectadas em carrapatos, ácaros e sangue de hospedeiros, e as sequências geradas foram similares à três espécies depositadas no GenBank (*Hepatozoon* sp. BT-2016, *Hepatozoon* sp. CCS-2010 e *Hepatozoon ayorgbor*) com hospedeiros e distribuição geográfica delimitadas. Três espécies de *Rickettsia* foram identificadas para o gene *gltA*, e quatro para o gene *OmpA* do Grupo da Febre Maculosa. Nenhuma das amostras de tecido dos hospedeiros testadas apresentou resultados positivos. *Rickettsia bellii* em *Amblyomma sculptum* é novo registro, e a presença no ácaro *Eutrombicula alfreddugesi* é um resultado inédito. *Rickettsia rhipicephali* foi detectada pela primeira vez em ácaros Mesostigmata e *Rickettsia amblyommatis* foi detectada pela primeira vez em *Amblyomma rotundatum*. A detecção de *Rickettsia aeschlimannii* em um ácaro macronissídeo (*Ophyonissus natricis*) é inédita, assim como *Rickettsia rickettsii* em ácaros Pterygosomatidae é também um novo relato. A detecção de espécies de *Rickettsia* do Grupo da Febre Maculosa em ácaros de répteis (Mesostigmata e Pterygosomatidae) destaca a importância de uma avaliação integrativa de ectoparasitos de répteis, principalmente devido à fragmentação do habitat, que, consequentemente, predispõe a um maior número de ocorrências entre humanos, herpetofauna e acarofauna.

Palavras-chave: Taxonomia. Acari. Herpetofauna. Anfíbios. Filogenia. Patógenos.

ABSTRACT

MENDOZA-ROLDAN, J. A. **Acarofauna of reptiles and amphibians of Brazil: Morphological and molecular studies and pathogens research [**Acarofauna de répteis e anfíbios do Brazil: Estudos morfológicos, moleculares e investigação de patógenos]. 2019.461 p. Thesis (Doctor in Science) – Faculdade de Medicina Veterinária e Zootecnia, Universidade de São Paulo, São Paulo. 2019

Brazil is a megadiverse country in herpetofauna, with 796 species of reptiles, and 1,080 species of amphibians. The high urbanization and the marked deforestation have increased the number of human-herpetofauna encounters. This fact has made some species to be considered currently as synanthropic. Reptiles and amphibians are known amplifiers and reservoirs of several pathogens, yet the role of these animals in the cycle of diseases and the vector potential of the ectoparasitic mites of these vertebrates are poorly known. These hosts are parasitized by more than 500 species of mites, distributed in 61 genera of 13 families that belong to the Trombidiformes (Acariformes), Mesostigmata and Ixodida (Parasitiformes) orders. In the Brazil context, the fragmentary records of species of mites of these orders, especially in the north and northeast regions, their taxonomic complexity and the scarce information regarding their role in the epidemiology of diseases, where the main reasons to pursue the proposition of the present study. Mites of reptiles and amphibians deposited in the Acari collection of the Instituto Butantan (IBSP) were reviewed and identified. Six other collections in various places where also visited (Argentina, Brazil, United States, France and Belgium). Also, mites collected at the animal reception site of the Instituto Butantan, and from field collections were also identified. Part of this material was prepared for molecular studies and phylogenetic inference using ribosomal and mitochondrial genes, and another part of the material was used to assess the presence of *Borrelia* spp., *Coxiella* spp., *Hepatozoon* spp. and *Rickettsia* spp. Of the subclass Acari, Six families, 12 genera and 32 species of Trombidiformes mites were identified, 23 occurring in Brazil, increasing six new species to the Brazilian territory. The Oribatid mite *A. longisetosus* was identified apparently parasitizing a frog, which is a new host-parasite association. Six families, 11 genera and 17 species of Mesostigmata mites were identified, wit 16 species occurring in Brazil, with one new species described (*Chironobius* n. sp.). Two families, four genera and 19 species of ticks were identified, 17 occurring in Brazil, with one new species of argasid tick registered in Brazil, with an argasid tick of the genus *Ornithodoros (Alectorobius)*. The total number of Acari parasites of herpetofauna in Brazil after this study is 56 species. Many

hosts are new records, as well as, some of the localities are new records of distribution. 4,515 reptiles and amphibians were examined, of which 170 were infested with mites and ticks. Assessing blood smears allowed to correlate hemoparasitic presence with ectoparasitic prevalence, and the histologic slides of amphibians helped better characterize the typical lesion produced by intradermic mites of the genus *Hannemania.* A phylogeny inference using the 18S V4 rRNA gene for Acari was proposed that inferred a polyphyletic Acari, with different bootstrap values for the monophyly of Acariformes and Parasitiformes. *Hepatozoon* was detected in mite ticks and hosts' blood. The sequences generated matched three main species with host and geographical delimitations (*Hepatozoon* sp. BT-2016, *Hepatozoon* sp. CCS-2010 and *Hepatozoon ayorgbor*). Three species were identified for the *gltA* gene for *Rickettsia*, and four species were identified for the *OmpA* gene for the Spotted Fever Group *Rickettsia* from ixodid ticks, trombiculid, pterygosomatid, and Mesostigmata mites. None of the hosts tissue samples tested yielded positive. *Rickettsia bellii* in *A. sculptum* is a new report and the presence in a *Eutrombicula alfreddugesi* mite, is unprecedented. *Rhickettisa rhipicephali* was detected for the first time on Mesostigmata mites. *Rickettisa amblyommatis* was detected for the first time in *A. rotundatum.* The detection of *R. aeschlimannii* from a macronyssid mite (*O. natricis*), is unprecedented, and *R. rickettsii* in Pterygosomatidae mites is also a new report. The detection of SFG *Rickettsia* species on reptile mites (Mesostigmata and Pterygosomatidae) highlights the importance of an integrative assessment of ectoparasites of reptiles mainly due to the fragmentation of the habitat, which, consequently, prompts to a greater number of occurrences between humans, herpetofauna and acarofauna.

Key Words: Taxonomy. Herpetofauna. Amphibians. Phylogeny. Pathogens.

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LIST OF ABBREVIATIONS AND ACRONYMS

µta: microtibiala leg I

1a, 3a: nautalae

1b: anterior setae

A: amplified

A: autolysis

A: axial

a: arm

AC: Acre (state)

AL: Alagoas (state)

AL: anterolateral setae

AM: anteromedial setae

AP: Amapá (state)

ASB: distance between the bases of the sensillas and the anterior margin of the dorsal shield

AW: anterior dorsal shield width

B.M: Arachnida department British Museum, United Kingdom

BA: Bayesian

BA: Bahia (state)

Bk: back

BLAST: Basic Local Alignment Search Tool

BM(NH): The Natural History Museum (formerly British Museum (Natural History), London, United Kingdom

BMNH: British Museum of Natural History, Londres, Inglaterra

bp: base pair

By: belly

C: central setae

Ca: circumanal setae

CAPI: capsular polysaccharide biosynthesis protein

Cb: chelicera

CE: Ceará (state)

CEUA: Comissão de Ética no Uso de Animais

CNAC: Colección Nacional de Ácaros del Instituto de Biología de la Universidad Nacional Autónoma de México en México, Distrito Federal

CNC: National Tick Collection (Coleção Nacional de Carrapatos Danilo Gonçalves Saraiva) of the School of Veterinary Medicine, University of São Paulo, São Paulo, Brazil

CNPq: Conselho Nacional de Desenvolvimento Científico e Tecnológico

COI: cytochrome oxidase subunit I

Ct: Connective tissue

DCa: Dorsal Celomatic area

dF: 564 ventral setae

dG: apical foliate seta

Di: Digits

Dmax: longest measure of dorsal idiosomal setae

Dmin: shortest measure of dorsal idiosomal setae

DNA: deoxyribonucleic acid

DoHa: Dorsal Head area

EM: examined material collected in this study

ES: Espírito Santo (state)

F: female

F: femur

f': microtarsala leg I

Fa: Forearm

FAPESP: Fundação de Amparo a Pesquisa do Estado de São Paulo

FCNyN UP: Facultad de ciencias naturales y Museo de la Universidad de la Plata, Argentina

FL: forelimb

FMVZ: Faculdade de Medicina Veterinária e Zootecnia

fSc: relation between lengths of the dorsal shield bristles (AL, AM and PL)

FZB: Fundação Zoobotanica, Porto Alegre, RS, Brazil

g: genital setae

G: Gland

Ga: galeala

ga: genuala

ga: genuala leg I

GA: Gular Area

Ge: genus

glTA: gene citrato sintase

gm: genuala leg II

GO: Goiás (state)

gp: genuala leg III

GT: guanidine isothiocyanate protocol

H: head

h1, h2, f1, f2: genital setae

HL: hindlimb

I: inguinal

I: Ixodida

I''G: tibial dorsal setae

IBSP: Acarological collection, of the Instituto Butantan, Special Zoological Collections Laboratory, São Paulo, Brazil

IBU: Instituto Butantan, São Paulo, SP, Brasil

ICMBio: Instituto Chico Mendes de Conservação da Biodiversidade

inP: inguinal mite Pocket

IRSN: Institut royal des Sciences naturelles de Belgique Brussels, Belgium

IRSNB: Institut royal des Sciences naturelles de Belgique Brussels, Belgium

IT: tarsal anterior setae

L: larvae

LAS: Lateral Anterior Scales

LECZ: Laboratório Especial de Coleções Zoológicas

LG: long gnatossoma

lnP: lateral nucal Pocket

LPS: Lateral Posterior Scales

m: latero-basal setae

M: male

M: Mesostigmata

MA: mean abundance

MA: Maranhão (state)

MI: mean intensity

MG: Minas Gerais (state)

MI: mean intensity

MLP: Facultad de Ciencias Naturales y Museo de la Universidad de la Plata, Argentina

MP: Maximum Parsimony

mRNA: mitochondrial ribonucleic acid

MS: Mato Grosso do Sul (state)

MTa: mastitarsala

mtDNA: mitochondrial DNA

ML: maximum likelihood

MZUC: Museo de Zoología, Universidad de Concepción, Concepción, Chile

N: naso

N: Nymphs

n: ventro-basal setae

NA: Not Amplified

NAL: No Aparent Lesion

NHMUK: United Kingdom, London, The Natural History Museum [formerly British Museum (Natural History)]

NL: Nodular Lesion

NMNH: National Museum of Natural History, Smithsonian Institution, Washington, D.C. 20560, U.S.A

NNP: nuchal non-pocket

nPl: nucal Pocket lateral

O: Oribatida

OSAL: Acarology Laboratory, The Ohio State University, Columbus, Ohio, United States

P-PL: distance from posterolateral to extreme posterior margin.

PA: Pará (state)

Pax: auxiliar mite Pocket

Pc: postcoxal setae

PCR: polymerase chain reaction

PE: Pernambuco (state)

Ph: posthypostomal setae

PI: post-inguinal

PI: prevalence index

PL: posterolateral setae

PM: posteromedian setae

Poa: Peri-ocular area

PR: Paraná (state)

ps1–2 and **ps3**: genital setae

PSB: distance from posterolateral to extreme posterior margin.

PU CSAV: Institute of Parasitology Academy of Sciences of the Czech Republic, Czech Republic

PW: posterior width

rDNA: ribosomal deoxyribonucleic acid

RJ: Rio de Janeiro (state)

RN: Rio Grande do Norte (state)

RM: Rijks Museum, Leiden, Holland

RML: Rocky Mountain Laboratories, Hamilton, Montana, USA

RO: Rondônia (state)

rpm: rotations per minute

rRNA: ribosomal ribonucleic acid

RS: Rio Grande do Sul (state)

S: sensilla

S: side

S1: tarsala leg I

S2: tarsala leg II

SB: Distance between the bases of sensillas

SBH: Brazilian Society of Herpetology (Sociedade Brasileira de Herpetologia)

sc: stratum compactum

SC: Santa Catarina (state)

scr: stratum corneum

scx: scapular setae

SD: standard deviation

SD: sum of ASB and PSB

SE: Sergipe (state)

SEM: Scanning electron microscopy

sg: stratum germinativum

Sisbio: Sistema de autorização e informação em Biodiversidade

SP: São Paulo (state)

spp.: várias espécies

SPR: Subtree-Pruning-Regrafting

ST: subterminala leg I

St: sternal setae

T: Trombidiformes

Ta: Tail

Ta: tarsal area

Ta: tarso

ta: tibiala leg I

TAE: Tris-acetate-EDTA

TE: Tris-EDTA

Th: Thigh

Ti: tibia

TIa: Tibial area

tm: tibiala leg II

TO: Tocantins (state)

tp: tibiala leg III

UK: United Kingdom

USNM: National Museum of Natural History, Smithsonian Institution, Washington, D.C. 20560, U.S.A

USNMENT: United States National Tick Collection, Georgia Southern University, Statesboro, USA

USP: Universidade de São Paulo

UV: ultraviolet light

v': ventral seta

VAS: Ventral Anterior Scales

VCa: Ventral Celomatic area

ve, vi, se, si, c1 – c3: dorsal anterior setae

vi, ve and sci: 3 pairs of peripectinate setae

Vmax: longest measure of ventral idiosomal setae

Vmin: shortest measure of ventral idiosomal setae

WG: wide gnatossoma

ZISP: Acarological collection of the Zoological Institute of the Russian Academy of Sciences, St. Petersburg, Russia

ZISP: Zoological Institute of the Russian Academy of Sciences, St. Petersburg, Russia

ZMH: Zoologisches Institut und Zoologisches Museum der Universitat, Hamburg, Germany

ZMUC: Zoological Museum, University of Copenhagen, Denmark

LIST OF SYMBOLS

-: not applied

%: percent

’: minute

’’: second

=: same

º C: degree Celsius

½: half

mL: mililitro

n: sample size

ng: nanogram

nm: nanometer

Nº: number

º: degree

pH: hydrogen potential

rpm: rotations per minute

µl: microliter

µm: micrometer

ζ: subterminala I

κ: microgenuala and microtibiala;

σ: genuala I, II and III;

Φ: tibiala I, II and III;

ω: tarsala I

SUMARY

1 INTRODUCTION

1.1 Reptiles and amphibians

1.1.1 Diversity and distribution of reptiles and amphibians

The classes Reptilia and Amphibia are both groups of ectothermic animals distributed almost worldwide (except the Antarctic and northern Nearctic regions). As of July 2018, more than 10,793 species of reptiles, distributed in 1,199 genera and 86 families, have been described (UETZ et al., 2018). On the other hand, currently there are 7,969 described species of amphibians, distributed in three orders: Anura (7,040 species), Caudata (717 species), and Gymnophiona (212 species) (AMPHIBIAWEB, 2019). Furthermore, one of the richest regions in herpetofauna biodiversity is the Neotropical region. The Neotropical region comprises the geographical area of South America, Central America, the Caribbean, up to central Mexico (MORRONE, 2014). This region has been divided in Sub-regions, depending of biogeographical, phytogeographical and zoogeographical data. One of the first divisions describes four sub-regions: Mexican, that comprises southern Mexico and Central America; Antillean, which englobes the Caribbean area; Brazilian, being the tropical part of South America; and Chilean, that is the temperate area of South America (Figure 1) (WALLACE, 1876). The reptile fauna of this region, to 2015, is composed by more than 4,049 species, represented by roughly 2,086 species distributed in South America (Figure 2A) (UETZ et al., 2018). For amphibians, the number of species described is 2,916 being the region of the world with the most species diversity (Figure 2B) (BOLAÑOS et al., 2008).

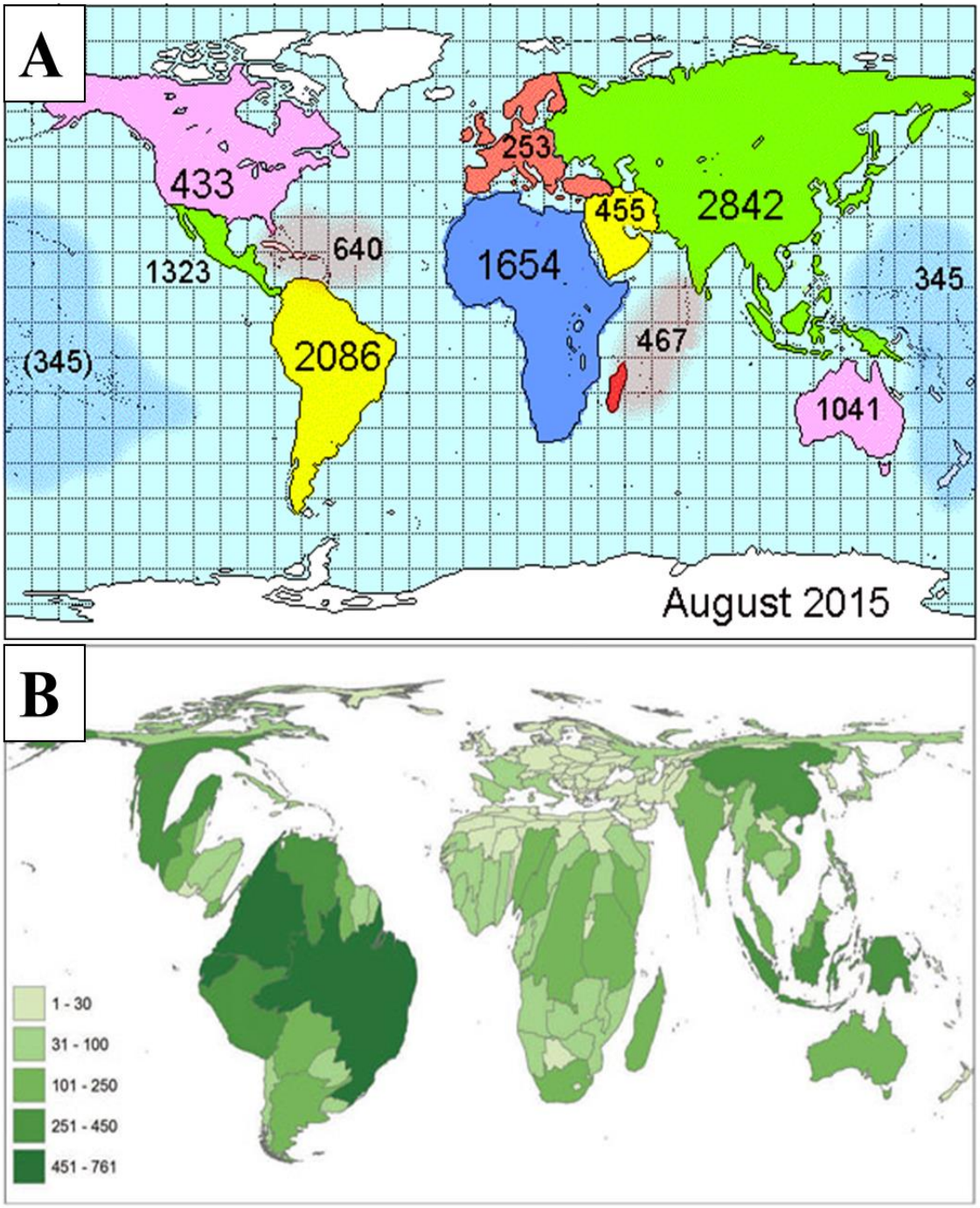
In Brazil, 796 species of reptiles, distributed in three orders [Testudines (36 species); Crocodylia (6 species) and Squamata (753 species, being 72 amphisbaenians, 276, Sauria and 405 Serpentes)] and 1,080 species of amphibians in three orders [Anura (1,039 species); Gymnophiona (36 species); and Caudata (5 species)] have been described and recorded (SEGALLA et al., 216; COSTA; BÉRNILS, 2018). This number of species turns Brazil one of the most megadiverse countries of the world, regarding herpetofauna (Figure 2).

Figure 1 - Sub-regions of the Neotropical region

Source: (Adapted from WALLACE, 1876).

Legend: 1) Chilian; 2) Brazilian; 3) Mexican; 4) Antillean.

Figure 2 - Diversity and distribution of reptiles and amphibians



Source: (Adapted from: UETZ et al., 2018, and WALLACE, 1876).

Legend: A) diversity and distribution of reptiles; B) diversity and distribution of amphibians, showing a high diversity of species in South America.

The herpetofauna in Brazil is distributed in the different environments of vegetal formations (biomes), that are categorized as follows: rain forests (Amazon basin and Atlantic forest), savannah or Cerrado and marshlands (central Brazil), Caatinga (northeastern region of Brazil), and Pampas (southern Brazil) (Figure 3) (VANZOLINI et al., 2010). Moreover, Brazil is considered a continental country given its territorial extension, that offers a diverse type of climate and soil conditions for each region. All the above, provides Brazil a great diversity of biomes, mainly defined by their unique vegetal coverage (COUTINHO, 2006).

Figure 3 - Biomes of Brazil

Source: (adapted from: http://www.geografia.seed.pr.gov.br)

1.1.2 Environmental modifications and the Brazilian herpetofauna

In the last decades, fragmentation, deforestation and urbanization are rising exponentially throughout the Brazilian territories (PIRES et al., 2006; TANUS et al., 2012). The Amazon rainforest, cerrado and atlantic forest are the biomes that have suffered the most due to the mentioned environmental modifications. The loss of habitat and proliferation of urban settlements has increased the number of encounters and accidents with reptiles and amphibians, consequently raising the number of venomous snakebite accidents in the last 20 years (BOCHNER et al., 2003; SILVA et al., 2015; GUERRA et al., 2016). Thus, species of the herpetofauna initially considered forested, have now developed synanthropic behaviors, therefore increasing the risk of pathogen and parasite transmission to other animals and humans (BARBO et al., 2011; RAGO et al., 2012; SOUSA et al., 2014; NOWAK-CHMURA, 2014; SILVA et al., 2017).

Some examples of these trends are records of parasitism of commonly ectoparasites from reptiles, now found on humans. This is the case of *Amblyomma fuscum* Neumann, 1907, that was recorded parasitizing humans as consequence of hosts new synanthropic behaviors (typically) reptiles and amphibians) (MARQUES et al., 2006). Another example of humans being affected by reptile ectoparasites, relates to cases of mite bites of snake parasites, such as *Ophionyssus natricis* (Gervais, 1844), which in human causes dermatitis and possibly can facilitate diseases transmission (SCHULTZ, 1975; AMANATFARD et al., 2014).

Furthermore, these anthropic pressures over the environment have affected the herpetofauna reducing species diversity in their natural habitats, endangering endemic species, and enabling the dispersion of diseases, as reptiles and amphibians are considered natural reservoirs for bacterial, viral and parasitic diseases (KRAUS et al., 2005, RABITSCH; SCHINDLER, 2017). One common route of disease transmission to humans is through vectors, such as mites and ticks (BURRIDGE, 2001; AMO et al., 2005). Thus, the importance of studying the species that parasitize the Brazilian herpetofauna and the pathogens they can harbor and transmit.

1.2 Acari of the herpetofauna

There are more than 500 species of mites and ticks (Acari) known to parasitize reptiles and amphibians worldwide. These ectoparasites species are distributed in 61 genera, and 13 families belonging to three orders: Trombiformes (Acariformes), Mesostigmata and Ixodida (Parasitiformes) (PIETZSCH et al., 2006; FAJFER, 2012; BARROS-BATTESTI et al., 2015; DIVERS; STAHL, 2018).

The superorder Acariformes includes the order Trombidiformes, which is the most numerous and diverse orders of mites, containing around 130 families with more than 22,000 species (ZHANG et al., 2011; REZENDE et al., 2012). Due to this diversity, this order clusters mites that are morphologically very different, thus having very few synapomorphies, which are apomorphic homologous characters shared by two or more taxa (LINDQUIST, 1996; DABERT et al., 2010). Trombidiformes mites that parasitize reptiles and amphibians are grouped in the suborder Prostigmata, in seven families: Cloacaridae, Ereynetidae, Harpirhynchidae, Leeuwenhoekiidae, Pterygosomatidae, Thermacaridae, and Trombiculidae. (FAIN, 1961; FAIN, 1964; CAMIN et al., 1967; BRENNAN; GOFF, 1977; MARTIN; SCHWOERBEL, 2002; BOCHKOV; OCONNOR, 2006). All the before mentioned families have been recorded in the Neotropical region. Amphibians are parasitized by Leeuwenhoekiidae, Ereynetidae, and Thermacaridae mites. This last family includes different genera, however, only four species of the genus *Thermacarus* (Sokolow, 1927), parasitize anurans (MARTIN; SCHWOERBEL, 2002).

On the other hand, the superorder Parasitiformes comprises the orders Mesostigmata, Holothyrida (a group of mites that feed of bodily fluids of dead arthropods, and it is a group more related to Ixodida) and Ixodida (ticks). Parasitiformes are characterized by having free coxae, covered anal opening by a pair of plaques, a sclerotized ring around the gnathosoma (capitulum), and they usually present a biflagellate tritosternum in Mesostigmata (WALTER; PROCTOR, 1988, LEHTINEN, 1991). The order Mesostigmata contains five families that infest reptiles and amphibians. These families are: Entonyssidae (endoparasitic mites of snakes), Heterozerconidae (mites that generally infest myriapods, with three species recorded on snakes and amphisbaenas), Ixodorhynchidae (ectoparasitic mites of reptiles), Omentolaelapidae (monotypic family of snakes); Macronyssidae (genus *Ophionyssus*, exclusive of lizards and snakes) (FAIN, 1961a; FAIN, 1962a; FAIN, 1962b; LIZASO, 1979; LIZASO, 1982; DE BELLOCQ; JOËLLE, 2007).

The order Ixodida groups ticks of three families. The most common and widely distributed families are Argasidae and Ixodida. The third family is Nuttalliellidae, a monospecific family recorded only in South Africa and Tanzania (MANS et al., 2012). Moreover, ticks that parasitize reptiles and amphibians belong mainly to the family Ixodidae (genera *Amblyomma*, *Bothriocroton*, *Dermacentor*, *Haemaphysalis*, *Hyalomma* and *Ixodes*). Parasitism by argasid ticks on the herpetofauna is more uncommon, nonetheless, some species of *Argas* and *Ornithodoros* infest reptiles and amphibians, with four records of parasitism on amphibians by the ge*nus Ornithodoros* in Brazil (BARROS-BATTESTI et al., 2006; DANTAS-TORRES et al., 2008; BARROS-BATTESTI et al., 2015, MUÑOZ-LEAL et al., 2017).

Moreover, in Brazil several studies have analyzed the species of mites and ticks that infest the herpetofauna. Regarding snake mites, Lizaso (1981; 1983; 1984) performed most of the ectoparasite arrays from non-venomous snakes of Brazil. Her studies evaluated the southeastern, central-western, and southern regions of the country, which resulted in the description of 8 new genera and 11 new species of Trombidiformes and Mesostigmata mites. However, after these studies there has not been updated research of mites in ophidian hosts until recently (MENDOZA-ROLDAN, 2015; MENDOZA-ROLDAN et al., 2017). The authors documented new records of occurrence and hosts, and new species of mites for the state of São Paulo (four new records of Trombidiformes mites and the description of a new species of *Ophioptes.* Thus, in Brazil, including all the information from literature and data from national collections, currently there are listed five families of mites of the Trombidiformes order (Pterygosomatidae, Harpirhynchidae, Trombiculidae, Leeuwenhoekiidae, and Ereynetidae), including 22 species in seven genera (FONSECA, 1934; FONSECA, 1940; FAIN, 1961b; FAIN, 1962; LIZASO, 1983; MENDOZA-ROLDAN, 2015; 2017), and four families distributed in eight genera and 15 species of Mesostigmata (Entonyssidae, Ixodorhynchidae, Heterozerconidae, and Macronyssidae) (FAIN, 1961; FAJFER, 2012; MENDOZA-ROLDAN, 2015).

Regarding ticks, more than 100 species of ticks are registered to parasitize reptiles and amphibians worldwide, with eight species for Brazil (BARROS-BATTESTI et al., 2006; BARROS-BATTESTI et al., 2015, MUÑOZ-LEAL et al., 2017). In some cases, reptile and amphibians are specific hosts for ticks (BARNARD; DURDEN, 1994; PIETZSCH et al., 2006). Some examples are *Ornithodoros transversus* (Klompen, 1992) found only in *Chelonoidis nigra* (HOOGSTRAAL; KOHLS, 1966; PIETZSCH et al., 2006), and in Brazil, the species

Ornithodoros faccinii Barros-Battesti et al., 2015, that infests the anuran *Thoropa miliaris* (BARROS-BATTESTI et al., 2015; SÁ-HUNGARO et al., 2017). This species was also recorded recently infesting *Rhinella* toads (LUZ et al., 2018).

Despite recent research, the Brazilian territory, given its wide extension, has still unknown information about the acari fauna of reptiles and amphibians, and some taxa of the herpetofauna have never been assessed for the presence of mites and ticks. In this context, venomous snakes and amphibians are the less studied groups. Another reason for the scarce information it the large number of species that are endoparasitic mites (Entonyssidae: Mesostigmata; Leewuenhoekidae: Trombidiformes), which makes it more difficult to study them (FAIN, 1961a FAIN, 1962; DUSZYNSKI; JONES, 1970; SILVA-DE LA FUENTE et al., 2016). Another fact to consider is the presence of exotic mites in Brazil, such as *Geckobia hemidactyli* (Lawrence, 1936), which is a mite introduced to the Neotropical region with its original host *Hemidactylus mabouia* (Moreau Jonnès, 1818) from Africa. Currently, this mite is adapted to endemic species of hosts in Brazil. However, the impact in native host populations is not clear, and more studies are required to better understand the effect of the parasitism in these populations (MENDOZA-ROLDAN, 2015). Other species such as *Geckobia bataviensis* (Vitztum, 1926) autor and *Geckobia keegani* Lawrence, 1953 (parasitizing *Hemidactylus frenatus* in Australia), colonized different environments of the Neotropical region (RIVERA et al., 2003, HOSKIN, 2011). Despite these introductions of invasive species of lizards and mites, there is scarce information of the real impact and the ecology of the diseases these mites can transmit given the unique features of the American continent.

As these mites, other species have been successfully introduced to the country, such is the case of *O. natricis* which now is widely distributed in captive populations of Squamata reptiles, and recently recorded in exotic species of snakes in Brazil (DA SILVA et al., 2018). Considering the adaptations upheld by exotic species for successful establishment, several species of ticks have been introduced to countries in Central America, United States, the United Kingdom, and other countries in Europe through the importation of reptiles and amphibians by the international pet trade (BURRIDGE, 2001; PIETZSCH et al., 2006; MIHALCA, 2015). Nevertheless, few are the species that adapted to the new environments and settled, as for example *Amblyomma dissimile* (Koch 1844) (from South America, introduced in Florida) and *Amblyomma variegatum* (Fabricius, 1794) (from Africa, introduced in the Caribbean). Hence, more information is needed to further

understand the impact and effect of exotic species of mites and ticks on the endemic populations of reptiles and amphibians in Brazil.

1.2.1 Effect of parasitism on the ectothermic host

Ectothermic or cold-blooded hosts have unique inflammatory, immunologic and metabolic responses, when confronted with a parasitic event, different from those observed in non-reptilian (birds) and mammals, and other animals considered endotherms. These responses depend on the species parasitizing, the number of parasites attached, the individual characteristics, and the environmental challenges (climate change) presented to the host (HARVELL et al., 2009; KLINGENBERG, 2012).

The negative effect of mites and ticks on the hosts fitness can be divided in the direct effect on the host health status, and the indirect effect, given by the vectoral capacity of the parasite to transmit pathogens. The direct effect generally results in anemia and, dehydration and emaciation of the host, when presented with hyper-infestation. Skin lesions are also common at the attachment site as edema, inflammation and erythema. Also, infestations lead to behavioral changes of the host, with more aggressive animals, moving repeatedly or remaining submerged (WOZNIAK; DENARDO, 2000; FAJFER, 2012). Finally, in reptiles, ectoparasite infestation promotes the ecdysis process, resulting in early molting, and when hyper-infested, the hosts can suffer from dysecdysis (MENDOZA-ROLDAN, et al., 2019). In amphibians, effects are similar to those seen on tepriles reptiles, and as most of the mites have skin-dwelling behavior (endoparasites), the capsule in which they develop promotes a granulomatous injury and deformation, which can lead to avascular necrosis and limb loss. In all cases hyper infestations affect negatively the health status of the ectothermic host which can finally result in death of the host (RODRIGUES et al., 2018).

On the other hand, the indirect effect is related to the parasite`s competence and capacity as a vector of pathogens (MORO et al., 2005). The pathogeny and development of diseases in ectothermic animals varies from that of the most commonly pathogenic patterns studied in mammals. Furthermore, reptiles and amphibians harbor a wide range of pathogens, which these animals play a role as natural reservoirs and amplifiers of microorganisms, that can be transmitted to other reptiles and in some cases even humans (FLAJNIK, 1996; POINAR; POINAR, 2004; OSTFELD; HOLT, 2004).

1.3 Pathogens associated with acari of reptiles and amphibians

Mites and ticks can have a significant role in transmitting pathogens from or to their hosts. Nonetheless, there is scarce information of the host-parasite relationship and the life-cycle of the diseases affecting the parasite and the ectothermic host (BALASHOV, 1984; KUO et al., 2000, INGOLE et al., 2015). Despite this lack of information, some families of reptiles and amphibians' mites and ticks have been pointed as suitable vectors of pathogenic agents (Macronyssidae, Trombiculidae, Pterygosomatidae, Ixodidae and Argasidae).

Some of diseases can be zoonotic, and the transmission to humans generally involves a blood-sucking arthropod, acting as vector. Of these, some species of mites and ticks have been reported as feasible vectors that can parasitize humans [Parasitiformes (ordens Ixodida, Mesostigmata) and Acariformes (ordem Trombidiformes)]. These mites have been indicated as vectors of bacterial, viral, protozoal, and even helminthic diseases (NADCHATRAM, 1970; BURRIDGE, 2001; FRANCES, 2005; VÁCLAV et al., 2011; BOWER et al., 2018).

Specifically, from the Tromidiformes order, mites of the family Pterygosomatidae (parasites of mainly Gekkonidae lizards) have been pointed as vectors and intermediate hosts of protozoa. The genus *Hirstiella* has been recorded as vector of hemogregarines and *Plasmodium* sp. (NEWELL; RYCKMAN, 1964). On the other hand, the species *Geckobiella texana* (Banks, 1904) was found naturally infected with *Schellackia occidentalis* (Bonorris & Ball, 1955), though its vectorial capacity has not been proven. Also, this family has been proven a vector of *Hepatozoon* spp. The transmission occurs by passive pathway when the host eats the mite (WALTER; PROCTOR, 2013).

Regarding the Mesostigmata order, the most studied species is the macronissid mite *O. natricis.* This mite has a worldwide distribution and has been suggested as vector of pathogens such as: *Arenavirus*, etiological agent of the inclusion bodies disease (IBD) in boid snakes (BECK et al., 2005; CHANG; JACOBSON, 2010; DIVERS; STAHL, 2018); it is the mechanical vector of *Aeromonas hydrophila* (Chester) (cited as *Proteus hydrophilus*), a gram-negative bacteria that causes a hemorrhagic disease in reptiles. In amphibians these bacteria cause an erythematous disease called "Red Leg disease" (KULP; BORDEN, 1942; MIRANDA et al., 2017). This mite was also found infected with *Anaplasma* spp., a rickettsial bacteria pathogenic in mammals

(REEVES et al., 2006). Furthermore, the species *Ophionyssus galloticolus* Fain & Bannert, 2000, is a known vector of the *Karyolysus* sp. protozoa, that infects lacertid lizards (BANNERT et al., 2000). However, it is not well known if this or other protozoa cause lesions to their ectothermic host. All the before mentioned highlights the importance of studying Mesostigmata mites of reptiles, particularly *Ophyonissus natricis* (Gervais, 1844), as it is a common parasite found in captive reptiles, which is a suitable life condition for diseases to spread and harm the facilities` overall health status, for example diminishing production of venom for sera production.

Regarding ticks, there is more rounded and updated knowledge of the epidemiological role of the Argasidae and Ixodidae families in the transmission of diseases. Concerning Argasid ticks, the species *Ornithodoros turicata* (Dugès, 1876), parasitizes mainly tortoises, among other hosts. This tick is the vector of *Borrelia turicatae*, bacteria that belong to the relapsing fever clade, of which tortoises are natural reservoirs. Other borrelial diseases are associated with ixodid ticks and reptiles (mainly lacertid lizards) and are one of the most widespread vector-borne diseases in the northern hemisphere. The *Borrelia burgdorferi* sensu lato group, which causes Lyme disease and other borreliosis, includes species such as *Borrelia lusitaniae* (pathogenic in humans), that use reptiles as natural reservoirs. Ticks of the genus *Ixodes* such as *Ixodes ricinus* (Linnaeus, 1758), *Ixodes scapularis* Say, 1821, *Ixodes persulcatus* Schulze, 1930, and *Ixodes pacificus* Cooley & Kohls, 1943 are vectors and reservoirs of *Borrelia burgdorferi* sensu lato (LANE, 1990; LEVIN et al., 1996; KUO et al., 2000; SZEKERES et al., 2016; MACDONALD et al., 2017; MENDOZA-ROLDAN et al., 2019). There is also a clade of reptile-associated *Borrelia*, with no demonstrated pathogenicity. This clade has been identified in species of ixodid ticks specialized in reptiles, such as the goanna tick *Bothriocroton undatum* (Fabricius, 1775) (PANETTA et al., 2017). In South America, several studies have revealed the presence of borrelial species in this region of the continent. However, no studies have shown the association of reptiles as reservoirs in the Neotropical region (NEED; ESCAMILLA, 1991; IVANOVA et al., 2014; MUÑOZ-LEAL et al., 2019).

Additionally, reptiles and amphibians can contribute to several bacterial diseases acting as reservoirs and having ticks and mites as vectors. A disease related to the presence of ectoparasites is the “viper plague” in Viperidae snakes, which causative agent is *Erlichia ruminatium.* This disease was introduced to the United States with the importation of a *Bitis gabonica* (Duméril, Bibron & Duméril, 1854) snake, from Ghana (KIEL et al., 2008). Other importation

events are the introduction of exotic species of ticks and mites to Florida, USA. Where four species of *Amblyomma* ticks, parasites of lizards and tortoises, were found infected with *E. ruminantium* or "Heartwater" disease and *Coxiella burnetti*, which produces Q fever (BURRIDGE et al., 2000). The genus *Coxiella* has been found to be a common symbiont of ticks (MACHADO-FERREIRA et al., 2011; ŠPITALSKÁ, et al., 2018).

Finally, other rickettsial agents of the spotted fever group have been detected in ticks that infest reptiles. In Australia, a novel *Rickettsia honei* was described infecting *Bothriocroton hydrosauri* (Denny, 1843) ticks (cited as *Aponomma hydrosauri*), that infest monitor lizards in the Flinder islands (STENOS et al., 2003; WHILEY et al., 2016). A similar *Rickettsia* to *Rickettsia anan* was detected in ticks from the species *Amblyomma exornatum* Koch, 1844, in varanid lizards imported to the USA (REEVES, 2006). In South America different species of *Rickettsia* have been detected linked to ticks that were infesting reptiles. For example, in the Colombian Caribbean, *Rickettsia* sp. strain Colombianensi was detected in *Amblyommma dissimile* Koch, 1844 ticks on Iguanas and other reptiles, as well as *Rickettsia belli* (MIRANDA et al., 2012; SANTODOMINGO et al., 2018). In Brazil, studies show the correlation between *R. belli* and species of ectothermic host-related ticks (*A. dissimile* and *Amblyomma rotundatum* Koch, 1844), which may be a symbiont of these parasites. Moreover, recent research found also *R. bellii* in both species of ticks, and *Rickettsia* sp. strain Colombianensi, *Hepatozoon*, and *Anaplasma* in *A. dissimile*, all these ticks from snakes of southeastern Brazil, and *Rickettsia* sp. strain Colombianensi in ticks from toads in the Brazilian amazon (OGRZEWALSKA, et al., 2018; LUZ et al., 2018).

Despite all this finding, the relation between ectoparasites, ectothermic hosts and the circulation of pathogenic agents is not fully known and understood, as well of the implications of these infections to the public human health in a one health concept.

1.4 Justification

The conception of this study comes from the need of an integrative research of the key elements of the epidemiological cycle, which in this specific context are the ectoparasites that infest reptiles and amphibians, the ectothermic hosts in Brazil, and the pathogenic agents that infect both Acari (mites and ticks) and their ectothermic hosts. These pathogens then can be transmitted to other animals and some even to humans. Furthermore, it is important also for this study to better understand the relationship between parasite-host-pathogen to elucidate the risks of zoonotic transmission and separate from symbiont and commensalism adaptations of the parasites and pathogens. Thus, it is significant to assess the effect the ectoparasites have in their hosts, and how the parasitic load affects their health status, welfare and response. Given all the above, despite recent efforts to identify and catalogue the mites and ticks of reptiles and amphibians of Brazil, there are still regions which have scarce information of the acari fauna. The northern, northeastern regions, and the Amazon, Caatinga and Pampas biomes have none to scarce records of mites and ticks infesting specifically reptiles and amphibians, given that almost all studies have been developed in the southeastern and southern regions. Furthermore, there is little information of the ectoparasites of amphibians and venomous reptiles, especially for Mesostigmata mites. Thus, there is still needed an extensive effort to describe, catalogue and revise new and known species of Acari, integrating morphology, taxonomy, and molecular biology. On the other hand, despite reptiles and amphibians are known to be natural reservoirs of some pathogens, there is almost no data of the different pathogens they can harbor in Brazil, and moreover of the implications of the natural infection, and if their ectoparasites have vectoral competence and capacity. For this reason, for this study, four pathogens (*Borrelia*, *Coxiella*, *Hepatozoon*, and *Rickettsia*) were picked to be assessed in the mites and ticks as well as in some hosts. Of all the possible pathogens, these were selected to assess vectoral competence and to difference between symbionts (*Coxiella*, *Hepatozoon*, some *Rickettsia*, reptile-associated *Borrelia*) and pathogenic agents. *Hepatozoon* was also chosen because it is widely found infecting reptiles and amphibians and can be a model of transmission from ectoparasites to hosts. In this way, updating the knowledge of the Acari fauna in reptiles and amphibians, and their associated pathogens. Considering their possible zoonotic potential, may aid future management plans, and epidemiological surveillance in the required areas, as well as support environmental and conservation efforts.

1.5 Thesis presentation

This thesis is divided in two parts. The first part is called “TAXONOMY AND MORPHOLOGY” and includes four chapters. Chapter 1 is named order Trombidiformes which provides morphological information of the mites of this order found in Brazil. It gives a catalogue of examined species, a taxonomical and morphological details of selected species of mites, and distribution of these parasites according to the findings of this study. It also includes an article published as follows: “MENDOZA-ROLDAN, J. A., BARROS-BATTESTI, D. M., BASSINI-SILVA, R. & JACINAVICIUS, F. C. A New Species of Pit Mite (Trombidiformes: Harpirhynchidae) from the South American Rattlesnake (Viperidae): Morphological and Molecular Analysis. **Entomol Ornithol Herpetol**, v. 6, n. 201, p. 2161-0983.1000201, 2017” (ATTACHMENT 1).

Chapter 2, order Mesostigmata gives morphological insights of the mites of this order found in of reptile and amphibians from Brazil. It provides a catalogue of examined species, a taxonomical and morphological details of selected and new species of mites and adds a distribution of these parasites according to the findings of this study.

Chapter 3, Order Ixodida, englobes taxonomic and morphological studies concerning both tick families (Ixodidae and Argasidae) of reptile and amphibians. It provides general information about these families, as well as data of examined species and new hosts and locations.

Chapter 4, host-parasite associations, focuses on the host-parasite relations. It provides information of the different examined hosts and presents insights about the parasitic load, the effect on the host, as well as new parasitic relations. It includes a published manuscript titled: “*Ixodes ricinus* infesting snakes: Insights from a new tick-host association in an endemic area for *Borrelia burgdorferi* sensu lato”, published in Acta Tropica, which provides new information about tick oral infestation in snakes (ATTACHMENT 2).

The second part of the thesis is called “MOLECULAR BIOLOGY” and it is divided in two chapters. Chapter 5, Phylogeny of Acari from reptiles and amphibians, provides information of the DNA extraction methods for the different types of Acari, assesses the molecular markers suiTable for molecular phylogeny of the different groups, and gives phylogenetic inferences using different models to elucidate the relations and clades of ectothermic host mites and ticks.

Chapter 6, molecular detection of associated pathogens discusses the detection of the selected pathogens (*Borrelia*, *Coxiella*, *Hepatozoon*, and *Rickettsia*) in mites and ticks, as well as blood and tissue samples of some of the collected hosts. This chapter provides molecular identification of *Hepatozoon* in mites and ticks and host tissues, and of *Rickettsia* in mites and ticks. It also includes a published manuscript titled: "MENDOZA-ROLDAN, J. A., COLELLA, V., LIA, R. P., NGUYEN, V. L., BARROS-BATTESTI, D. M., IATTA, R., ... & OTRANTO, D. *Borrelia burgdorferi* (sensu lato) in ectoparasites and reptiles in southern Italy. Parasites & vectors, v. 12, n. 1, p. 35, 2019", which was a parallel study, performed to compare borrelial agents in known regions with the Neotropical region (ATTACHMENT 3).

Finally, this thesis gives general conclusions to highlight the important findings, as well as their contribution to the acari (mites and ticks) of reptiles and amphibians. Thus, pointing the implications of mites and ticks` parasitism and association of pathogens with ectothermic animals.

2 OBJECTIVES

2.1 General

Identify mites (Trombidiformes and Mesostigmata) and ticks (Ixodida) that parasitize reptiles and amphibians in Brazil based on morphological and molecular studies and assess the impact of the ectoparasites on their hosts, as well as detect associated pathogens.

2.2 Specific

- PART I
  - Chapter 1, 2 and 3:
    - Assess the mites and ticks of reptiles and amphibians deposited in the acarological collection of the Instituto Butantan (IBSP), and in other reference collections;
    - Identify the species of mites and ticks found in reptiles and amphibians through optic and electronic scanning microscopy and genetic sequencing;
  - Chapter 4:
    - Asses the host-parasite relations and the impact of the parasitic load through the prevalence, mean intensity and abundance of the different species of mites and ticks related to their hosts.
- PART II
  - Chapter 5:
    - Asses the phylogenetic relationships of the mites and ticks associated to ectothermic hosts applying molecular phylogeny of selected molecular markers;
  - Chapter 6:
    - Detect the presence of selected pathogens (*Borrelia*, *Coxiella*, *Hepatozoon*, and *Rickettsia*) in the studied mites and ticks, and in the collected hosts using molecular biology.

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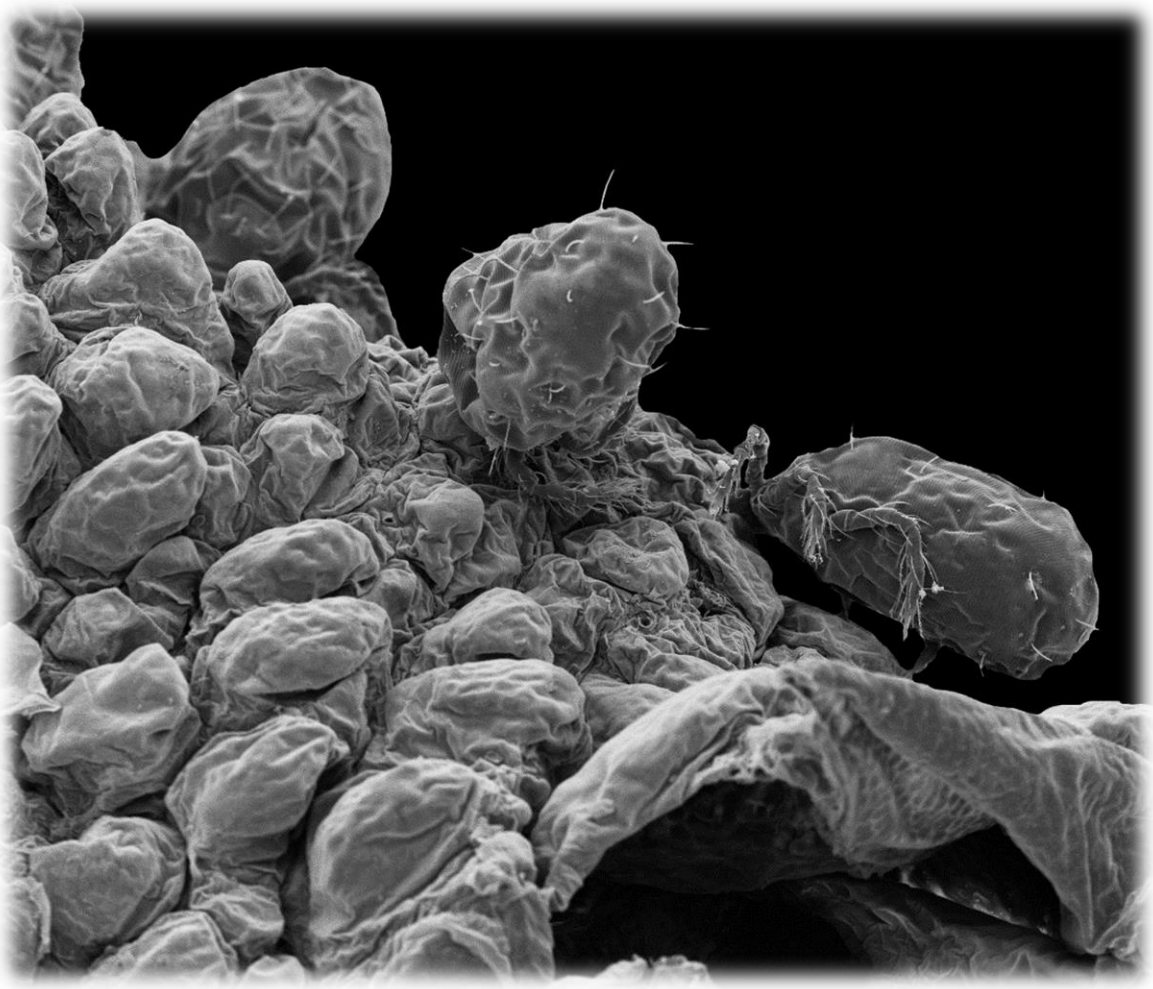
PART I:

TAXONOMY AND MORPHOLOGY



(Mendoza-Roldan, 2018)

CHAPTER I: Order Trombidiformes

1 INTRODUCTION

1.1 Trombidiformes mites of reptiles and amphibians

The order Trombidiformes belongs to the Acariformes superorder, which also includes mites of the order Sarcoptiformes (Oribatida and Endeostigmata). This superorder has more than 30, 000 described species, inhabiting almost all biomes and having a myriad type of behaviors (parasitic, predator, soil-based, herbivore, etc.) (LINDQUIST, 1984; KLIMOV et al, 2018). Moreover, Trombidiformes is the biggest and the most diverse order of mites, with more than 26,000 species described, englobed in 151 families and 2, 235 genera (REZENDE et al., 2012). Also, there are 24 fossil records for this order (ZHANG et al., 2011).

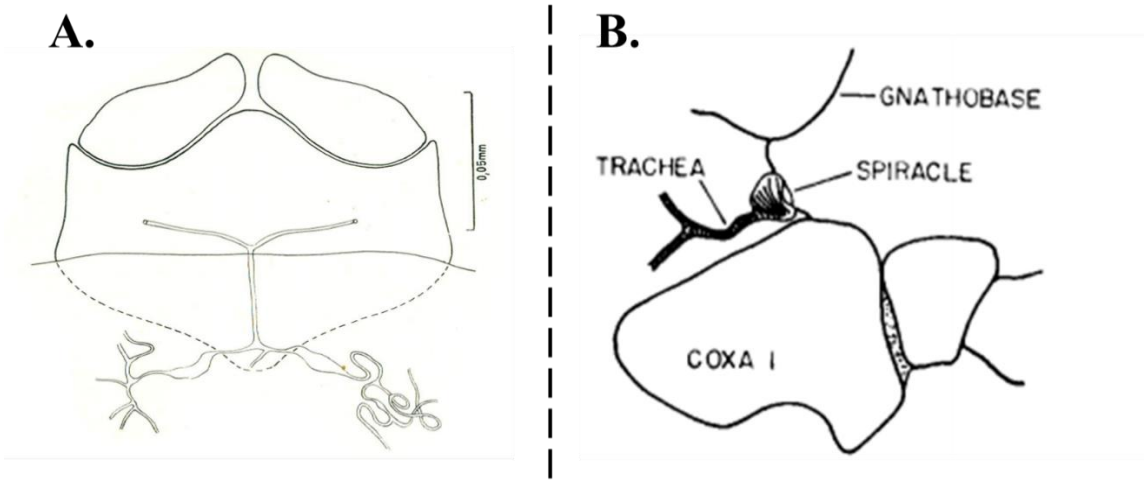
Given this number of species and diversity, this order has few synapomorphies. These are apomorphic homologous characters that are found in some or all terminal taxa of a given clade, that are shared from a common ancestor, for which it was an autapomorphic characteristic, meaning a unique apomorphy to that taxon (NOVICK; CATLEY, 2007). This lack of common features has proven to be challenging for taxonomists who work on this group`s systematics. Thus far, Trombidiformes is divided in two major groups: Sphaerolichida OConnor, 1984 and Prostigmata Kramer, 1877 (KRANTZ; WALTER, 2009; ZHANG et al., 2011). All of the mite parasites of reptile and amphibians are grouped in the suborder Prostigmata. Furthermore, Prostigmata is divided in three infraorders: Anystina, Eleutherengonides, and Eupodina. Acari from this latter group, with some exceptions, have common characteristics such as tracheal openings (stigmata and peritremes) (Figure 4) located in the anterior portion [gnathosoma (Figure 4A, or in between gnathosoma and first coxae (Figure 4B)]. Also, most of the group has styliform chelicerae, and well developed palpi. The idiosoma is generally poorly chitinized, bearing some type of scutum or plates, that can be ornamented. Some Prostigmata can have worm-like or vermiform shape, thus in general, all the Trombidiformes share a lack of primary segmentation (GRIFFITHS, BOWMAN, 1984; OCONNOR, 1984; BERTRAND, 2002).

Moreover, Trombidiformes can be distinguished (in their majority) from the Sacoptiformes order, because Trombidiformes mites have movable digits with hook or stylet-

shaped, cushioned empodium at the end of the legs, and mainly due to the absent stigmas and, and stigmatic opening located anteriorly to the dorsal prosoma.

Differently, Sarctoptiformes mites have chelated chelicerae as in ancestral mites, and mainly the empodium and the stigmas and peritremes are absent (LINDQUIST, 1996; OCONNOR, 2009).

Figure 4 - Stigmata (tracheal openings) and peritremes of Prostigmata



Source: (Adapted from: FAIN., 1964, and BRENNAN et al., 1976).

Legend: A) stigmata and peritremes located in the gnathosoma of an *Ophioptes* mite; B) stigmata (spiracle) and peritremes (trachea) located in between the gnathosoma and first coxae in a trombiculid mite.

Prostigmata mites, parasites of reptiles and amphibians, are grouped in seven families, that are distributed in the three main infraorders or super cohorts, thus having varied morphologies and life cycles and development. They are grouped as follows: super cohort Eleutherengonides (superfamily Cheyletoidea: **Cloacaridae**, **Harpirhynchidae**; superfamily Pterygosomatoidea: **Pterygosomatidae**), super cohort Anystina, cohort Parasitengona (superfamily Trombidioidea: **Leeuwenhoekiidae**, **Trombiculidae;** superfamily Hydryphantoidea: **Thermacaridae**), cohort Eupodina (superfamily Tydeoidea: **Ereynetidae**). All these families have been recorded in the neotropical region.

1.2 Super cohort Eleutherengonides (superfamily Cheyletoidea)

1.2.1 Cloacaridae family

The cloacaridae family encompasses two subfamilies: Cloacarinae and Pneumophaginae, with six genera and 15 species. The Pneumophaginae subfamily has one genus (*Pneumophagus*) with one species (*Pneumophagus bubonis* Fain & Smiley, 1989), that parasitized avian lungs, mainly *Bubo virginianus* (FAIN; SMILEY, 1989). Reptiles are parasitized by mites of the Cloacarinae subfamily, which belong to five genera of highly specialized endoparasites. These mites live and develop in the cloacal region of testudinate reptiles (turtles and tortoises) of both suborders (Cryptodira and Pleurodira), which would suggest an ancestral origin of the parasitism behavior (CAMIN et al., 1967; PENCE; WRIGHT, 1998). Probably, this monophyletic group originated, before the divergence of Pleurodira and Cryptodira, in the Jurassic or Cretacean periods (BOCHKOV; OCONNOR, 2008). This family has been recorded in the Nearctic region (FAIN, 1968; PENCE; CASTO, 1975) Palearctic, Australasian regions (FAIN, 1968), and in the Ethiopian region (FAIN, 1968; PENCE; CASTO, 1975; PENCE; WRIGHT, 1998; BOCHKOV; OCONNOR, 2008).

The genera that parasitize turtles and tortoises are: *Cloacarus* Camin & Singer (1967), *Caminacarus* Fain (1968), *Emyduracarus* Fain (1968), *Theodoracarus* Fain (1968) e *Chelonodacarus* Pence e Wright (1998), and it is believed that they are highly specialized venereal transmitted mites. Studies indicate that they reproduce parthenogenetically, and non-fertilized eggs develop in to males (arrhenotoky) (PENCE; CASTO, 1975; FAJFER, 2012). Species of *Cloacarus* and *Caminacarus* occur in the continental United States, and *Chelonacarus* is distributed in Panama in *Chelonia mydas* Linnaeus, 1758. There are no records of ocurrence of this family in South America (PENCE; WRIGHT, 1998; FAJFER, 2012).

1.2.1.1 Harpirhynchidae family

The Harpirhynchidae family includes three subfamilies (Harpirhynchinae, Harpypalpinae e Ophioptinae), and 14 genera widely distributed. These family of mites, are highly specialized, having monoxenous intradermal development, and feeding of tissue detritus and lymph.

(BOCHKOV, 2002). The first two mentioned subfamilies infest the epidermis and plumose follicle of birds. Meanwhile, the subfamily Ophioptinae, infest exclusively snakes. This subfamily groups two genera *Ophioptes* Sambon, 1928 and *Afrophioptes* Fain, 1962 (FAIN, 1964; 1965; BERON, 1974; LIZASO, 1981; BOCHKOV et al., 1999). Ophioptinae was considered a valid family (LOMBERT; MOSS 1983), until cladistic studies categorized it as a subfamily inside Harpirhynchidae (BOCHKOV et al., 1999).

The life cycle of the Ophioptinae, occurs mostly inside the skin of their host, thus these mites are usually called "pit mites", as they penetrate scales and skin. The life cycle includes eggs, larvae (apod stage), nymphs (with three stages, last two with developed legs), and adults. Mature stages are active, and dwell freely over the host, where they copulate. These mites do not have a direct connection of the midgut to the anal opening; thus, the debris is deposited as guanine inside the adult (FAIN, 1964; MENDOZA-ROLDAN et al., 2017).

Mites from the genus *Ophioptes* occur in the Neotropical, Palearctic, Australasian, and Ethiopian regions, and the *Afrophioptes* genus is restricted to the Ethiopian region (FAIN, 1964). In the Neotropical region, six species of *Ophioptes* occur. *Ophioptes dromicus* Allred 1958 was recorded in Cuba, and the remaining species have been described in South America (FAIN, 1964; LIZASO, 1980; MENDOZA-ROLDAN et al., 2017). Information regarding the Neotropical species published before the present study can be observed in the Table 1 and Figure 5.

Table 1 - Species of Ophioptinae mites of the Neotropical region, hosts and localities

No.		Holotype	Host	Locality	Reference
1	*Ophioptes parkeri* Sambon, 1928	Non-specified	*Erythrolamprus aesculapii* Linnaeus, 1758	Buenavista, Bolivia	Sambon (1928)
			Erythrolamprus poecilogyrus poecilogyrus (Wied-Neuwied, 1825) (cited as *Liophis poecilogyrus poecilogyrus*, sin.)	Norte, Paraguay	Fain (1964)
			E. poecilogyrus poecilogyrus (cited as *L. poecilogyrus poecilogyrus*, sin.)	Centro-Oeste, Brazil	Fain (1964)
			Lygophis anomalus (Günther, 1858)	Brazil	Fain (1964)
			Spilotes pullatus Linnaeus, 1758.	Bélem, Pará, Brazil	Lizaso (1981)
			E. poecilogyrus poecilogyrus (cited as *L. poecilogyrus poecilogyrus*, sin.)	Itumbara, Goiás, Brazil	Lizaso (1981)
			Xenodon merremii (Romano & Hoge, 1972) (cited as *Waglerophis merremiii*, sin.)	Uberlândia, Minas Gerais, Brazil	Lizaso (1981)
			E. poecilogyrus poecilogyrus (cited as *L. poecilogyrus poecilogyrus*, sin.)	Juiz de Fora, Minas Gerais, Brazil	Lizaso (1981)

(Continues)

No.	Holotype	Host	Locality	Reference	Holotype
	O. parkeri		*E. aesculapii*	Lambari, Minas Gerais, Brazil	Lizaso (1981)
			S. pullatus	Sapucaí, Minas Gerais, Brazil	Lizaso (1981)
			E. aesculapii	Três Corações, Minas Gerais, Brazil	Lizaso (1981)
			Leptodeira annulata annulata Linnaeus, 1758	Colatina, Espírito Santo, Brazil	Lizaso (1981)
			E. poecilogyrus poecilogyrus (cited as *L. poecilogyrus poecilogyrus*, sin.)	Presidente Venceslau, São Paulo, Brazil	Lizaso (1981)
			Chironius foveatus Bailey, 1955	Arujá, São Paulo, Brazil	Lizaso (1981)
			E. aesculapii	Biritiba-Mirim, São Paulo, Brazil	Lizaso (1981)
			E. aesculapii	Inúbia Paulista, São Paulo, Brazil	Lizaso (1981)
			X. merremii (cited as *Waglerophis merremiii*, sin.)	Jaú, São Paulo, Brazil	Lizaso (1981)
			X. merremii (Romano & Hoge) (cited as *Waglerophis merremiii*, sin.)	São Carlos, São Paulo, Brazil	Lizaso (1981)
			E. poecilogyrus poecilogyrus (cited as L. *poecilogyrus poecilogyrus*, sin.)	Pelotas, Rio Grande do Sul, Brazil	Lizaso (1981)

(Continues)

No.		Holotype	Host	Locality	Reference
	O. parkeri (cited as *Ophioptes oudemansi*, sin.)	B.M. Coll 19552.9.24-123	*Clelia rustica* (Cope, 1878)	Ajo, East, Argentina	Fain (1964)
2	*Ophioptes tropicalis* Ewing, 1933	USNM 1080 ♀	*Erpetodryas carinatus* Wagler, 1830 (cited as *Chironius carinatus* sin.)	British Guiana	Ewing (1933)
3	*Ophioptes longipilis* Lizaso, 1981	IBSP 6070 ♀	*Oxyrhopus trigeminus trigeminus* Duméril, Bibron & Duméril, 1854	Itú, São Paulo, Brazil	Lizaso (1981)
			O. trigeminus trigeminus	Guararapes, Pernambuco, Brazil	Lizaso (1981)
			O. trigeminus trigeminus	Itumbara, Goiás, Brazil	Lizaso (1981)
			E. poecilogyrus poecilogyrus (cited as *L. poecilogyrus poecilogyrus*, sin.)	Domingos Martins, Espírito Santo, Brazil	Lizaso (1981)
			O.trigeminus trigeminus	Itú, São Paulo, Brazil	Lizaso (1981)
			Oxyrhopus petola Lönnberg, 1896 (cited as *Oxyrhopus petolarius petolarius* sin.)	Foz do Areia, Paraná, Brazil	Lizaso (1981)
4	*Ophioptes brevipilis* Lizaso, 1981	IBSP 3627 ♀	*Chironius flavolineatus* (Jan, 1863)	Goiânia, Goiás, Brazil	Lizaso (1981)

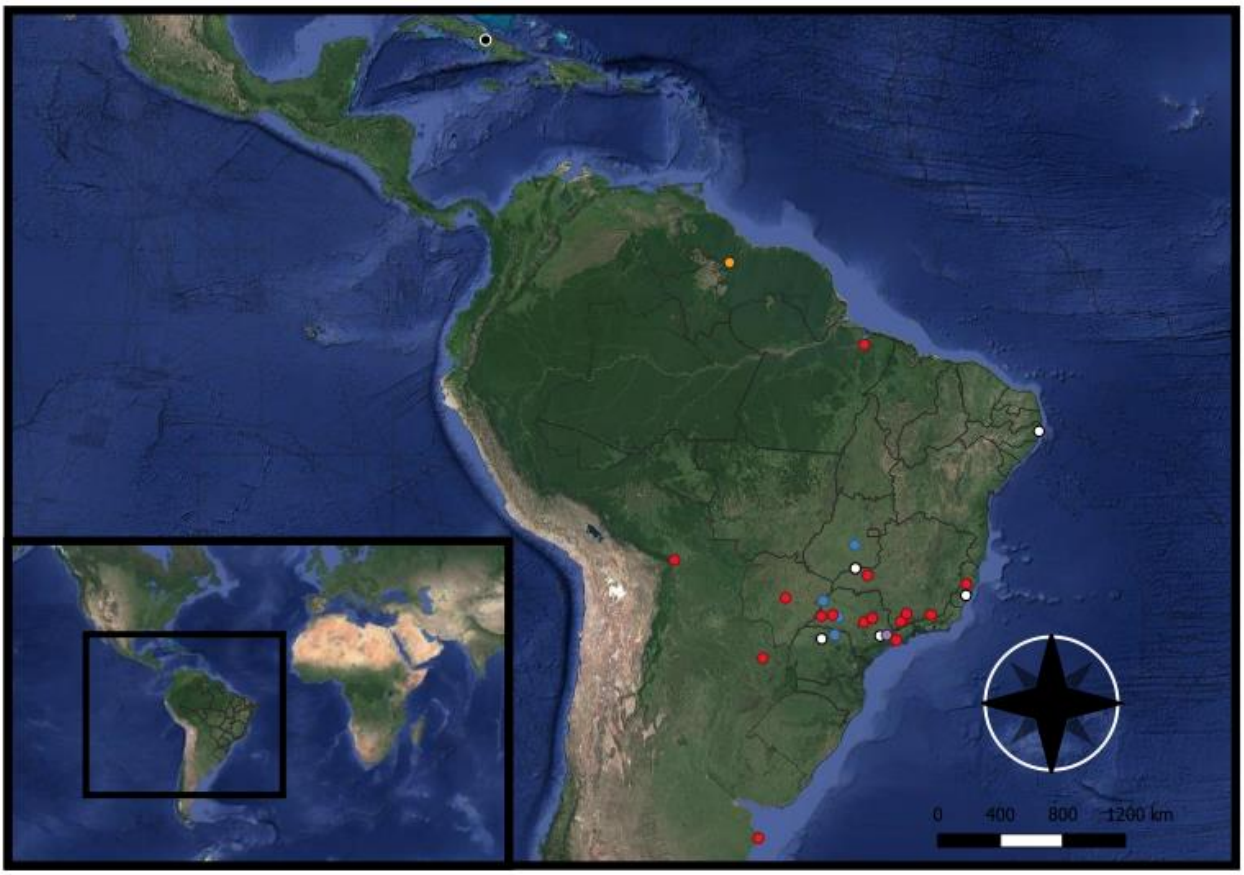
(Conclusion)

No.		Holotype	Host	Locality	Reference
	O. brevipilis		*Philodryas olfersii olfersii* (Lichtenstein, 1823)	Itumbara, Goiás, Brazil	Lizaso (1981)
			Mastigodryas bifossatus bifossatus (Raddi, 1820)	Três Lagoas, Mato Grosso Sul, Brazil	Lizaso (1981)
			E. poecilogyrus poecilogyrus (cited as *L. poecilogyrus poecilogyrus*, sin.)	Colatina, Espírito Santo, Brazil	Lizaso (1981)
			M. bifossatus bifossatus	Tupã, São Paulo, Brazil	Lizaso (1981)
			P. olfersii olfersii	Uraí, Paraná, Brazil	Lizaso (1981)
5	*Ophioptes ekans* Mendoza-Roldan & Barros-Battesti, 2017	IBSP 12078 ♀	*Crotalus durissus terrificus* (Laurenti, 1768)	Campo Limpo Paulista, São Paulo, Brazil	Mendoza-Roldan et al. (2017)
6	*Ophioptes dromicus* Allerd, 1958	USNM ♀	*Caraiba andreae* (Reinhardt & Lütken, 1862) (cited as *Dromicus andreae orientalis*)	Banes, Oriente Province, Cuba	Allerd (1958)

Source: (MENDOZA-ROLDAN, J. A., 2019)

Legend: IBSP (Acarological collection, of the Instituto Butantan, Special Zoological Collections Laboratory, São Paulo, Brazil), USNM (National Museum of Natural History, Smithsonian Institution, Washington, D.C. 20560, U.S.A), B.M (Arachnida department British Museum, United Kingdom).

Figure 5 – Distribution map of species of Ophioptinae



Source: (MENDOZA-ROLDAN, J. A., 2019).

Legend: (red circles) *Ophioptes parkeri*, (blue circles) *Ophioptes brevipilis*, (white circles) *Ophioptes longipilis*, (black circle) *Ophioptes dromicus*, (purple circle) *Ophioptes ekans*, (yellow circle) *Ophioptes tropicalis*.
Source: Literature cited in Table 1.

1.2.2 Super cohort Eleutherengonides (Superfamily Pterygosomatoidea)

1.2.2.1 Family Pterygosomatidae

This family includes ten genera and 182 species, with worldwide distribution (except Antarctica). Seven of those genera are parasitic mites of lizards, except for *Geckobia enigmatica* Bertrand and Pedrono, 2000, which parasitizes tortoises. The other two genera are monotypic and parasitize beetles and birds (BERTRAND; PEDRONO, 1999; PAREDES-LEON et al., 2012; FAJFER, 2015; 2018).

The genera *Bertrandiella*, *Pterygosoma*, *Geckobia*, *Geckobiella* , *Zanurobia* , *Ixoderma* , *Scaphothrix* , and *Tequisistlana*, are highly specialized, mono- or stenoxeous, of the suborder Sauria (lizards) (BERTRAND et al., 1999; PAREDES-LEON et al., 2012). It is likely that this parasitism originated from the feeding habits of reptiles, which ate arthropods that were the original hosts of the family (BOCHKOV; OCONNOR, 2006).

As mentioned, the whole life cycle occurs on the host, and most of the species are parthenogenetic. Mites develop fixated to the connective tissue under the scales. Depending on the degree of adaptation, morphology varies. Highly adapted mites have bodies shaped as the scales of their hosts, as for example *Geckobia* mites (BERTRAND, 2002; BERTRAND; MODRÝ, 2004).

In the Neotropical region, there are five recorded genera: *Bertrandiella*, *Geckobia*, *Geckobiella*, *Pterygosoma*, and *Tequisistlana* (DE LA CRUZ et al., 2004; FAJFER, 2012; 2015; PAREDES-LEON et al., 2012). *Geckobiella* is distributed from North America to Brazil, and *Tequisistlana* is restricted to Mexico (PAREDES-LEON et al., 2012; FAJFER, 2012). *Bertrandiella* has one species recorded in Colombia, parasitizing Sphaerodactylidae lizards, and one species in Peru, parasitizing Phyllodactylidae geckos (PAREDES-LEON et al., 2012; QUIROZ-GUTIÉRREZ et al., 2015).

Among the family Pterygosomatidae, the genus *Geckobia* has the most diversity and taxonomic complexity, with 73 described species. These species are divided in four species groups based on leg chaetotaxy features (FAJFER, 2015; 2018). Still, due to morphological intricacy and succinct descriptions most of the species have not been included in those groups.

Species of *Geckobia* parasitize lizards from the families Gekkonidae, Phyllodactylidae, Carphodactylidae, Diplodactylidae, Eublepharidae, and Liolaemidae (FAJFER, 2012; 2015; 2018). Mites of this genus have a prodorsal scutum or scutum, eyes, exposed peritreme and coxae with thorns. Four species are registered in South America (FAJFER, 2015). Of those species, one was introduced from Africa with its host (*H. mabouia*) and is currently widespread throughout the American continent, and one is endemic from Peru parasitizing Phyllodactylidae geckos (RIVERA et al., 2003; QUIROZ-GUTIÉRREZ et al., 2015). The genus *Geckobiella* has one species *Geckobiella harrisi* Davidson, 1958 registered in South America, infesting Tropiduridae lizards in Brazil (PAREDES-LEON et al., 2012). Finally, the genus *Pterygosoma* includes 56 species, of which six are described from South America (BERTRAND et al., 1999; FAJFER; GONZÁLES-ACUÑA, 2013). Detailed information of the species that occur in South America, that parasitize reptiles, can be observed in Table 2, with genera distribution in Figure 6.

1.2.3 Super cohort Anystina, cohort Parasitengona (superfamily Trombidioidea)

1.2.3.1 Families Leeuwenhoekiidae, Trombiculidae (chigger mites)

The families Leeuwenhoekiidae a Trombiculidae, although are considered separate group, share morphological and life-cycle similarities, thus both families are called chigger mites, among other names. The main morphological difference is the segmentation of the legs. Trombiculidae have 7-7-7 or 7-6-6 leg segmentation, and Leeuwenhoekiidae have 6-6-6, except for the genus *Comatacarus* Ewing, 1942, which has 7-6-6 leg segmentation (KOLEBINOVA, 1992). Due to the mentioned similarities, both families are discussed together.

Table 2 - Species of Pterygosomatidae recorded in South America, with host and localities information

No.	Species	Holotype	Host	Locality	Reference
1	*Geckobia aureae* Quiroz-Gutiérrez, Paredes-León, Roldán-Rodríguez y Pérez, 2015	CNAC007250	*Phyllodactylus microphyllus* Cope, 1876	Cerro Campana, Trujillo, Perú.	Quiroz-Gutiérrez, Paredes-León, Roldán-Rodríguez y Pérez, 2015
2	*Geckobia hemidactyli* Lawrence, 1936	Iziko Museum, Cape Town	*Hemidactylus tasmani* Hewitt	Zimbawe	Lawrence (1936)
			Hemidactylus mabouia (Moreau De Jonnès, 1818)	Leticia, Colombia	Martínez-Rivera (2003)
			H. mabouia	Brazil	Martínez-Rivera (2003)
3	*Geckobia nitidus* Fajfer, 2015	ZISP (Reg. No. ZISP AVB 14-2710-001) ♀	*Liolaemus nitidus* Wiegmann	Pan de Azucar National Park, Chile	Fajfer (2015)
4	*Geckobia zapallarensis* Fajfer, 2015		*Liolaemus zapallarensis zapallarensis* Müller & Hellmich	Llanos de Challe, Chile	Fajfer (2015)
5	*Geckobia gerrhopygus* Fajfer, 2015		*Phyllodactylus gerrhopygus* Wiegmann	Santa Maria Island, Chile	Fajfer (2015)
6	*Geckobiella harrisi* Davidson, 1958	USNM 1860, ♀	*Plica plica* Linnaeus	Santarem, Pará, Brazil	Davidson (1958)
7	*Pterygosoma patagonica* Dittmar de la Cruz, Morando & Avila, 2004	(Lost Holotype)	*Liolaemus buergeri* Werner	Paso de Indios, Chubut, Argentina	Dittmar de la Cruz et al. (2004)
			Liolaemus bibroni Bell	Paso de Indios, Chubut, Argentina	Dittmar de la Cruz et al. (2004)
			Liolaemus petrophilus Donoso-Barros & Clei	Paso de Indios, Chubut, Argentina	Dittmar de la Cruz et al. (2004)
			Liolaemus austromendocinus Cei	Catamarca, Argentina	Dittmar de la Cruz et al. (2004)
			Liolaemus elongatus Koslowsky	Catamarca, Argentina	Dittmar de la Cruz et al. (2004)
			Liolaemus gracilis Bell	Catamarca, Argentina	Dittmar de la Cruz et al. (2004)
			Liolaemus rothi Koslowsky	Telsen, Chubut, Argentina	Dittmar de la Cruz et al. (2004)

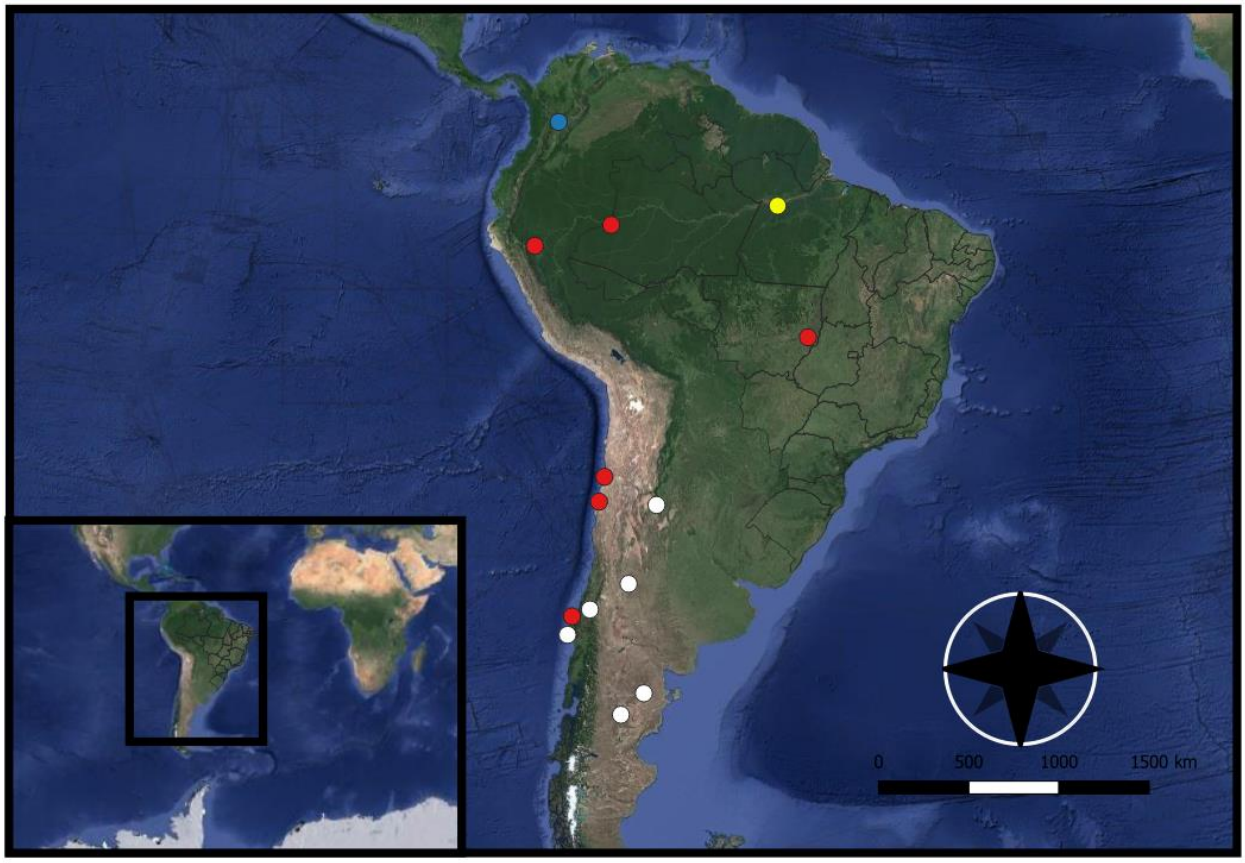
(Conclusion)

No.	Species	Holotype	Host	Locality	Reference
7			*L. austromendocinus*	San Rafael, Mendoza, Argentina	Fajfer (2014)
8	*Pterygosoma* (*Pterygosoma*) *ligare* Fajfer & González-Acuña, 2013	ZISP T-Pt-4	*Liolaemus pictus* (Duméril &Bibron)	Isla Mocha, Tirúa, Arauco, Chile	Fajfer & Gonzáles-Acuña (2013)
9	*Pterygosoma* (*Pterygosoma*) *formosus* Fajfer & González-Acuña, 2013	ZISP T-Pt-5	*L. pictus*	Isla Mocha, Tirúa, Arauco, Chile	Fajfer & Gonzáles-Acuña (2013)
10	*Pterygosoma* (*Pterygosoma*) *ovata* Fajfer & González-Acuña, 2013	ZISP T-Pt-6	*L. pictus*	Isla Mocha, Tirúa, Arauco, Chile	Fajfer & Gonzáles-Acuña (2013)
11	*Pterygosoma* (*Pterygosoma*) *levissima* Fajfer & González-Acuña, 2013	ZISP T-Pt-7	*L. pictus*	Isla Mocha, Tirúa, Arauco, Chile	Fajfer & Gonzáles-Acuña (2013)
12	*Pterygosoma* (*Pterygosoma*) *chilensis* Fajfer & González-Acuña, 2013	ZISP T-Pt-8	*Liolaemus chilensis* (Lesson)	Rio Ñuble, Chile	Fajfer & Gonzáles-Acuña (2013)
13	*Pterygosoma* (*Pterygosoma*) *cyanogasteri* Fajfer & González-Acuña, 2013	ZMUC (ZMUC-R37901)	*Liolaemus cyanogaster* (Duméril & Bibron)	Chile	Fajfer & Gonzáles-Acuña (2013)
14	*Bertrandiella tenuipes* (Hirst, 1917)	(BM(NH) Deutonymph	*Gonatodes albogularis* (Duméril & Bibrón)	Honda, Magdalene River, Colombia	Hirst 1917
15	*Bertrandiella campanensis* Quiroz-Gutiérrez, Paredes-León, Roldán-Rodríguez,& Pérez, 2015	CNAC (007251-007254	*Phyllodactylus microphyllus* Cope, 1876	Cerro Campana, Trujillo, Perú.	Quiroz-Gutiérrez, Paredes-León, Roldán-Rodríguez y Pérez, 2015

Source: (MENDOZA-ROLDAN, J. A., 2019).

Legend: BM(NH) (The Natural History Museum (formerly British Museum (Natural History), London, United Kingdom), CNAC (Colección Nacional de Ácaros del Instituto de Biología de la Universidad Nacional Autónoma de México en México, Distrito Federal), ZISP (Zoological Institute of the Russian Academy of Sciences, St. Petersburg, Russia), USNM (National Museum of Natural History, Smithsonian Institution, Washington, D.C. 20560, U.S.A), ZMUC (Zoological Museum, University of Copenhagen, Denmark).

Figure 6 – Distribution map of species of genera of Pterygosomatidae



Source: (MENDOZA-ROLDAN, J. A., 2019).

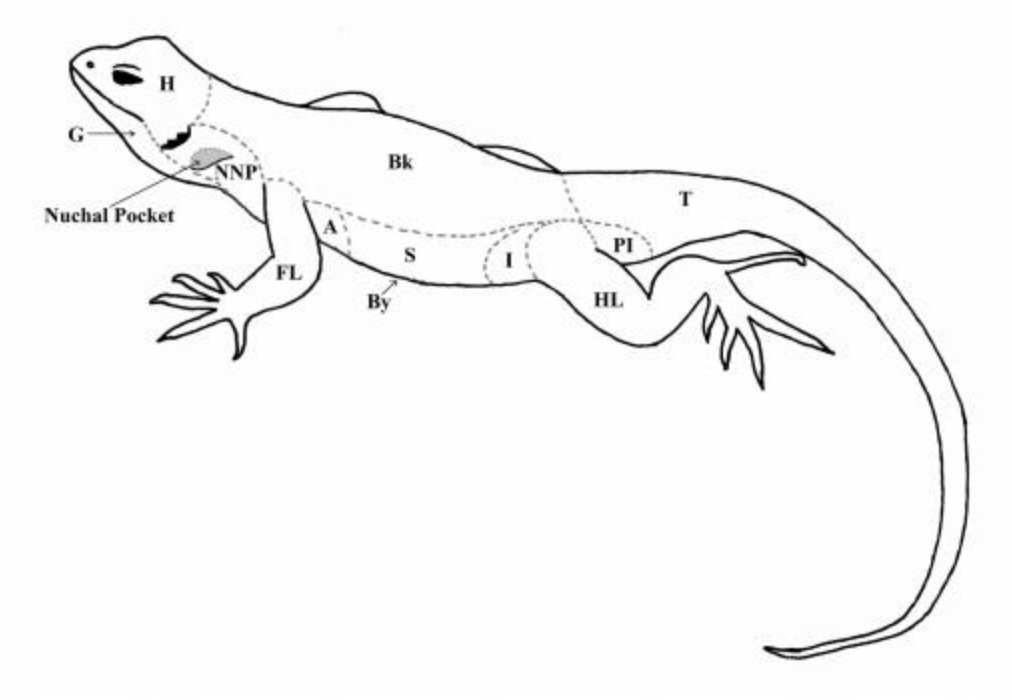
Legend: (blue circle) *Bertrandiella* genus, (red circles) *Geckobia* genus, (yellow circle) *Geckobiella* genus, (white circles) *Pterygosoma* genus.
Source: Literature cited in Table 2.

Mites from these families have parasitic behavior in the larval stage, and infest arthropods and vertebrates, including reptiles and amphibians. For most of the described species, only the larval stage is known, and to date more than 3,000 species have been described. The other life stages (nymphs and adults) are free living predators (WHARTON; FULLER 1952; BRENNAN; GOFF 1977; FAJFER, 2012; JACINAVICIUS et al., 2018).

As other Parasitengona mites, chiggers from the Trombiculidae family have low host specificity and rather infest the different possible hosts in a given environment (nests, urbanized areas, forested areas, etc.) (O'CALLAGHAN et al., 1994). Specifically, in ectothermic hosts, these mites can be found fixated in soft tissues of the skin and depending of the association of the mite-

hosts, some animals have developed microhabitats, also called “mite pockets”, that can be from cavities to folds of the skins (located generally in the armpit, groin and neck regions) (Figure 7). These distinct morphological adaptations are common in lizards and some anuran amphibians (AUDY, 1954; ARNOLD, 1986; 1993; REED, 2014). In other cases, trombiculid mites fixate in between the scales, which also protects them from falling off.

Figure 7 - Division of body regions, microhabitats and “mite pokets” on the saurian host



Source: (Adaptaded from REED, 2014).

Legend: **A** – axial; **Bk** – back; **By** – belly; **FL** – forelimb; **G** – gular; **H** – head; **HL** – hindlimb; **I** – inguinal; **NNP** – nuchal non-pocket; **PI** – post-inguinal; **S** – side; **T** – tail. In all Sceloporus the nuchal pocket occupies the central nuchal region roughly midway between ear and shoulder (grey).

Mites from the Trombiculidae family are generally ectoparasites, though some exceptions exist. Moreover, the genera *Vatacarus* and *Iguanacarus* are endopatasitic mites of the respiratory tract of marine snakes (*Laticauda* sp.), and iguana (*Amblyrhynchus* sp.) (NADCHATRAM, 1980; 2006). Larvae of this family can have deleterious effects on their hosts, producing dermatitis,

immune response, anemia, dehydration and dysecdysis (GOLDBERG; BURSEY, 1991; BRENNAN et al., 2008).

In the Americas the following genera of Trombiculidae that infest reptiles and amphibians are registered: *Paratrombicula* Goff & Whitaker (1984); *Hyponeocula* Vercammen-Grandjean (1967); *Parasecia* Loomis (1966); *Neotrombicula* Hirst (1925); *Vatacarus* Southcott (1957)*; Fonsecia* Radford (1942); *Kayella* Vercammen-Grandjean, (1960); *Microtrombicula* Ewing (1950); Hexidionis Vercammen-Grandjean (1967); and *Vercammenia* Audy & Nadchatram, 1957 (BRENNAN; GOFF 1977; JACINAVICIUS et al., 2018). In South America, the Family is represented by: *Paratrombicula* (five species), *Parasecia* (12 species), *Eutrombicula* (80 species), *Vatacarus* (one species), *Iguanacarus* (five species), *Fonsecia* (seven species), and *Vercammenia* (one species). Data of distribution of these species is encompassed in the Table 3 and Figure 8.

On the other hand, mites from the family Leeuwenhoekiidae that parasitize reptiles and amphibians in the Neotropical are distributed in four genera: *Hannemania* Oudemans (1911); *Morelacarus* Vercammen-Grandjean (1974); *Acamatacarus* Ewing (1942) and *Odontacarus* Ewing (1929). In South America, only *Hannemania* occurs. This genus has 27 valid species, 26 occurring in America and one in Oceania. Still, the status of 11 of these species is uncertain due to succinct descriptions and most of the type material is lost (SILVA-DE LA FUENTE et al., 2016). In South America 13 species were registered and 11 of them have poor original descriptions or no type material, difficulty taxonomical studies and new species description. Moreover, this genus is composed by highly specific and specialized intradermic mites. Larvae penetrate the skin and develop inside a capsule. the mite feeds of the debris and lysates of skin tissue, and it can remain for weeks and even months inside the capsule. This capsular process produces and inflammatory response, that can result in cysts, pustules, and limb loss due to avascular necrosis (WOHLTMANN; KOHLER; MATIN, 2006; XUE; ZHANG, 2008). Species distribution of the South American species of *Hannemania* is shown in Table 4 and Figure 9.

Table 3 – Species of Trombiculidae mites distributed in South America, with hosts and localities

No.	Species	Holotype	Host	Locality	Reference
1	*Parasecia manueli* Brennan & Jones, 1960	Lost	Reptiles	Peru	Brennan (1969)
			Reptiles	Colombia	Brennan (1969)
2	*Parasecia longicalcar* Brennan & Jones, 1960	Lost	Snakes	Trinidad and Tobago	Brennan (1969)
3	*Paratrombicula chillensis* Stekolnikov & González-Acuña, 2012	ZISP no. 7728, T-Tr.-54	*Liolaemus chillanensis* Muller & Hellmich	Chillán Mts, Biobío, Chile	Stekolnikov & González-Acuña (2012)
4	*Paratrombicula goffi* Stekolnikov & González-Acuña, 2012	ZISP no. 7696, T-Tr.-55	*Liolaemus lemniscatus* Gravenhorst	Bellavista, O'Higgins, Chile	Stekolnikov & González-Acuña (2012)
			Liolaemus chillanensis Muller & Hellmich	Shangri-la, Chile	Stekolnikov & González-Acuña (2012)
5	*Vatacarus ipoides* Southcott, 1957	NMHH	*Laticauda* sp.	Galápagos, Ecuador	Southcott (1957)
6	*Iguanacarus alexfaini* Nadchatram, 1980	NMHH	*Amblyrhynchus* sp.	Galápagos, Ecuador	Nadchatram (1980)
7	*Iguanacarus amblyrhynchus* Vercammen-Grandjean, 1965	NMHH	*Amblyrhynchus* sp.	Galápagos, Ecuador	Vercammen-Grandjean (1965)
8	*Iguanacarus amersoni* (Brennan, 1965) (cited as *Blankaartia amersoni*, sin.)	NMHH	*Amblyrhynchus* sp.	Galápagos, Ecuador	Brennan (1965) Syn. Nadchatram (1980)

(Continues)

No.	Species	Holotype	Host	Locality	Reference
9	*Iguanacarus danieli* Dusbabek & Cerny, 1970 Syn. Nadchatram 1980	PU CSAV Acarol.Coll. N – 1642	*Amblyrhynchus* sp.	Galápagos, Ecuador	Dusbabek & Cerny (1970) sin. Nadchatram (1980)
10	*Fonsecia ewingi* Fonseca, 1932	Cótipos IBSP 27	*X. merremii* (Romano & Hoge) (citedas *Ophis merremiii*, sin.)	Correntes, Mato Grosso, Brazil	Brennan & Loomis (1959)
		IBSP 12071	*Rhinella ornata* Spix	Sete Barras, São Paulo, Brazil	Mendoza-Roldan (2015)
11	*Fonsecia travassosi* Fonseca, 1936	IBSP 30	*Spilotes pullatus* L.	Angra dos Reis, Rio de Janeiro, Brazil	Brennan & Loomis (1959)
12	*Fonsecia lachesis* Brennan, 1974	RML no. 50193	*Lachesis muta* Schinz	Guayaramerin, Rio Mamore, Beni, Bolivia	Brennan (1970)
13	*Fonsecia ophidica* (Fonseca, 1932)	IBSP 86	*X. merremii* (Romano & Hoge) (cited as *Ophis merremiii*, sin.)	Promissão, São Paulo, Brazil	Radford (1942)
			X. merremii (Romano & Hoge) (cited as *Ophis merremiii*, sin.)	Matão, São Paulo, Brazil	Radford (1942)
14	*Eutrombicula butantanensis* Fonseca, 1932	IBSP 83	*Homo sapiens* L.	Instituto Butantan, São Paulo, Brazil	Radford (1942)

(Continues)

No.	Species	Holotype	Host	Locality	Reference
	E. butantanensis	IBSP 83	*X. merremii* (Romano & Hoge) (cited as *Ophis merremiii*, sin.)	Instituto Butantan, São Paulo, Brazil	Brennan & Reed (1974)
15	*Eutrombicula batatas* Linnaeus, 1758	NMHH	Lagartos	Merida, Venezuela	Brennan & Reed (1974)
16	*Eutrombicula goeldii* Oudemans, 1910	Desconhecido	Lagartos	Amazonas, Venezuela	Brennan & Reed (1974)
17	*Eutrombicula tropica* Ewing, 1925	Desconhecido	Lagartos	Carabobo, Venezuela	Brennan & Reed (1974)
18	*Eutrombicula chillanensis* Stekolnikov & González-Acuña, 2010	ZISP 7711, T-Tr.-41	*Liolaemus chillanensis* Müller et Hellmich	Chillán, Chile	Stekolnikov & González-Acuña (2010)
29	*Eutrombicula araucanensis* Stekolnikov & González-Acuña, 2010	ZISP 685, T-Tr.-42	*Liolaemus pictus* Dumeril & Bibron	Mocha island, Chile	Stekolnikov & González-Acuña (2010)
20	*Eutrombicula liolaemi* Stekolnikov & González-Acuña, 2010	ZISP 7717, T-Tr.-43	*L. chillanensis*	Chillán, Chile	Stekolnikov & González-Acuña (2010)
21	*Eutrombicula paula* Stekolnikov & González-Acuña, 2010	ZISP 7694, T-Tr.-44	*Liolaemus monticola* Müller & Hellmich	Bellavista, Chile	Stekolnikov & González-Acuña (2010)
22	*Eutrombicula alfreddugesi* Oudemans, 1910	RM	Lagartos	Venezuela	Brennan & Reed (1974)
			Tropidurus torquatus Wied	Jurubatiba, Rio de Janeiro, Brazil	Cunha-Barros et al. (2003)

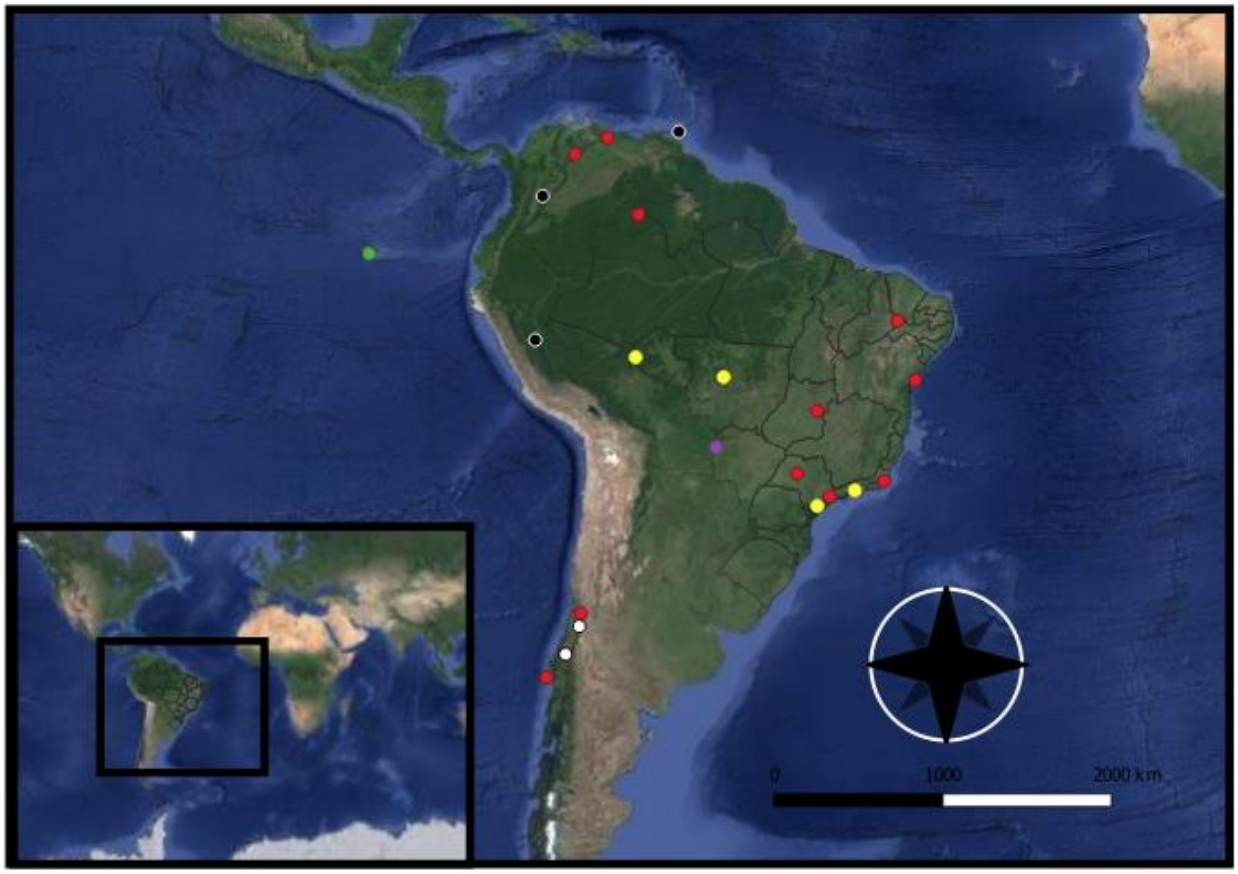
(Conclusion)

No.	Species	Holotype	Host	Locality	Reference
22	*E. alfreddugesi*	RM	*Ameivula littoralis* (Rocha, Araújo, Vrcibradic & Costa) (cited as *C. littoralis*, sin.)	Jurubatiba, Rio de Janeiro, Brazil	Cunha-Barros et al. (2003)
			Mabuya agilis (Raddi)	Jurubatiba, Rio de Janeiro, Brazil	Cunha-Barros et al. (2003)
			Psychosaura macrorhyncha (Hoge, 1946) (cited as *Mabuya macrorhyncha*, sin.)	Jurubatiba, Rio de Janeiro, Brazil	Cunha-Barros et al. (2003)
			Tropidurus hispidus (Spix)	Chapada do Araripe, Ceará, Brazil	Delfino et al. (2011)
			Tropidurus cocorobensis Rodrigues *Tropidurus erythocephalus* Rodrigues *Tropidurus semitaeniatus* Spix *T. hispidus*	Morro do Chapéu, Bahia, Brazil	Menezes et al. (2011)
			T. torquatus *Copeoglossum nigropunctatum* (Spix) (cited as *Mabuya agilis*, sin.) *P. macrorhyncha*(cited as *Mabuya macrorhyncha*, sin.) *A. littoralis* (Rocha, Araújo, Vrcibradic & Costa) (cited as *C. littoralis*, sin.)	Brasília, Brazil	De Carvalho et al. (2006)
			Mabuya (two species)	Barra de Maricá, Rio de Janeiro, Brazil	Cunha-Barros et al. (2003)
23	*Vercammenia yorkei* (Sambon, 1928):	NHMUK n° 147-9	*Scinax funereus* Cope	Urucum, Mato Grosso do Sul Brazil	Sambon, (1928)

Source: (MENDOZA-ROLDAN, J. A., 2019)

Legend: RM (Rijks Museum, Leiden, Holland), RML (Rocky Mountain Laboratories, Hamilton, Montana, USA), NMNH (National Museum of Natural History, Smithsonian Institution, Washington, D.C. 20560, U.S.A), ZISP (Acarological collection of the Zoological Institute of the Russian Academy of Sciences, St. Petersburg, Russia), PU CSAV (Institute of Parasitology Academy of Sciences of the Czech Republic, Czech Republic), IBSP (Acarological collection, of the Instituto Butantan, Special Zoological Collections Laboratory, São Paulo, Brazil), NHMUK (United Kingdom, London, The *Natural History Museum* [formerly British Museum (Natural History)].

Figure 8 – Distribution map of species of genera of Trombiculidae



Source: (MENDOZA-ROLDAN, J. A., 2019).

Legend: (Red circles) *Eutrombicula* genus, (black circules) *Parasecia* genus, (yellow circle) *Fonsecia* genus, (green circle) *Vatacarus* and *Iguanacarus* genera, (white circles) *Paratrombicula* genus, (purple circle) *Vercammenia* genus.
Source: Literature cited in Table 3.

Table 4 – Species of *Hannemania* (Leeuwenhoekiidae), distributed in South America

No.	Species	Holotype	Host	Locality	Reference
1	*Hannemania hepatica* Fonseca, 1935	IBSP 31	*Leptodactylus ocellatus* Girard *Leptodactylus latrans*	Butantã, São Paulo, Brazil	Fonseca (1935)
		IBSP 12076	*Physalaemus spiniger* Miranda-Ribeiro	Sete Barras, São Paulo, Brazil	Mendoza-Roldan (2015)
2	*Hannemania minor* Alzuet & Mauri, 1985	FCNyM UP	*L. ocellatus*	Buenos Aires, Benavídez, Argentina	Alzuet & Mauri (1985)
		IBSP 12065	*L. latrans*	Sete Barras, São Paulo, Brazil	Mendoza-Roldan (2015)
3	*Hannemania achalai* Alzuet & Mauri, 1987	FCNyM UP	*Pleurodema kriegi* Müller	Córdoba, Pampa de Achala, Argentina	Alzuet & Mauri (19857)
			Odontophrynus occidentalis Berg	Córdoba, Pampa de Achala, Argentina	Alzuet & Mauri (1987)
4	*Hannemania edwardsi* Sambon, 1928	Unknown	*Bufo variegatus* (Günther) (cited as *Nannophryne variegate*, sin.)	Lago Nahuel Huapi, Puerto Blest, Argentina	Sambon (1928)
5	*Hannemania dayi* Sambon, 1928	Unknown	*Pleurodenna hufonina* Schudi	Rio Negro, Argentina	Sambon (1928)
6	*Hannemania hylodeus* Oudemans, 1910	Unknown	*Hylodes* sp.	Brazil	Oudemans (1911)

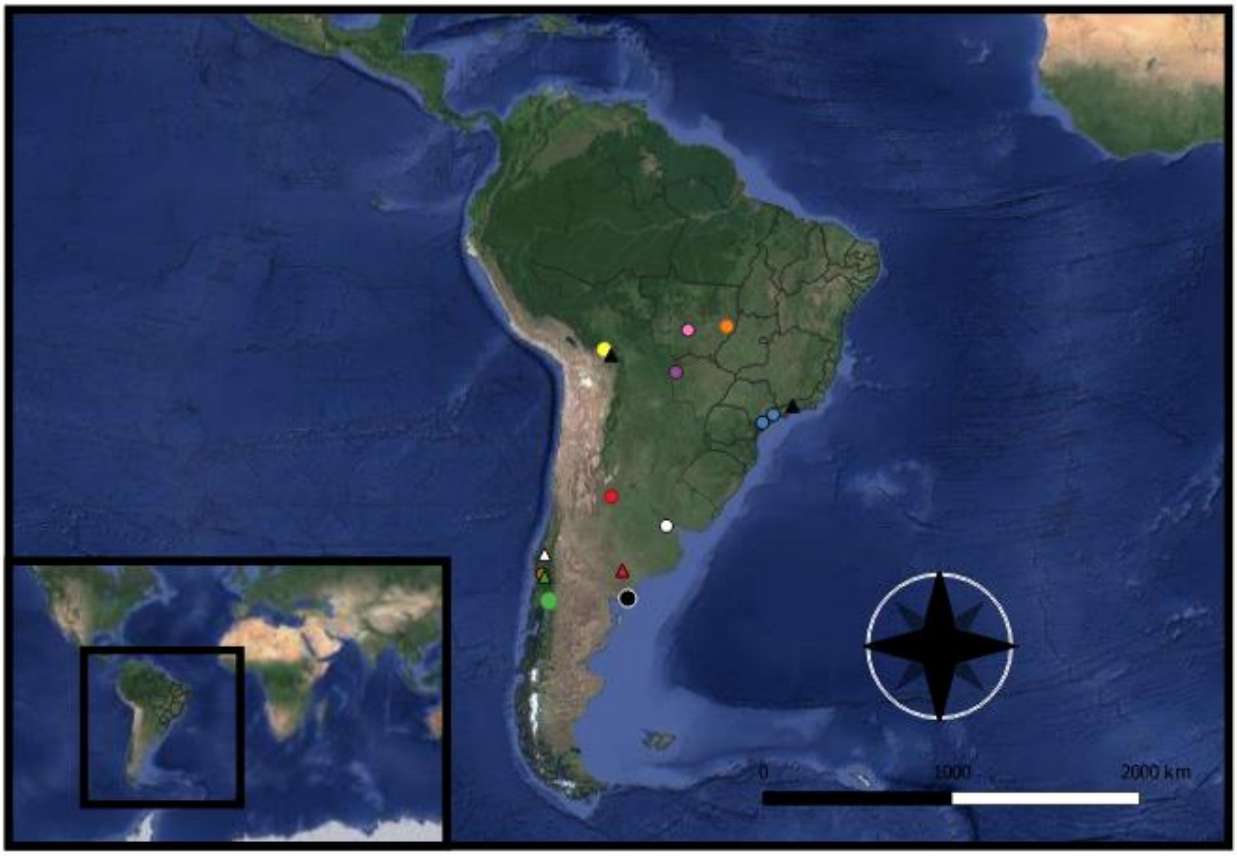
(Conclusion)

No.	Species	Holotype	Host	Locality	Reference
7	*Hannemania newsteadi* Sambon, 1928	Unknown	*Hyla rubra* Laurenti	Urucum, Mato Grosso, Brazil	Sambon (1928)
8	*Hannemania pattoni* Sambon, 1928	Unknown	*Barhorocoetes taeniatus*	Temuco, Chile	Sambon (1928)
9	*Hannemania. samboni* Ewing, 1931 (cited as *Hannemania argentina* Sambon, 1928, non *Hannemania argentina* Lahille, 1927)	FCNyM UP	*Pleurodema bibroni* Tshudi	Rio Negro, Argentina	Alzuet & Mauri (1985)
10	*Hannemania stephensi* Sambon, 1928	Unknown	*Eleutherodactylus gohlneri*	Tombador, Mato Grosso, Brazil	Sambon (1928)
11	*Hannemania yungicola* Wohltmann & Köhler, 2006	ZMH A7/05	*Yunganastes bisignatus* (Stejeneger) (cited as *Eleutherodactylus gollmeri*, sin.)	Cochabamba, Carrasco, Bolivia	Wohltmann & Köhler (2006)
		IBSP 12049	*Fritziana fissilis* Miranda-Ribeiro	São Jose do Barreiro, São Paulo, Brazil	Mendoza-Roldan (2015)
12	*Hannemania chaparensis* Wohltmann & Köhler, 2006	ZMH A5/05	*Rhinella quechua* (Gallardo, 1961) (cited as *Bufo quechua*, sin.)	Paractito, Cochabamba, Chapare, Bolivia	Wohltmann & Köhler (2006)
13	*Hannemania argentina* Lahille, 1927	Unknown	Anuros	Argentina	Lahille (1927)
14	*Hannemania* sp.		*Hylodes phyllodes* Heyer & Cocroft	Ilha Grande, Rio de Janeiro, Brazil	Attademo et al. (2012)
15	*Hannemania ortizi* Silva–de la Fuente, Moreno-Salas & Castro-Carrasco, 2016	MZUC 44557	*Pleurodema thaul* Ortiz, Ibarra-Vidal & Formas	Araucanía, Chile	Silva-De la Fuente et al. (2016)
16	*Hannemania gonzaleacunae* Silva–de la Fuente, Moreno-Salas & Castro-Carrasco, 2016	MZUC 44561	*Eupsophus nahuelbutensis* Ortiz, Ibarra-Vidal & Formas	Biobío, Chile	Silva-De la Fuente et al. (2016)

Source: (MENDOZA-ROLDAN, J. A., 2019)

Legend: IBSP (Coleção Acarológica Instituto Butantan, Laboratório Especial de Coleções Zoológicas, Instituto Butantan, São Paulo, Brazil), FCNyN UP (Facultad de ciências naturales y Museo de la Universidad de la Plata, Argentina), ZMH (Zoologisches Institut und Zoologisches Museum der Universitat, Hamburg, Germany), MZUC (Museo de Zoología, Universidad de Concepción, Concepción, Chile).

Figure 9 – Distribution map of species of *Hannemania* in South America



Source: (MENDOZA-ROLDAN, J. A., 2019).

Legend: (Red circle) *H. achalai*, (yellow circle) *H. chaparensis, (*green circle) *H. edwadrsi*, (blue circle) *H. hepatica*, (black circle) *H. hobdayi*, (orange circle) *H. hylodeus, (*White circle) *H. minor*, (purple circle) *H. newsteadi*, (pink circle) *H. stepnhensi*, (brown circle) *Hannemania pattoni*, (black triangle) *H. yungicola (*red triangle) *H. argentina*, (green triangle) *Hannemania ortizi*, (White triangle) *Hannemania gonzaleacunae*.
Source: Literature cited in Table 4.

1.2.4 Super cohort Anystina, cohort Parasitengona (Superfamily Hydryphantoidea)

1.2.4.1 Family Thermacaridae

The family Thermacaridae includes a monogeneric group of water mites, specialized in inhabiting hot-spring waters. The genus *Thermacarus* has four valid species distributed in North America (two species), South America (one species), and Asia (one species) (HERON; SHEFFIELD, 2016).

These mites have a multifaceted life cycle which includes a larval stage, that can be parasitic of invertebrates or amphibians, different nymphal stages (quiescent protonymph, predatory deutonymph, and quiescent tritonymph), and adults that are predators. In the Neotropical region only one species has been described, *Thermacarus andinus* Martin & Schwoerbel, 2002, as previous record of another species (*Thermacarus nevadensis* Marshall, 1928) reported in Chile, were later confirmed to be also *T. andinus*. This species was described infesting toads in El Tatio, Chile (SCHWOERBEL, 1987 MARTIN; SCHWOERBEL 2002).

Only two species of *Thermacarus* have parasitic larvae infesting amphibians (Anura). The species *Tandinus* is confirmed to parasitize *Rhinella spinulosa* (Wiegmann, 1834), and other toads in South America (MARTIN; SCHWOERBEL 2002; THORP; COVICH, 2009; WALTER; PROCTOR, 2013). Also, *T. nevadensis* could possibly parasitize *Anaxyrus boreas* Baird and Girard, 1852, and other amphibians in Canada. Furthermore, *Thermacarus* mites could also infest other vertebrate, including humans that visit hot springs (MITCHELL, 1960; HERON; SHEFFIELD, 2016).

1.2.5 Cohort Eupodina (superfamily Tydeoidea)

1.2.5.1 Family Ereynetidae

This family includes 29 genera and 180 species grouped in three subfamilies (PARKER, 1982; MAURI et al., 1984; ZHANG et al., 2011). Of these subfamilies, Lawrencarinae Fain, 1957, comprises three genera that parasitize the upper respiratory tract of amphibians (*Batracarus* Fain, 1961; *Lawrencarus* Fain, 1957; and *Xenopacarus* Fain, Baker & Tinsley, 1969) (FAIN, 1957; 1961; ZHANG; ZHI-QIANG; WEN, 2000). Of these, *Lawrencarus* has three species and two subspecies with distribution in the Neotropical region (MAURI et al., 1984, FAIN, 1961; 1962).

The *Lawrencarus* mites are also called nasal mites, and can be considered endoparasitic mites of anuran amphibians. They are characterized by having two pair of sensillae on the dorsal idiosoma, males do not present genital suckers, and female with vestigial genital suckers. All the species have smooth perigenital discs (FAIN, 1961). The life cycle of this genus is still poorly understood, although three developmental stages are distinguished: larvae, nymphs (protonymph and deutonymph) and adults, and they can be viviparous or oviparous. It is believed these mites

are monoxenous, and the whole development occurs inside the upper respiratory tract of their hosts. The deleterious effect these parasites can have on the health status of their hosts is still unknown (FAIN, 1957; 1961; 1962). Species distribution of the neotropical species of *Lawrencarus* is shown in Table 5 and Figure 10.

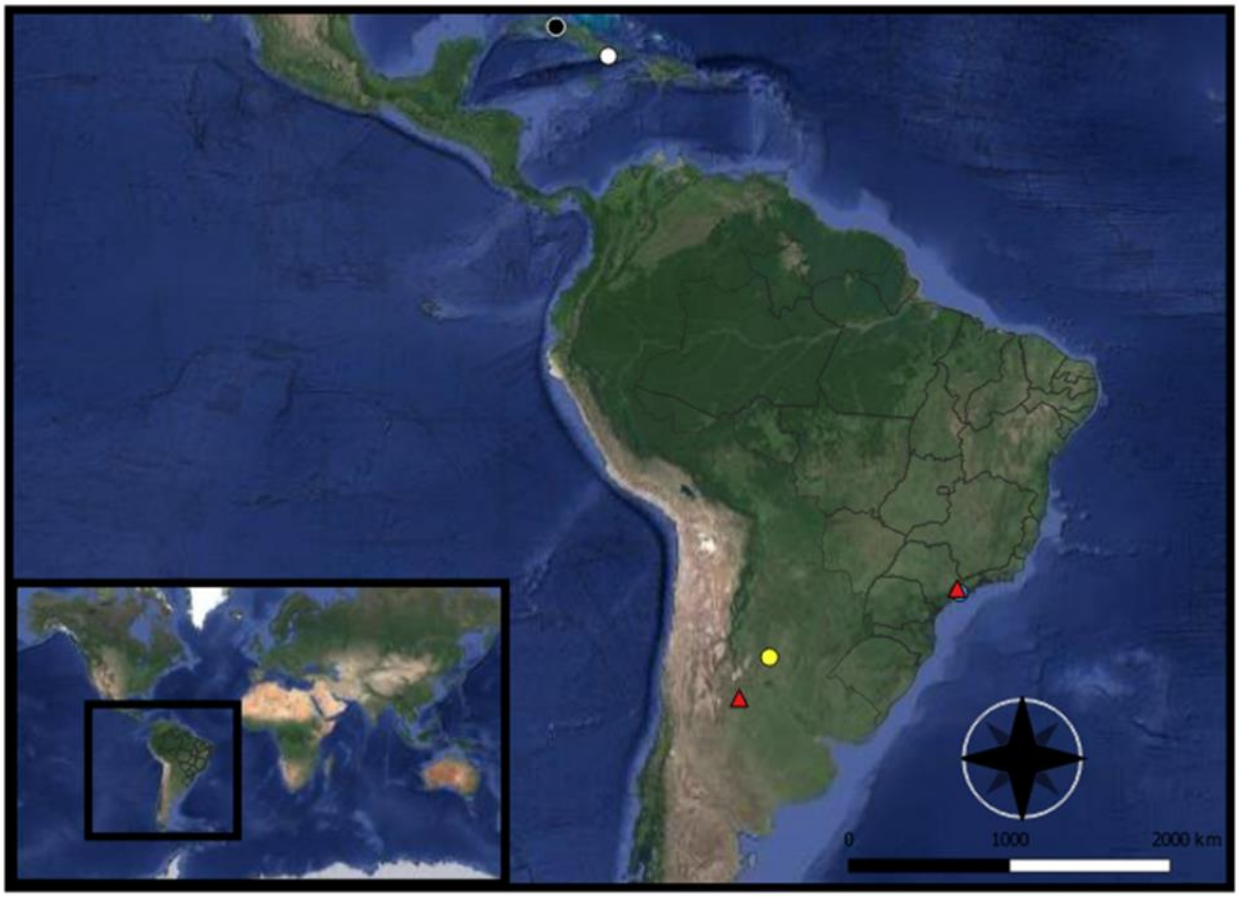
Table 5 – Species of *Lawrencarus* (Ereynetidae), distributed in the Neotropical region

No.	Species	Holotype	Host	Locality	Reference
1	*Lawrencarus brasiliensis* Fain, 1961	IRSNB - ♀	*Cycloramphus asper* Werner	Cubatão, São Paulo, Brazil	Fain, (1961)
2	*Lawrencarus brasiliensis desantisi* Mauri & Alzuet, 1984	FCNyN UP 4007/1 - ♀	*Rhinella arenarum* (Hensel) (cited as: *Bufo arenarum*)	Santiago del Estero, Averías, Argentina	Mauri & Alzuet, (1984)
		FCNyN UP 4007/13-15	*Rhinella schneideri* (Werner) (cited as *Bufo paracnemis*)	Santiago del Estero, Averías, Argentina	Mauri & Alzuet, (1984)
3	*Lawrencarus hylae intermedius* Fain, 1961	IRSNB - ♀	*Scinax hayii* (Barbour) (cited as *Hyla hayii*)	São Paulo, Brazil	Fain (1961)
			Pleurodema sp.	Pampa de Achala, Cordoba, Argentina	Mauri & Alzuet, (1984)
4	*Lawrencarus eweri cubanus* Cruz, 1971	Unknown	*Peltophyne peltacephala*	Cienfuegos, Soledad, Cuba	Cruz (1971)
5	*Lawrencarus hollandsae* Cruz, 1971	Unknown	*Eleutherodactylus dimidiatus* Cope	Santiago de Cuba, Culantrillo, Sierra Maestra, Cuba	Cruz (1971)

Source: (MENDOZA-ROLDAN, J. A., 2019)

Legend: FCNyN UP (Facultad de ciencias naturales y Museo de la Universidad de la Plata, Argentina), IRSN (Institut royal des Sciences naturelles de Belgique Brussels, Belgium).

Figure 10 – Distribution map of Neotropical species of *Lawrencarus*



Source: (MENDOZA-ROLDAN, J. A., 2019)

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Legend: (Red triangle) *Lawrencarus hylae intermedius*, (black circle), *Lawrencarus eweri cubanus*, (white circle) *Lawrencarus hollandsae*, (yellow circle) *Lawrencarus Braziliensis desantisi*, (blue circle) *Lawrencarus Braziliensis.*

Source: Literature cited in Table 5.

2 OBJECTIVES

- Assess the Trombidiformes mites of reptiles and amphibians deposited in the acarological collection of the Instituto Butantan (IBSP), and in other reference collections;
- Identify the Trombidiformes mites found in reptiles and amphibians through optic and electronic scanning microscopy and genetic sequencing (Part II, Chapter 5);
- Update distribution of Brazilian species of Trombidiformes mites, according to recent collections.

3 MATERIAL AND METHODS

3.1 Trombidiformes mites' material

The mite species of the order Trombidiformes that infest reptiles and amphibians that were assessed, collected, identified, and evaluated, came from three possibilities: material deposited in collections; mites that were brought upon their hosts to the different laboratories of the Instituto Butantan, or to the Venomous Animals Reception site of the same institute; and material that was collected from reptiles and amphibians in different field trips at various locations in Brazil. New or fresh material of mites and hosts were used for molecular biology studies (Part II of this thesis).

3.1.1 Material from collections

This study was based on the revision of the mite material deposited in the acarological collection of the Instituto Butantan (IBSP). Other reference collections were also revised to asses type material of some groups of Trombidiformes mites of reptiles and amphibians.

Acarological Collection of the Instituto Butantan (IBSP) – curator: Valeria Castilho Onofrio. It is one of the oldest collections of mites and ticks of Latin America. Trombidiformes mites of reptiles and amphibians are represented in this collection with 424 lots, being 16 type material. The mites are conserved in alcohol or mounted in slides, and part of the collections remains unidentified.

Acarological Collection of the Smithsonian Institute, Beltsville, Maryland, EUA (USNM Smithsonian Acari Collection - BARC-USDA-ARS) - curator: Ronald Ochoa. The Acarological Collection of Smithsonian Institute is located at the Laboratory of Entomologic Systematics of the Agricultural Department of USA. This collection harbors paratype material of several parasitic mites of reptiles and amphibians, and a holotype of a species of Harpirhynchidae. It is also the biggest collection of Trombiculidae, having in its lots most of the type material of this family for the Neotropical region (GOFF, 1989).

Acarological Collection of the La Plata Museum, La Plata, Argentina (MLP Museo de La Plata) – curator: Ana Salazar Martínez. Harbors 21 species and one subspecies. Mites of amphibians are represented by three type series of two families (Ereynetidae, Leeuwenhoekiidae) (SALAZAR-MARTINEZ et al., 2014).

Acarological Collection of the Fundação Zoobotanica, Porto Alegre, RS (FZB) - curator: Ricardo Ott. Although it does not have type material, it possesses material of parasitic mites of amphibians of the Rio Grande do Sul state, Brazil.

Fain Acari Collection of the Royal Belgian Institute of Natural Sciences –IRSN – curator: Wouter Dekoninck. One of the widest European collections held together by Dr. Alexander Fain. It harbors more than 100,000 slides, with 300,000 type material representing 2,407 species of Acari. Mites of reptiles and amphibians are embodied by more than 30 type series of six families.

3.1.2 Laboratories of the Instituto Butantan (IBSP)

3.1.2.1 Venomous Animals Reception site of the Herpetological collection of the Special Zoological Collections Laboratory (LECZ)

The Special Zoological Collections Laboratory (LECZ) of the Instituto Butantan, has a Venomous Animals Reception site, which receives snakes, amphibians, spiders, scorpions, acari (mites and ticks), insects, among other animals, that come from varied localities of Brazil and from other countries. Reptiles and amphibians are then routed to the laboratories from the Instituto Butantan (Herpetology, Cellular Biology, Biological Museum, Ecology and Evolution, among others). Spiders and scorpions are routed to the Arthropods Laboratory, and Acari are deposited in the Acarological collection of the LECZ. Venomous animals (vertebrates and invertebrates) are used first for venom extraction and in some cases reproduction. When these animals die they are deposited in the collections of the LECZ, which has five collections (Herpetology, Arachnids, Acarology, Entomology and, Myriapoda).

Mites and ticks from reptiles and amphibians that arrived from different regions of Brazil, herein studied, were collected whenever possible before being sent to the different laboratories or collections.

3.1.2.2 Laboratories of the Instituto Butantan

To assess infestation in captivity conditions, the laboratories that harbor live reptiles and amphibians for different purposes in the Instituto Butantan, were visited and the animals were examined for mites and ticks. Laboratories visited were: Cellular Biology, Ecology and Evolution, and the Biological Museum.

3.1.2.3 Material collected in field trips

Mites and ticks' material that was collected from reptiles and amphibians in different field trips at various locations in Brazil. The listed field trips are from projects this study collaborated in fieldwork, or material that was revised from the hosts. The projects also comprise three biomes.

Atlantic forest

- Project “Herpetofauna of the Brazilian south and southeast: diversity and conservation”. Coordinated by Dr. Francisco L. Franco (FAPESP Proc/n 2011/50313-0), ICMBio/SISBio (protocol nº 23225-1, nº 21526-1 e nº 37820). During this Project, collaborative fieldwork occurred in a campaign in Vale do Ribeira, Paraná (April 2016).

- Project “Diversity and distribution of snakes and lizards in the Curucutu Nucleus”. Coordinated by Msc. Silara Fatima Batista. SISBIO/ICMBio protocol nº 44913-3 (São Paulo). During this Project, collaborative fieldwork occurred in a campaign in Parque Estadual Serra do Mar Nucleo Curucutu (March 2016).

Amazon rainforest

- Project “Epidemiology of *Toxoplasma gondii* in domestic and wild animals if the Amazon fauna”. Coordinated by Dr. Solange M. Gennari. SISBI/ICMBIO protocol nº 44913-3 (PARÁ). During this Project, collaborative fieldwork occurred in three campaigns (August, December 2016, October 2017).

- Project “Scales of Biodiversity: Estudos integrados de evolução e função de venenos ofídicos nos múltiplos níveis da diversidade". Coordinated by Felipe Gobbi Grazziotin. SISBI/ICMBIO protocol nº 65653 (Resex Cazumbá-Iracema, Acre). Animals from the October expedition (2018) were examined.

Cerrado

- Project “Diversity and effects of fire in Squamata reptiles of Cerrado. Coordinated by Msc. Bruno Ferreto. Águas de Santa Barbara (São Paulo). During this Project, collaborative fieldwork occurred in one campaign (October 2017).

3.2 Collection of Trombidiformes mites from reptiles and amphibians

Depending on de family of mite, different collection methods were used, as well as specific areas of the host were examined depending on the species of the hosts and the parasitic habits of the mite. In all cases, mites were extracted delicately through scarification (mite removal using a needle) (FAIN 1964; LIZASO, 1981; 1983; 1984; MENDOZA-ROLDAN et al., 2017). All animals were visually examined, some under stereo microscope, and a complete physical exam from the cranial portion to the caudal (posterior) portion was held for each animal.

For the family Cloacaridae, which are found mainly inside the cloaca of Testudinata (turtles and tortoises), cloacal swabs were performed to the turtles and tortoises received in the Venomous Animals Reception site (LECZ) or maintained in the Ecology and Evolution Laboratory (IB), as well as for those animals found in field trips (Figure 11).

Figure 11 - Cloacal swab in common snapping turtle (*Chelydra serpentina*)

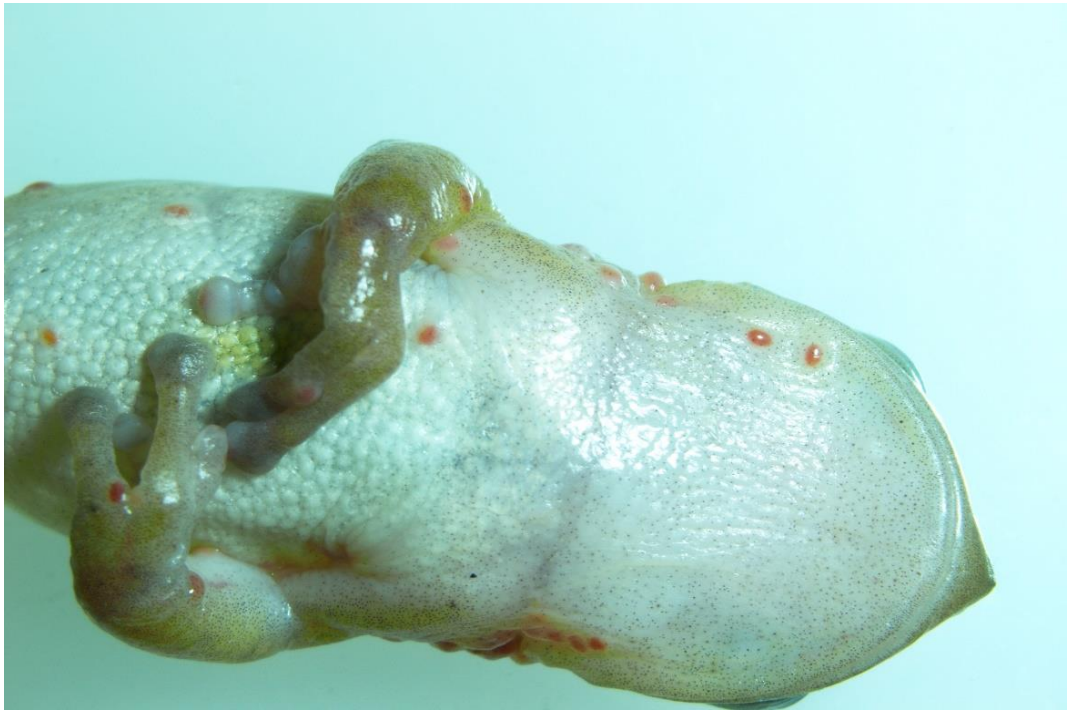
Source: (MENDOZA-ROLDAN, J. A., 2016).

When mites from the families Pterygosomatidae and Tombiculidae, which are ectoparaties that fixate mainly in the connective tissue in between the scales of reptiles and in skinfolds of amphibians, scarification was performed to safely extract the mites (Figure 12). Scarification was also performed by opening the capsules and extracting the mites in amphibians, which are generally parasitized by Leeuwenhoekiidae, which are embedded under the skin (Figure 13). Also, in amphibians, the oral cavity, nostrils and choana were examined for nasal mites of the Ereynetidae family (Figure 14).

Figure 12 - Scarification in green iguana (*Iguana iguana*)

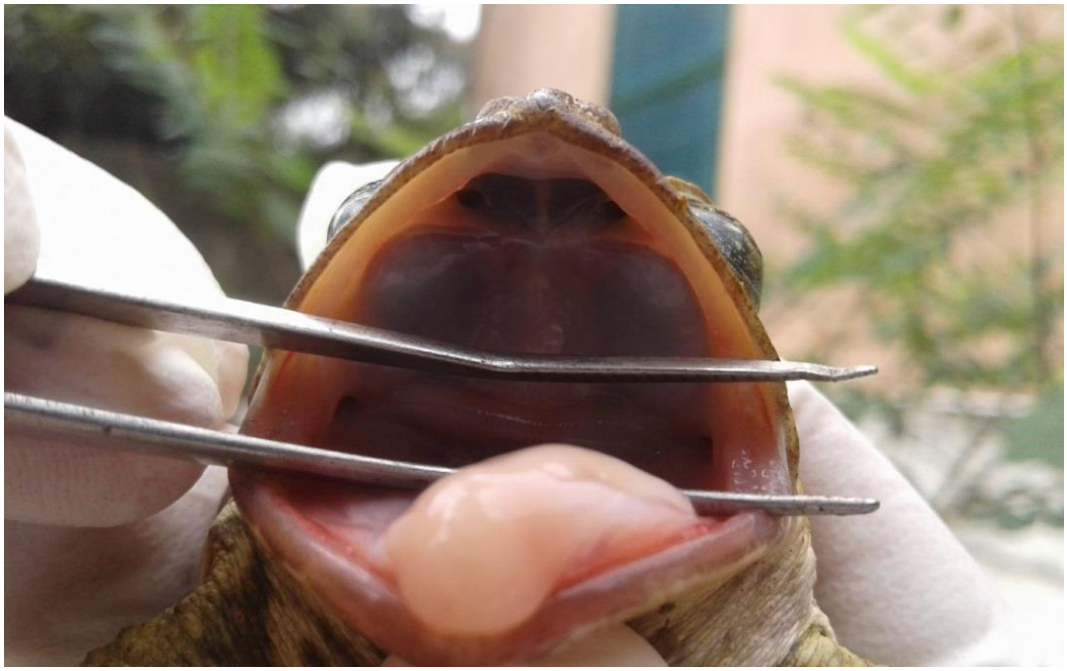
Source: (MENDOZA-ROLDAN, J. A., 2018).

Figure 13 – Capsules of Leeuwenhoekiidae mites (red spots) in *Fritziana fissilis*



Source: (MENDOZA-ROLDAN, J. A., 2018).

Figure 14 – Oral cavity exam in toad (*Rhinella icterica*)



Source: (MENDOZA-ROLDAN, J. A., 2017).

Identification of hosts (reptile and amphibians) used in this study, was performed by the team of herpetologists of the Herpetological collection of the Special Zoological Collections Laboratory (LECZ) of the Instituto Butantan (LECZ). The host nomenclature was updated by consulting the "Reptile Database" (http://www.reptile-database.org) (UETZ, 2010) as well as the database of the Brazilian Society of Herpetology (Sociedade Brasileira de Herpetologia - SBH), for reptiles (COSTA; BÉRNILS, 2018), and amphibians (SEGALLA et al., 2016).

3.3 Storage and conservation of mites and host tissue

Collected mites were stored in microtubes in absolute alcohol, and after some of those mites were used for slide mounting (this chapter), DNA extraction and molecular studies (Chapter 5 and 6). Eventually, some tissue samples (blood or liver) were obtained (techniques detailed in chapters 4) from parasitized hosts in the laboratories of the Instituto Butantan or in field trips. These blood samples were used to evaluate hemoparasites in smears (Chapter 4) and for pathogen detection (Chapter 6). Mites and tissue were collected with approval of the Ethics Committee of Animal Use (Comissão de Ética no Uso de Animais - CEUA) of the Faculty of Vetetinary

Medicine of the University of de São Paulo (Faculdade de Medicina Veterinária e Zootecnia da Universidade de São Paulo - FMVZ/USP), protocol nº 7491300715.

3.4 Morphological identification

Dichotomous keys (FAIN, 1964) as well as original descriptions (FAIN, 196; LIZASO, 1981; MENDOZA-ROLDAN et al., 2017) were used for morphological identification of Trombidiformes mites of the family Harpirhynchidae. For the Pterygosomatidae family, recent dichotomous keys for genera (MONTGOMERY, 1996), and species of *Geckobia*, *Geckobiella,* and *Bertrandiella* (PAREDES-LEON et al., 2012; 2013).

In case of Parasitengona mites from the superfamily Trombidioidea (Trombiculidae and Leeuewnhoekiidae), original description and keys for genera were used (WHARTON et al., 1951; BRENNAN; GOFF, 1977; GOFF et al., 1982; KUDRYASHOVA, 1998). Keys of Brennan & Loomis (1959) were used for identification of species of the genus *Fonsecia.* Identification of species of the genus *Eutrombicula* was performed using keys of Brennan & Reed (1974). Species of the *Hannemania* genus were identified using original descriptions and recent keys of Silva De La Fuente et al. (2016). For some species, as diagnosis, measurements (mean, standard deviation) are given in µm. Holotype measurements are shown in parenthesis.

Some of the mites from field trip collections, were clarified and mounted in slides. Clarification was made using lactic acid, at 55º C. Mites usually were monitored until achieved desired results (3 – 5 days). After, material was prepared in modified Berlese`s médium (Hoyer`s medium), according to Krantz & Walter (2009). Mites that had guanine agglutinations inside the idiosoma (*Ophioptes* spp.) were clarified using protocols of Fain (1964), described as follows.

Mites were placed in a Petri dish with 1 – 2 mL of potassium hydroxide (KOH) 5%, at 50 ºC, monitored every 3 minutes to assess guanine disintegration. After total guanine disintegration, mites were transferred, using a fine brush, to acetic acid (10%) for 10 minutes. Finally, mites were transferred to lactic acid till final clarification. Each mite was then mounted in slides. Once the slides were totally dried, coverslips were sealed using ISOQUID-4571 (Glyptal) resin and deposited in the IBSP collection.

3.4.1 Illustrations

Anatomic features with taxonomic importance of some species of mites with scarce taxonomical information were drawn to better illustrate species diagnosis and differences between species. Illustrations were made using a LEICA DM 400B microscope, then scanned, digitalized, edited and compiled in Photoshop CS6 and Corel Draw X7.

3.4.2 Scanning Electron Microscopy (SEM)

Whenever possible, one to four mites of each species were selected for scanning electron microscopy. The material was first dehydrated for 30 minutes, in a crescent alcohol concentration (70%, 80%, 90%, 95%, 100%, 100%, 100%, 100%), then maintained in Hexamethyldisilane for 24 hours. Metallization was performed leaving the specimens in a chemical cabinet with Hexamethyldisilane, at room temperature, until the material was completely dry. Each specimen was mounted on a ½-inch aluminum metal plate and metallized with gold. Scanning electron microscopy was performed at the Cellular Biology Laboratory of the Butantan Institute, under a digital scanning microscope, of the FEI model Quanta 250 (Multiuser Equipment).

3.5 Distribution

Distribution maps were generated using QGIS version 3.4.4-Madeira, to compare new distribution localities with those reported in literature (QGIS DEVELOPMENT TEAM, 2015).

4 RESULTS

Information of the identified species of mites (from collections and recent field trips) can be observed in Tables 5 and 6. Examined species are summarized in the Catalogue of examined species (item 4.3), which also includes information about specimens that were used for molecular biology (phylogeny and pathogen detection in part II). Host information, as well as parasite-hosts associations and parasitic impact, are discussed in chapter 4.

4.1 Species of Trombidiformes mites identified

In this study six families, 12 genera and 32 species of Trombidiformes mites were identified. These species were identified from the IBSP collection (and other examined collections), and from ectothermic hosts examined in the laboratories of the Instituto Butantan, as well as those examined in recent field trips (Table 6). Species identified are: super cohort Eleutherengonides superfamily Cheyletoidea: **Cloacaridae** (*Cloacarus faini* Camin, Moss, Oliver & Singer, 1967; *Caminacarus chrysemys* Pence & Casto, 1975; *Caminacarus deirochelys* Fain, 1968; *Caminacarus costai* Fain 1968; *Theodoracarus testudinis* Fain 1968); **Harpirhynchidae** (*Ophioptes brevipilis* Lizaso (1981); *Ophioptes longipilis* Lizaso, 1981; *Ophioptes parkeri* Sambon, 1928; *Ophioptes tropicalis* Ewing, 1933; *Ophioptes dromicus* Allred, 1958; *Ophioptes beshkovi* Beron, 1974; and *Ophioptes ekans* Mendoza-Roldan & Barros-Battesti, 2017); superfamily Pterygosomatoidea: **Pterygosomatidae** (*Bertrandiella jimenezi* (Paredes-León & Morales-Malacara, 2009); *Geckobia hemidactyli* Lawrence, 1936; *Geckobia bataviensis* (Vitzthum, 1926), *Geckobiella harrisi* Davidson, 1958)]; super cohort Anystina, cohort Parasitengona, superfamily Trombidioidea: **Leeuwenhoekiidae** (*Hannemania achalai* Alzuet & Mauri, 1987; *Hannemania hepatica* Fonseca, 1935; *Hannemania minor* Alzuet & Mauri, 1985; *Hannemania Yungicola* Wohltmann & Kohler, 2006); **Trombiculidae** [(*Eutrombicula alfreddugesi* (Oudemans, 1920); *Eutrombicula butantanensis* (Fonseca, 1932); *Eutrombicula ophidica* (Fonseca, 1935); *Eutrombicula tropica* (Ewing, 1925); *Fonsecia ewingi* Fonseca (1932); *Fonsecia coluberina* Radford, 1946; *Fonsecia anguina* Brennan & Loomis, 1959; *Fonsecia travassosi* (Fonseca, 1935); *Eutrombicula hirsti* (Sambon, 1927); *Neotrombicula microti* (Ewing, 1928)]; and cohort Eupodina, superfamily Tydeoidea: **Ereynetidae** (*Lawrencarus braziliensis desantisi* Mauri e Alzuet, 1984). Finally, a species of the order Sarcoptiformes, suborder Oribatida (family Trhypochthoniidae: *Archegozetes longisetosus* Aoki, 1965 was identified. Though it is not a Trombidiformes mites it is included in the Acariformes superorder (Sarcoptiformes order).

Of the 32 species identified in this study, 23 occur in Brazil. The Brazilian species are shown in Table 6 in bold. Hosts for each species of mites are shown in Table 7 (new hosts records are shown with **X**). Parasite-host associations are discussed in chapter 4.

Table 6 - Trombidiformes types and material examined of reptiles and amphibians: collection, field trips and laboratories of the IBSP

Family	Species	Collections					Field trips and laboratories of the IBSP				
		IBSP	MLP	FZB	RBINS	USDA	North	Northeast	Central-west	Southeast	South
Cloacaridae	*Cloacarus faini* Camin, Moss, Oliver & Singer, 1967				2						
	Caminacarus chrysemys Pence and Casto, 1975				1						
	Caminacarus deirochelys Fain, 1968				1						
	Caminacarus costai Fain 1968				1						
	Theodoracarus testudinis Fain 1968				1						
Harpirhynchidae	***Ophioptes brevipilis* Lizaso, 1981**	9									
	***Ophioptes longipilis* Lizaso, 1981**	8									
	***Ophioptes parkeri* Sambon, 1928**	54			9					1	
	Ophioptes tropicalis Ewing,1933					1					
	Ophioptes dromicus Allred, 1958				3						
	Ophioptes beshkovi Beron, 1974				1						
	***Ophioptes ekans* Mendoza-Roldan & Barros-Battesti, 2017**	3								1	
Pterygosomatidae	***Geckobia hemidactyli* Lawrence, 1936**	19								9	
	***Geckobia bataviensis* (Vitzthum, 1926)**								1		
	***Geckobiella harrisi* Davidson, 1958**									9	
Leeuwenhoekiidae	***Bertrandiella jimenezi* (Paredes-León & Morales-Malacara, 2009)**							18			
	***Hannemania achalai* Alzuet & Mauri, 1985**		8								9
	***Hannemania hepatica* Fonseca, 1935**	17						1		3	
	***Hannemania yungicola* Wohltmann e Kohler, 2006**	1									
	***Hannemania minor* Alzuet & Mauri, 1985**	1	10								
	***Eutrombicula alfreddugesi* (Oudemans, 1920)**	33					8	1	3	16	
	***Eutrombicula butantanensis* (Fonseca, 1932)**	3									

(Conclusion)

Family	Species	Collections					Field trips and laboratories of the IBSP				
		IBSP	MLP	FZB	IRSN	US NM	North	Northeast	Central-west	Southeast	South
Trombiculidae	***Eutrombicula ophidica* Fonseca, 1935**	4					2			1	
	***Eutrombicula tropica* Ewing, (1925)**									1	
	***Fonsecia ewingi* Fonseca (1932)**	11									
	Fonsecia coluberina Radford, 1946	1									
	***Fonsecia anguina* Brennan &. Loomis, 1959**						1				
	***Fonsecia travassosi* Fonseca (1935**	1									
	Trombicula hirsti Sambon (1927)	1									
	***Neotrombicula microti* (Ewing, 1928)**	1									
Ereynetidae	***Lawrencarus Braziliensis desantisi* Mauri e Alzuet, 1984**		15								
Oribatida	***Archegozetes longisetosus* Aoki 1965**							1			

Source: (MENDOZA-ROLDAN, J. A., 2019)

Legend: IBSP (Acarological collection, of the Instituto Butantan, Special Zoological Collections Laboratory, São Paulo, Brazil), MLP (Facultad de ciências naturales y Museo de la Universidad de la Plata, Argentina), FZB (Fundação Zoobotanica, Porto Alegre, RS, Brazil), IRSN (Institut royal des Sciences naturelles de Belgique Brussels, Belgium), USNM (National Museum of Natural History, Smithsonian Institution, Washington, D.C. 20560, U.S.A).

Table 7 – Species of hosts and species of Trombidiformes infesting mites

Class	Host	*O. parkeri*	*O. ekans*	*G. hemidactyli*	*G. bataviensis*	*G. harrisi*	*B. jimenezi*	*H. achalai*	*H. hepatica*	*E. alfreddugesi*	*E. ophidica*	*E. tropica*	*F. anguina*	*A. longisetosus*
Serpentes	*Bothrops jararaca*		**X**											
	Chironius bicarinatus	**X**												
	Chironius multiventris									**X**				
	Chironius scurrulus									**X**				
	Erythrolamprus typhlus												**X**	
	Drymoluber brazili									**X**				
	Philodryas nattereri									**X**				
	Spilotes pullatus									**X**				
Sauria	*Anolis meridionalis*									**X**				
	Arthrosaura reticulata									**X**				
	Aspronema dorsivittatum									**X**				
	Cercosauria eigenmani									**X**				
	Copeoglossum nigropunctatum									**X**				
	Enyalius iheringii									**X**				
	Gymnodactylus geckoides						**X**							
	Hemidactylus mabouia			x										
	Kentropyx calcarata									**X**	**X**			
	Phyllopezus pollicaris						**X**							
	Psychosaura macrorhyncha											**X**		
	Thecadactylus rapicauda				**X**					**X**				
	Trachylepis atlantica									**X**				
	Tropidurus catalanensis					**X**								
	Tropidurus itambere									**X**				
	Tropidurus montanus										**X**			
	Tropidurus torquatus					**X**								

(Conclusion)

Class	Host	*O. parkeri*	*O. ekans*	*G. hemidactyli*	*G. bataviensis*	*G. harrisi*	*B. jimenezi*	*H. achalai*	*H. hepatica*	*E. alfreddugesi*	*E. ophidica*	*E. tropica*	*F. anguina*	*A. longisetosus*
Anura	*Cycloramphus boraceiensis*								**X**					
	Corythomantis greeningi								**X**					
	Cycloramphus dubius								**X**					
	Leptodactylus latrans							**X**						
	Melanophryniscus admirabilis							**X**						
	Phyllomedusa iheringii									**X**				
	Rhinella major													**X**
	Scinax squalirostris							**X**						
	Thoropa megatympanum								**X**					

Source: (MENDOZA-ROLDAN, J. A., 2019).

Legend: New records of hosts are highlighted with **X**

4.2 Catalogue of examined species

Information regarding identified species of Trombidiformes mites (from collections and recent field trips) are detailed in this section. Material used for molecular biology (Part II) is highlighted with *, new host record with **, and new localities with***.

Order TROMBIDIFORMES

Super cohort Eleutherengonides

Superfamily Cheyletoidea

Family Cloacaridae

***Cloacarus faini* Camin**, **Moss, Oliver & Singer, 1967**

Kansas, USA: IRSN 24714, 1 Protonymph paratype, *Chelydra serpentina*, 20.XI.1965; IRNS, 1 Female paratype, *Chelydra serpentina*, 20.XI.1965, coll. George Singer.

***Caminacarus chrysemys* Pence & Casto, 1975**

Louisiana, USA: IRSN, 1 female, *Trachemys scripta elegans*, 15.I.1972, coll. D. B. Pence.

***Caminacarus deirochelys* Fain, 1968**

Florida, USA: IRSN, 1 female paratype, *Deirochelys reticularia*, 15.III.1938, coll. A. Fain.

***Caminacarus costai* Fain 1968**

Benjamina, Israel: IRSN, 1 female paratype, *Mauremys caspica*, 15.XII.1966, coll. O. Theodor.

***Theodoracarus testudinis* Fain 1968**

Jerusalem, Israel: IRSN, 1 female paratype, *Testudo graeca ibera*, 17.V.1967, coll. O. Theodor.

Family Harpirhynchidae

***Ophioptes parkeri* Sambon, 1928**

Southeast Region: São Paulo state - Araçoiaba da Serra, SP - IBSP 6205, 2 males, *Chironius foveatus*, 27.II.1978. **Arujá, SP** - IBSP 6037, 5 females, 2 males, *C. foveatus*, 22.XI.1976. **Birita-Mirim, SP -** IBSP 6204, 18 females, 9 males, *Erythrolamprus aesculapii*, 20.II.1978. **Inúiba Paulista, SP** - IBSP 6266, 7 females, 9 males, *E. aesculapii*, 22.IX.1978. **Itapecerica da Serra, SP** - IBSP 12044, 1 female, *Pilodryas patagoniensis*, 12.XI.2013. **Morro Agudo, SP** - IBSP 6468, 5 females, 2 males, *E. aesculapii*, 7.XII.1981. **Presidente Wenceslau, SP** - IBSP 5981, 5 females, 2 males, *Erythrolamprus poecilogyrus*, 14.IV.1976. **Rancharia, SP** - IBSP 6222, 7 females, 2 males, *E. aesculapii*, 22.V.1978. **São Carlos, SP** - IBSP 6480, 1 macho, *Xenodon merremii*, 7.XII.1981. **São Paulo, SP -** IBSP 12043, 1 female, *T. dorsatus*, 13.XI.2013; IBSP 12908, 4 females, 4 males *Chironius bicarinatus*, 25.I.2016, coll Jairo Mendoza-Roldan*, **.

***Ophioptes brevipilis* Lizaso, 1981**

Central-West Region: Goiás state - Goiânia, GO - IBSP 6327, 1 female holotype, *C. flavolineatus*, 30.III.1979, coll. Nelida Lizaso.

Southeast Region: Espírito Santo state - Colatina, ES - IBSP 6202, 1 female, 4 males partypes, *E. poecilogyrus*, 17.II.1978. **São Paulo state - Tupã, SP -** IBSP 6299, 9 female, 4 males paratypes, *Mastigodryas bifossatus*, 1.XII.1978.

South Region: Paraná state - Uraí, PR - IBSP 6351 1 male paratype, *Philodryas olfresii*, 11.IX.1979.

***Ophioptes longipilis* Lizaso, 1981**

Southeast Region: São Paulo state - Itú, SP - IBSP 6070, 1 female holotype, *Oxyrhopus trigeminus*, 7. I.1977; IBSP 6111, 3 females, 4 males paratypes, *O. trigeminus*, 25.IX.1978; IBSP 6267, 2 females, 2 males paratypes, *O. trigeminus*, 25.IX.1978, coll Nelida Lizaso.

South Region: Paraná state - Foz do Areia, PR - IBSP 6418, 2 males paratypes, *Oxyrophus pelota*, 25.IV.1980.

***Ophioptes tropicalis* (Ewing, 1933)**

British Guiana: USNM 1081, 1 female lectotype, *Chironius carinatus* 15.I.1931, coll. C Ewing.

***Ophioptes dromicus* Allred, 1958**

Oriental province, Cuba: IRSN, 2 females, *Caraiba andreae*, 15.IV.1956, coll. Alex Fain.

***Ophioptes beshkovi* Beron, 1974**

Frolosh, Bulgaria: IRSN, 1 female paratype, *Platyceps najadum*, 11.VI.1968, coll. P Beron.

***Ophioptes ekans* Mendoza-Roldan & Barros-Battesti, 2017**

Southeast Region: São Paulo state - Campo Limpo Paulista, SP - IBSP 12078, 1 female holotype, *Crotalus durissus terrificus*, 6.I.2014; IBSP 12079, 2 males, 2 deutonymphs paratypes, *Crotalus durissus terrificus*, 6.I.2014*. **São Paulo, SP –** IBSP 14907, 2 females, *Bothrops jararaca*, 22.I.2018, coll Jairo Mendoz-Roldan*, **, ***.

Superfamily Pterygosomatoidea

Family Pterygosomatidae

***Bertrandiella jimenezi* (Paredes-León & Morales-Malacara, 2009)**

Northeast Region: Alagoas state - Piranhas, AL - IBSP 14846, 2 females, 2 deutonymphs, *Gymnodactylus geckoides*, 25.V.2017*, **, ***; IBSP 14847, 1 female, *G. geckoides*, 25.V.2017*, **, ***; IBSP 14848, 6 females, 2 deutonymphs, *G. geckoides*, 25.V.2017*, **, ***; IBSP 14849, 2 females, 8 deutonymphs, *G. geckoides*, 25.V.2017*, **, ***; IBSP 14857, 2 females, 2 deutonymphs *Phyllopezus pollicaris*, 27.V.2017*, **, ***; IBSP 14858, 2 females, 2 deutonymphs *P. pollicaris*, 28.V.2017*, **, ***; IBSP 14859, 3 females, 6 deutonymphs *P. pollicaris*, 27.V.2017*, **, ***; IBSP 14860, 2 females, 2 deutonymphs *P. pollicaris*, 29.V.2017; *, **, *** IBSP 14875, 2 females, 8 deutonymphs *P. pollicaris*, 30.V.2017*, **, ***; IBSP 14862, 2 females, 3 deutonymphs *P. pollicaris*, 30.V.2017, Coll Valdir Germano*, **, ***. **Sergipe state - Caniné de São Francisco, SE -** IBSP 14850, 2 females, 4 deutonymphs, *G. geckoides*, 25.V.2017 *, **, ***; IBSP 14851, 2 females, 3 deutonymphs, *G. geckoides* 25.V.2017 *, **, ***; ***; IBSP 14852, 3 females, 3 deutonymphs, *G. geckoides*, 25.V.2017 *, **, ***; IBSP 14853, 3 females, 3

deutonymphs, *P. pollicaris*, 25.V.2017 *, **, ***; IBSP 14854, 3 females, 3 deutonymphs, *P. pollicaris*, 25.V.2017 *, **, ***; IBSP 14855, 3 females, 3 deutonymphs, *P. pollicaris*, 25.V.2017 *, **, ***; IBSP 14856, 3 females, 3 deutonymphs, *P. pollicaris*, 26.V.2017, Coll Valdir Germano *, **, ***. **Rio Grande do Norte state – Angicos, RN -** IBSP 14897, 1 female, 4 deutonymphs, *G. geckoides*, 7.XII.2018 *, **, ***.

***Geckobia hemidactyli* Lawrence, 1936**

North Region: Pará state - **Tucuruí, PA** - IBSP 6784, 1 Female, *Thecadactylus rapicauda*, VII 1984.

Southeast Region: São Paulo state – Paulo Assis, SP - IBSP 4788, 1 female, 1 deutonymph, *Hemidactylus mabouia*, 20.XI.1951. **Ilha do Bom abrigo, SP** - IBSP 2117, 6 females, *H. mabouia*, no collection data. **São Paulo, SP** - IBSP 1938, 2 females, *Mabuya mabouya*, 14.VI.1940; IBSP 3852, 2 females, gecko, 2.VI.1956; IBSP 12087, 2 females, *H. mabouia*, 15.VIII.2013; IBSP 12047, 1 female, *H. mabouia*, 11.XI.2013; IBSP 12048, 2 females, *H. mabouia*, 11.XI.2013; IBSP 12077, 2 females, *H. mabouia*, 15.VIII.2013; IBSP 12081, 2 females, *H. mabouia*, 15.III.2014; IBSP 12082, 3 females, *H. mabouia*, 15.VI.2014; IBSP 12083, 2 females, *H. mabouia*, 11.IX.2014; IBSP 12084, 1 female, *H. mabouia*, SP, 22.IX.2014; IBSP 12085, 2 females, *H. mabouia*, 15.XII.2014; IBSP 12086, 2 females, *H. mabouia*, 23.XII.2014; IBSP 12097, 2 females, *H. mabouia*, 12.III.2015; IBSP 12098, 2 females, *H. mabouia*, 7.IV.2015; IBSP 12911, 2 Females, 3 Deutonymphs, 1 egg, *H. mabouia*, 29.IX.2015*; IBSP 12912, 3 Females, 2 Deutonymphs *H. mabouia* 14.XII.2015*; IBSP 14837, 2 females, 8 deutonymphs, *H. mabouia*, 11.XI.2015*; IBSP 12913 2 females, 3 deutonymphs, *H. mabouia*, 14.XII.2015*; IBSP 12916, 1 Females, 1 Deutonymph, *H. mabouia*, 15.XII.2015*; IBSP 12930, 5 Females, 3 Deutonymphs, *H. mabouia*, 15.XII.2015*; IBSP 12931, 2 Females, 1 Deutonymph, *H. mabouia*, 17.II.2016*; IBSP 12933, 7 Females, 2 Deutonymph, *H. mabouia*, 30-III-2016*; IBSP 12940, 3 females, 6 deutonymphs, *H. mabouia*, 2.VIII.2016, coll Jairo Mendoza Roldan*.

Sete Barras, SP - IBSP 12045, 3 females, 7.VIII.2013; IBSP 12072, 4 females, *H. mabouia*, 13.XII.2013.

***Geckobia bataviensis* (Vitzthum, 1926)**

Central-West Region: Mato Grosso state - Vale de São Domingos, MT - IBSP 12975, 3 females, 5 deutonymphs, *T. rapicauda*, 15.VII.2012, coll Drausio Honorio Morais**, ***.

***Geckobiella harrisi* Davidson, 1958**

Southeast Region: São Paulo state – Serra da Cantareira, SP - IBSP 14867, 2 females, *Tropidurus torquatus* 26.II.2018, coll Drausio Honorio Morais**, ***. **São Paulo, SP -** IBSP 14887, 15 females, 18 deutonymphs, *Tropidurus catalanensis*, 15. X.2018*, **, ***; IBSP 14888, 15 females, 18 deutonymphs, *T. catalanensis*, 14.XI.2018*, **, ***; IBSP 14889, 10 females, 7 deutonymphs, *T. catalanensis*, 14.XI.2018*, **, ***; IBSP 14890, 5 females, 10 deutonymphs, *T. catalanensis*, 14.XI.2018*, **, ***; IBSP 14891, 15 females, 10 deutonymphs, *T. catalanensis*, 14.XI.2018*, **, ***; IBSP 14892, 6 females, 8 deutonymphs, *T. catalanensis*, 14.XI.2018*, **, ***; IBSP 14893, 3 females, 10 deutonymphs, *T. catalanensis*, 14.XI.2018*, **, ***; IBSP 14894, 5 females, 8 deutonymphs, *T. catalanensis*, 14.XI.2018, coll Jairo Mendoza Roldan*, **, ***.

Super cohort Anystina

Cohort Parasitengona

Superfamily Trombidioidea

Family Leeuwenhoekiidae

***Hannemania achalai* Alzuet & Mauri, 1987**

Pampa de Achala, Córdoba. Argentina- MLP 4006/1, 1 larva holotype, *Pleurodema* sp. 15.XII.1970, coll Barrio; MLP 4006/3-4, 3 larvae paratypes, *Pleurodema kriegi*, same collecting data as holotype; MLP 4006/5-11, 48 larvae paratypes, same collecting data as holotype; MLP 4006/12-14, larvae 10 paratypes, *Odontophrynus occidentalis*, 2.XI.1983, coll Kehr; MLP 4006/15, 1 larva paratype, same collecting data.

South Region: Rio Grande do Sul state – Arvorezinha, RS - IBSP 12918, 2 larvae, *Melanophryniscus admirabilis*, 26.I.2016*, **, ***; IBSP 12919, 3 larvae, *M. admirabilis* 26.I.2016*, **, ***; IBSP 12920, 2 larvae, *M. admirabilis*, 26.I.2016*, **, ***; IBSP 12921, 4 larvae, *M. admirabilis*, 26.I.2016*, **, ***; IBSP 12922, 3 larvae, *M. admirabilis*, 26.I.2016*, **, ***; IBSP 12923, 3 larvae, *M. admirabilis* 26.I.2016*, **, ***; IBSP

12924, 3 larvae, *M. admirabilis* 26.I.2016, coll Ricardo Ott*, **, ***. **Itapuã, RS** IBSP 12925, 2 larvae, *Leptodactylus latrans*, 27.I.2016*, **, ***; IBSP 12926, 2 larvae, *L. latrans*, 27.I.2016*, **, ***; IBSP 12927, 2 larvae, *L. latrans*, 27.I.2016*, **, ***; BSP 12928, 2 larvae, *L. latrans*, 27.I.2016*, **, ***; IBSP 12929, 1 larva, *Scinax squalirostris*, 27.I.2016, coll Ricardo Ott *, **, ***.

***Hannemania hepatica* Fonseca, 1935**

Southeast Region: São Paulo state – São Paulo, SP (Bairro Butantã) - IBSP 31, 1 larva holotype, *L. latrans*, 28.X.1933. **Sete Barras, SP -** IBSP 12050, 1 larva, *Physalaemus spiniger*, 12.XII.2013; IBSP 12051, 1 larva, *P. spiniger*, 12.XII.2013; IBSP 12058, 2 larvas, *P. spiniger*, 13.XII.2013; IBSP 12059, 1 larva, *P. spiniger* 13.XII.2013; IBSP 12060, 1 larva, *P. spiniger*, 14.XII.2013; IBSP 12060, 2 larvas, *P. spiniger*, 16.XII.2013; IBSP 12061, 1 larva, *P. spiniger*, XII.2013; IBSP 12062, 1 larva, *P. spiniger*, 12.XII.2013; IBSP 12063, 1 larva, *P. spiniger*; 16.XII.2013; IBSP 12066, 1 larva, *P. spiniger*; IBSP 12064, 1 larva, *P. spiniger*, 16.XII.2013; IBSP 12069, 2 larvas, *P. spiniger*, 17.XII.2013; IBSP 12073, 1 larva, *P. spiniger*, 19.XII.2013; IBSP 12074, 1 larva, *P. spiniger*, 17.XII.2013; IBSP 12075, 1 larva, *P. spiniger*, 19.XII.2013; IBSP 12076, 1 larva, *P. spiniger*, 19.XII.2013, coll Jairo Mendoza Roldan. **Cubatão, SP –** IBSP 12957, 1 Larva, *Cycloramphus dubius*, 17.I.2017*, **, ***. **Ilhabela, SP -** IBSP 12935, 1 larva, *C. boraceiensis*, 26.IV.2016, coll Felipe Toledo*, **, ***. **Minas Gerais state – Diamantina, MG -** IBSP 12934, 6 Larvas, *Thoropa megatympanum*, 27.I.2016, coll Hermes Ribeiro*, **, ***. **Northeast Region: Rio Grande do Norte state – Angicos, RN –** IBSP 14896, 5 larvae, *Corythomantis greeningi*, 7.XII.2018, coll Bruno Rocha*, **, ***.

***Hannemania minor* Alzuet & Mauri, 1987**

Argentina: Benavídez, Buenos Aires- MLP 4005/1, 1 larva holotype, *Leptodactylus ocellatus*, 4.V.1978, no collector; MLP 4005/1, 6 larvae paratypes, *L. ocellatus*, same collecting data as holotype; MLP 4005/3, 18 larvae paratypes, *L. ocellatus*, same collecting data as holotype. **Santiago del Estero -** MLP 4005/4, 7 larvae paratypes, *L. ocellatus*, 15.V.1978, no collector.

Southeast Region: São Paulo state – Sete Barras, SP - IBSP 12065, 2 larvae, *L. latrans*, 14.XII.2013.

Hannemania yungicola **Wohltmann & Köhler, 2006**

Southeast Region: São Paulo state – São Jose do Barreiro, SP - IBSP 12049, 6 larvae, *Fritziana fissilis*, 1.XII.2013.

Family Trombiculidae

Eutrombicula alfreddugesi **(Oudemans, 1910)**

Central-West Region: Mato Grosso state - IBSP 11666, 2 larvae, *Ameivula* sp., 24.III.2013; IBSP 11667, 4 larvae, *Tropidurus oreadicus*, 24.III.2013; IBSP 11668, 2 larvae, *M. atticolus*, 24.III.2013; IBSP 11669, 1 larva, *Kentropyx paulensis*, 28.III.2013; IBSP 11678, 1 larva, *Norops meridionalis*, 28.III.2013; IBSP 11682, 4 larvae, *M. atticolus*, 2.III.2013; IBSP 11684, 2 larvae, *M. atticolus*, 1.IV.2013; IBSP 11687, 5 larvae, *M. atticolus*, 31.III.2013. **Guaporé, MT -** IBSP 12972, 2 larvae, *Copeoglossum nigropunctatum*, 26.VII.2012**, IBSP 12974, 2 larvae, *Thecadactylus rapicauda*, 12.VIII.2012, coll Drausio Honorio Morais**. **Universidade Federal de Mato Grosso, MT -** IBSP 12976, 4 larvae, *Drymoluber brazili*, 27.VIII.2012**. **Vale de são domingos, MT –** IBSP 12971, 3 larvae, *Cercosauria eigenmani*, 18.III,2017**.

North Region: Acre state – Iracema, AC – IBSP 14876, 6 larvae, *Chironius multiventris*, 10.X.2018*, **, ***; IBSP 1488, 3 larvae, *Chironius scurrulus*, 10.X.2018, coll Flora Roncolatto Ortiz*, **, ***. **Pará state** - **Tucuruí, PA-** IBSP 12950, 4 larvae, *Arthrosaura reticulata*, 27. VIII.2016*, **, ***; IBSP 12951, 4 larvae, *Kentropyx calcarata*, 22. VIII.2016*, **, ***; IBSP 12952, 6 larvae, *K. calcarata*, 28.VIII.2016 coll Jairo Mendoza Roldan*, **, ***; IBSP 14829, 5 larvae, *Copeoglossum nigropunctatum*, 9.X.2017*,**,***; IBSP 14835, 3 larva, *C. nigropunctatum*, 9.X.2017*, **,***; IBSP 14836, 4 larvae, *C. nigropunctatum*, 9.X.2017, coll Jairo Mendoza Roldan*, **, ***; IBSP 12949, 3 larvae, *Rhinella icterica*, 18.VIII.2016, coll Jairo Mendoza Roldan. **Northeast Region: Pernambuco state - Fernando de Noronha, PE –** IBSP 14828, 2 larvae, *Trachylepis atlantica*, 15.XI.2017, coll Vinicius Gasparotto*, **, ***.

Southeast Region: São Paulo state - Barragem Paraitinga, SP - IBSP 12596, 2 larvae, *Phyllomedusa iheringii*, 15.V.2004, coll Patricia B. Bertola **, ***; IBSP 12565, 3 larvae, *P. iheringii* , 03.V.2004**;IBSP 12566, 3 larvae, same host and date; IBSP 12567, 4 larvae, same host and date; IBSP 12569, 3 larvae, same host and date; IBSP 12584, 4 larvae, same host and date; IBSP 12590. 3 larvae, same host and date; IBSP 12591, 4 larvae, same host and date; IBSP

12593, 3 larvae, same host and date; IBSP 12595, 4 larvae, same host and date, coll Patricia B. Bertola. **Cananéia, SP** - IBSP 12917, 2 larvae, *Spilotes pullatus*, 27.VIII.2016*, ***. **Sete Barras, SP -** IBSP 12067, 1 larva, *Placosoma glabellum*, 12.XII.2013; IBSP 12072, 2 larvae, *R. icterica*. 14.XII.2013, coll Jairo Mendoza Roldan. **Santa Barbara, SP -** IBS P14831, 5 larvae, *Aspronema dorsivittatum*, 20.X.2017*, **, ***; IBSP 14833, 3 larvae, *A. dorsivittatum*, 23.X.2017, coll Jairo Mendoza Roldan*, **, ***; IBSP 14834, 1 larva, *Anolis meridionalis*, 24.X.2017, coll. Jairo Mendoza Roldan*, **, ***; **São Bernardo do Campo, SP –** IBSP 14839, 4 larvae, *Philodryas nattererii*, 22.IX.2017, coll Jairo Mendoza Roldan*, **, ***. **São Paulo, SP –** IBSP 14840, 1 larva, *Tropidurus itambere*, 10.VII.2017*, **, ***; IBSP 14863, 1 larva, *Enyalius iheringii*, 31.I.2018 coll Arlei Marcili*, **, ***.

Eutrombicula butantanensis (**Fonseca, 1932)**

Southeast Region: São Paulo state – São Paulo, SP - IBSP 28, 1 larva holotype – (sin. *T. butantanensis*), *Homo sapiens*, 17.II.1932; IBSP 83, IBSP 84, 3 larvae, *X. merremii* (cited as *O. merremii*), 26.III.1932.

Eutrombicula ophidica **(Fonseca, 1932)**

North Region: Pará state - **Tucuruí, PA -** IBSP 12955, 4 larvae, *K. calcarata*, 1.XII.2016*, **, ***; IBSP 12956, 4 larvae, *K. calcarata*, 01.XII.2016, coll Jairo Mendoza Roldan*, **, ***.

Southeast Region: Minas Gerais state - Diamantina, MG - IBSP 12914, 4 larvae, *Tropidurus montanus*, 7-XII-2015, coll Bruno Rocha **, ***; **São Paulo state - Promissão, SP -** IBSP 29, 1 larva holotype – (sin. *T. ophidica*), *X. merremii*, 18.V.1932; IBSP 88, 2 larvae, *X. merremii*, 19.V.1932. **São Paulo, SP -** IBSP 86, 3 larvae, *X. merremii*, 3.IV.1932; IBSP 87, 3 larvae, *X. merremii*, 3.IV.1932.

Eutrombicula tropic**a (Ewing, 1925)**

Southeast Region: São Paulo state – Ilha da Queimada Grande, SP - IBSP 12906, 2 larvae, *Psychosaura macrorhyncha*, 28.V.2015, coll Ricardo Augusto Dias*, **, ***.

***Foncesia ewingi* (Fonseca, 1932)**

Central-West Region: Mato Grosso state - Correntes, MT - IBSP 27, 1 larva holotype – (sin. *T. ewingi*), *X. merremii*, 13.IV.1932; IBSP 392, 3 Larvae paratypes, *X. merremii*, 30.IV.1932. **Southeast Region: São Paulo state - Birigui, SP -** IBSP 378, 3 larvae, *X. merremii*, 19.V.1932. **Penápolis, SP -** IBSP 335, 1 larva, *X. merremii*, 13.IV.1932. **Promissão, SP -** IBSP 331, 3 larvae, *E. aesculapii*, 30.VIII.1933; IBSP 4683, 3 larvae, *X. merremii*, 19.V.1932. **São Paulo, SP -** IBSP 329, 3 larvae paratypes, *X. merremii*, 03.VI.1932. **Sete Barras, SP -** IBSP 12071, 2 larvae, *R. ornata*, 12.XII.2013 coll Jairo Mendoza Roldan.

***Fonsecia coluberina* Radford, 1946**

Imphal, Manipur, India: IBSP 4365, 1 larva paratype, *Coelognathus radiatus* 10.V.1945, coll Charles D. Radford.

***Fonsecia anguina* Brennan &. Loomis, 1959**

North Region: Acre state – Iracema, AC- IBSP 14886, 5 larvae, *Erythrolamprus typhlus*, 28.X.2018, coll Flora Roncolatto Ortiz*, **, ***.

***Fonsecia travassosi* (Fonseca, 1936)**

Southeast Region: Rio de Janeiro state - Angra dos Reis, RJ - IBSP 30, 1 larva holotype – (sin. *T. travassosi*), *S. pullatus*, 13.II.1932.

***Eutrombicula hirsti* (Sambon, 1927)**

Imphal, Manipur, India: IBSP 4377, 1 larva, *C. radiatus* 17.V.1945, coll Charles D. Radford.

***Neotrombicula microti* (Ewing, 1928)**

Southeast Region: Paraná state, Ponta Grossa, PR - IBSP 4377, 1 larva, *Masticophis schotti*, 22.XI.1940, coll Aristoreris Teixeira Leão.

Cohort Eupodina

Superfamily Tydeoidea

Family Ereynetidae

***Lawrencarus braziliensis desantisi* Mauri e Alzuet, 1984**

Argentina: Averías, Santiago del Estero - MLP 4007/1, 1 female, Holotype, *Rhinella* sp., 10.I.1971, No collector; MLP 4007/2 1 male, Allotype, same collecting data as holotype; MLP 4007/5, 1 male, 1 female paratypes, same collecting data as holotype; MLP 4007/6, 2 females paratypes, same collecting data as holotype; MLP 4007/6-7, 5 males paratypes, same collecting data as holotype. **La Plata, Buenos Aires** - MLP 4007/8, 1 female paratype, *Rhinella arenarum*, 5.XI.1983, coll Mauri. **Las Cejas, Tucumán -** MLP 4007/13, 1 female paratype, *Rhinella schneideri*, 5.III.1971 coll Mauri-Alzuet; MLP 4007/14, 2 females paratypes, *R. schneideri*, 5.III.1971, coll Mauri-Alzuet; MLP 4007/15, male paratype, *R. schneideri*, 5.III.1971 coll Mauri-Alzuet. **La Posta, Córdoba -** MLP 4007/4, 2 nymphs, 1 male, 1 female paratypes, *Rhinella* sp., 20-IV-1971. Mauri coll; MLP 4007/9-10, 4 females paratypes, *Rhinella* sp., 20.IV.1971. coll Mauri. **Tigre, Buenos Aires -** MLP 4007/3, 1 larva, Paratype, *Rhinella* sp., 15.IX.1970. Mauri coll.

Order Sarcoptiformes

Suborder Oribatida

Family Trhypochthoniidae

***Archegozetes longisetosus* Aoki, 1965**

Northeast Region: Rio Grande do Norte state - UFERSA, Mossoró, RN - IBSP 12992, 8 females, 3 males, 5 nymphs, *Rhinella major*, 16.IV.2017, coll Josivania Soares Pereira*, **.

4.3 Morphological and taxonomical details

In this section 14 species of Tombidiformes mites, and one species of Sarcoptiformes mite, are detailed morphologically as follow. Species not mentioned in this section were detailed before (MENDOZA-ROLDAN, 2015.

Order TROMBIDIFORMES

Super cohort Eleutherengonides

Superfamily Cheyletoidea

Family Harpirhynchidae

4.3.1 *Ophioptes parkeri* Sambon, 1928 p. 141

Type material - Holotype male, *Erythrolamprus aesculapii* Linnaeus 1766, Buena Vista, Bolivia. Paratypes 2 females, *Erythrolamprus poecilogyrus* (Wied-Neuwied, 1825) and *Liophis anomalus* Günther, 1858, Brazil.

Synonym: *Ophioptes oudemansi* Sambon, 1928: p. 141; Fain 1964, p. 31; host *Paraphimophis rusticus* (COPE, 1878), Ajo, Argentina.

Diagnosis. Type species of the genus (Figure 15), and of the "*parkeri*" group due to a nude ventral seta (v') in the femur III. Adults legs chaetotaxy: tarsi (10-9-8-8); tibia (3-3-2-2); genu (3-3-0-0); femur (2-1-1-0); trochanter (1-1-2-2); coxa (1-1-1-0). Tibiae I – IV with a barbed seta (l') and the other nude (v'). 3 – 4 pairs of dorsal posterior setae in female (d1, d2, e1, e2). Pulvilles of male with 12 – 14 barbs. Comparative measurements in Table 8.

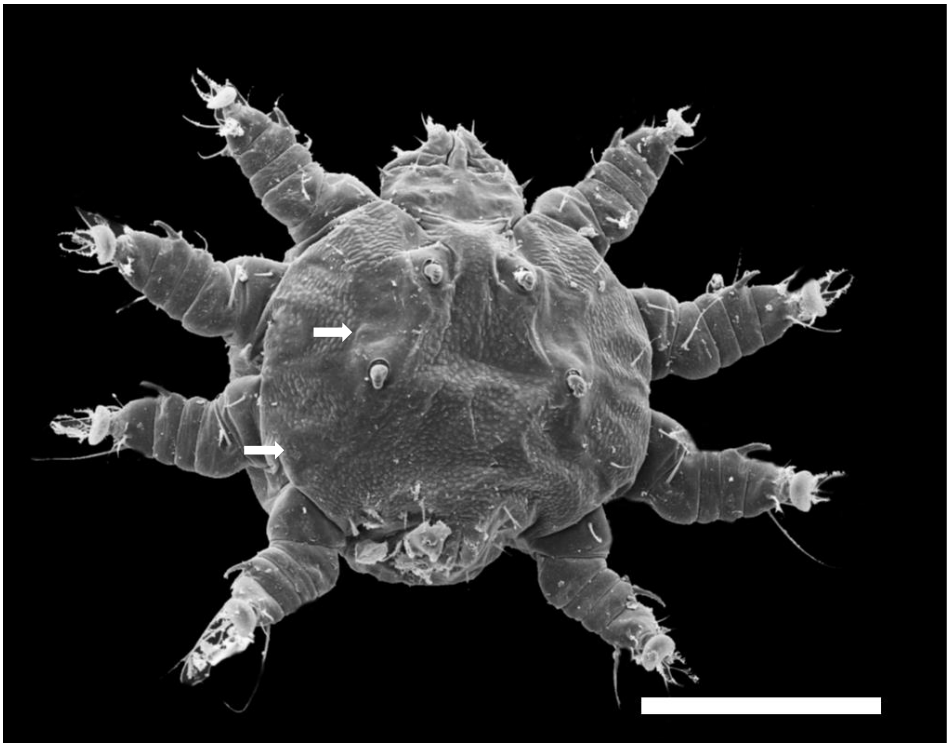
Table 8 – Comparative measurements of *Ophioptes parkeri*

	Gnathosoma				Idiosoma				
Type	**Ventro-basal setae**	**lateral-basal setae**	**tarsal anterior setae**	**tarsal posterior setae**	**Scapular setae**	**Dorsal anterior setae**	**Dorsal posterior setae**	**Genital setae**	**Coxal setae**
Holotype ♂	15 -18	12 -15	18	9 -11	13 -15	18 -30	8-10	-	18 - 20
Paratype ♀	18	18	10	15 -18	10 - 12	15	8 -10	11 -18	18 - 20
n = 2 ♀	17 - 18	18	9 - 11	15 - 17	10 - 12	14 - 16	8 -10	10 -15	17 - 20
EM ♀ = 10 mites	16 - 18	18	10.5 - 11	16 – 17	10 - 12	15 - 16	8 - 10	10 - 18	15 - 19

Source: (MENDOZA-ROLDAN, J. A., 2019)

Legend: Holotype and paratype according to Fain (1964); EM, examined material collected in this study.

Figure 15 – Scanning electron microscopy of female *Ophioptes parkeri*, natualae setae pointed by arrows.



Source: (MENDOZA-ROLDAN, J. A., 2015)

Legend: Scale bar 100 µm.

4.3.2 *Ophioptes tropicalis* (Ewing, 1933) p. 53

Type material – Lectotype 1 female, *Chironius carinatus* (Linnaeus, 1758) (U.S.N.M. no. 1081), 1031, from British Guiana (collected at Washington D.C.).
Synonym: *Ophioptes tropicalis* Allerd, 1958: p. 287.

Diagnosis. Only female is known (Figure 16A) and belongs to the "*parkeri*" group due to a nude ventral seta (v') in the femur III. Adults legs chaetotaxy: tarsi (6-9-8-4); tibia (3-3-2-2); genu (3-3-0-0); femur (2-1-1-0); trochanter (1-1-2-2); coxa (1-1-1-0). Pulvilles of female with 10 – 11 barbs (Figures 16 - 17). Gnathosoma (LG 130µm, WG 310µm) larger that *O. parkeri* (LG 115µm, WG 96µm), measurements of type in Table 9.

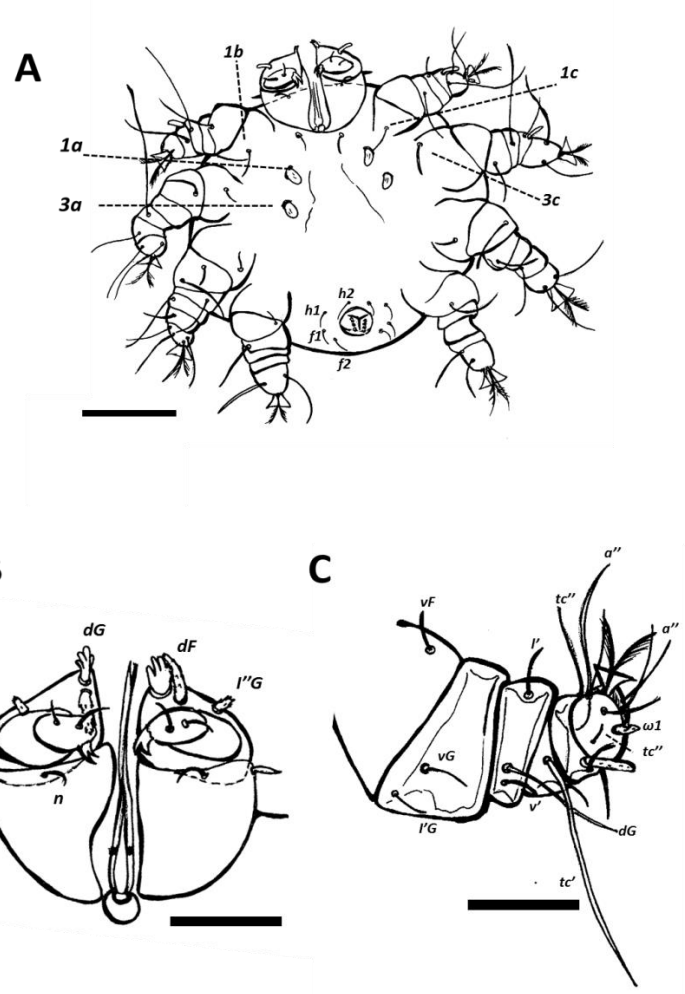
Table 9 – Type measurements of *Ophioptes tropicalis*

	Gnathosoma				Idiosoma				
Type	**Ventro-basal setae**	**lateral-basal setae**	**tarsal anterior setae**	**tarsal posterior setae**	**Scapular setae**	**Dorsal anterior setae**	**Dorsal posterior setae**	**Genital setae**	**Coxal setae**
lectotype ♀	16 -17	9 - 10	17	11 -12	12	35 - 43	-	12 -18	22

Source: (MENDOZA-ROLDAN, J. A., 2019)

Legend: Holotype and paratype according to Fain (1964); EM, examined material collected in this study.

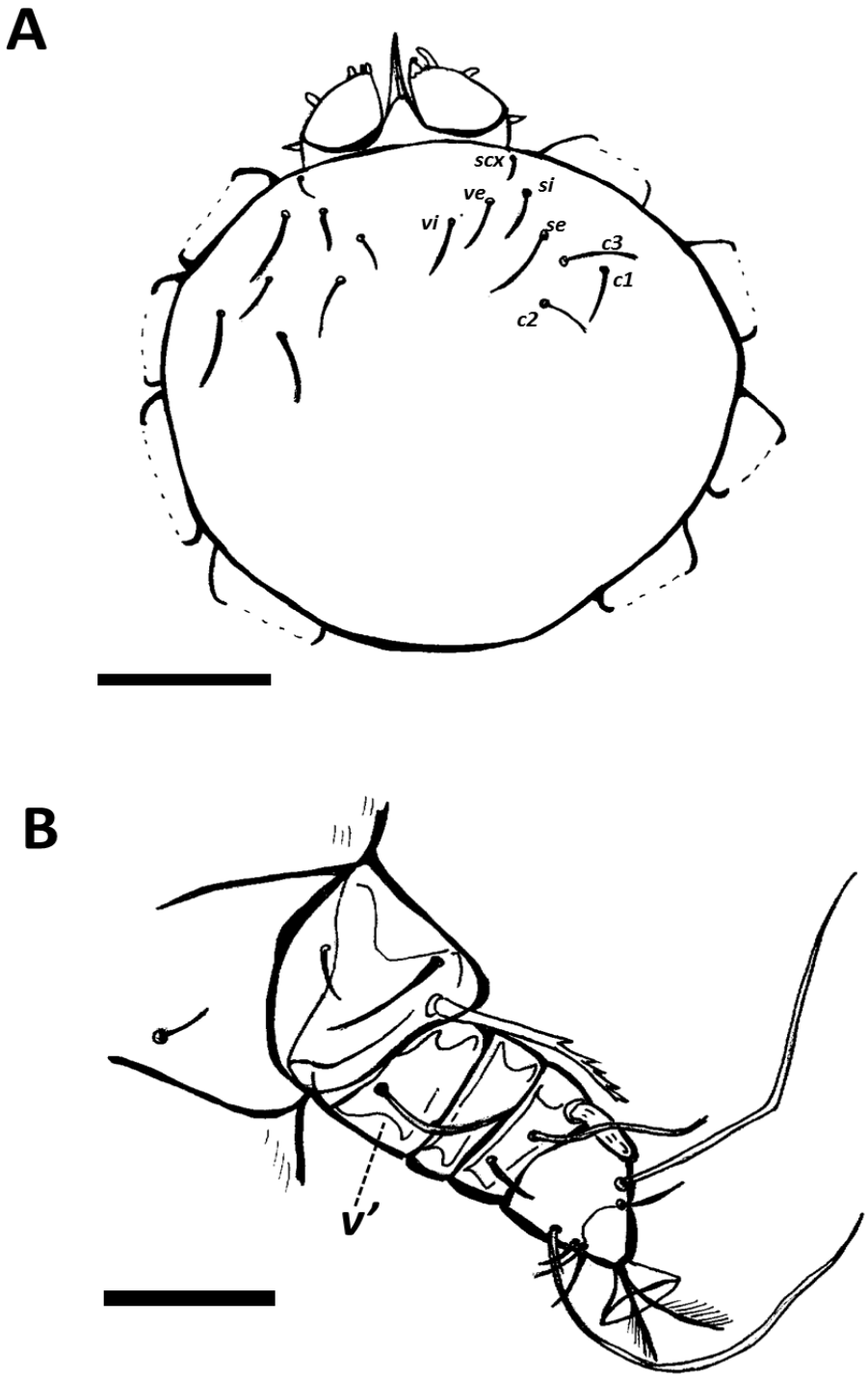
Figure 16- Illustrations of female *Ophioptes tropicalis*, ventral view, gnathosoma and Leg I



Source: (MENDOZA-ROLDAN, J. A., 2019)

Legend: A. female idiosoma ventral view; B. gnathosoma, ventral and dorsal view of male and female; C. leg I Abbreviations: dG: apical foliate seta; 1b: anterior setae; 1a, 3a: nautalae; h1, h2, f1, f2: genital setae; m: latero-basal setae; IT: tarsal anterior setae; I''G: tibial dorsal setae; dF: 564 ventral setae; n: ventro-basal setae. Scale bar: A, 100µm; B, 50µm; C, 50µm.

Figure 17- Illustrations of female *Ophioptes tropicalis*, dorsal view and leg III



Source: (MENDOZA-ROLDAN, J. A., 2019)

Legend: A. Idiosoma setae of female; B. leg III. Abbreviations: ve, vi, se, si, c1 – c3: dorsal anterior setae; scx: scapular setae; Scale bar: A, 100µm B, 50µm.

4.3.3 *Ophioptes dromicus* Allred, 1958 p. 107

Type material –Holotype 1 female, *Caraiba andreae* (Reinhardt & Lütken,1862) (cited as *Dromicus andreae orientalis*), from Banes, Oriente Province, Cuba. Collected by Grant Chapman, 1956. Paratypes 14 males, 8 females,1 nymph and 3 larvae, same host and locality. Deposited (National Museum of Natural History, Smithsonian Institution, Washington, D.C., U.S.A (USNM).

Diagnosis. This species belongs to the "*parkeri*" group due to a nude ventral seta (v') in the femur III (Figure 18), and differs from te other species of the genus by having a short latero-basal setae (*m*), solenidia in genus III, and all the setae from tibiae are barbed (Figure 19). Adults legs chaetotaxy: tarsi (9-9-8-8); tibia (3-3-2-2); genu (3-3-0-0); femur (2-1-1-0); trochanter (1-1-2-2); coxa (1-1-1-0). Pulvilles with 10 - 12 barbs. Measurements of type in Table 10.

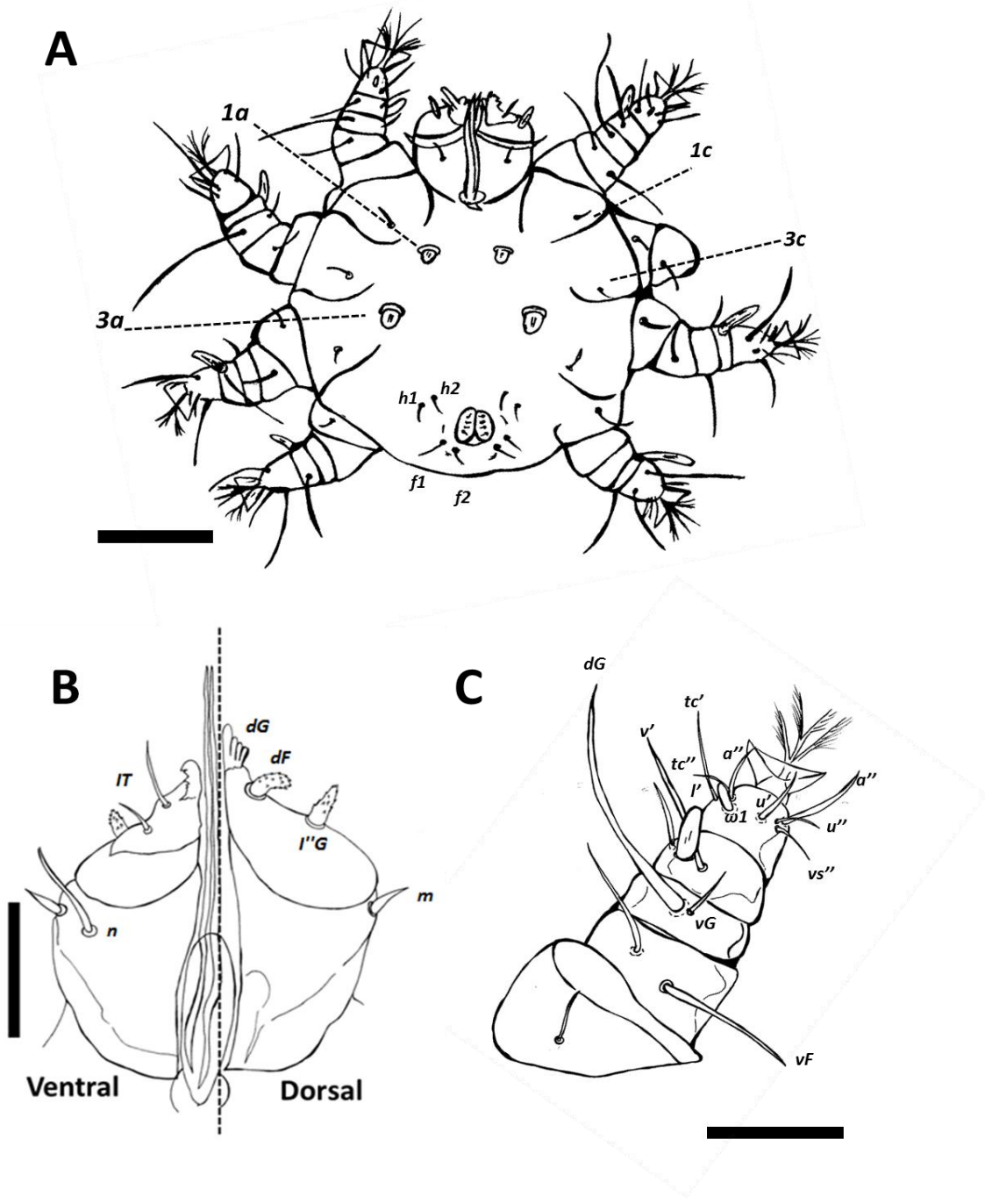
Table 10 – Comparative measurements of *Ophioptes dromicus*

	Gnathosoma				**Idiosoma**				
Type	**Ventro-basal setae**	**lateral-basal setae**	**tarsal anterior setae**	**tarsal posterior setae**	**Scapular setae**	**Dorsal anterior setae**	**Dorsal posterior setae**	**Genital setae**	**Coxal setae**
Paratype ♀	23	7	19	11	12 - 13	25 - 32	7 - 8	10 - 18	18 - 19

Source: (MENDOZA-ROLDAN, J. A., 2019)

Legend: Paratype according to Fain (1964).

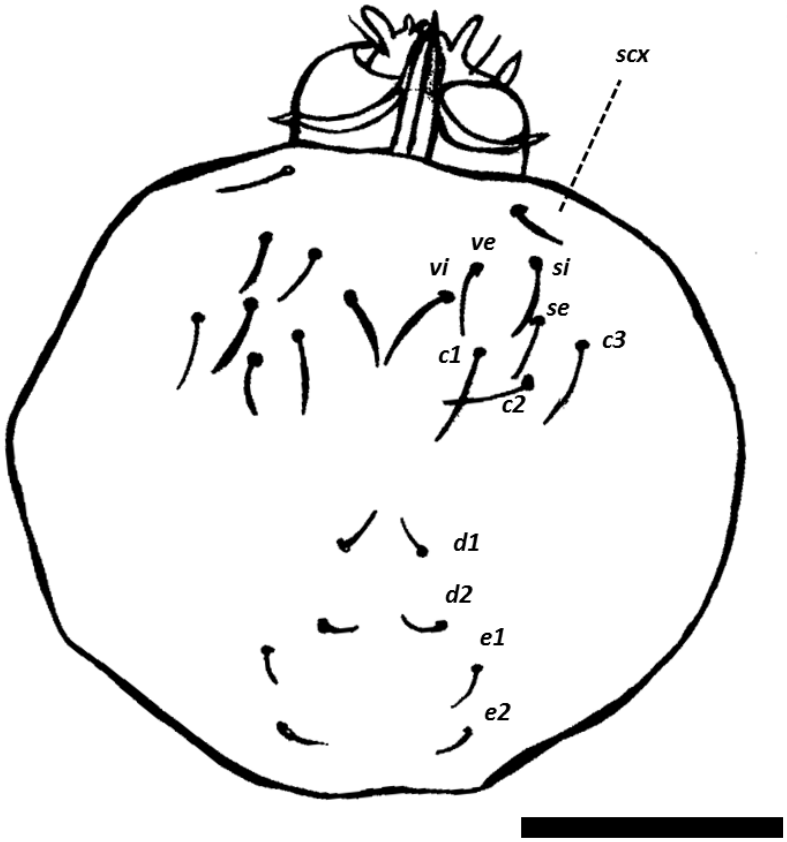
Figure 18 - Illustrations of female *Ophioptes dromicus*, ventral view, gnathosoma and Leg I



Source: (MENDOZA-ROLDAN, J. A., 2019)

Legend: A. female idiosoma ventral view; B. gnathosoma, ventral and dorsal view of male and female; C. leg I Abbreviations: dG: apical foliate seta; 1b: anterior setae; 1a, 3a: nautalae; h1, h2, f1, f2: genital setae; m: latero-basal setae; IT: tarsal anterior setae; I''G: tibial dorsal setae; dF: 564 ventral setae; n: ventro-basal setae. Scale bar: A, 100µm; B, 50µm; C, 50µm.

Figure 19- Illustrations of female *Ophioptes dromicus*, dorsal view



Source: (MENDOZA-ROLDAN, J. A., 2019)

Legend: Idiosoma setae of female. Abbreviations: ve, vi, se, si, c1 – c3; dorsal anterior setae; scx: scapular setae; Scale bar: A, 100µm.

4.3.4 *Ophioptes ekans* Mendoza-Roldan & Barros-Battesti, 2017 p. 1

Type material – Holotype 1 female, (IBSP 12078) 2 female, 2 male and 2 nymphs paratypes (IBSP 12079), from a single female specimen of *Crotalus durissus terrificus* (Linnaeus, 1758), (IBSP 85008) Brazil: Campo Limpo Paulista, State of São Paulo, 6 January 2014, coll. Jairo Mendoza-Roldan. The entire type series is deposited in the Acari collection of the Laboratório Especial de Coleções Zoológicas of the Instituto Butantan, São Paulo, State of São Paulo, Brazil.

Diagnosis. This species belongs to the "*parkeri*" group due to a nude ventral seta (v') in the femur III. It differs from the other five species known in the "parkeri" group, *O. brevipilis*, *O. dromicus*, *O. longipilis*, *O. parkeri*, and *O. tropicalis* by the presence in all stages of long ventro-basal (*n*) setae (2 to 3 times longer than in other species), and by 3 pair of genital-anal setae in females (Figure 20). The new species is closest to *O. parkeri* due to their similar size and leg chetotaxy. *O. ekans* differs from *O. parkeri* species due to the body lengths, including gnathosoma of the male and female, which are 357 – 559 and 360–380 and, respectively (vs. 330–350 and 380–390 long in *O. parkeri*). Leg chaetotaxy is tarsus (10 – 7 – 5- 5) in female and (7 – 7- 5- 5) in male; tibia (3-2-2-2) in female and (2-2-2-2) in male; genu 193 (3-3-0-0); femur (2-1-1-0); trochanter (1-1-2-2); coxa (1-1-1-0) . Comparative measurements in Table 11.

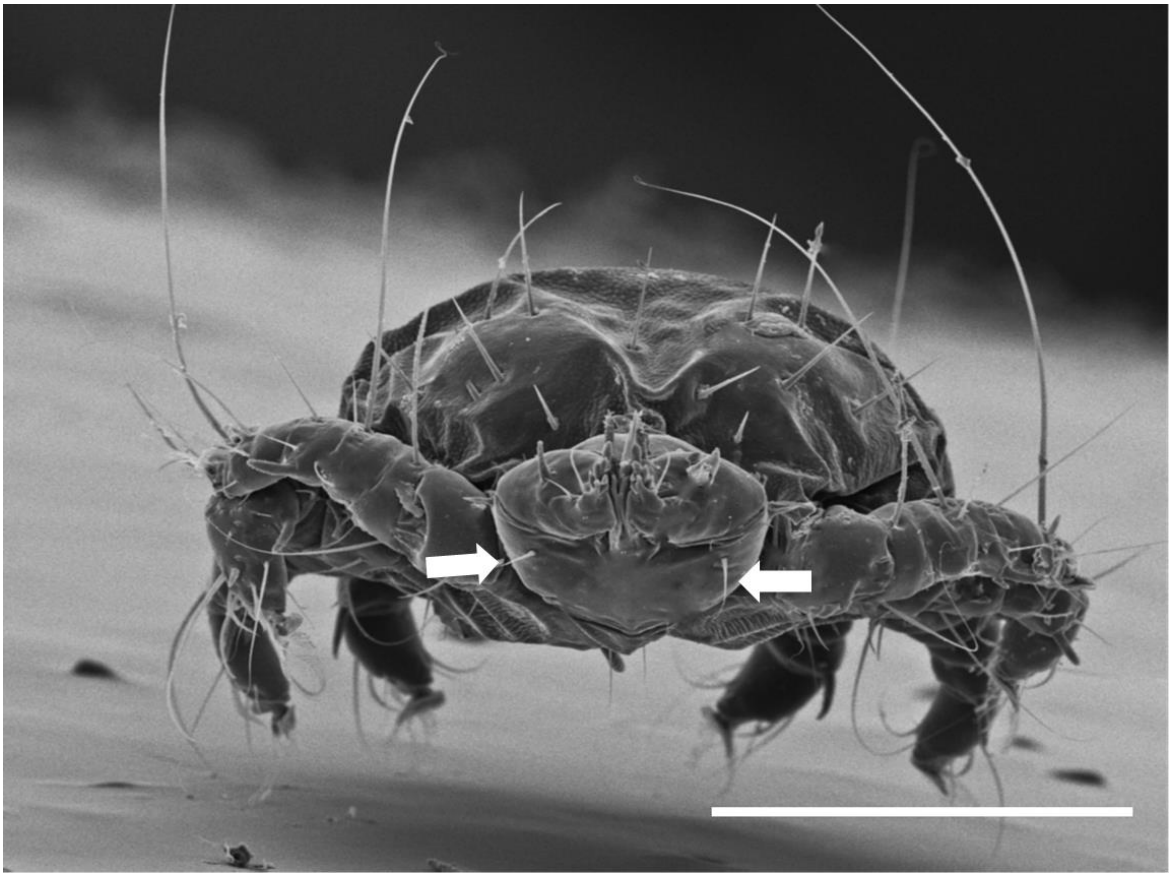
Table 11 – Comparative measurements of *Ophioptes ekans*

	Gnathosoma				**Idiosoma**				
Type	**Ventro-basal setae**	**lateral-basal setae**	**tarsal anterior setae**	**tarsal posterior setae**	**Scapular setae**	**Dorsal anterior setae**	**Dorsal posterior setae**	**Genital setae**	**Coxal setae**
Holotype ♀	39 - 40	13 - 14	27 -28	15 - 16	11 - 12	43 - 50	12 -13	22 - 23	8 - 9
Paratype ♂	24 - 25	13	18 - 19	14 - 16	12 - 13	11 - 34	10 - 13	-	22 - 24
EM ♀ = 6 mites	40	13	26 - 27	16	11 - 12	44 - 49	12	22 - 23	8 - 10

Source: (MENDOZA-ROLDAN, J. A., 2019)

Legend: Holotype and paratype according to Mendoza-Roldan & Barros-Battesti (2017); EM, examined material collected in this study.

Figure 20 – Scanning electron microscopy of female *Ophioptes ekans*, *n* setae pointed by arrows.



Source: (MENDOZA-ROLDAN, J. A., 2017)

Legend: Scale bar 100 µm.

4.3.5 Key of species of *Ophioptes* of the Neotropical region

Females

1(0).
- Genital plate near coxae I and I.....genus ***Afrophioptes***
- Genital plate near coxae IV.....genus ***Ophioptes***……**(2)**

2(1).
- Ventral barbed seta in femur III, present.....……...……***parkeri*** group…….. **(3)**
- Ventral barbed seta in femur III, absent…….. ……..…… ***schoutedeni*** group

3(2).
- Seta *v'* of genus II, spined shaped. A pair of genital setae inside the genital plate (*f1*, *f2*) ……...***Ophioptes brevipilis***
- Seta *v'* of genus II, piriform. A pair of genital setae around the posterior area of the genital plate (*f1*, *f2*) ……**……(4)**

4(3).
- Laterobasal setae (*m*), piliform…….....……...…….....……***Ophioptes longipilis***
- Laterobasal setae (*m*), robust and spine-like …….....**(5)**

5(4)
- Ventro-basal setae (*n*) > 39 µm, 3 pair of genital-anal setae. Hosts vipers…….....***Ophioptes ekans***
- Ventro-basal setae (*n*) < 39 µm, 4 pair of genital-anal setae. Hosts Colubrids ……...**(6)**

6(5)
- WG > 126 µm. Laterobasal setae (*m*) 10 µm. Distributed in British Guiana…..……***Ophioptes tropicalis***
  WG < 105 µm. Laterobasal setae (*m*) > 12 µm. Distributed in Argentina, Bolivia and Brazil……***Ophioptes parkeri***
- WG >12 µm. Laterobasal setae (*m*) < 7 µm. Distributed in Cuba……***Ophioptes dromicus***

Males

1(0).
- Genital plate near coxae I and I.....genus ***Afrophioptes***
- Genital plate near coxae IV.....genus ***Ophioptes***…….... **(2)**

2(1).
- Dorsal- posterior setae, absent. L < 304 µm.....***Ophioptes brevipilis***
- Dorsal- posterior setae, absent. L >304 µm ….....**(3)**

3(2).
- Laterobasal setae (*m*), piliform.....***Ophioptes longipilis***
- Laterobasal setae (*m*), robust and spine-like……...**(4)**

4(3)
- Laterobasal setae (*m*) > 13 µm, ventro-basal setae (*n*) > 25 µm, hosts vipers.....***Ophioptes ekans***
- Laterobasal setae (*m*) < 7 µm, ventro-basal setae (*n*) < 23 µm, colubrids.....***Ophioptes dromicus***
- Laterobasal setae (*m*) >15 µm, ventro-basal setae (*n*) < 18 µm, hosts colubrids.....……...***Ophioptes parkeri***

Superfamily Pterygosomatoidea

Family Pterygosomatidae

4.3.6 *Bertrandiella jimenezi* (Paredes-León & Morales-Malacara, 2009) p. 443

Type material - Holotype female (CNAC005885), *Phyllodactylus bordai* Taylor, 1942, from. Zapotitlán de las Salinas, Puebla, México. Deposited at Colección Nacional de Ácaros, Instituto de Biología, UNAM, Distrito Federal, Mexico (CNAC). Paratypes female, male, deutonymphal and larva deposited at CNAC and the Acarology Laboratory, Ohio State University. Columbus, Ohio, USA (OSAL).
Synonym: *Hirstiella jimenezi* Paredes-León and Morales-Malacara, 2009

Diagnosis. Seta *ft* shorter than solenidion *ω*2 on tarsus I. Female (Figure 21) - Prodorsal scutum triangular in shape, with very acute posterior margin, and 3 pairs of peripectinate setae (*vi*, *ve* and *sci*) (Figure 22). Male - Prodorsal scutum trapezoid in shape with anterior margin wider than posterior and with 4 pairs of long peripectinate setae (*vi*, *ve*, *sci* and *c1*). Protruding stigma between gnathosoma and coxa I.

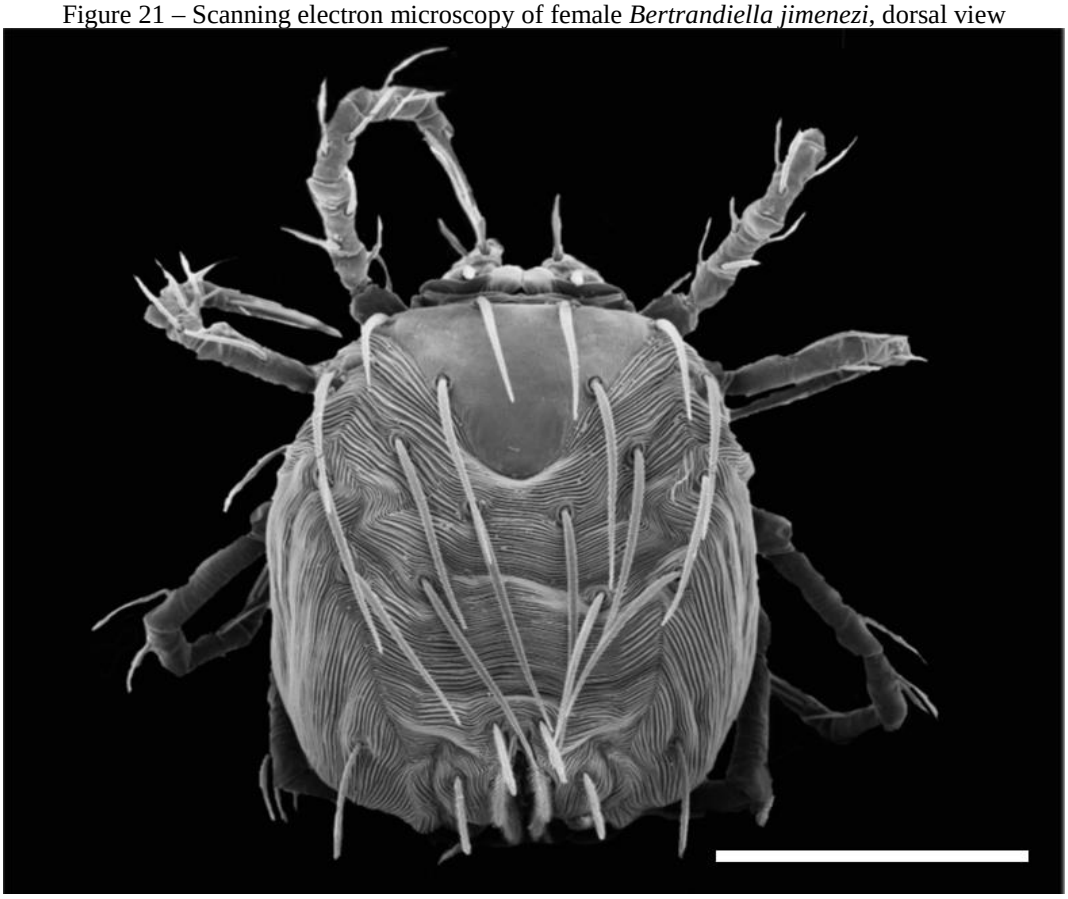
4.3.7 *Geckobia hemidactyli* Lawrence, 1936 p. 14

Type material – Holotype (Lost) - host *Hemidactylus tasmani* Hewitt 1932, Driesourcein, Rhodesia (nowadays Zimbawe). Type material seemed to be deposited initially in the Iziko museum, Cape Town, South Africa.

Diagnosis. Female with hypertrichous idiosoma (Figure 23), prodorsal scutum with, 15 – 17 pairs of pectinated short setae, one pair of eyes in the antero-lateral margins of the prodorsal scutum. Chaetotaxy of the trochanters and tibiae of legs I- IV corresonding with group 1 or *haplodactyli* group (*G. haplodactyli* Womersley, 1941): 1-1-1-1, 3-2-2-2, 1(k)-0-0-1, 55-5-5; chaetotaxy of tarsi corresponding to group I-IV A: 14(ω)-10(ω)-10-10; Coxae I-IV: 2-2-2-3. Setae *v'* on the genus of leg IV. Protruding stigma between gnathosoma and coxa I.

Egg (**IBSP 12911).** Eggs were found being laied by a female *G. hemidactyli* on a female *H. mabouia*. Length: 179 µm, width: 159 µm. eggs were round shaped and apparently fertilized, which confirms oviparous reproduction in this species (Figure 24).

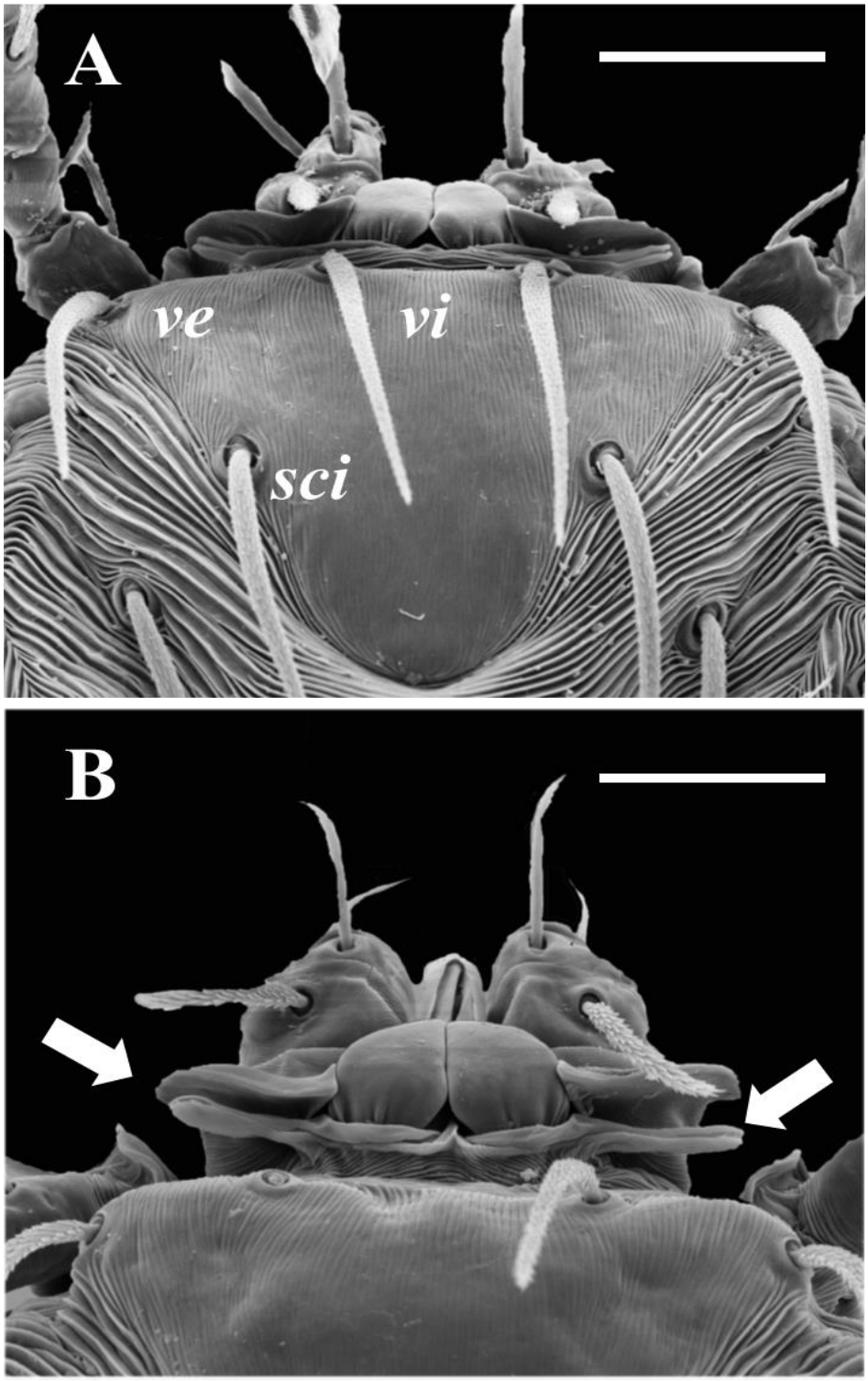
Figure 21 – Scanning electron microscopy of female *Bertrandiella jimenezi*, dorsal view



Source: (MENDOZA-ROLDAN, J. A., 2018)

Legend: Prodorsal scutum triangular, with 3 pairs of peripectinate setae. Scale bar 200 µm.

Figure 22 – Scanning electron microscopy of female *Bertrandiella jimenezi*



Source: (MENDOZA-ROLDAN, J. A., 2018)

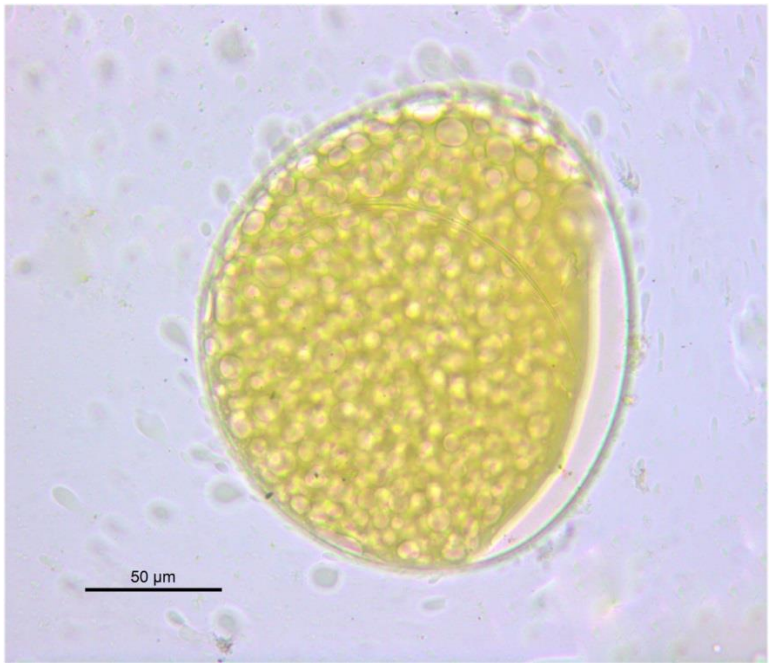
Legend: A. Prodorsal scutum triangular. B Protruding stigma between gnathosoma and coxa I. Abbreviations *vi*, *ve* and *sci*: 3 pairs of peripectinate setae. Scale bar A, 50 µm; B, 40 µm.

Figure 23 – Scanning electron microscopy of female *Geckobia hemidactyli*

Source: (MENDOZA-ROLDAN, J. A., 2016)

Legend: hypertrichous idiosoma. Scale bar 200µm

Figure 24 – Optic microscopy of egg of *Geckobia hemidactyli*



Source: (MENDOZA-ROLDAN, J. A., 2018)

Legend: Scale bar 50 µm

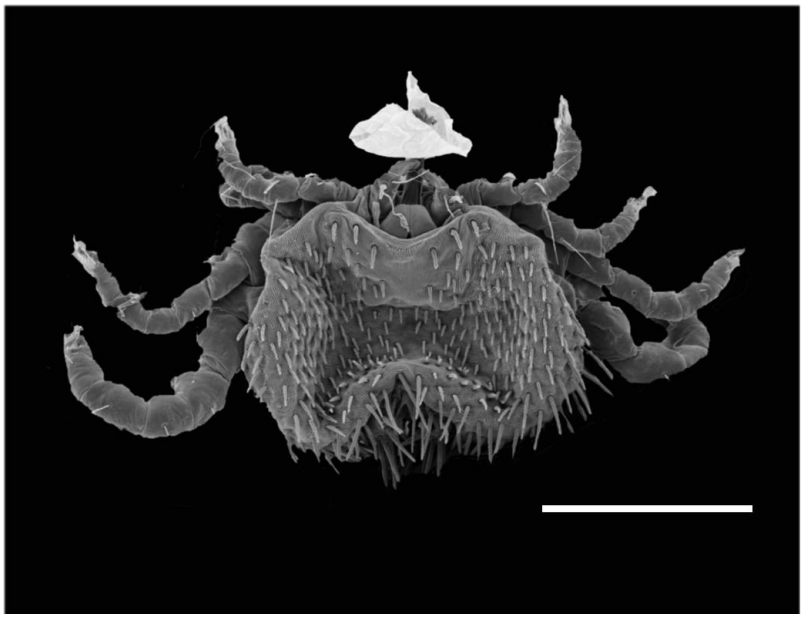
4.3.8 *Geckobia bataviensis* (Vitzthum, 1926) p. 122

Type material - One adult female lectotype, One nymph, paralectotype. *Hemidactylus frenatus* (Duméril & Bibron, 1836), from Batavia (Jakarta), deposited in the Zoologische Staatssammlung, München (ZSM).

Synonyms: *Geckobia gleadoviana* Hirst, 1926: 185; Jack, 1964: 8; *Geckobia nepalii* Hiregaudar, Joshee & Soman, 1959: 66; *Geckobia*. *cosymboti* Cuy, 1979: 156.

Diagnosis. Species with very high morphological variability, given by three species which are synonyms, but could be subspecies. This species belongs to the group of species with short enlarged setae on the scutum (Figure 25). The eyes are borne on extensions of the dorsal scutum. In the nymph, the eyes are on unisetose scutumlets free of the dorsal scutum. Hypertrichous idiosoma (> 300), lack of short setae near posterior border of scutum, palpal femur with thin slightly ciliate setae. 5 setae on tibiae I—IV. Dorsal genual seta on legs I and IV are absent or strongly reduced. Genus I and IV without setae. Protruding stigma between gnathosoma and coxa I (Figure 26).

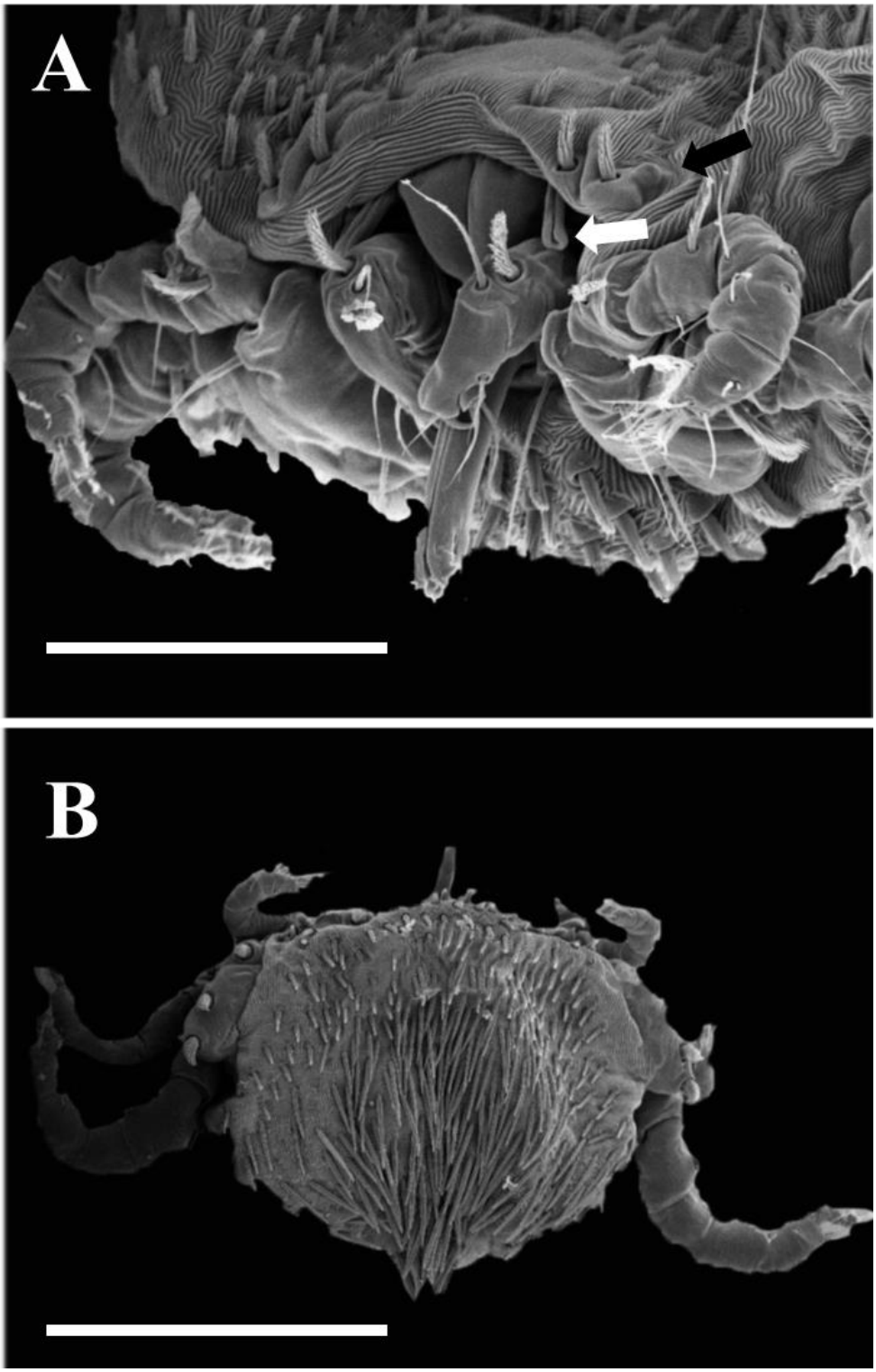
Figure 25 – Scanning electron microscopy of female *Geckobia bataviensis*



Source: (MENDOZA-ROLDAN, J. A., 2019)

Legend: short enlarged setae on the scutum. Scale bar 200µm.

Figure 26 – Scanning electron microscopy of female *Geckobia bataviensis*



Source: (MENDOZA-ROLDAN, J. A., 2018)

Legend: A. Gnathosoma. Black arrow showing eyes, white arrow showing protruding stigma between gnathosoma and coxa I. B. Ventral view of female with hypertrichous idiosoma. Scale bar A, 50 µm; B, 200 µm.

4.3.9 *Geckobiella harrisi* Davidson, 1958 p. 75

Type material - Holotype female (USNMC 1860) and male allotype (USNMC), *Plica plica* (Linnaeus 1758). Santarém, Pará, Brazil.

Synonyms: *Geckobia gleadoviana* Hirst, 1926: 185; Jack, 1964: 8; *Geckobia nepalii* Hiregaudar, Joshee & Soman, 1959: 66; *Geckobia cosymboti* Cuy, 1979: 156.

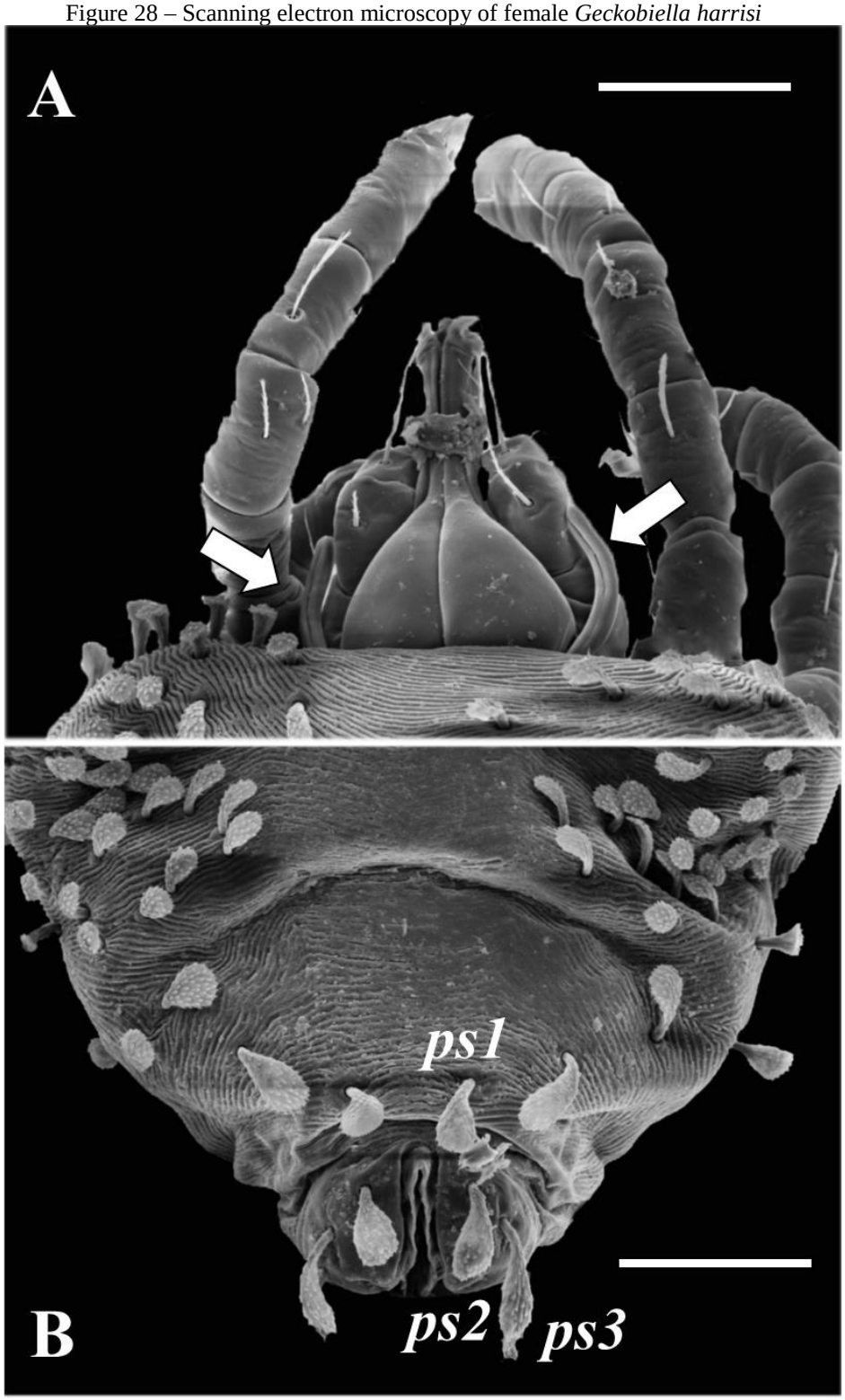
Diagnosis. Hypertrichous idiosoma. Female. Idiosoma laterally compressed (Figure 27); dorsal setae short club-like, occurring in patches; short peritremes which do not extend to second palpal segment. Setae ps1–2 spinose spatulate (club-like) and ps3 sparsely barbed (Figure 28). Male. Idiosoma dorso-ventrally flattened; club-like setae present, most abundant anteriorly on margin of dorsum. Specific ectoparasite of Tropiduridae lizards.

Figure 27– Scanning electron microscopy of female *Geckobiella harrisi*, lateral view

Source: (MENDOZA-ROLDAN, J. A., 2019)

Legend: Hypertrichous idiosoma. Idiosoma laterally compressed. Scale bar 200µm.

Figure 28 – Scanning electron microscopy of female *Geckobiella harrisi*



Source: (MENDOZA-ROLDAN, J. A., 2019)

Legend: A. Gnathosoma. Protruding stigma between gnathosoma and coxa I. B. Dorsal view of genital area (genital setae ps1–2 and ps3). Scale bar A, B 50 µm.

Super cohort Anystina

Cohort Parasitengona

Superfamily Trombidioidea

Family Leeuwenhoekiidae

4.3.10 *Hannemania achalai* Alzuet & Mauri, 1987 p. 114

Type material – Larva holotype (MLP 4006/1), *Pleurodema* sp. Larvae paratypes, *Pleurodema kriegi* (Müller, 1926), *Odontophrynus occidentalis* (Berg, 1896), and *Odontophrynus* sp. from Pampa de Achala, Córdoba, Argentina.

Diagnosis. SIF = 5B–B–3–2111.0000; fPp = B/B/BBB. Pc = 3; Gn = 2; fSc = PL > AL ≥ AM; PL/SB; fCx = 2.1.1; fSt = 0.1; DS = 53 – 75; VS = 49 – 69; NDV = 132; Ip = 765 – 865; AW = 50 – 60; PW = 65 – 80; SB = 20 – 30; ASB = 45 – 55; PSB = 20 – 25; SD = 75 – 80; AP = 15 – 20; AM = 30 – 40; AL = 30 – 40; PL = 60 – 70; S = 75 – 115; H = 40 – 45; Dmin = 30 – 45; Dmax = 50 – 65; Vmin = 30 – 35; Vmax = 35 – 45; pa = 280 – 320; pm = 240 – 275; pp = 250 – 290. Can be separated of the other species of the genus by having 2 - 4 genuala on leg I. (Table 12 and Figure 29– 31).

Gnathosoma. Palpal claw trifurcate, galeala branched; cheliceral blade expanded distally with a series of teeth. Gnathobasal setae branched.

Idiosoma. Eyes 2/ 2, Scutum with naso. Ventrally with only one pair of posterior sternal setae.

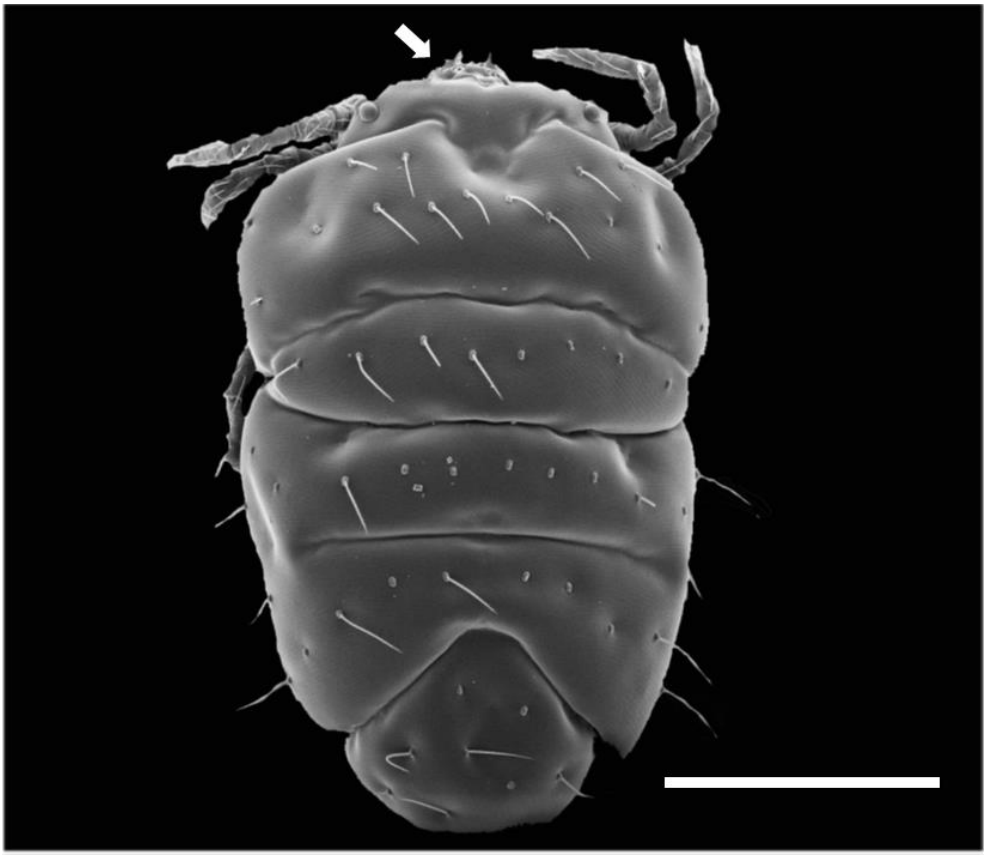
Legs. 6-6-6.

Table 12 – Morphometrics of 10 larvae of *Hannemania achalai*

	AW	PW	SB	ASB	PSB	SD	AP	AM	AL	PL	H
MIN	52	63	24	46	24	77	15	30	30	66	40
MAX	60	79	27	55	25	79	20	40	40	70	44
Mean	56	71	25.5	50.5	24.5	78	17.5	35	35	68	42
SD	4	8	1.5	4.5	0.5	1	2.5	5	5	2	2
Holotype	50 - 60	65 - 80	20 – 30	45 - 55	20- 25	75 - 80	15 -20	30 - 40	30 - 40	60 - 70	40 - 45

Source: (MENDOZA-ROLDAN, J. A., 2019)

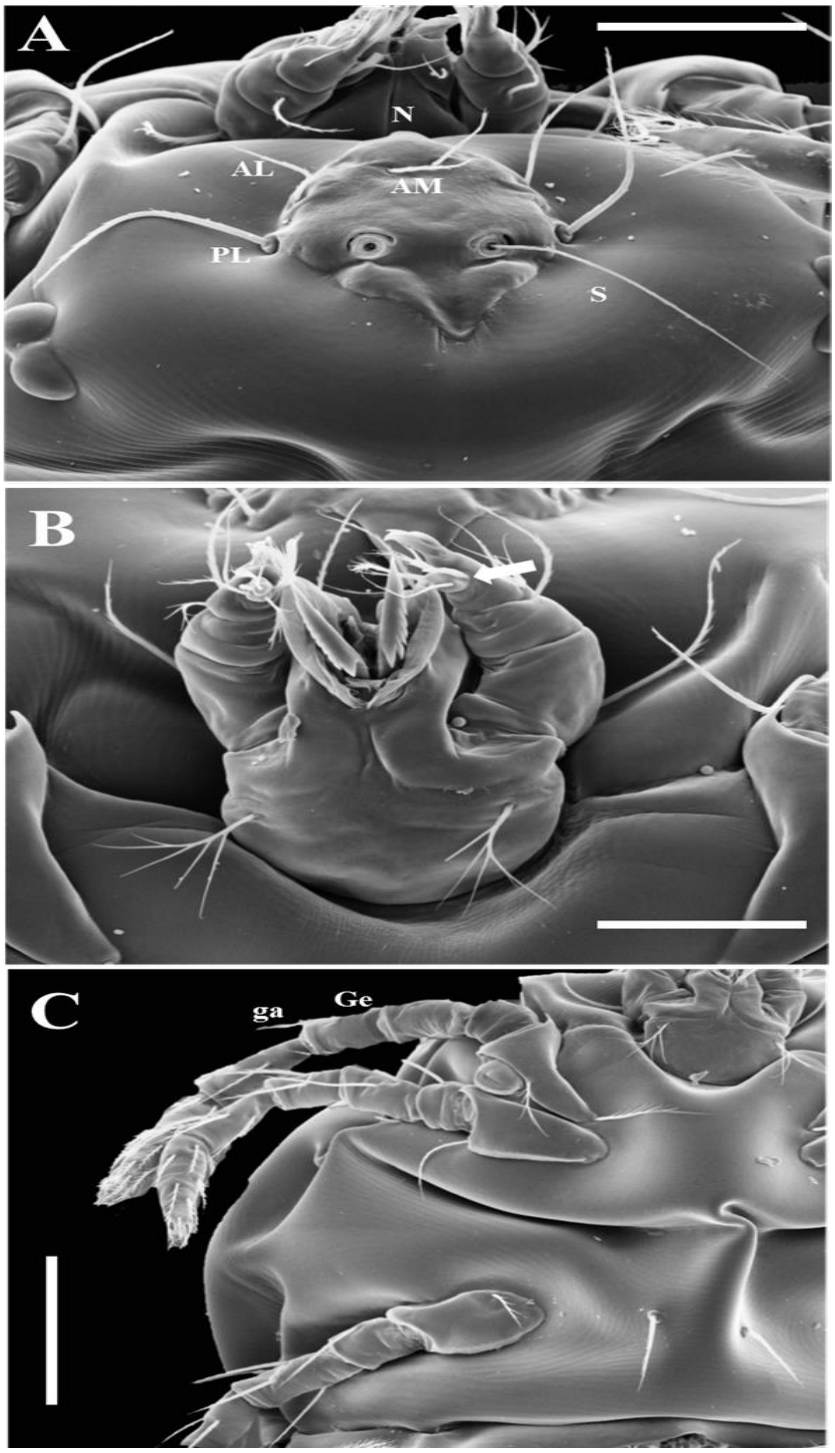
Figure 29 – Scanning electron microscopy of larva *Hannemania achalai*, dorsal view



Source: (MENDOZA-ROLDAN, J. A., 2019)

Legend: white arrow showing the dorsal scutum. Scale bar 200µm.

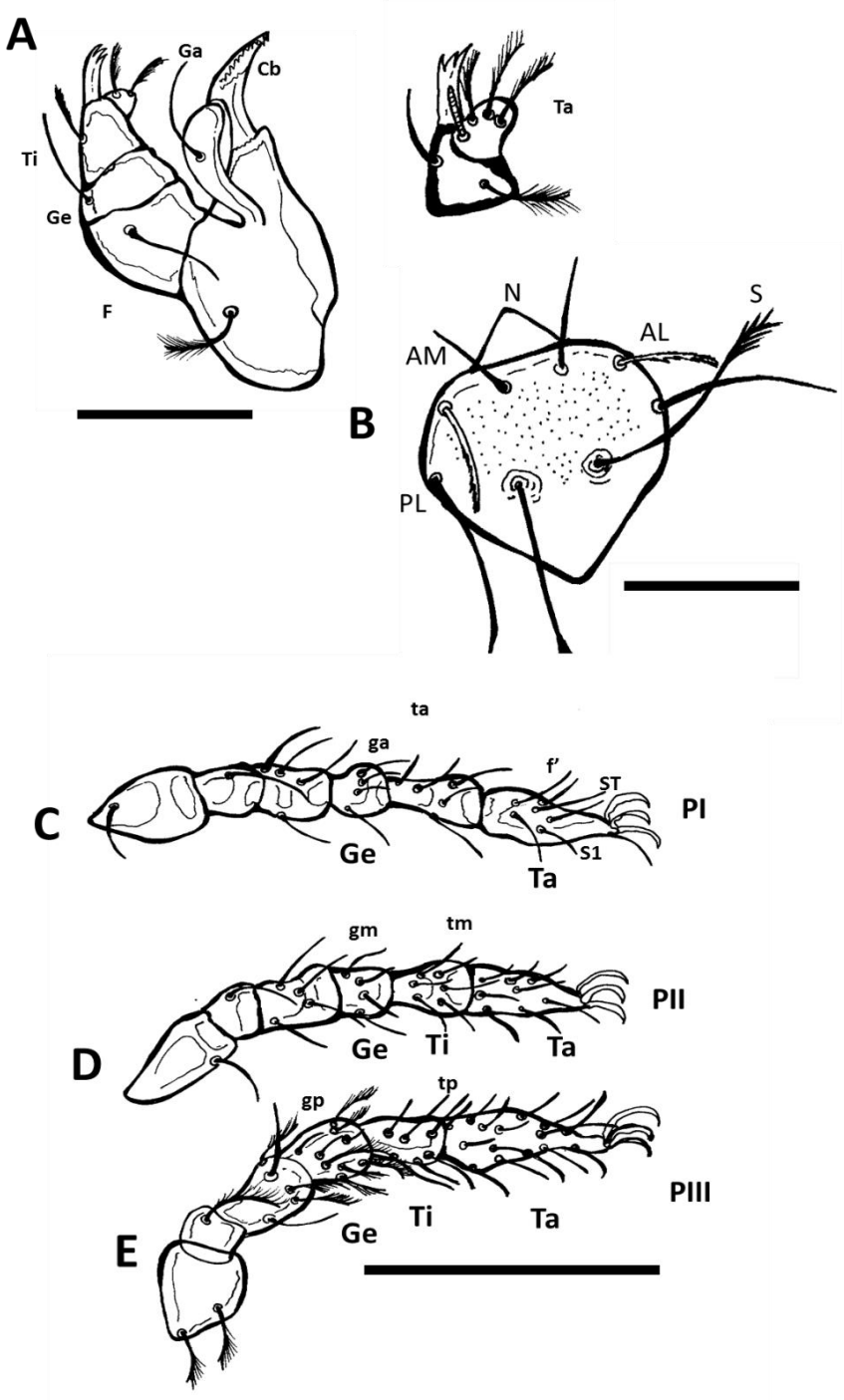
Figure 30 – Scanning electron microscopy of larva *Hannemania achalai*



Source: (MENDOZA-ROLDAN, J. A., 2019)

Legend: A. Dorsal scutum; B Gnathosoma, arrow showing palpal tarsus; C. Leg I ventral view. Abbreviations: AM: anteromedial seta; AL: anterolateral seta; ga: genuala; Ge: genus; N: naso; PL: posterolateral seta; S: sensilla. Scale bar: A, 50 µm; B, 40 µm; C, 50 µm.

Figure 31 – Illustrations with morphological features of larva *Hannemania achalai*



Source: (MENDOZA-ROLDAN, J. A., 2018)

Legend: A. gnathosoma, dorsal view and palp tarsus, ventral view; B. dorsal scutum; C. Leg I; D. Leg II; E. Leg III. Abbreviations: AM: anteromedial seta; AL: anterolateral seta; Cb: chelicera; ga: genuala leg I; gm: genuala leg II; F: femur; f': microtarsala leg I; Ga: galeala; Ge: genus; gp: genuala leg III; N: naso; PL: posterolateral seta; S1: tarsala leg I; S2: tarsala leg II; ST: subterminala leg I; µta: microtibiala leg I; Ta: tarso; ta: tibiala leg I; tm: tibiala leg II; tp: tibiala leg III; Ti: tibia. Scale bar: A, 50 µm; B, 30 µm; C, D, E 50 µm.

4.3.11 *Hannemania hepatica* Fonseca, 1935 p. 49

Type material – Larva holotype (IBSP 31), *Leptodactylus latrans* (Steffen, 1815), Instituto Butantan, São Paulo, 28.X.1933

Diagnosis. SIF: 5BS-N-3-4111.0000; fPp: B/B/BBN; fCx: 1.1.1; fSc: PL>AL>AM. Can be separated of the other species of the genus by having 4 – 5 genuala um leg I (Table 13 and Figure 32-33).

Gnathosoma. Chelicera trifurcated, tarsus of palp 5BS; palpal claw trifurcate. Palp setae formula B/B/BBN.

Idiosoma. Ellipsoidal. Eyes 2/2. Scutum with naso, one pair of humeral setae; anus at the same height as the ventral setae.

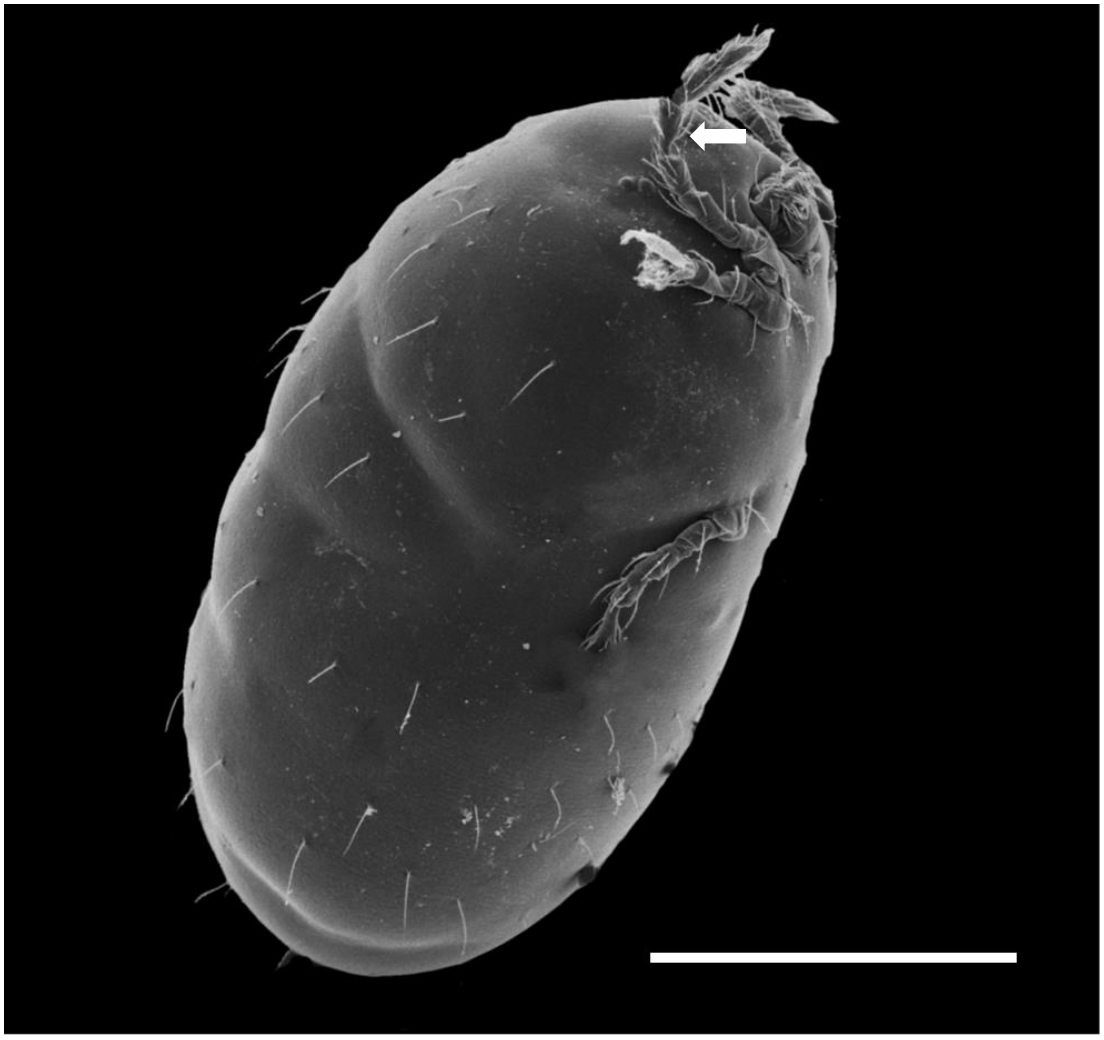
Legs. 6-6-6.

Table 13 – Morphometrics of 10 larvae of *Hannemania hepatica*

	AW	PW	SB	AS B	PSB	SD	P-PL	AP	AM	AL	PL	H
MIN	49	60	24	16	6	22	9	10	19	15	34	40
MAX	58	66	27	29	12	35	29	12	22	36	62	42
Mean	53.5	63	25.5	22.5	9	28.5	19	11	20.5	25.5	48	41
SD	4.5	3	1.5	6.5	3	6.5	10	1	1.5	10.5	14	1
Holotype	53.11	67.95	28.13	36.53	8.07	44.6	23.3	15.98	15.5	25.94	41.32	42.27

Source: (MENDOZA-ROLDAN, J. A., 2019)

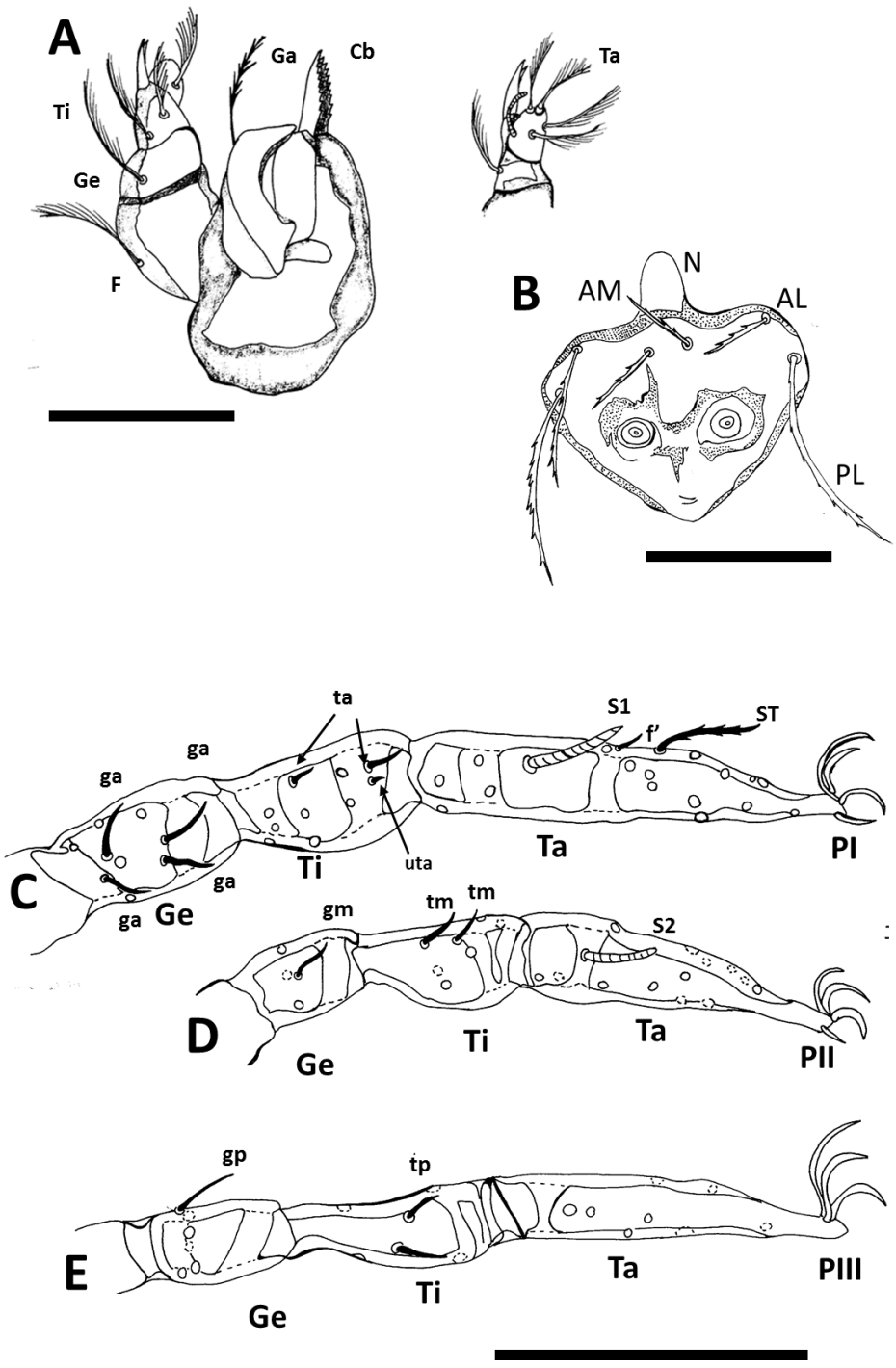
Figure 32 – Scanning electron microscopy of larva *Hannemania hepatica*, lateral view



Source: (MENDOZA-ROLDAN, J. A., 2019)

Legend: white arrow showing the dorsal scutum. Scale bar 100 µm.

Figure 33 – Illustrations with morphological features of larva *Hannemania hepatica*



Source: (MENDOZA-ROLDAN, J. A., 2017)

Legend: A. gnathosoma, dorsal view and palp tarsus, ventral view; B. dorsal scutum; C. Leg I; D. Leg II; E. Leg III. Abbreviations: AM: anteromedial seta; Al: anterolateral seta; Cb: chelicera; ga: genuala leg I; gm: genuala leg II; F: femur; f': microtarsala leg I; Ga: galeala; Ge: genus; gp: genuala leg III; N: naso; PL: posterolateral seta; S1: tarsala leg I; S2: tarsala leg II; ST: subterminala leg I; µta: microtibiala leg I; Ta: tarso; ta: tibiala leg I; tm: tibiala leg II; tp: tibiala leg III; Ti: tibia. Scale bar: A, 50 µm; B, 30 µm; C, D, E 50 µm.

Family Trombiculidae

4.3.12 *Eutrombicula alfreddugesi* (Oudemans, 1910)

Type material - *Leptus irritans* (Riley, 1873), *Homo sapiens sapiens*, from United States.

Synonyms: *Tretanychus tlalsahuate* Murray, 1877; *Microthombidium alfreddugesi* Oudemans, 1910: 84; *Microthombidium tlalzahuatl* Oudemans, 1912: 18; *Trombicula cinnabaris* Ewing, 1920; *Trombicula tlalzahuatl* Ewing, 1923; *Trombicula irritans* André, 1930; *Trombicula vanomereni* Shierbeck, 1937; *Leptus rileyi* Oudemans, 1939: 80; *Trombicula alfreddugesi* Fitch, 1954; *Eutrombicula alfreduggesi* Ewing, 1939; Hoffman, 1949; Brennan & Yunker, 1966; Vercammen-Granjean, 1968; Loomis, 1969; Brennan, 1970; Brennan & Goff, 1977; *Eutrombicula alfreddugesi alfreddugesi* Brennan & Jones, 1960.

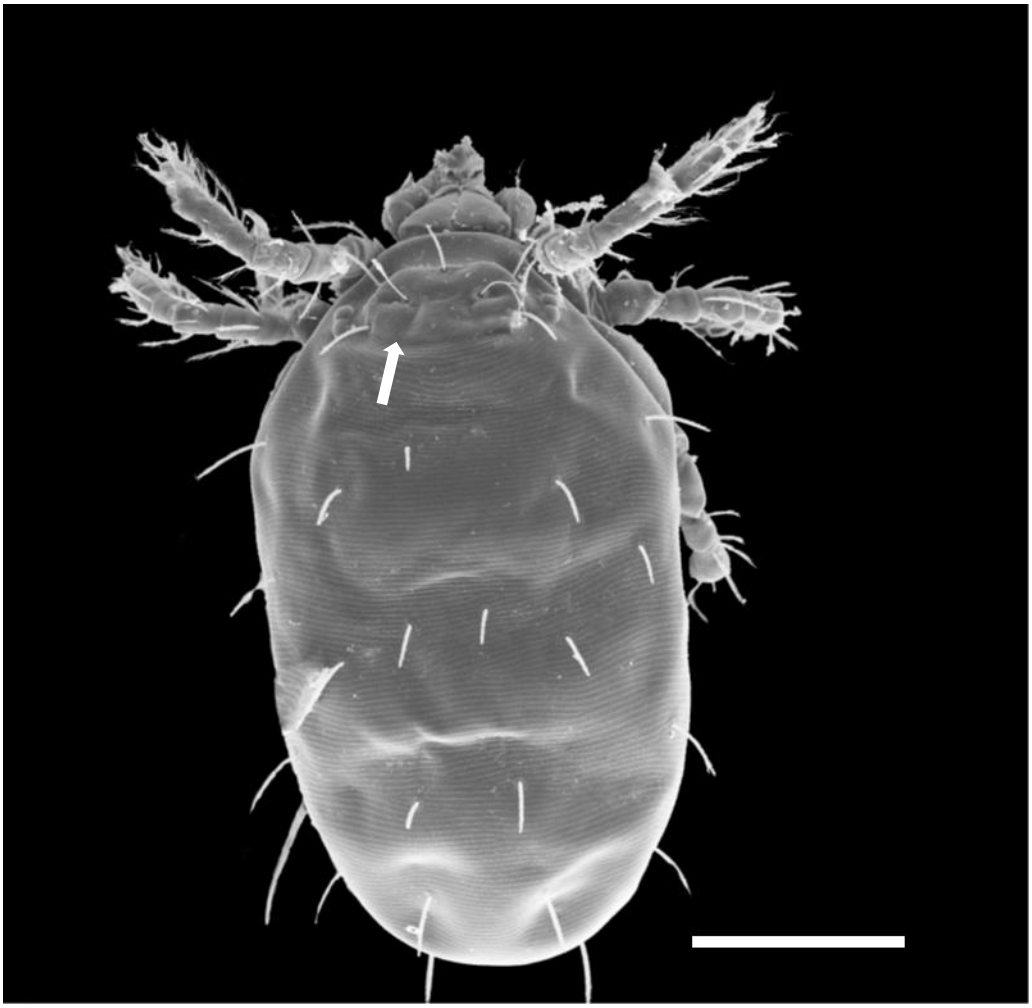
Diagnosis. SIF: 7BS-N-2-3311.1000; fPp: B/N(B)/NNB; fCx: 1.1.1; fSc: PL>AL>AM; fD: 2-6-6-4-2-2; fV: 2-2-2-2-2-2-2. 22 dorsal setae, 14 ventral setae. Tibia III with one tibiala; Tarsi III with mastitarsala (Table 14 and Figure 34).

Table 14 – Morphometrics of 20 larvae of *Eutrombicula alfreddugesi*

	AW	PW	SB	ASB	PSB	SD	P-PL	AP	AM	AL	PL	H
MIN	73	87	42	22	30	56	22	25	32	26	33	35
MAX	79	90	46	26	34	59	23	32	33	33	39	41
Mean	76	88.5	44	24	32	57.5	22,5	28.5	32.5	29.5	36	38
SD	3	1.5	2	2	2	1.5	0.5	3,5	0.5	3.5	3	3
(Jenkins, 1949)	81	90	43	23	26	42	-	27	28	29	49	-
Wolfenbarger (1952)	77	88	43	23	26	-	-	27	28	29	40	-
Daniel & Stekolnikov (2004)	77	90	43	25	32	57	21	28	31	32	37	39

Source: (MENDOZA-ROLDAN, J. A., 2019)

Figure 34 – Scanning electron microscopy of larva *Eutrombicula alfreddugesi*, dorsal view



Source: (MENDOZA-ROLDAN, J. A., 2019)

Legend: white arrow showing the dorsal scutum. Scale bar 100 µm.

4.3.13 *Eutrombicula ophidica* (Fonseca, 1932) p. 27

Type material -Larva holotype (IBSP 29), in *Xenodon merremiii* (Wagler, 1824), from Promissão, São Paulo state, Brazil.

Synonyms: *Trombicula ophidica* Fonseca, 1932: 27; *Eutrombicula ophidica* Radford, 1954: 261

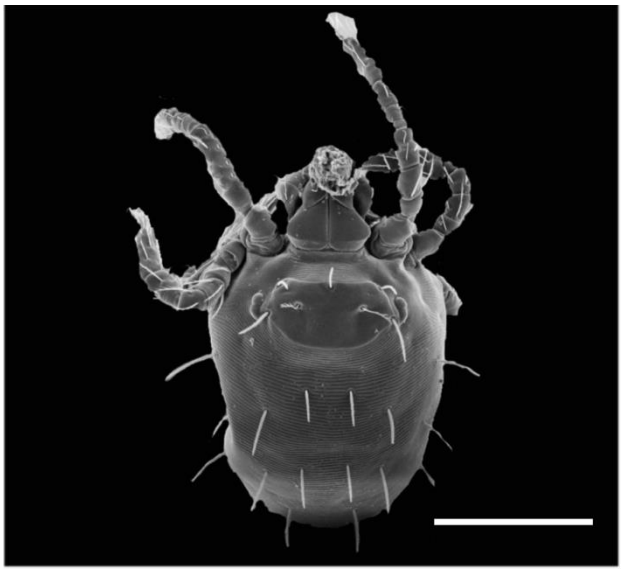
Diagnosis. SIF: 6BS-N-3-4111.0000; fPp: B/N/NNN; fCx: 1.1.1; fSc: PL>AM>AL; fD: 2-6-6-4-2-2; fV: 2-2-4-2-2-2-2. 22 dorsal setae, 20 ventral setae. Tibia III with 2 tibiala; Tarsi III without mastitarsala (Table 15 and Figure 35- 36).

Table 15 – Morphometrics of 10 larvae of *Eutrombicula ophidica*

	AW	PW	SB	ASB	PSB	SD	P-PL	AP	AM	AL	PL	H
MIN	70	80	35	10	22	47	20	25	32	23	40	33
MAX	79	90	46	26	34	59	23	32	33	33	49	41
Mean	74.5	85	40.5	18	28	53	21.5	28.5	32.5	28	44.5	74.5
SD	4.5	5	5.5	8	6	6	1.5	3.5	0.5	5	4.5	4.5
(Fonseca, 1932)	71	80	33	13	26	42	-	30	28	39	49	-

Source: (MENDOZA-ROLDAN, J. A., 2019)

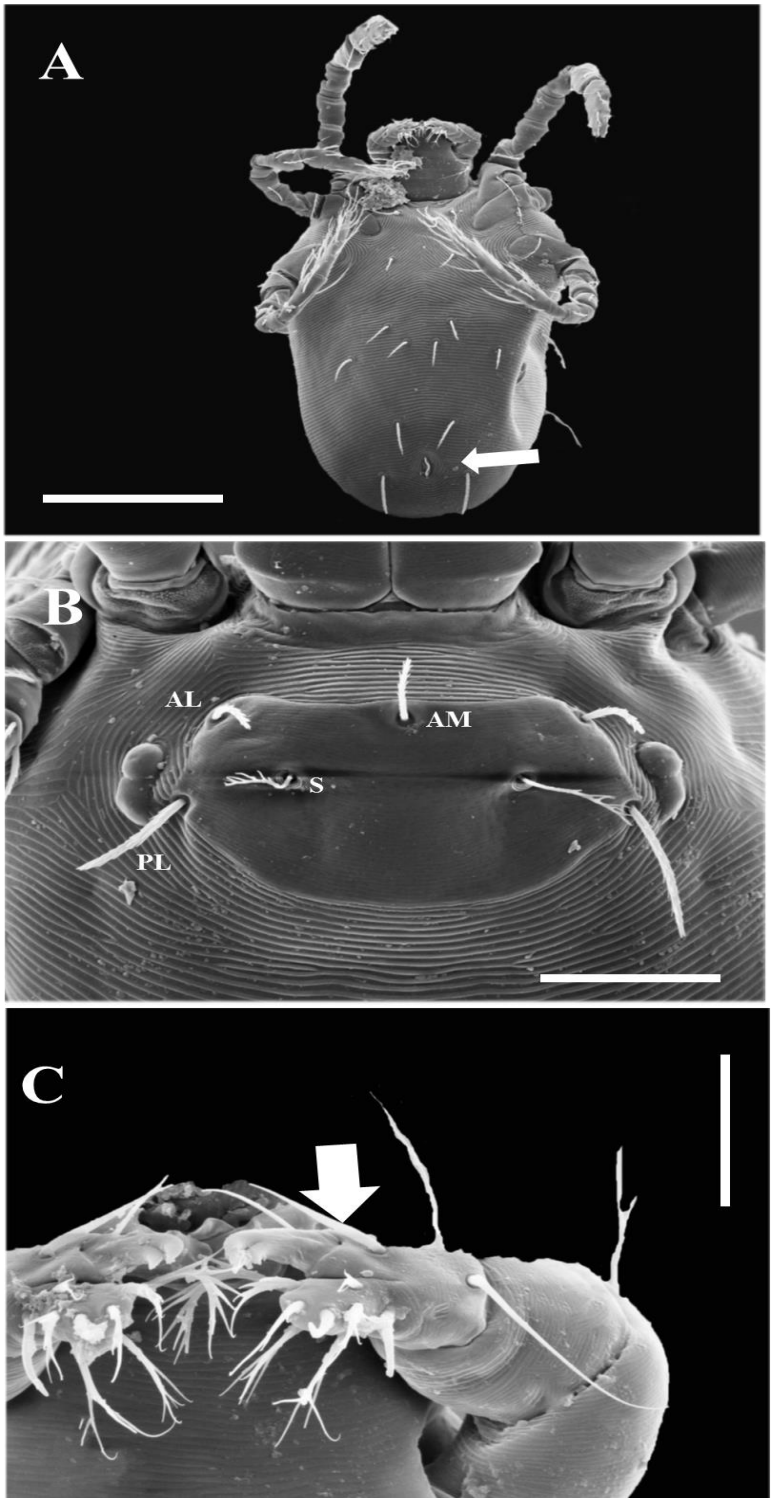
Figure 35 – Scanning electron microscopy of larva *Eutrombicula ophidica*, dorsal view



Source: (MENDOZA-ROLDAN, J. A., 2019)

Legend: white arrow showing the dorsal scutum. Scale bar 100 µm.

Figure 36 – Scanning electron microscopy of larva *Eutrombicula ophidica*



Source: (MENDOZA-ROLDAN, J. A., 2019)

Legend: A. Larva dorsal view, arrow showing the anus; B. Dorsal Scutum; C. Gnathosoma, arrow showing palpal tarsus. Abbreviations: AM: anteromedial seta; AL: anterolateral seta; PL: posterolateral seta; S: sensilla. Scale bar: A, 50 µm; B, 40 µm; C, 20 µm.

4.3.14 *Eutrombicula tropic*a (Ewing, 1925) p. 258

Type material – Larva, *Anadia bitaeniata* Boulenger, 1903, from Chama River, Venezuela

Synonyms: *Trombicula irritans* var. *tropica* Ewing, 1925: 258; *Eutrombicula tropica* Radford, 1942: 66

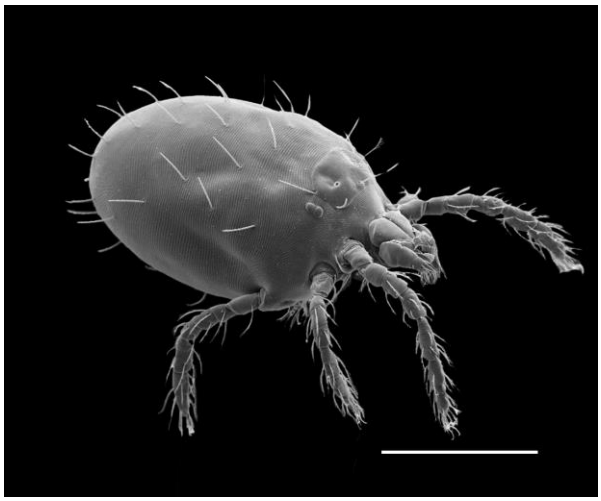
Diagnosis. SIF: 7BS-N-2-3311.1000; fPp: B/N/NNN; fCx: 1.1.1; fSc: PL>AL>AM; fD: 2-6-6-4-2-2; fV: 2-2-2-2-2-2-2. 22 dorsal setae, 14 ventral setae. Three genuala on leg I; tarsi III with mastitarsala; mastitibiala III absent. Accessory prong of palpal tibial claw arises subapically from axial prong. Six setae in first posthumeral row (Table 16 and Figure 37 - 39).

Table 16 – Morphometrics of 20 larvae of *Eutrombicula tropica*

	AW	PW	SB	ASB	PSB	SD	P-PL	AP	AM	AL	PL	H
MIN	70	88	38	23	33	55	21	26	30	27	33	70
MAX	79	90	46	26	34	59	23	32	33	33	39	79
Mean	74.5	89	42	24.5	33.5	57	22	29	31.5	30	36	74.5
SD	4.5	1	4	1.5	0.5	2	1	3	1.5	3	3	4.5
(Ewing, 1925)	70	88	38	23	33	55	21	26	30	27	33	70

Source: (MENDOZA-ROLDAN, J. A., 2019)

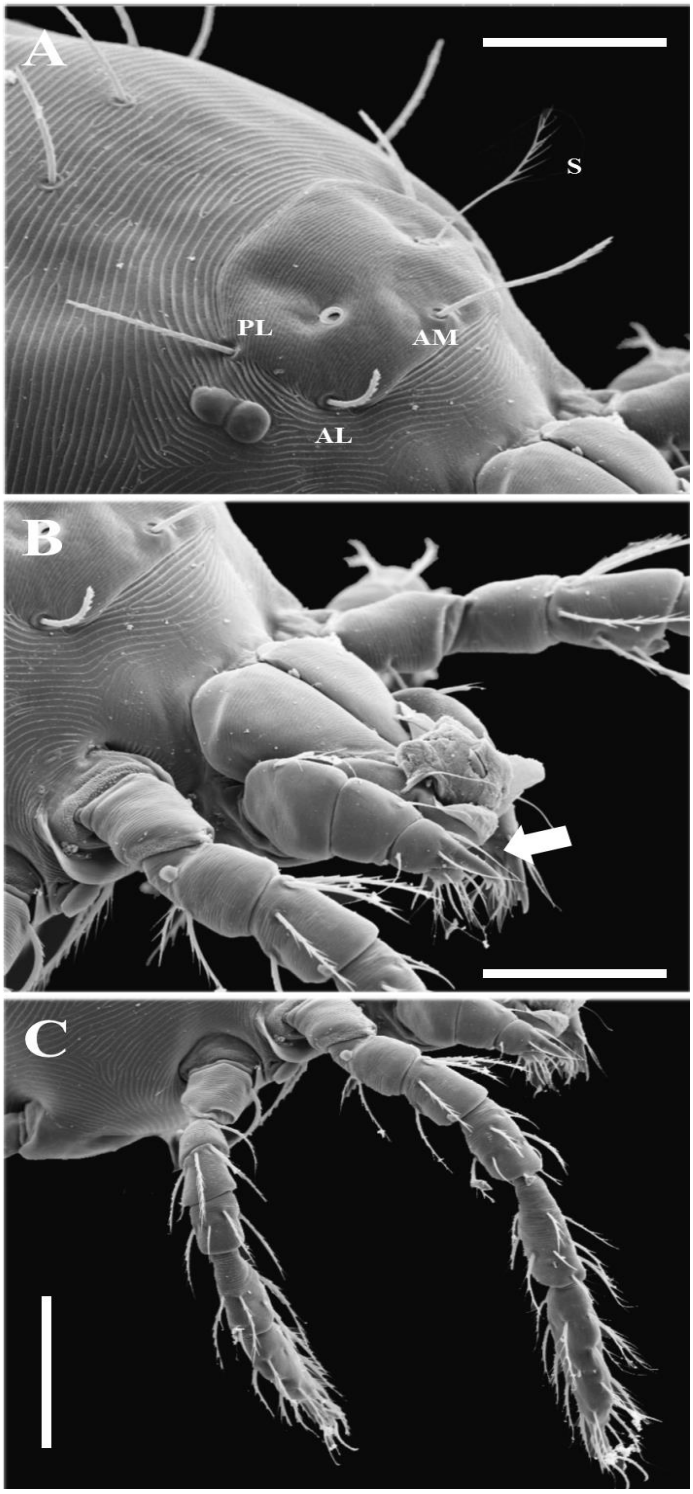
Figure 37 – Scanning electron microscopy of larva *Eutrombicula tropica*, lateral view



Source: (MENDOZA-ROLDAN, J. A., 2017)

Legend: Scale bar 100 µm.

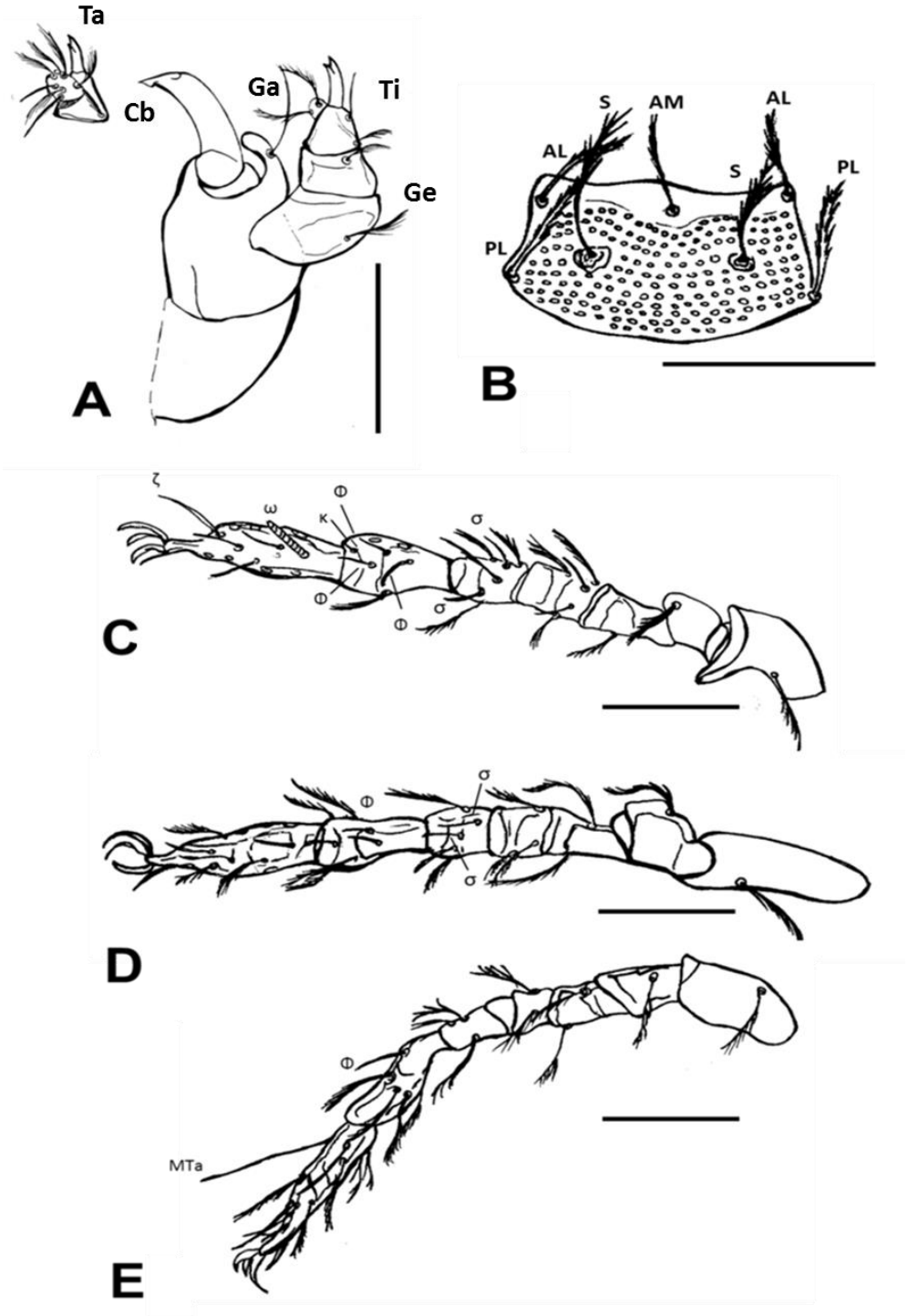
Figure 38 – Scanning electron microscopy of larva *Eutrombicula tropica*



Source: (MENDOZA-ROLDAN, J. A., 2019)

Legend: A. Dorsal scutum; B Gnathosoma, arrow showing palpal tarsus; C. Leg I, lateral view. Abbreviations: AM: anteromedial seta; AL: anterolateral seta; PL: posterolateral seta; S: sensilla. Scale bar: A, 50 µm; B, 40 µm; C, 50 µm.

Figure 39 – Illustrations with morphological features of larva *Eutrombicula tropica*



Source: (MENDOZA-ROLDAN, J. A., 2017)

Legenda: A. gnathosoma, dorsal view and palp tarsus, ventral view; B. dorsal scutum; C. Leg I; D. Leg II; E. Leg III. Abbreviations: AM: anteromedial seta; AL: anterolateral seta; σ = genuala I, II and III; κ = microgenuala and microtibiala; Φ = tibiala I, II and III; ω = tarsala I; ζ = subterminala I; MTa = mastitarsala. Scale bar: A-C 50µm; D-E 100µm.

4.3.15 *Fonsecia anguina* Brennan & Loomis, 1959 p. 61

Type material – larvar holotype and 2 paratypes, RML No. 28004, off "snake", from Yepocapa, Chimal- tenango, Guatemala. Deposited in the collection of the Rocky Mountain Laboratory.

Diagnosis. SIF: 6BS-N-3-4111.0000; fPp: B/N/NNB; fCx: 1.1.1; fSc: PL>AL>AM; fD: 2-8-6-4-2-2.; fV: 2-2-4-2-2-4-2-2. 8 setae in the first dorsal row. Branched palpal ventro- tibial seta and conspicuously larger terminal digituli of the anterolateral seta; large scutum. AM seta normal. Dorsal palpal tibial seta nude. Preanal setae not swollen basally (Table 17 and Figure 40 - 42).

Gnathosoma. Cheliceral bases and capitular sternum punctate. Cheliceral blade with tricuspid cap. Palpal claw trifurcate.

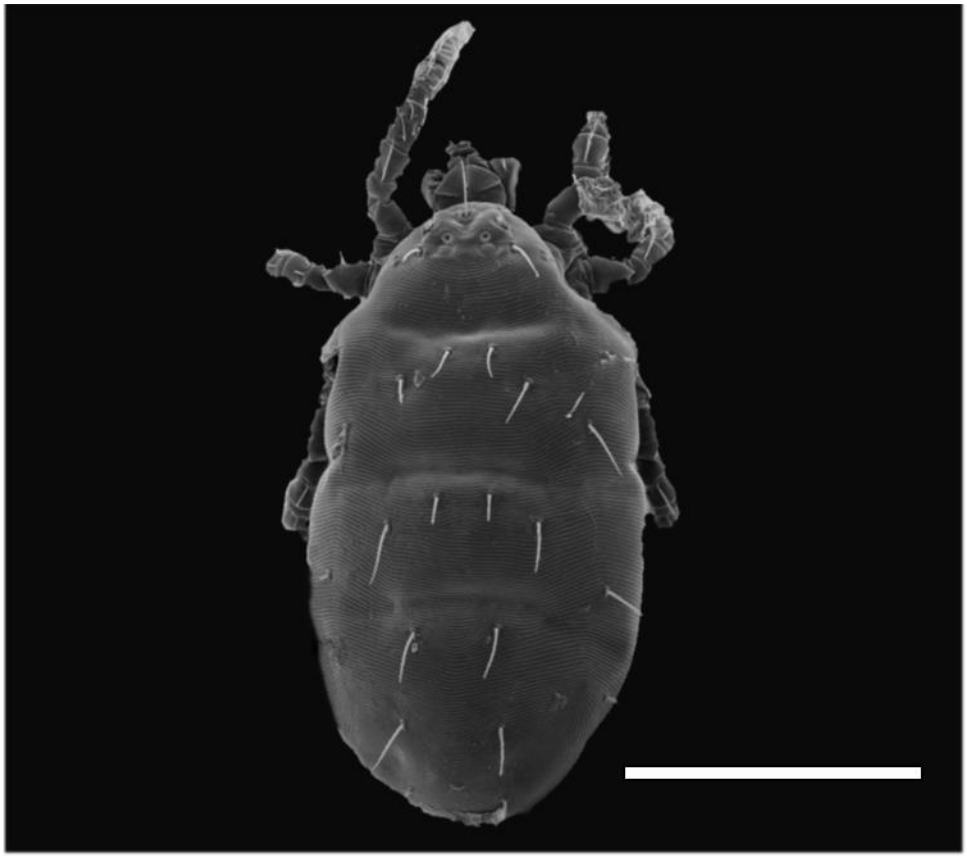
Idiosoma. Eyes 2/2, small, indistinct, not on a plate. Anus at level of 4th row of ventral setae.

Table 17 – Morphometrics of 10 larvae of *Fonsecia anguina*

	AW	PW	SB	ASB	PSB	SD	P-PL	AP	AM	AL	PL	H
MIN	59	78	30	30	28	33	10	17	41	10	40	67
MAX	68	81	32	31	33	40	11	22	44	15	46	69
Mean	63.5	79.5	31	30.5	30.5	36.5	10.5	19.5	42.5	12.5	43	68
SD	4.5	1.5	1	0.5	2.5	3.5	0.5	2.5	1.5	2.5	3	1
(Brennan & Loomis, 1954)	59	78	30	30	28	33	10	17	41	10	40	67

Source: (MENDOZA-ROLDAN, J. A., 2019)

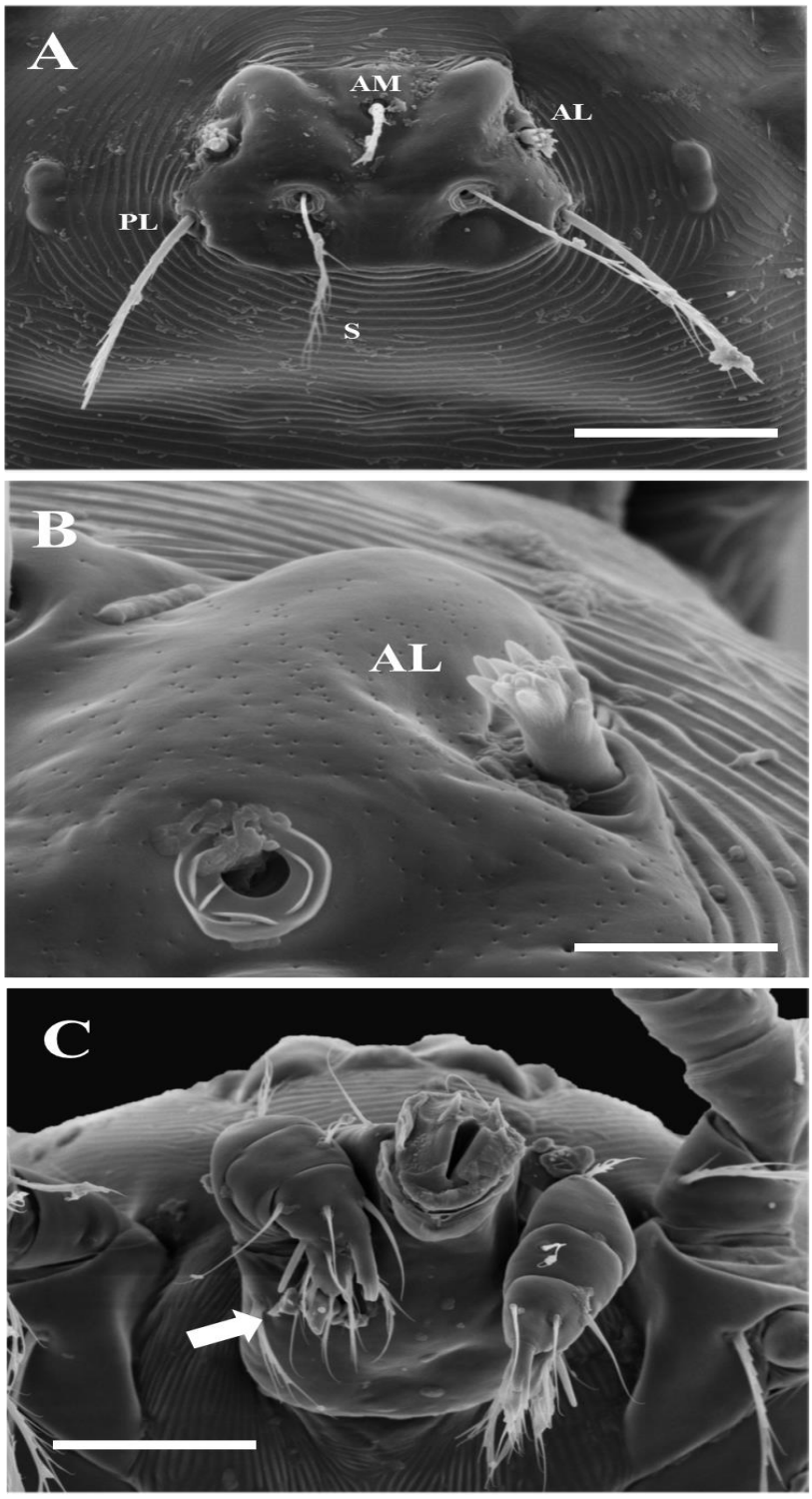
Figure 40 – Scanning electron microscopy of larva *Fonsecia anguina*, dorsal view



Source: (MENDOZA-ROLDAN, J. A., 2019)

Legend: Scale bar 100 µm.

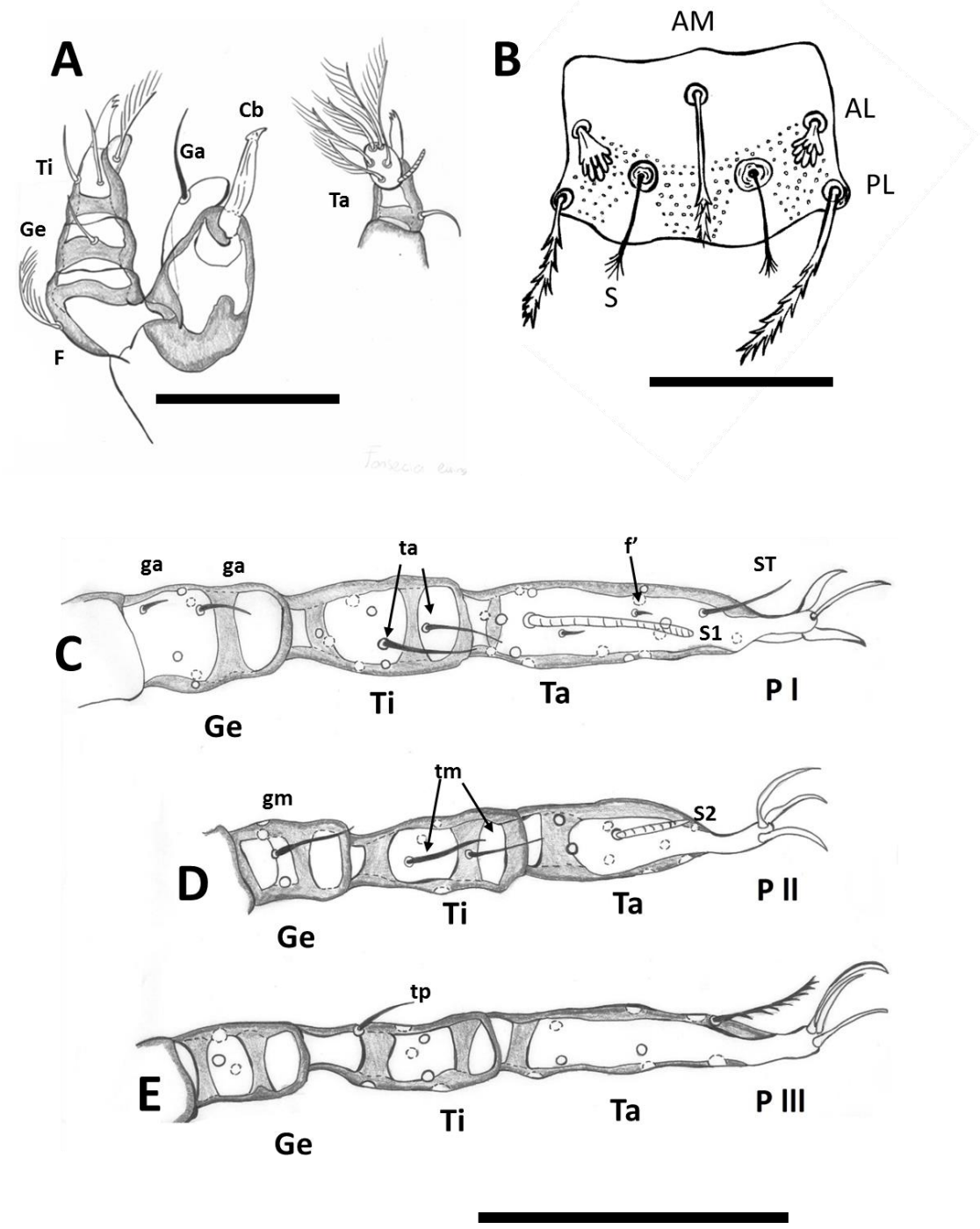
Figure 41 – Scanning electron microscopy of larva *Fonsecia anguina*



Source: (MENDOZA-ROLDAN, J. A., 2019)

Legend: A.; Dorsal scutum; B. AL setae peg-like; C. Gnathosoma, arrow showing palpal tarsus. Abbreviations: AM: anteromedial seta; AL: anterolateral seta; PL: posterolateral seta; S: sensilla. Scale bar: A, 50 µm; B, 5 µm; C, 20 µm.

Figure 42 – Illustrations with morphological features of larva *Fonsecia anguina*



Source: (MENDOZA-ROLDAN, J. A., 2019)

Legend: A. gnathosoma, dorsal view and palp tarsus, ventral view; B. dorsal scutum; C. Leg I; D. Leg II; E. Leg III. Abbreviations: AM: anteromedial seta; Al: anterolateral seta; Cb: chelicera; ga: genuala leg I; gm: genuala leg II; F: femur; f': microtarsala leg I; Ga: galeala; Ge: genus; gp: genuala leg III; N: naso; PL: posterolateral seta; S1: tarsala leg I; S2: tarsala leg II; ST: subterminala leg I; µta: microtibiala leg I; Ta: tarso; ta: tibiala leg I; tm: tibiala leg II; tp: tibiala leg III; Ti: tibia. Scale bar: A, 50 µm; B, 30 µm; C, D, E 50 µm.

Order Sarcoptiformes

Suborder Oribatida

Family Trhypochthoniidae

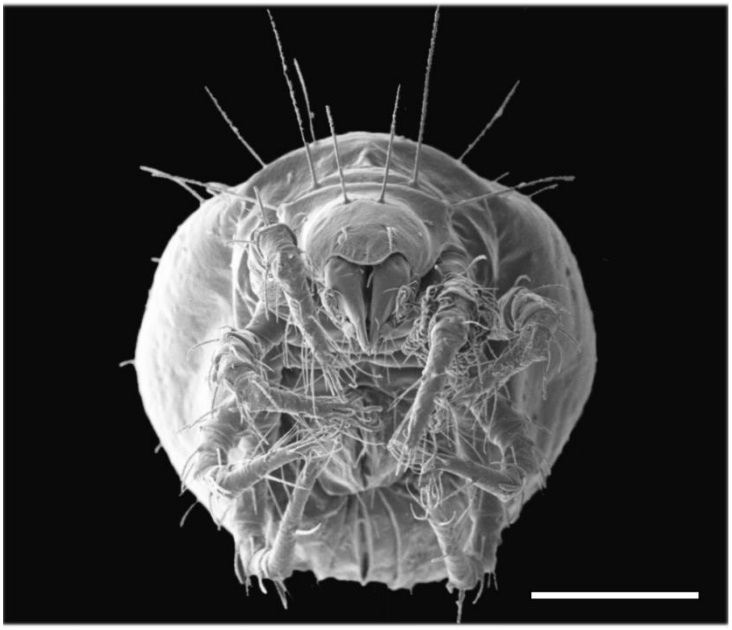
4.3.16 *Archegozetes longisetosus* Aoki, 1965 p .147

Type material – Tritonymph holotype, from Nakon Pathom, Thailand. Deposited in the Nacional Science Museum, Tokyo.

Synonyms: *Archegozetes longisetosus* Aoki, 1656: 147; *Archegozetes chamelenesisi* Palacios-Vargas & Iglesias, 1997: 46.

Diagnosis. Prodorsum punctated; prodorsal and notogastral setae long, fine, densely beset with fine bristles; sensillus long, fine, densely covered with bristles; *d1* longer than their mutual distance; genital setae 7 pairs; *4a* about 1/2 as long as *4b*; solenidia on palp sharp. (Figures 43 - 44).

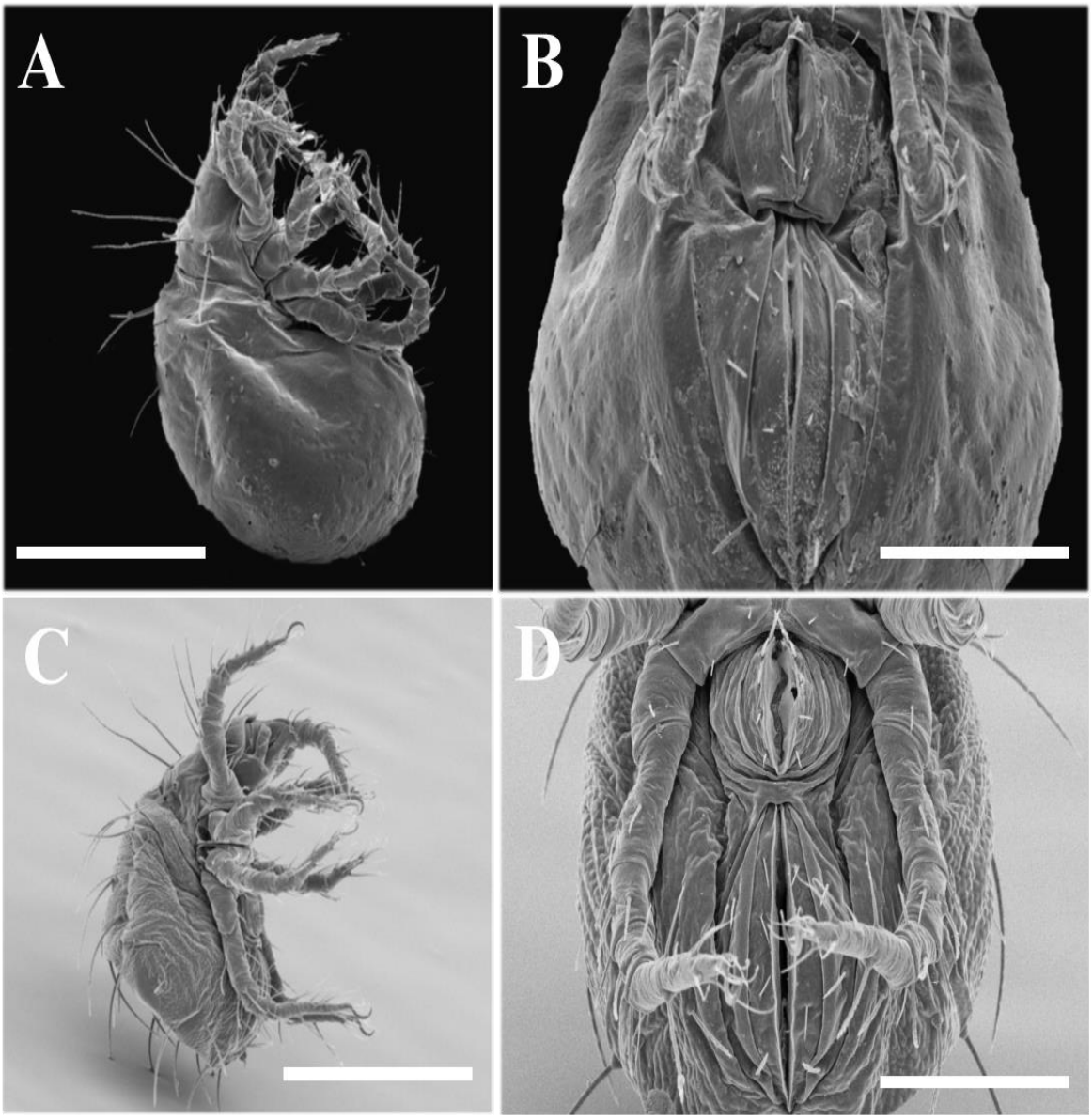
Figure 43 – Scanning electron microscopy of female *Archegozetes longisetosus*, frontal view



Source: (MENDOZA-ROLDAN, J. A., 2019)

Legend: Scale bar 400 µm.

Figure 44 – Scanning electron microscopy of female and tritonymph of *Archegozetes longisetosus*



Source: (MENDOZA-ROLDAN, J. A., 2019)

Legend: A. female lateral view, B. female ventral view, anal and genital opening; C. tritonymph lateral view; D. tritonymph ventral view, anal and genital opening Scale bar: A, 400 µm; B, 100 µm; C, 300 µm; D, 200 µm.

4.4 Geographical distribution

Maps of geographical distribution of the species of Trombidiformes mites examined in this study are shown in Figures 45 to 55. Geographic coordinates of each locality for each species are detailed hereafter, including information from literature and collections.

***Ophioptes parkeri*:** Brazil – **Pará:** Belém (23° 33' 52" S, 46° 43' 15" W); **Goiás:** Itumbara (18° 20' 32" S, 49° 9' 29" W); **Minas Gerais:** Uberlândia (18° 57' 15" S, 48° 18' 54" W); Juiz de Fora (21° 43' 44" S, 43° 22' 57" W); Lambari (21° 57' 57" S, 45° 23' 13" W); Sapucaí (22° 13' 1" S, 45° 43' 4" W); Três Corações (21° 41' 6" S, 45° 15' 6" W); **Espírito Santo:** Colatina (19° 27' 51" S, 40° 34' 22" W); **São Paulo:** Araçoiaba da Serra (23° 32' 32.4888" S, 47° 38' 56.9976" W); Morro Agudo (20° 43' 39.6084" S, 48° 3' 10.062" W); Presidente Venceslau (21° 52' 7" S, 51° 49' 49" W); Arujá (23° 22' 16" S, 46° 19' 36" W); Biritiba-Mirim (23° 33' 51" S, 46° 3' 10" W); Inuíba (23° 40' 18" S, 47° 12' 44" W); Jaú (22° 18' 7" S, 48° 32' 22" W); São Carlos (22° 0' 13" S, 47° 53' 24" W); São Paulo (23° 40' 56" S, 46° 35' 43" W); Itapecerica da Serra (23° 44' 3" S, 46° 51' 4" W); Rancharia (22° 13' 46.1928" S, 50° 53' 32.0388" W); **Rio Grande Do Sul:** Pelotas (31° 38' 58" S, 52° 21' 26" W). Paraguay – **Alto Paraguay** (19° 43' 56" S, 60° 43' 1" W). Bolivia – **Buena Vista** (17° 27' 18" S, 63° 39' 9" W) (Figure 45).

***Ophioptes ekans*:** Brazil **- São Paulo:** Campo Limpo Paulista (23° 12' 21.8844" S, 46° 47' 0.9168" W; São Paulo (23° 40' 56" S, 46° 35' 43" W) (Figure 46).

***Bertrandiella jimenezi*:** Brazil – **Alagoas:** Piranhas (9° 36' 25.38" S, 37° 45' 41.688" W); **Sergipe:** Caniné de São Francisco (9° 39' 37.908" S, 37° 47' 21.048" W); **Rio Grande do Norte:** Angicos (5° 40' 42.672" S, 36° 36' 34.056" W). Mexico – **Puebla:** Salinas (18° 19' 38.964" N, 97° 28' 24.6" W) (Figure 47).

Geckobia hemidactyli: Colombia – **Leticia** (3° 56' 42" S e 70° 9' 31" W). Brazil- **São Paulo:** Assis (22° 39' 37" S, 50° 25' 7" W); Sete Barras (24° 24' 58" S, 47° 55' 10" W); São José do Barreiro (22° 39' 5" S, 44° 33' 33" W); São Paulo (23° 33' 52" S, 46° 43' 15" W); **Pará:** Tucuruí (3° 46' 10.632" S, 49° 40' 25.7808" W) (Figure 48).

***Geckobiella harrisi*:** Brazil - **São Paulo:** São Paulo (23° 33' 1.872" S, 46° 37' 59.9088" W); Serra da Cantareira (23° 27' 2.988" S, 46° 35' 38.472" W); **Pará:** Santarém (2° 26' 21.984" S, 54° 41' 55.464") (Figure 49).

***Hannemania achalai*:** Argentina – **Córdoba:** Pampa de Achala (31° 43' 6.204" S, 64° 59' 59.424" W). Brazil - **Rio Grande do Sul:** Arvorezinha (28° 55' 22.62" S, 52° 8' 18.204" W); Itapuã (30° 1' 57.1836" S, 51° 7' 11.1036" W) (Figure 50).

Hannemania hepática: Brazil - **São Paulo:** São Paulo (23° 33' 1.872" S, 46° 37' 59.9088" W); Sete Barras (24° 22' 53.6124" S, 47° 55' 48.9756" W); Cubatão (23° 53' 5.8236" S, 46° 25' 15.0564" W); Ilhabela (23° 46' 43.4964" S, 45° 21' 29.8764" W); **Minas Gerais:** Diamantina (18° 14' 29.6844" S, 43° 36' 6.8328" W); **Rio Grande do Norte:** Angicos (5° 40' 42.672" S, 36° 36' 34.0524" W) (Figure 51).

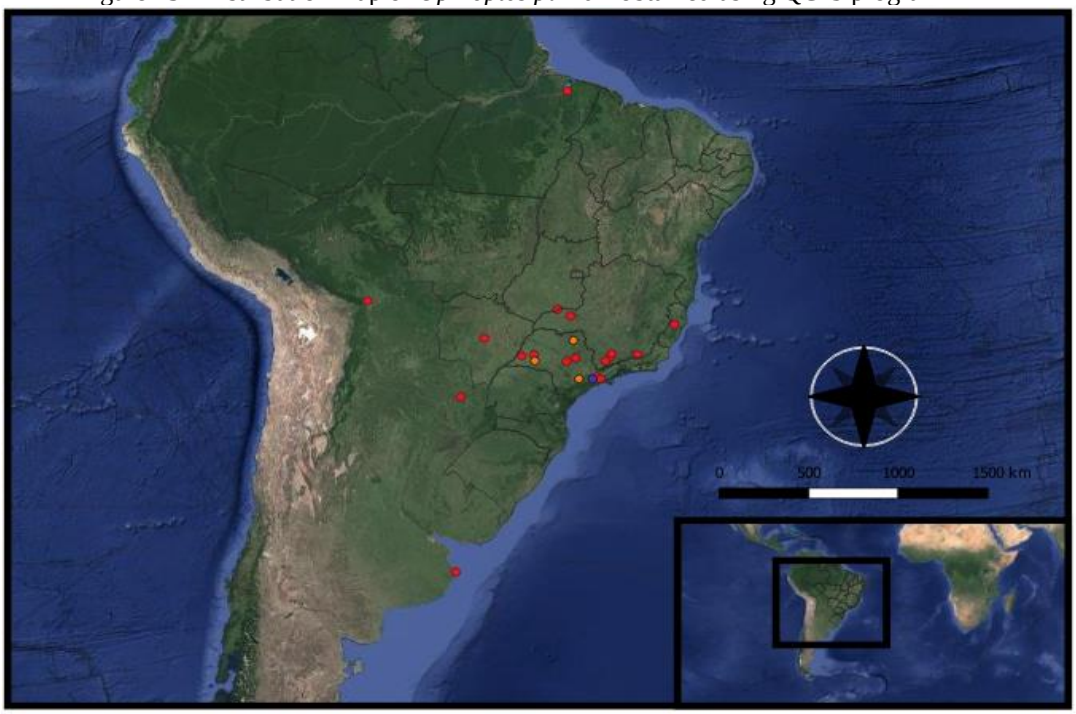
***Eutrombicula alfreddugesi*:** United States (37° 5' 24.864" N, 95° 42' 46.4076" W). Brazil – **Acre:** Iracema (9° 57' 30.708" S, 67° 49' 15.924" W); **Bahia:** Morro de Chapéu (11° 32' 43" S, 41° 9' 34" W); **Ceará:** Chapada do Criador (7° 23' 14" S, 40° 12' 58" W); **Brazilia:** Distrito Federal (15° 43' 18" S, 47° 56' 17" W); **Mato Grosso:** (12° 40' 54." S, 56° 55' 154" W); Guaporé (19° 28' 15.564" S, 44° 15' 31.716" W); Universidade Federal de Mato Grosso (15° 38' 54.204" S, 56° 7' 56.856" W); **Pará:** Tucuruí (3° 46' 10.632" S, 49° 40' 25.7808" W); **Pernambuco:** Fernando de Noronha (3° 50' 45.744" S, 32° 24' 43.344" W); **Rio de Janeiro:** Jurubatiba (22° 10' 23 " S, 41° 26' 34" W); Barra da Marica (22° 55' 11" S, 42° 54' 12" W); **São Paulo:** Sete Barras (24° 24' 58" S, 47° 55' 10" W); Cananéia (24° 54' 27.432" S, 47° 58' 25.644" W); Santa Barbara (22° 52' 43.572" S, 49° 14' 23.172" W); São Bernardo do Campo (23° 41' 40.02" S, 46° 33' 56.88" W); São Paulo (23° 33' 1.872" S, 46° 37' 59.9088" W); Barragem Paraitinga (23° 30' 5.904" S, 46° 24' 32.076" W); Sete Barras (24° 24' 58" S, 47° 55' 10" W). Venezuela – (6° 25' 25.5" N, 66° 35' 23.0244" W) (Figure 52).

***Eutrombicula ophidica*:** Brazil **– São Paulo:** Promissão (21° 32' 18.276" S, 49° 51' 27.7632" W); São Paulo (23° 33' 1.872" S, 46° 37' 59.9088" W); **Minas Gerais: Diamantina** (17° 53' 19.1616" S, 42° 54' 7.7724" W); **Pará:** Tucuruí (3° 46' 10.632" S, 49° 40' 25.7808" W) (Figure 53).

***Eutrombicula tropica*:** Brazil - **São Paulo:** Ilha Queimada (24° 29' 15.5544" S, 6° 40' 25.1328" W). Venezuela - Chama River (6° 25' 25.5" N, 66° 35' 23.0244" W) (Figure 54).

***Fonsecia anguina*:** Brazil – **Acre:** Iracema (9° 57' 30.708" S, 67° 49' 15.924" W). Guatemala - **Chimal:** Yepocapa (14° 30' 9.972" N, 90° 57' 15.228" W) (Figure 55).

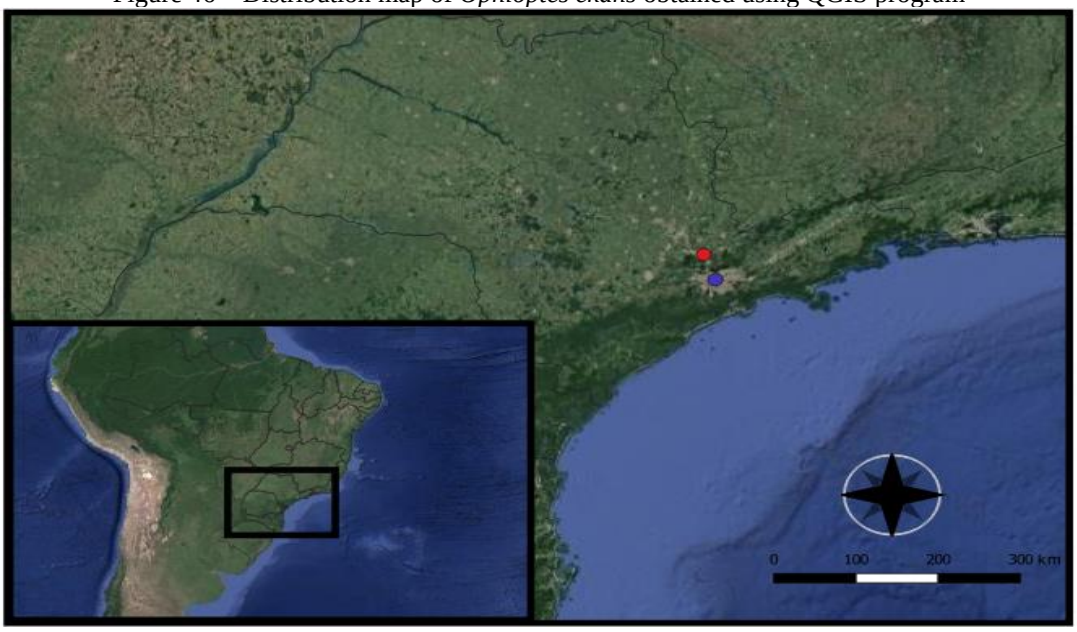
Figure 45– Distribution map of *Ophioptes parkeri* obtained using QGIS program



Source: (MENDOZA-ROLDAN, J. A., 2019).

Legend: (red circles) information cited in literature, (orange circles) material examined deposited in collections, (blue circle) material from this study.

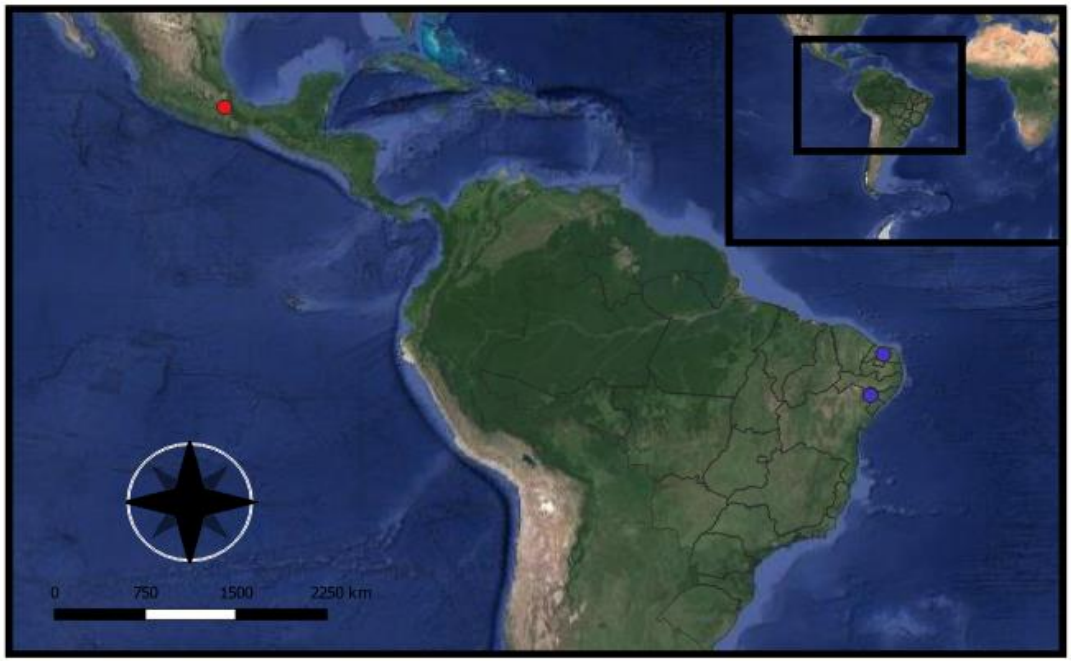
Figure 46 – Distribution map of *Ophioptes ekans* obtained using QGIS program



Source: (MENDOZA-ROLDAN, J. A., 2019).

Legend: (red circles) information cited in literature, (blue circle) material from this study.

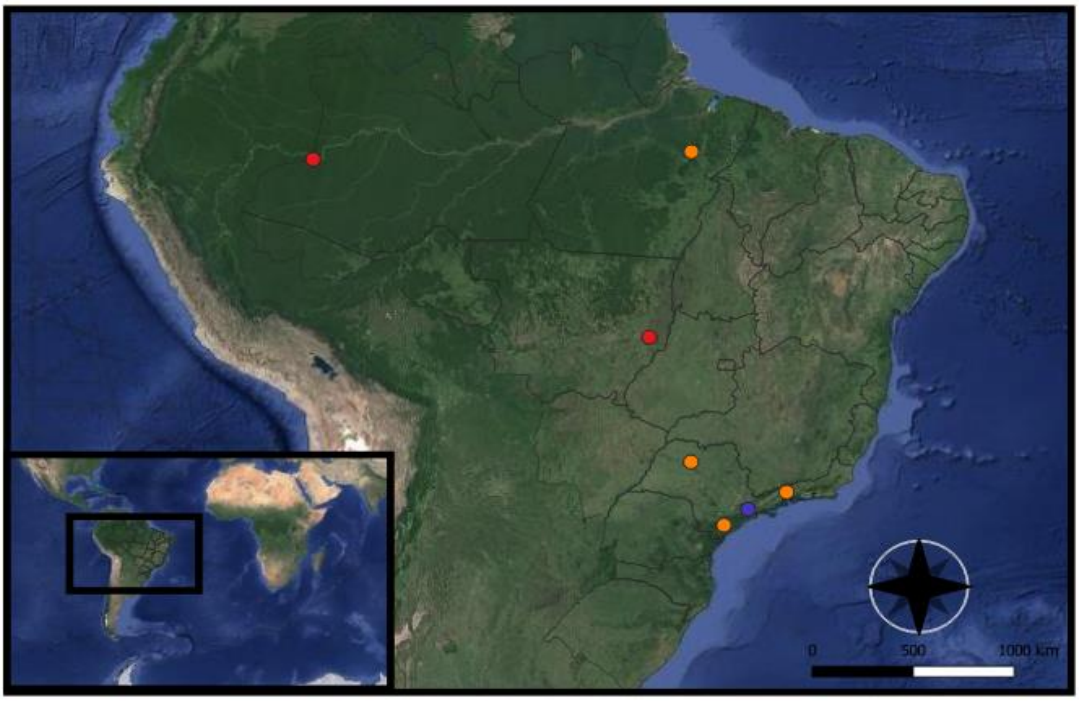
Figure 47 – Distribution map of *Bertrandiella jimenezi* obtained using QGIS program



Source: (MENDOZA-ROLDAN, J. A., 2019).

Legend: (red circles) information cited in literature, (blue circle) material from this study.

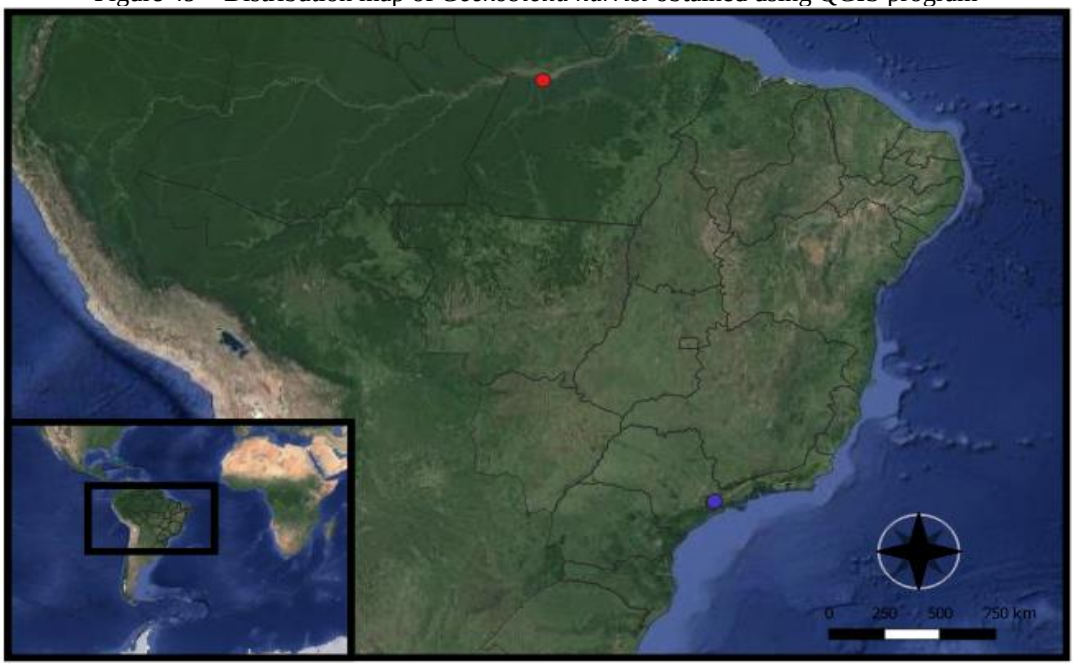
Figure 48 – Distribution map of *Geckobia hemidactyli* obtained using QGIS program



Source: (MENDOZA-ROLDAN, J. A., 2019).

Legend: (red circles) information cited in literature, (orange circles) material examined deposited in collections, (blue circle) material from this study.

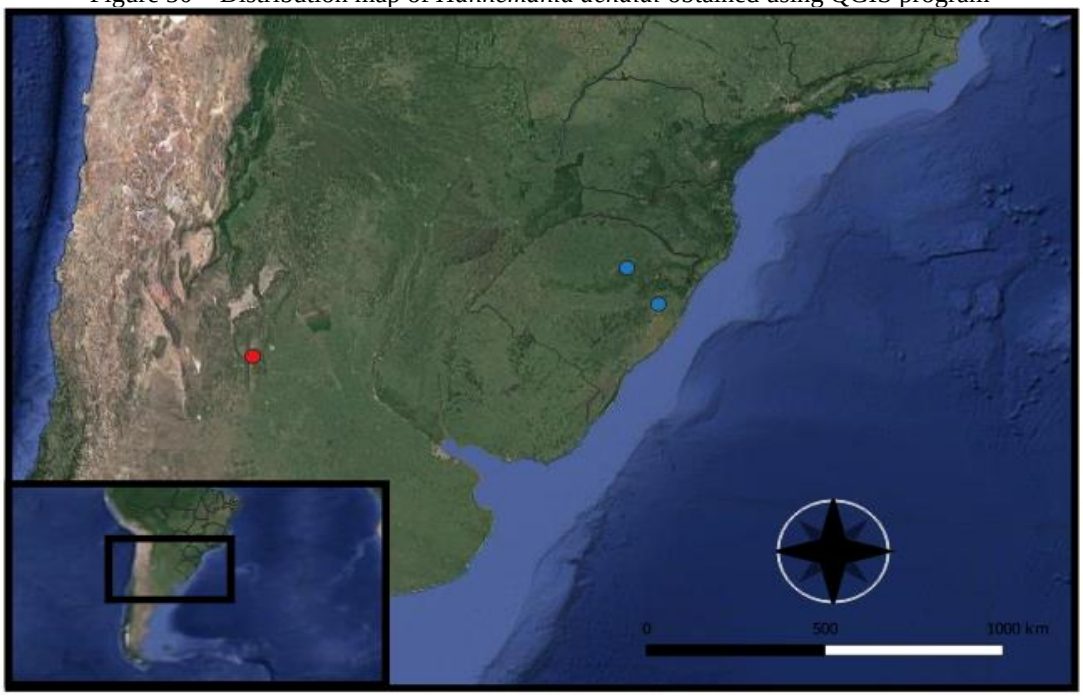
Figure 49 – Distribution map of *Geckobiella harrisi* obtained using QGIS program



Source: (MENDOZA-ROLDAN, J. A., 2019).

Legend: (red circles) information cited in literature, (blue circle) material from this study.

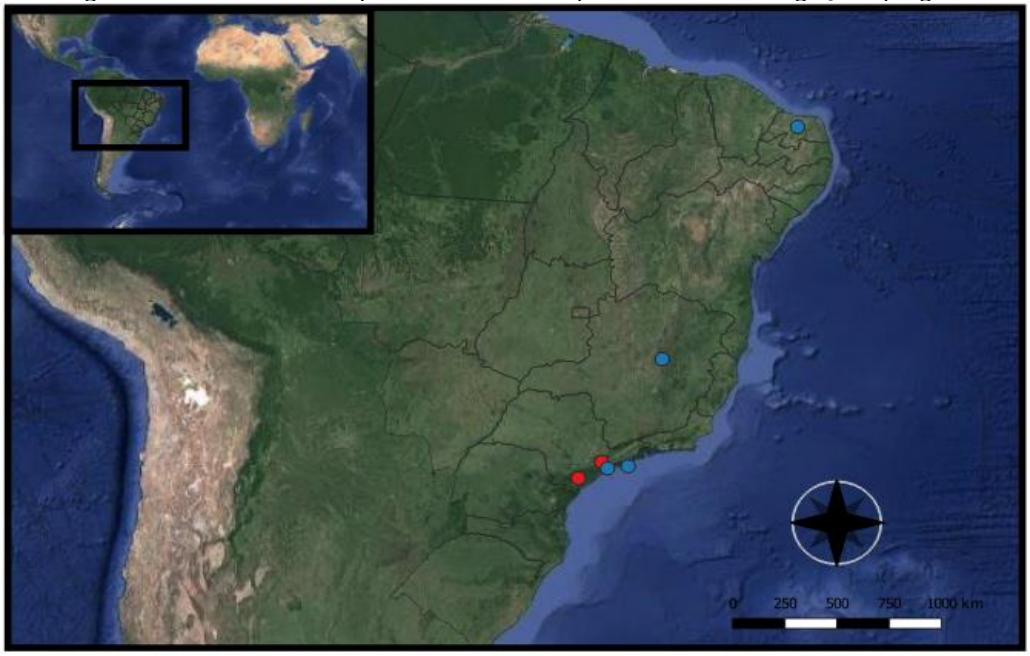
Figure 50 – Distribution map of *Hannemania achalai* obtained using QGIS program



Source: (MENDOZA-ROLDAN, J. A., 2019).

Legend: (red circles) information cited in literature, (blue circle) material from this study.

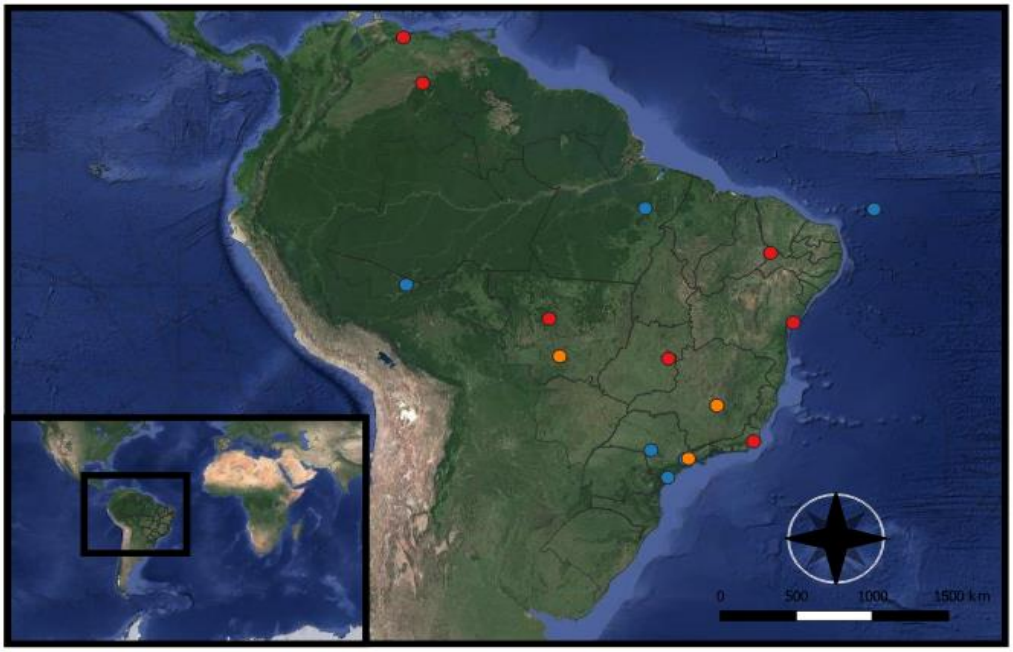
Figure 51 – Distribution map of *Hannemania hepatica* obtained using QGIS program



Source: (MENDOZA-ROLDAN, J. A., 2019).

Legend: (red circles) information cited in literature, (blue circle) material from this study.

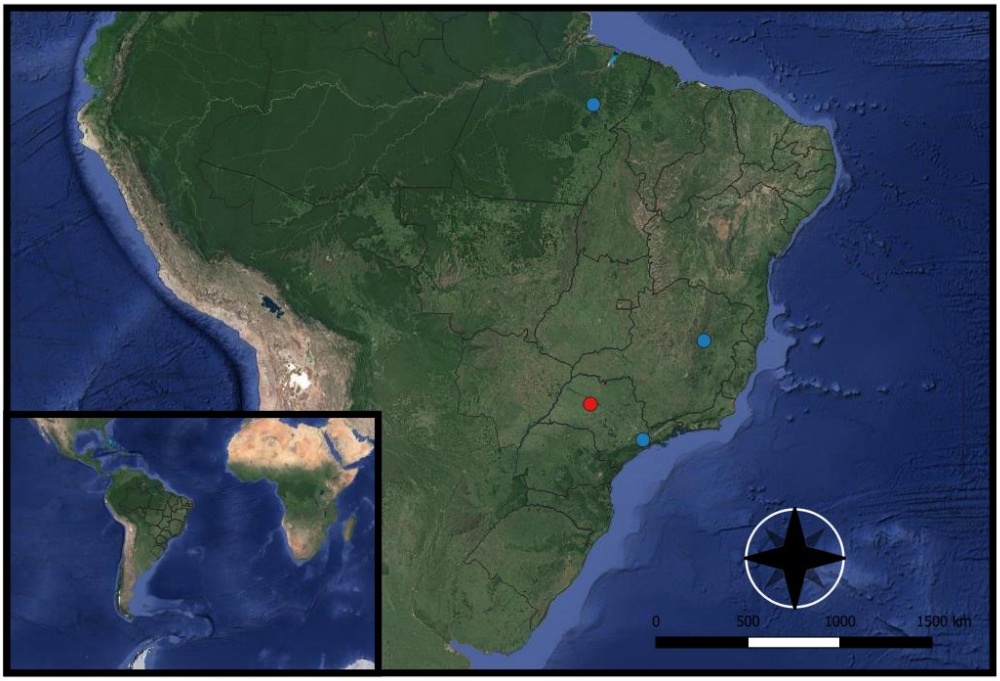
Figure 52– Distribution map of *Eutrombicula alfreddugesi* obtained using QGIS program



Source: (MENDOZA-ROLDAN, J. A., 2019).

Legend: (red circles) information cited in literature, (orange circles) material examined deposited in collections, (blue circle) material from this study.

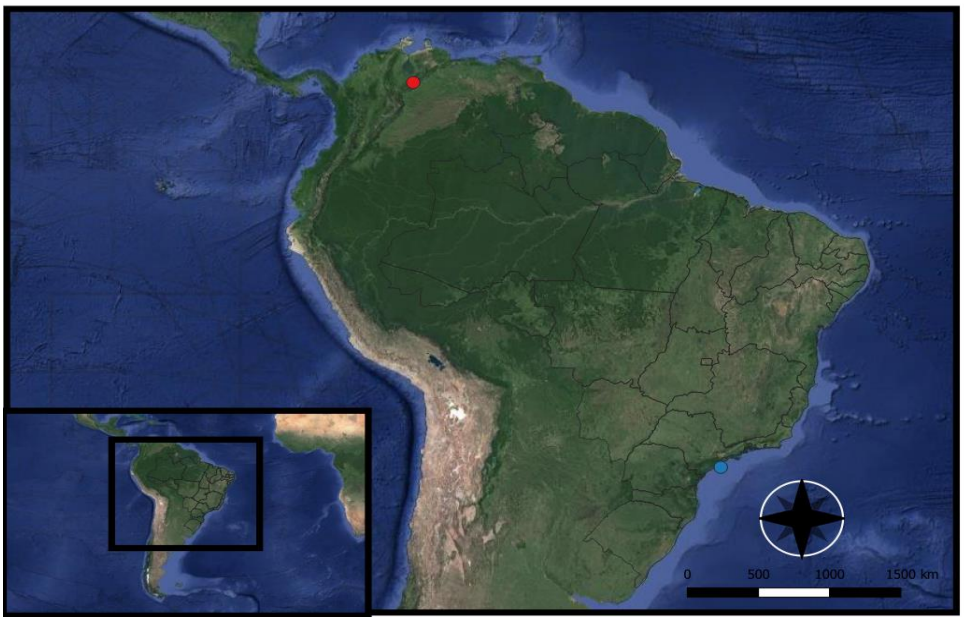
Figure 53 – Distribution map of *Eutrombicula ophidica* obtained using QGIS program



Source: (MENDOZA-ROLDAN, J. A., 2019).

Legend: (red circles) information cited in literature, (blue circle) material from this study.

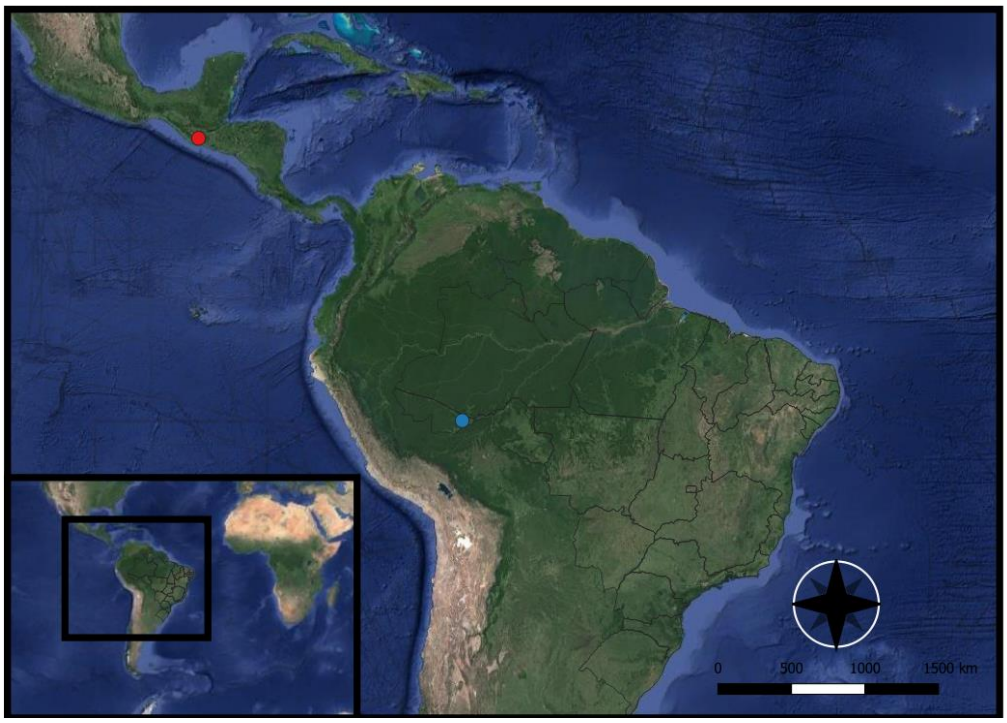
Figure 54 – Distribution map of *Eutrombicula tropica* obtained using QGIS program



Source: (MENDOZA-ROLDAN, J. A., 2019).

Legend: (red circles) information cited in literature, (blue circle) material from this study.

Figure 55 – Distribution map of *Fonsecia anguina* obtained using QGIS program



Source: (MENDOZA-ROLDAN, J. A., 2019).

Legend: (red circles) information cited in literature, (blue circle) material from this study.

5 DISCUSSION

In the present study six families, 12 genera and 32 species of Trombidiformes mites were identified, and 23 occur in Brazil. Before, former studies catalogued 17 species of Trombidiformes mite parasites of reptiles and amphibians for all the Brazilian territory (LIZASO, 1984; MENDOZA-ROLDAN, 2015; MENDOZA-ROLDAN et al., 2017; JACINAVICIUS et al., 2018).

The Trombidiformes species of superfamily Cheyletoidea (**Cloacaridae**, **Harpirhynchidae)**, superfamily Pterygosomatoidea: (**Pterygosomatidae)**, superfamily Trombidioidea (**Leeuwenhoekiidae**, **Trombiculidae)** superfamily Hydryphantoidea (**Thermacaridae)** and superfamily Tydeoidea (**Ereynetidae)** have been recorded in the Neotropical region. Of the aforementioned families, Cloacaridae, Thermacaridae and Ereynetidae were not found in the recent field trips or in live reptiles and amphibians examined. Moreover, Cloacaridae mites have been found in Panama in *Chelonia mydas* Linnaeus, 1758, yet, there are no records of occurrence of this family in South America (PENCE; WRIGHT, 1998; FAJFER, 2012). In the present study, quelonians from both of Pleurodira and Cryptodira orders were assessed for Cloacaridae mites, though no marine turtles were examined and some species were examined at the Fain Acari Collection of the Royal Belgian Institute of Natural Sciences –IRSN (It is likely that if *C. mydas* specimens are examined, they could be infested by these mites. Nonetheless, other important factor to take into consideration is the collection method. Here, cloacal swabs were performed on turtles and tortoises. Other authors have found these mites when performing necropsy of the quelonian hosts. Thus, finding these family of mites would require assessment of the connective tissue and muscle of the cloacal area of deceased animals, which were not available for the present study (FAIN, 1968; CAMIN et al., 1967; PENCE; WRIGHT, 1998).

Regarding the Thermacaridae family, as this is a monogeneric group of water mites specialized in inhabiting hot-spring waters, we were unable to examine this kind of habitat, thus restricting the possibility of finding this mite in the Brazilian territory. There is only one species of *Thermacarus* (*T. andinus*) recorded in South America *R. spinulosa* Chile (SCHWOERBEL, 1987 MARTIN; SCHWOERBEL 2002). Nontheless, *Thermacarus* mites could also infest other vertebrate, including humans that visit hot springs (MITCHELL, 1960; HERON; SHEFFIELD, 2016). Thus, the importance of assessing the presence of these mites.

On the other hand, Ereynetidae mites, which also infest amphibians (mainly of the order Anura), were also not found in this study. Type material of these nasal mites from the Museo de La Plata in La Plata, Argentina of the species *Lawrencarus braziliensis desantisi* Mauri & Alzuet, 1984, were examined to better understand the morphology of these mites. Oral and nasal cavities of amphibians were assessed for the presence of *Lawrencarus* mites, but none were found. The last record of *Lawrencarus* mites in Brazil is from the early 60s' (*Lawrencarus hylae intermedius*) in *Scinax hayii* (Barbour, 1909) frog from São Paulo, and *Lawrencarus braziliensis* Fain, 1961 in *Cycloramphus asper* Werner, 1899 from Cubatão, São Paulo) (FAIN, 1961). Furthermore, little is known of the life cycle and development of these mites and though the type hosts species were examined in this study, we did not find this family of mites, thus, these mites seem to be rare and have a low prevalence.

Concerning the superfamily Cheyletoidea, family Harpirhynchidae, the six Neotropical species and one Palearctic species were examined. Of these, *O. parkeri, O. tropicalis*; *O. dromicus*; *O. beshkovi* and *O. ekans* were morphologically detailed. *Ophioptes brevipilis and O. longipilis* were already described in a former study (MENDOZA-ROLDAN, 2015). Herein, *O. parkeri* was recorded in São Paulo on *C. bicarinatus*. This is the second record of this species in São Paulo municipality and the first record in this species of snake. Furthermore, *O. parkeri* is the most common species of *Ophioptes* found in South America, distributed in Argentina, Brazil, Bolivia, and Paraguay, infesting colubrid snakes of seven genera (FAIN, 1964; LIZASO, 1981). Additionally, *O. ekans* was described in a recent study as the only species of *Ophioptes* that parasitizes vipers. It was described on South-American rattlesnake (*Crotalus durissus terrificus Laurenti*) form Campo Limpo Paulista, São Paulo state (ATTACHMENT 1) (MENDOZA-ROLDAN et al., 2017). It was compared with other neotropical species and the main difference from the rest of the "*parkeri*" group" is by having three pair of genital-anal setae in the female instead of four. In this study, *O. ekans* was found in *Bothrops jararaca* (Wied-Neuwied, 1824) from São Paulo municipality. This is the second record of this species in a viper snake and the first record in this locality. All the hosts records show that the Ophioptinae subfamily has an ancestral origin in the Colubriodea superfamily. Last cladistic studies suggest that these mites might have originated when their ancestors passed from birds to the snakes that predated on them. In some cases, such as elapid snakes, pit mite ancestors passed from colubrid snakes to elapid snakes by their ophiophagous behavior (LOMBERT; MOSS, 1983; FAIN; BOCHKOV; MIRONOV, 1999).

Viper snakes in general do not have birds as part of their diet, nonetheless, as they belong to the Colubroidea super family, explains pit mite parasitism on them (SAZIMA, 1992; SANT'ANNA, 2007). Finally, after revision of the neotropical species of *Ophioptes*, a key of females and males was proposed (Item 4.3.5), considering morphological, host specificity (colubrid and viper snakes), and distribution information. *O. parkeri, O. ekans, O. brevipilis and O. longipilis* represent the five species distributed in Brazil, most of them recorded in the southeast region.

Regarding the superfamily Pterygosomatoidea, family Pterygosomatidae, three genera (*Bertrandiella*, *Geckobia*, and *Geckobiella*) were found parasitizing lizards in the central-west, northeast and southeast regions. Most of these findings are new hosts and distribution records.

The species *B. jimenezi* was described as *H. jimenezi* on lizard *P. bordai*, from México (PAREDES-LEÓN; MORALES-MALACARA, 2009). In the present study, this species was found on *G. geckoides* and *P. pollicaris* in three localities of three different states on the northeastern region (Alagoas, Sergipe, and Rio Grande do Norte). This is the first record of this species in Brazil, and the second species of *Bertrandiella* recorded in South America. The other is *Bertrandiella campanensis* Quiróz-Gutiérrez, Gene, Paredes-León, Roldan-Rodriguez & Perez, 2015 from Peru (QUIRÓZ-GUTIÉRREZ et al., 2015). *Bertrandiella jimenezi* differs from *B. campanensis*, by the latter species has bifurcated idiosomal setae (unique characteristic for this species). This genus has high specificity for lizards of gekkotan families (Sphaerodactylidae, Phyllodactylidae and Eublepharidae) (PAREDES-LEON; KLOMPEN; PEREZ, 2012). The lizards *G. geckoides* and *P. pollicaris* are a new host records from the family *Phyllodactylidae* and show the high specificity of this mite for this family of host.

The exotic species *G. hemidactyli*, was first described from Zimbawe on *H. tasmani*. From there on, the species spread almost worldwide, with records in various localities in the neotropical region. This species expansion success is due to the widespread occurrence of the tropical house gecko *H. mabouia* (Moreau de Jonnès, 1818), which is native to sub-Saharan Africa, but now it is found in North, Central and South America and the Caribbean, where it has been inadvertently introduced by humans (RIVERA et al., 2003; PAREDES-LEÓN et al., 2013). In the present study, this species was found in the southeastern region (São Paulo state) on *H. mabouia* and from the northern region (Pará state) from *T. rapicauda* (MENDOZA-ROLDAN, 2015). This species of mite is considered venereal as it is transmitted when adult geckoes are copulating. Nonetheless, as it has been found in endemic species (*T. rapicauda*), this mite could affect the ecology of diseases

between invasive species and endemic vulnerable animals. Moreover, the life cycle of this species (and most likely of all species of *Geckobia*) occurs entirely on the host. Some of the biological stages are quiescent stages -protonymphs, tritonymphs and females; and other stages are the active infesting forms- deutonymphs and males (BERTRAND; KUKUSHKIN; POGREBNYAK 2013; RIVERA et al., 2003). They reproduce generally through parthenogenesis, where generally most of the generations are females, and eventually a male can surge. Males are considered neotenic deutonymphs, meaning they are sexually mature, yet remain with immature characteristics (JACK, 1961). In the present study, in addition to finding the known different stages of *G. hemidactyli*, fertilized eggs from this species were also identified being laid by a female as the collection of the mites was performed from the host. This is the first description of an egg of *G. hemidactyli*, and adds information to their life cycle and reproduction, being viviparous and oviparous mites.

The other species of *Geckobia* identified in the present study, was *G. bataviensis*. This species was originally described from Batavia (Jakarta), in *H. frenatus* (VITZTHUM, 1926). Since then, it has been recorded in New Guinea and in Asia also infesting *Hemidactylus brookii* Gray, 1845 (BOCHKOV; MIRONOV, 2000), and also on *Hemidactylus platyurus* (Schneider, 1797) and *Hemidactylus garnotii* Duméril & Bibron, 1836 from Indonesia (PRAWASTI; FARAJALLAH; RAFFIUDIN, 2013). Herein, *G. bataviensis* was found on *T. rapicauda* from Vale de São Domingos, Mato Grosso state **(**central-west region). This finding represents the first record of this exotic mite in South America and signifies the first record of this species parasitizing and endemic species of gecko. Thus, this is the second exotic species of Pterygosomatidae mite infesting native species of lizards from Brazil. The implications of the parasitism of exotic mites on endemic reptiles are still unknown, thus require further studies to better understand the impact on native populations.

The last species of Pterygosomatidae mite identified is the genus *Geckobiella*, *G. harrisi*, initially described in *P. plica*, from Santarém, Pará, northern Brazil (DAVIDSON, 1958). Here, it was recorded parasitizing *T. catalanensis*, and *T. torquatus*, from the state of São Paulo, southeast region. These records represent new hosts and localities for this mite, and as reported in former studies this species appears to be a specific ectoparasite of Tropiduridae lizards (PAREDES-LEÓN; KLOMPEN; PEREZ, 2012). Probably the distribution of this species englobes all the Brazilian territory, or where Tropiduridae lizards occur.

The largest group of mites identified in this study is the super cohort Anystina, cohort Parasitengona, superfamily Trombidioidea. Which is represented by two main families: Leeuwenhoekiidae (genus *Hannemania*), and Trombiculidae (*Eutrombicula*, *Fonsecia* and *Neotrombicula*).

Regarding the family Leeuwenhoekiidae, four species of *Hannemania* were identified, being one species recorded for the first time in Brazil (*H. achalai* from the south region), and *H. hepatica* was recorded from São Paulo and Minas Gerais states (Southeastern region). *Hannemania minor* and *H. Yungicola* were recorded in amphibians in a previous study (MENDOZA-ROLDAN, 2015). The genus *Hannemania* includes 26 species in America and 1 species in Oceania (New Caledonia) (SILVA-DE LA FUENTE; MORENO-SALAS; CASTRO-CARRASCO, 2016). In South America, 13 species have been described and 11 of them have poor original descriptions or no type material, hampering taxonomical studies and new species description. Of those, Sambon (1928) described eight species of *Hannemania*, but the status of these species currently is uncertain because the descriptions are insufficient and / or type material is lost (SAMBON, 1928). This makes the identification of these mites in the Americas problematic, thus in this work it was preferred to identify the specimens to known species rather than to add more species without first correction and revising the genus, which is almost impossible as almost all the south American species do not have available type material. Before this study, six species were recorded in Brazil (MENDOZA-ROLDAN, 2015; JACINAVICIUS et al., 2018). One additional species is here added for the south region (*H. achalai).* Furthermore, *H. achalai* was recently redescribed from type frog host (*P. kriegi*) and an additional host (*Pleurodema cordobae* Valetti, Salas, and Martino, 2009) in the type locality of this mite (Pampa de Achala, Córdoba province, Argentina) (PAREDES-LEÓN et al., 2018). Here, most of the mites collected from Rio Grande do Sul state (Arvorezinha and Itapuã) were identified as *H. achalai* from *Melanophriniscus admirabilis* Di-Bernardo, Maneyro & Grillo, 2006, *Scinax squalirostris* Lutz, 1925, and *L. latrans*. All these hosts are new records. It is important to mention that the host species *M. admirabilis* (Bufonidae), is a critically endengared amphibian, that can only be found in a 700 meters area of the Forqueta river in the Arvorezinha municipality described in 2006 (DI-BERNARDO et al., 2006). As this species, many species of amphibians are currently threatened. Most causes of the declining amphibian populations are related to the destruction and modification of habitats. Also, this decline of populations could be due to the spreading of several diseases, such as fungus, viruses

and parasites (HOULAHAN et al., 2000; SOTO et al., 2012). Therefore, the study of amphibian associated parasitic fauna has become an important subject that needs more in-depth research efforts.

The other species of *Hannemania* identified in field trips and collected in this study is *H. hepatica.* This species was described from *L. latrans*, collected in Instituto Butantan, São Paulo, in the early 30's (FONSECA, 1935). It was then recorded on *Physalaemus spiniger* (Miranda-Ribeiro, 1926) from Sete Barras, São Paulo (MENDOZA-ROLDAN, 2015). Thus, this species had a restricted distribution to the state of São Paulo. In the present study, *H. hepatica* was identified in other localities of São Paulo state, such as Cubatão, on *Cycloramphus dubius* (Miranda-Ribeiro, 1920), and Ilhabela on *Cycloramphus boraceiensis* Heyer, 1983. Also, it was identified on *Thoropa megatympanum* Caramaschi & Sazima, 1984 from Diamantina, Minas Gerais state, and on *Corythomantis greeningi* Boulenger, 1896 from Angicos, Rio Grande do Norte state, which is one of the few species of frogs that have skull spines capable of injecting venom in other animals or human hands via headbutting (JARED et al., 2015). All these hosts and localities represent new information and records for *H. hepatica.* These results also increase the distribution of *H. hepatica* to the southeastern region up to the northeastern region, making this species the most common and less host-specific species of the genus. Moreover, *Hannemania* species show high ecological specificity and low physiological specificity (host-specific). This means, one species of these mites can parasitize different amphibians in the same biome, thus avoiding competition for host with other *Hannemania* species. This was seen in this study as some species shared same hosts but were not found in the same localities. This behavior is seen in most of the parasitic species of Parasitengona mites (WOHLTMANN, 2001; WOHLTMANN et al., 2006).

Concerning the Trombiculidae family, 10 species were identified from the ISBP collection, other reference collection, and from recent field trips and laboratories of the Instituto Butantan. Of these species, eight were recorded for Brazil, and four have new records of host and localities.

From collections, trombiculid species identified from Brazilian reptiles and amphibians are the genera *Eutrombicula*, *Fonsecia*, and *Neotrombicula*: *E. alfreddugesi, E. butantanensis, F. ewingi*; *F. travassosi*, and *N. microti*. *Eutrombicula tropica* and *F. anguina* are recorded for the first time in Brazil. Moreover, *N. microti* was collected in *M. schotti*, from Ponta Grossa, Paraná state, in the early 40's and deposited in the IBSP collection. Unfortunately, the only slide available

was darkened which forbid the identification of the mite and the morphological detailing. Still, it is included in the catalogue as it is important information from the IBSP collection.

Of the other species identified of trombiculid mites, the most abundant species was *E. alfreddugesi*. This species forms a complex of species, that has low host specificity, and can parasitize amphibians, reptiles, birds, and mammals, including humans (DANIEL; STEKOLNIKOV, 2004). Also, it is widely distributed through South America (MENEZES et al., 2011). In Brazil, this species has been previously recorded parasitizing lizards in the North region (DELFINO et al. 2011; MENEZES et al., 2011), central-west region (CARVALHO et al., 2006), and in the south region (CUNHA-BARROS et al., 2003; JACINAVICIUS et al., 2018). Herein, *E. alfreddugesi* had a wide distribution (found in Central-West, North, Northeast, and southeastern regions). If was identified from Guaporé, Mato grosso state on *Copeoglossum nigropunctatum* (Spix, 1825), and *T. rapicauda*; from Universidade Federal de Mato Grosso on the snake *Drymoluber brazili* (Gomes, 1918). From the northern region, it was identified in Iracema, Acre state, from the snake *Chironius multiventris* Schmidt & Walker, 1943, and *Chironius scurrulus* (Wagler, 1824); and Tucuruí, Pará state, on *Kentropyx calcarata* Spix, 1825, and *Arthrosaura reticulata* (O'shaughnessy, 1881), and *C. nigropunctatum*. From the Northeast Region, if was identified from Fernando de Noronha island on *Trachylepis atlantica* (Schmidt, 1945), also known as the Noronha skink. This species of lizard is endemic to this island thus is a vulnerable species due to human activity and invasive species predation (ROCHA et al., 2009). Many parasitic helminths have been identified in this species of lizard (RAMALHO), but this is the first record of parasitic mites of tis endemic saurian.

Eutrombicula alfreddugesi was also identified in material collected from the southeastern region (São Paulo state). It was identified from the Barragem Paraitinga on *Phyllomedusa iheringii* Boulenger, 1885; from Cananéia, on *Spilotes pullatus* (Lineu, 1758); from Santa Barbara, on *Aspronema dorsivittatum* (Cope, 1862); from São Bernardo do Campo, on *Philodryas nattereri* Steindachner, 1870; and from São Paulo, on *Tropidurus itambere* Rodrigues, 1987, and *Enyalius iheringii* Boulenger, 1885. This information increases the distribution of *E. alfreddugesi* to almost all the Brazilian territory, and all the hosts are new record of ectoparasite association. It is important to highlight that this is one of the first studies that identifies *E. alfreddugesi* mites on snakes form South America, being the former study performed in the early 80's (LIZASO, 1984), that only identified the mites as trombiculid larvae.

Eutrombicula butantanensis, *E. ophidica*, *F. ewingi*, and *F. travassosi* were all identified from the collection IBSP. Of these, *E. ophidica*, that was initially described as *Trombicula ophidica* from Promissão, São Paulo state, on *X. merremii* (FONSECA, 1932), was identified in this study from Tucuruí, Pará state (North Region) on *K. calcarata* and from Diamantina, Minas Gerais state **(**Southeast Region), on *Tropidurus montanus* Rodrigues, 1987. These hosts and localities are new records for this species. Furthermore, *E. ophidica* was referenced recently as *F. ophidica* (JACINAVICIUS et al., 2018), nonetheless, Radford (1942), placed this species in the *Eutrombicula* genus (RADFORD, 1942). Examining the specimens of this species, indeed the species belongs to *Eutrombicula*, as *Fonsecia* mites have stubby, peg-like anterolateral setae (AL), which *E. ophidica* does not have (BRENNAN; LOOMIS, 1959).

Eutrombicula tropica was herein recorded for the first time in Brazil. It was first described on *Anadia bitaeniata* Boulenger, 1903, from Chama River, Venezuela as *Trombicula irritans* var. *tropica* (EWING, 1925). Here, it was identified in *Psychosaura macrorhyncha* lizard (Hoge, 1946), from Queimada Grande island, São Paulo state. Besides being the first record of this species in Brazil, it is the first parasitic mite recorded from this island on reptiles. It is not clear how did the mite arrive to the island, though it is possible that it arrived as other parasitic acari (*Amblyomma rotundatum* Koch, 1844), when the island was still part of the continent (ARAGÃO, 1936; DUARTE.; PUORTO; FRANCO, 1995). Another possibility is the mites were transported by migratory birds that occasionally land in the island (BRENNAN; REED, 1974).

Finally, it was recorded for the first time *Fonsecia anguina* in Brazil. This species was described on an unknown snake from Yepocapa, Chimal- tenango, Guatemala (BRENNAN; LOOMIS, 1959). Here, it was identified in *Erythrolamprus typhlus* (Linnaeus, 1758) from Iracema, Acre state. Thus, there are three species of this recorded for Brazil, *F. anguina* from the north region, *F. travassosi* from the southeast region, and *F. ewingi* from the central-west region, and reported in *Rhinella ornata* (Spix, 1824) in the southeast region on a previous study (MENDOZA-ROLDAN, 2015).

In this chapter it was included a species of Oribatid mite (Order Sarcoptiformes, Suborder Oribatida, Family Trhypochthoniidae), that although it does not belong to the Trombidiformes order, it belongs to the higher superorder Acariformes, of which Trombidiformes is also included. The species of oribatid mite here identified as *A. longisetosus* was apparently parasitizing *R. major* from Mossoró, Rio Grande do Norte state. This result would the first record of an oribatid mite

having parasitic behavior. Although, it could also be a case of Phoretic behavior, which has been recorded before on a frog *Engystomops pustulosus* (Cope, 1864) by *Archegozetes magnus* (Sellnick, 1925) from Panama (BEATY et al., 2013). The implications of this new host association are discussed in chapter 4. This mite was described from tritonymphs from Nakon Pathom, Thailand (AOKI, 1965), and it is widely distributed across the Oriental, Australian and Neotropical region (HEETHOFF et al., 2013).

6 CONCLUSIONS

1. Six families, 12 genera and 32 species of Trombidiformes mites, parasites of reptiles and amphibians, were here identified, and 23 of them occur in Brazil, increasing six new species to the Brazilian territory.
2. Cloacaridae, Thermacaridae and Ereynetidae were not found in the recent field trips or in live reptiles and amphibians examined in the different laboratories of the Instituto Butantan.
3. Four species of Cloacaridae (*Caminacarus chrysemys, Caminacarus deirochelys, Caminacarus costai*, *Theodoracarus testudines*), and one species of Ereynetidae (*Lawrencarus braziliensis desantisi*), were identified from reference collections (Belgium and Argentina).
4. An updated illustrated key for females and males of *Ophioptes* from the Neotropical region is here proposed.
5. The species *O. parkeri* on *Chironius bicarinatus* is the second record of this species in São Paulo municipality and the first record in this species of snake.
6. The species *O. ekans* was registered for the first time in *B. jararaca* from São Paulo municipality. This is the second record of this species in a viper snake and the first record in this locality.
7. Three genera (*Bertrandiella, Geckobia,* and *Geckobiella*) were found parasitizing lizards in the central-west, northeast and southeast regions. Most of of them are new hosts and distribution records.
8. The species *B. jimenezi* is recorded for the first time in Brazil on *Gymnodactylus geckoides* and *P. pollicaris* in three localities of three different states on the northeastern region.

9. Fertilized eggs layed by a female of *G. hemidactyli* were registered and described for the first time.
10. The species *G. bataviensis* found on *T. rapicauda* from Vale de São Domingos, Mato Grosso state **(**central-west region), represents first record of this exotic mite in South America and first record of parasitism on endemic species of gecko.
11. The species *G. harrisi* parasitizing *T. catalanensis*, and *Tropidurus T. torquatus* from the state of São Paulo, represents new host and locality records.
12. Four species of *Hannemania* were identified and *H. achalai* is recorded for the first time in Brazil.
13. *Hannemania achalai* collected from Rio Grande do Sul state (Arvorezinha and Itapuã) from *M. admirabilis*, *S. squalirostris*, and *L. latrans* are new records of hosts and localities.
14. The species *H. hepatica* was identified from São Paulo state (Cubatão, on *C. dubius* and Ilhabela on *C.* boraceiensis); Minas Gerais (on *Thoropa* from Diamantina); and from Rio Grande do Norte state (from Angicos on *C. greening*). All these hosts and localities are new records.
15. Eight species of Trombiculidae mite were recorded for Brazil, and four have new records of host and localities.
16. The species *E. alfreddugesi* has been registered for the first time on snakes from South America.
17. The species *E. ophidica*, was identified from Tucuruí, Pará state (North Region) on *K. calcarata* and from Diamantina, Minas Gerais state **(**Southeast Region), on *Tropidurus montanus*, all these hosts and localities are new records for this species.
18. The species *E. ophidica* was placed here in the *Eutrombicula* genus and not in *Fonsecia.*
19. *Eutrombicula tropica*, was recorded for the first time in Brazil, in *Psychosaura macrorhyncha* lizard from Queimada Grande island, São Paulo state.
20. The species *F. anguina* was recorded for the first time in Brazil, in *Erythrolamprus typhlus* from Iracema, Acre state.
21. There are three species of *Fonsecia* Brazil: *F. anguina* from the north region, *Fonsecia travassosi* from the southeast region, and *Foncesia ewingi* from the central-west and southeast region.

22. The species of oribatid mite *A. longisetosus* was identified apparently parasitizing *R. major* from Mossoró, Rio Grande do Norte state. This is the first record of an oribatid mite having parasitic behavior.

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ATTACHMENT 1 - A New Species of Pit Mite (Trombidiformes: Harpirhynchidae) from the South American Rattlesnake (Viperidae): Morphological and Molecular Analysis[1]

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A New Species of Pit Mite (Trombidiformes: Harpirhynchidae) from the South American Rattlesnake (Viperidae): Morphological and Molecular Analysis

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Abstract

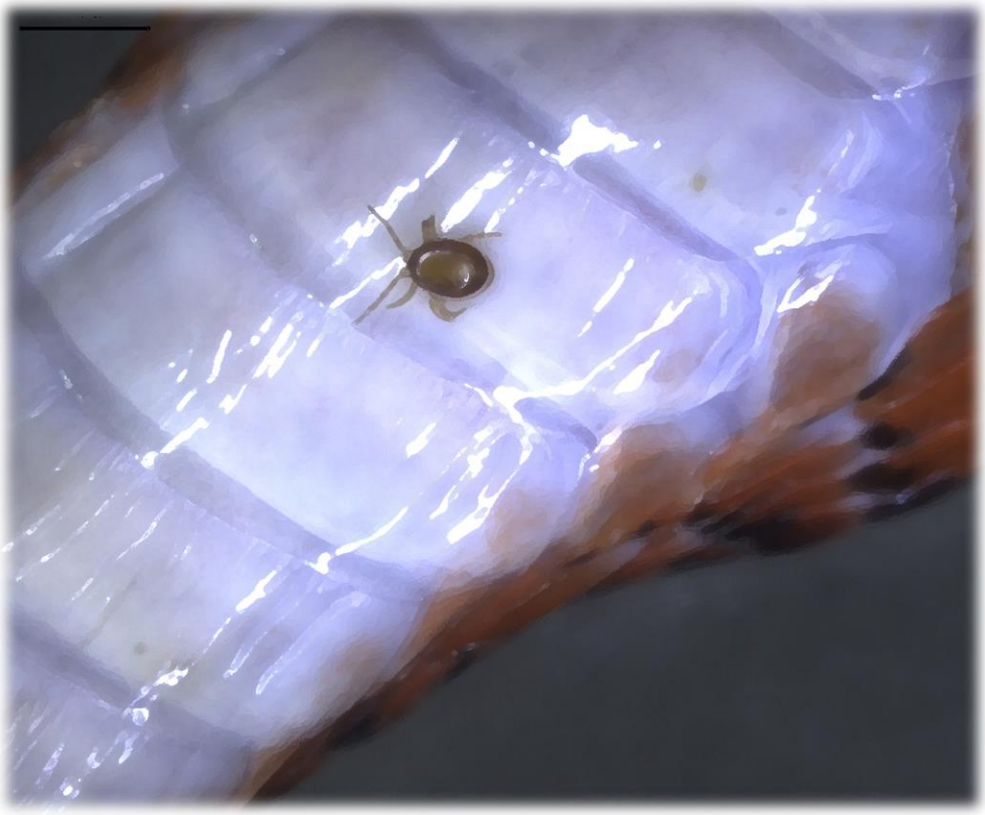
Background: Mites of the genus *Ophioptes*, parasitize a wide range of snakes' species worldwide. Pit mites develop in capsules inside the connective tissue or scales of their hosts and all stages have a genital-anal opening with no connection to the midgut. To this date, there are 15 known species, of which five occur in the Neotropical region. In South America four species have been described from Colubrid snakes.

Methods: Mites were collected from the chin shields and infralabial area of the head, and the anterior third portion of the snake. Comparisons of South American species of pit mites are provided for identification purposes. SEM imaging and illustration were made to provide morphological details of the new species. DNA extraction, sequencing, and phylogeny inference were performed of the new mite species and other species of Trombidiformes mites found on reptiles and amphibians.

Results: *Ophioptes ekans* n. sp. is described from the pits made by the mite on the scales and skin of a South American rattlesnake (*Crotalus durissus terrificus*) in Campo Limpo Paulista, São Paulo state, Brazil, captured on January 2014. The Genbank accession numbers of the new species are KU891263, KU891264 and KU891265. DNA sequences were used for molecular phylogenetic inference. Three nymhpal stages were observed for this species.

Conclusion: This is the first record of a viper snake from the sub-family Crotaline parasitized by *Ophioptes* mites. Molecular analyses showed that molecular systematic of Trombidiformes mites is still unclear and more sequences and other genes are needed do better elucidate the relationships within the group. These are the first DNA sequences (18rRNA V4 region) of mites from the Ophioptinae subfamily.

[1] MENDOZA-ROLDAN, J. A., BARROS-BATTESTI, D. M., BASSINI-SILVA, R., & JACINAVICIUS, F. C. A New Species of Pit Mite (Trombidiformes: Harpirhynchidae) from the South American Rattlesnake (Viperidae): Morphological and Molecular Analysis. **Entomol Ornithol Herpetol**, v. 6, n. 201, p. 2161-0983.1000201, 2017.



(Mendoza-Roldan, 2018)

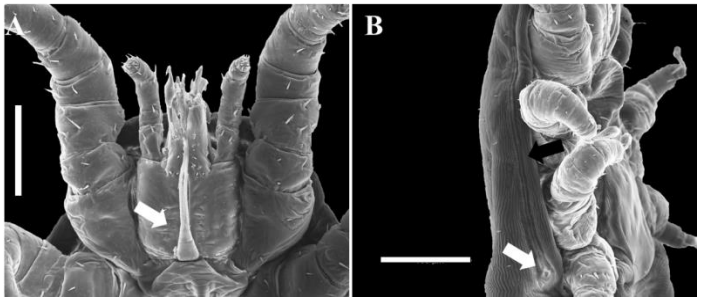
CHAPTER II: Order Mesostigmata

1 INTRODUCTION

1.1 Mesostigmata mites of reptiles and amphibians

The order Mesostigmata belongs to the Parasitiformes superorder, which also includes the orders Holothyrida (a group of mites that feed of bodily fluids of dead arthropods, and it is a group more related to Ixodida), and Ixodida (ticks) (WALTER; PROCTOR, 1988; LEHTINEN, 1991). Mesostigmata includes more than 100 families, with 900 genera, and over 8,000 species. These mites are characterized by having a biflagellate tritosternum (Figure 56A), mid-body stigmata (spiracular openings), located behind legs III to IV, and connected to forward-pointing peritremes (Figure 56B). The chelicerae are long and have terminal scissor-like processes. In the. The palpi are developed, usually five-segmented. Eyes absent. The legs have free coxae and usually end with two claws and an empodium (ZUMPT, 1958; SAUNDERS, 1975; MOSS, 1978, PHILIPS, 2000; DOWLING, 2015). Furthermore, this order is divided in two suborders: Trigynaspida, group of poorly known mites associated to insects, and reptiles (Paramegistidae); and Monogynaspida, of which one-quarter of all Mesostigmata mite species belong to this suborder (WALTER; KRANTZ, 2009; MULLEN; OCONNOR, 2019). To this date, there are no records of Mesostigmata mites permanently parasitizing amphibians.

Figure 56 – Anatomical features of Mesostigmata mites



Source: (MENDOZA-ROLDAN, J. A., 2019)

Legend: Female *Ixobiodes butantanensis*. A. white arrow showing biflagellate tritosternum; B. white arrow showing respiratory stigma, black arrow showing peritreme. Scale bar 100 µm.

Concerning reptiles, seven families of Mesostigmata mites can be temporary or permanent parasites of them. Three of these families do not occur in South America, these are: Laelapidae, Paramegistidae and Omentolaelapidae. The Laelapidae family is represented by one species of nasal inhabiting mites (*Mabuyonyssus freedmani* Till, 1957), collected inside the nasal cavity of the lizard *Trachylepis margaritifera* (Peters, 1854), from Botswana (TILL, 1954). It was first considered a species belonging to the Entonyssidae family, but it was later transferred to the Laelapidae family (FAIN, 1961a). Also, belonging to the Laelapidae family, is the genus *Haemolaelaps*, which has one species parasite of snakes in Europe (FEIDER; SOLOMON, 1960). The Paramegistidae family is associated mainly with invertebrates (insects and myriapods), and one genus (*Ophiomegistus)* has specificity with reptiles (snakes and skinks). This genus has 21 species described mainly from New Guinea, and other parts of Oceania and Asia, of which only adults have been described associated to fossorial reptiles (GOFF, 1980; KLOMPEN; AUSTIN, 2007; BAKER; SEEMAN, 2008). Finally, the family Omentolaelapidae is monotypic, containing the species *Omentolaelaps mehelyae* Fain, 1961, described on *Mehelya* genus of snakes, now known as *Limaformosa capensis* (Smith, 1847) and *Gonionotophis poensis* (Smith, 1849) species, collected from Congo, Africa (FAIN,1961b). These mites are highly specialized with a large sucker on the ventral idiosoma which helps the mite attach firmly onto the body surface of the snake (FAIN, 1963; FAJFER, 2012).

In the Neotropical region, four families of Mesostigmata mites have been recorded parasitizing reptiles, especially snakes. These families are: Entonyssidae (endoparasitic mites of snakes' respiratory system), Heterozerconidae (mites that generally infest myriapods, with three species recorded on snakes and amphisbaenas), Ixodorhynchidae (ectoparasitic mites of reptiles), and Macronyssidae (genus *Ophionyssus*, exclusive of lizards and snakes) (FAIN, 1961a; FAIN, 1962a; FAIN, 1962b; LIZASO, 1979; LIZASO, 1982; DE BELLOCQ; JOËLLE, 2007).

The aforementioned families are all included in the suborder Monogynaspida. Furthermore, they are divided in two superfamilies: Dermanyssoidea, a large group with more than 273 genera and 1,360 species (includes the families: Entonyssidae, Ixodorhynchidae and Macronyssidae); and Heterozerconoidea, which contains 11 genera and 17 species (includes the family Heterozerconidae).

1.2 Superfamily Dermanyssoidea

1.2.1 Entonyssidae family

The family Entonyssidae contains 24 described species, distributed in eight genera. These mites have been all described from the respiratory tract of snakes from families if the superfamily Colubroidea (Colubridae, Lamprophiidae, Elapidae, Homalopsidae, and Viperidae (FAIN, 1961a). this family of mites is characterized by being poorly chitinized, and small to medium size bodies. Dorsal scutum well developed and covering the entire idiosoma. Sternal, genital and anal scuta present in all species. Peritrema absent or smaller and lying forward (present in Pneumophionyssinae subfamily). Legs are long with a pair of curved claws. Tritosternum is generall vestigial or absent. Chelicera are well developed and in form of tongs, with the fixed digit generally rudimentary and the mobile digit well developed and without teeth. The few males known have a dorsal stucum as females (FAIN, 1961a; TURK, 1974). The life cycle of this family consists, in general, of egg, larva, protonymph, deutonymph, and adults (males are rare), and they have been recorded in almost all continents except Antarctica and Australia (FAIN, 1961a). The eight genera are comprised in two subfamilies: Entonyssinae, which englobes six genera - ***Entonyssus***, with three species in North America, and three in Asia; ***Entophionyssus***, with five species in North America; ***Entophiophaga***, with three species in Africa and one species in Europe; ***Cobranyssus***, one species in Asia; ***Hamertonia***, three species in Africa; and ***Viperacarus***, with one species in Europe, and Pneumophionyssinae, which includes two genera described from South America, ***Pneumophionyssus*** and ***Entophioptes*****.** (FONSECA, 1940; FAIN, 1961a; FAIN; YUNKER, 1972). Information regarding the Neotropical species can be observed in the Table 18.

1.2.2 Ixodorhynchidae family

Ixodorhynchidae consists of six genera (*Chironobius, Lxobioides, Ixodorhynchus, Hemilaelaps, Ophiogongylus,* and *Strandtibbettsia*), englobing 31 species, ectoparasitic on snakes worldwide, excluding Australia. This family of mites is found attached beneath the snake's scales, often on the head, around the eyes and gular area.

Table 18 – Species of Pneumophionyssinae, distributed in the Neotropical region

No.	Species	Holotype	Host	Locality	Reference
1	*Pneumophionyssus aristoterisi* Fonseca, 1940	IBSP 1887 - ♀	*Erythrolamprus aesculapii* (Linnaeus)	Botucatú, São Paulo, Brazil	Fonseca (1940)
2	*Pneumophionyssus jellisoni* Fain & Yunker, 1972	USNM No. 34911 - ♀	Unidentified snake	Azul, Argentina	Fain & Yunker (1972)
3	*Entophioptes liophis* Fain, 1961	IRSNB 114-047 - ♀	*Scinax hayii* (Barbour) (cited as *Hyla hayii*)	South America	Fain (1960)

Source: (MENDOZA-ROLDAN, J. A., 2019)

Legend: IBSP (Coleção Acarológica Instituto Butantan, Laboratório Especial de Coleções Zoológicas, Instituto Butantan, São Paulo, Brazil), USNM (National Museum of Natural History, Smithsonian Institution, Washington, D.C. 20560, U.S.A).

Ixodorhynchidae mites have been recorded exclusively in snakes of the families Colubridae, Elapidae, Lamprophiidae, and Viperidae. They are often found attached beneath the (FONSECA, 1934; FAIN 1962a). These mites are characterized by being small or medium size. In most species the body is flattened, short and stocky, elliptical in shape or oval. The gnathosoma is long and the dorsal scutum covers most of the idiosoma or can also be two separate scuta (podosomal and opisthosomal). The anal scutum is generally well developed and chitinized, with various "drawings", and with the 3 anal setae. Cribrum always present, which is a spiculate area posterior to and often lateral to the anal opening on the scutum bearing the anal opening and circumanal setae. Stigmata e located laterally or ventro-laterally at the level of the IV coxa and extended anteriorly by a peritreme of variable length in some genera the cornicles have one or two harpoon hooks near their apical end (these hooks do not exist in the male). Tritosternum with 2 lacinae normally developed and finely hairy. Legs short and all tarsi end with a suction cup. Coxae have usually strong spurs with rounded or bifid tops replacing an ordinary seta. The life cycle of these family, as in most parasitic Mesostigmata mites, occurs mostly on the host and it has five biological stages (egg, larva, protonymph, deutonymph, and adult) (FAIN 1962a; LIZASO, 1982; DOWLIN, 2009).

The Ixodorhynchidae have two types of reproductive strategies: oviparous (Figure 57A) and ovoviviparous (Figure 57B). Almost all species are known to have one or both reproductive strategies, still, little is known of their life cycle (LIZASO, 1988).

Finally, the genera of these ectoparasitic mites are distributed worldwide, excluding Australia. Of the six genera, four occur in South America (*Chironobius,* with two species from Brazil; *Lxobioides,* with three species from Brazil; *Ophiogongylus*, with two species from Brazil and *Strandtibbettsia*, with one species from Brazil). Information regarding the Brazilian species can be observed in the Table 19 and Figure 58.

Table 19 – Species of Ixodorhynchidae distributed in the Neotropical region

No.	Species	Holotype	Host	Locality	Reference
1	*Chironobius alvus* Lizaso, 1983	IBSP 6083 - ♀	*Chironius bicarinatus* (Wied, 1820)	Palmeiras, São Paulo, Brazil	Lizaso (1983)
2	*Chironobius nordestinus* Lizaso, 1983	IBSP 6030 - ♀	*Chironius carinatus* (Linnaeus, 1758)	Mirinzal, Maranhão, Brazil	Lizaso (1983)
3	*Ixobioides butantanensis* Fonseca, 1934	IBSP 26 - ♀	*Xenodon merremi* (Wagler, 1824)	Balsa Nova, Paraná, Brazil	Fonseca (1934)
		IBSP 222	*Xenodon merremi* (Wagler, 1824)	Uberlândia, Minas Gerais, Brazil	Fonseca (1934)
		IBSP 520	*Xenodon merremi* (Wagler, 1824)	Colômbia, São Paulo, Brazil	Fonseca (1934)
		IBSP 3577	*Xenodon merremi* (Wagler, 1824)	Rio Branco, Mato Grosso, Brazil	Fonseca (1934)
4	*Ixobioides fonsecae* Fain, 1961	IRSNB 1214-042 - ♀	*Xenodon guentheri* Boulenger, 1894	Mato Grosso, Brazil	Fain (1961)
5	*LIxobioides brachispinosus* Lizaso, 1983	IBSP 61232- ♀	*Xenodon neuwiedii* Gunther, 1866	Juquitiba, São Paulo, Brazil	Lizaso (1983)
		IBSP	*Chironius bicarinatus* (Wied, 1820)	Pindorama, São Paulo, Brazil	Lizaso (1983)
		IBSP	*Thamnodynastes strigatus* (Günther, 1858)	Rio Azul, Paraná, Brazil	Lizaso (1983)
6	*Ophiogongylus breviscutum* Lizaso, 1983	IBSP 6067 - ♀	*Liophis poecilogyrus* (Wied-Neuwied, 1825)	Votuporanga, São Paulo, Brazil	Lizaso (1983)
7	*Ophiogongylus rotundus* Lizaso, 1983	IBSP 6091 - ♀	*Xenodon neuwiedii* Gunther, 186	Santa Isabel, São Paulo, Brazil	Lizaso (1983)
		IBSP	*Erythrolamprus aesculapii* (Linnaeus, 1758)	Miracatu, São Paulo, Brazil	Lizaso (1983)

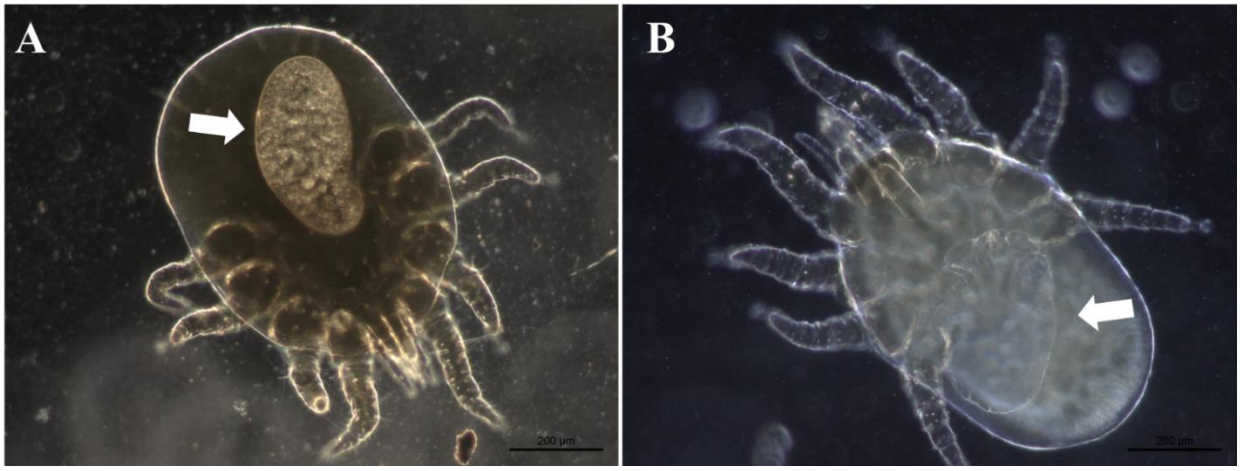
(Conclusion)

No.	Species	Holotype	Host	Locality	Reference
7	*Ophiogongylus rotundus* Lizaso, 1983	IBSP	*Leptodeira annulata* (Linnaeus, 1758)	Colatina, Espírito Santo, Brazil	Lizaso (1983)
		IBSP	*Xenodon neuwiedii* Gunther, 186	Curitiba, Paraná, Brazil	Lizaso (1983)
8	*Strandtibbettsia Braziliensis* Fain, 1961	IRSNB 1214-031 - ♀	*Siphlophis cervinus* (Laurenti, 1768)	Juquiá, São Paulo Brazil	Fain (1961)

Source: (MENDOZA-ROLDAN, J. A., 2019)

Legend: IBSP (Coleção Acarológica Instituto Butantan, Laboratório Especial de Coleções Zoológicas, Instituto Butantan, São Paulo, Brazil), IRSNB (Institut royal des Sciences naturelles de Belgique Brussels, Belgium).

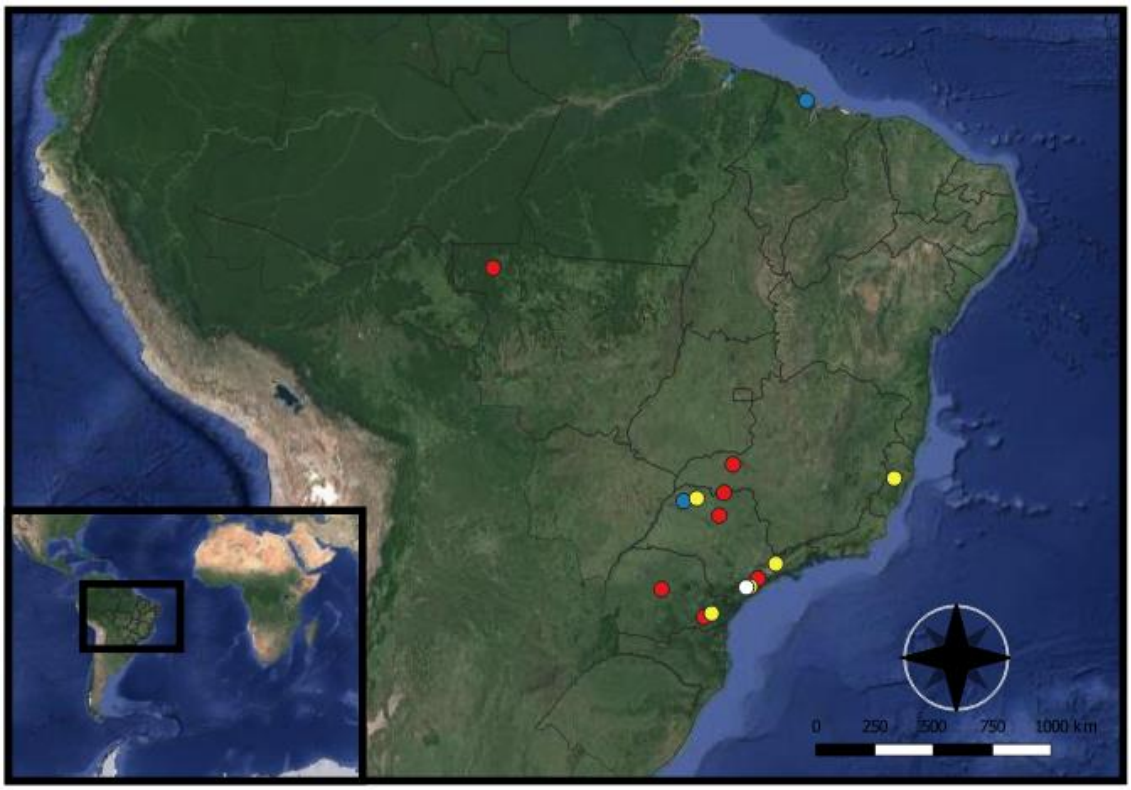
Figure 57 – Anatomical features of Ixodorhynchidae mites



Source: (MENDOZA-ROLDAN, J. A., 2019)

Legend: Female *Ophiogongylus rotundus*. A. white arrow showing egg inside female (oviparous); B. white arrow larva inside female (ovoviviparous). Scale bar 200 µm.

Figure 58 – Distribution map of species of Ixodorhynchidae obtained using QGIS program



Source: (MENDOZA-ROLDAN, J. A., 2019).

Legend: (blue circle) *Chironobius* genus, (red circles) *Lxobioides* genus, (yellow circle) *Ophiogongylus* genus, (white circles) *Strandtibbettsia* genus.

1.2.3 Macronyssidae family

Mite species of the Macronyssidae family that infest reptiles, are all encompassed in the genus *Ophionyssus*. This genus has 17 species of ectoparasitic mites mainly of lizards, snakes and other Squamata reptiles (Sauria: Agamidae, Scincidae, Lacertidae, Cordylidae, Diplodactylidae, and Serpentes), still, there are some reports on species parasitizing mammals (FAIN; BANNERT, 2000; FAIN; BANNERT, 2002; DE BELLOCQ; JOËLLE GOÜY, 2007). Of these 17 species, only one has been described in the neotropical region, *Ophionyssus natricis* (Gervais, 1844) (FAIN; BANNERT, 2002; DE BELLOCQ, 2007; FAJFER, 2012). All *Ophionyssus* species have been found in the palearctic region, except for *O. natricis* which is a cosmopolitan inhabitant of captive snakes, but also infest captive lizards, turtles, crocodiles and other reptiles (WOZNIAK; DENARDO, 2000).

The species *O. natricis* is commonly found in between the scales on the soft tissues, around the eyes, gular region, under the scales or around the cloaca of their hosts (WOZNIAK; DENARDO, 2000; BANNERT et. al. 2000). The life cycle of this species consists on egg, larva, protonymph, deutonymph, and adults. The Protonymphs and females are parasitic. Deutonymphs do not feed and display only low activity (quiescent stages). Females lay eggs and can be be parthenogenetic. The virgin females generate only male offspring (arrhenotokous parthenogenesis), while inseminated females can produce offspring of both sexes (BANNERT, 2002; DE BELLOCQ, 2007).

1.3 Superfamily Heterozerconoidea

1.3.1 Heterozerconidae family

The Family Heterozerconidae is a scarcely studied group of mites that are associated mostly to Diplopoda from tropical and subtropical regions worldwide. Morphologically, this family is characterized by their enlarged ventral suckers. In the Heterozerconidae, the suckers are present in most species and only during the adult stage when they are found on the hosts. These suckers, as well as in Omentolaelapidae, are thought to help the mites attach to their fossorial hosts (FAIN, 1962a; FAIN,1989; FLECHTMANN; JOHNSTON, 1990). Species associated to reptiles are

known only for the neotropical region. It is believed the mites passed to the Squamata reptiles (Amphisbaenia and Serpentes suborders), that shared fossorial habits and habitats with myriapods (FLECHTMANN; JOHNSTON, 1990). All the neotropical species of Heterozerconidae occur in Brazil, and *Heterozercon oudemansi* (Finnegan, 1931) on *Epicrates cenchria* (Linnaeus, 1758) from the Amazon, was the first species described (FINNEGAN, 1931). Later, *Heterozercon elegans* (Lizaso, 1979) was described on *Xenodon merremii*, *Mastigodryas bifossatus*, and *Erythrolamprus aesculapii* (LIZASO, 1979). These two species were synonymized and transferred to the genus *Amheterozercon* (FAIN, 1989), and finally *Zeterohercon amphisbaenae* Flechtmann & Johnston, 1990 was described on *Amphisbaena alba* from São Paulo state (FLECHTMANN; JOHNSTON, 1990). The species *Amheterozercon oudemansi* was transferred to *Zeterohercon* as well. Thus, two species occur in Brazil to date. Information regarding the Brazilian species can be observed in the Table 20 and Figure 59.

2 OBJECTIVES

- Assess the Mesostigmata mites of reptiles and amphibians deposited in the acarological collection of the Instituto Butantan (IBSP), and in other reference collections;
- Identify the Mesostigmata mites found in reptiles and amphibians through optic and electronic scanning microscopy and genetic sequencing (Part II, Chapter 5);
- Update distribution of Brazilian species of Mesostigmata mites, according to recent collections.

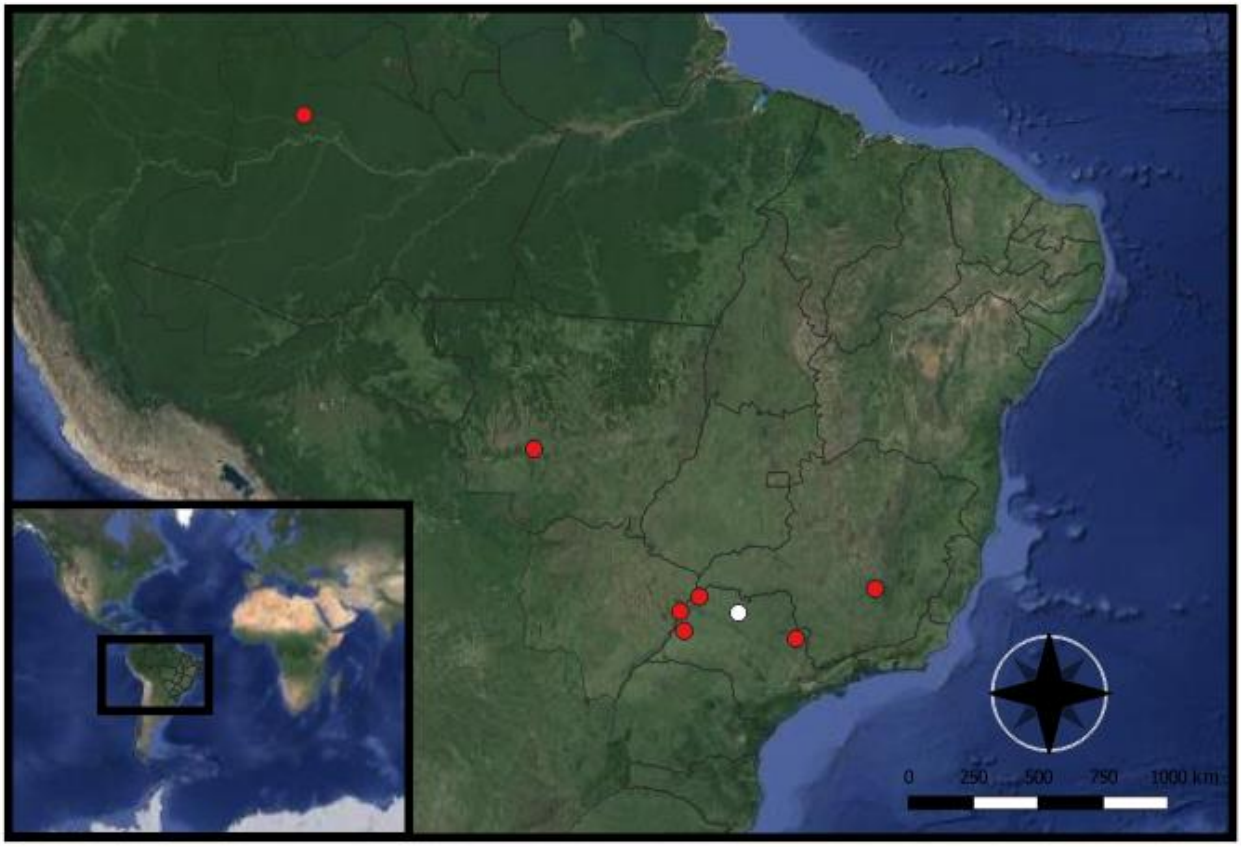
Table 20 – Species of Heterozerconidae distributed in the Neotropical region

No.	Species	Holotype	Host	Locality	Reference
1	*Zeterohercon oudemansi* (Finnegan, 1931)	BM(NH) - ♀	*Epicrates cenchria* (Linnaeus)	Upper Amazon, Brazil	Finnegan, (1931)
		IBSP 6290 - ♀	*Xenodon merremii* (Wagler)	Santa Fe do Sul, São Paulo, Brazil	Lizaso (1979)
		IBSP 6186	*Xenodon merremii* (Wagler)	Dracena, São Paulo, Brazil	Lizaso (1979)
		IBSP	*Mastigodryas bifossatus* (Raddi)	Tangará da Serra Santa Catarina, Brazil	Lizaso (1979)
		IBSP	*Mastigodryas bifossatus* (Raddi)	Belo Horizonte, Minas Gerais, Brazil	Lizaso (1979)
		IBSP	*Erythrolamprus aesculapii* (Linnaeus)	Três Lagoas, Mato Groso, Brazil	Lizaso (1979)
		IBSP	*Erythrolamprus aesculapii* (Linnaeus)	Casa Branca, São Paulo, Brazil	Lizaso (1979)
2	*Zeterohercon amphisbaenae* Flechtmann & Johnston, 1990	OSAL - ♀	*Amphisbaena alba* Linnaeus	São José do Rio Preto, São Paulo, Brazil	Flechtmann & Johnston (1990)

Source: (MENDOZA-ROLDAN, J. A., 2019)

Legend: BM(NH) (The Natural History Museum (formerly British Museum (Natural History), London, United Kingdom, IBSP (Coleção Acarológica Instituto Butantan, Laboratório Especial de Coleções Zoológicas, Instituto Butantan, São Paulo, Brazil), OSAL (Acarology Laboratory, The Ohio State University, Columbus, Ohio, United States), IRSNB (Institut royal des Sciences naturelles de Belgique Brussels, Belgium).

Figure 59 – Distribution map of species of Heterozerconidae obtained using QGIS program



Source: (MENDOZA-ROLDAN, J. A., 2019).

Legend: (red circles) *Z. oudemansii*, (white circles) *Z. amphisbaenae*.
Source: Literature cited in Table 20.

3 MATERIAL AND METHODS

3.1 Mesostigmata mites' material

The mite species of the order Mesostigmata that infest reptiles and amphibians that were assessed, collected, identified, and evaluated, came from three possibilities: material deposited in collections; mites that were brought upon their hosts to the different laboratories of the Instituto Butantan, or to the Venomous Animals Reception site of the same institute; and material that was collected from reptiles and amphibians in different field trips at various locations in Brazil. New or fresh material of mites and hosts were used for molecular biology studies (Part II of this thesis).

3.1.1 Material from collections

This study was based on the revision of the mite material deposited in the acarological collection of the Instituto Butantan (IBSP). Other reference collections were also revised to asses type material of some groups of Trombidiformes mites of reptiles and amphibians.

Acarological Collection of the Instituto Butantan (IBSP) – curator: Valeria Castilho Onofrio. It is one of the oldest collections of mites and ticks of Latin America. Mesostigmata mites of reptiles and amphibians are represented in this collection with 555 lots, being 11 type material. The mites are conserved in alcohol or mounted in slides, and part of the collections remains unidentified.

Fain Acari Collection of the Royal Belgian Institute of Natural Sciences –IRSN – curator: Wouter Dekoninck. One of the widest European collections held together by Dr. Alexander Fain. It harbors more than 100, 000 slides, with 300,000 type material representing 2,407 species of Acari. Mites of reptiles and amphibians are embodied by more than 30 type series of six families.

3.1.2 Laboratories of the Instituto Butantan (IBSP)

3.1.2.1 Venomous Animals Reception site of the Herpetological collection of the Special Zoological Collections Laboratory (LECZ)

The Special Zoological Collections Laboratory (LECZ) of the Instituto Butantan, has a Venomous Animals Reception site, which receives snakes, amphibians, spiders, scorpions, acari (mites and ticks), insects, among other animals, that come from varied localities of Brazil and from other countries. Reptiles and amphibians are then routed to the laboratories from the Instituto Butantan (Herpetology, Cellular Biology, Biological Museum, Ecology and Evolution, among others). Spiders and scorpions are routed to the Arthropods Laboratory, and Acari are deposited in the Acarological collection of the LECZ. Venomous animals (vertebrates and invertebrates) are used firs for venom extraction and in some cases reproduction. When these animals die they are deposited in the collections of the LECZ, which has five collections (Herpetology, Arachnids, Acarology e Entomology and, Myriapoda).

Mites and ticks from reptiles and amphibians that arrived from different regions of Brazil, herein studied, were collected whenever possible before being sent to the different laboratories or collections.

3.1.2.2 Laboratories of the Instituto Butantan

To assess infestation in captivity conditions, the laboratories that harbor live reptiles and amphibians for different purposes in the Instituto Butantan, were visited and the animals were examined for mites and ticks. Laboratories visited were: Cellular Biology, Ecology and Evolution, and the Biological Museum.

3.1.2.3 Material collected in field trips

Mites and ticks' material that was collected from reptiles and amphibians in different field trips at various locations in Brazil. The listed field trips are from projects this study collaborated in fieldwork, or material that was revised from the hosts. The projects also comprise three biomes.

The projects for each area (Atlantic forest, Amazon rainforest, and Cerrado) are presented with details in Chapter I (pages 101-103 of this Thesis).

3.2 Collection of Mesostigmata mites from reptiles and amphibians

Mites were extracted delicately through scarification (mite removal using a needle) according to Fain (1962A), Lizaso (1983) and Mendoza-Roldan et al. (2019). All animals were visually examined, some under stereo microscope, and a complete physical exam from the cranial portion to the caudal (posterior) portion was held for each animal.

Some mite species are endoparasites (Entonyssidae), thus, snakes from various species [mainly *Erythrolamprus aesculapii* (Linnaeus, 1758) for being the type host of *Pneumophionyssus aristoterisi*], deposited in the Herpetological collection or recently euthanized in the same laboratory, were examined and their trachea to lungs were dissected (Figure 60).

Figure 60 – Examination of the celomatic cavity (trachea – lungs) of *Erythrolamprus aesculapii*

Source: (MENDOZA-ROLDAN, J. A., 2017).

Identification of hosts (reptile and amphibians) used in this study, was performed by the team of herpetologists of the Herpetological collection of the Special Zoological Collections Laboratory (LECZ) of the Instituto Butantan (LECZ). The host nomenclature was updated by consulting the "Reptile Database" (http://www.reptile-database.org) (UETZ, 2010) as well as the

database of the Brazilian Society of Herpetology (Sociedade Brasileira de Herpetologia - SBH), for reptiles (COSTA; BÉRNILS, 2018).

3.3 Storage and conservation of mites and host tissue

Collected mites were stored in microtubes in absolute alcohol, and after some of those mites were used for slide mounting (this chapter), DNA extraction and molecular studies (Chapter 5 and 6). Eventually, some tissue samples (blood or liver) were obtained (techniques detailed in chapters 4) from parasitized hosts in the laboratories of the Instituto Butantan or in field trips. These blood samples were used to evaluate hemoparasites in smears (Chapter 4) and for pathogen detection (Chapter 6). Mites and tissue were collected with approval of the Ethics Committee of Animal Use (Comissão de Ética no Uso de Animais - CEUA) of the Faculty of Vetetinary Medicine of the University of de São Paulo (Faculdade de Medicina Veterinária e Zootecnia da Universidade de São Paulo - FMVZ/USP), protocol nº 7491300715.

3.4 Morphological identification

Dichotomous keys (FAIN, 1962b) as well as original descriptions (FONSECA, 1940; FAIN, 1690; FAIN; YUNKER, 1972) were used for morphological identification of Mesostigmata mites of the family Entonyssidae. For the Ixodorhynchidae family, dichotomous keys for genera and species were also used (FAIN, 1962b, LIZASO, 1982; DOWLIN, 2009).

In case of Macronyssidae family original keys for species of *Ophionyssus* were used (FAIN; BANNERT, 2002). For the Family Heterozerconidae keys of the genus *Zeterohercon* were used (FLECHTMANN; JOHNSTON, 1990).

Some of the mites from field trip collections were clarified and mounted in slides. Clarification was made using lactic acid, at 55ºC. Mites usually were monitored until achieved desired results (3 – 5 days). After, material was prepared in modified Berlese`s medium (Hoyer`s medium), according to Krantz & Walter (2009). Once the slides were totally dried, coverslips were sealed using ISOQUID-4571 (Glyptal) resin and deposited in the IBSP collection.

3.4.1 Illustrations

Anatomic features with taxonomic importance of some species of mites with scarce taxonomical information were drawn to better illustrate species diagnosis and differences between species. Illustrations were made using a LEICA DM 400B microscope, then scanned, digitalized, edited and compiled in Photoshop CS6 and Corel Draw X7.

3.4.2 Scanning Electron Microscopy (SEM)

Whenever possible, one to four mites of each species were selected for scanning electron microscopy. The material was first dehydrated for 30 minutes, in a crescent alcohol concentration (70%, 80%, 90%, 95%, 100%, 100%, 100%, 100%), then maintained in Hexamethyldisilane for 24 hours. Metallization was performed leaving the specimens in a chemical cabinet with Hexamethyldisilane, at room temperature, until the material was completely dry. Each specimen was mounted on a ½-inch aluminum metal plate and metallized with gold. Scanning electron microscopy was performed at the Cellular Biology Laboratory of the Butantan Institute, under a digital scanning microscope, of the FEI model Quanta 250 (Multiuser Equipment).

3.5 Distribution

Distribution maps were generated using QGIS program, version 3.4.4-Madeira, to compare new distribution localities with those reported in literature (QGIS DEVELOPMENT TEAM, 2015).

4 RESULTS

Information of the identified species of mites (from collections and recent field trips) can be observed in Tables 20 and 21. All the species of mites collected in this study were incorporated to the acarological collection of the IBSP. Examined species are summarized in the Catalogue of examined species (item 4.2), which also includes information about specimens that were used for

molecular biology (phylogeny and pathogen detection in part II). Host information, as well as parasite-hosts associations and parasitic impact, are discussed in chapter 4.

4.1 Species of Mesostigmata mites identified

In this study, 11 genera and 17 species of Mesostigmata mites were identified. These species were identified from the IBSP collection (and other examined collections), and from ectothermic hosts examined in the laboratories of the Instituto Butantan, as well as those examined in recent field trips (Table 21). Species identified are: Dermanyssoidea superfamily: **Entonyssidae** - *Entophioptes liophis* Fain, 1961; *Entophionyssus glasmacheri* Vitzthum, 1935; *Pneumophionyssus aristoterisii* Fonseca, 1940; **Ixodorhynchidae** - *Chironobius nordestinus* Lizaso, 1983; *Chironobius alvus* Lizaso, 1983; *Chironobius* sp. n.; *Ixobioides butantanensis* Fonseca, 1934; *Ixobioides fonsecae* Fain 1961; *Ixobioides branchispinosus* Lizaso 1983; *Ophiogongylus rotundus* Lizaso, 1983; *Ophiogongylus breviscutum* Lizaso 1983; *Strandtibbettsia braziliensis* Fain, 1961; *Strandtibbettsia gordoni* (Tibbetts, 1957); **Macronyssidae** - *Ophionyssus natricis* (Gervais, 1844); **Laelapidae** - *Haemolaelaps natricis* Feider & Solomon, 1960; **Omentolaelapidae** - *Omentolaelaps mehelyae* Fain, 1961; and Superfamily Heterozerconoidea: **Heterozerconidae** - *Zeterohercon oudemansi* (Finnegan, 1979).

Of the 17 species identified in this study, 13 occur in Brazil. The Brazilian species are shown in Table 20 in bold. Hosts for each species of mites are shown in Table 22 (new hosts records are shown with **X**). Parasite-host associations are discussed in chapter 4.

Table 21 - Mite types and material examined of reptiles and amphibians: collection, field trips and laboratories of the IBSP

Family	Species	Collections		Field trips and laboratories of the IBSP				
		IBSP	RBINS	North	Northeast	Central-west	Southeast	South
	Entophioptes liophis		2					
Entonyssidae	*Entophionyssus glasmacheri*	1						
	***Pneumophionyssus aristoterisii* Fonseca, 1940**	2	5					
Ixodorhynchidae	***Chironobius nordestinus* Lizaso, 1983**	1						
	***Chironobius alvus* Lizaso, 1983**	1						
	***Chironobius* sp. n.**			2				
	***Ixobioides butantanensis* Fonseca, 1934**	39						
	***Ixobioides fonsecae* Fain 1961**	8						
	***Ixobioides branchispinosus* Lizaso 1983**	19						
	***Ophiogongylus rotundus* Lizaso, 1983**	23					2	
	***Ophiogongylus breviscutum* Lizaso 1983**	3						
	***Strandtibbettsia Braziliensis* Fain, 1961**		3					
	Strandtibbettsia gordoni (Tibbetts, 1957)		1					
Macronyssidae	***Ophionyssus natricis* (Gervais, 1844)**	9					3	
Heterozerconidae	***Zeterohercon oudemansi* (Finnegan, 1979)**	15		1			1	
Laelapidae	***Haemolaelaps natricis* Feider & Solomon, 1960**	1						
Omentolaelapidae	*Omentolaelaps mehelyae* Fain, 1961		4					

Source: (MENDOZA-ROLDAN, J. A., 2019)

Legend: IBSP (Acarological collection, of the Instituto Butantan, Special Zoological Collections Laboratory, São Paulo, Brazil), IRSN (Institut royal des Sciences naturelles de Belgique Brussels, Belgium

Table 22 – Species of hosts and species of Mesostigmata infesting mites

Class	Host	*P. aristoterisii*	*C. nordestinus*	*C. alvus*	*Chironobius* **sp. n.**	*I. butantanensis*	*I. fonsecae*	*I. branchispinosus*	*O. rotundus*	*O. breviscutum*	*S. Braziliensis*	*O. natricis*	*Z. oudemansi*	*H. oudemansi*
Serpentes	*Erythrolamprus aesculapii*	x				x	x		x					
	Erythrolamprus typhlus	x												
	Erythrolamprus poecilogyrus					x			x	x				
	Chironius carinatus		x											
	Chironius bicarinatus			x										
	Chironius multiventris				**X**									
	Lygophis anomalus					x								
	Tomodon dorsatus					x								
	Xenodon merremi					x	x						x	
	Xenodon guentheri						x							
	Xenodon neuwiedii							x	x					
	Leptodeira annulata								x					
	Syphlophis pulcher										x			
	Epicrates Cenchria											x		
	Corallus hortullanus											**X**		
	Crotalus durissus terrificus											**X**		
	Oxyrhopus trigeminus													x
	Oxyrhopus melanogenys												**X**	
	Mastigodryas bifossatus												x	
	Micrurus sp.												**X**	
	Pseudoboa nigra												**X**	
Sauria	*Enyalius iheringii*											**X**		
	Pogona vitticeps											**X**		

Source: (MENDOZA-ROLDAN, J. A., 2019).

Legend: New records of hosts are highlighted with **X**

4.2 Catalogue of examined species

Informations regarding identified species of Mesostigmata mites (from collections and recent field trips) are detailed in this section. Material used for molecular biology (Part II) is highlighted with *, new host record with **, and new localities with***.

Order MESOSTIGMATA

Superfamily Dermanyssoidea

Family Entonyssidae

***Entophioptes liophis* Fain, 1961**

South America: IRSNB 1114-047, 1 female paratype, *Lygophis anomalus*, 15.VI.1960, coll. Alex Fain.

***Entophionyssus glasmacheri* (Vitzthum, 1935)**

London, UK: IBSP 1611, 1 female, *Pantherophis alleghaniensis*, 21.VI.1938, coll. Charles D. Radford.

***Pneumophionyssus aristoterisii* Fonseca, 1940**

Brazil: IRSNB 1114-043, 1 protonymph, *Erythrolamprus aesculapii*, 18.VIII.1931, coll. Alex Fain; IRSNB 1114-044, 1 protonymph and 1 female, *E. aesculapii*, 18.VIII.1931, coll. Alex Fain; IRSNB 1114-045, 1 female, *Erythrolamprus typhlus*; IRSNB, 1 female, *E. aesculapii*, 18.VIII.1931, coll. Alex Fain.

Southeast Region: São Paulo state – Botucatu, SP – IBSP 1887, 1 female holotype, *E. aesculapii*, 23.IV.1940, coll. Flávio da Fonseca; IBSP 1956, 1 female paratype, host data and locality same as holotype; IBSP 1956, 1 female paratype, *E. aesculapii*, 15. VI. 1940, coll. Flávio da Fonseca; IRSN 869, 2 females, *E. typhlus*, 18.VIII.1921, coll. Alex Fain; IRSN 1114-044, 1 female and 1 protonymph, *E. aesculapii*, 18.VIII.1921, coll. Alex Fain; IRSN 1114-043, 1 female and 1 protonymph, host data and locality, coll. Alex Fain.

Family Ixodorhynchidae

***Chironobius nordestinus* Lizaso, 1983**

North East Region: Maranhão state – Mirinzal, MA – IBSP 6030, 1 female holotype, *Chironius carinatus*, 16.XI.1976; 39 nymphs, 10 females, 29 males paratypes, host data and locality same as holotype, coll. Nélida Lizaso.

***Chironobius alvus* Lizaso, 1983**

Southeast Region: São Paulo state – Palmeiras, SP – IBSP 6083, 1 female holotype, *Chironius bicarinatus*, 04.IV.1977; 3 females, 2 males paratypes; host data and locality same as holotype; coll. Nélida Lizaso.

***Chironobius* sp.n.**

North Region: Acre state – Iracema, AC – IBSP 14877, 1 female holotype, *Chironius multiventris*, 10.X.2018, coll. Jairo Mendoza-Roldan; IBSP 14878, 2 females paratypes, host data and locality same as holotype; coll. Jairo Mendoza-Roldan *, **, ***.

***Ixobioides butantanensis* Fonseca, 1934**

Brazil: IRSNB 1214-030, 1 female, *Lygophis anomalus*, 15. VI.1961, coll. Alex Fain; IRSNB 1214-039, 1 female, *Tomodon dorsatus*, 18.VIII.1931, coll. Alex Fain.

Center-West Region: Mato Grosso do Sul state – Campo Grande, MS – IBSP 108, 2 females, *Xenodon merremi*, 16.VI.1932, coll. Flávio da Fonseca.

South Region: Paraná state – Balsa Nova, PR – IBSP 26, 1 female holotype, *X. merremi*, 08.V.1933, coll. Flávio da Fonseca; IBSP 103, 1 male paratype, *X. merremi*, 19.V.1932, coll. Flávio da Fonseca. **Maringá, PR –** IRSNB 1214-038, 1 female, *T. dorsatus*, 30.VIII.1897, coll. Alex Fain. **Rio Grande do Sul state – Cruz Alta, RS –** IRSNB 1214-033, 1 female, *Erythrolamprus poecilogyrus*, 04.XI.1947, coll. Alex Fain.

Southeast Region: São Paulo state – Guararema, SP – IBSP 6120, 1 female, *X. merremi*, 04.III.1936, coll. Nélida Lisazo. **Guaratinguetá, SP –** IRSNB 1214-037, 1 female, *Erythrolamprus aesculapii*, 30.XII.1959, coll. Alex Fain. **São Paulo, SP –** IRSNB 1214-036, 1 female, *X. merremi*, 16.I.1956, coll. Uchoa.

***Ixobioides fonsecae* Fain, 1961**

Center-West Region: Mato Grosso state, MT – IRSNB 1214-042, 1 female holotype, *Xenodon guentheri*, 15.VI.1961, coll. Alex Fain.

South Region: Paraná state – Porto Vitória, PR – IBSP 6457, 1 female and 1 male, *Xenodon guentheri*, 30.III.1981, coll. Nélida Lizaso. **Santa Catarina state – Caçador, SC –** IBSP 6331, 3 larvae, 14 nymphs, 27 females and 33 males, *X. guentheri*, 06.IX.1979, coll. Nélida Lizaso; IBSP 6302, 6 females, *X. merremi*, 06.XI.1978, coll. Nélida Lisazo. **Porto União, SC – ** IBSP 6282, 9 females, *X. guentheri*, 06.XI.1978, coll. Nélida Lizaso.

Southeast Region: São Paulo state – Assis, SP – IBSP 6340, 2 females and 1 male, *E. aesculapii*, 01.VI.1979, coll. Nélida Lizaso.

***Ixobioides branchispinosus* Lizaso, 1983**

South Region: Paraná state – Mallet, PR – IBSP 6193, 1 male, *Xenodon neuwiedii*, 20.I.1978, coll. Nélida Lizaso.

Southeast Region: São Paulo state – Juquitiba, SP – IBSP 6123, 1 female holotype, *X. neuwiedii*, 27.VIII.1977, coll. Nélida Lizaso.

***Ophiogongylus rotundus* Lizaso, 1983**

Northeast Region: Bahia state – Alagoinhas, BA – IBSP 6638, 1 female and 1 male, *E. poecilogyrus*, 15.VIII.1985, coll. Lombert & Moss.

South Region: Paraná state – Curitiba, PR – 21 eggs, 22 nymphs, 12 females and 1 male paratypes, *X. neuwiedii*, 16.XII.1977, coll. Nélida Lizaso. **Mallet, PR –** 1 female paratype, *X. neuwiedii*, 20.I.1978, coll. Nélida Lizaso.

Southeast Region: Espírito Santo state – Colantina, ES – 1 female paratype, *Leptodeira annulata*, 17.II.1978, coll. Nélida Lizaso. **Pedro Nolasco, ES –** 15 nymphs, 6 females and 1 male paratypes, *X. neuwiedii*, 20.I.1978, coll. Nélida Lizaso. **São Paulo state – Embu Guaçu, SP –** 2 females paratypes, *E. aesculapii*, 10.II.1978, coll. Nélida Lizaso. **Juquiá, SP –** 7 eggs, 1 larva and 3 nymphs paratypes, *X. neuwiedii*, 21.XII.1977, coll. Nélida Lizaso. **Juquitiba, SP –** 1 female paratype, *X. neuwiedii*, 23.VI.1977, coll. Nélida Lizaso; IBSP 14868, 20 females and 5 males, *X. neuwiedii*, 03.VII.2018, coll. Jairo Mendoza-Roldan*; IBSP 13660, 42 females and 3 males, *X. neuwiedii*, 08.I.2018, coll. Jairo Mendoza-Roldan*. **Miracatu, SP –** 4 eggs, 1 nymph and 2

females paratypes, *E. aesculapii*, 17.I.1979, coll. Nélida Lizaso. **Ribeirão Pires, SP –** 8 females paratypes, *X. neuwiedii*, 29.VIII.1977, coll. Nélida Lizaso. **Santa Isabel, SP –** IBSP 6091, 1 female holotype, *X. neuwiedii*, 30.IV.1977, coll. Nélida Lizaso; 12 nymphs and 10 males paratypes, host data and locality same as holotype, coll. Nélida Lizaso. **Santos, SP –** 15 nymphs and 12 females paratypes, *X. neuwiedii*, 08.III.1976, coll. Nélida Lizaso. **São Roque, SP –** IBSP 5987, 3 nymphs, 17 females and 8 males paratypes, *X. neuwiedii*, 26.V.1976, coll. Nélida Lizaso.

***Ophiogongylus breviscutum* Lizaso, 1983**

Southeast Region: São Paulo state – Presidente Prudente, SP – 2 larvae, 1 nymph and 1 female paratypes, *E. poecilogyrus*, 14.IV.1976, coll. Nélida Lizaso. **Votuporanga, SP –** IBSP 6067, 1 female holotype, *E. poecilogyrus*, 04.II.1977, coll. Nélida Lizaso; 7 eggs and 20 females paratypes, host data and locality same as holotype, coll. Nélida Lizaso; 1 nymph paratype, *E. poecilogyrus*, 10.XII.1976, coll. Nélida Lizaso.

***Strandtibbettsia Braziliensis* Fain, 1961**

São Paulo state – Juquiá, SP – IRSNB 1214-031, 1 female holotype and 1 female paratype, *Syphlophis pulcher*, 09.VIII.1944, coll. Alex Fain.

***Strandtibbettsia gordoni* (Tibbetts, 1957)**

Myanmar, Yangon: IRSNB 1214-030 1 female, *Rhabdophis subminiatus*, 15.VI.1932, coll. Alex Fain.

Family Macronyssidae

***Ophionyssus natricis* (Gervais, 1844)**

Manchester, UK: IBSP 4353, 1 female, *Epicrates Cenchria*, 01.VI.1940, coll. Gervais.

Southeast Region: São Paulo state – Barragem Paraitinga, SP – IBSP 12686, 2 females, *Enyalius iheringii*, 15.V.2004, coll. Patricia B. Bertola, **. **São Paulo, SP –** IBSP 2135, 2 females and 1 male, *Epicrates Cenchria*, 28.IX.1954, coll. Flávio da Fonseca; IBSP 4389, 1 male, *X. merremi*, 22.XI.1947, coll. Flávio da Fonseca; IBSP 12907, 4 females, 1 male and 1 deutonymph, *Crotalus durissus terrificus*, 06.XI.2015, *, **; IBSP 12983, 2 females, 2 males and 1 deutonymph,

Corallus hortullanus, 20.IV.2017, coll. Jairo Mendoza-Roldan, *,**; **Zoo Bauru, SP –** IBSP 14874, 8 females and 2 males, *Pogona vitticeps*, 10.XII.2018, coll. Bruna Simonato*,**, ***.

Family Laelapidae

***Haemolaelaps natricis* Feider & Solomon, 1960**

Northeast Region: Pernambuco state – Guararapes, PE – IBSP 6217, 1 female, *Oxyrhopus trigeminus*, 14.IV.1978, coll. Nélida Lizaso.

Family Omentolaelapidae

***Omentolaelaps mehelyae* Fain, 1961**

Congo: IRSNB, 1 protonymph paratype, *Limaformosa capensis*, 15.VI. 1955, coll. Alex Fain; 1 female paratype, host data and locality same as before; 1 male paratype, *L. capensis*, 25.I.1950, coll. Alex Fain; 1 larvae paratype and 1 nymph paratype, *L. capensis*, 15.VI.1955, coll. Stan.

Heterozerconoidea superfamily

Heterozerconidae family

***Zeterohercon oudemansi* Finnegan (1979)**

Center-West Region: Mato Grosso do Sul state – Três Lagoas, MS – IBSP 6308, 2 males, *Mastigodryas bifossatus*, 29.XII.1978, coll. Nélida Lizaso.

North Region: Acre state – Iracema, AC – IBSP 14884, **2 females,** *Oxyrhopus melanogenys*, 10.X.2018, coll. Jairo Mendoza-Roldan*,**, ***; **Pará state –Tucuruí, PA –** IBSP 6760, 1 female, *Micrurus* sp., 15.VI.1984, coll. Angela Mingozzi**.

South Region: Santa Catarina state – Tangará da Serra, SC – IBSP 6110, 1 female, *X. merremi*, 01.VII.1977, coll. Nélida Lizaso.

Southeast Region: São Paulo state – Santa Fé do Sul, SP – IBSP 6290, 1 female holotype, *X. merremi*, 24.XI.1978, coll. Nélida Lizaso; **São Paulo, SP –** IBSP 12953, 4 females and 4 males, *Pseudoboa nigra*, 26.IX.2016**, ***.

4.3 Morphological and taxonomical details

In this section seven species of Mesostigmata mites are detailed morphologically. These species are: **Entonyssidae** *Pneumophionyssus aristoterisii* Fonseca, 1940; **Ixodorhynchidae** *Chironobius nordestinus* Lizaso, 1983; *Chironobius alvus* Lizaso, 1983; *Chironobius* sp. n.; *Ophiogongylus rotundus* Lizaso, 1983*;* **Macronyssidae** *Ophionyssus natricis* (Gervais, 1844); and **Heterozerconidae** *Zeterohercon oudemansi* (Finnegan, 1979).

Order MESOSTIGMATA

Superfamily Dermanyssoidea

Family Entonyssidae

4.3.1 *Pneumophionyssus aristoterisii* Fonseca, 1940: 54

Type material - Holotype female (IBSP 1887) and two female paratype (IRSNB), *Erythrolamprus aesculapii* (Linnaeus, 1758), Botucatú, São Paulo, Brazil.

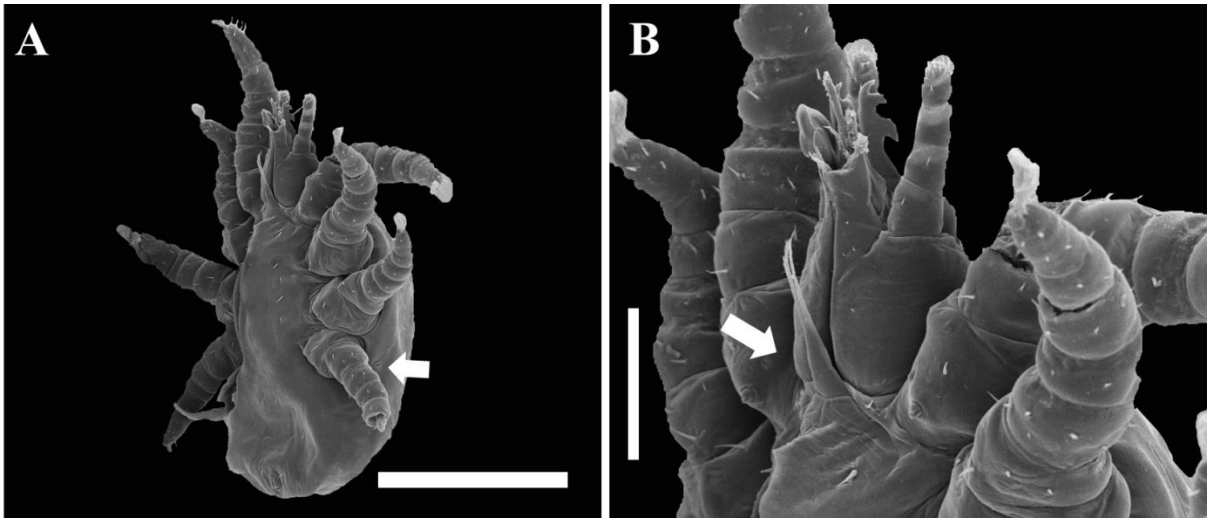
Diagnosis. Only female is known. Fixed digit of rudimentary chelicera; little mobile digit chitinized, long and falciform; the two digits without teeth or thorns. Tritosternum with 2 well developed lacinae reaching approximately the base of palpi. Latero-ventral stigmata with a short peritreme lying anteriorly. Well-developed deutosternal teeth arranged in a longitudinal row. Females have 3 pairs of sternal setae. Dorsal patch with 5 to 7 pairs of setae.

Female (redescription)[2]. Idiosoma oval and 680 µm (670 – 775) long, and 340 (340 – 400) µm wide. Total length, including gnathosoma, 780 µm. **Ventral idisoma**: (Figure 61A). Stigmata located near the coxae IV, in ventro-lateral position, anteriad of these is a short peritreme. Tritosternum ends in two short barbed lacinae. Sternal scutum wider than large (130 µm wide and 90 µm long), bearing 6 setae (Figure 61B).

[2] Award for best oral presentation (Postgraduate), II Congresso latinoamericano de acarologia, Montenegro, Quindio, Colombia, 2016.

Genital scutum small, 125 µm long and 50 µm wide, and bears I pair of setae that are in the soft cuticule next to the margin of the scutum. Soft cuticle of opisthosoma has 4 pairs of setae arranged 2-2-4. Anal scutum narrow, subterminally located, and bears 3 setae (Figure 62A, D, E). **Dorsal idiosoma**: An elongate scutum on dorsum 320 µm long, and 196 µm wide, bears 5 - 7 pairs of setae. **Gnathosoma**: Palps much longer (110 µm) than base of gnathosorna (45 µm); Seven deutosternal teeth disposed in a single row. Chelicerae 85 µm long and at most 22 µm wide, movable digit is poorly sclerotized and triangular, fixed digit is cylindrical and as long as movable one (Figure 62 B, C).

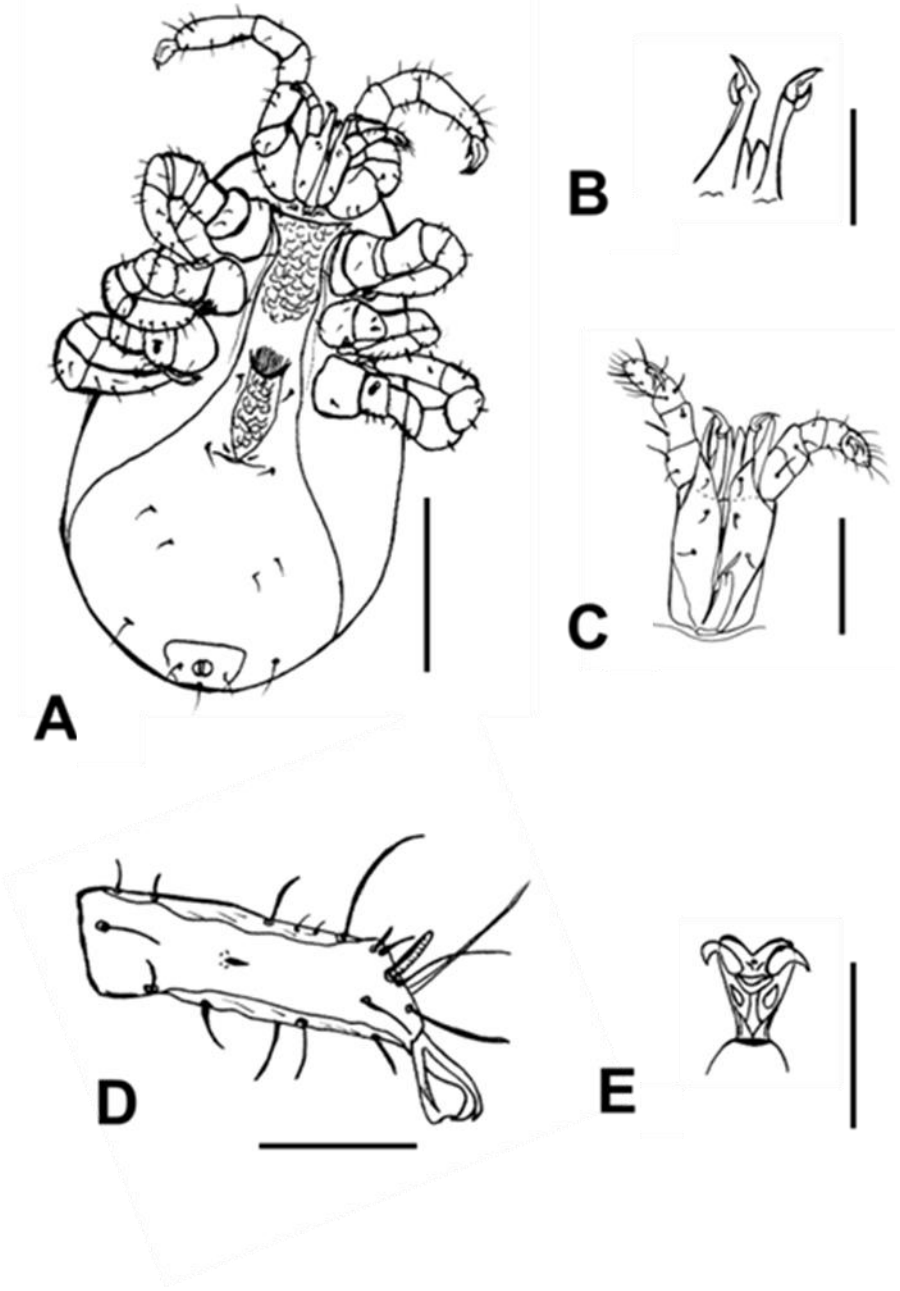
Figure 61 – Scanning electron microscopy of female *Pneumophionyssus aristoterisii*



Source: (MENDOZA-ROLDAN, J. A., 2016)

Legend: Female *Pneumophionyssus aristoterisii*. A. white arrow showing respiratory stigma; B. white arrow showing biflagellate tritosternum. Scale bar: A 300 µm, B 100 µm.

Figure 62 – Illustrations with morphological features of female *Pneumophionyssus aristoterisii*



Source: (MENDOZA-ROLDAN, J. A., 2016)

Legend: A. Female ventral view; B. *Digitus mobilis*; C. Gnathosoma; D. Tarsus leg I; E. claws e *Pulvillus*; A 200µm; B-E 50µm.

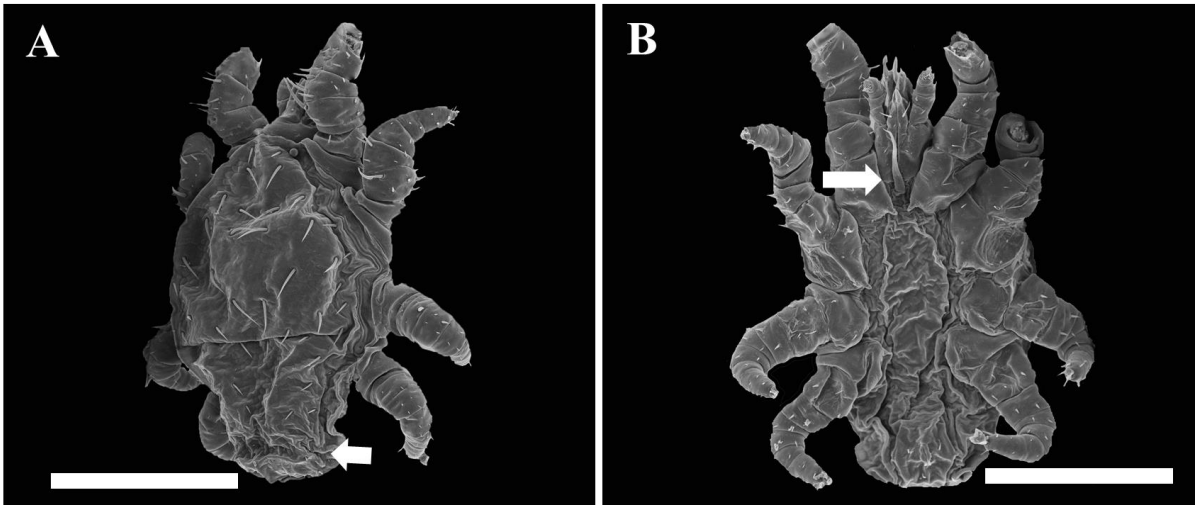
Family Ixodorhynchidae

4.3.2 *Chironobius nordestinus* Lizaso, 1983: 201

Type material - Holotype female, and 29 female, 10 males and 39 nymhps paratypes (IBSP 6030), on *Chironius carinatus* Linnaeus, 1758, from Mirinzal, Maranhão, Brazil.

Diagnosis. Large mites with divided dorsal scutum, poorly chitinized, long setae on dorsal scutum, coxae I and II with a strong spur. Chelicerae without fixed digit, mobile digit with 3 teeth.
Female. Large, robust, white colored, with a chitinized band in shape of half moon in the dorsal idiosoma, in the posterior margin of the dorsal scutum (Figure 63A, Figure 64B). Dorsal scutum divided at coxae IV level. Sternal scutum with diffuse margins, genital scutum slightly reticulated, and anal scutum rounded and reticulated. Bifid tritosternum with pilous lascinae (Figure 63B). Leg Chaetotaxy: coxae 2-2-2-1, trochanter 6-5-5-5, femur 11-8-5-6, genu 12-9-8-7, tibia 12-10-7-6 (Figure 64A). **Male.** Smaller than female, slightly more chitinized, scutum with no chitinized band. Genital scutum reticulated.

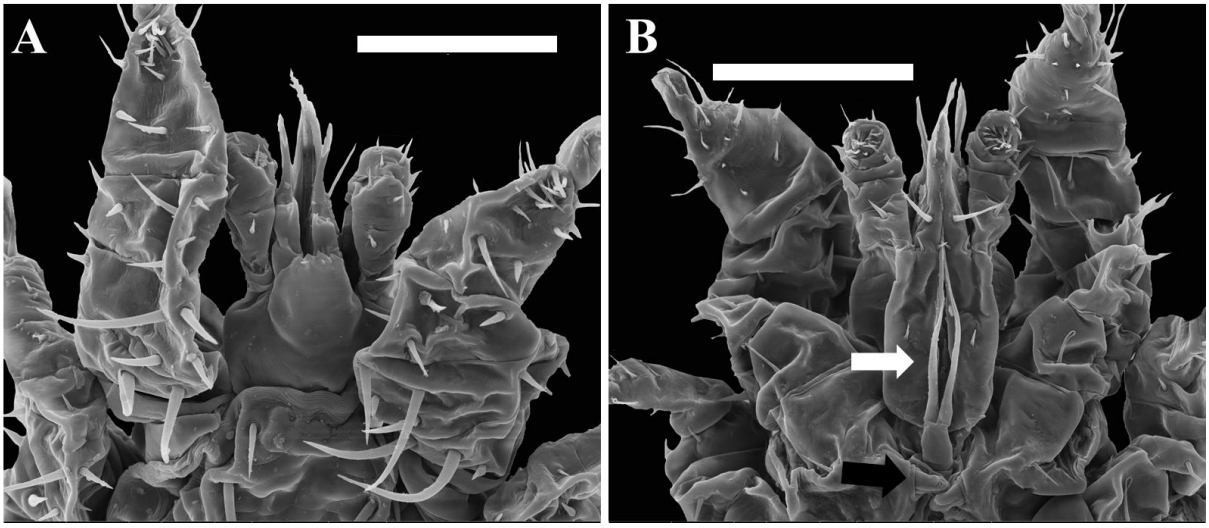
Figure 63 – Scanning electron microscopy of female *Chironobius nordestinus*



Source: (MENDOZA-ROLDAN, J. A., 2018)

Legend: Female *Chironobius nordestinus*. A. chitinized band in shape of half moon; B. white arrow showing biflagellate tritosternum. Scale bar 200 µm.

Figure 64 – Scanning electron microscopy of female *Chironobius nordestinus*



Source: (MENDOZA-ROLDAN, J. A., 2018)

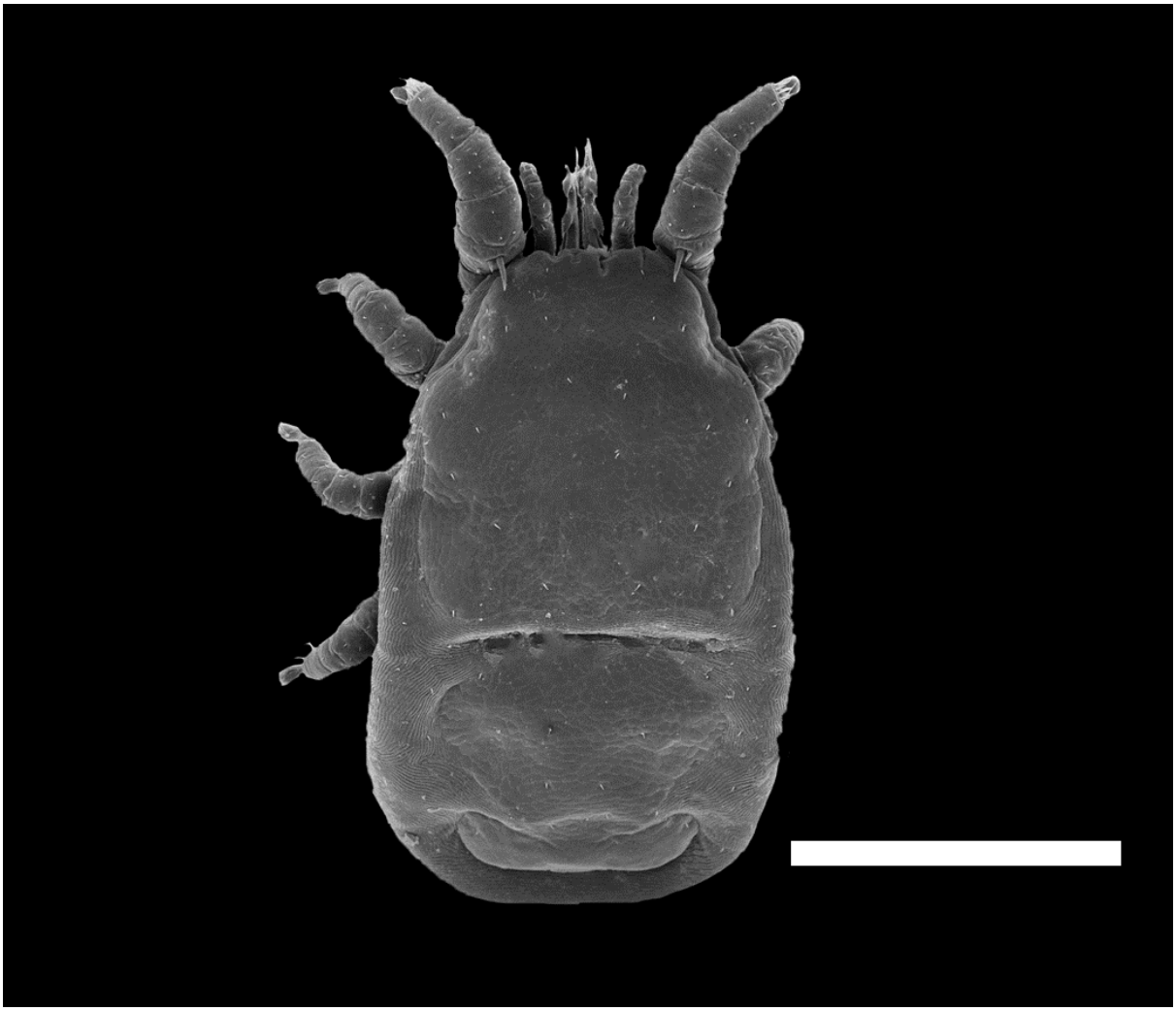
Legend: Female *Chironobius nordestinus*. A. gnathosoma dorsal view; B. white arrow showing biflagellate tritosternum, black arrow showing stron spur in coxa I. Scale bar 50 µm.

4.3.3 *Chironobius alvus* Lizaso, 1983: 197

Type material - Holotype female, and 3 female, 2 males paratypes (IBSP 6083), on *Chironius bicarinatus* (Wied, 1820), from Santa Isabel, São Paulo, Brazil.

Diagnosis. Large mites with divided dorsal scutum, poorly chitinized, long setae on dorsal anterior scutum, coxae I and II with a strong spur. Chelicerae without fixed digit, mobile digit with 3 teeth. Femurs I and II with long dorsal setae. **Female.** Dorsal scutum divided at coxae IV level (Figure 65). Long dorsal setae in the anterior region of the dorsal scutum. Dorsal scutum with lateral and posterior margin strongly chitinized. Sternal scutum reticulated, and margins poorly shapen (Figure 66C). Genital scutum reticulated as well as the anal scutum, which is poorly chitinized (Figure 66A). Small tritosternum, bifid and with pilous lascinae (Figure 66C). Leg Chaetotaxy: coxae 2-2-2-1, trochanter 6-6-5-5, femur 11-8-4-7, genu 12-9-5-6, tibia 12-10 -5-6 (Figure 66D). **Male.** Smaller than female, slightly more chitinized, scutum with no chitinized band. Genital scutum reticulated. Large anal scutum. Femur I with large dorsal setae.

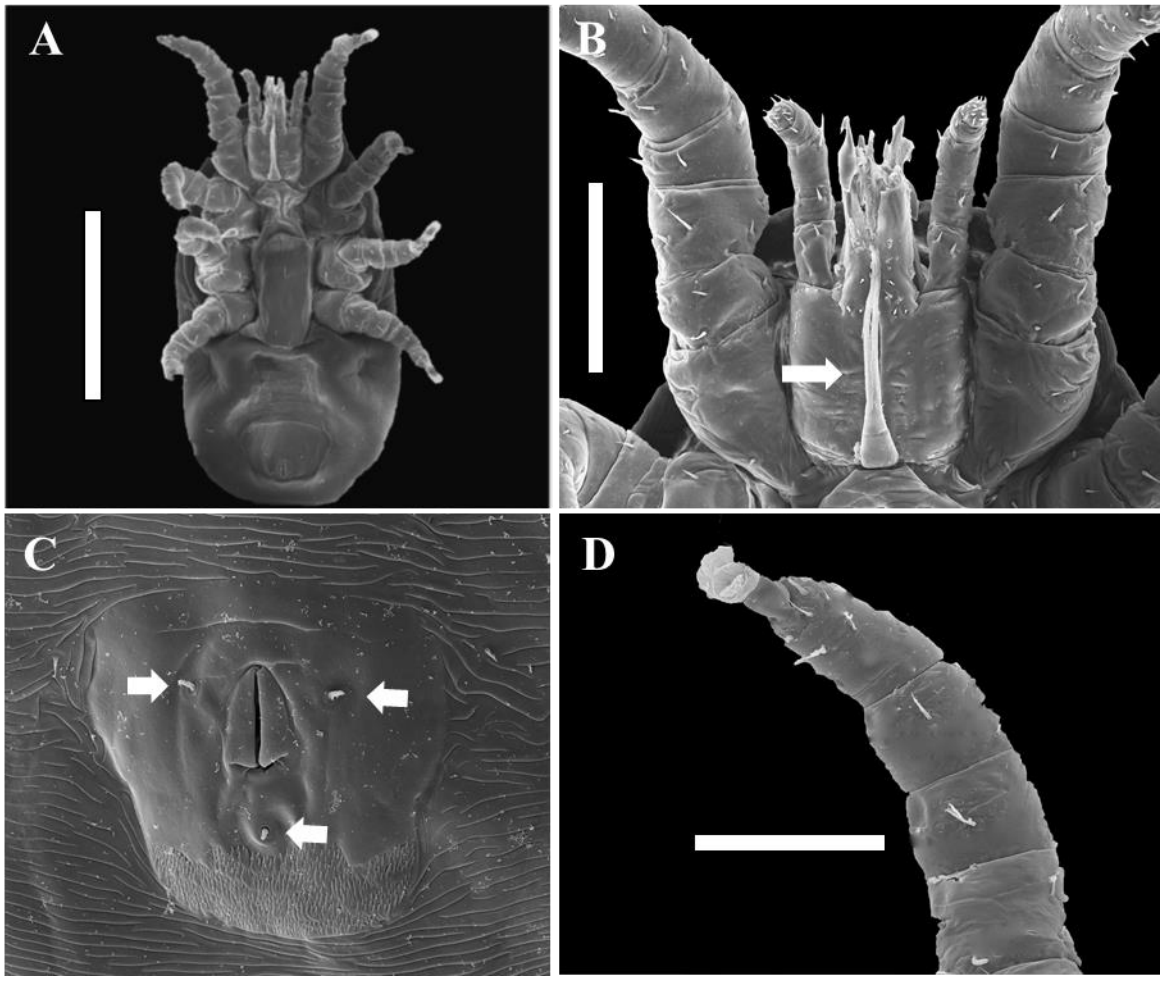
Figure 65 – Scanning electron microscopy of female *Chironobius alvus*, dorsal view



Source: (MENDOZA-ROLDAN, J. A., 2018)

Legend: Female *Chironobius alvus*, dorsal view. Divided dorsal scutm. Scale bar 300 µm.

Figure 66 – Scanning electron microscopy of female *Chironobius alvus*



Source: (MENDOZA-ROLDAN, J. A., 2018)

Legend: Female *Chironobius alvus*. A. ventral view; B. white arrow showing biflagellate tritosternum; C. white arrows shogin genital setae on genital scutum; D. leg I. Scale bars: A 300 µm, B 100 µm, C, D 40 µm.

4.3.4 *Chironobius* sp. n. Mendoza-Roldan & Barros-Battesti, 2019

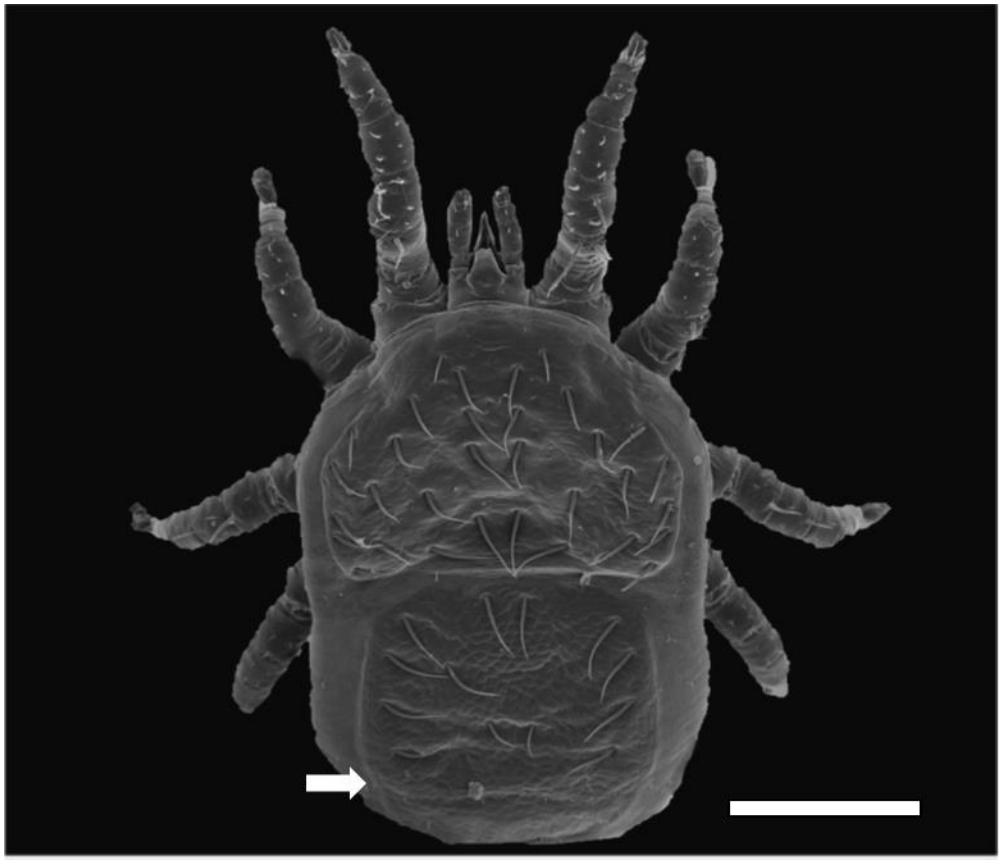
Type material - Holotype female (IBSP 14877), and 2 females paratypes (IBSP 14878) on *Chironius multiventris* Schmidt & Walker, 1943, Iracema, Acre, Brazil.

Diagnosis. Only females are known. Large mites with divided dorsal scutum, poorly chitinized, long setae on all the dorsal scuta, coxae I and II with a strong spur (Figures 67, 69A). Chelicerae without fixed digit, mobile digit with 3 teeth. Femurs I and II with long dorsal setae. Posterior dorsal scutum with a chitinized dark band in the posterior and lateral margins. Genital scutum reticulated and chitinized in the lateral margins. Anal scutum strongly chitinized in the lateral posterior margins.

Female description. Idiosoma rounded, 780 µm (770 – 780) long, and 514 (500 – 520) µm wide. Total length, including gnathosoma, 969 µm. **Ventral idiosoma**: (Figure 68A). Stigmata located after the coxae IV, in ventro-lateral position, anterial of these is a long and cheratinized peritreme. Tritosternum ends in two long barbed lacinae (Figure 68B). Sternal scutum with margins poorly shapen and 110 µm (80 – 115) long, and 103 (100 – 105) µm wide (Figures 68B, 69B). Genital scutum long, 205 µm and 93 µm wide, and bears 2 setae, reticulated and chitinized in the lateral margins. Anal scutum rounded, long, 139 µm long and 125 µm wide subterminally located, and bears 3 setae, strongly chitinized in the lateral posterior margins (Figures 68C, 69B). Leg Chaetotaxy: coxae 2-2-2-1, trochanter 5-5-3-4, femur 9-4-4-3, genu 9-6-3-2, tibia 8-4 -4-3. Strong spurs on coxae I and II. **Dorsal idiosoma**: Dorsal scutum divided at coxae IV level, 675 µm (anterior 311 – posterior 367) long, and anterior 456 – posterior 348 µm wide, with a chitinized dark band in the posterior and lateral margins of the dorsal scutum (Figures 68D, 69A). Dorsal scutum with 26 pairs of setae, 16 pairs of long setae on the anterior scutum and 7 pairs of long, and 3 pairs of short setae on the posterior scutum. Most setae on the dorsal long, thick and setiform, 105–120 µm long. Short setae of posterior scutum 10 - 15 µm long. **Gnathosoma**: Chelicerae without fixed digit, mobile digit with 3 teeth. Pedipalps two-tined and of general form found in many dermanyssoids.

Female with larva inside idiosoma (ovoviviparity) (Figure 70).

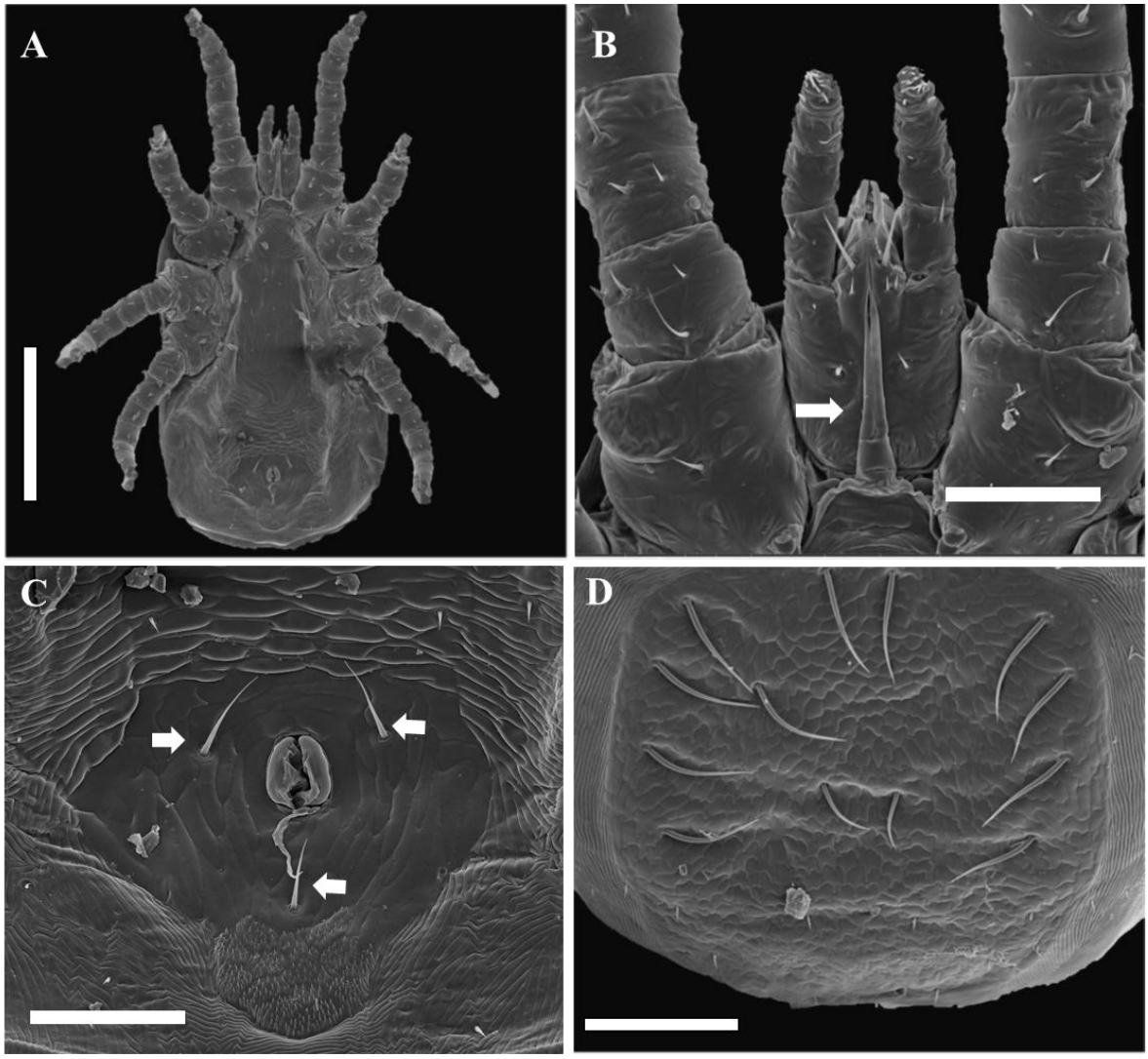
Figure 67 – Scanning electron microscopy of female *Chironobius* sp. n., dorsal view



Source: (MENDOZA-ROLDAN, J. A., 2019)

Legend: Female *Chironobius* sp. n. white arrow showing Posterior dorsal scutum with a chitinized dark band in the posterior and lateral margins. Scale bar 300 µm.

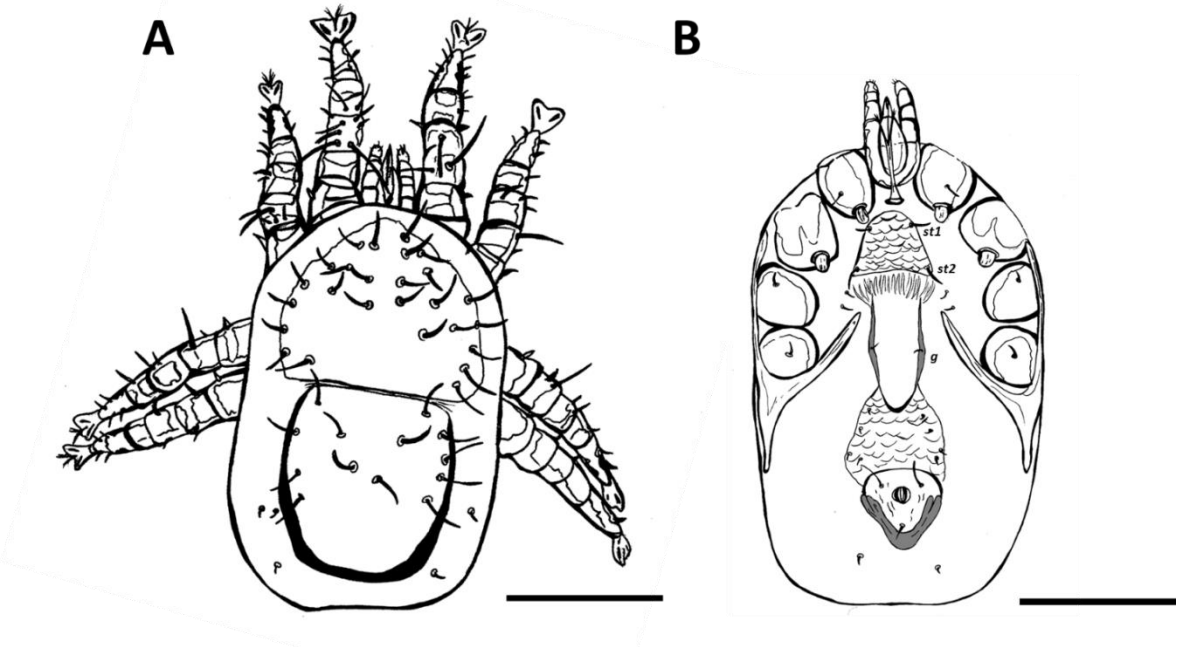
Figure 68 – Scanning electron microscopy of female *Chironobius* sp. n.



Source: (MENDOZA-ROLDAN, J. A., 2019)

Legend: Female *Chironobius* sp. n. A. ventral view; B. white arrow showing biflagellate tritosternum; C. white arrows showing setae on anal scutum; D. posterior dorsal scutm. Scale bars: A 300 µm, B-D 50 µm.

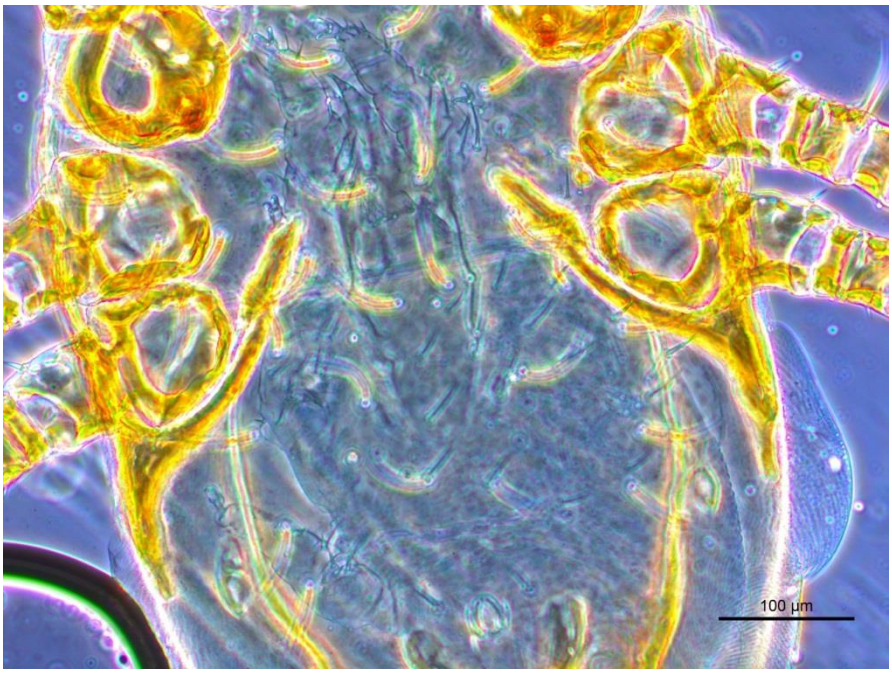
Figure 69 – Illustrations with morphological features of female *Chironobius* sp. n.



Source: (MENDOZA-ROLDAN, J. A., 2019)

Legend: A. Female dorsal view; B. Female ventral view. Abbreviations: *st1*- *st2* sternal setae, *g* genital setae. Scale bars: 300 µm.

Figure 70 – Optic microscopy female with larva of *Chironobius* sp. n.



Source: (MENDOZA-ROLDAN, J. A., 2019)

Legend: Larva inside idiosoma. Scale bar 100 µm

4.3.5 Key of species of *Chironobius*

Females

1(0).
- Corniculi elongate and pointed with retrorse hooks or barbs, resembling a harpoon .....genera ***Ixodorhynchus, Ophiogongylus, Ixobioides***
- Corniculi smooth with no barbs or hooks.....genera ***Hemilaelaps, Strandtibbettsia***, ***Chironobius*** **(2)**

2(1).
- Chelicerae with both movable and fixed digits present.....…….....…….……***Hemilaelaps***
- Chelicerae with fixed digit reduced to small nub or completely absent.....……**(3)**

3(2).
- Chelicerae with fixed digit reduced to nub, but pilus dentilis still present; sternal shield with heavily sclerotized band across anterior portion; genitoventral shield narrow, but never tapering to a point……***Strandtibbettsia***
- Chelicerae with fixed digit and pilus dentilis absent; sternal shield subtriangular without heavily sclerotized region; genitoventral shield tapering to a point. ……..……. ***Chironobius*** **(4)**

4(3).
- Posterior dorsal scutum with chitinized band only in the posterior margin.....……..……..C***hironobius nordestinus***
- Posterior dorsal scutum with chitinized band the posterior and lateral margins……..**(5)**

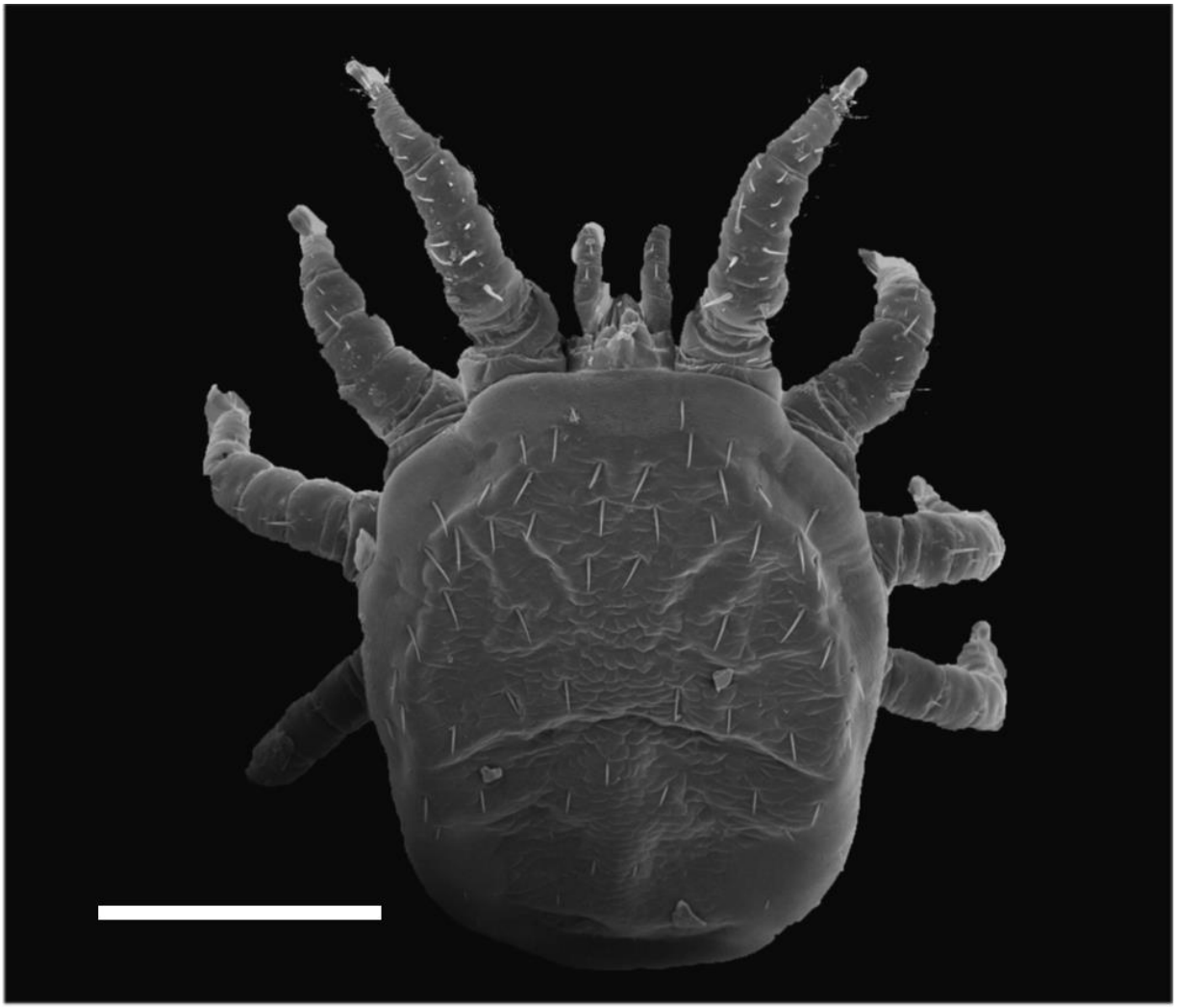
5(4)
- Genital scutum reticulated as well as the anal scutum, which is poorly chitinized, long setae on the anterior dorsal scutum.....……..……..……***Chironobius alvus***
- Genital scutum long, reticulated and chitinized in the lateral margins; anal scutum triangular, strongly chitinized in the lateral posterior margins, long setae on all the dorsal scuta…..…. ***Chironobius*** **sp.n.**

4.3.6 *Ophiogongylus rotundus* Lizaso, 1983

Type material - Holotype female, (IBSP 6091) and 10 female, 8 males and 12 nymphs (IBSP 5987) paratypes, on *Xenodon neuwiedii* Günther, 1863, from Santa Isabel, São Paulo, Brazil.

Diagnosis. Stubby, small, whitish, and rounded mites. One dorsal scutum, poorly distinguished, narrowed sternal scutum (Figure 71). Strong bifid spur in coxae I, II, III (Figure 72C). Long setae in femurs I and II. **Female.** Idiosoma with few setae. Sternal scutum with irregular margins; genital scutum well defined and dotted; anal scutum slightly reticulated and long. Tritosternum short and bifid (Figure 72). Gnathosoma with strong chelicera. Leg Chaetotaxy: coxae 2-2-2-1, trochanter 6-5-5-5, femur 11-8-5-5, genu 10-7-7-5, tibia 12-7 -6-6. **Male.** Smaller than female (Figure 72A), slightly less chitinized. Genital scutum joined to the anal scutum (Figure 72D).

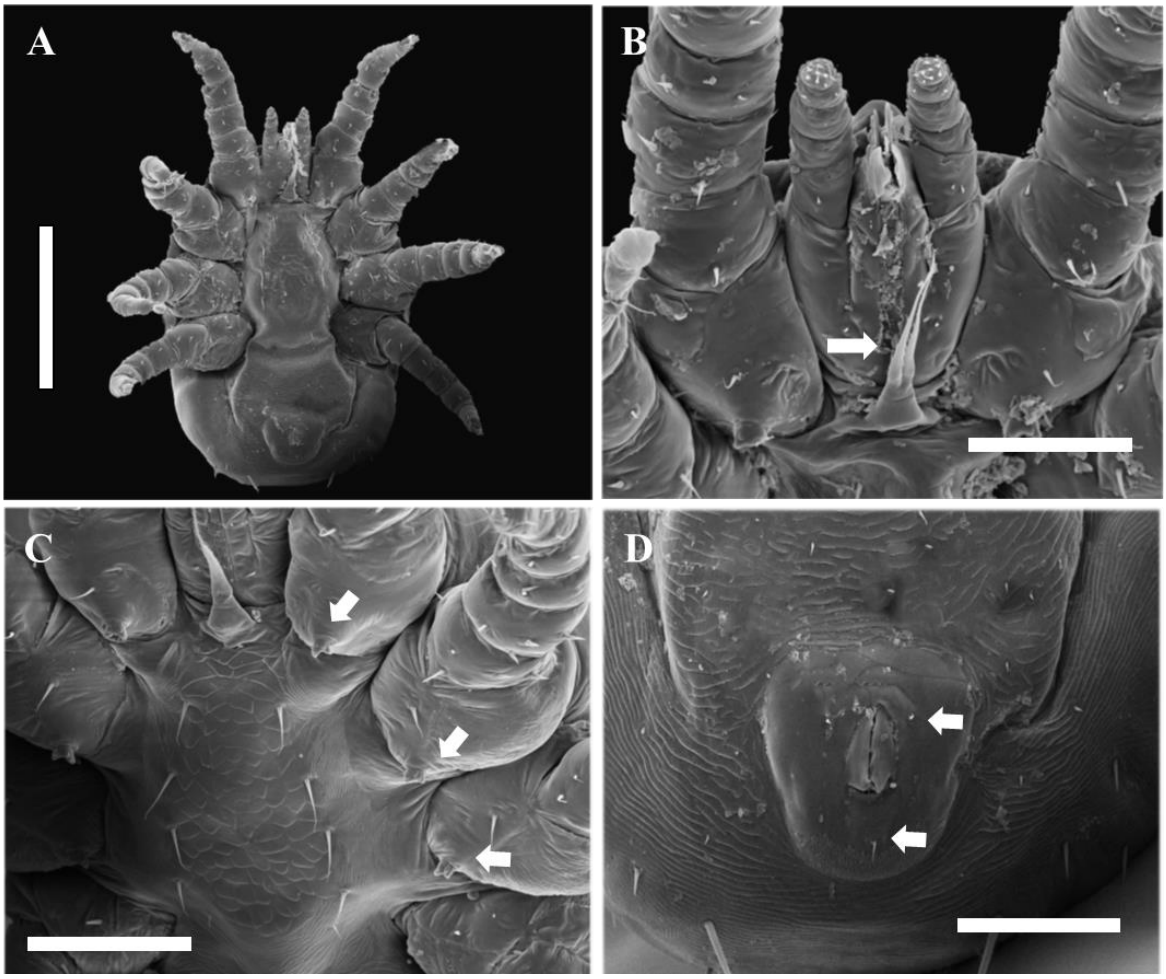
Figure 71 – Scanning electron microscopy of male *Ophiogongylus rotundus*, dorsal view



Source: (MENDOZA-ROLDAN, J. A., 2019)

Legend: male *Ophiogongylus rotundus*, dorsal view. Scale bar 200 µm.

Figure 72 – Scanning electron microscopy of male *Ophiogongylus rotundus*



Source: (MENDOZA-ROLDAN, J. A., 2019)

Legend: Male *Ophiogongylus rotundus*. A. ventral view; B. white arrow showing biflagellate tritosternum; C. white arrows showing bifid spurs on coxae I, II, III; D. white arrows showing genital setae on genital scutum. bar: A 200 µm, B-D 30 µm.

Family Macronyssidae

4.3.7 *Ophionyssus natricis* (Gervais, 1844): 223

Type material - Holotype female (lost) on *Hierophis viridiflavus* (Lacépède, 1789). Probably collected from Europe.

Synonyms: *Dermanyssus natricis* Gervais, 1844: 223. *Ophionyssus natricis*, Megnin, 1884: 107; André, 1937: 63; Piekarski 1936: 615; Cooreman, 1943: 1; Fonseca, 1948: 260; Camin, 1949: 583; 1953: 5; Zemskaya, 1951: 51; Bregetova, 1956: 160 et 223; Womersley, 1956: 599; Baker et al., 1956: 33; Keegan, 1956; Yunker, 1956; Till, 1957: 126; Strandtmann & Wharton, 1958. *Liponyssus natricis*, Berlese, 1918: 55; Hirst, 1921. *Liponyssus arabics* Hirst, 1921: 365. *Ophionyssus arabicus*, Camin, 1949: 584. *Liponyssus monodi* Hirst, 1925: 95. *Ichoronyssus serpentium* Hirst, 191: 383. *Liponyssus serpentium*, Hirst, 1921: 773. *Serpenticola serpentium*, Ewing, 1923: 12. *Serpenticola easti* Ewing, 1925: 18; Camin, 1949: 587; 1953: 4. *Ophionyssus serpentium*, André, 1937: 62; Ferris, 1942: 77; Radford, 1942; Camin, 1948: 345; 1949: 583. *Ophionyssus easti*, Fonseca, 1948: 313; Camin, 1949: 587.

Diagnosis. (Figure 73). Dorsal scutum of female divided into a large anterior scutum and a smaller posterior scutum (Figure 74A); sternal plate provided with only two pairs of setae; male dorsal scutum undivided; genito-ventral scutum of male divided into a sterno- genital and an isolated anal scutum (Figure 74B, C). On snakes in Africa; in vivaria all over the world. **Female**. Anterior dorsal scutum with 10 pairs of setae, two pairs of minute mesonotal scutellae and posterior dorsal scutum with 1 or 2 setae or nude; sternal shield ratio width/length: 2.5; peritreme extending to posterior margin of coxae II (Figure 74CD). **Male.** Femur III ventral spur absent. Genito-ventral scutum with 2 pairs of setae (*st1*, *st2*). Dorsal scutum with 14 pairs of setae; tarsus II–IV without ventral setae. Femur III and IV without modified ventral setae; dorsal scutum with 17 pairs of setae. Peritremes extend to anterior border of coxae III; at least 11 pairs of ventral setae.

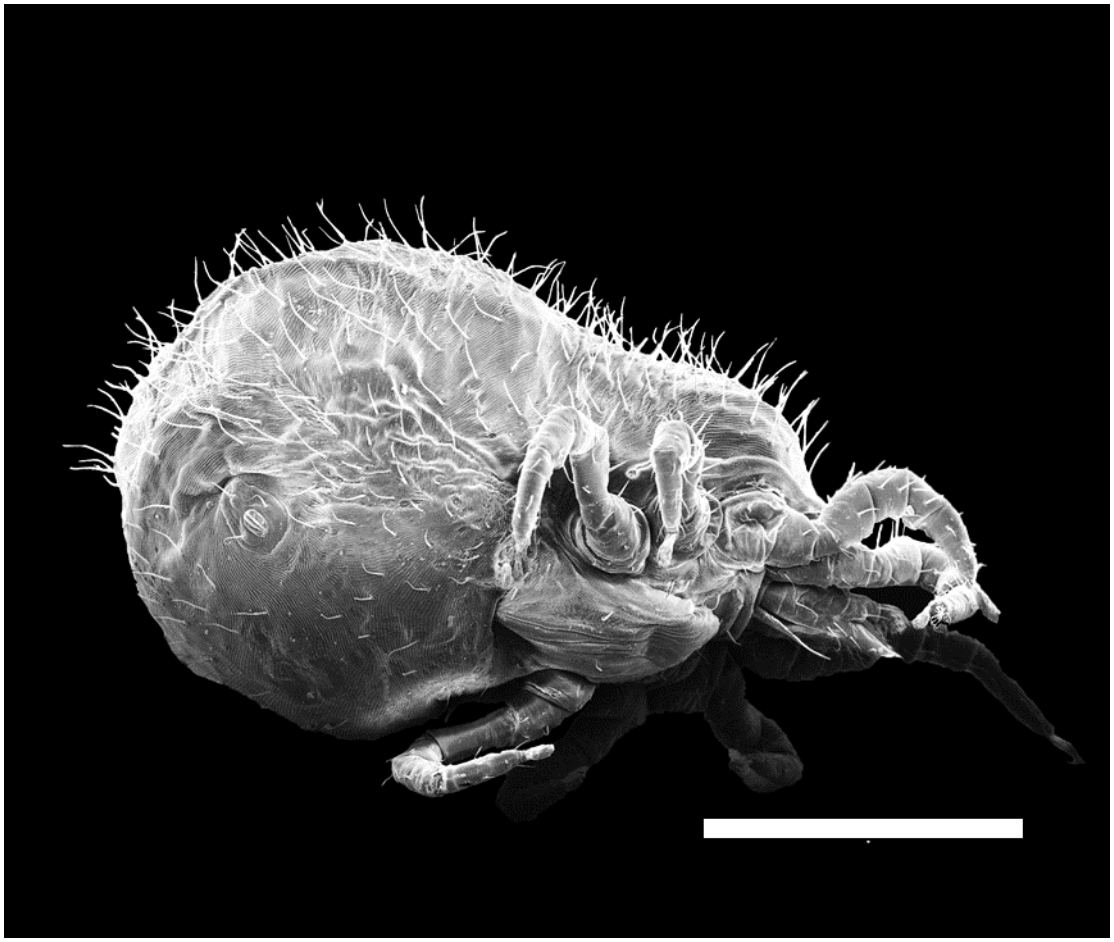
Heterozerconoidea superfamily
Family Heterozerconidae

4.3.8 *Zeterohercon oudemansi* Finnegan (1979): 1349

Type material - Holotype female (BMNH) and 3 females and two males paratypes, on *Epicrates cenchria* (Linnaeus, 1758), from Upper Amazon, Brazil.

Synonyms: *Heterozercon oudemansi* Finnegan, 1979: 1349 *Heterozercon elegans* Lizaso, 1979: 139. *Zeterohercon oudemansi* Flechtmann & Johnston 2009: 143.

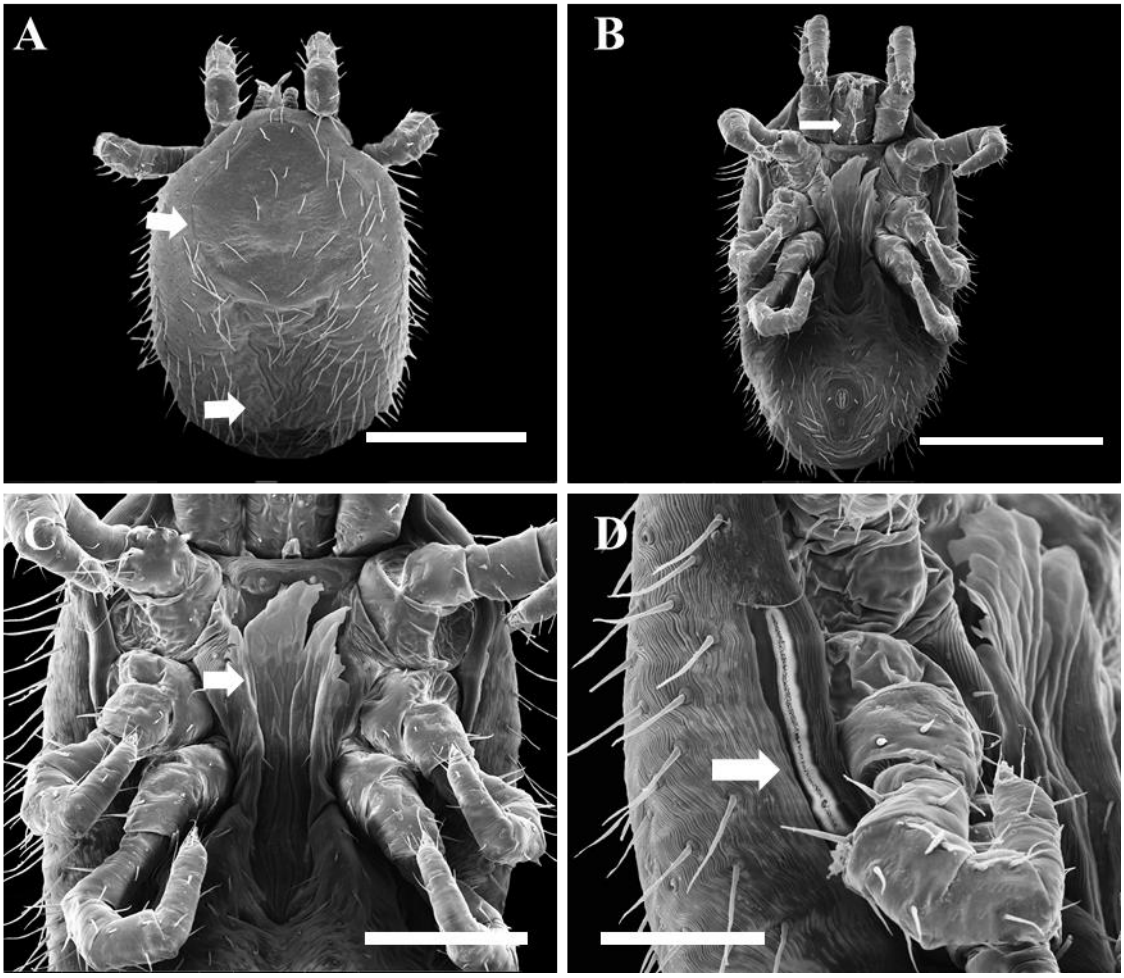
Figure 73 – Scanning electron microscopy of female *Ophionyssus natricis,* lateral view



Source: (MENDOZA-ROLDAN, J. A., 2018)

Legend: Female *Ophionyssus natricis,* lateral view. Scale bar 300 µm.

Figure 74 – Scanning electron microscopy of female *Ophionyssus natricis*



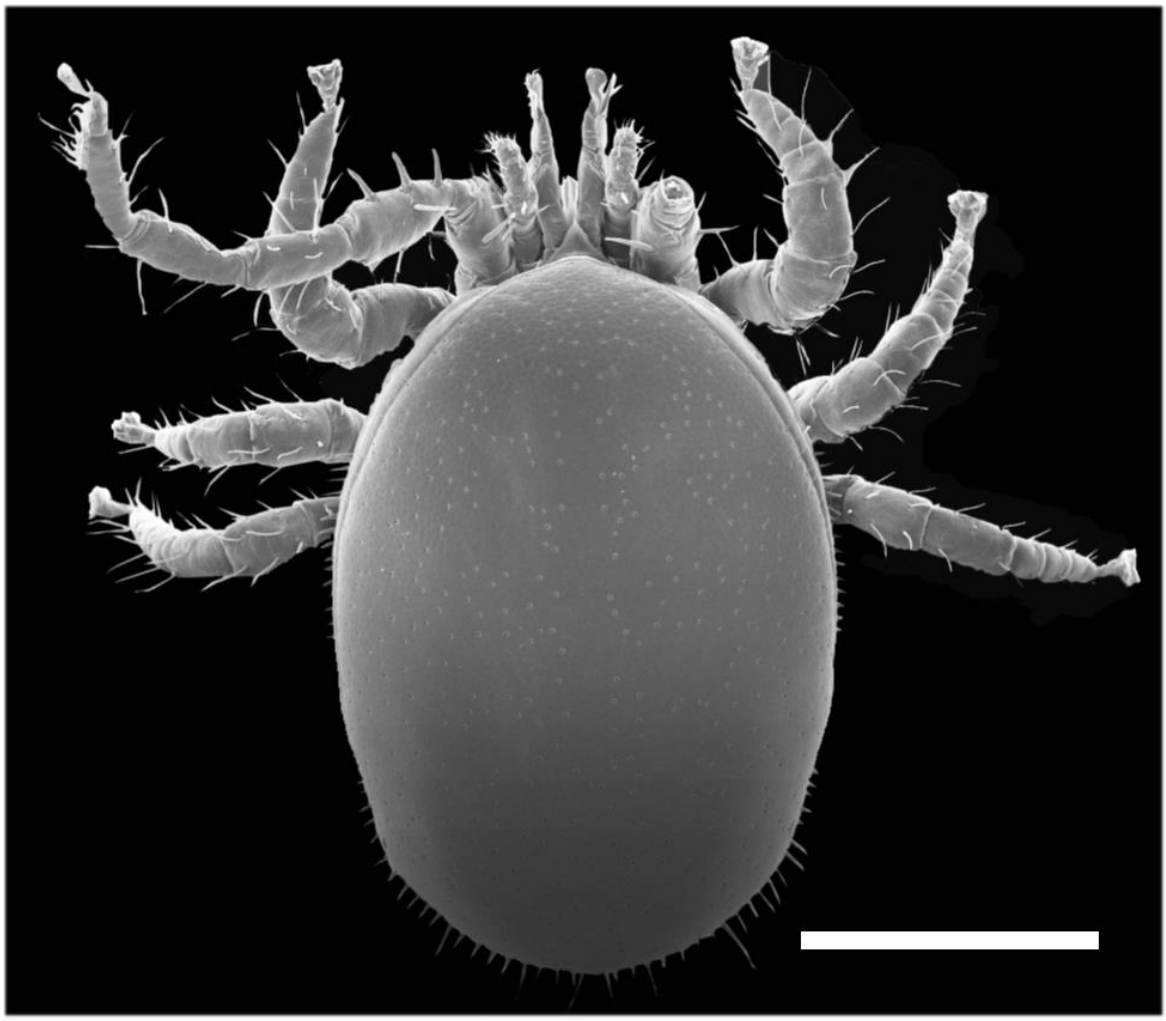
Source: (MENDOZA-ROLDAN, J. A., 2019)

Legend: female *Ophionyssus natricis*. A. dorsal view, white arrows showing posterior and anterior dorsal scuta; B. ventral view, white arrow showing tritosternum; C. white arrows showing genital scutum; D. white arrows showing peritreme. Scale bars: A, B 300 µm, C-D 100 µm.

Diagnosis. Very large mites(>400µm). Tarsus and tibia I only slightly narrower than the other segments of leg I. Anterior region of dorsum with one pair of short setae. Dorsal shield with only microsetae and bearing a network of lines less developed in the female (Figure 75). Posterior margins of body with a short membrane bearing 28-33 setae attenuated apically (Figure 76A). Anal shield with 3 setae. Margin of ventral surface armed with a row of strong movable setae extending forward from the hind margin to the level of the third pair of legs (Figure 76A, D); nine similar setae in serial arrangement occur on the lateral portions of the ventral surface

approximately midway between posterior legs and posterior margin of body (Figure 76B, C, D). **Female.** Femur of anterior pair of legs with three spines on anterior margin. Body subovate, somewhat produced posteriorly, greatest width just behind fourth pair of legs. Dorsal shield ovate, scarcely covering two-thirds of the dorsal area of the body, surface of shield with minute setae, dotted at irregular intervals and sculptured between into regular polygons, giving the whole the appearance of being coarsely granular. **Male**. Femur, genu and tibia with spinous setae, not thick spines, on their ventral surface.

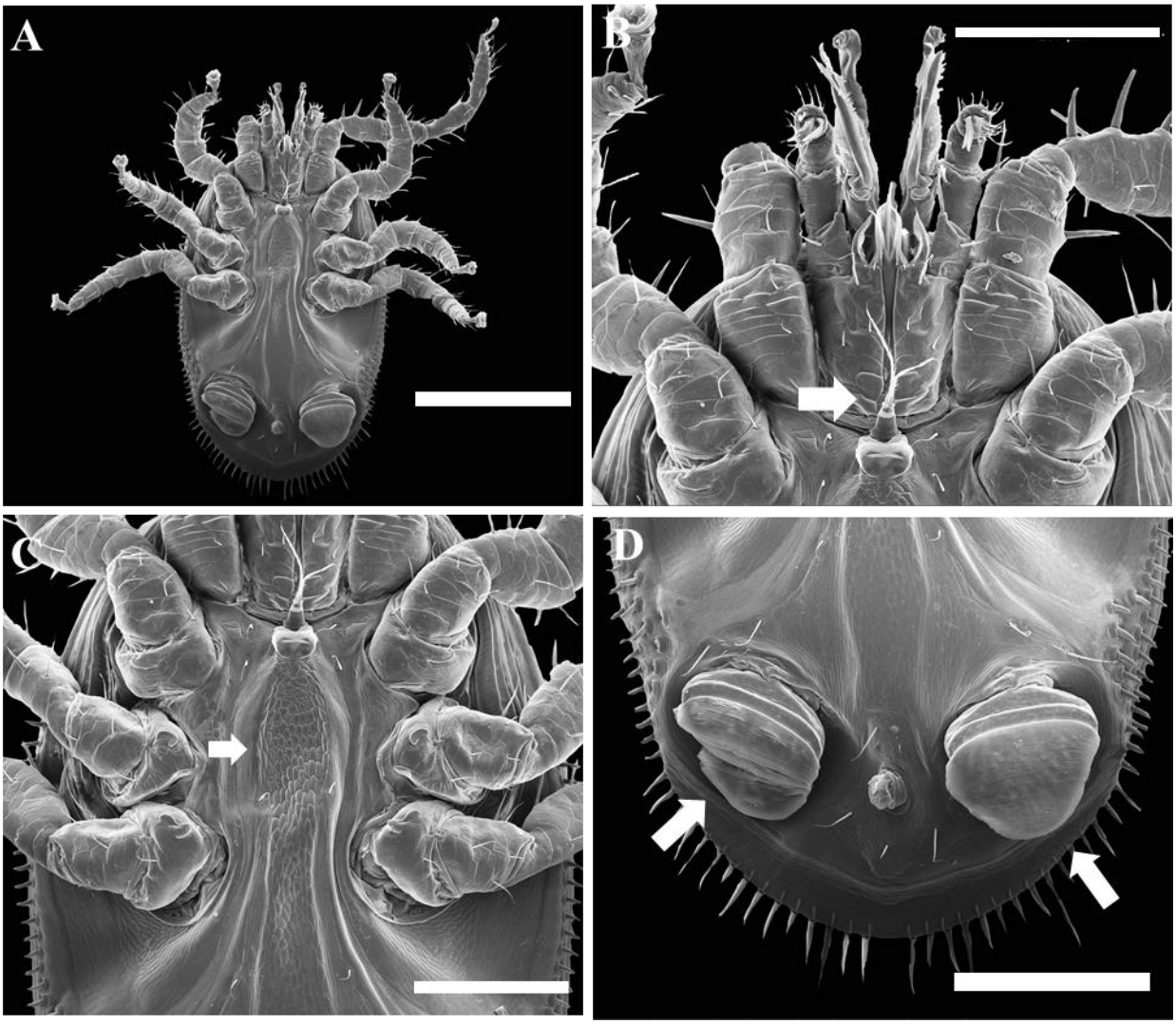
Figure 75 – Scanning electron microscopy of female *Zeterohercon oudemansi*, dorsal view



Source: (MENDOZA-ROLDAN, J. A., 2019)

Legend: Scale bar 500 µm.

Figure 76 – Scanning electron microscopy of female female *Zeterohercon oudemansi*



Source: (MENDOZA-ROLDAN, J. A., 2019)

Legend: female *Zeterohercon oudemansi* A. ventral view; B. ventral view, white arrow showing tritosternum; C. white arrows showing genita-sternal scutum; D. white arrows showing modified sucker setae. Scale bars: A 500 µm, B, C, D 200 µm.

4.4 Geographical distribution

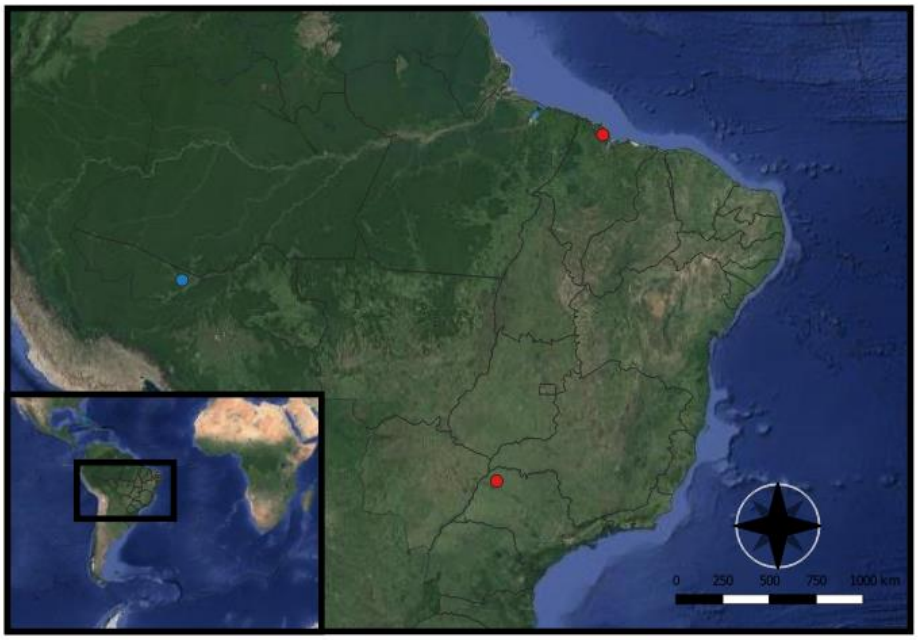
Maps of geographical distribution of the species of mesostigmata mites examined in this study are shown in Figures 77 to 79. Geographic coordinates of each locality for each species are detailed hereafter, including information from literature and collections.

Chironobius* sp.n.:** Brazil – **Acre:** Iracema (9° 57' 30.708" S, 67° 49' 15.924" W); **Maranhão:** Mirinzal (2° 3' 54.288" S, 44° 47' 1.104" W). ***Chironobius nordestinus; **São Paulo:** Palmeiras (21° 49' 5.628" S, 47° 15' 16.164" W). ***Chironobius alvus***, Santa Isabel **(**23° 18' 59" Sul, 46° 13' 25" W) (Figure 77).

***Ophionyssus natricis*:** Brazil - **Paraíba:** Puxinanã (7° 8' 49.56" S 35° 58' 8.58" W). **São Paulo:** Barragem Paraitinga (23° 13' 25.932" S, 45° 18' 38.88" W); São Paulo (23° 33' 1.872" S 46° 37' 59.9088" W); Soracaba (23° 30' 22.572" S, 47° 27' 20.268" W); Zoo Bauru (22° 19' 19.4916" S, 49° 4' 16.1472" W) (Figure 78).

***Zeterohercon oudemansi*:** Brazil – **Upper Amazon, Brazil** (1° 11' 45.78" S66° 34' 56.7552" W). **Acre:** Iracema (9° 57' 30.708" S, 67° 49' 15.924" W). **Mato Grosso do Sul:** Três Lagoas (20° 45' 37.872" S, 51° 41' 37.896" W); **Pará:** Tucuruí (3° 46' 10.632" S, 49° 40' 25.7808" W).
São Paulo: Santa Fé do Sul (20° 12' 44.6076" S, 50° 55' 33.9924" W); São Paulo (23° 33' 1.872" S 46° 37' 59.9088" W); Dracena (21° 29' 2.364" S, 51° 32' 2.0436" W); Casa Branca (21° 46' 11.244" S, 47° 5' 30.444" W). **Minas Gerais:** Belo Horizonte (19° 55' 2.2764" S, 43° 56' 4.4124" W) (Figure 79).

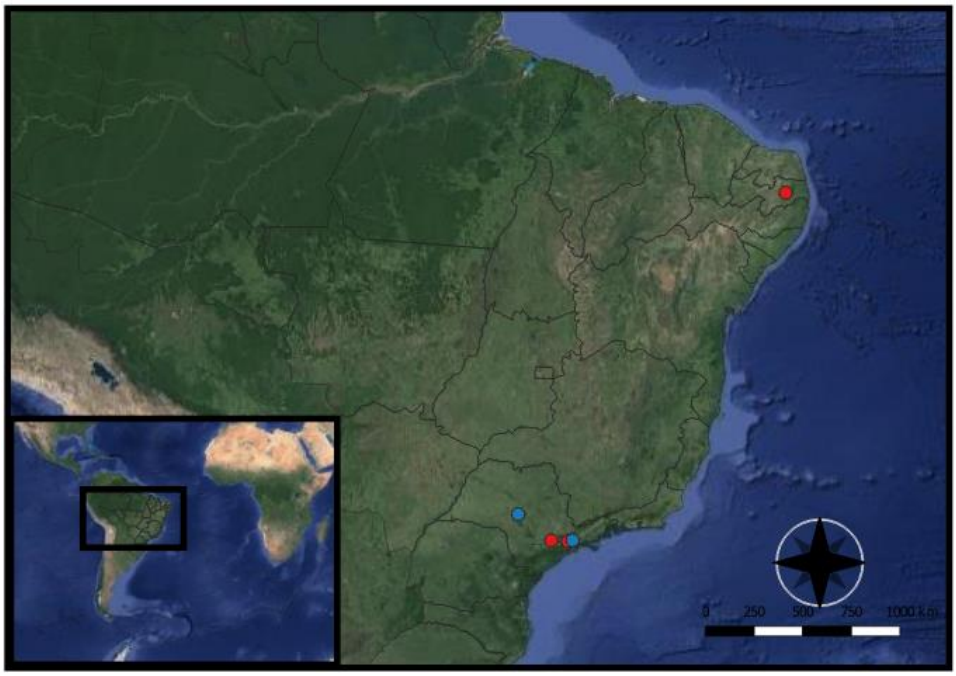
Figure 77 – Distribution map of *Chironobius* obtained using QGIS program



Source: (MENDOZA-ROLDAN, J. A., 2019).

Legend: (red circles) information cited in literature of *Chironobius* genus, (blue circle) material of *Chironobius* sp. n.

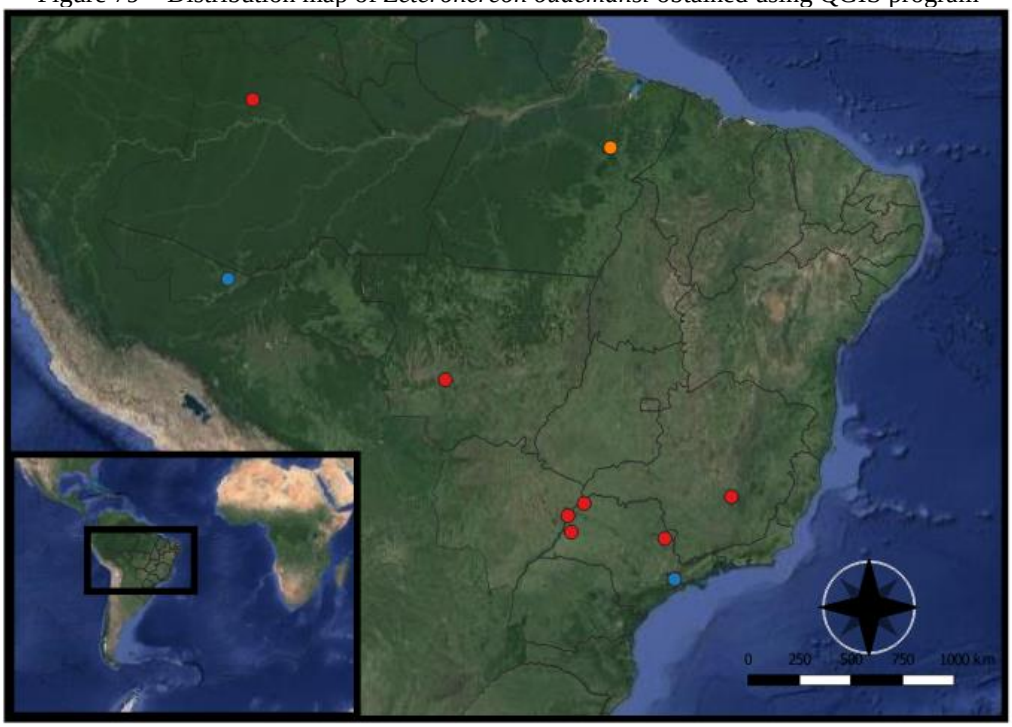
Figure 78 – Distribution map of *Ophionyssus natricis* obtained using QGIS program



Source: (MENDOZA-ROLDAN, J. A., 2019).

Legend: (red circles) information cited in literature; (blue circle) material of this study.

Figure 79 – Distribution map of *Zeterohercon oudemansi* obtained using QGIS program



Source: (MENDOZA-ROLDAN, J. A., 2019).

Legend: (red circles) information cited in literature, (orange circles) material examined deposited in collections, (blue circle) material from this study.

5 DISCUSSION

In this study, six families, 11 genera and 17 species of Mesostigmata mites were identified. Of the 17 species, 14 occur in Brazil. Former studies catalogued 14 species of Mesostigmata mites of reptiles, mainly snakes, in Brazil. (FONSECA, 1940; FAIN, 1961a, 1962a; 1962b; LIZASO, l979; 1982; FLECHTMANN; JOHNSTON, 1999; FAJFER, 2012). Herein, after 20 years of almost no cataloguing of Mesostigmata mites of reptiles, new records of hosts and localities are discussed, and a new species is described from the northern region of Brazil, increasing the number to 16 species of Brazilian Mesostigmata mites on reptiles. No amphibians were found parasitized, which was an expected result as no previous reports of Mesostigmata mites infesting amphibians were found, and there is no material deposited in the examined collections of Mesostigmata on amphibians. As in former studies, Squamata reptiles were found parasitized by Mesostigmata mites, though their prevalence is much lower than of Trombidiformes mites (infestation rates and host-parasite association are better discussed in chapter 4).

The Mesostigmata species of mites identified here are grouped as follows: Dermanyssoidea superfamily: **Entonyssidae, Ixodorhynchidae, Macronyssidae, Laelapidae, Omentolaelapidae,** and Superfamily Heterozerconoidea: **Heterozerconidae.** Of these families, Omentolaelapidae is restricted to Africa (Congo) (FAIN, 1962b), and Laelapidae has been recorded in Africa, Asia, Europe, North America, and Oceania (FAIN, 1962b). Here, material deposited in the acarological collection of the IBSP, was compatible with *Haemolaelaps natricis* Feider & Solomon, 1960, on *Oxyrhopus trigeminus*, from Guararapés, Pernambuco state. This would be the first report if this species and overall genus in the Neotropical region. This species was described on *Natrix natrix* from Romania (FEIDER; SOLOMON, 1960). Unfortunately, the only slide, collected by Lizaso, in 1978, has darkened and better identification or morphological detailing was further unachievable. Thus, further confirmation or collection of similar material in the same locality, can aid a better deification. However, as is was deposited as Laelapidae, it will remain as so, until further confirmation.

Regarding the superfamily Dermanyssoidea, species of all the four families before mentioned were identified. The family Entonyssidae, which has two genera distributed in South America, was represented by *E. liophis*, and *P. aristoterisii*. Despite efforts of finding more mites in the type host and other snakes, no new material was collected. The species *E. liophis* was described in the early 60's in *Lygophis anomalus* (Günther, 1858), from a nonspecified locality in South America (FAIN, 1961a); and *P. aristoterisii* has only been recorded twice. In the 40`s from São Paulo, when it was described un a *E. aesculappii* snake, after it was euthanized by beheading (FONSECA, 1940). And later, it was recorded in the early 60´s from the same species of host also from Brazil (FAIN, 1961a). It was possible to observe the type material in IBSP collection, and the material deposited in the IRSNB collection, and redescribed the female of *P. aristoterisii*. Fain (1961) reported nymphs and adults, nonetheless, the male specimen was not found in the collection, and the protonymphs were not in good conditions, thus further redescritption was unachievable. The life cycle of snake lung mites has been scarcely detailed, and consists of egg, larva, protonymph, deutonymph, and adults (males are rare) (FAIN, 1961a). However, almost nothing is known of the development of the Pneumophionyssinae subfamily, that occurs in south America. These species seem to be very rare, and further efforts should be held to assess the type hosts for mites.

On the other hand, the Ixodorhynchidae family was the most abundant in this study, with nine Brazilian species and one from Asia [*Strandtibbettsia gordoni* (Tibbetts, 1957)]. The brazilian species were distributed in 4 genera. *Chironobius* was described in the early 80`s, together with *Ophiogongylu*s (Lizaso, 1983). After examining the two known species of *Chironobius* (*C. nordestinus* Lizaso, 1983, and *C. alvus* Lizaso, 1983), it was possible to describe a new species on *Chironius multiventris* Schmidt & Walker from Iracema, Acre state. This is the first record of this species from the northern region, and it differs from the other species by having a posterior dorsal scutum with a chitinized dark band in the posterior and lateral margins; a genital scutum reticulated and chitinized in the lateral margins; and an anal scutum strongly chitinized in the lateral posterior margins. Also, the chaetotaxy differs greatly from the other two species. Furthermore, *Chironobius* are highly specialized mites that only parasitize snakes of the genus *Chironius*. Thus, as more snakes are examined from different localities, probably new species of these mites will be found. Subsequently, further morphological detailing of the three species of *Chironobius*, allowed to update and include them in a dichotomous key proposed by Dowling (2009). Also, *Chironobius* species were described as ovoviviparous or viviparous (unique larva develops inside the female idiosoma) (LIZASO. 1988). The new species of *Chironobius*, which only females were found, is also ovoviviparous, and possibly parthenogenetic as no males have been described. Finally, the distribution of the three species is very distinctive as well as for their hosts. This result highlights the importance of collecting material from the northern region of the country, as the amazon forest biome is one of the most biodiverse environments of the world, and before this study there was few to no information of Mesostigmata mites parasitic of reptiles (COSTA; BERLINS, 2018). The other genus described by Lizaso (1983), herein examined, was *Ophiogongylus*. This genus is represented by two species: *O. breviscutum*, described on *Liophis poecilogyrus* (Wied-Neuwied, 1825), from Votuporanga, São Paulo state. This species was only examined in the IBSP collection, as no mites were found in recent field trips. On the other hand, *O. rotundu*s was described on *Xenodon neuwiedii* Gunther, 1866, from Santa Isabel, São Paulo state. It was also collected in *Erythrolamprus aesculapii* (Linnaeus, 1758), *Leptodeira annulata* (Linnaeus, 1758), from various localities in São Paulo state, Espírito Santo, and Paraná (LIZASO, 1983). This species was idenfitied here on *X. neuwiedii* from Juquitiba, São Paulo state. Both species are very similar, nonetheless can be distinguished morphologically comparing the sternal scutum, which in females of *O. breviscutum* is narrow and has the *st2* setae in the posterior margins

of the scutum, while *O. rotundu*s as a wider sternal scutum and the *st2* setae are in the center of the scutum. Also *O. breviscutum*, has a smaller tritosternum (half the size of *O. rotundu*s) (LIZASO, 1983). Moreover, *O. rotundu*s has a wider distribution than *O. breviscutum* and seems to be less specific. This genus can be oviparous or ovoviviparous, and it was observed here both reproductive patterns (LIZASO, 1988).

The other two genera examined in this study (*Ixobioides* and *Strandtibbettsia*), were only found in the IBSP and IRSNB collections. *Ixobioides* has three South American species, and one North American species, *I. truncatus* (Johnson, 1962), described on *Pantherophis vulpinus*, *P. obsoletus,* and *Thamnophis sirtalis,* from the United States (JOHNSTON, 1962). This species was redescribed and transferred to *Ixobioides* (DOWLING, 2009). The three South American species were described in the early 30`s (*I. butantanensis*), the early 60`s (*I. fonsecae*), and early 80`s (*I. brachispinosus*). These species were described from the Central-west, South, and Southeast regions from various colubrid snakes (FONSECA, 1934; FAIN, 1961c; LIZASO, 1983). These species are distinguished by the differences in the dorsal scutum. In *I. fonsecae* the scutum is not divided, with only a slight constriction medially, *I. brachispinosus* also has an undivided scutum with lateral incisions near the midline and a posterior portion of the shield that is much narrower than the anterior portion, and *I. butantanensis* has a divided scutum, with the anterior position much larger than the posterior scutum, and appears almost divided into two separate scuta (DOWLING, 2009). Although in this study snakes from the same type host species and localities were examined, no new records were found.

Strandtibbettsia genus was represented by two species: *Strandtibbettsia braziliensis* Fain, 1961, and *Strandtibbettsia gordoni* (Tibbetts, 1957). The *S. braziliensis* was described on *Siphlophis cervinus* (Laurenti, 1768), from Juquiá, São Paulo state (FAIN, 1961b). The specimens examined here, are from the IRSNB collection, and no further information is known for this genus.

An important family examined in here, is the Macronyssidae, which is a very diverse family of parasitic mites, and many of them have medical and veterinary importance (MICHERDZINSKI, 1980; REEVES et al., 2007). The only genus that parasitizes reptiles is *Ophionyssus* (WOZNIAK; DENARDO, 2000; FAIN; BANNERT, 2000). Here, new records of hosts and localities are reported for *O. natricis*. This species is found mainly in captive snakes worldwide. In Brazil, it has been officially recorded on *Boa constrictor constrictor* Linnaeus, 1758, from Puxinanã, Paraíba state (BARBOSA et al., 2006); and Soracaba, São Paulo stare, on *Python*

reticulatus (Schneider, 1801) (DA SILVA et al., 2018). Here, *O. natricis* is recorded from snakes *Crotalus durissus terrificus* Lineu, 1758; *Corallus hortullanus* (Linnaeus, 1758); and lizards *Enyalius iheringii* Boulenger, 1885; and *Pogona vitticeps* Ahl, 1926. All these hosts represent new records. These animals were all examined in São Paulo, state, and all except *E. iheringii* were captive kept. The species *O. natricis* is normally found in captive Squamata, thought it has been found in some rare occasions in wild populations (SIMONOV; ZINCHENKO, 2010). This is the first record of this mite in wild lizards in the neotropics. Also, all the species of host recorded are endemic to South America, except for *P. vitticeps*. Thus, this would be the first record of an exotic saurian infested by *O. natricis.* This mite is opportunistic, thus if in need, it can infest mammals and even humans that work at facilities where the mite is thriving. It is important to maintain a constant vigilance and examination of facilities that harbor reptiles, as this mite can colonize quickly these spaces (WOZNIAK; DENARDO, 2000).

Finally, the superfamily Heterozerconoidea was represented by the family Heterozerconidae, and with the species *Zeterohercon oudemansi* (Finnegan, 1979), which has new records of hosts and localities. This species was described on *Epicrates cenchria* (Linnaeus, 1758), from the Brazilian amazon. Later, the species *Zeterohercon elegans*, was synonymized to *Z. oudemansi*, thus adding other snakes as hosts (*Xenodon merremii*, *Mastigodryas bifossatus*, and *Erythrolamprus aesculapii*), all recorded from the central west, south, and southeast regions (FINNEGAN, 1931; LIZASO, 1979). Here, *Z. oudemansi* was recorded infesting *Oxyrhopus melanogenys* (Tschudi, 1845) from Iracema, Acre state; and *Pseudoboa nigra* (Duméril, Bibron & Duméril, 1854), from São Paulo. In the material examined from the IBSP collection, mites were identified from *Micrurus* sp., from Tucuruí, Pará state. These are all new host and locality records. The other species of *Zeterohercon*, is *Z. amphisbaenae* described on *Amphisbaena alba* from São José do Rio Preto, São Paulo state. Despite examining various specimens of *Amphisbaena*, no mites were collected from this fossorial host. Heterozerconidae species associated to reptiles are known only for the Neotropical region, and it is believed the mites passed to the Squamata reptiles (Amphisbaenia and Serpentes suborders), that shared fossorial habits and habitats with myriapods (FLECHTMANN; JOHNSTON, 1990).

6 CONCLUSIONS

1. Six families, 11 genera and 17 species of Mesostigmata mites, parasites of reptiles were identified.
2. Of the 17 species of Mesostigmata mites identified in this study, 14 occur in Brazil. Increasing two new species to the Brazilian territory, totalizing 16 species of Brazilian Mesostigmata mites.
3. No amphibians were found parasitized, which was expected as there are no previous reports of Mesostigmata mites infesting amphibians.
4. Squamata reptiles were found parasitized by Mesostigmata mites, though their prevalence is much lower than Trombidiformes mites.
5. Omentolaelapidae was not found in field trips as it is restricted to Africa.
6. It was possible to observe the type material in IBSP collection, and the material deposited in the IRSNB collection, and redescribed the female of *P. aristoterisii*.
7. The Ixodorhynchidae family was the most abundant in this study, with nine Brazilian species and one from Asia.
8. After examining the two known species of *Chironobius*, it was possible to describe a new species on *Chironius multiventris* from Iracema, Acre state.
9. *Chironobius* sp. n. is the first record of this genus from the northern region.
10. After morphological detailing of the three species of *Chironobius*, the species were included in an updated dichotomous key.
11. The new species of *Chironobius*, is also ovoviviparous, and possibly parthenogenetic.
12. Species of *Ixobioides* and *Strandtibbettsia* were only found in the IBSP and IRSNB collections.
13. The species *O. natricis* is recorded from snakes (*C. durissus terrificus*, *C. hortullanus*) and lizards (*E. iheringii*, *P. vitticeps*), from São Paulo state, and all hosts and localities are new.
14. The species *O. natricis* on *E. iheringii* is the first record of infestation of wild lizards in the neotropics.
15. The *O. natricis* on *P. vitticeps* is the first record of an exotic saurian infested in Brazil.
16. The species *Zeterohercon oudemansi* has new records of hosts and localities. It was recorded infesting *O. melanogeny* from Iracema, Acre state, and *P. nigra* from São Paulo.

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(Mendoza-Roldan, 2017)

CHAPTER III: Order Ixodida

1 INTRODUCTION

The order Ixodida belongs to the Parasitiformes superorder, which includes the orders Holothyrida (a group of mites that feed of bodily fluids of dead arthropods), and Mesostigmata mites. Parasitiformes are characterized by having free coxae, covered anal opening by a pair of plaques, and a sclerotized ring around the gnathosoma also called capitulum (WALTER; PROCTOR, 1988, LEHTINEN, 1991). Ixodida are also known as ticks. These invertebrates are hematophagous parasites of vertebrates (mammal, birds, amphibians, and reptiles). Generally, they are larger than other Acari, with engorged female ticks of some species being able to be more than 30 mm in size (Figure 80) (EVANS, 1992; NATUSCH, 2018). The Ixodida share some synapomorhpies, such as, latigynial scuta reduced (a pair of lateral scuta in some female parasitiform mites that help protect the genital opening); tarsi of the palps reduced (IV article of the palp), and the hypostome protrudes anteriorly and in shape of a saw (LEHTINEN, 1991). Other characteristic that distinguish the Ixodida, is the presence of the Haller's organ on tarsus I (a sensorial apparatus) (CARR et al., 2017).

Figure 80 – Comparative sizes of Ixodida and Mesostigmata

Source: (MENDOZA-ROLDAN, J. A., 2019).

Legend: female *Amblyomma rotundatum* (left), female *Chironobius alvus* (right).

The Ixodida is currently represented 944 species which are distributed into families Nuttalliellidae Bedford, 1931 (monospecific), Argasidae (214 species), and Ixodidae (729 species) (DANTAS-TORRES, 2018; DU ET AL., 2018; KWAK, 2018).

Generally, ticks have low host specificity. Nevertheless, in some species, reptiles or amphibians are the main hosts. For example, almost all the species of the genus *Bothriocroton* parasitize Squamata reptiles and tuataras; and some species of *Amblyomma* prefer to parasitize cold-blooded animals (KLOMPEN; DOBSON; BARKER, 2002). Moreover, ticks that parasitize reptiles and amphibians belong mainly to the family Ixodidae (genera *Amblyomma, Bothriocroton, Dermacentor, Haemaphysalis, Hyalomma* and *Ixodes*). Additionally, the genera *Argas* and *Ornithodoros,* from the Argasidae family, infest reptiles and amphibians (BARROS-BATTESTI et al., 2006; DANTAS-TORRES et al., 2008; BARROS-BATTESTI et al., 2015).

1.1 Ticks of reptiles and amphibians from Brazil

1.1.1 Family Ixodidae

The family Ixodidae, also known as hard ticks, are characterized by a dorsal scutum in all biological stages (larva, nymph and adult), and a prominent capitulum, that protrudes anteriorly (BARROS-BATTESTI et al., 2006). In Brazil, the genus reported to infest reptiles and amphibians is *Amblyomma,* with around 32 species recorded in the Brazilian territory (DANTAS-TORRES; ONOFRIO; BARROS-BATTESTI, 2009). Of these, six species have been recorded on reptiles and amphibians, and three species that also parasitize these hosts, have a doubtful status in Brazil, and requires confirmation (*Amblyomma albopictum* Neumann 1899, *Amblyomma fulvum* Neumann, 1899, and *Amblyomma scutatum* Neumann, 1899) (BARROS-BATTESTI et al., 2006; SZABÓ; OLEGÁRIO; SANTOS, 2007; DANTAS-TORRES; ONOFRIO; BARROS-BATTESTI, 2009).

The most common species of *Amblyomma* of the herpetofauna are *Amblyomma rotundatum* Koch 1844 and *Amblyomma dissimile* Koch 1844. Furthermore, *A. rotundatum* is parthenogenetic, with mostly records of females, and some records of males collected from a *Tropidurus* lizard, and a tortoise (LABRUNA; TERRASSINI; CAMARGO, 2005; ; GIANIZELLA et al., 2019). Females of *A. rotundatum* and *A. dissimile* are morphologically very similar, thus making it difficult to

distinguish each species. Nonetheless, they differ in the form and form of spurs of coxae I to IV and in the scutal punctations (GUGLIELMONE; NAVA, 2010). These two species can infest a wide range of vertebrates, having a unique relationship with anuran amphibians with a possible evolutionary relevance (OLIVER, 1989). On the other hand, the myriad of possible hosts of both species of ticks could imply that they colonize environments were infestation of different types of vertebrates is possible, as well as occasional visitors, that aid dispersion to other areas (GUGLIELMONE; NAVA, 2010).

Furthermore, *A. dissimile* has a wider host range than *A. rotundatum.* Moreover, *A. rotundatum* has been recorded on 12 species of anuran amphibians, and 63 species of reptiles (42 snakes, 11 lizards and 10 turtles and tortoises). On the other hand, *A. dissimile* has been recorded parasitizing five species of anuran amphibians, and 86 species of reptiles (51 snakes, 20 lizards, and 15 turtles and tortoises) (LAMPO; RANGEL; MATA, 1998; GUGLIELMONE; NAVA, 2010; TORRES et al., 2018).

The other species of *Amblyomma* recorded in the herpetofauna of Brazil are *Amblyomma sculptum* Berlese, 1888, recorded on rattlesnake in Goiás State; *Amblyomma humerale* Koch, 1844, mainly infesting Testudinata, but can also infest lizards, anurans and mammals; *Amblyomma fuscum* Neumann, 1907, recorded on snakes, mammals and humans; and *Amblyomma goeldii* Neumann 1899, infesting anteaters, and boid snakes (LABRUNA et al., 2002; BARROS-BATTESTI et al., 2005; SZABÓ; OLEGÁRIO; SANTOS, 2007; MARTIN et al., 2015).

1.1.2 Family Argasidae

The Argasidae family, are also called soft ticks, due to the lack of dorsal scutum (larvae have a rudimentary small dorsal scutum). These ticks also have capitulum that is poorly visible dorsally, as it is located on the underside of the tick body. These family, differently of Ixodidae, have more than one nymphal stage and feed intermittently not remaining attached to the host. Also, they may feed numerous times on several different hosts and may have more than one gonotrophic cycle. Furthermore, this family of ticks can have a remarkable longevity, with species surviving for and for many years and after long periods of starvation (CLIFFORD; KOHLS; SONENSHINE, 1964; VIAL, 2009; RAMIREZ et al., 2016).

In Brazil, the herpetofauna has been recorded infested by species of the genus *Ornithodoros*, with 129 representatives worldwide (MUÑOZ-LEAL et al., 2016; MUÑOZ-LEAL et al., 2017). The species of *Ornithodoros* associated with amphibians are: *Ornithodoros faccinii* Barros-Battesti, Landulfo & Luz, 2015, described on *Thoropa miliaris* (Spix, 1824), from Rio de Janeiro State, and later also recorded on *Rhinella ornata* (Spix, 1824), from the same locality (BARROS-BATTESTI et al. 2015; LUZ et al., 2018); and *Ornithodoros saraivai* Muñoz-Leal & Labruna, 2017, described on *Cycloramphus boraceiensis* Heyer, 1983 from São Paulo state. This species was later recorded on *Thoropa taophora* (Miranda-Ribeiro, 1923), from three localities in the state of São Paulo: two continental and one insular (MUÑOZ-LEAL et al., 2017; SÁ-HUNGARO et al., 2018). Furthermore, other species of *Ornithodoros* recorded from snakes are: *Ornithodoros mimon* Kohls, Clifford & Jones, 1969, on *Corallus hortulanus* (Linnaeus, 1758), from Ceará state; *Ornithodoros rietcorreai* Labruna, Nava & Venzal, 2016, on *Leptodeira annulata* (Linnaeus, 1758), also from Ceará state; and various of *Ornithodoros* sp. on captive *Boa constrictor constrictor* Linnaeus, 1758 in Rio Grande do Norte state, and *L. annulata*, *Oxyrhopus trigeminus* Duméril, Bibron & Duméril, 1854, and *Philodryas olfersii* from Ceará state (PEREIRA et al., 2012; DE ALCANTARA, et al., 2018). The distribution of the Brazilian species of *Ornithodoros* from reptiles and amphibians is shown in Table 23 and Figure 81.

2 OBJECTIVES

- Assess the Ixodida ticks of reptiles and amphibians deposited in the acarological collection of the Instituto Butantan (IBSP), and in other reference collections;
- Identify the Ixodida ticks found in reptiles and amphibians through optic and electronic scanning microscopy and genetic sequencing (Part II, Chapter 5);
- Update distribution of Brazilian species of Ixodida ticks of reptiles and amphibians, according to recent collections.

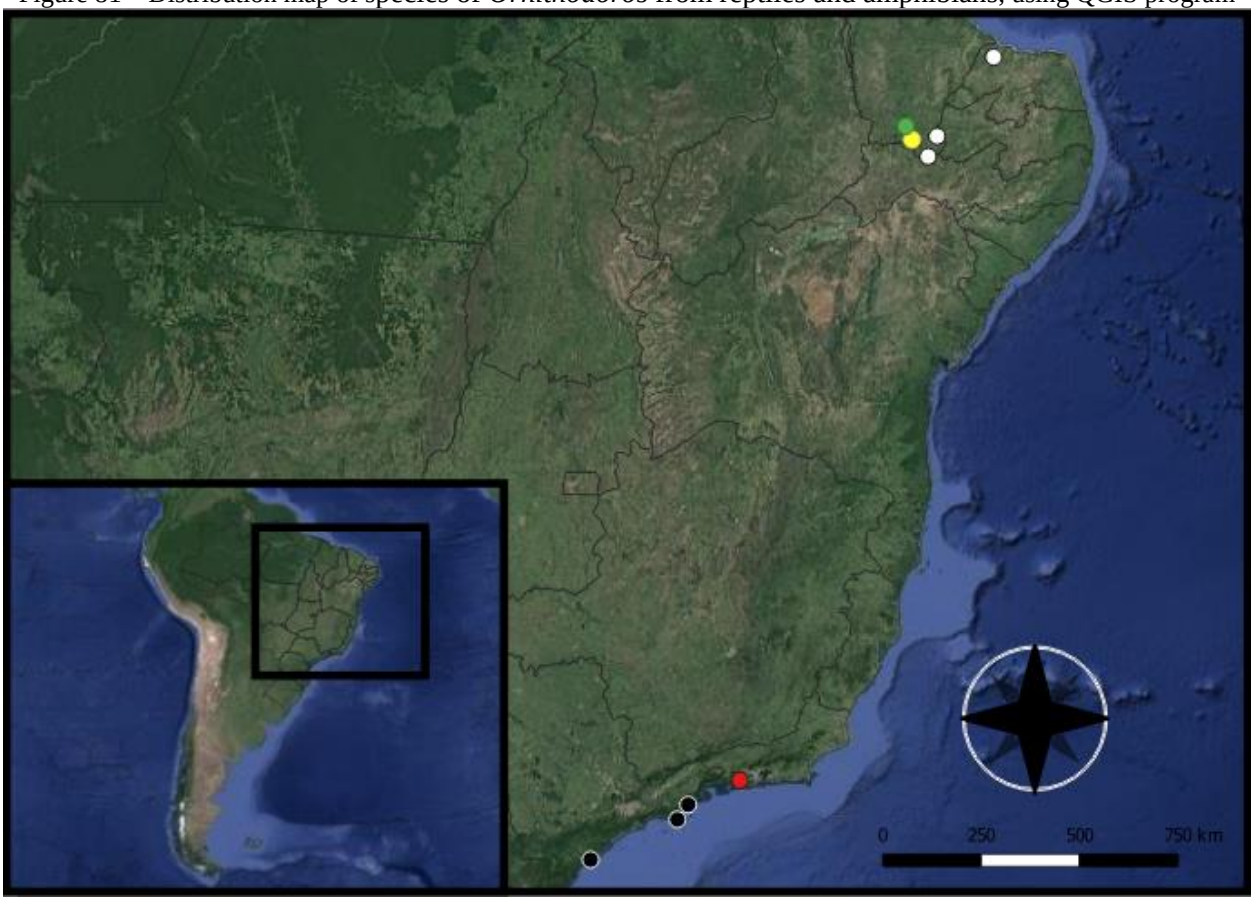
Table 23 – Species of *Ornithodoros* from reptiles and amphibians, distributed in Brazil

No.	Species	Holotype	Host	Locality	Reference
1	*Ornithodoros faccinii* Barros-Battesti, Landulfo & Luz, 2015	IBSP 10316 -Larva	*Thoropa miliaris* (Spix, 1824)	Itaguaí, Rio de Janeiro	Barros-Battesti et al. (2015)
		CNC-3514	*Rhinella ornata* (Spix, 1824	Itaguaí, Rio de Janeiro	Luz et al. (2018)
2	*Ornithodoros saraivai* Muñoz-Leal & Labruna, 2017	USNMENT00862468 Larva	*Cycloramphus boraceiensis* Heyer, 1983	Ilhabela, São Paulo	Muñoz-Leal et al. (2017)
		ZUEC- UNICAMP	*Thoropa taophora* (Miranda-Ribeiro, 1923)	Ilhabela, São Paulo	Sá-Hungaro et al. (2018)
		ZUEC- UNICAMP	*T. taophora*	Ubatuba, São Paulo	Sá-Hungaro et al. (2018)
		ZUEC- UNICAMP	*T. taophora*	Iguape, São Paulo	Sá-Hungaro et al. (2018)
3	*Ornithodoros mimon* Kohls, Clifford & Jones, 1969	CNC	*Corallus hortulanus* (Linnaeus, 1758)	Chapada do Araripe, Crato, Ceará	De Alcantara, et al. (2018)
4	*Ornithodoros rietcorreai* Labruna, Nava & Venzal, 2016	CNC	*Leptodeira annulata* (Linnaeus, 1758)	Farias Brito, Ceará	De Alcantara, et al. (2018)
5	*Ornithodoros* sp.	CNC	*Oxyrhopus trigeminus* Duméril, Bibron & Duméril, 1854	Barro, Ceará	De Alcantara, et al. (2018)
		CNC	*Philodryas olfersii* (Lichtenstein, 1823)	Barro, Ceará	De Alcantara, et al. (2018)
		CNC	*L. annulate*	Jati, Ceará	De Alcantara, et al. (2018)
		-	*Boa constrictor constrictor* Linnaeus, 1758	Mossoró, Rio Grande do Norte	Pereira et al. (2012)

Source: (MENDOZA-ROLDAN, J. A., 2019)

Legend: IBSP (Acarological collection, of the Instituto Butantan, Special Zoological Collections Laboratory, São Paulo, Brazil), USNMENT (United States National Tick Collection, Georgia Southern University, Statesboro, USA), (CNC) National Tick Collection (Coleção Nacional de Carrapatos Danilo Gonçalves Saraiva) of the School of Veterinary Medicine, University of São Paulo, São Paulo, Brazil).

Figure 81 – Distribution map of species of *Ornithodoros* from reptiles and amphibians, using QGIS program



Source: (MENDOZA-ROLDAN, J. A., 2019).

Legend: (Red circles) *Ornithodoros faccinii*, (black circules) *Ornithodoros saraivai*, (yellow circle) *Ornithodoros mimon*, (green circle) *Ornithodoros rietcorreai*, (white circles) *Ornithodoros* sp.

Source: Literature cited in Table 22.

3 MATERIAL AND METHODS

3.1 Ixodida ticks` material

The tick species of the order Ixodida that infest reptiles and amphibians that were assessed, collected, identified, and evaluated, came from three possibilities: material deposited in collections; ticks that were brought upon their hosts to the different laboratories of the Instituto Butantan, or to the Venomous Animals Reception site of the same institute; and material that was collected from reptiles and amphibians in different field trips at various locations in Brazil. New or fresh material of mites and hosts were used for molecular biology studies (Part II of this thesis).

3.1.1 Material from collections

This study was based on the revision of the tick material deposited in the acarological collection of the Instituto Butantan (IBSP). Other reference collections were also revised to asses type material of some groups of ticks of reptiles and amphibians.

Acarological Collection of the Instituto Butantan (IBSP) – curator: Valeria Castilho Onofrio. It is one of the oldest collections of mites and ticks of Latin America. Ixodida ticks of reptiles and amphibians are represented in this collection with 360 lots, being one type material. The ticks are conserved in alcohol or mounted in slides (larvae).

Ixodologic collection of the L'École nationale vétérinaire de Toulouse (ENVT), Toulouse, France (França (Neumann collection of the École nationale Vétérinaire de Toulouse – ENV) – curator: Michel Franc. Belongs to the parasitology school of the L'École nationale vétérinaire and harbors six type material described by Neumann (two types of the genus *Amblyomma* parasites of reptiles and amphibians).

National Tick Collection (Coleção Nacional de Carrapatos Danilo Gonçalves Saraiva) of the School of Veterinary Medicine, University of São Paulo, São Paulo, Brazil – CNC – curator:

Marcelo Bahia Labruna. This reference collection has more than 300 lots of ticks from reptiles and amphibians, including two type material from argasid ticks from amphibians.

3.1.2 Laboratories of the Instituto Butantan (IBSP)

3.1.2.1 Venomous Animals Reception site of the Herpetological collection of the Special Zoological Collections Laboratory (LECZ)

The the Special Zoological Collections Laboratory (LECZ) of the Instituto Butantan, has a Venomous Animals Reception site, which receives snakes, amphibians, spiders, scorpions, Acari (mites and ticks), insects, among other animals, that come from varied localities of Brazil and from other countries. Reptiles and amphibians are then routed to the laboratories from the Instituto Butantan (Herpetology, Cellular Biology, Biological Museum, Ecology and Evolution, among others). Spiders and scorpions are routed to the Arthropods Laboratory, and Acari are deposited in the Acarological collection of the LECZ. Venomous animals (vertebrates and invertebrates) are used first for venom extraction and in some cases reproduction. When these animals die they are deposited in the LECZ). Mites and ticks from reptiles and amphibians that arrived from different regions of Brazil, herein studied, were collected whenever possible before being sent to the different laboratories or collections.

3.1.2.2 Laboratories of the Instituto Butantan

To assess infestation in captivity conditions, the laboratories that harbor live reptiles and amphibians for different purposes in the Instituto Butantan, were visited and the animals were examined for mites and ticks. Laboratories visited were: Cellular Biology, Ecology and Evolution, and the Biological Museum.

3.1.2.3 Material collected in field trips

Tick material was collected from reptiles and amphibians in different field trips at various locations in Brazil.

The listed field trips are from projects this study collaborated in fieldwork, or material that was revised from the hosts. The projects for each area (Atlantic forest, Amazon rainforest, and Cerrado) are presented with details in Chapter I (pages 101-103 of this Thesis).

3.2 Collection of ticks from reptiles and amphibians

Depending on the biological state of the tick, different collection methods were used, as well as specific areas of the host were examined depending on the species of the hosts and biological stage of the ticks. Larvae were extracted delicately through scarification (tick removal using a needle), and nymphs and adults were extracted using special tweezers to remove the tick without damaging the hypostome (Figure 82) (LIZASO, 1983; MENDOZA-ROLDAN et al., 2019). All animals were visually examined, some under stereo microscope, and a complete physical exam from the cranial portion to the caudal (posterior) portion was held for each animal.

Figure 82 – Removal of ticks with special tweezers in *Podarcis siculus*

Source: (MENDOZA-ROLDAN, J. A., 2018).

Identification of hosts (reptile and amphibians) used in this study, was performed by the team of herpetologists of the Herpetological collection of the LECZ. The host nomenclature was updated by consulting the "Reptile Database" (http://www.reptile-database.org) (UETZ, 2010) as well as the database of the Brazilian Society of Herpetology (Sociedade Brasileira de Herpetologia - SBH), for reptiles (COSTA; BÉRNILS, 2018).

3.3 Storage and conservation of ticks and host tissue

Collected ticks were stored in microtubes in absolute alcohol, and after, some of those ticks (nymphs and larvae) were used for slide mounting (this chapter), DNA extraction and molecular studies (all the biological stages) (Chapter 5 and 6). Eventually, some tissue samples (blood or liver) were obtained (techniques detailed in chapters 4) from parasitized hosts in the laboratories of the Instituto Butantan or in field trips. These blood samples were used to evaluate hemoparasites in smears (Chapter 4) and for pathogen detection (Chapter 6). Ticks and tissue were collected with approval of the Ethics Committee of Animal Use (Comissão de Ética no Uso de Animais - CEUA) of the Faculty of Veterinary Medicine of the University of de São Paulo (Faculdade de Medicina Veterinária e Zootecnia da Universidade de São Paulo - FMVZ/USP), protocol nº 7491300715.

3.4 Morphological identification

For Ixodidae ticks, dichotomous keys for adults and nymphs were used morphological identification (BARROS-BATTESTI et al., 2006; MARTINS et al, 2010). For argasid ticks, original descriptions were used, in most cases, for morphological identification ((BARROS-BATTESTI et al., 2015; MUÑOZ-LEAL et al., 2017). Measurements of species description are in µm. Also, DNA from larvae was extracted for molecular identification (chapter 5).

3.4.1 Illustrations

Anatomic features with taxonomic importance of some species of ticks with scarce taxonomical information were drawn to better illustrate species diagnosis and differences between

species. Illustrations were made using a LEICA DM 400B microscope, then scanned, digitalized, edited and compiled in Photoshop CS6 and Corel Draw X7.

3.4.2 Scanning Electron Microscopy (SEM)

Whenever possible, one to four ticks were selected for scanning electron microscopy. The material was first dehydrated for 30 minutes, in a crescent alcohol concentration (70%, 80%, 90%, 95%, 100%, 100%, 100%, 100%), then maintained in Hexamethyldisilane for 24 hours. Metallization was performed leaving the specimens in a chemical cabinet with Hexamethyldisilane, at room temperature, until the material was completely dry. Each specimen was mounted on a ½-inch aluminum metal plate and metallized with gold. Scanning electron microscopy was performed at the Cellular Biology Laboratory of the Butantan Institute, under a digital scanning microscope, of the FEI model Quanta 250 (Multiuser Equipment).

3.5 Distribution

Distribution maps were generated using QGIS program, version 3.4.4-Madeira, to compare new distribution localities with those reported in literature (QGIS DEVELOPMENT TEAM, 2015).

4 RESULTS

Information of the identified species of ticks (from collections and recent field trips) can be observed in Tables 24 and 25. All the species of ticks collected in this study were incorporated to the acarological collection of the IBSP. Examined species are summarized in the Catalogue of examined species (item 4.2), which also includes information about specimens that were used for molecular biology (phylogeny and pathogen detection in part II). Host information, as well as parasite-hosts associations and parasitic impact, are discussed in chapter 4.

4.1 Species of ticks identified

In this study, four genera and 19 species of ticks were identified. These species were identified from the IBSP collection (and other examined collections), and from ectothermic hosts examined in the laboratories of the Instituto Butantan, as well as those examined in recent field trips (Table 24). Species that were collected in field trips in this study are highlighted in Bold. Species identified: Order Ixodida: **Argasidae** (*O.* faccinii, *O. mimon, O. rietcorreai*, *O. saraivai* Muñoz-Leal & Labruna 2017; ***Ornithodoros* (*Alectorobius*) sp. Ixodidae** [*A.* cajennense, ***A.*** *dissimile, Amblyomma fuscum* Neumann 1899; *Amblyomma goeldii* Neumann, 1899; ***Amblyomma humerale* (Koch 1844); *Amblyomma nodosum* Neumann, 1899**; *Amblyomma oblongoguttatum* Kock 1844; ***A. rotundatum*; *Amblyomma sculptum* Berlese,** 1888; *Amblyomma flavomaculatus* (Lucas, 1846); *Amblyomma quadricavum* (Schulze**,** 1941); ***Rhipicephalus sanguineus* s.l. (Latreille, 1806); *Rhipicephalus* (*Boophilus*) *microplus* (Canestrini, 1887); *Dermacentor nitens* (Neumann, 1897)**].

Of the 19 species identified in this study, 17 occur in Brazil. The Brazilian species are shown in Table 24 in bold. Hosts for each species of tick are shown in Table 25 (new hosts records are shown with **X**). Parasite-host associations are discussed in chapter 4.

Table 24 - Tick types and material examined of reptiles and amphibians: collection, field trips and laboratories of the IBSP

Family	Species	Collections			Field trips and laboratories of the IBSP				
		IBSP	ENV	CNC	North	Northeast	Central-west	Southeast	South
Argasidae	***Ornithodoros faccinii***	2		2					
	Ornithodoros mimon			1					
	Ornithodoros rietcorreai			1					
	Ornithodoros saraivai	2		1					
	***Ornithodoros* (*Alectorobius*) sp.**							1	
Ixodidae	***Amblyomma cajennense***	1		1					
	Amblyomma dissimile	58	2	43		1	14	2	
	Amblyomma fuscum	7		54					
	Amblyomma goeldii	3		9					
	Amblyomma humerale	1	2	77	1				
	Amblyomma nodosum			136			1		
	Amblyomma oblongoguttatum			86					
	Amblyomma rotundatum	171		190	8	2	5	18	
	Amblyomma sculptum			4				1	
	Amblyomma flavomaculatus	1							
	Amblyomma quadricavum	1							
	***Rhipicephalus sanguineus* s.l.**						1		
	Rhipicephalus microplus						1		
	Dermacentor nitens						1		

Source: (MENDOZA-ROLDAN, J. A., 2019)

Table 25 – Species of hosts and species of ticks from collections and field trips

Class	Host	*Ornithodoros (Alectorobius)* sp.	*A. cajennense*	*A. dissimile*	*A. humerale*	*A. nodosum*	*A. rotundatum*	*A. sculptum*	*R. sanguineus* s.l.	*R. (Boophilus) microplus*	*Dermacentor nitens*
Serpentes	*Philodryas nattereri*	**X**									
	Bothrops asper			x							
	Bothrops atrox						x				
	Bothrops leucurus						x				
	Bothrops jararaca						x				
	Bothrops jararacussu						x				
	Bothrops alternatus						x				
	Bothrops insularis						x				
	Porthidium lansbergii			**X**							
	Spilotes pullatus			x			x				
	Pseustes sulphureus						**X**				
	Phalotris matogrossensis			**X**							
	Crotalus durissus terrificus			x			x				
	C*hironius laurenti*			**X**							
	Chironius multiventris						**X**				
	Chironius scurrulus						**X**				
	Boa constrictor constrictor			x		**X**	x			**X**	
	Xenodon merremii						x				
	Xenodon severus			x							
	Dipsas indica bucephala,			**X**							
	Pseudoboa nigra		**X**	**X**							
	Eunectes murinus,			x							
	Helicops angulatus						**X**				
	Oxyrhopus melanogenys						**X**				
	Oxyrhopus trigeminus						**X**				
	Leptodeira annulata						**X**				
	Dipsas turgidus						**X**				
	Dipsas neuwiedi						**X**				
	Corallus hortulanus						x				
	Philodryas viridissima						**X**				
	Lachesis muta						x				
	Epicrates cenchria						x				
	Bothrops moojeni				**X**						

(Conclusion)

Class	Host	*Ornithodoros (Alectorobius)* sp.	*A. cajennense*	*A. dissimile*	*A. humerale*	*A. nodosum*	***A. rotundatum***	*A. sculptum*	*R. sanguineus* s.l.	*R. (Boophilus) microplus*	*Dermacentor nitens*
Sauria	*Kentropix calcarata*				x						
	Ameiva ameiva						x				
	Iguana iguana			x			x				
	Trachylepis atlantica						**X**				
	Tropidurus hispidus						**X**				
	Salvator merianae							**X**			
	Plica umbra				x						
Testudinata	*Chelonoidis carbonaria*			**X**	x		x				
	Chelonoidis denticulata						x				
	Kinosternon scorpioides						x				
	Rhinoclemmys pulcherrima										
	Kinosternon scorpioides						x				
	Phrynops geoffroanus							**X**			
Crocodylia	*Caiman latirostris*						x				
	Caiman crocodilus				x		x				
	Paleosuchus trigonatus						x				
Anura	*Rhinella crucifer*										
	Rhinella granulosa										
	Rhinella icterica										
	Rhinella schneideri			x			x		**X**		**X**
	Rhinella marina			x			x				
	Rhinella jimi			**X**			**X**				
	Rhinella margaritifera						x				
	Rhaebo guttatus						**X**				

Source: (MENDOZA-ROLDAN, J. A., 2019).

Legend: New records of hosts are highlighted with **X**

4.2 Catalogue of examined species

Information regarding identified species of ticks (from collections and recent field trips) are detailed in this section. Material used for molecular biology (Part II) is highlighted with *, new host record with **, and new localities with***.

Order IXODIDA

Family Argasidae

***Ornithodoros faccinii* Barros-Battesti, Landulfo & Luz, 2015**

Southeast region: Rio de Janeiro state –Itaguaí, RJ – IBSP 10316, 1 larvae holotype, *Thoropa miliaris*, 26.XI.2010, coll. Gabriel Landulfo; IBSP 10317, 7 larvae paratypes, host data and locality same as holotype; CNC 3002, 2 larvae paratypes, host data and locality same as paratype. **Mangaratiba, RJ –** CNC 3519, 3 larvae, *Rhinella ornata*, 15.I.2017.

***Ornithodoros mimon* Kohls, Clifford & Jones 1969**

Northeast region: Ceará state – Crato, CE – CNC 3511, 6 larvae, *Corallus hortulanus*, 15.VII.2017.

***Ornithodoros rietcorreai* Labruna, Nava & Venzal 2016**

Northeast region: Ceará state – Farias Brito, CE – CNC 3512, 1 larva, *Leptodeira annulata*, 15.VII.2017.

***Ornithodoros saraivai* Muñoz-Leal & Labruna 2017**

Southeast region: São Paulo state – Ilhabela, SP – CNC 3910, 2 larvae, 2 females and 1 nymph paratypes, *Cycloramphus boraceiensis*, 22.III.2016.

***Ornithodoros* (*Alectorobius*) sp.**

Southeast region: São Paulo state – São Bernardo do Campo, SP – IBSP 14838, 7 larvae, *Philodryas nattereri*, 22.IX.2017, coll. Jairo Mendoza-Roldan, *,***, ***.

Family Ixodidae

***Amblyomma cajennense* (Fabricius 1777)**

Central-West region: Mato Grosso state - Zoológico da Universidade Federal de Mato Grosso – UFMT, Cuiabá, MT – IBSP7702, 1 female, *Iguana iguana*, 12.IV.1997

North region: Tocantins state – Lagoa da Confusão, TO – CNC 4256, 1 nymph, *Pseudoboa nigra*, 01.IV.2013.

***Amblyomma dissimile* (Koch 1844)**

Cocoli, Panamá: CNC 1750, 2 males, *Bothrops asper*, 15.III.2008, coll. David Correa. CNC 1751, 1 nymph, host data and locality same as before.

Honduras: CNC 3042, 2 nymphs, 1 female and 1 male, *Iguana iguana*, 15.VIII.2014.

Colombia: Atlantico state: Barranquilla - IBSP12521, 1 male, *Porthidium lansbergii*, 15.VI.2016. coll Jairo Alfonso Mendonza Roldan**. **Santander-** CNC 2729, 5 nymphs and 1 female, *Spilotes pullatus*, 03.I.2014; CNC 2730, 2 nymphs, *Iguana iguana*, 20.I.2014.

Toulouse, France: ENV 835, 1 female holotype and 4 males.

Central-West region: Mato Grosso state – Paranatinga, MT – IBSP 12984, 2 females, *Chelonoidis carbonarius*, 18.III.2017; IBSP 12985, 1 female, *Phalotris matogrossensis*, 18.III.2017**; IBSP 12986, 1 nymph, *P. nigra*, 18.III.2017; IBSP 12989, 1 nymph, *Crotalus durissus terrificus*, 18.III.2017. **Pirezal, MT –** IBSP 12960, 1 male, *Rhinella schneideri*, 18.III.2017. **Mato Grosso do Sul state – RPRN Fazendinha, MS –** IBSP 12973, 1 nymph and 1 female, C*hironius laurenti* 18.III.2017**.

North region: Amapá state – Macapá, AP – CNC 3674, 1 female, *R. marina*, 15.XI.2017; CNC 3675, 1 female, *R. marina*, 15.V.2017; CNC 3875, 1 female, *R. marina*, 10.IV.2017. **Santana, AP –** CNC 3676, 1 female, *R. marina*, 15.III.2017. **Pará state – Belterra, PA –** IBSP 12553, 5 females and 4 males, *Rhinella marina*, 4.V.2010. Zoológico Santarém, PA – CNC 3535, 239 nymphs, 127 females and 387 males, *Boa constrictor*,07.VI.2012; CNC 3536, 3 nymphs, 8 females and 13 males, *Eunectes murinus*, 03.I.2010; CNC 3537, 1 female and 4 males, *Iguana iguana*, 06.II.2010; CNC 3538, 6 females and 2 males, *Xenodon severus*, 06.II.2012.

Northeast region: Bahia state – Ilhéus, BA – IBSP 12581, 20 females and 8 machos, *Rhinella jimi*, 19.V.2016, coll. Marta Maria Antonniazi**.

Southeast region: São Paulo state –Pompeia, SP – IBSP 12932, 1 nymph and 1 female, *Dipsas indica bucephala*, 3.III.2016, coll Jairo Mendoza Roldan*, **. **São Paulo, SP –** IBSP 12953, 4 females and 4 males, *P. nigra*, 26.IX.2016, **.

***Amblyomma flavomaculatus* (Lucas, 1846)**

Guiné-Bissau: IBSP 4921, 2 females and 5 males, *Varanus exanthematicus*, 15.VII.1949, coll. Tendeiro.

***Amblyomma fuscum* Neumann 1899**

South region: Rio Grande do Sul state – Estação Ecológica do Taim, RS – IBSP 9261, 1 female, *Tupinambis* sp., 15.VII.1994, coll. Afonso L. Sinkoc. **Três Barros, RS –** IBSP 10670, 1 female, *Tupinambis teguixin*.

Southeast region: São Paulo state – Cananéia, SP – IBSP 5796, 1 female, *Clelia clelia*, 17.I.1961, coll. Mario Nogueira. **Pedro de Toledo, SP –** IBSP 9202, 1 female and 1 male, *Spilotes pullatus*, 26.XI.2004. **Peruíbe, SP –** CNC 2959, 3 males, *T. teguixin*, 01.XI.2010. **Rosana, SP –** IBSP 9272, 4 females and 1 male, *Rhinella* sp., 15.I.1994, coll. Afonso L. Sinkoc. **Santos, SP –** IBSP 4593, 1 male, *T. teguixin*, 10.XI.1952.

***Amblyomma goeldii* Neumann, 1899**

No locality: IBSP 1396, 1 female, *R. schneideri*, coll. Alcides Prado.; IBSP 1754, 1 female, *Bothrops jararaca*, coll Flávio da Fonseca.

North region: Pará state – Serra dos Carajás, PA – IBSP 6855, 2 males, *B. constrictor*, 15.XII.1970.

***Amblyomma humerale* (Koch 1844)**

Central-West region: Goiás state – Nova Crixas, GO – CNC 3900, 1 nymph, *Caiman crocodilus*, 24.VI.2017. **Mato Grosso state – Cláudia, MT –** CNC 3898, 2 nymphs, *Kentropix calcarata*, 01.V.2015. **Cotriguaçu, MT –** CNC 2837, 16 males, *Chelonoidis denticulata*,

06.VI.2014; CNC 3576, 1 female and 24 males, *C. denticulata*, 27.III.2016. **Santa Terezinha, MT –** CNC 2 nymphs, *Bothrops moojeni*, 01.VI.2012**.

France, Toulouse: ENV 1803, 2 females.

North region: Pará state – Belém, PA – IBSP 12910, 4 females and 4 males, *C. carbonarius*, 28.V.2015, *, coll. Bruno Rocha. **Oriximiná, PA –** CNC 3064, 2 nymphs, *Plica umbra*, 19.IV.2014. **Parauapebas, PA –** CNC 3283, 1 female and 4 males, *C. denticulata*, 02.X.2014. **Rondônia state – Monte Negro, RO –** CNC 1346, 1 male, *C. crocodilus*, 17.XII.2004. **Tocantins state – Araguaína, TO –** CNC 3811, 2 males, *C. carbonaria*, 25.VI.2018.

Northeast region: Bahia state – Ilhéus, BA – CNC 2809, 5 males, *C. carbonaria*, 12.V.2014.

Southeast region: Espírito Santo state – Pinheiros, ES – CNC 2999, 3 males, *C. denticulata*, 05.II.2012. **Sooretama, ES –** CNC 3375, 2 females and 1 male, *C. carbonaria*, 19.VIII.2016.

***Amblyomma nodosum* Neumann, 1899**

Central-West region: Goiás state – Centro de Triagem de Animais Silvestres (CETAS), Goiânia, GO – IBSP 12469, 4 females and 4 males, *Boa constrictor constrictor*, 29.III.2013, coll. Adriana Marques Faria**.

***Amblyomma oblongoguttatum* Kock 1844**

North region: Pará state – Reserva Extrativista Verde para Sempre, PA – CNC 3950, 1 nymph, C. *denticulata*, 01.III.2013.

***Amblyomma quadricavum* (Schulze, 1941)**

Cuba: IBSP 8730, 1 male, *Chilabothrus angulifer*, coll. Mercedes Hernandez.

***Amblyomma rotundatum* (Koch 1844)**

Central-West region: Goiás state – Nova Crixas, GO – CNC 3900, 4 females, *C. crocodilus*, 24.VI.2017. **Mato Grosso state – Alta Floresta, MT –** CNC 2574, 10 females and 4 nymphs, *Paleosuchus trigonatus*, 06.XII.2013. **Aripuanã, MT –** IBSP 12970, 1 nymph, *Rhinella marina*, 18.III.2017. **Cláudia, MT –** CNC 3573, 75 nymphs, *R. marina*, 04.XII.2016. **Confresa, MT –** CNC 2991, 2 females, *Kinosternon scorpioides*, 15.IX.2010. **Cotriguaçu, MT –** CNC 2838, 1 female, *Rhaebo guttatus*, 23.XI.2013**; CNC 2839, 17 nymphs and 13 females, *R. marina*,

03.VII.2014; CNC 3558, 3 nymphs and 1 female, *Rhinella margaritifera*, 22.XI.2016; CNC 3576, 3 nymphs and 3 females, *R. marina*, 20.I.2015; CNC 3619, 4 nymphs and 4 females, *R. marina*, 21.VII.2015; CNC 3896, 1 female, *Helicops angulatus*, 03.VIII.2011**; CNC 3899, 3 females, *R. marina*, 25.I.2018-22.I.2018-22.II.2012. **Cuiabá, MT –** IBSP 14865, 2 nymphs, *Oxyrhopus melanogenys*, 28.II.2018, coll. Jairo Mendoza-Roldan, *,**; IBSP 14866, 3 nymphs, *Oxyrhopus trigeminus*, 28.II.2018, coll. Jairo Mendoza-Roldan, *,**. **Parque Estadual do Cristalino, MT –** CNC 3574, 3 nymphs, *R. marina*, 10.X.2015. **Pontes e Lacerda, MT –** IBSP 14864, 1 nymph, *O. melanogenys*, 28.II.2018, coll. Jairo Mendoza-Roldan, *,**. **Mato Grosso do Sul state – Aquidauana, MS –** CNC 3194, 1 female, *C. carbonaria*, 15.XII.2015. **Caracol, MS –** IBSP 14869, 1 nymph and 3 females, *Leptodeira annulata*, 03.III.2018, coll. Jairo Mendoza-Roldan, *, **; IBSP 14870, 2 nymphs and 1 female, *Dipsas turgidus*, 03.XII.2018, coll. Jairo Mendoza-Roldan, *,**. **Miranda, MS –** CNC 3501, 1 female, *Chelonoidis denticulata*, 17.I.2017.

North region: Acre state – Iracema, AC – IBSP 14875, 5 larvae and 5 nymphs, *Chironius multiventris*, 10.X.2018, coll Flora Roncolatto*, **; IBSP 14879, 3 nymphs and 1 female, *Chironius scurrulus*, 10.X.2018, coll Flora Roncolatto Ortiz*, **; IBSP 14880, 4 nymphs and 2 females, *Chironius multiventris*, 10.X.2018, coll Flora Roncolatto*, **; IBSP 14882, 4 nymph and 2 females, *Corallus hortulanus*, 10.X.2018, coll. Jairo Mendoza-Roldan, *; IBSP 14883, 4 nymphs, *O. melanogenys*, 10.X.2018, coll. Jairo Mendoza-Roldan, *,**; IBSP 14885, 1 nymph, *Philodryas viridissima*, 15.X.2018, coll. Jairo Mendoza-Roldan, *,**. **Zoológico Rio Branco, AC –** CNC 3830, 8 nymphs and 4 females, *C. hortulanus*, 15.V.2018; CNC 3831, 83 nymphs and 26 females, *Pseustes sulphureus*, 15.V.2018**. **Amapá state: Macapá, AP –** CNC 3674, 1 female, *R. marina*, 15.XI.2017. **Santana, AP –** CNC 3676, 1 female, *R. marina*, 15.III.2017. **Pará state – Marabá, PA –** CNC 3287, 2 nymphs, *Tropidurus hispidus*, 05.V.2016**. **Monte Alegre, PA –** IBSP 14898, 3 nymphs, *Bothrops atrox*, 20.XII.2018, coll. Jairo Mendoza-Roldan, *; IBSP 14899, 3 nymphs, *C. hortulanus*, 20.XII.2018, coll. Jairo Mendoza-Roldan, *. **Ourolândia do Norte, PA –** CNC 2933, 1 female, *Rhinoclemmys pulcherrima*, 15.VI.2014. **Pacajá, PA –** CNC 3664, 2 females, *R. schneideri*, 15.XI.2017. **Parauapebas, PA –** CNC 3282, 4 females, *R. marina*, 02.X.2014. **Placas, PA –** CNC 3179, 1 nymph and 1 female, *C. denticulata*, 15.XI.2015. **Santarém, PA –** CNC 3174, 11 nymphs and 3 females, *R. jimi*, 15.XI.2015. **Tucuruí, PA –** CNC 2828, 1 nymph, *R. marina*, 15.VII.2014. **Rondônia state – Monte Negro, RO –** CNC 3613, 12 nymphs and 4 females, *Lachesis muta*, 08.II.2017. **Porto Velho, RO –** CNC 2845, 1 nymph and

1 female, *Epicrates cenchria*, 15.VI.2014; CNC 2946, 1 nymph and 5 females, *B. constrictor*, 01.V.2013; CNC 3452, 1 nymph and 1 female, *Chironius* sp., 27.X.2015; CNC 3454, 2 females, *B. atrox*, 21.X.2016; CNC 3456, 5 nymphs, *Spilotes pullatus*, 15.VI.2014. **Tocantins state – Araguaína, TO –** CNC 2506, 1 female, *Paleosuchus trigonatus*, 15.IV.2013; 3 nymphs, *Rhinella* sp., 15.IX.2013; CNC 2752, 2 females, *Iguana iguana*, 26.III.2014; CNC 2754, 6 females, *B. constrictor*, 03.IV.2013. **Chapada da Natividade, TO –** CNC 3812, 2 nymphs and 2 females, *Xenodon merremii*, 18.VII.2018; CNC 3814, 4 nymphs, *B. constrictor*, 17.VIII.2018.

Northeast region: Bahia state – Ilhéus, BA – CNC 2809, 1 female, *C. carbonaria*, 12.V.2014; IBSP 12941, 1 nymph, *Atractus guentheri*, 8.X.2016; IBSP 12942, 2 nymphs, *Rhinella crucifer*, 8.X.2016; IBSP 12943, 1 female, *Rhinella schneideri*, 8.X.2016; IBSP 12944, 2 females, *Rhinella schneideri*, 8.X.2016; IBSP 12945, 1 female, *Rhinella* sp., 8.X.2016; IBSP 12946, 1 nymph, *Rhinella granulosa*, 8.X.2016; IBSP 12947, 2 females, 1 nymph, *Rhinella crucifer*, 8.X.2016; IBSP 12948, 8 females, 10 nymphs, *Rhinella jimi*, 8.X.2016. **Maranhão state – Arari, MA –** CNC 3213, 3 nymphs, *C. hortulanus*, 15.VII.2015. **Chapadinha, MA –** CNC 3114, 1 female, *Rhinella* sp., 15.III.2013. **São Luís, MA –** CNC 2601, 1 nymph and 3 females, *K. scorpioides*, 15.XII.2013; CNC 3278, 2 females and 1 male, *B. atrox*, 15.VIII.2015. **Pernambuco state – Fernando de Noronha, PE –** CNC 2613, 1 female, *R. jimi*, 27.I.2014; CNC 2919, 16 nymphs and 8 females, *R. jimi*, 15.IX.2014; CNC 3130, 3 nymphs and 1 female, *R. jimi*, 15.VIII.2015; CNC 3300, 1 nymph, *Trachylepis atlantica*, 19.II.2016**; CNC 3770, 1 nymph and 3 females, *R. jimi*, 17.XI.2015; IBSP12980, 1 female, *Rhinella jimi*, 20.IV.2017; IBSP12982, 1 female, *Rhinella jimi*, 20.IV.2017**.

Southeast region: Espírito Santo state – Anchieta, ES – IBSP 14871, 4 nymphs and 1 female, *Dipsas neuwiedi*, 09.V.2018, coll. Jairo Mendoza-Roldan, *, **; IBSP 14873, 3 nymphs, *Bothrops leucurus*, 10.V.2018, coll. Jairo Mendoza-Roldan, *. **Linhares, ES –** CNC 3825, 1 female, *Rhinella* sp., 20.X.2018; CNC 3826, 1 female, *Caiman latirostris*, 01.IX.2017. **São Roque do Canaã, ES –** CNC 3307, 16 nymphs, *Ameiva ameiva*, 11.IV.2017. **Serra, ES –** CNC 3660, 1 female, *C. latirostris*, 03.XII.2017. **Sooretama, ES –** CNC 3374, 1 female, *Rhinella* sp, 19.VIII.2016; CNC 3375, 3 nymphs and 2 females, *C. carbonaria*, 19.VIII.2016; CNC 3684, 3 females, *C. latirostris*, 15.XII.2017. **Vitória, ES –** CNC 3683, 1 female, *C. latirostris*, 15.XII.2017. **Minas Gerais state – Estação Ecológica Pirapitinga, Três Marias, MG –** CNC 2524, 5 nymphs and 3 females, *Rhinella* sp., 15.IX.2013; CNC 2534, 1 female and 43 nymphs,

host data and locality same as before, 15.X.2013. **Montes Claros, MG –** IBSP 13768, 1female, *Xenodon merremii*, 27.VII. 2017, coll. Bruno Rocha*. **Varginha, MG –** IBSP 12978, 10 larvae and 8 nymph, *C. durissus terrificus*, 18.III.2017, *; IBSP 12954, 1 nymph, *Bothrops jararaca*, 24.XI.2016, *; IBSP 14845, 10 nymphs, *Bothrops alternatus*, 06.VII.2017, coll. Jairo Mendoza-Roldan, *. **Rio de Janeiro State – São João da Barra, RJ –** IBSP 13766**,** 1 female, *Bothrops jararacussu*, 11.IV.2018 coll. Bruno Rocha*; IBSP 13767, 5 females, 2 nymphs, *Bothrops jararacussu*, 11.IV.2018 coll. Bruno Rocha*. **São Paulo state – Guararapes, SP –** CNC 3557, 3 females, *R. schneideri*, 15.I.2017. **Ilha Queimada Grande, SP –** IBSP 12936, 6 females, *Bothrops insularis*, 5.VI.2016, coll. Bruno Rocha, *; IBSP 12937, 5 females, host data and locality same as before, 06.I.016, coll. Bruno Rocha, *; IBSP 12938, 3 females, host data and locality same as before, 24.VI.2016, coll. Bruno Rocha, *; IBSP 12939, 7 females, host data and locality same as before, 24.VI.2016, coll. Bruno Rocha, *; IBSP 14830, 4 nymphs, *B. insularis*, 10.IX.2017, coll. Bruno Rocha, *. **Indaiatuba, SP –** IBSP 12915, 1 nymph, *C. durissus terrificus*, 12.X.2015, *. **Pompeia, SP –** IBSP 14895, 3 larvae, *P. nigra*, 12.VI.2018, coll. Jairo Mendoza-Roldan, *. **Santana do Parnaíba, SP –** IBSP 12909, 10 larvae and 1 nymph, *C. durissus terrificus*, 28.VIII.2015, *. **São Paulo, SP –** IBSP 12979, 1 female, *Epicrates cenchria*, 20.IV.2017.

***Amblyomma sculptum* Berlese, 1888**

Southeast region: São Paulo state – Santa Bárbara, SP – IBSP 14832, 1 male, *Salvator merianae*, 27.X.2017, coll. Jairo Mendoza-Roldan, *,**. **Zoológico Sorocaba, SP –** CNC 3057, 1 female, *Phrynops geoffroanus*, 11.III.2015**; CNC 5090, 1 male, *P. geoffroanus*, 01.IV.2015.

***Dermacentor nitens* (Neumann, 1897)**

Central-West region: Mato Grosso state – Pirezal, MT – IBSP 12962, 1 male, *R. schneideri*, 18-III-2017**.

***Rhipicephalus* (*Boophilus*) *microplus* (Canestrini, 1887)**

Central-West region: Goiás state – Centro de Triagem de Animais Silvestres (CETAS), Goiânia, GO – IBSP 12469, 2 females, *B. constrictor constrictor*, 29.III.2013, coll. Adriana Marques Faria**.

***Rhipicephalus sanguineus* s.l. (Latreille, 1806)**

Central-West region: Mato Grosso state – Pirezal, MT – IBSP 12962, 1 male, *R. schneideri*, 18.III.2017**.

4.3 Morphological and taxonomical details

In this section two species of ticks are detailed morphologically. One of them is a possible new species and is detailed theirhein.

Order IXODIDA
Family Argasidae

4.3.1 *Ornithodoros* (*Alectorobius*) sp.

Examined material – 7 larvae (IBSP 14838), *Philodryas nattereri* Steindachner, 1870, São Bernardo do Campo, São Paulo state, Brazil

Diagnosis: Idiosoma with 22 pairs of dorsal setae, being 7 anterolateral, four central and 12 posterolateral, usually barbed; ventral surface with 8 pairs of setae plus a posteromedian seta; hypostome pointed, dentition 2/2 from basis to median part, and 3/3 then 2/2 to 1/1 (from median to apex), number of denticles in external, median and internal rows, respectively, 22, 21, and 9 denticles; dorsal plate pyriform, anteriorly narrowed and rounded, then expanded laterally, but narrowing from the median part to the posterior margin, slightly concave posteriorlly.

Description

Larva (Figures 83-86)

Idiosoma dorsal, oval (Figures 83, 85A, 86A), length including capitulum 1.727 – 1.730 (1.729), length excluding capitulum 1.321 – 1330 (1.326), width 1.199 – 1.201 (1.200). Dorsal plate pyriform, anteriorly narrowed and rounded, then expanded laterally, but narrowing from the median part to the posterior margin, slightly concave posteriorlly, length 0.342 – 0.346 (0.344) width 0.257 – 0.260 (-0.259) (Figures 83, 84A, 85A, 86B). Dorsal chaetotaxy as follow: 22 pairs of setae (7 anterolateral, 4 central, and 12 posterolateral). Anterolateral setae (*Al*): *Al1* length 0.085–0.090 (0.088), *Al2* length 0.105–0.110 (0.108), *Al3* length 0.108–0.109 (0.109), *Al4* length 0.121–0.126 (0.124), *Al5* length 0.117–0.119 (0.118), *Al6* length 0.125–0.126 (0.126), *Al7* length 0.180 - 0.130 (0.129). Central setae (*C*): *C1* length 0.111–0.113 (0.112), *C2* length 0.107–0.109 (0.108), *C3* length 0.099–0.089 (0.080), *C4* length 0.096–0.097 (0.098). Posterolateral setae (*Pl*): *Pl1* length 0.067–0.073 (0.070), *Pl2* length 0.080–0.081 (0.081), *Pl3* length 0.081, *Pl4* length 0.082–0.083 (0.083), *Pl5* length 0.086, *Pl6* length 0.091, *Pl7* length 0.097–0.098 (0.098), *Pl8* length 0.102–0.103 (0.103), *P9* length 0.0102–0.104 (0.103), *Pl0* length 0.107–0.0108 (0.108), *Pl1* length 0.108–0.109 (0.109), *Pl2* length 0.111–0.113 (0.112)(83A) (Figure 85A). ***Idiosoma ventral***, with seven pairs of setae, and 1 pair on anal valves, one posteromedian seta present (Figure 83B, 85B). Three pairs of sternal setae (*St*): *St1* length 0.098–0.099 (0.099), *St2* length 0.084–0.087 (0.086), *St3* length 0.088–0.089 (0.089); three pairs of circumanal setae (*Ca*): *Ca1* length 0.082–0.084 (0.083), *Ca2* length 0.072–0.073 (0.072), *Ca3* length 0.062–0.064 (0.063); posteromedian setae (*PM*) length 0.104–0.106 (0.105), postcoxal setae (*Pc*) length 0.062–0.066 (0.064) (Figure 85B).

Gnathosoma (Figure 86C), basis capituli hexagonal; Length from posterior margin of basis capituli to posthypostomal setae: *Ph1* 0.280–0.300 (0.290), length from posterior margin of basis capituli to insertion of hypostome 0.170–0.180 (0.170), width 0.129–0.135 (0.132). Two pairs of posthypostomal setae; *Ph1* length 0.013–0.016 (0.015), *Ph2* length 0.015–0.018 (0.017), distance between *Ph1* setae 0.028–0.030 (0.029), distance between *Ph2* setae 0.082–0.083 (0.083). Palpal total length 0.352–0.360 (0.355), segmental length from I-IV: (I) 0.078 (II) 0.120, (III) 0.102, (IV) 0.046. Hypostome pointed: length from *Ph1* to apex 0.250–0.256 (0.254), length from basis toothed portion to apex 0.212–0.220 (0.214), width in medial basis portion of hypostome 0.040–0.044 (0.043). Dentition 2/2 from basis to median part, and 3/3 then 2/2 to 1/1 (from median to

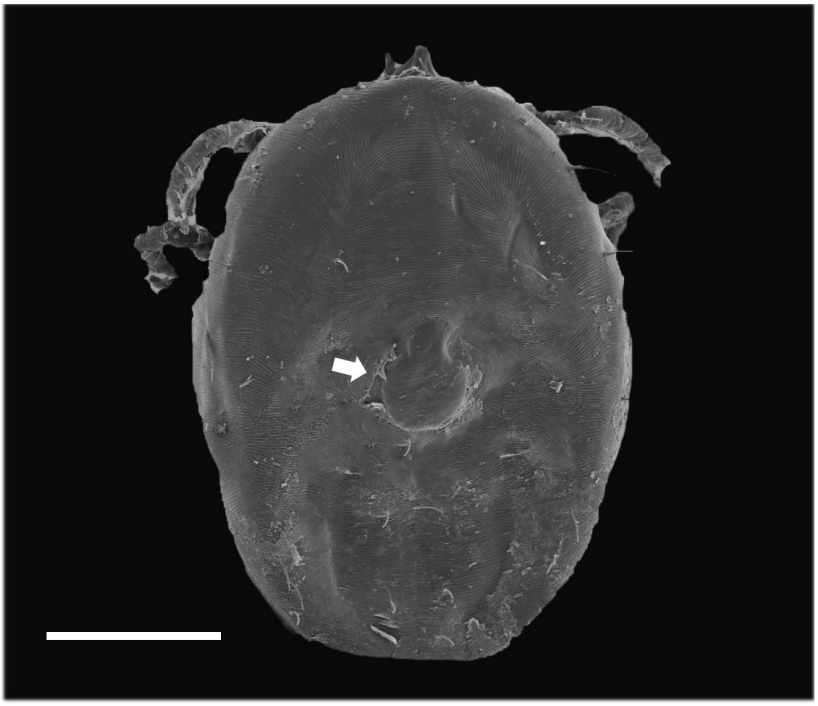
apex), number of denticles in external, median and internal rows, respectively, 22, 21, and 9 denticles (Figure 86C).

Legs: Tarsus I (Figures 84C, 86D), length 0.217–0.218 (0.217), width 0.075–0.080 (0.077) (84C, 86D). Tarsus I setal formula: 1 pair A (anterior), 1 DM (dorsomedian), 5 PC (paracapsular), 1 PM (posteromedian), 1 pair B (basal), 1 pair AV (anteroventral), 1 pair MV (midiventral), 1 pair BV (basiventral), and 1 pair PL (posterolateral).

Species relationship

The larva of *Ornithodoros* sp. is close to *Ornithodoros* (*Alectorobius*) *rioplatensis* Venzal, Estrada Peña & Mangold, 2008 mainly because of the morphology of the dorsal plate and hypostome pointed. It also resembles *Ornithodoros* (*Alectorobius*) *puertoricensis* (Fox, 1947) because both characteristics, but differs from them by having hypertrichy dorsally, and dorsal plate larger. Besides, the hypostome of the new species is more acute and has less denticle in each row. Furthermore, these species also differ molecularly (chapter 5).

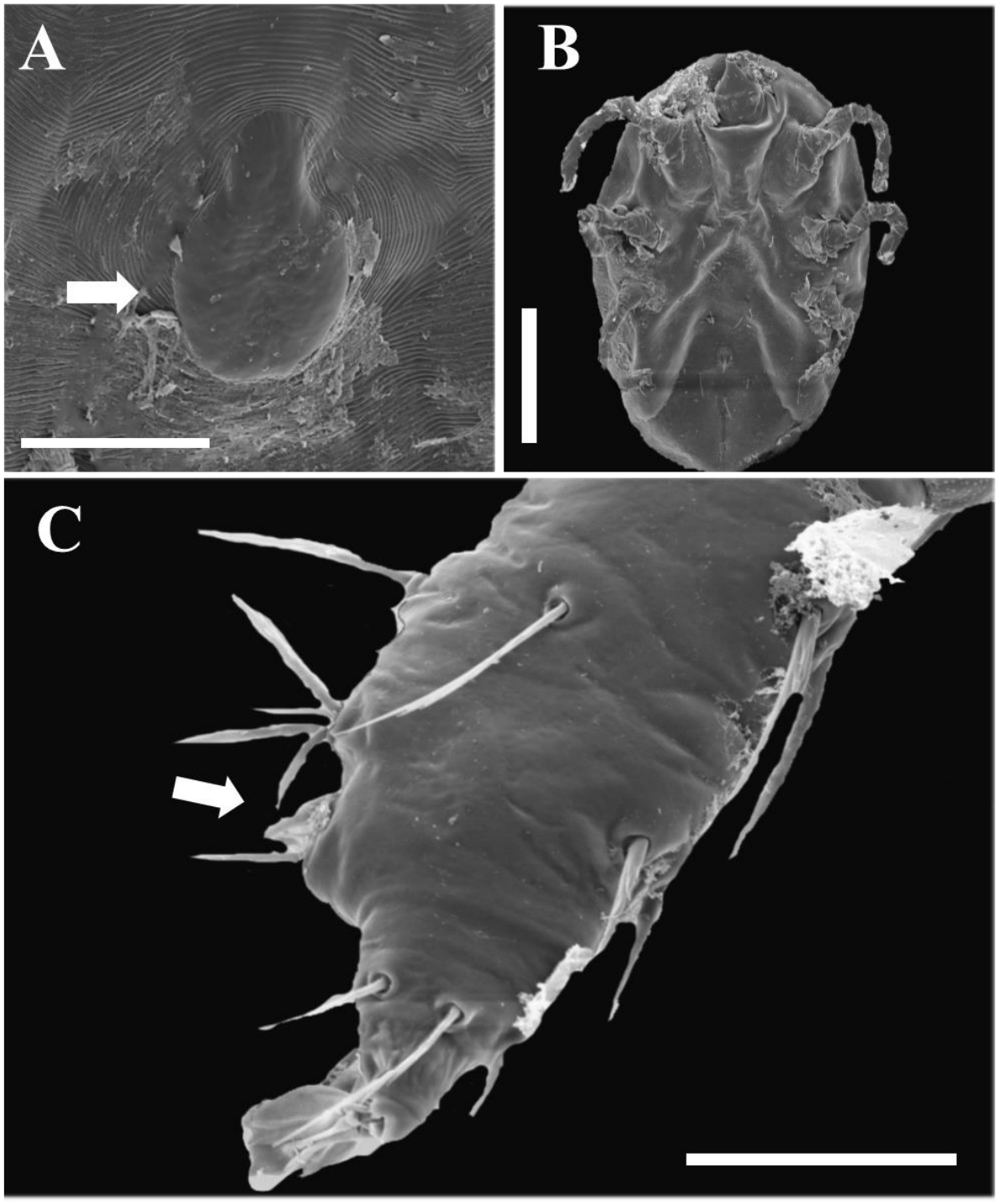
Figure 83 – Scanning electron microscopy of larva *Ornithodoros* (*Alectorobius*) sp, dorsal view



Source: (MENDOZA-ROLDAN, J. A., 2019)

Legend: larva *Ornithodoros (Alectorobius)* sp. white arrow showing dorsal scutum. Scale bar 500 µm.

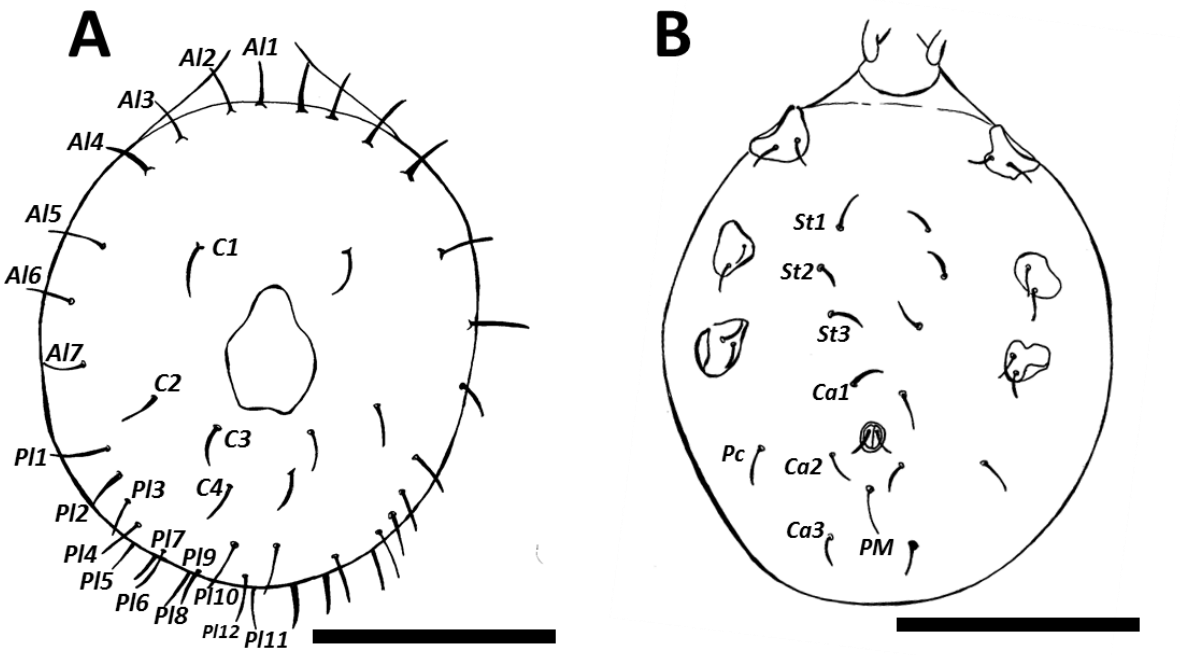
Figure 84 – Scanning electron microscopy of larva *Ornithodoros* (*Alectorobius*) sp.



Source: (MENDOZA-ROLDAN, J. A., 2019)

Legend: Larva of *Ornithodoros* (*Alectorobius*) sp. A. white arrow showing dorsal scutum; B. ventral view; C. white arrow showing Haller's organ. Scale bar A: 50 µm, B 500 µm, C 50 µm.

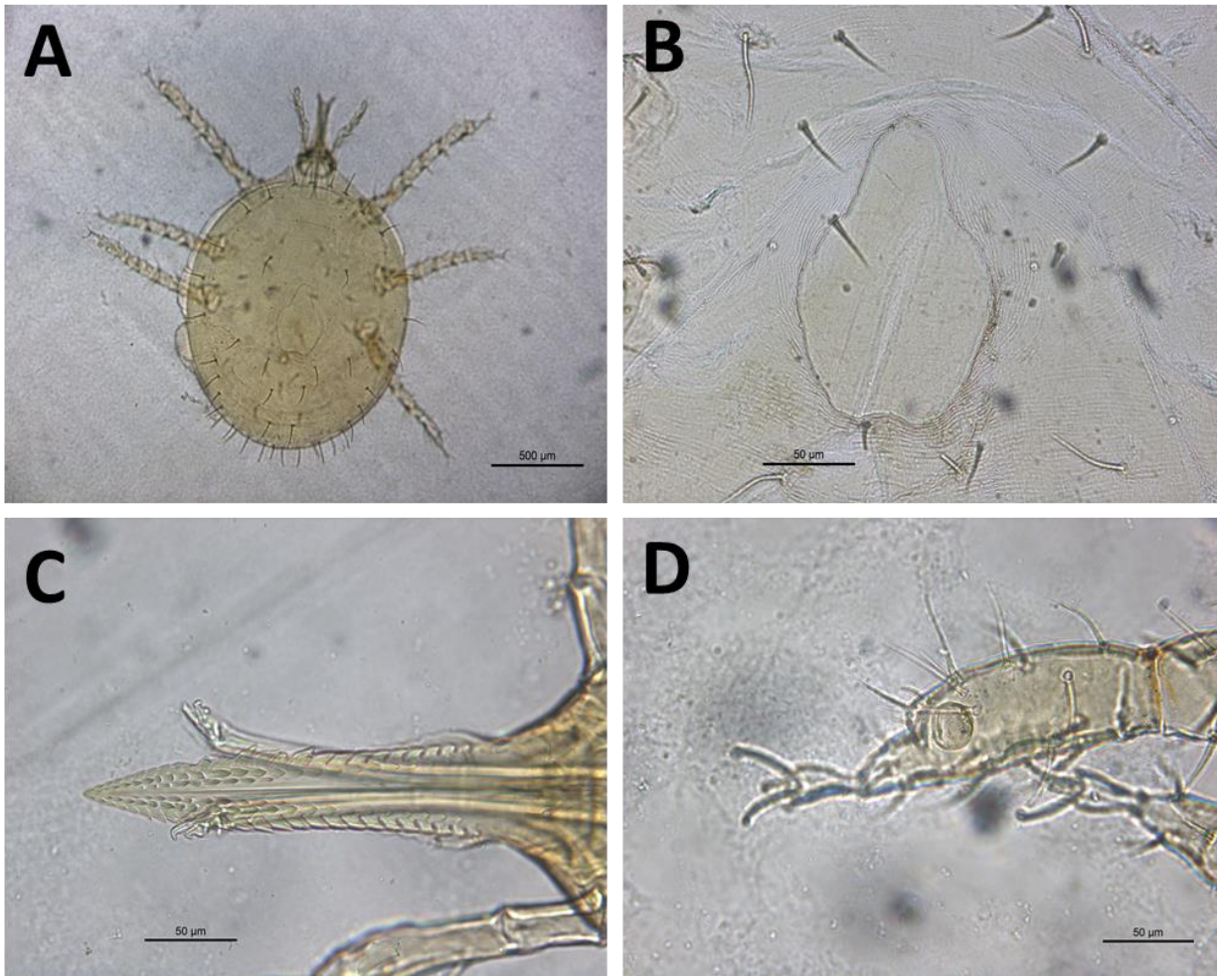
Figure 85 – Illustrations with morphological features of larva of *Ornithodoros* (*Alectorobius*) sp. n.



Source: (MENDOZA-ROLDAN, J. A., 2019)

Legend: A. Larva dorsal view; B. ventral dorsal view. Abbreviations: *Al1 – Al7* (anterolateral setae), *C1 – C3* (central setae), *Pl1 – Pl12* (posteriorlateral setae). Scale bar 500 μm.

Figure 86 – Optic microscopy of larva of *Ornithodoros* (*Alectorobius*) sp. n.



Source: (MENDOZA-ROLDAN, J. A., 2019)

Legend: A. larva dorsal view; B. dorsal scutum; C. Hypostomal dentition; D. Haller's organ. Scale bar: A Scale bar 500 µm, B-D 50 µm.

Order IXODIDA

Family Ixodidae

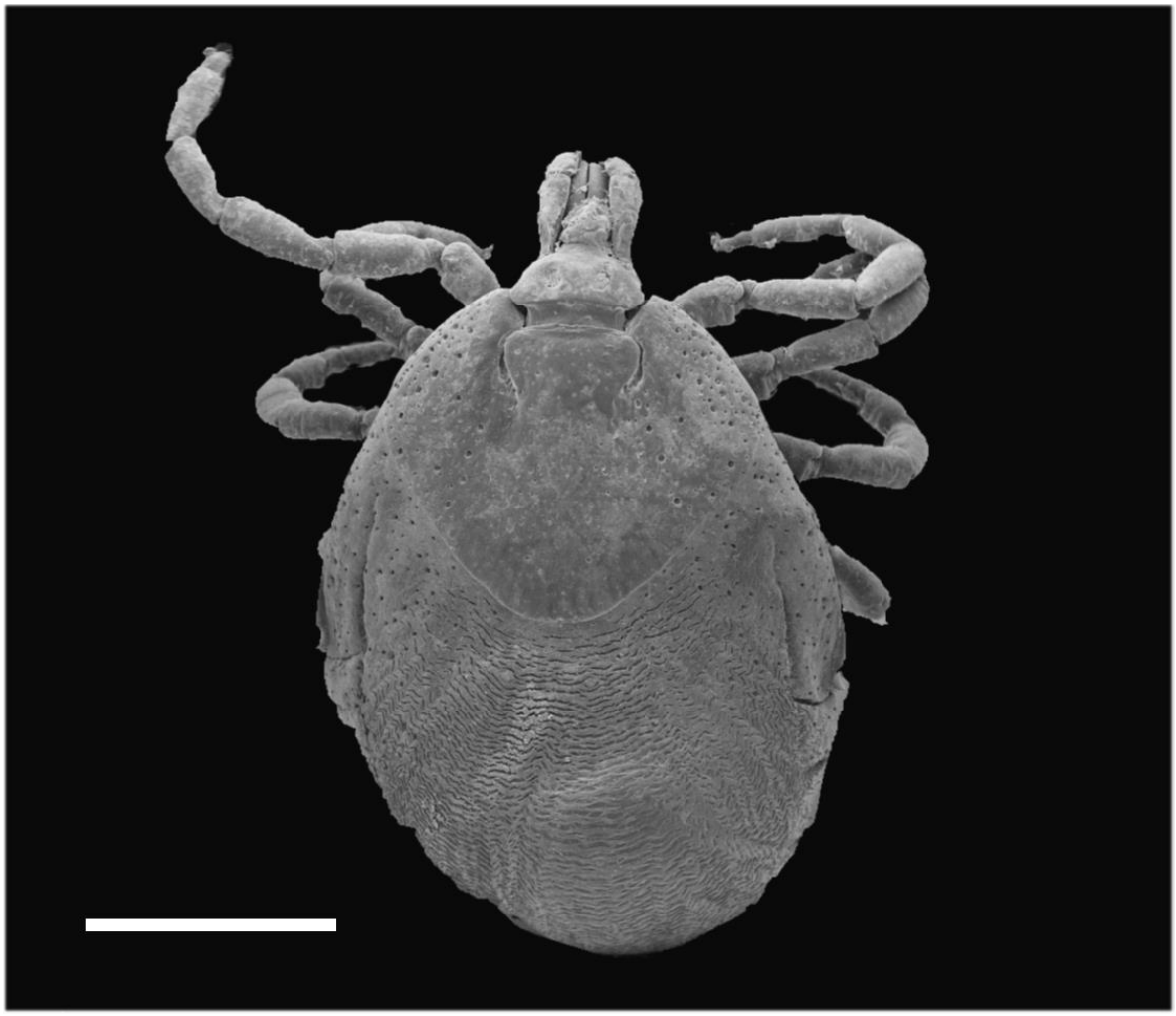
4.3.2 *Amblyomma rotundatum* (Koch 1844)

Type material - Holotype female, (ZMB 1065), ZMB - Zoologischen Museums Berlin, Berlin, Germany

Synonyms: *Ixodes fuscomaculatus* Lucas, 1873; *Amblyomma rotundatum* Koch, 1844; *Ixodes rotundatum* Neumann, 1892; *Amblyomma göldii* Neumann, 1899; *Amblyomma agamum* Aragão, 1912 *Amblyomma goeldii* Robinson, 1926:49; *Amblyomma kerberti* Oudemans, 1927; *Amblyomma fuscomaculatum* Santos Dias, 1958; *Amblyomma (Filippovanaia) fuscomaculatum* Santos Dias, 1993; *Amblyomma (Macintoshiella) rotundatum* Santos Dias, 1993; *Amblyomma (Walkeriana) rotundatum* Camicas et al., 1998.

Diagnosis. Female (Figure 87): Ornamented scutum with macular fort areason the lateral margind and the central region, being whiter on the posterior margin. Few large punctations, concentrated on the anterior lateral portions, and smaller punctations are numerous (Figure 88A). Porous areas transversely elongated and separate. Cornua absent. Dental formular 3/3. Coxa I with two subequal spurs, short and rounded. Coxa II – IV with two spurs, also rounded (Figure 88B). Base of the capitulum, subtriangular.

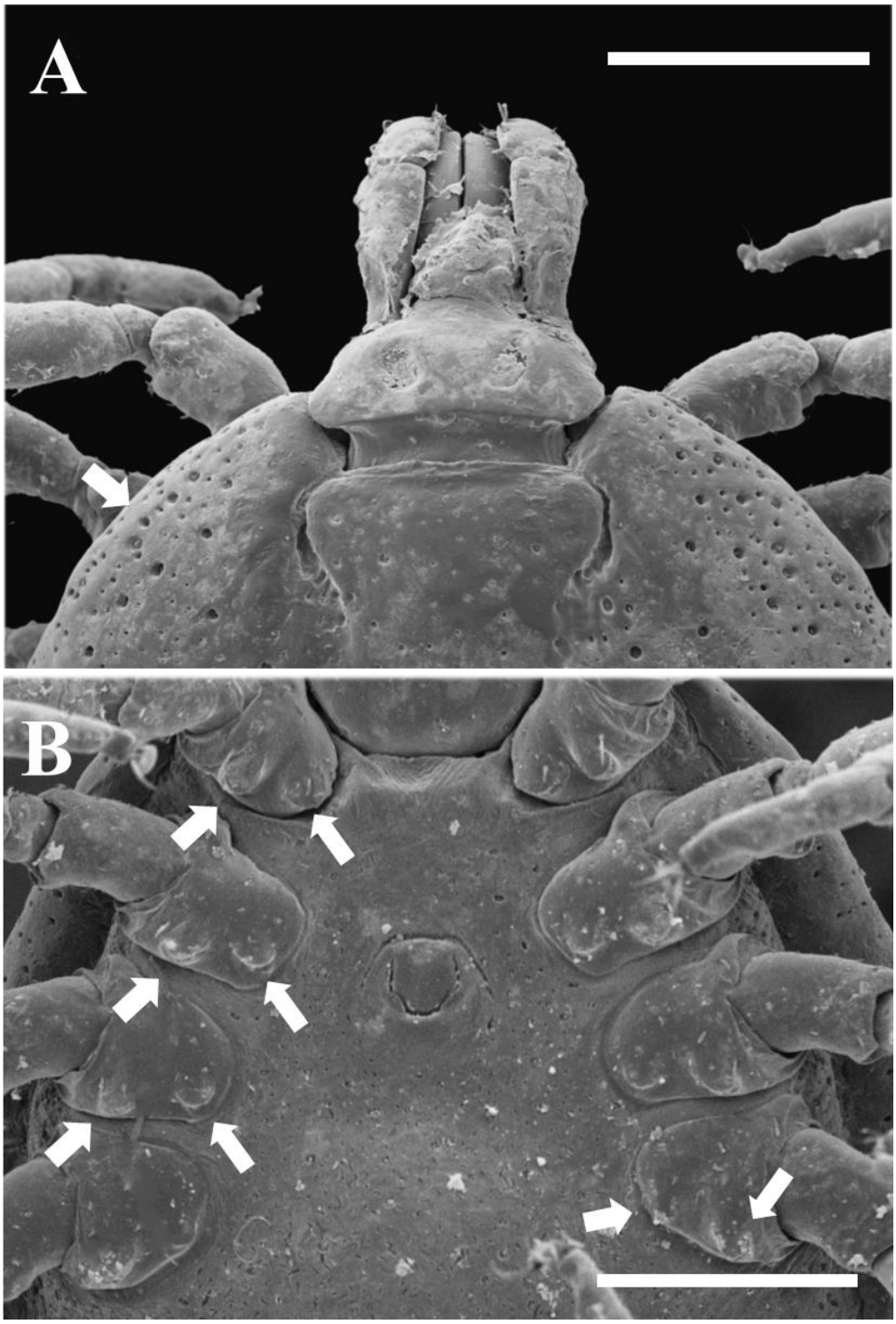
Figure 87 – Scanning electron microscopy of female *Amblyomma rotundatum*, dorsal view



Source: (MENDOZA-ROLDAN, J. A., 2018)

Legend: Female *Amblyomma rotundatum* dorsal view. Scale bar 2000 µm.

Figure 88 – Scanning electron microscopy of female *Amblyomma rotundatum*



Source: (MENDOZA-ROLDAN, J. A., 2018)

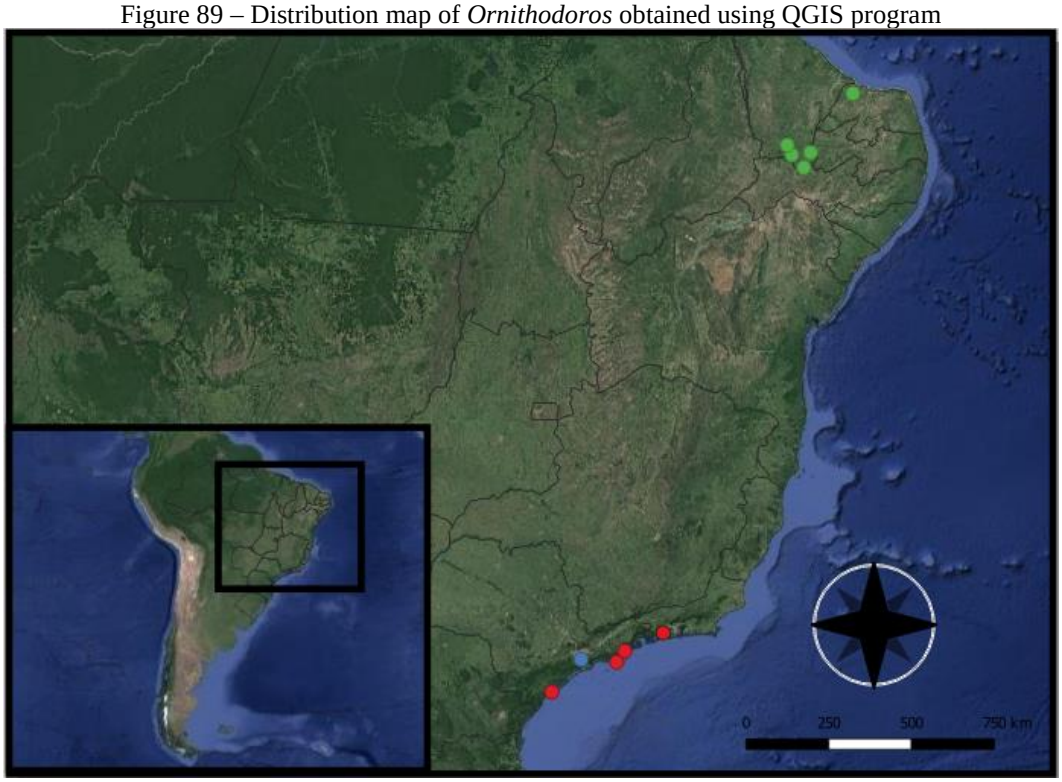
Legend: Female *Amblyomma rotundatum*. A. white arrow showing punctations on dorsal scutum; B. white arrow showing spurs, short and rounded. Scale bar: A, B 500 µm.

4.4 Geographical distribution

Maps of geographical distribution of the species of *Ornithodoros* examined in this study are shown in Figure 89. Geographic coordinates of each locality for each species are detailed hereafter, including information from literature and collections. Ixodidae ticks examined have wide distribution in the Neotropical region, thus maps are not informative.

Ornithodoros* (*Alectorobius*) sp.:** Brazil – **São Paulo:** São Bernardo do Campo (23° 41' 40.02" S, 46° 33' 56.88" W); ***Ornithodoros saraivai Ilhabela (23° 46' 43.4964" S, 45° 21' 29.8764" W). **Ceará state:** ***Ornithodoros mimon*** Crato (7° 14' 13.416" S, 39° 24' 57.888" W); ***Ornithodoros rietcorreai*** Farias Brito (6° 55' 45.084" S, 39° 34' 14.376" W). **Rio de Janeiro:** ***Ornithodoros faccinii*** Itaguaí (22° 51' 59.5476" S, 43° 46' 37.9992" W); Mangaratiba (22° 57' 39.2796" S, 44° 2' 29.4432" W) (Figure 89).

Figure 89 – Distribution map of *Ornithodoros* obtained using QGIS program



Source: (MENDOZA-ROLDAN, J. A., 2019).

Legend: (red circles) information of species of *Ornithodoros* parasitizing amphibians in Brazil; (blue circle) material collected of *Ornithodoros* (*Alectorobius*) sp.n. in this study; (green circles) information in literature of *Ornithodoros* collected from snakes in Brazil.

5 DISCUSSION

In the present study two families, four genera and 19 species of ticks were identified, from IBSP collection and from other collections well as those observed in recent field trips. Of the 19 species identified here, 17 occur in Brazil. Five of them correspond to argasid species, including possible new species, and 14 ixodid species, including two exotic species deposited in IBSP. New hosts records for collected species (host-parasite associations) are discussed in-depth in chapter 4).

The exotic species are *Amblyomma flavomaculatum* (Lucas, 1846) and *Amblyomma quadricavum* (Schulze, 1941). These species were deposited belonging to the genus *Aponomma* as *Aponomma halli* and *Aponomma quadricavum*. The species *A. halli* was synonymized with *A. flavomaculatum*, and it was initially described in varanid lizards of Guiné-Bissau (TENDEIRO, 1950; CAMICAS et al. 1998; KLOMPEN; DOBSON; BARKER, 2002). Here a material collected on *Varanus exanthematicus* from Guiné-Bissau was observed from the IBSP collection. The other exotic species, *A. quadricavum*, was found on *Chilabothrus striatus* (Fischer, 1856) from Haiti, and later synonymized (KEIRANS; KLOMPEN, 1996). Here we examine *A. quadricavum* collected on *Chilabothrus angulifer* (Cocteau and Bibron, 1840), from Cuba. This species has been recorded on boid snakes and iguanid lizards in the neotropics, mainly in the Caribbean. It has also been reported in the Nearctic and Palearctic region, but there is no evidence of adaptation to these regions (GUGLIELMONE et al., 2014).

Of the ixodid collected in the present study four species normally do not parasitize reptiles or amphibians. These are: *R. sanguineus* s.l and *D. nitens*, collected on toads (*R. schneideri*) from Pirezal, Mato Grosso state; and *R. microplus* and *A. nodosum*, collected on *B. constrictor constrictor* from the Centro de Triagem de Animais Silvestres (CETAS), Goiânia, Goiás state. These ticks were collected and sent to the Acarological collection of the IBSP for identification, thus confirmation of parasitism remains not established. Possibly, if the ticks did attach to these uncommon hosts, it could be an accidental parasitism due to scarce main hosts, or in case of the *B. constrictor constrictor*, as it was a captive animal maintained in a facility with other wild animals, ticks could parasitize it due to the proximity with main hosts. Nonetheless, this is speculative, and further collections with photographic evidence should be performed to better register these new hosts. Normally these species infest mammals, *R. sanguineus* s.l. is a common parasite of domestic dogs (BARROS-BATTESTI et al., 2018); *R. microplus* infest domestic and

wild ruminants (CONSTANTINOIU et al., 2010); and *D. nitens* parasitized equines (DESPINS, 1992). The species *A. nodosum* has been recorded on birds (mainly larvae), anteaters, and even dogs (accidental parasitism) (MAZIOLI et al., 2012).

Regarding the Argasidae family, one species was collected in field trips and four others were examined in two collections (IBSP, CNC). The ticks *Ornithodoros* species described from ambiphians are *O. faccinii* and *O. saraivai*, both from the southeast region on anurans (BARROS-BATTESTI et al., 2015; MUÑOZ-LEAL et al, 2017). The other records of *Ornithodoros* are all from the northeastern region, and from snakes. *Ornithodoros mimon*, on *Corallus hortulanus*, from Ceará state; *O. rietcorreai*, on *L. annulata*, also from Ceará state; and various of *Ornithodoros* sp. on captive *B. constrictor constrictor* from Rio Grande do Norte state, and *L. annulata*, *O. trigeminus*, and *P. olfersii* from Ceará state (PEREIRA et al., 2012; DE ALCANTARA, et al., 2018). The species collected in this study is the first record of an argasid tick infesting a snake in the southeastern region of Brazil, here identified as *Ornithodoros* (*Alectorobius*) sp. It is close to *O. puertoricensis* and *O. rioplatensis* in having dorsal plate pyriform and hypostome pointed with dental formula similar, but the new species is more hypertrichous, with 22 pairs of dorsal setae, while *O. puertoricensis* has 18 pairs of setae on the dorsum (14 dorsolateral and 4 central), 7 pairs on the ventral idiosoma, 1 pair on the anal valves, and a posteromedian seta. Moreover, *O. puertoricensis* is one of the few species of *Ornithodoros* described from reptiles in the neotropics, as it was described on *Phymaturus palluma* (Molina, 1782), from Chile (FOX, 1947). The species *O. rioplatensis* has 19 -20 dorsal setae (VENZAL, ESTRADA-PEÑA, 2006; VENZAL et al, 2008; BERMÚDEZ et al, 2013) and the dorsal plate is very similar to *Ornithodoros* sp. However, the new species differs from other *Alectorobius g*roup species by having 22 pairs of dorsal setae, 7 anterolateral, four central and 12 posterolateral setae. Besides, these species differ molecularly (see discussion on chapter 5).

Concerning the Ixodidae family, the *Amblyomma* was the most abundant genus, with 10 species identified. Of these, four were collected in recent field trips, and four were only examined from material deposited in collections: (*A. cajennense*, *A. fuscum*, *A. oblongoguttatum* and *A. goeldii*). The species *A. cajennense* has been recorded on *Drymarchon corais*, *Chelionoidis carbonaria*, *Podocnemys vogli*, and *Iguana iguana* (MOISSANT DE ROMÁN, 2016). It was examined material from Lagoa da Confusão, Tocantins state on *Pseudoboa nigra* Duméril, Bribon & Duméril, 1854 (deposited in the CNC collection), which would be a new host record. The

species *A. fuscum* (restricted to Brazil) and *A. goeldii* have been recorded parasitizing reptiles, canids and Xenanthra mammals (BARROS-BATTESTI et al., 2006). The species *A. oblongoguttatum* has been recorded in a myriad type of hosts including humans (MENDOZA-URIBE; CHÁVEZ-CHOROCCO, 2004). Here it was examined in a material from the CNC collection on *C. carbonaria*, from Pará state. Nonetheless, it is most possible that this species has already been recorded on this tortoise (BARROS-BATTESTI et al., 2006). *Amblyomma sculptum* was collected on *Salvator merianae* (Duméril & Bibron, 1839) from a cerrado biome reserve in Santa Bárbara, São Paulo state**,** which is a new host record. This tick is a common species of the Cerrado biome and Atlantic Forest areas with anthropic modifications, and it prefers mammal hosts, such as, capivaras and horses (NAVA et al., 2014). It has also been recorded in birds from the cerrado Biome (LUZ et al., 2016).

Finally, three species which have high specificity for the herpetofauna were idenfitied: *Amblyomma humerale* was identified from material collected on *C. carbonarius* from Belém, Pará state. Material was examined from collections (IBSP and CNC), with material from the Central-west, North, Northeast and Southeast regions, generally associated with tortoises (*C. carbonária* or *C. denticulata*) (LABRUNA et al, 2002b). Other material was deposited in CNC, and was published with records of this species infesting lizards (*Plica plica*, *Plica umbra*, and *Kentropyx calcarata*). On the other hand, two nymphs were identified from *Bothrops moojeni* Hoge, 1966 (material deposited in the CNC), which would be a new host record. This tick species is an endemic species of South America, mainly parasitic of Testudinata (*Chelionoidis*) when adult, and immature stages can infest small vertebrates (reptiles and mammals) (LABRUNA et al., 2002b; MORAIS et al., 2017). *Amblyomma dissimile* and *Amblyomma rotundatum* were the most abundant species in field trip collections, as well as in reference collections (IBSP and CNC). These species of ticks are established in the Nearctic and Neotropical regions from northern Argentina to southern U.S.A, therefore, mapping of distribution was not performed. These species have some difficulties when attempting to separate both. Firstly, morphological diagnosis is complex due to the slightly differences between females (spurs of coxae I to IV and scutal punctations) (BARROS-BATTESTI et al., 2006; ONOFRIO et al., 2007). On the other hand, there is a large number of host records, thus it was attempted to register new hosts records based on last studies (DANTAS-TORRES et al., 2008; GUGLIELMONE; NAVA, 2010; DE ALCANTARA et al., 2018). Moreover, *A. dissimile* was collected from different field trips from the Central-west

region (*Phalotris matogrossensis* Lema, D'agostini & Cappelari, 2005; and *Chironius laurenti* Dixon, Wiest & Cei, 1993 being two new host records), Northeast region on *Rhinella jimi* (Stevaux, 2002), which is a new host record for amphibians; and from the Southeast region [(*Dipsas indica bucephala* (Shaw, 1802) and *P. nigra*, are new host records)]. It was also collected on a *Porthidium lansbergii* (Schlegel, 1841), from Barranquilla Colombia, being a new host record of a viper snake in Colombia. Furthermore, collection material examined here shows the wide distribution of this tick from Honduras, to Southeastern Brazil infesting reptiles and anuran amphibians. On the other hand, in the present study, the most abundant tick of the herpetofauna was *A. rotundatum*. This tick had the greatest number of lots deposited and it was the most collected species of this study. It was collected in recent field trips, from the central-west region, *Helicops angulatus* (Lineus, 1758), *O. melanogenys*, *Oxyrhopus guibei* Hoge & Romano, 1977, *L. annulata*, and *Dipsas turgidus* (Cope, 1868) are new host records. From the North region, *Chironius scurrulus* (Wagler, 1824); and *Philodryas viridissima* (Linnaeus, 1758), are new host records, and this is one of the first studies to collect *A. rotundatum* from the state of Acre. Recent studies from the state of Rondônia (also Amazon forest), recorded infestations of ticks in snakes, lizards and amphibians, with four species of snakes infested, including a species recorded here for *A. rotundatum* (*Chironius multiventris* Schmidt & Walker, 1943), and one infested with *A. dissimile* (ZIMMERMANN et al., 2018). Also, *A. dissimile* was identified in various species of reptiles and amphibians, including *C. scurrulus* (TORRES et al., 2018).

From the northeastern region, *Trachylepis atlantica* (Schmidt, 1945), and *R. jimi* are new host records from the Fernando de Noronha island. Finally, from the southeastern region, *Dipsas neuwiedi* (Ihering, 1911), is a new host record. Moreover, examining the material deposited in the collections, new host records were identified in the CNC collection: from the north region on *Pseustes sulphureus* (Wagler, 1824) and *Tropidurus hispidus* (Spix, 1825). These results are in accordance with former studies, on which the wide host range of both *A. dissimile* and *A. rotundatum* is highlighted, and these ticks are not just related to anura (VOLTZIT, 2007; GUGLIELMONE; NAVA, 2010). Also, both species can parasitize other vertebrates including mammals and birds (GUGLIELMONE; NAVA, 2010; SCOTT; DURDEN, 2015a). These hosts may help maintain adult populations in a given area or even help the dispersion of the ticks via migratory birds and colonize other habitats (SCOTT; DURDEN, 2015b). The host range of these two species has been widen and is supported by recent studies (DANTAS-TORRES et al., 2008;

DE ALCANTARA et al., 2018) in which the host range of *A. rotundatum* is wider than *A. dissimile*, and also it is more abundant throughout the Brazilian territory, thus parthenogenesis is not a disadvangate. Finally, these findings suggest differently from former studies, in which it was stated that *A. dissimile* has a wider host range and a wider distribution (LAMPO et al., 1997; GUGLIELMONE; NAVA, 2010). These studies also stated the strong relationship between anurans and these species of ticks and suggested it could have evolutionary relevance. Nonetheless, the wide host range of both species indicates that these species can colonize new environments and infest the different types of of hosts that inhabit them.

6 CONCLUSIONS

1. Two families, four genera and 19 species of ticks, parasites of reptiles and amphibians, were identified.
2. Of the 19 species of ticks identified, 17 occur in Brazil, with one new species of *Ornithodoros* described in Brazil, as well as new hosts records for collected species, totalizing 17 species (five Argasidae and 12 Ixodidae).
3. Four species of ticks were collected in the central-west region (Goiás and Mato Grosso states), that normally do not parasitize reptiles or amphibians. *R. sanguineus* s.l. and *D. nitens* on toads *R. schneideri* from Mato Grosso state; and *R. microplus*, and *A. nodosum* on *B. constrictor constrictor* from Goiás state.
4. Of the Argasidae family, one species was collected in field trips, *Ornithodoros* (*Alectorobius*) sp. n., and four others were examined in two collections (IBSP, CNC). The species collected in this study is the first record of an argasid tick infesting a snake (*P. nattereri*) in the southeastern region of Brazil.
5. The species *A. cajennense* was idendified on *P. nigra* (deposited in the CNC collection), which would be a new host record.
6. The species *A. sculptum* was collected on *Salvator merianae* from a cerrado biome reserve in Santa Bárbara, São Paulo state**,** being a new host record.
7. The species *A. humerale* on *B. moojeni* (deposited in the CNC), is a new host record.
8. The species *A. dissimile* and *A. rotundatum* were the most abundant species in field trip collections, as well as in reference collections (IBSP and CNC).

9. The species *A. dissimile* was collected from the Central-west region (*P. matogrossensis* and C*hironius laurenti*), the Northeast region on *Rhinella jimi* and from the Southeast region (*D. indica bucephala* and *P. nigra*), all are new host records.
10. The species *A. dissimile* was also collected on a *P. lansbergii,* from Barranquilla Colombia, being a new host record of a viper snake in Colombia.
11. Collection material examined here shows the wide distribution of this tick from Honduras, to Southeastern Brazil infesting reptiles and anuran amphibians.
12. The species *A. rotundatum* collected in recent field trips, from the central-west region, on five colubrid snakes as new host records; from the North region, on three colubrid snakes as new host records. From the southeastern region, *D. neuwiedi* is a new host record.
13. The species *A. rotundatum* from the material deposited in the collections, new host records were identified in the CNC collection: in the Central-west (anuran) and from the north region (lizard).
14. The host range of these two species has been widen and supports the wider the host range of *A. rotundatum* than *A. dissimile*, and *A. rotundatum* is more abundant throughout the Brazilian territory.

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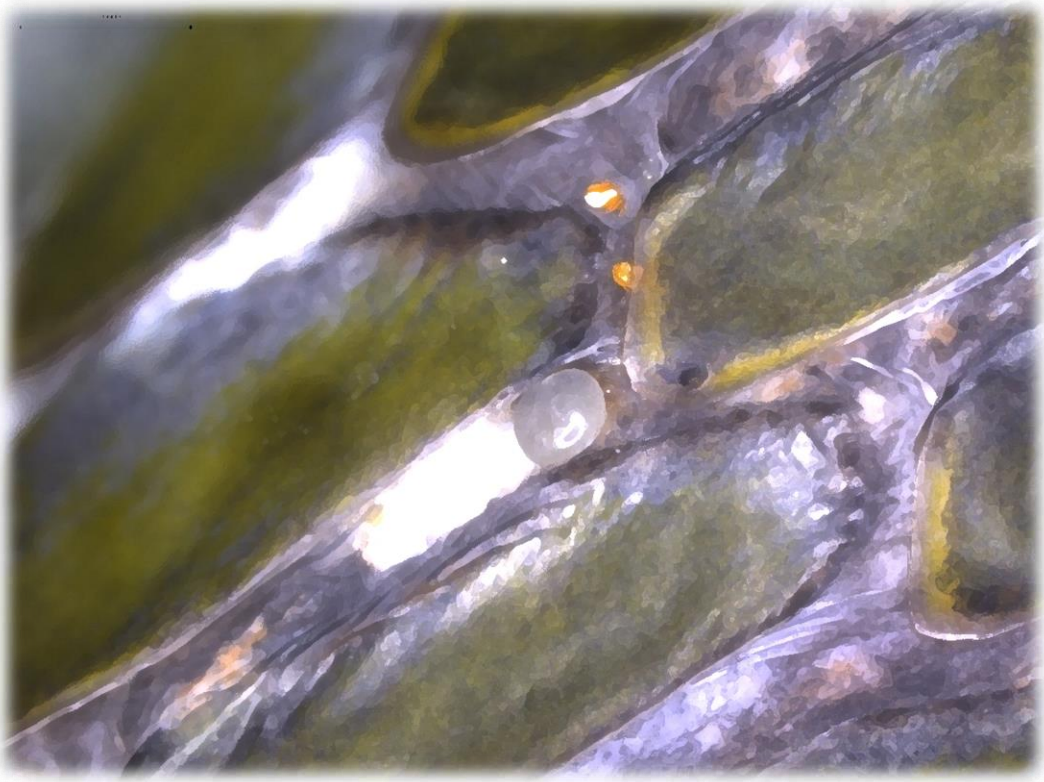


(Mendoza-Roldan, 2018)

CHAPTER IV: Host – parasite associations

1 INTRODUCTION

Reptiles and amphibians, as ectothermic or cold-blooded hosts, have unique inflammatory, immunologic and metabolic responses to a parasitic event. These responses differ from those observed in endotherms (birds and mammals) (HUEY, 1982; BOWER et al., 2018). These responses depend on the species of parasite, the number of parasites attached, the individual characteristics, and the environmental challenges (climate change) presented to the host (HARVELL et al., 2009; LÓPEZ-ALCAIDE; MACIP-RÍOS, 2011; KLINGENBERG, 2012).

Regarding mites and ticks, host-parasite relationships can be divided in parasites that are facultative (organism that does not rely on parasitic behavior to complete its life cycle, but might resource to it in specific cases), and those who are obligate parasites (temporary or permanent parasites) (MITCHELL; BAKER 2007). Cases of facultative parasitism on herpetofauna are scarce, and generally are accidental findings, of unintentional phoresy. Such is the case of *Benoinyssus najae* Fain, 1958 mite, described in the nasal area of *Naja melanoleuca* Hallowell, 1857 (Squamata: Elapidae), and later it was confirmed that in fact these mites are free-living on soil and, given the chance can resource to parasitic activities (FAIN, 1958; OLIVIER et al., 1997). Obligate Acari parasites of reptiles and amphibians are divided in temporary parasites (ecto and endoparasites), and permanent parasites (ecto and endoparasites) (FAJFER, 2012).

Temporary parasites are those who develop one or more stages on the host, but not necessarily, remain all their life on the host. These include the Ixodida order (ticks), with some exceptions of Argasidae species that develop their whole life cycle on the host (*Argas* (*Microargas*) *transversus* Banks, 1902 from Galapagos giant tortoises (*Chelonoidis nigra*) (HOOGSTRAAL et al., 1973). Furthermore, some families of the Mesostigmata order are also considered temporary parasites (Macronyssidae, and Heterozerconidae); and from the Trombidiformes order, the Parasitengona cohort mites are only parasitic in their larval stages (Trombiculoidea). All these parasites have a low host specificity, thus the response to the parasitism of the ectothermic hosts can be in general exacerbated, as parasites are not fully adapted to these kind of host (ALEKSEEV,1998; HARKEWICZ, 2001).

Permanent parasites are species of Acari that are fully adapted and have a very dependent relationship with their host. These adaptations can be observed in the mite morphology, as species adapt more and more, their bodies modify to better adjust to their host's anatomy (BERTRAND, 2002; BERTRAND, 2004; BOCHKOV et al., 2017). Permanent mite parasites of reptiles and amphibians include Trombidiformes mites of the superfamily Cheyletoidea (Cloacaridae, Harpirhynchidae), the superfamily Pterygosomatoidea (Pterygosomatidae), and the Superfamily Tydeoidea (Ereynetidae). All of these families of mites have an ancestral parasitic origin, which means most of them do not cause a deleterious effect on the host, when the intensity of the infestations is low (BOCHKOV, 2009; FAJFER et al., 2014; ŠLAPETA et al., 2017). Also, permanent mites include some families of the Mesostigmata order (Entonyssidae, Ixodorhynchidae and Omentolalepidae), all parasitic of snakes (FAIN, 1962a; FAIN, 1962b; FAJFER et al., 2012). Differently from Trombidiformes mites, the permanent parasitism of some the Mesostigmata mites can be deleterious to the host, given the hematophagous behavior and the areas in which these mites fixate (connective tissue, lungs) (RADOVSKY, 1994; CERVONE et al., 2016).

The negative effect of mites and ticks on the hosts fitness can be divided in the direct effect on the host health status, and the indirect effect, given by the vectoral capacity of the parasite to transmit pathogens. The direct effect generally results in anemia and, dehydration and emaciation of the host, when presented with hyper-infestation. Skin lesions are also common at the attachment site as edema, inflammation and erythema. Also, infestations lead to behavioral changes of the host (WOZNIAK; DENARDO, 2000; FAJFER, 2012). In reptiles, ectoparasite infestation promotes the ecdysis process, resulting in early molting, and when hyper-infested, the hosts can suffer from Dysecdysis (MENDOZA-ROLDAN, et al., 2019). In amphibians, tick infestation consequences are like that from reptiles, and as most of the mites have skin-dwelling behavior (endoparasites), the capsule in which they develop promotes a granulomatous injury and deformation, which can lead to avascular necrosis and limb loss. In all cases hyper infestations affect negatively the health status of the ectothermic host which can result in death of the host (RODRIGUES et al., 2018).

1.1 Host specificity and preferred areas on host

Some families of Acari are restricted to a particular host order. For example, most of the Mesostigmata mites are specific of snakes (FAIN, 1962b). Thus, permanent parasites have a higher host specificity (Ereynetidae, Pterygosomatidae, Harpirhynchidae, and Cloacaridae from the Trombidiformes order; and Entonyssidae, Omentolaelapidae, and Ixodorhynchidae from the Mesostigmata order). Of these, Ixodorhynchidae is the least host specific and Cloacaridae the most (FAJFER, 2012).

Permanent and temporary mites and ticks can colonize different areas of the host body (called parasitic niches), and the higher the host specificity the narrower the niche is. For example, Cloacaridae mites are restricted to cloaca of Testudinata (DOWLING, 2016). In reptilian hosts, most parasites attach on the connective tissue underneath the scales. In the amphibian host, parasites occur in areas of less exposure, or even inside the connective tissue of the skin (DÍAZ-PÁEZ et al., 2016). Mites and ticks with convergent evolution, can live completely under the scales of their reptile hosts (Omentolalepidae and some species of Pterygosomatidae, and Argasidae ticks) (FAIN, 1969; 1994), or in some families they hide in unexposed areas like the arm pits, the lateral anterior area, gular area, and the head (Ixodorhynchisae, Heretozeronidae, and Ixodidae). On the other hand, some families resource to penetrate the skin (Harpirhynchidae in snakes and Leeuwenhoekiidae in amphibians) (CHILTON et al., 1992a; SILVA-DE LA FUENTE et al., 2016).

Preferred niches depend on the ability and size of the mite or tick. Large parasites (Ixodidae and Macronyssidae) choose areas that are unreachable after producing pruritus, such as, the head, nasal area, the axillae, joints, toes and cloaca. In snakes they can attach on the gular area, eyes, and the anterior lateral portion (CHILTON et al., 1992b; BANNERT et al., 2000). Smaller mites (Trombiculidae, Pterygosomatidae, and smaller Macronyssidae) can also attach to the beforementiones niches, and some species of lizards have developed structures called mite-pockets, which are skin folds that have a vast lymphal irrigation and promotes parasitic aggregation (BERTRAND, 2002; 2004).

Finally, Endoparasitic mite species are adapted to the respiratory system of their hosts, from the upper tract (Ereynetidae, in amphibians), to lower portions, such as lungs and air sacs

(Entonyssidae and Trombiculidae). Intradermal mites can also be considered endoparasitic, as well as cloacal mites (CAMIN et al., 1967; FAIN; YUNKER, 1972; NADCHATRAM, 2006).

1.2 Infestation rates

The infestation rates can be measured in prevalence (number of hosts in a population who have are infested, usually expressed as a percentage of the population), mean intensity (number of parasites found in infected hosts in a particular population), and mean abundance (number of parasites found in all the hosts in a particular population) (RÓZSA et al., 2000; REICZIGEL; RÓZSA, 2005). Generally, permanent parasites have a low prevalence. Some studies from museum material showed a prevalence of 2 to 3% (of 2180 snakes) (FAIN, 1961; 1962a). On the other hand, temporary parasites have high and varied infestation rates, with some studies on Tombiculidae and Pterygosomatidae having 100% of the population infested (DELFINO et al., 2011).

Studies where permanent and temporary parasites were assessed together, also showed moderate to high infestation rates. In Brazil, analyzing non-venomous snakes from the State of Sao Paulo, showed prevalence of 13 to 16% (Ixodidae, Mesostigmata, Trombiculidae) (LIZASO, 1982). Moreover, a recent study in Southern Italy with captive and wild reptiles, showed a prevalence of 82% of 211 examined reptiles infested with Ixodidae, Macronyssidae, Ptergosomatidae and Trombiculidae (MENDOZA-ROLDAN et al., 2019). Thus, examining host on their natural habitat or in captive conditions, would allow to find a higher number of parasites. Some species of Mesostigmata and Trombidiformes mites have only been found once, when described, making it unclear of the status of these species and if they can be considered endangered or even extinct. Furthermore, some species of ticks that were described on vulnerable or critically endangered reptilian hosts, are also considered endangered (MILLER, et al., 2011; MIHALCA, et al., 2011)

1.3 Effects of parasitim

The negative effect of mites and ticks on the hosts fitness can be divided in the direct effect on the host health status, and the indirect effect, given by the vectoral capacity of the parasite to

transmit pathogens (chapter 6). The direct effect generally results in anemia and, dehydration and emaciation of the host, when presented with hyper-infestation. Skin lesions are also common at the attachment site as edema, inflammation and erythema (WOZNIAK; DENARDO, 2000; FAJFER, 2012).

Additionally, in wild populations, the negative effect on hosts seems to be very low, as hosts are adapted to high parasitic load, without metabolic costs, minimum tissue damage, and overall fair population health status (HANLEY et al., 1995; MORITZ et al., 2001). On the other hand, in captive animal, the negative effect of parasitism in the host fitness is very noticeable, as animals cannot avoid being infested and captive conditions allow parasites to thrive. Infested reptiles can have behavioral changes and become irritated, aggressive and with intense pruritus, spending long periods inside water (WOZNIAK et al, 2000; MENDOZA-ROLDAN, et al., 2019; MENDOZA-ROLDAN; COLELLA, 2019).

The indirect effect is related to the parasite`s competence and capacity as a vector of pathogens (MORO et al., 2005). The pathogeny and development of diseases in ectothermic animals varies from that of the most commonly pathogenic patterns studied in mammals. Furthermore, reptiles and amphibians harbor a wide range of pathogens, which these animals play a role as natural reservoirs and amplifiers of microorganisms, that can be transmitted to other reptiles and in some cases even humans (FLAJNIK, 1996; OSTFELD; HOLT, 2004).

2 OBJECTIVES

- Asses the host-parasite relations and the impact of the parasitic load through the infestation rates of the different species of mites and ticks related to their hosts;
- Calculate the prevalence index (PI), mean intensity (MI) and mean abundance (MA), of the different species of mites and ticks related to their hosts;
- Assess parasitic niches of the different species of mites and ticks related to their hosts;
- Evaluate lesions produced by the Acari through hematological and histological studies, to describe the impact of the different species of mites and ticks on their hosts.

3 MATERIAL AND METHODS

3.1 Mites and tick's material

The mites and ticks species of the orders Trombidiformes, Mesostigmata, and Ixodida that infest reptiles and amphibians that were collected, identified, and evaluated, came from two places: mites and ticks that were brought upon their hosts to the different laboratories of the Instituto Butantan, or to the Venomous Animals Reception site of the same institute; and material that was collected from reptiles and amphibians in different field trips at various locations in Brazil. New or fresh material of mites and hosts were used for molecular biology studies (Part II of this thesis).

3.1.1 Laboratories of the Instituto Butantan (IBSP)

3.1.1.1 Venomous Animals Reception site of the Herpetological collection of the Special Zoological Collections Laboratory (LECZ)

The Special Zoological Collections Laboratory (LECZ) of the Instituto Butantan, has a Venomous Animals Reception site, which receives snakes, amphibians, spiders, scorpions, Acari (mites and ticks), insects, among other animals, that come from varied localities of Brazil and from other countries. Reptiles and amphibians are then routed to the laboratories from the Instituto Butantan (Herpetology, Cellular Biology, Biological Museum, Ecology and Evolution, among others). Spiders and scorpions are routed to the Arthropods Laboratory, and Acari are deposited in the Acarological collection of the LECZ. Venomous animals (vertebrates and invertebrates) are used firs for venom extraction and in some cases reproduction. When these animals die the are deposited in the collections of the LECZ, which has five collections (Herpetology, Arachnids, Acarology e Entomology and, Myriapoda).

Mites and ticks from reptiles and amphibians that arrived from different regions of Brazil, herein studied, were collected whenever possible before being sent to the different laboratories or collections.

3.1.1.2 Other laboratories of the Instituto Butantan

To assess infestation in captivity conditions, the laboratories that harbor live reptiles and amphibians for different purposes in the Instituto Butantan, were visited and the animals were examined for mites and ticks. Laboratories visited were: Cellular Biology, Ecology and Evolution, and the Biological Museum.

3.1.1.3 Material collected in field trips

Mites and ticks were collected from reptiles and amphibians in different field trips at various locations in Brazil. The listed field trips are from projects this study collaborated in fieldwork, or material that was revised from the hosts. The projects also comprise three biomes. The projects for each area (Atlantic forest, Amazon rainforest, and Cerrado) are presented with details in Chapter I (pages 101-103 of this Thesis).

3.2 Collection of mites and ticks from reptiles and amphibians

Collection methods of mites and ticks of examined reptiles are discussed in chapters 1 to 3. All animals were visually examined, some under stereo microscope, and a complete physical exam from the cranial portion to the caudal (posterior) portion was held for each animal.

Identification of hosts (reptile and amphibians) used in this study, was performed by the team of herpetologists of the Herpetological collection of the Special Zoological Collections Laboratory (LECZ) of the Instituto Butantan (LECZ). The host nomenclature was updated by consulting the "Reptile Database" (http://www.reptile-database.org) (UETZ, 2010) as well as the database of the Brazilian Society of Herpetology (Sociedade Brasileira de Herpetologia - SBH), for reptiles (COSTA; BÉRNILS, 2018).

3.3 Infestation rates

To assess the parasitic load of mites and ticks, descriptive statistics was calculated using Quantitative Parasitology software, version 3.0 (RÓZSA et al., 2000). Prevalence (PI), mean

abundance (MA) (number of Acari per total number of hosts) and mean intensity (MI) (number of Acari per number of infested hosts) of infestation were determined.

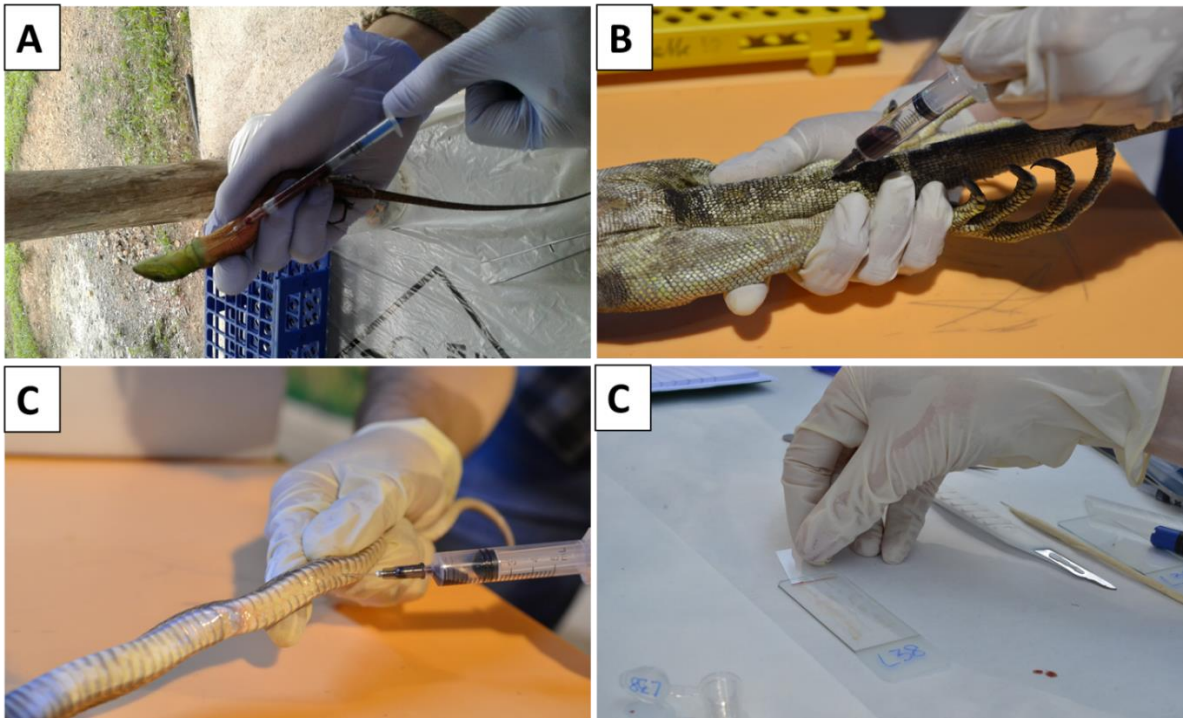
Whenever possible, parasitic niches or microhabitats were assessed and number of parasites per area were quantified. Each host had different niches or microhabitats that are named and detailed in the results.

3.4 Reptile and amphibian sample collection

Whenever possible, blood samples were obtained from reptiles. In lizards and small snakes, a small amount of blood was obtained by cardiocentesis, when animals were adults and non-gravid females (Figure 90A). In larger reptiles, such as snakes and larger Sauria, blood samples were obtained from the ventral coccygeal vein. All samples were stored at -20 °C (Figure 90B, C) (MENDOZA-ROLDAN et al., 2019). Part of the samples was used for molecular studies (Chapter 6), and part was used to perform blood smears for evaluation of hemoparasites and hematological examination (Figure 90D) (POINAR; Telford, 2009 NARDINI et al., 2013). Blood smears were prepared using Diff-Quik stain (commercial Romanowsky stain variant, based on a modification of the Wright Giemsa stain). The protocol used was as follows: smears we fixated in "Diff Quick" Fixative for 30 seconds, then stained with "Diff Quick" solution II for 30 seconds, finally counterstained with "Diff Quick" solution III for 30 seconds. Smears were rinsed in tap water to remove excess stain, and later evaluated in optical microscope (LEICA DM 400B microscope) (SKIPPER; DESTEPHANO, 1989).

When animals were euthanized or brought dead to the laboratories, skin tissue samples where mites and ticks were attached, were collected. These samples were fixated in formalin 10%, and later processed into histological slides in the "Laboratório de Patologia Comparada de Animais Silvestres – LAPCOM/ FMVZ/ USP" (samples processed by PhD candidate Pedro Navas). Lesions were categorized according to herpetological medicine protocols (WRIGHT, 2001; JACOBSON, 2007; DIVERS; MADER, 2005).

Figure 90 – Blood collection techniques and blood smear peparation



Source: (MENDOZA-ROLDAN, J. A., 2019).

Legend: A. Blood draw by cardiocentesis in *Kentropyx calcarata*; B, C. Blood draw from ventral coccygeal vein in *Iguana iguana* and *Pantherophis guttatus*; D. blood smear preparation

4 RESULTS

4.1 Examined hosts

From august 2015 to December 2018, a total of 4,515 animals were examined in the laboratories of the Instituto Butantan (IBSP) and from recent field trips. Of these, 3,596 were reptiles from the orders Squamata (subordens Amphisbaenia, Serpentes, and Sauria), Crocodylia and Testudinata. The other 919 animals were amphibians (Anura and Gymnophiona). Of the reptiles from the order Squamata, 51 individuals of two genera and four species of Amphisbaenia were revised. In total 3,104 specimens from 32 genera and 60 species of Serpentes suborder were examined; and 246 individuals of 20 genera distributed in 23 species of Sauria were examined. Three specimens of one species of one genus of Crocodylia order were examined. Furthermore, of

the order Testudinata, 195 specimens of four genera and nine species were examined (Table 25). Amphibians from the orders Anura and Gymnophiona were examined, and 919 specimens distributed in 13 genera and 22 species of amphibians were assessed for mites and ticks (Table 26).

For lung mite studies (Entonyssidae family parasitic of snakes), six species were examined. Animals deposited in the IBPS herpetological collection were dissected (trachea to air sacs), totalizing 650 examined animals (information of examined species is detailed in bold in Table 25).

4.1.1 Infested hosts

In total 4,515 individuals were examined, of which 170 were infested with mites and ticks (overall PI of 3.8%; 95% CI: 3.2–4.3%). These infested hosts were distributed as follows: 121 infested reptiles of 3596 (3.4%; 95% CI: 2.7 - 4%), and 49 infested amphibians of 919 examined (5.3%; 95% CI: 3.9- 6.9%). From the reptiles infested, 46 of 3,104 were infested snakes (1.5%; 95% CI: 1- 1.9%), 72 of 246 lizards were infested (29.3%; 95% CI: 23.6- 35.3%), and 3 of 195 turtles and tortoises were infested (1.5%; 95% CI: 0.3- 4.4%). Moreover, the 5.3% of infested amphibians were all anurans (Tables 26 and 27).

The prevalence of the different orders of mites and ticks in reptiles was as follows: of the 121 reptiles infested, Trombidiformes mites on Serpentes suborder were identified in 7 (5.7%) snakes, and 67 (55.3%) lizards. Mesostigmata mites on snakes were identified in 5 (4.3%) snake, and 3 (2.4%) lizards; Ixodida ticks were identified on 30 (24.7%) snakes, 2 (1.6%) lizards, and 3 turtles (2.4%). Also, co-infestations were observed on snakes, with four co-infestations recorded (one Trombidiformes with Mesostigmata, one Trombidiformes with Ixodida, one Mesostigmata with Ixodida, and one Trombidiformes, Mesostigmata and Ixodida). Additionally, the prevalence of the different orders of mites and ticks in amphibians was as follows: of 49 infested anurans, Trombidiformes mites were identified on 17 (34.6%) specimens, Ixodida ticks were identified on 31 (63.2%) individuals, and one (2%) specimen was infested with Oribatida mites. No Mesostigmata mites were identified on amphibians, and no co-infestations were observed (Tables 26 and 27).

Table 26– Species of reptiles examined and infested

Order (suborder)	Host species	No. Examined	No. Infested
Squamata (Amphisbaenia)	*Amphisbaena alba* Linnaeus, 1758	5	0
	Amphisbaena dubia Müller, 192	12	0
	Amphisbaena mertensii Strauch, 1881	14	0
	Leposternon microcephalum (Wagler, 1824)	20	0
Squamata (Serpentes)	*Apostolepis assimilis* (Reinhardt, 1861)	23	0
	Atractus pantosticus Fernandes & Puorto1993	11	0
	Atractus crassicaudatus (Duméril, Bibron & Duméril, 1854)	7	0
	Atractus guentheri (Wucherer, 1861*)*	9	1I
	Boa constrictor amarali Stull, 1932	2	0
	***Boa constrictor constrictor* Linnaeus, 1758**	60 **(50)**	0
	Bothrops alternatus (Duméril, Bibron & Duméril, 1854)	40	1I
	Bothrops atrox Linnaeus, 1758	1	I1
	Bothrops diporus Cope, 1862	50	0
	Bothrops insularis Amaral, 1922	5	5I
	***Bothrops jararaca* Wied, 1824**	456 **(150)**	1T, 1I
	Bothrops jararacussu Lacerda, 1884	50	2I
	Bothrops leucurus Wagler, 1824	10	1I
	Bothrops moojeni Hoge, 1966	10	0
	Chironius bicarinatus Hollis, 2006	68	1T
	Chironius brazili Hamdan & Fernandes 2015	2	0
	Chironius exoletus Linnaeus, 1758	23	0
	Chironius laurenti Dixon, Wiest & Cei 1993	5	1I
	Chironius multiventris Schmidt & Walker, 1943	5	1TMI, 1I
	Chironius scurrulus Wagler, 1824	1	1TM
	Crotalus durissus collilineatus (Amaral, 1926)	13	0
	Crotalus durissus terrificus* Laurenti, *1768	580 **(150)**	1M, 4I
	Corallus hortulanus (Linnaeus, 1758)	10	1M, 3I
	Dipsas bucephala (SHAW, 1802)	11	0
	Dipsas indica Laurenti, 1768	30	1I
	Dipsas mikanii (Schlegel, 1837)	100	0
	Dipsas neuwiedi (Ihering, 1911)	97	1I
	Dipsas turgidus (Cope, 1868)	1	1I
	Drymoluber brazili (Gomes, 1918)	5	1T
	Epicrates cenchria Linnaeus, 1758	11	1I
	***Erythrolamprus aesculapii* (Linnaeus 1758)**	256 **(150)**	0
	***Erythrolamprus miliaris* Linnaeus, 1758**	133 **(100)**	0
	Erythrolamprus poecilogyrus Wied-Neuwied 1825	20	0
	Erythrolamprus typhlus (Linnaeus, 1758)	10	1T

(Continues)

Order (suborder)	Host species	No. Examined	No. Infested
Squamata (Serpentes)	*Helicops carinicaudus* Pontes et al., 2008	23	0
	Lampropeltis getula (Linnaeus, 1766)	8	0
	Leptodeira annulata (Linnaeus, 1758)	16	1I
	Micrurus corallinus Merrem 1820	6	0
	Micrurus surinamensis (Cuvier, 1817)	1	0
	Oxyrhopus clathratus Duméril, Bibron & Duméril, 1854	8	0
	Oxyrhopus guibei Hoge & Romano, 1977	198	0
	Oxyrhopus trigeminus Duméril, Bibron & Duméril, 1854	15	1I
	Oxyrhopus melanogenys (Tschudi, 1845)	8	1MI, 2I
	Pantherophis gutatus Linnaeus, 1766	150	0
	Phalotris matogrossensis Lema, D'agostini & Cappelari, 2005	15	1I
	Phalotris mertensi (Hoge, 1955)	9	0
	Philodryas nattereri Steindachner, 1870	1	1TI
	Philodryas olfersii Lichtenstein, 1823	33	0
	Philodryas patagoniensis Grazziotin et al., 2012	46	0
	Philodryas viridissima (Linnaeus, 1758)	1	1I
	Porthidium lansbergii (Schlegel, 1841)		1I
	Psomophis joberti (Sauvage, 1884)	11	0
	Pseudoboa nigra (Duméril, Bibron & Duméril, 1854)	32	1M, 2I
	Python bivittatus Kuhl, 1820	2	0
	Python molurus (Linnaeus, 1758)	4	0
	Python reticulatus (Schneider, 1801)	2	0
	Simophis rhinostoma (Schlegel, 1837)	3	0
	***Spilotes pullatus* Linnaeus, 1758**	69 **(50)**	1T
	Thamnodynastes strigatus Gunther,1858	12	0
	Tomodon dorsatus Starace, 1998	178	0
	Tropidodryas striaticeps Vrcibradic et al., 2011	2	0
	Xenodon merremi Boulenger, 1894	73	1I
	Xenodon neuwiedii Günther, 1863	60	2M
Squamata (Sauria)	*Anolis meridionalis* Boettger, 1885	6	1T
	Arthrosaura reticulata (O'shaughnessy, 1881)	16	1T
	Aspronema dorsivittatum (COPE, 1862)	4	2T
	Cercosaura eigenmanni (Griffin, 1917)	2	1T
	Chatogekko amazonicus (Andersson, 1918)	6	0
	Colobodactylus taunayi Amaral, 1933	3	0
	Copeoglossum nigropunctatum (Spix, 1825)	15	4T

(Conclusion)

Order (suborder)	Host species	No. Examined	No. Infested
Squamata (Sauria)	*Enyalius iheringii* Boulenger 1885	30	11T, 1M
	Gymnodactylus geckoides Spix, 1825	11	8T
	Hemidactylus mabouia Moreau De Jonnès 1818	16	10T
	Kentropyx calcarata Spix, 1825	30	4T
	Ophiodes fragilis Raddi,1820	29	0
	Placosoma glabellum Peters 1870	1	0
	Pogona vitticeps Ahl, 1926	2	2M
	Phyllopezus pollicaris (Spix, 1825)	15	10T
	Psychosaura macrorhyncha (Hoge 1946)	1	1T
	Thecadactylus rapicauda (Houttuyn, 1782)	4	2T
	Tropidurus montanus Rodrigues, 1987	6	1T
	Salvator merianae (Duméril & Bibron, 1839)	35	1I
	Trachylepis atlantica (Schmidt, 1945)	2	1T, 1I
	Tropidurus catalanensis Gudynas & Skuk, 1983	8	8T
	Tropidurus itambere Rodrigues, 1987	3	1T
	Tropidurus torquatus (Wied-Neuwied, 1820)	1	1T
Crocodylia	*Caiman latirostris* Daudin, 1802	3	0
Testudinata	*Chelonoidis carbonarius* (Spix, 1824)	45	3I
	Chelonoidis denticulatus (Linnaeus, 1766)	11	0
	Chelydra acutirostris Peters, 1862	3	0
	Chelydra serpentina (Linnaeus, 1758)	10	0
	Hydromedusa tectifera Cope, 1869	16	0
	Phrynops geoffroanus (Schweigger, 1812)	6	0
	Trachemys dorbigni (Duméril & Bibron, 1835)	23	0
	Trachemys scripta (Thunberg & Schoepff, 1792)	56	0
	Trachemys scripta elegans (Wied 1838)	25	0
	Total	3596 **(650)**	121 (74T, 8M, 35I) 1TM, 1TI, 1MI, 1TMI

Source: (MENDOZA-ROLDAN, J. A., 2019)

Legend: animals examined internally, and quantity examined are highlighted in bold. Abbreviations: T (Trombidiformes), M (Mesostigmata), I (Ixodida). Co-infestations are shown as: TM, TI, MI, TMI.

Table 27 – Species of amphibians examined and infested

Order	Host species	No. Examined	No. Infested
	Adelphobates galactonotus (Steindachner, 1864)	5	0
	Cycloramphus boraceiensis Heyer, 1983	2	1T
	Cycloramphus dubius (Miranda-Ribeiro, 1920)	3	1T
	Corythomantis greeningi		1T
	Fritziana fissilis Miranda-Ribeiro, 1920	6	0
	Hylodes lateristrigatus Myers, 1962	1	0
	Hypsiboas polytaenius Faivovich, 2005	3	0
Anura	*Leptodactylus latrans* Steffen, 1815	12	4T
	Melanophryniscus admirabilis Di-Bernardo, Maneyro & Grillo, 2006	7	7T
	Phylomedusa distincta Heyer, 1978	1	0
	Phyllomedusa iheringii Boulenger, 1885	2	1T
	Physalaemus spiniger Miranda-Ribeiro, 1926	10	0
	Physalaemus centralis Bokermann, 1962	89	0
	Physalaemus cuvieri Fitzinger, 1826	76	0
	Physalaemus nattereri (Steindachner, 1863)	156	0
	Rhinella crucifer Wied-Neuwied, 1821	43	5I
	Rhinella granulosa Spix, 1824	33	2I
	Rhinella icterica Spix, 1824	67	1I
	Rhinella jimi Stevaux, 2002	56	6I
	Rhinella major (Müller & Helmich, 1936)	16	1O
Anura	*Rhinella marina* Linnaeus, 1758	56	3I
	Rhinella ornata Spix, 1824	87	0
	Rhinella sncheideri (Werner, 1894)	164	14I
	Scinax duartei Duellman & Wiens, 1992	20	0
	Scinax squalirostris Lutz, 1925	1	1T
	Thoropa megatympanum Caramaschi & Sazima, 1984	3	1T
Gymnophiona	*Siphonops annulatus* Mikan, 1820	5	0
Total		919	49 (17T, 31I, 1O)

Source: (MENDOZA-ROLDAN, J. A., 2019)

Legend: Abbreviations: T (Trombidiformes), I (Ixodida), O (Oribatida).

4.2 Infestation rates

Mites of the order Trombidiformes were represented by 13 species, being the most abundant *E. alfreddugesi* (n=510), infesting 33 individuals of 16 species (5 species of 5 genera of

snakes, 25 individuals of 10 genera of lizards, 1 amphibian). The other of species of Trombidiformes mites, identified in the laboratories of the IBSP and field trips, were: *O. parkeri* (n=45, on one species of snake), *O. ekans* (n=2, on one species of snake), *G. hemidactyli* (n=80, on 10 specimens of one species of lizard), *G. bataviensi* (n=2=35, on one species of lizard), *G. harrisi* (n=384, on 9 specimens of two species of lizards), *B. jimenezi* (n=74, 18 on 18 specimens of two species of lizards), *H. achalai* (n=46, on 13 individuals of three species of anurans), *H. hepatica* (n=19, on four species of anurans), *E. ophidica* (n=46, on three specimens of two species of lizards), *E. tropica* (n=39, on one speciesof lizard), and *F. anguina* (n=30, on one species of snake). *A. longisetosus*, a species of Oribatida mite was found possibly infesting on species of anuran (n=87) (Table 28).

Furthermore, four species mites of the order Mesostigmata were only found in lizards or snakes. The species identified were: *Chironobius* sp. n. (n=4, on one species of snake), *O. rotundus* (n=43, on one species of snake), *O. natricis* (n=18, on five individuals of two species of snakes and two species of lizards), and *Z. oudemansi* (n=5, on two species of snakes) (Table 29).

Finally, six species of ticks were identified on the examined hosts. The most abundant species was A. *rotundatum* (n=172, on 52 specimens of 18 species of snakes, one species of lizards, and six species of anurans). The other species of ticks were: *Ornithodoros (Alectorobius)* sp. (n=7, on one species of snake), *A. dissimile* (n=40, on eight specimens of six species of snakes and one species of anurans), *A. nodosum* (n=8, on one species of snake), and *A. sculptum* (n=1, on one species of lizards) (Table 30).

Table 28 – Species of hosts and number of infesting Trombidiformes mites

Class	Host	*O. parkeri*	*O. ekans*	*G. hemidactyli*	*G. bataviensis*	*G. harrisi*	*B. jimenezi*	*H. achalai*	*H. hepatica*	*E. alfreddugesi*	*E. ophidica*	*E. tropica*	*F. anguina*	*A. longisetosus*
Serpentes	*Bothrops jararaca*		2											
	Chironius bicarinatus	45												
	Chironius multiventris									10				
	Chironius scurrulus									20				
	Erythrolamprus typhlus												30	
	Drymoluber brazili									6				
	Philodryas nattereri									8				
	Spilotes pullatus									4				
Sauria	*Anolis meridionalis*									1				
	Arthrosaura reticulata									10				
	Aspronema dorsivittatum									10				
	Cercosauria eigenmani									2				
	Copeoglossum nigropunctatum									60				
	Enyalius iheringii									120				
	Gymnodactylus geckoides						30							
	Hemidactylus mabouia			80										
	Kentropyx calcarata									230	35			
	Phyllopezus pollicaris						44							
	Psychosaura macrorhyncha											39		
	Thecadactylus rapicauda				35					20				
Sauria	*Trachylepis atlantica*									1				
	Tropidurus catalanensis					350								
	Tropidurus itambere									6				
	Tropidurus montanus										11			
	Tropidurus torquatus					34								
Anura	*Cycloramphus boraceiensis*								1					
	Corythomantis greeningi								10					
	Cycloramphus dubius								3					
	Leptodactylus latrans							20						

(Conclusion)

Class	Host	*O. parkeri*	*G. hemidactyli*	*G. bataviensis*	*G. harrisi*	*B. jimenezi*	*H. achalai*	*H. hepatica*	*E. alfreddugesi*	*E. ophidica*	*E. tropica*	*F. anguina*	*A. longisetosus*
	Melanophryniscus admirabilis						23						
	Phyllomedusa iheringii								2				
	Rhinella major												87
	Scinax squalirostris						3						
	Thoropa megatympanum							5					
	Total mites	45	2	80	35	384	74	46	19	510	46	39	30	87

Source: (MENDOZA-ROLDAN, J. A., 2019).

Table 29 – Species of hosts and number of infesting Mesostigmata mites

Class	Host	*Chironobius* sp. n.	*O. rotundus*	*O. natricis*	*Z. oudemansi*
Serpentes	*Chironius multiventris*	4			
	Corallus hortullanus			3	
	Crotalus durissus terrificus			4	
	Oxyrhopus melanogenys				2
	Pseudoboa nigra				3
	Xenodon neuwiedii		43		
Sauria	*Enyalius iheringii*			3	
	Pogona vitticeps			8	
Total mites		4	43	18	5

Source: (MENDOZA-ROLDAN, J. A., 2019).

Table 30– Species of hosts and number of infesting Ixodida ticks

Class	Host	*Ornithodoros (Alectorobius) sp.*	*A. dissimile*	*A. humerale*	*A. nodosum*	*A. rotundatum*	*A. sculptum*
Serpentes	*Philodryas nattereri*	7L					
	Bothrops atrox					3N	
	Bothrops leucurus					3N	
	Bothrops jararaca					1N	
	Bothrops jararacussu					2N, 6F	
	Bothrops alternatus					2N, 1F	
	Bothrops insularis					4N, 21F	
	Porthidium lansbergii		1M				
	Phalotris matogrossensis		1F				
	Crotalus durissus terrificus		1N			11N	
	Chironius laurenti		1N,1F				
	Chironius multiventris					5L, 9N, 2F	
	Chironius scurrulus					3N, 1F	
	Boa constrictor constrictor				4F, 4M		
	Xenodon merremii					1F	
	Dipsas indica bucephala		1N, 1F				
	Pseudoboa nigra		4F,4 M				
	Oxyrhopus melanogenys					7N	
	Oxyrhopus trigeminus					3N	
	Leptodeira annulata					1N, 3F	
	Dipsas turgidus					2N, 1F	
	Dipsas neuwiedi					4N, 1F	
	Corallus hortulanus					7N, 2F	
	Philodryas viridissima					1N	
	Epicrates cenchria					1F	
Sauria	*Trachylepis atlantica*					1N	
	Salvator merianae						1M
Testudinata	*Chelonoidis carbonaria*			4F,4 M			
	Rhinella crucifer					3N	
	Rhinella granulosa					1N	

Class	Host	*Ornithodoros (Alectorobius) sp.*	*A. dissimile*	*A. humerale*	*A. nodosum*	*A. rotundatum*	*A. sculptum*
Anura	*Rhinella icterica*					5L	
	Rhinella schneideri					2F	
	Rhinella marina					1N	
	Rhinella jimi		20F,8M			44N, 26F	
	Total ticks		3N,27F, 11M	4F,4 M	4F,4M	10L,113 N, 49F	1M

Source: (MENDOZA-ROLDAN, J. A., 2019).

Legend: Abbreviations: L (larvae), N (Nymphs), F (female), M (male).

Considering each order, and species of mite and tick, prevalence index (PI), mean intensity (MI), and mean abundance (MA) were calculated for species that infested more than one specimen of host, that more than one individual of the same species was examined (Table 30). Moreover, of the Trombidiformes order, the highest infestation rates were from the family Pterygosomatidae, with *G. harrisi* being the most prevalent considering the examined/infested hosts. Furthermore, considering its host range, *E. alfreddugesi* had the highest infestation rates of the Trombiculidae family, with moderate intensity in the infested hosts. The species of the Harpirhynchidae family were the least prevalent of all the Trombidiformes. In general, Mesostigmata mites had the lowest infestation rates of all the Acari orders examined (MA of 0.13 to 0.8). Finally, the Ixodida order showed low infestation rates considering the species of reptiles and amphibians examined (most species had a high examined number vs infested host) (Table 31).

4.3 Parasitic niches and preferred locations

Parasitic niches, or microhabitats, were assessed and counted in mite and tick species where number of hosts examined made it possible (generally more than one infested host that was

examined and parasitic niches described). When it was not possible to describe the parasitic niche, preferred location of ectoparasites on the host was recorded.

Table 31 – Infestation rates of mite and tick species

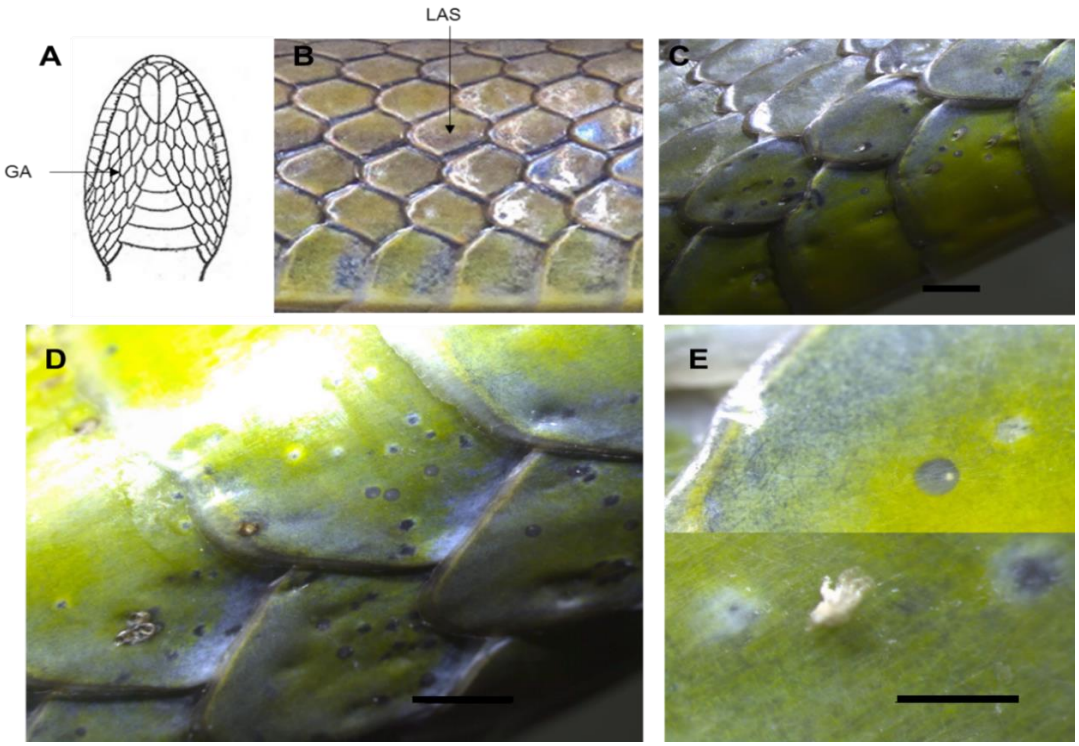
	Species	PI	MI	MA
Trombidiformes	***O. parkeri***	1.5%	-	0.66
	O. ekans	0.2%	-	-
	G. hemidactyli	62.5 %	8.1	5.06
	G. bataviensis	25%	-	8.7
	G. harrisi	100 %.	42.8	42.8
	B. jimenezi	69.2%	4.4	3.08
	H. achalai	65%	3.62	2.35
	H. hepatica	44.4%	4.5	2
	E. alfreddugesi	19.9 %	15.4	3
	F. anguina	10%	-	3.9
	E. ophidica	8.3%	15.6	1.3
Mesostigmata	***Chironobius* sp. n.**	20%	-	0.8
	O. rotundus	3.3%	16.5	0.55
	O. natricis	6%	3.6	0.22
	Z. oudemansi	5%	2.5	0.13
Ixodida	***A. dissimile***	5%	1	0.05
	A. rotundatum	3.1%	2	0.10
	A. humerale	6.7%	3	0.2
	A. sculptum	2.9%	-	0.03

Source: (MENDOZA-ROLDAN, J. A., 2019)

Legend: PI: prevalence index; MI: mean intensity; MA: mean abundance.

Parasitic niches of seven species of Trombidiformes mites, and one Oribatid species, were described, and preferred location of three species were recorded. The two species of the Harpirhynchidae family had the preferred location described. *O. ekan*s (n=2, PI 0.2%) was collected on the gular area (**GA**) of *B. jararaca* (Figure 91A), and *O. parkeri* (n=45, PI 1.5%) was collected from the lateral anterior scales (**LAS**) of *C. bicarinatus*, producing cavitations on the scales (Figure 91B-E).

Figure 91 – Preferred locations on hosts *Bothrops jararaca* and *Chironius bicarinatus* infested with *Ophioptes ekan*s and *Ophioptes parkeri*, respectively

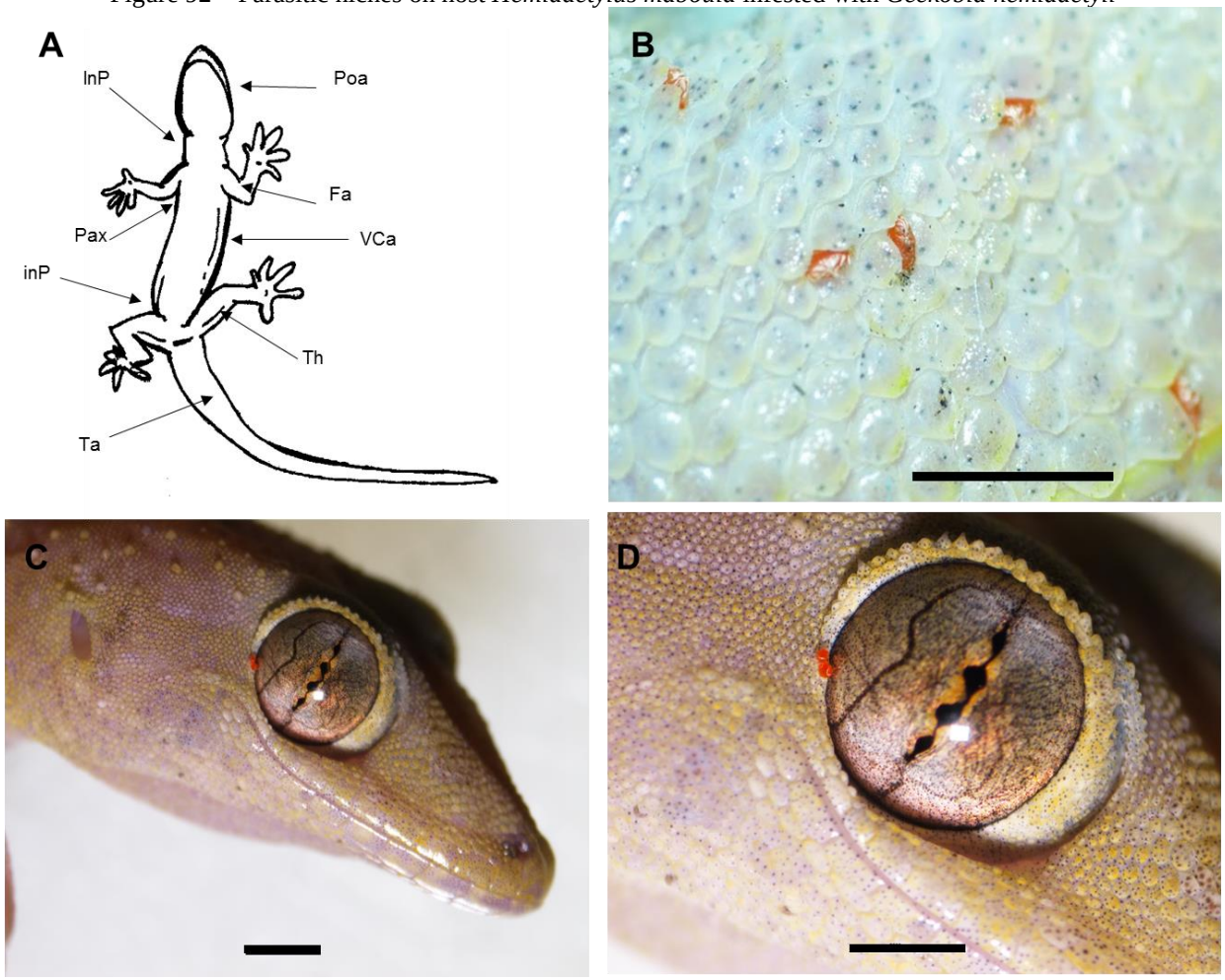


Source: A, drawings from (http://users.dickinson.edu/~nicholsa/Romnat/animacole.htm); B-E: (MENDOZA-ROLDAN, J. A., 2019)

Legends: A) preferred locations on *B. jararaca;* B) preferred locations on host *C. bicarinatus*; C-E *C. bicarinatus* infested with *O. parkeri*. Abbreviations: Gular Area (**GA**), Lateral Anterior Scales (**LAS**). Scale bars: C, D 2000 µM; E 1000 µM.

On the other hand, the three species of the Ptegysomomatidae family had their parasitic niches described. The species *G. hemidactyli* (n=80, PI 62.5%) was infesting 10 *H. mabouia* distributed in the following parasitic niches: **Poa** (Peri-ocular area) 12.5 % (n = 10); l**nP** (lateral nuchal Pocket) 7.5% (n = 6); **Fa** (Forearm) 10 % (n = 8); **Th** (Thigh) 25 % (n = 20); **VCa** (Ventral Celomatic area) 37.5 % (n =30); **Ta** (Tail) 2.5 % (n = 2); **inP** (inguinal mite Pocket) 3.7 % (n = 3); **Pax** (auxiliary mite Pocket) 1.25 % (n = 1) (Figure 92).

Figure 92 – Parasitic niches on host *Hemidactylus mabouia* infested with *Geckobia hemidactyli*

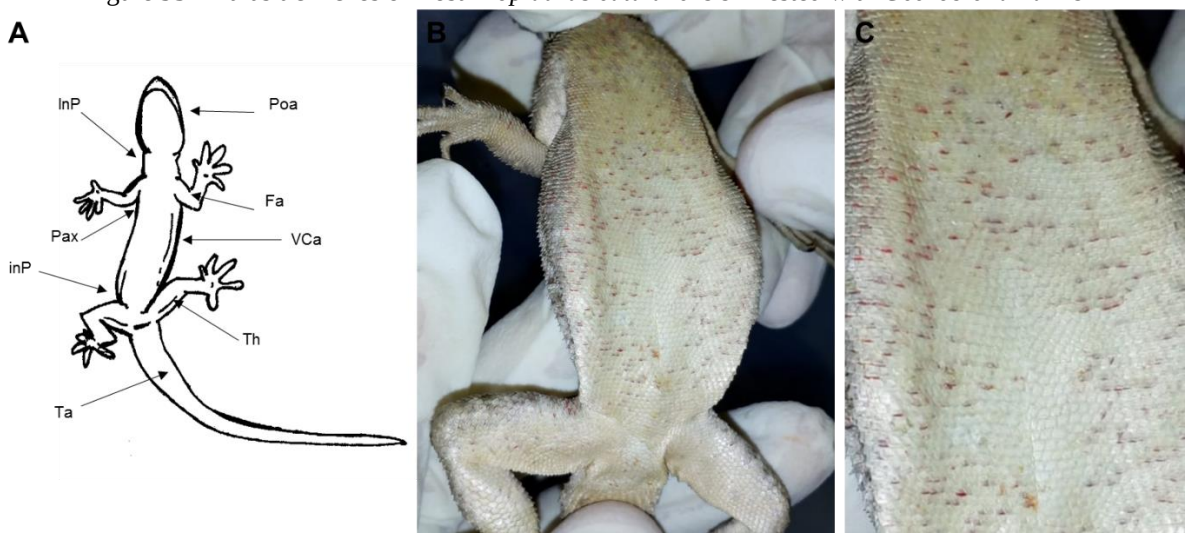


Source: (MENDOZA-ROLDAN, J. A., 2019)

Legend: A) parasitic niches: **Poa** (Peri-ocular area) 12.5 % (n = 10); **lnP** (lateral nuchal Pocket) 7.5% (n = 6); **Fa** (Forearm) 10 % (n = 8); **Th** (Thigh) 25 % (n = 20); **VCa** (Ventral Celomatic area) 37.5 % (n =30); **Ta** (Tail) 2.5 % (n = 2); **inP** (inguinal mite Pocket) 3.7 % (n = 3); **Pax** (auxiliary mite Pocket) 1.25 % (n = 1). B) mites on **VCa**. C, D) mites on **Poa**. Scales bar: B, 1000 µm; C, D 2000 µm.

The species *G. harrisi* (n=384, PI 100%) was infesting 8 *T. catalanensis* and one *T. torquatus* distributed in the following parasitic niches: **Poa** (Peri-ocular area) 0 % (n = 0); l**nP** (lateral nuchal Pocket) 1.5% (n = 6); **Fa** (Forearm) 1.5% (n =6); **Th** (Thigh) 15.6 % (n = 60); **VCa** (Ventral Celomatic area) 77.6% (n =298); **Ta** (Tail) 0 % (n = 0); **inP** (inguinal mite Pocket) 3.3 % (n = 13); **Pax** (auxiliary mite Pocket) 0.5 % (n = 2) (Figure 93).

Figure 93 – Parasitic niches on host *Tropidurus catalanensis* infested with *Geckobiella harrisi*

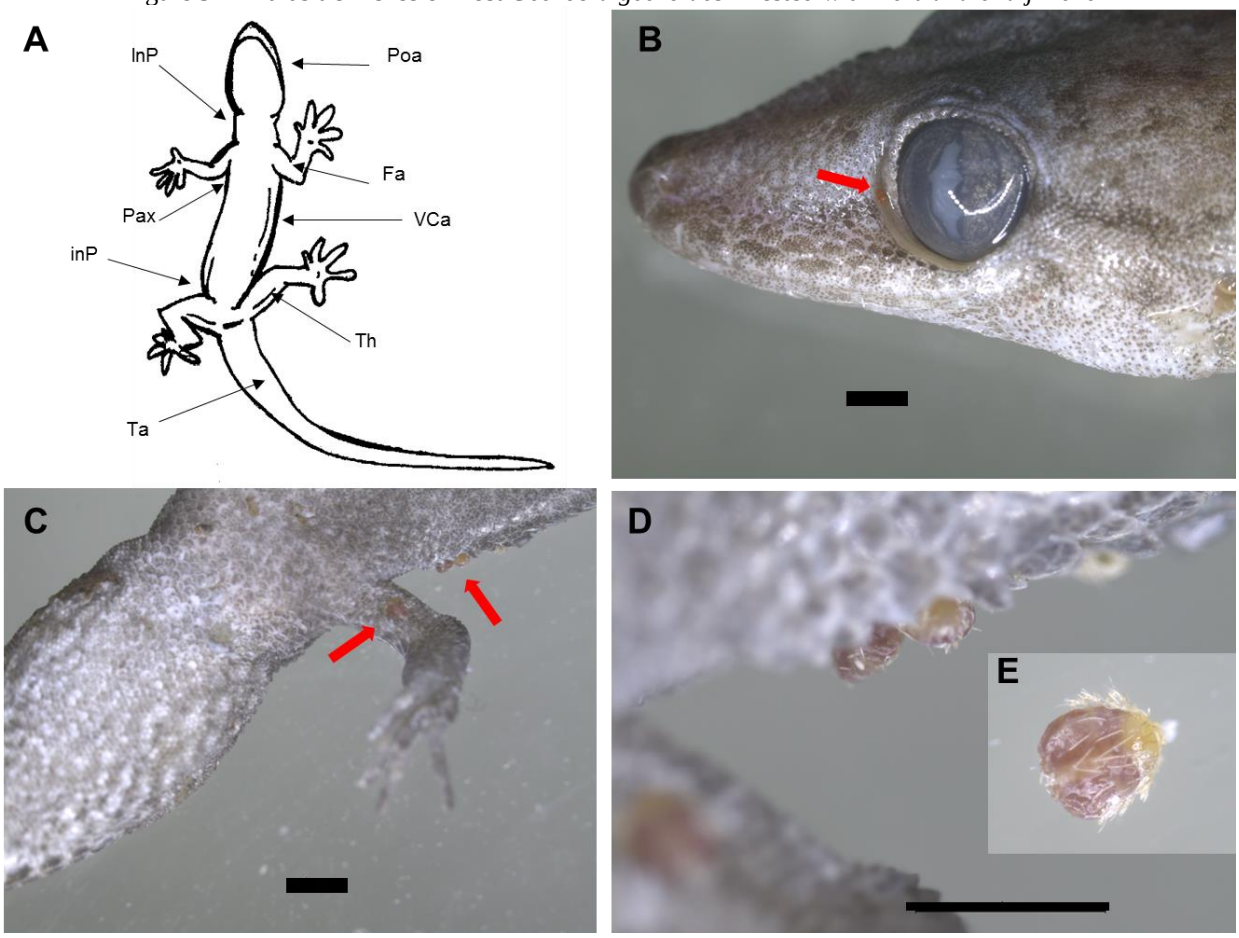


Source: (MENDOZA-ROLDAN, J. A., 2019)

Legend: A) parasitic niches: **Poa** (Peri-ocular area) 0 % (n = 0); **lnP** (lateral nuchal Pocket) 1.5% (n = 6); **Fa** (Forearm) 1.5% (n =6); **Th** (Thigh) 15.6 % (n = 60); **VCa** (Ventral Celomatic area) 77.6% (n =298); **Ta** (Tail) 0 % (n = 0); **inP** (inguinal mite Pocket) 3.3 % (n = 13); **Pax** (auxiliary mite Pocket) 0.5 % (n = 2). B, C) mites on **VCa**.

The species *B. jimenezi* (n=74, PI 69.2%) was infesting 8 *G. geckoides* and 10 *P. pollicaris* distributed in the following parasitic niches: **Poa** (Peri-ocular area) 16.2 % (n = 12); l**nP** (lateral nuchal Pocket) 17.5% (n = 13); **Fa** (Forearm) 24.3% (n =18); **Th** (Thigh) 5.4 % (n = 4); **VCa** (Ventral Celomatic area) 10.81% (n =8); **Ta** (Tail) 4 % (n = 3); **inP** (inguinal mite Pocket) 8.1 % (n = 6); **Pax** (auxiliary mite Pocket) 13.5 % (n = 10) (Figure 94).

Figure 94 – Parasitic niches on host *Geckobia geckoides* infested with *Bertrandiella jimenezi*

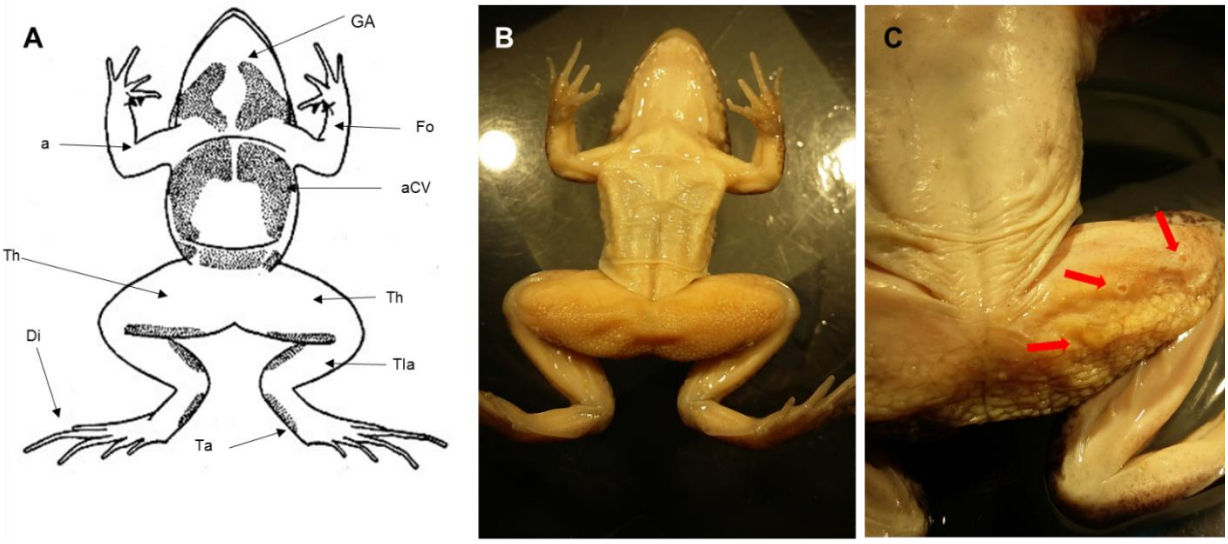


Source: (MENDOZA-ROLDAN, J. A., 2019)

Legend: A) parasitic niches: **Poa** (Peri-ocular area) 16.2 % (n = 12); l**nP** (lateral nuchal Pocket) 17.5% (n = 13); **Fa** (Forearm) 24.3% (n =18); **Th** (Thigh) 5.4 % (n = 4); **VCa** (Ventral Celomatic area) 10.81% (n =8); **Ta** (Tail) 4 % (n = 3); **inP** (inguinal mite Pocket) 8.1 % (n = 6); **Pax** (auxiliary mite Pocket) 13.5 % (n = 10) (Figure 92). B) mites on **Poa**; C, D) mites on **Fa** and **Pax**; E) female mite. Scales bars: 1000 µm.

On the other hand, two species of the Leeuwenhoekiidae family had their parasitic niches described. *H. achalai* (n=46, PI 65%) was infesting four *L. latrans*, seven *M. admirabilis*, and one *S. squalirostris* distributed in the following parasitic niches: **Fo** (Forearm) 13% (n =6); **VCa** (Ventral Celomatic area) 21.7 % (n = 10); **a** (arm) 4.3 % (n = 2); **Th** (Thigh) 17.3% (n = 8); **GA** (Gular Area) 2.1 % (n = 1); **TIa** (Tibial area) 6,55 (n=3); **Ta** (tarsal area) 13 % (n=6); **Di** (Digits) 21.7% (n=10) (Figure 95).

Figure 95 – Parasitic niches on host *Scinax squalirostris* infested with *Hannemania achalai*

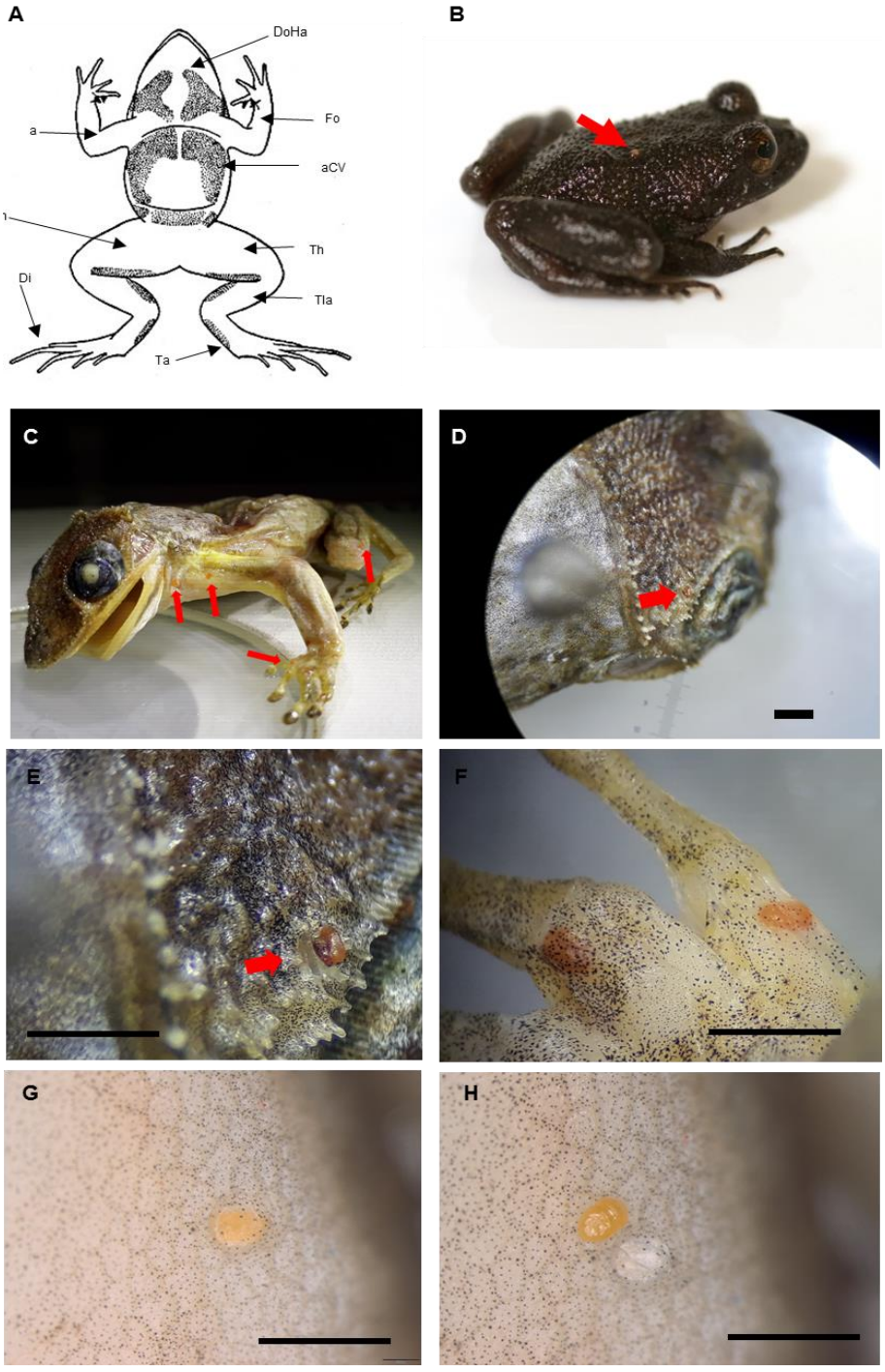


Source: (MENDOZA-ROLDAN, J. A., 2019)

Legend: A) parasitic niches: **Fo** (Forearm) 13% (n =6); **VCa** (Ventral Celomatic area) 21.7 % (n = 10); **a** (arm) 4.3 % (n = 2); **Th** (Thigh) 17.3% (n = 8); **GA** (Gular Area) 2.1 % (n = 1); **TIa** (Tibial area) 6,55 (n=3); **Ta** (tarsal area) 13 % (n=6); **Di** (Digits) 21.7% (n=10). B, C) mites on **Th**.

The species *H. hepatica* (n=19, PI 44.4%) was infesting one *C. boraceiensis*, one *C. greeningi*, one *C. dubius* and one *T. megatympanum*, distributed in the following parasitic niches**:** **DCa** (Dorsal Celomatic area) 5.2% (n =1); **Fo** (Forearm) 10.5% (n = 2); **VCa** (Ventral Celomatic area) 10.5 % (n = 2); **a** (arm) 10.5 % (n = 2); **Th** (Thigh) 0% (n = 0); **DoHa** (Dorsal Head area) 15.7 % (n = 3); **TIa** (Tibial area) 0% (n=0); **Ta** (tarsal area) 10.5 % (n=2); **Di** (Digits) 21% (n=4) (Figure 96).

Figure 96 – Parasitic niches on hosts *Cycloramphus boraceiensis*, *Corythomantis greeningi*, *Cycloramphus dubius*, and *Thoropa megatympanum*, infested with *H. hepatica*



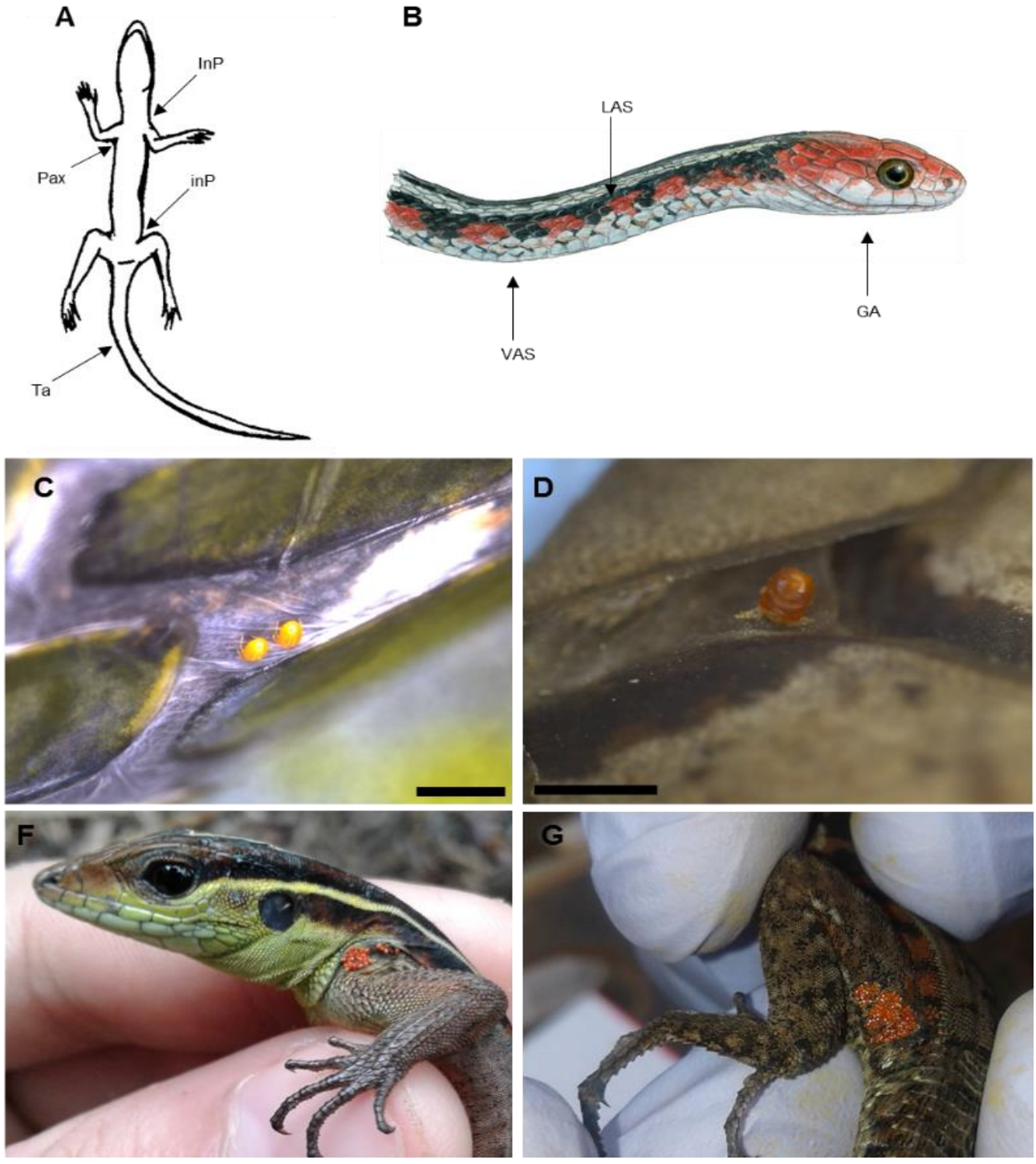
Source: A: (MENDOZA-ROLDAN, J. A., 2019); B: (TOLEDO F, 2016); C-F: (CORREA L, 2018); G, H: (LUZ H, 2016)

Legend: A) parasitic niches: **DCa** (Dorsal Celomatic area) 5.2% (n =1); **Fo** (Forearm) 10.5% (n = 2); **VCa** (Ventral Celomatic area) 10.5 % (n = 2); **a** (arm) 10.5 % (n = 2); **Th** (Thigh) 0% (n = 0); **DoHa** (Dorsal Head area) 15.7 % (n = 3); **TIa** (Tibial area) 0% (n=0); **Ta** (tarsal area) 10.5 % (n=2); **Di** (Digits) 21% (n=4). B) mite on **DCa** on *C. boraceiensis*; C-F) mites on *C. greeningi;* G, H) mites on **Th** on *T. megatympanum*. Scale bars: D, 1000 µm; E- H 500 µm.

Additionally, two species of the Trombiculidae family had their parasitic niches described and one species had its preferred locations on its host documented. *E. alfreddugesi* (n=510, of which 375 were counted on their parasitic niches, PI 19.9%) was infesting one *C. multiventris*, one *C. scurrulus,* one *P. nattereri*, one *S. pullatus,* one *A. meridionalis,* two *A. reticulata,* two *A. dorsivittatum*, one *C. eigenmani*, four *C. nigropunctatum*, and three *K. calcarata*, distributed in the following parasitic niches**:** l**nP** (lateral nuchal Pocket) 26.6% (n =100); **inP** (inguinal mite Pocket) 28.6 % (n =106); **Pax** (auxiliary mite Pocket) 30.4% (n = 114); **Ta** (Tail) 2.6% (n =10); **GA** (Gular Area) 0.53% (n =2); **LAS** (Lateral Anterior Scales) 10.6% (n =40); **VAS** (Ventral Anterior Scales) 2.6% (n =10) (Figure 97). The species *E. ophidica* had similar parasitic niches of *E. alfreddugesi*, infesting *K. calcarata* (n = 35, PI 8.3%), l**nP** (lateral nuchal Pocket) 57.14% (n =20); **inP** (inguinal mite Pocket) 28.5 % (n =10); and **Pax** (auxiliary mite Pocket) 12.4% (n = 5).

The species *F. anguina* (n=30; PI 10%) preferred location was the **VAS** (Ventral Anterior Scales) of *E. typhlus* (Figure 98A). Additionally, *A. longisetosus* (n=78), an Oribatid species, preferred location was the **DCa** (Dorsal Celomatic area) on *R. major* (Figure 98B, C).

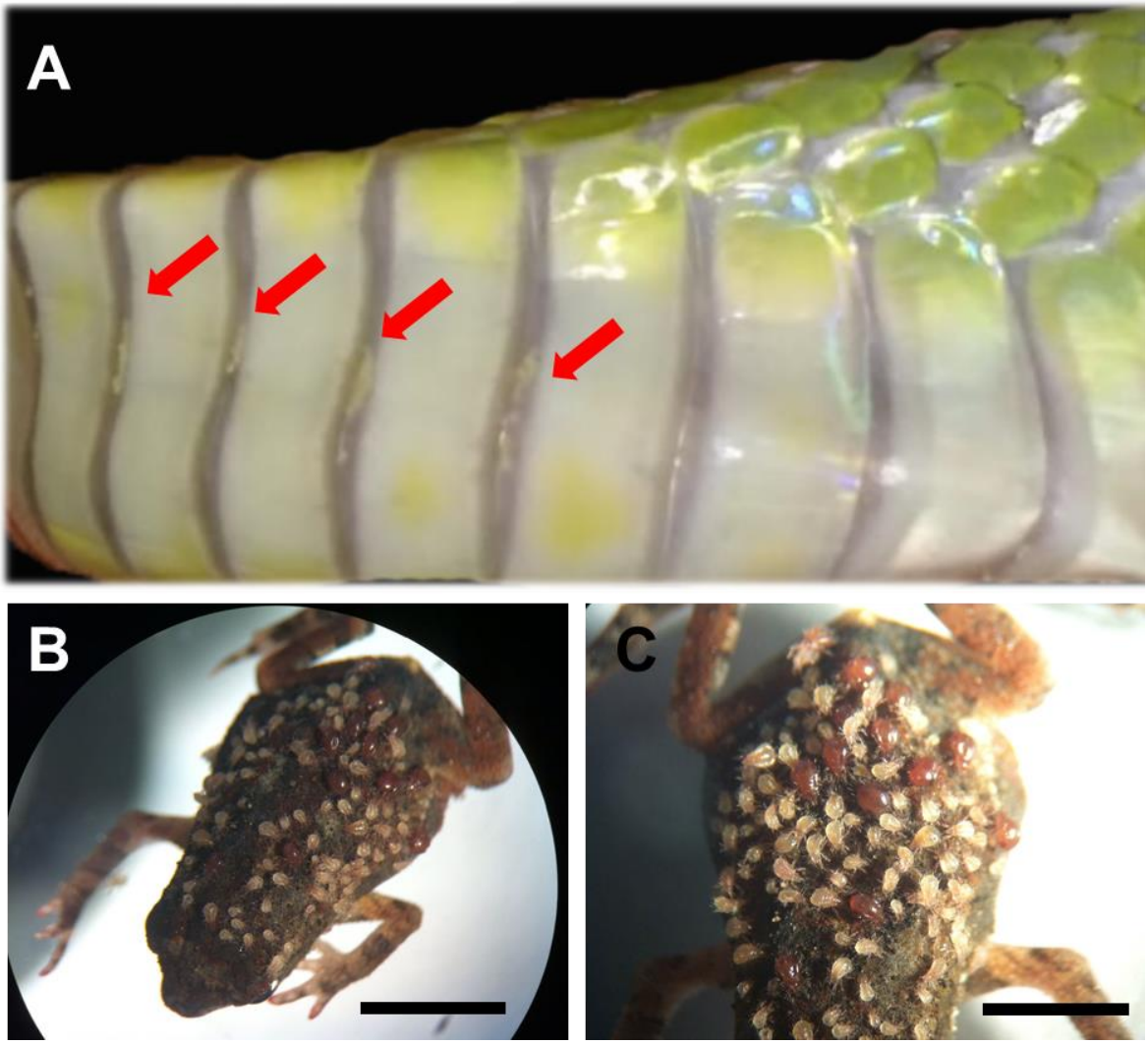
Figure 97 – Parasitic niches on hosts *Chironius multiventris*, *Phylodrias nattereri*, and *Kentropyx calcarata*, infested with *Eutrombicula alfreddugesi*



Source: A: (MENDOZA-ROLDAN, J. A., 2019); B: https://johnmuirlaws.com/drawing-scales/

Legend: A) parasitic niches on lizards: **lnP** (lateral nuchal Pocket) 26.6% (n =100); **inP** (inguinal mite Pocket) 28.6 % (n =106); **Pax** (auxiliary mite Pocket) 30.4% (n = 114); **Ta** (Tail) 2.6% (n =10); B) parasitic niches on snakes: **GA** (Gular Area) 0.53% (n =2); **LAS** (Lateral Anterior Scales) 10.6% (n =40); **VAS** (Ventral Anterior Scales) 2.6% (n =10) C, D) mite on **LAS** of *C. multiventris*, and *P. nattereri;* E-F) mites on **lnP** and **inP** of *E. alfreddugesi*. Scale bars: C 1000 µm; D 500 µm.

Figure 98 – Preferred locations on hosts *Erytrolamprus typhlus* infested with *Fonsecia anguina*, and *Rhinella major* with *Archegozetes longisetosus*



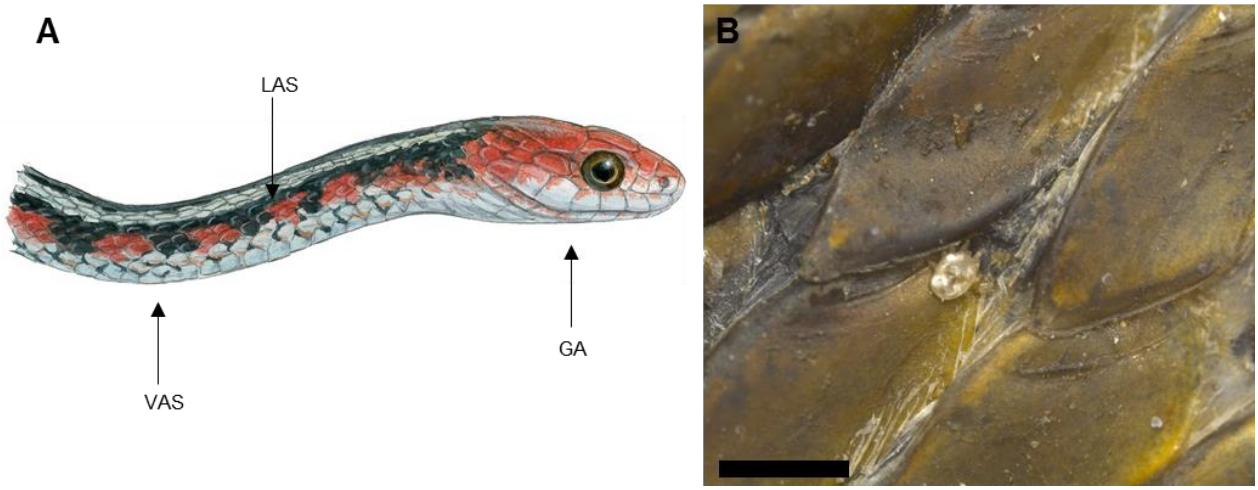
Source: A (ROCHA B, 2018); B, C (SOARES PEREIRA J, 2017)

Legends: A) preferred locations on *E. typhlus;* B, C) preferred locations on host *R. major*. Scale bars: B 3000 µM; C 1000 µM.

Regarding Mesostigmata mites, the parasitic niches of two species were recorded (*O. rotundus* and *Z. oudemansi*). The four females of *Chironoius* sp.n., infesting *C. multiventris* (PI 20%), were all collected from the **VAS** (Ventral Anterior Scales), almost near the head. On the other hand, the 18 specimends of *O. natricis* (PI 6%), infesting one *C. hortullanus*, one *C. durissus terrificus*, one *E. iheringii*, and two *P. vitticeps*, were identified upon material collected by other researchers, thus preferred locations or parasitic niches were not recorded.

The parasitic niches of *O. rotundus* (n= 43, PI 3.3%), on two *X. neuwiedii*, were recorded as follows: **GA** (Gular Area) 6.9% (n = 3); **LAS** (Lateral Anterior Scales) 69.7% (n =30); **VAS** (Ventral Anterior Scales) 23.2% (n =10) (Figure 99).

Figure 99 – Parasitic niches on hosts *Xenodon neuwiedii* infested with *Ophiogongylus rotundus*



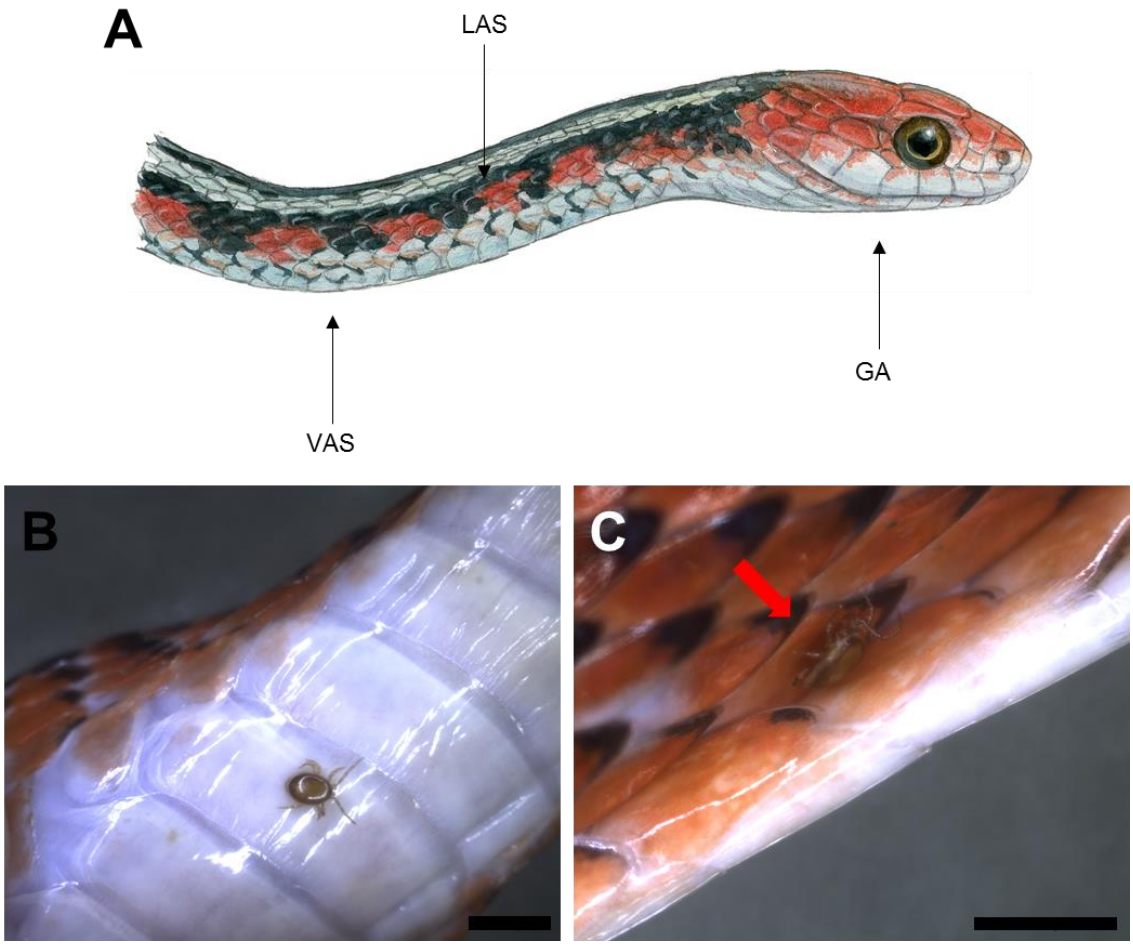
Source: A: https://johnmuirlaws.com/drawing-scales/; B: (MENDOZA-ROLDAN, J. A., 2018)

Legend: A) parasitic niches on snakes: **GA** (Gular Area) 6.9% (n = 3);**LAS** (Lateral Anterior Scales) 69.7% (n =30); **VAS** (Ventral Anterior Scales) 23.2% (n =10). B) mite on **LAS** of *X. neuwiedii.* Scale bar: 1000 µm.

Additionally, *Z. oudemansi* (n=5, PI 5%) was collected from one *O. melanogenys*, and one *P. nigra*, distributed in the following parasitic niches**:** **GA** (Gular Area) 20% (n =1); **LAS** (Lateral Anterior Scales) 60% (n =3); **VAS** (Ventral Anterior Scales) 20% (n =1) (Figure 100).

Finally, regarding the order Ixodida, the parasitic niches of one species were recorded (*A. rotundatum*). On the other hand, the preferred locations of *A. sculptum*, and *Ornithodoros* (*Alectorobius*) sp. n. were established. The male of *A. sculptum* (PI 29%), infesting *S. merianae* was collected from **lNa** (Lateral neck area) (Figure 101A, C). Also, the seven larvae of *Ornithodoros* (*Alectorobius*) sp. n., infesting the only specimen of *P. nattereri* examined in this study, were collected solely from the **LAS** (Lateral Anterior Scales) (Figure 101B, D-F).

Figure 100 – Preferred locations on hosts *Oxyrhopus melanogenys* infested with *Zeterhercon oudemansi*



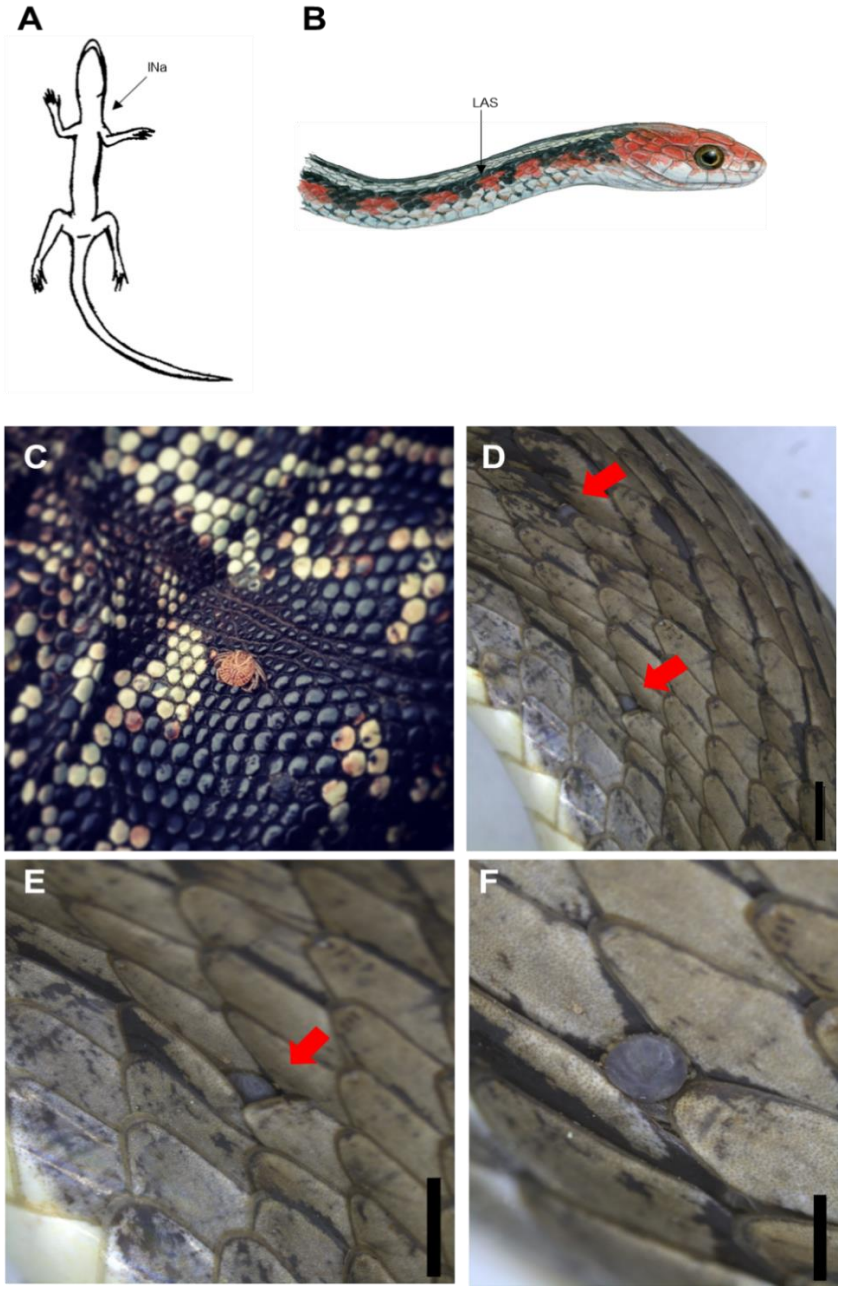
Source: A: https://johnmuirlaws.com/drawing-scales/; B, C: (MENDOZA-ROLDAN, J. A., 2018)

Legend: A) parasitic niches on snakes: **GA** (Gular Area) 20% (n =1); **LAS** (Lateral Anterior Scales) 60% (n =3); **VAS** (Ventral Anterior Scales) 20% (n =1). B) mite on **VAS** of *O. melanogenys*. C) mite on **LAS** of *O. melanogeny*. Scale bar: 2000 µm.

In addition, *A. dissimile* (n=41, PI 5%) was collected from one *P. lansbergii*, one *P. matogrossensis*, one *C. durissus terrificus*, one *C. laurenti*, one *D. indica bucephala*, two *P. nigra*, and one *R. jimi*. Nonetheless, most of the identifies tick were collected by other researchers, thus parasitic niches and preferred locations were not recorded. Lastly, the parasitic niches of *A. rotundatum* (n= 165, PI 3.1 %), were documented only on 11 species of snakes (n= 69 ticks): one *B. alternatus*, one *B. leucurus*, one *B. jararaca*, two *B. jararacussu*, three *C. durissus terrificus*,

two *C. multiventris*, one *C. scurrulus*, one *X. merremii*, one *L. annulate*, one *D. turgidus*, *one D. neuwiedi*, and three *C. hortulanus*. The parasitic niches were as follows: **Poa** (Peri-ocular area) 2.8 % (n = 2); **GA** (Gular Area) 1.4% (n = 1); **LAS** (Lateral Anterior Scales) 75.3% (n =52); **LPS** (Lateral Posterior Scales) 14.9% (n =10); **VAS** (Ventral Anterior Scales) 5.9% (n =4) (Figure 102).

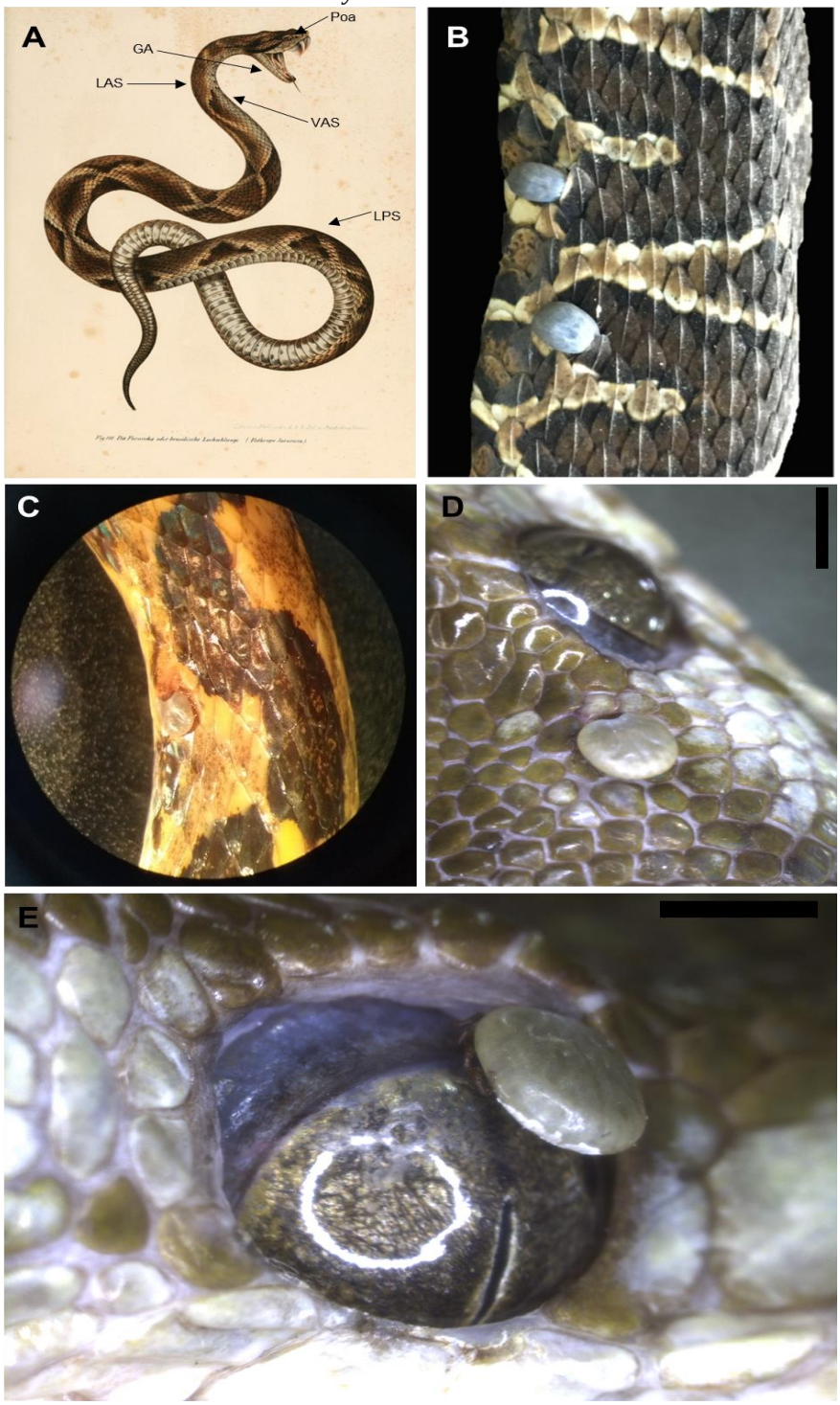
Figure 101 – Preferred locations on hosts *Salvator merianae* infested with *Amblyomma sculptum*, and *Philodryas nattereri* with *Ornithodoros* (*Alectorobius*) sp. n.



Source: A,C-F: (MENDOZA-ROLDAN, J. A., 2019); B: https://johnmuirlaws.com/drawing-scales/

Legends: A) preferred locations on *S. merianae;* B, C) preferred locations on host *P. natterei*. Scale bars: 3000 µm.

Figure 102 – Parasitic niches on hosts *Bothrops alternatus*, *Dipsas turgidus* and *Corallus hortulanus* infested with *Amblyomma rotundatum*



Source: A: weheartit.com; B - E: (MENDOZA-ROLDAN, J. A., 2019)

Legend: A) parasitic niches on snakes: **Poa** (Peri-ocular area) 2.8 % (n = 2); **GA** (Gular Area) 1.4% (n = 1); **LAS** (Lateral Anterior Scales) 75.3% (n =52); **LPS** (Lateral Posterior Scales) 14.9% (n =10); **VAS** (Ventral Anterior Scales) 5.9% (n =4**).** B) ticks on **LAS** of *B. alternatus.* C) ticks on **LAS** of *D. turgidus*. D, E) ticks on **Poa** of *C. hortulanus.* Scale bars: D, E 2000 µm.

4.3.1 Co-infestations

Infestations by different species of mites and ticks in the same host, occurred in four snakes. Three of them from Iracema, Acre state; and one snake from São Bernardo do Campo, São Paulo state. The snakes from Iracema from Acre state were: *C. multiventris* infested with *E. alfreddugesi* (Trombidiformes), *Chironobius* sp. n. (Mesostigmata), and *A. rotundatum* (Ixodida) (Figure 103); *C. scurrulus* infested with *E. alfreddugesi* (Trombidiformes), and *A. rotundatum* (Ixodida); and *O. melanogenys* infested with *Z. oudemansi* (Mesostigmata), and *A. rotundatum* (Ixodida). The snake co-infested in São Paulo state was a *P. natteteri* infested with *E. alfreddugesi* (Trombidiformes), and *Ornithodoros (Alectorobius)* sp.n. (Ixodida) (Table 32).

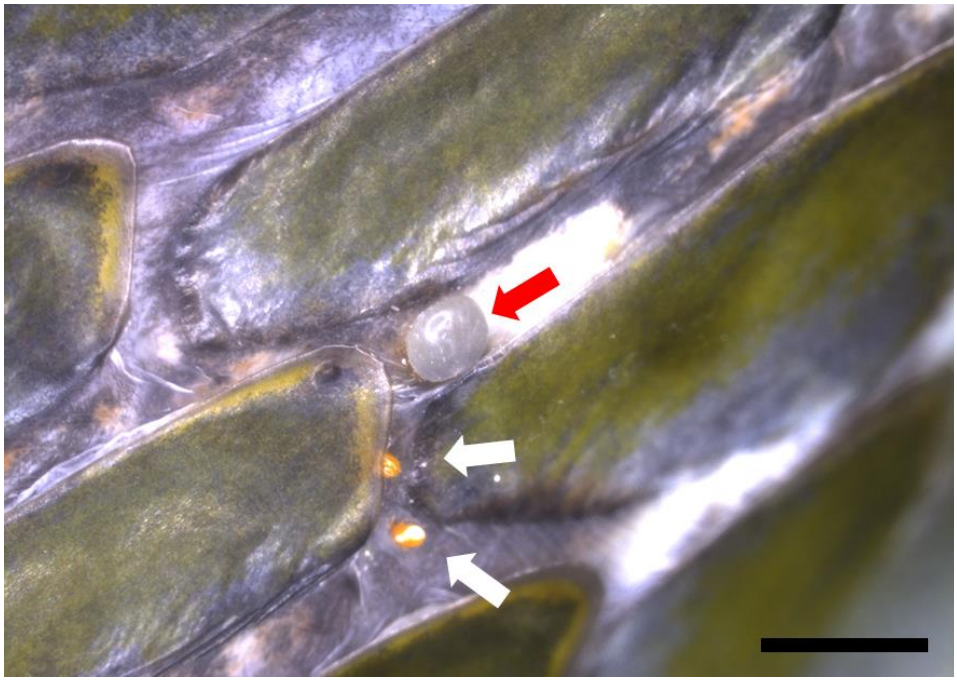
Table 32 – Co-Infestations on snakes

Host	Parasite Species	Locality
C. multiventris	*E. alfreddugesi* (T) *Chironobius* sp. n. (M) *A. rotundatum* (I)	Iracema, AC
C. scurrulus	*E. alfreddugesi* (T) *A. rotundatum* (I)	Iracema, AC
O. melanogenys	*Z. oudemansi* (M) *A. rotundatum* (I)	Iracema, AC
P. natteteri	*E. alfreddugesi* (T) *Ornithodoros* (*Alectorobius*) sp. n. (I)	São Bernardo do Campo, SP

Source: (MENDOZA-ROLDAN, J. A., 2019).

Legend: Abbreviations: T (Trombidiformes), M (Mesostigmata), I (Ixodida).

Figure 103 – Co-infestation of *Eutrombicula alfreddugesi* (T) and *Amblyomma rotundatum* (I) on *Chironius multiventris*



Source: (MENDOZA-ROLDAN, J. A., 2018)

Legend: *E. alfreddugesi* (T) (white arrows) and *A. rotundatum* (I) (red arrow) on *C. multiventris.* Scale bar: 2000 µm.

4.4 Blood smear assessment

Blood draws were only performed in reptiles, and from the 121 infested reptiles, 48 blood smears were performed (39.6% of all the reptile hosts). These blood samples were used for DNA extraction and pathogen testing, which is discussed on chapter 6.

Of these smears, six showed intraerythrocytic parasites (12.5% of the smears). Moreover, four had *Hepatozoon* gamonts (*C. multiventris*, *C. scurrulus*, *C. hortullanus*, and *P. viridissima*) (Figure 104), one had *Hepatozoon* gamonts and inclusions compatible with *Iridovirus* (*C. multiventris*) (Figure 105A, B), and one (*O. melanogenys*) had gamonts compatible with *Hepatozoon* yet with larger size and basophilic (Figure 105C, D). Molecular diagnosis of the protozoa parasites is further discussed in chapter 6. Information of hosts with which Acari was infesting them, and blood smear results, are shown in Table 33.

Table 33 – Host and Acari parasites with blood smears information

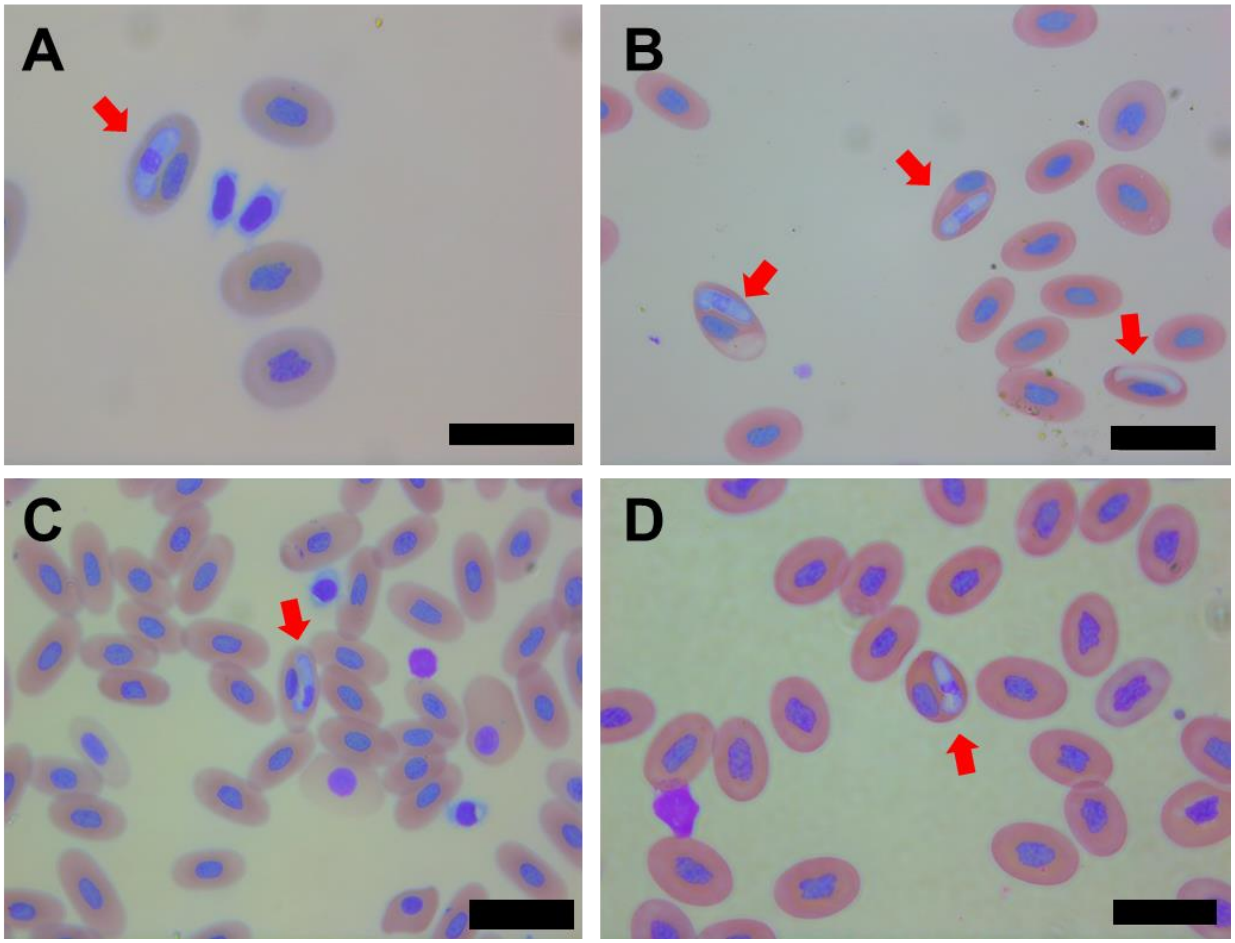
IBSP of Acari	Host	Species of Acari	Blood smear
12907	*C. durissus terrificus*	*O. natricis*	Negative
12908	*C. bicarinatus*	*O. parkeri*	Negative
12909	*C. durissus terrificus*	*A. rotundatum*	Negative
12911	*H. mabouia*	*G. hemidactyli*	Negative
12912	*H. mabouia*	*G. hemidactyli*	Negative
12913	*H. mabouia*	*G. hemidactyli*	Negative
12915	*C. durissus terrificus*	*A. rotundatum*	Negative
12916	*H. mabouia*	*G. hemidactyli*	Negative
12917	*S. pullatus*	*E. alfreddugesi*	Negative
12930	*H. mabouia*	*G. hemidactyli*	Negative
12940	*H. mabouia*	*G. hemidactyli*	Negative
12950	*A. reticulata*	*E. alfreduggesi*	Negative
12951	*K. calcarata*	*E. alfreduggesi*	Negative
12952	*K. calcarata*	*E. alfreduggesi*	Negative
12954	*B. jararaca*	*A. rotundatum*	Negative
12955	*K. calcarata*	*E. ophidica*	Negative
12956	*K. calcarata*	*E. ophidica*	Negative
12933	*H. mabouia*	*G. hemidactyli*	Negative
12940	*H. mabouia*	*G. hemidactyli*	Negative
12978	*C. durissus terrificus*	*A. rotundatum*	Negative
14829	*C. nigropunctatum*	*E. alfreduggesi*	Negative
14831	*A. dorsivittatum*	*E. alfreduggesi*	Negative
14832	*S. merianae*	*A. sculptum*	Negative
14833	*A. dorsivittatum*	*E. alfreduggesi*	Negative
14834	*A. meridionalis*	*E. alfreduggesi*	Negative
14835	*C. nigropunctatum*	*E. alfreduggesi*	Negative
14836	*C. nigropunctatum*	*E. alfreduggesi*	Negative
14837	*H. mabouia*	*G. hemidactyli*	Negative
14838	*P. nattererii*	*E. alfreddugesi* *Ornithodoros* (*Alectorobius*) sp.n.	Negative
14871	*D. neuwiedi*	*A. rotundatum*	Negative
14873	*B. leucurus*	*A. rotundatum*	Negative
14874	*P. vitticeps*	*O. natricis*	Negative
14874	*P. vitticeps*	*O. natricis*	Negative

(Conclusion)

IBSP of Acari	Host	Species of Acari	Blood smear
14875	*C. multiventris*	*E. alfreddugesi* *Chironobius* sp. n. *A. rotundatum*	*Hepatozoon* *Iridovirus*
14879	*C. multiventris*	*A. rotundatum*	*Hepatozoon*
14880	*C. scurrulus*	*E. alfreddugesi* *A. rotundatum*	*Hepatozoon*
14882	*C. hortullanus*	*A. rotundatum*	*Hepatozoon*
14883	*O. melanogenys*	*Z. oudemansi* *A. rotundatum*	*Hepatozoon* (Larger)
14885	*P. viridissima*	*A. rotundatum*	*Hepatozoon*
14886	*E. typhlus*	*E. alfreddugesi*	Negative
14887	*T. catalanensis*	*G. harrisi*	Negative
14888	*T. catalanensis*	*G. harrisi*	Negative
14889	*T. catalanensis*	*G. harrisi*	Negative
14890	*T. catalanensis*	*G. harrisi*	Negative
14891	*T. catalanensis*	*G. harrisi*	Negative
14892	*T. catalanensis*	*G. harrisi*	Negative
14893	*T. catalanensis*	*G. harrisi*	Negative
14894	*T. catalanensis*	*G. harrisi*	Negative

Source: (MENDOZA-ROLDAN, J. A., 2019)

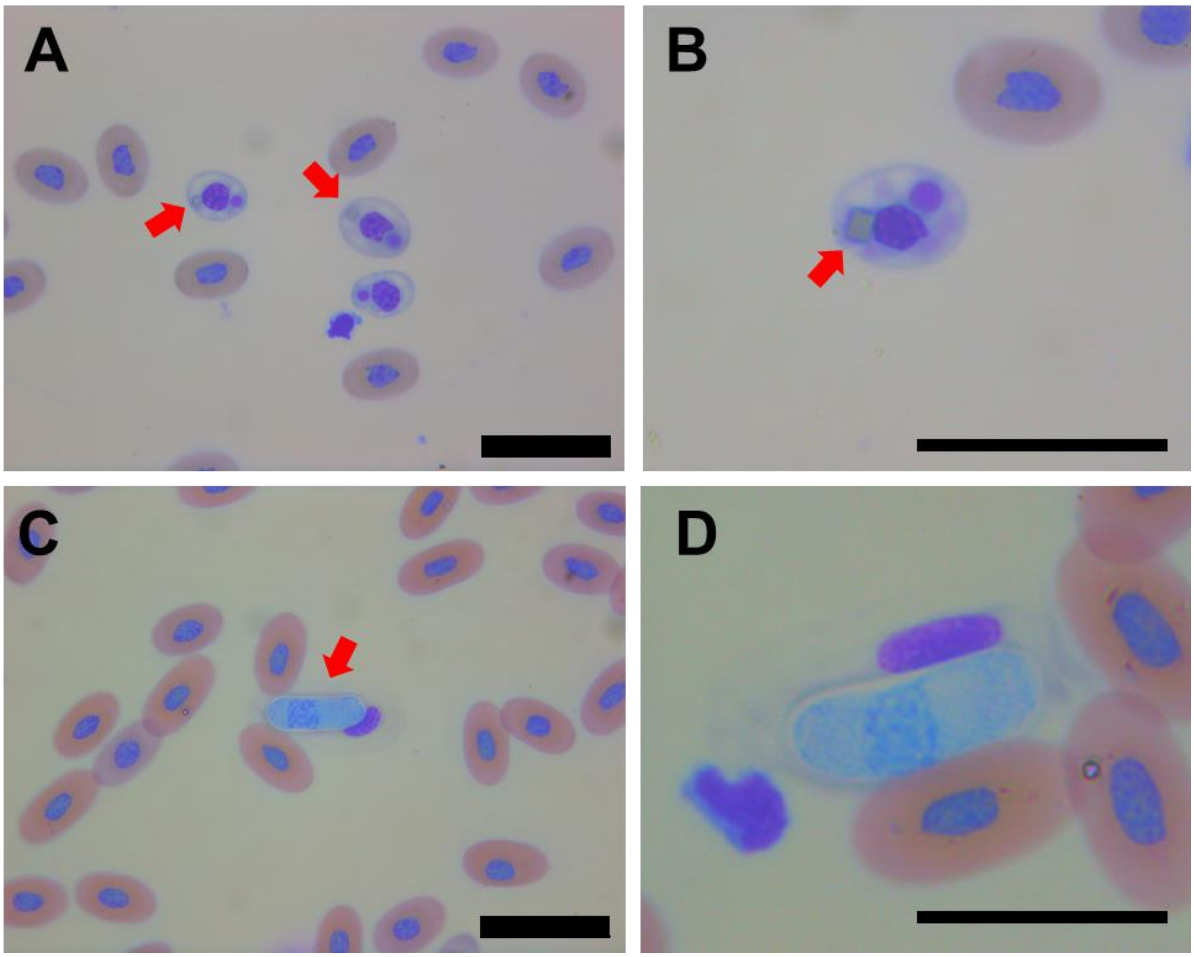
Figure 104 – *Hepatozoon* gamonts on bloods smears of snakes



Source: (MENDOZA-ROLDAN, J. A., 2018)

Legend: *Hepatozoon* gamonts (red arrows). A) *Hepatozoon* on *C. multiventris*. B) *Hepatozoon* on *C. scurrulus*. C) *Hepatozoon* on *C. hortullanus*. D) *Hepatozoon* on *P. viridissima*. Scale bars: 20 µm.

Figure 105 – *Iridovirus* inclusions and *Hepatozoon* on bloods smears of snakes



Source: (MENDOZA-ROLDAN, J. A., 2018)

Legend: *Iridovirus* inclusions and *Karyolysus* (red arrows). A) *Iridovirus* inclusions on *C. multiventris.* B) *Karyolysus* on *O. melanogenys.* Scale bars: 20 µm.

4.5 Histology of lesions

Tissue samples of skin where the mites and ticks were attached, and possibly had lesions associated to the fixation, were collected whenever possible. Of the 121 infested reptiles, only 18 skin tissue samples were collected (one snake *C. bicarinatus*, nine *H. mabouia*, one *A. reticulata*, four *K. calcarata*, three *C. nigropunctatum*, and one *P. nattereri*). On the other hand, skin tissue samples were collected from 14 of the 49 infested amphibians (seven *M. admirabilis*, four *L. latrans*, one *S. squalirostris*, one *T. megatympanum* Figure 103A-B, and one *C. boraceiensis*) (Table 33).

Furthermore, skin samples from reptiles did not show any evidence of lesions associated to the fixation of Acari. The sample from *C. bicarinatus* was with a high state of autolysis, which prevented lesions to be observed. On the other hand, samples from amphibians had similar type of lesions associated with intradermic mites of the genus *Hannemania.* This lesion was characterized as a capsular reaction, located generally within the stratum spongiosum layer of the dermis (Figure 106B, C).

The lesions were observed with several alterations: acanthosis (hypertrophy or thickening) of the epidermis (cells of the stratum corneum and germinativum); proliferation of connective tissue that forms the outer layer of the capsule; and a there is a distortion of the stratum compactum directly beneath the capsule (Figure 106B-F). Information of hosts with which Acari was infesting them, and histology results, are shown in Table 34.

Table 34 – Host and Acari parasites with histology information

IBSP of Acari	Host	Species of Acari	Blood smear
12908	*C. bicarinatus*	*O. parkeri*	A
12911	*H. mabouia*	*G. hemidactyli*	NAL
12912	*H. mabouia*	*G. hemidactyli*	NAL
12913	*H. mabouia*	*G. hemidactyli*	NAL
12916	*H. mabouia*	*G. hemidactyli*	NAL
12930	*H. mabouia*	*G. hemidactyli*	NAL
12940	*H. mabouia*	*G. hemidactyli*	NAL
12950	*A. reticulata*	*E. alfreduggesi*	NAL
12951	*K. calcarata*	*E. alfreduggesi*	NAL
12952	*K. calcarata*	*E. alfreduggesi*	NAL
12955	*K. calcarata*	*E. ophidica*	NAL
12956	*K. calcarata*	*E. ophidica*	NAL
12933	*H. mabouia*	*G. hemidactyli*	NAL
12940	*H. mabouia*	*G. hemidactyli*	NAL
14829	*C. nigropunctatum*	*E. alfreduggesi*	NAL
14835	*C. nigropunctatum*	*E. alfreduggesi*	NAL
14836	*C. nigropunctatum*	*E. alfreduggesi*	NAL
14837	*H. mabouia*	*G. hemidactyli*	NAL

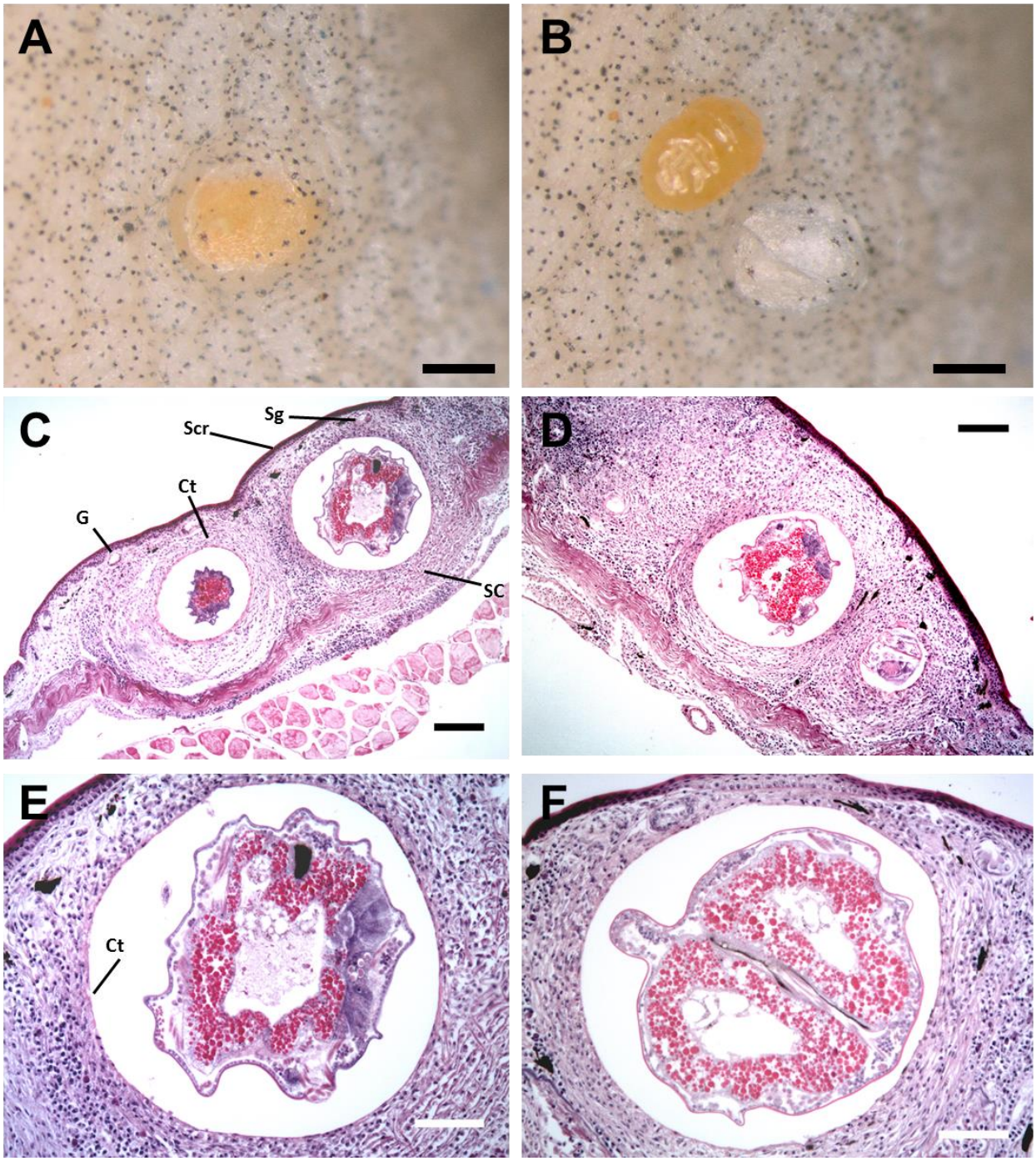
(Conclusion)

IBSP of Acari	Host	Species of Acari	Blood smear
14838	*P. nattererii*	*E. alfreddugesi* *Ornithodoros (Alectorobius)* sp.n.	NAL
	M. admirabilis	*H. achalai*	NL
12919	*M. admirabilis*	*H. achalai*	NL
12920	*M. admirabilis*	*H. achalai*	NL
12921	*M. admirabilis*	*H. achalai*	NL
12922	*M. admirabilis*	*H. achalai*	NL
12923	*M. admirabilis*	*H. achalai*	NL
12924	*M. admirabilis*	*H. achalai*	NL
12925	*L. latrans*	*H. achalai*	NL
12926	*L. latrans*	*H. achalai*	NL
12927	*L. latrans*	*H. achalai*	NL
12928	*L. latrans*	*H. achalai*	NL
12929	*S. squalirostris*	*H. achalai*	NL
12934	*T. megatympanum*	*H. hepatica*	NL
12935	*C. boraceiensis*	*H. hepatica*	NL

Source: (MENDOZA-ROLDAN, J. A., 2019)

Legend: A (Autolysis); NAL (No Aparent Lesion); NL (Nodular Lesion).

Figure 106 –*Hannemania* capsular lesion on amphibian hosts



Source: (NAVAS-SUAREZ P., 2016)

Legend: A, B) Capsule of *H. hepática* in *T. megatympanum*; C-F skin of *M. admirabilis* with capsules of *H. achalai.* Abbreviations: scr =stratum corneum; sg = stratum germinativum; sc =stratum compactum; Ct = Connective tissue; G =Gland. Scale bars. A, B 500 µm;C, D 300 µm; E, F 50µm.

5 DISCUSSION

The deleterious effect of mites and ticks parasitizing the herpetofauna in the present study, was investigated using a multifocal approach. Thus, many reptiles and amphibians were examined (4,515) to ensure representativeness of the Brazilian herpetofauna megadiversity. Consequently, the overall prevalence index was calculated to better understand which the preferred hosts for each order of Acari were (Trombidiformes, Mesostigmata and Ixodida), and to determine which of these orders can be more frequently found parasitizing reptiles and amphibians in Brazil, and the host specificity of the different orders of Acari. Next, infestation rates were calculated (PI, MI, MA) for each order and for each species to determine which of the identified mites and ticks are more abundant and more likely to be found parasitizing the herpetofauna. Also, infestation rates, parasitic niches and preferred locations were calculated and identified, to measure parasitic loads, that when high can manifest in detriment of the health status of the host. Additionally, parasitic niches and preferred locations were recorded to help identify specific places where to search for the different species of mites and ticks, and to determine the host-parasite adaptations, specificity, and relationships (convergent evolution). On the other hand, blood smear assessment was used to determine hemoparasitic presence in infested hosts. Finally, histological evaluation of skin tissue of infested hosts allowed to determine the type of lesions associated with specific kind of mites and assess the impact on the host.

Regarding the type of infested host, of the 4,515 examined hosts, most were snakes (68.7%, 3104/4515). This was because of the large number of snakes that were received by the IBPS laboratories (average of 20 snakes per day). Nonetheless, 42.3% of all the 170 infested individuals were lizards, 29.3% of the 246 lizards examined. The Trombidiformes order (Trombiculidae and Pterygosomatidae) was the main order parasitizing lizards. This could be since almost all lizards were examined from wild environments. Former studies showed that lizards (Tropiduridae, Gekkonidae, and Scincidae) in Brazil have a high prevalence (50% to almost 100%) of mite infestations (CUNHA-BARROS et al., 2003; DE CARVALHO et al., 2006; DELFINO et al., 2011). On the other hand, ticks were only found in two species of lizards (PI 1.6%). Other studies showed a prevalence of 2.3% to 50% of assessed lizards infested with ticks (mainly *A. dissimile*), in the northern region of Brazil (TORRES et al., 2018). No co-infestations were seen in lizards here, yet on other regions co-infestations are common (Trombidiformes, Mesostigmata and

Ixodida) in lacertid lizards from the northern hemisphere (MENDOZA-ROLDAN et al., 2019). On other words, of reptiles, the most examined hosts were snakes, yet the most infested types of hosts were lizards.

Moreover, of the examined hosts, 3.4% (121/3,596) were infested reptiles, and 5.3% were infested amphibians (49/919). These prevalence rates are similar to those seen in former studies, where prevalence was low. Some studies from museum material showed a prevalence index of 2 to 3% in snakes (Mesotigmata) (FAIN, 1961; 1962a). Field caught hosts studies showed high prevalence, with some studies on Trombiculidae and Pterygosomatidae having 100% of the population infested (CUNHA-BARROS et al., 2003; DE CARVALHO et al., 2006; DELFINO et al., 2011). Moreover, studies where Trombidiformes, Mesostigmata and Ixodida parasites were assessed together, also showed moderate to high infestation rates. In Brazil, analyzing non-venomous snakes from the State of São Paulo, showed prevalence rates of 13 to 16% (Ixodidae, Mesostigmata, Trombiculidae) (LIZASO, 1982). Also, a recent study in Southern Italy with captive and wild reptiles, showed a prevalence of 82% reptiles infested with Ixodidae, Macronyssidae, Ptergosomatidae and Trombiculidae (MENDOZA-ROLDAN et al., 2019). Thus, low infestation rates found in the present study can be because most of the hosts were examined in the IBSP laboratories. Although, not considered captive, these animals were collected from their environment, days before arriving to the IBSP laboratories. Hence, giving some time for the ectoparasites to detach from the host. Considering the amphibian species of hosts, most of the infested anurans came from field trips, therefore, the PI was consequently slightly higher (5.3%).

Concerning the prevalence of each order of Acari (mites and ticks), in reptiles, the Trombidiformes order was the most prevalent order on lizards, followed by Ixodida on snakes. The order Mesostigmata was the less prevalent order of ectoparasites identified, and it was identified only on Squamata reptiles. No Mesostigmata mites were found on amphibians, which was expected as no records of this kind of parasitic relationship was found in literature. Also, in amphibians, Ixodida was the most prevalent order, followed by Trombidiformes, and lastly Oribatida. Co-infestations were rare and seen only in snakes. These findings were expected as most studies show Trombidiformes as the main parasitic order in Lizards (CUNHA-BARROS et al., 2003; DE CARVALHO et al., 2006; DELFINO et al., 2011). Also, studies show a higher prevalence of ticks in snakes through the Brazilian territory (OGRZEWALSKA et al., 2018; TORRES et al., 2018). In addition, Mesostigmata of the three orders examined, was the less

prevalent in reptiles (7.4%, 9/121). This order is highly diverse in Brazil, with four families parasitizing snakes and lizards. The last study performed in the State of São Paulo, stated that Mesostigmata mites, specifically the genus *Ixobioides*, was the most prevalent order (66.5%, 212/241), out of the three that infest snakes (LIZASO, 1982). However, in the present study *Ixobioides* species were not found in the examined hosts, and the prevalence was very low. This could be due to the fact that Mesostigmata mites, in general, do not attach to its hosts for long periods of time, and can move quickly, thus snakes received in the IBSP could have lost their mites from the moment they were captured, until they were examined in the LECZ laboratory reception site. Despite this fact, two of the mites found on snakes were collected from animals captured and brought to the laboratory (*Chironobius* and *Zeterohercon* species), so examining animals for ectoparasites that arrive in the laboratories of the IBSP, should be added to the quarantine protocols. This procedure should be continued also because the other Mesostigmata mites, identified here, came from snakes and lizards kept in captive conditions (mostly infested by the Macronyssidae mite *O. natricis*). This species when present in a facility can spread fast and its control is difficulted by its biological cycle, which in part is performed in the substrate. Therefore, treatment and control should include the hosts and the entire facility and staff (WOZNIAK; DENARDO, 2000; CASTRO et al, 2019). Nonetheless, some species of Mesostigmata mites have only been found once when described or haven't been found for more than 30 years. This makes it unclear if they can be considered endangered or even extinct. Considering ticks, some species that were described on vulnerable or critically endangered reptilian hosts, are also considered endangered, thus possibly mites highly specific to a host (Entonyssidae) can be consider endangered as well (MILLER et al., 2011; MIHALCA et al., 2011; DERNE et al., 2018).

Furthermore, in amphibians, Ixodida was the most prevalent order (63.2%), followed by Trombidiformes of the family Leeuwenhoekiidae (genus *Hannemania*) (34.6%). Anuran amphibians, mainly the genus *Rhinella* of the family Bufonidae, have a strong relationship with two *Amblyomma* species (*A. rotundatum* and *A. dissimile*) (GUGLIELMONE; NAVA, 2010; LUZ et al., 2013; LUZ; FACCINI. 2013). Nevertheless, these species of ticks have a low host specificity, being able to infest many other classes of hosts, as seen in 24.7% of infested reptiles were infested snakes with ticks of these species. Thus, the low specificity allows the species to colonize diverse biomes, making its distribution wide throughout the Brazilian territory. However, it is more likely to find an infested toad (*Rhinella*), than any other type of infested host. Concerning

the *Hannemania* genus, species found infested with these intradermic mites were all anurans considered as frogs, which are species that usually long legs and smooth skins covered in mucus. Differently from toads, that generally have shorter legs and rougher, thicker skins (TURNER, 1962). This may be associated with the penetrative capacity of these mites. Though, toads of different species have been found infested with *Hannemania* mites previously in other countries (Chile and Mexico) (DUSZYNSKI; JONES, 1973; DÍAZ-PÁEZ et al., 2016). In Brazil, all records in infestations are on frogs, and *Rhinella* toads have not been indicated as hosts in the country (HATANO et al., 2007; JACINAVICIUS et al., 2018). This suggests that *Rhinella* toads are likely to be infested with *Amblyomma* ticks, and frogs are more prone to be infested with *Hannemania* larvae, in humid areas of the southeastern and south regions of Brazil. However, in this study *H. hepatica* was found infesting *C. greeningi*, in the northeastern region. The only former record of *Hannemania* in the northeastern region was from Rio Grande do Norte state on *Leptodactylus macrosternum* Miranda-Ribeiro, 1926 (RODRIGUES et al., 2018). It is noteworthy to state, that the present study is one of the firsts to identify *Hannemania* to a species level, as past studies only cited *Hannemania* as sp.

In addition, one specimen of *R. major* was found infested with an oribatid mite (2%, 1/49). This could be the first record of an oribatid being parasitic. However, it was not possible to establish true parasitism, and some Oribatid mites can have phoretic behavior. Phoretic oribatid mites have been recorded on insects, mainly beetles. They attach by grasping a hair between the aspis and genital plate (NORTON, 1980). Additionally, phoretic oribatid mites have also been recorded on triatomine bugs and harvestmen (TOWNSEND et al., 2008; WALECKX et al., 2018). But most importantly, a species from the same genus as the one identified here was found on *E. pustulosus* from Panama. The species of mite was *Archegozetes magnus* (Sellnick, 1925) (BEATY et al., 2013). Nevertheless, this phoretic behavior has not been reported before in *A. longisetosus*, which is pantropical and has similar behaviors and life cycle as *A. magnus*. Moreover, differently that was seen on the *E. pustulosus*, where the frog was apparently healthy, and the skin seemed undamaged skin, in the *R. major* examined here, the skin seemed thicker, and had a lichenification aspect. Mites (48 adults and nymphs) appeared to be attached to this layer over the skin. Thus, it is still possible that mites were feeding on the skin exudates, which would imply them being parasitic. Still, further in-depth investigation should be held to establish the relationship between

the anuran and the oribatid mite species, and whether it implies a true parasitism or an active phoresy.

Regarding the infestation rates of each species identified from examined hosts, species of mites from the Trombidiformes order that had high infestation rates (Pterygosomatidae), also were the least harmful to their hosts, despite some species (*G. harrisi*) having high MI and MA (>40 mites per host). The other species and orders of mites showed low parasitic loads (MI of 1 to 16 and MA from 0.03 to 2.35). Furthermore, from the 13 species of Trombidiformes identified, *E. alfreddugesi* was the most abundant in terms of number of host species and individuals infested (33 individuals of 16 species of reptiles and amphibians). Also, considering the number of examined and infested hosts, the PI for this species was high (19.9%) as well as its MI (15.4 mites per host). Mesostigmata mites, though rare, seemed to have an impact on the overall health status of their ophidian host. And Ixodida, yet slightly more common, also showed low preferred locations, but also different degrees of deleterious effects on the host. Also, the genus that presented the highest apparent negative impact of the hosts were the species of the genus *Hannemania*, considering the preferred locations of infestation and the MI (3.62 to 4.5).

Considering the infestation rates, and parasitic niches and preferred locations, mites and ticks generally attached to the anterior portion of snakes, mainly on the lateral anterior scales (**LAS**). Species of Acari that preferred this location were: *O. parkeri*, *E. alfreddugesi*, and *F. anguina* (Trombidiformes); of *Chironoius* sp.n., *O. rotundus*, and *Z. oudemansi* (Mesotigmata); *Ornithodoros* (*Alectorobius*) sp. n., *A. dissimile* and *A. rotundatum* (Ixodida). This location on the host has various advantages for the infesting parasites. For the smallest species (Trombidiformes and Mesostigmata), the space in-between scales of this portion of the body is wider and more elastic, thus providing full cover of the mite, and well as a well irrigated spot, with enough soft connective tissue to attach. Also, the lateral or anterior area near the head is the least reachable area of the entire body of the snake, which makes the host unable to remove the parasites (FAIN, 1994; FAFJER, 2012). For the larger species (Ixodida), larvae and some nymphs can occupy the **LAS**, but the engorged nymphs and adults are not fully protected and thus prefer the regions of the gular area or head (NOWAK, 2010). Sometimes, ticks can be seen infesting areas, such as the periocular area (**Poa**), which can lead to vision impairment, inflammation, and lenticular scale retention (LAWTON, 2006). Furthermore, another protected area recently recorded, is the oral cavity of snakes (ATTACHMENT 2), that some larvae of ticks can infest and remain attached.

Ticks have a long survival time under water, thus this environment low on oxygen can offer a secure space for the parasite to develop (MENDOZA-COLELLA, 2019). Thus, in the routine examination, oral cavity should be assessed for possible ectoparasites.

Overall, snakes did not show signs of health issues related to infestation of mites and ticks. Nonetheless, *Ophioptes* mites produce a cavitary lesion on the scales, which can lead to infections, and dysecdysis (retention of the molted skin) (MENDOZA-ROLDAN et al., 2017). On the other hand, co-infestations (presented only on snakes), also were implied in the detriment of the health status of the hosts. The snake *P. natteteri* infested with *E. alfreddugesi* (Trombidiformes), and *Ornithodoros (Alectorobius)* sp. n., at the moment of examination, was dehydrated and debilitated, and died few days after. Snakes can maintain a very low parasitic load and remain healthy, thus why most of the infested snakes did not show signs of sickness (HARKEWICZ, 2001; UJVARI; MADSEN, 2005; PANDIT et al., 2011). Also, snakes are very susceptible to ticks and there are some scarce cases of tick paralysis or toxicosis (HANSON et al., 2007). However, reports of tick toxicosis and paralysis in reptiles are few and unconvincing. Even so, high parasitic loads of ticks can kill the snake host very fast. Animals usually present anorexia, weakening, dysecdysis, anemia, oral congestion, edema and caseous exudate, mucous oral and nasal discharges, diarrhea, dermatitis and cutaneous abscesses (ARAGÃO, 1912; RODRIGUES et al., 2010). Moreover, this myriad of clinical manifestations can occur in any case of ectoparasitism in reptiles. High infestations are associated with loss of appetite, depression, dysecdysis, dermatitis, ulcers and abscesses (CASTRO et al., 2019). This leads to immunosuppression and finally death (JACOBSON, 2007; MADER; DIVERS, 2013).

Regarding lizard mites, Trombidiformes mites (Trombiculidae and Pterygosomatidae) were mainly associated to the ventral celomatic area, and some species to the pocket-like structures. From the Pterygosomatidae, *G. hemidactyli*, *G. bataviensi* and *G. harrisi* occurred mostly on the **VCa** (Ventral Celomatic area), and *B. jimenezi* on the anterior region (Peri-ocular area and forearm). The pocket-like structures were occupied in general by Trombiculidae mites *E. alfreddugesi* and *E. ophidica* were mainly found in the **Pax** (auxiliary mite Pocket). Former investigations have revealed this phenomenon of distribution of parasitic mites. Trombiculidae mites have a high aggregation to mite-pocket structures, and Pterygosomatidae and Macronyssidae mites are spread throughout the host body, under the scales (HEATH; WHITAKER, 2015; DE OLIVEIRA et al., 2019; MENDOZA-ROLDAN et al., 2019). Lizards, differently from snakes,

have adapted to endure high parasitic loads with minimum effects on their health. Former research has demonstrated this, with some species of lizards (Lacertidae, Gekkonidae, Tropiduridae) being able to sustain a high parasitic load, with no apparent negative impact on the animals, and also high prevalence with almost all the population of lizards infested (MORITZ et al., 199; ROCHA et al., 2008; DE OLIVEIRA et al., 2019; MENDOZA-ROLDAN et al., 2019). Moreover, in some studies on other regions such as the Palearctic, Nearctic and Ethiopic regions, showed high parasitic loads of ticks on lizards, also with no apparent negative effect on the host health (PRENDEVILLE; HANLEY, 2000; SOUALAH-ALILA et al., 2015; DUDEK et al., 2016; MENDOZA-ROLDAN et al., 2019). Nonetheless, in the present work, ticks were not found in high parasitic loads on lizards, which can mean that differently from other regions, lizards are not intermediate hosts used in immature stages, as some species of *Ixodes* infest as larvae and nymphs (*Ixodes pacificus* in the Nearctic region, and *Ixodes ricinus* in the Palearctic region). This may also mean that lizards do not have an important role on the epidemiological chain of some important vector-borne diseases, such as Lyme disease (*Borrelia Burgdorferi* sensu lato), on the Neotropical region. Which in other hand, could imply that natural reservoirs for this disease in the region are other highly infested animals such as birds and small mammals (BARBIERI et al., 2013; OGRZEWALSKA et al., 2016; DE OLIVEIRA et al., 2018).

Additionally, assessing blood smears allows to correlate hemoparasitic presence with ectoparasitic prevalence, parasitic load and host overall health status (AMO et al., 2005; CERVONE et al., 2016; TELFORD, 2016). Contrarily of what expected, blood parasites were found in snakes rather than in lizards. Generally, high ectoparasitic loads of mites and ticks on lizards are followed by hemoparasites, as these protozoa depend on the invertebrate vector to perform their sexual reproduction and sporogonic development (LAINSON et al., 2003; HARRIS et al., 2015; TELFORD, 2016). Also, lizards have a higher diversity of species of hemoparasites (HARRIS, et al., 2015).

In snakes, it is common not to find parasites present in blood. Some studies show a low prevalence of less than 1% of infected erythrocytes (SANTOS et al., 2005; GLASER et al., 2008). Nonetheless, here six snakes had intraerythrocytic parasites (12.5% of the smears) Molecular diagnosis of the protozoa parasites is further discussed in chapter 6. Four had *Hepatozoon* gamonts (*C. multiventris, C. scurrulus, C. hortullanus,* and *P. viridissima*). *Hepatozoon* is an Apicomplexa, obligate intraerythrocytic parasite that uses a numerous type of invertebrate vectors.

Acari vectors, for reptiles and amphibians that have been identified are: the Trombidiformes mites (Pterygosomatidae) *Hirstiella*; the Mesostigmata mites (Macronyssidae) *Ophionyssus* (Ramanandan Shanavas and Ramachandran, 1990); and the Ixodidae ticks *A. dissimile*, and *Hyalomma cf. aegyptium* (TELFORD, 2016). Generally, infection of the vertebrate host is by passive transmission ingesting the invertebrate vector. However, on snakes, the mechanism of infection is still an open question. Possibilities include passive transmission, ingestion of other vertebrate hosts with monozoic or dizoic cysts in their tissues, or salivary transmission during feeding by mosquito vectors in nature, and even vertical transmission is possible (O'DWYER et al., 2003; FERGUSON et al., 2013; KAUFFMAN et al., 2017; CALIL et al., 2019). Here, the snakes that had *Hepatozoon* gamonts, were infested by one species (*C. multiventris* and *C. hortullanus* with *A. rotundatum* ticks), or co-infested (*C. multiventris* and *C. scurrulus* with three species and two species, respectively). Also, it is noticeable that all the positive samples came from the north region (Acre state), and all snakes were wild caught. Despite the infection of *Hepatozoon* and the infestation of ectoparasites, none of the snakes had sings of disease related to the parasitic load. Generally, pathology, even in massive infection, usually appears mild. Even so, in snakes, *Hepatozoon* infections can cause negative effects such as granulomatous hepatitis (WOZNIAK et al., 1996; 1998). Furthermore, morphological identification of *Hepatozoon* is rather difficult, as the gamonts of most of the species are very similar and can even be confused with other hemoparasites such as *Karyolysus* and *Hemolivia*. Thus, final identification must be accompanied by molecular diagnosis. This part is discussed further in chapter 6. Moreover, one snake (*O. melanogenys*) had gamonts compatible with *Hepatozoon*, yet as stated before, *Karyolysus* and *Hemolivia* species are very similar to *Hepatozoon*, and morphological identification by its own is not enough. At first, the gamonts seen on this snake were categorized as *Karyolysus*, due to the severe distortion and sometimes apparent lysis of the nucleus inside erythrocytes. However, when identifying hemogregarines of snakes, most of the times they are *Hepatozoon* (TELFORD, 2016).

Finally, one snake (*C. multiventris*) in addition to having *Hepatozoon* gamonts, also had intraerythrocytic inclusions compatible with *Iridovirus*, also known as snake erythrocytic Virus. This virus was described as a protozoon called *Toddia.* It was then described as a virus that produces erythrocytic inclusions associated with crystalloid bodies (rectangular, square, hexagonal, or rod-like) (FRANÇA, 1911). In Brazil, this virus has been reported in viper snakes

(four species of *Bothrops*), and one species of *Chironius* (*Chironius flavolineatus*) (DE SOUSA et al., 2013). Here, the crystalloid inclusions were quadrangular, as in the *C. flavolineatus*, while on the *Bothrops* they were hexagonal. Moreover, the clinical significance of this virus is not well understood, though it is believed to induce anemia, immunosuppression, and septicemia (WELLEHAN et al., 2008). Also, the ecology of erythrocytic iridoviruses is also unclear. Given the location in the erythrocytes could mean that this virus had a blood-borne transmission. Thus, it is possible that hematophagous arthropods have some role in virus transmission (JOHNSRUDE, 1997; WELLEHAN et al., 2008; TELFORD, 2016). Hence, the presence of three species of Acari is important in this case and should be further studied. Thus, here a new host for this virus is reported for Brazil and furthers the theory of vector-borne transmission.

The histologic slides of amphibians helped better characterized the typical lesion produced by intradermic mites of the genus *Hannemania*. Considering the infestation rates and parasitic niches on frogs and the lesions produced, these mites can have a negative impact on their host. Firstly, both species identified, *H. achalai* and *H. hepatica*, had high prevalence among the examined frogs (65% and 44% respectively). Also, one of the preferred locations on the host was the Digits **(Di)**, with both species having 21% of the mites there. Generally, the inflammation associated with these mites is frequently mild and consists of macrophages and fibrous connective tissue. However, a high parasitic load of intradermic mites in amphibians is associated with disruption of normal physiologic mechanisms such as transdermal respiration, and secondary bacterial, viral, or fungal infections. In the case of the digits, as the size of the mite increases when engorged, and with the high number of mites in the digits, avascular necrosis can be seen and host can suffer from digit to limb loss (BROWN et al., 2006; ESPINO DEL CASTILLO et al., 2011). Furthermore, it is important to note that some of the species of anurans infested are critically endangered, thus a high parasitic load can affect the population overall health status. This requires further investigation, as mites can be used as ecological sensors of population health.

6 CONCLUSIONS

1. Reptiles and amphibians totalizing 4,515 specimens were examined, of which 170 were infested with mites and ticks (overall PI of 3.8%).
2. Of all the examined hosts, 3.4% (121/3,596) were infested reptiles, and 5.3% were infested amphibians (49/919).
3. Of the 4,515 examined hosts, most were snakes, due to the large number of snakes that were received by the IBPS laboratories. Nonetheless, 42.3% of all the 170 infested individuals were lizards.
4. The Trombidiformes order (Trombiculidae and Pterygosomatidae) was the main order parasitizing lizards, and no co-infestations were seen in lizards.
5. Of reptiles, the most examined hosts were snakes, yet the most infested type of hosts were lizards.
6. The order Mesostigmata was the less prevalent order of ectoparasites identified, and it was identified only on Squamata reptiles.
7. In amphibians, Ixodida was the most prevalent order (63.2%), followed by Trombidiformes of the family Leeuwenhoekiidae (genus *Hannemania*) (34.6%), and lastly Oribatida.
8. Examining animals for ectoparasites that arrive in the laboratories of the IBSP should be added to the quarantine protocols. This procedure should be continued also because the other Mesostigmata mites, identified here, came from snakes and lizards kept in captive conditions (mostly infested by the Macronyssidae mite *O. natricis*).
9. Species of ticks found here have a low host specificity, being able to infest many other classes of hosts. Thus, the low specificity allows the species to colonize diverse biomes, making its distribution wide throughout the Brazilian territory.
10. In Brazil, all records in infestations of *Hannemania* are on frogs.
11. *Rhinella* toads are likely to be infested with *Amblyomma* ticks, and frogs are more prone to be infested with *Hannemania* larvae.
12. The present study is one of the firsts to identify *Hannemania* to a species level, as past studies only cited *Hannemania* as sp.

13. *R. major* was found infested with an oribatid mite and this could be the first record of an oribatid being parasitic. However, it was not possible to establish true parasitism, and some Oribatid mites can have phoretic behavior.
14. Species of mites from the Trombidiformes order that had high infestation rates (Pterygosomatidae), also were the least harmful to their hosts (Lizards).
15. From the 13 species of Trombidiformes identified, *E. alfreddugesi* was the most abundant in terms of number of host species and individuals infested.
16. Mesostigmata mites, though rare, seemed to have an impact on the overall health status of their ophidian host. And Ixodida, yet slightly more common, also showed low preferred locations, but also different degrees of deleterious effects on the host. Moreover, the genus that presented the highest apparent negative impact of the hosts were the species of the genus *Hannemania*.
17. Mites and ticks generally attached to the anterior portion of snakes, mainly on the lateral anterior scales (**LAS**), and snakes did not show signs of health issues related to infestation of mites and ticks. Snakes can maintain a very low parasitic load and remain healthy, thus why most of the infested snakes did not show signs of sickness.
18. Co-infestations (presented only on snakes), were implied in the detriment of the health status of the hosts.
19. Lizard mites generally attached to the ventral celomatic area (Pterygosomatidae), and some species to the pocket-like structures (Trombiculidae).
20. Lizards, differently from snakes, have adapted to endure high parasitic loads with minimum effects on their health.
21. Assessing blood smears allows to correlate hemoparasitic presence with ectoparasitic prevalence, parasitic load and host overall health status, and contrarily of what expected, blood parasites were found in snakes rather than in lizards.
22. Five snakes had *Hepatozoon* gamonts, and despite the infection of *Hepatozoon* and the infestation of ectoparasites, none of the snakes had sings of disease related to the parasitic load.
23. One snake (*C. multiventris*) in addition to having *Hepatozoon* gamonts, also had intraerythrocytic inclusions compatible with *Iridovirus*, also known as snake erythrocytic Virus. Given the location of the virus in the erythrocytes could mean that this virus had a

blood-borne transmission. Thus, it is possible that hematophagous arthropods have some role in virus transmission species of acari is important in this case and should be further studied.

24. The histologic slides of amphibians helped better characterized the typical lesion produced by intradermic mites of the genus *Hannemania*, and, these mites can have a negative impact on their host, due to had high prevalence among the examined frogs, and because one of the preferred locations on the host was the Digits **(Di)**.
25. In the case of the digits, as the size of the mite increases when engorged, and with the high number of mites in the digits, avascular necrosis can be seen, and host can suffer from digit to limb loss.
26. Some of the species of anurans infested are critically endangered, thus a high parasitic load can affect the population overall health status.

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ATTACHMENT 2 - *Ixodes ricinus* infesting snakes: Insights on a new tick-host association in a *Borrelia burgdorferi* sensu lato endemic area[3]

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Ixodes ricinus infesting snakes: Insights on a new tick-host association in a *Borrelia burgdorferi* sensu lato endemic area

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ABSTRACT

The castor bean tick *Ixodes ricinus* is one of the most abundant tick species in Europe, being able to parasitize a wide number of vertebrate hosts, including mammals, birds, and reptiles. This tick species has an important role as vector of zoonotic pathogens, including the causative agents of Lyme borreliosis (i.e. *Borrelia burgdorferi* sensu lato). Here, we provide insights on a new tick-host association (i.e. *I. ricinus* infesting snakes) in an area recently recognized as endemic for reptile-associated zoonotic species of *Borrelia burgdorferi* s.l.

1. Introduction

Reptiles are parasitized by more than 500 species of mites and ticks,

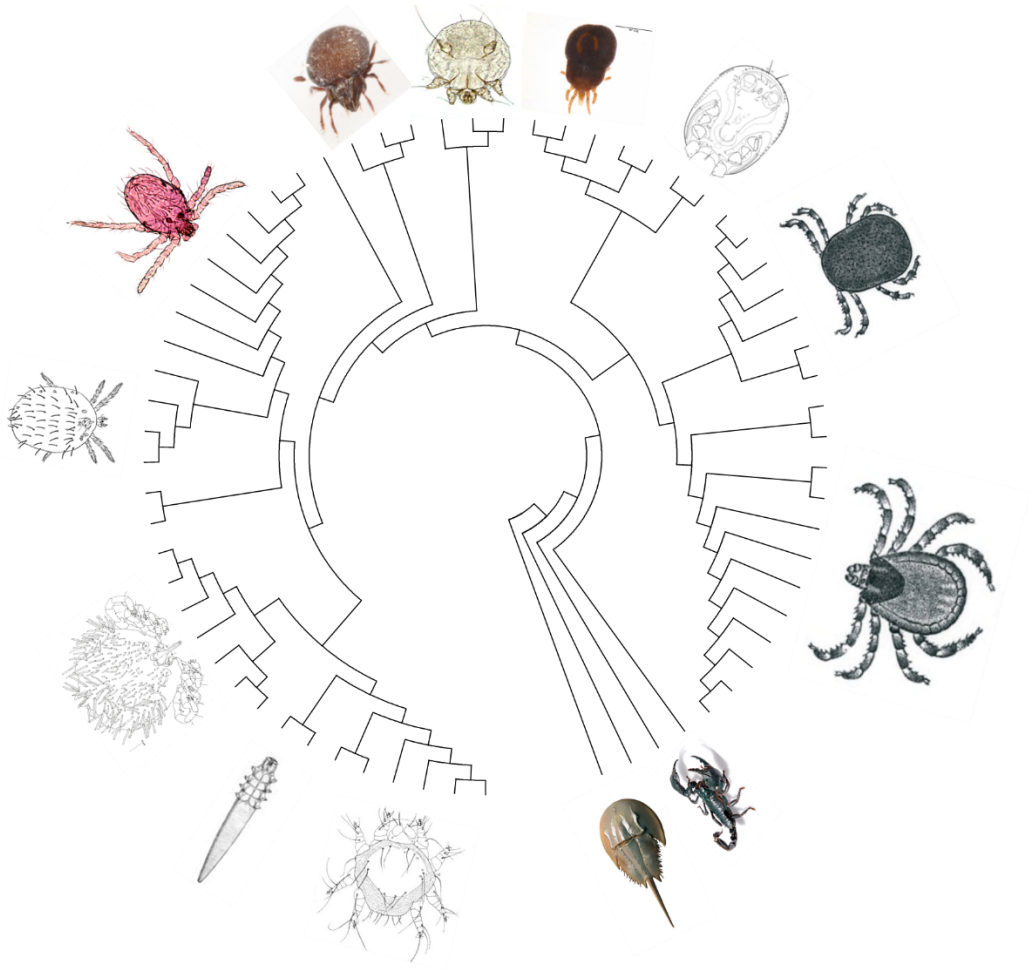
2. Material and methods

2.1. Ethics statement

[3] MENDOZA-ROLDAN, Jairo Alfonso; COLELLA, Vito. *Ixodes ricinus* infesting snakes: Insights on a new tick-host association in a *Borrelia burgdorferi* sensu lato endemic area. **Acta Tropica**, v. 193, p. 35-37, 2019.

PART II:

MOLECULAR BIOLOGY



(Mendoza-Roldan, 2019)

CHAPTER V: Phylogeny of Acari from reptiles and amphibians

1 INTRODUCTION

The subclass Acari (includes mites and ticks) is a highly diverse group inside the Arachnida class, of the subphylum Chelicerata. More than 50,000 specie are known to science, though this number can be unrepresentative of the estimated 1,000,000 species yet to discover (ALBERTI, 2005; DUNLOP; ALBERTI, 2008). Despite this diversity, and the medical and veterinary importance some of these species have (parasitic mites and ticks), the origin of this morphologically diverse group is still open for debate. It is still not clear if they originated from a single ancestor or from two or more arachnid ancestors with morphological characters shared by a set of species but not present in their common ancestor (homoplasies) (PEPATO et al., 2010). Moreover, there are records of fossils as old as the early Devonian period. Furthermore, Acari is grouped morphologically, depending on the following characters: larvae are generally hexapod; nymphal stages can be one or three and are mostly octopod; the hypostome is formed by the fusion of the ends of the palpal coxae; and no evident idiosomal segmentation (VARMA, 1993; ALBERTI, 2000). These few synapomorphies is one of the reasons the monophyly of this group is still unclear, as well as the relationships of mites and ticks with other arachnids.

Additionally, the taxonomy and systematics of the Acari is quite complex, with a wide set of taxonomic ranks to classify the different groups. Modern systematics considers Acari as a subclass of the Arachnida, which is divided in two main superorders: Acariformes and Parasitiformes, and some consider Opilioacariformes as a superorder the latter or also a subgroup within the Parasitiformes (KRANTZ, 2009; DHOORIA, 2016). Moreover, the Opilioacariformes consists of a single order and family (Opilioacarida, Opilioacaridae). The Acariformes is the most diverse group containing more than 300 families and over 30,000 species. This group is divided in the Sarcoptiformes (Oribatida and Astigmata) and Trombidiformes. Also, there is the Endeostigmata group that is usually considered a suborder of the Prostigmata. Additionally, the Parasitiformes is divided in three mayor orders: Ixodida, Holothyrida, and Mesostigmata (FULLER,1956; ZHANG, 2018). This diversity raises another question, on what would be considered a mite. Many of the characteristics mentions before for

Acarina are seen in other arachnids and even in other higher Chelicerata orders, and many apomorphic characteristics have been proposed for the Acari, though most of them are not shared with the Parasitiformes (WHEELER; HAYASHI, 1998; SHARMA, 2018).

1.1 Molecular phylogeny of Acari

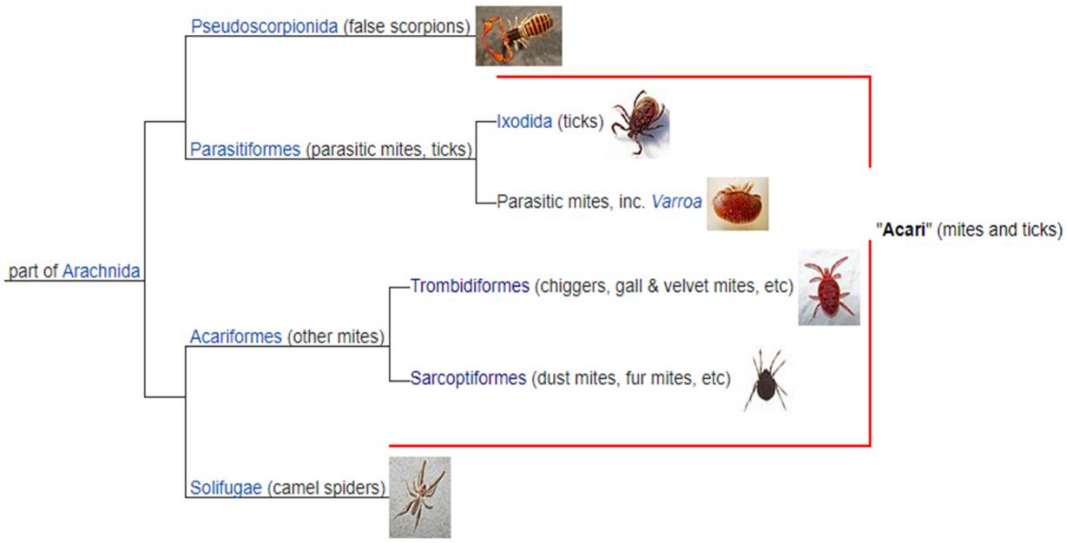
Molecular phylogeny is based on the preposition that the difference in nucleotides of a given number of sequences should show for how long those sequences share a common ancestor (divergence) (FIELD et al., 1988). DNA markers have shown over and over that they are the most reliable source of data for phylogenetic analyses. Though, some difficulties need to be addressed when performing such analyses, for example which fragment of which genome should be used. Additionally, sequences of different fragments have a myriad of functions, causing differences in rates of nucleotide substitutions and deletion. Thus, choosing the molecular marker to use, depending on the phylogenetical question to address, is the most important matter, therefore avoids producing misleading data (DABERT, 2006).

Furthermore, The phylogeny of the Acari is still on debate, with different taxonomic systems currently used. The most accepted classification divides the Acari of six orders, grouped into three superorders: Opilioacariformes, Parasitiformes (Holothyrida, Ixodida and Mesostigmata), Acariformes, divided in Trombidiformes (Sphaerolichida and Prostigmata) and Sarcoptiformes (Oribatida and Astigmata) (KRANTZ, 2009).

Recent molecular studies have pointed out that that Acari is polyphyletic (set of organisms, grouped together but do not share a common ancestor), with ticks and spiders being more related than ticks and mites (KLOMPEN et al., 2007; DABERT et al., 2010; PEPATO et al., 2010). These analyses were based on the use of the 18S rDNA, and strongly supported grouping the Acari into two monophyletic groups: Acariformes and Parasitiformes. These molecular analyses revealed that the order Solifugae is a sister group of Acariformes. Also analyses showed Pseudoscorpionida as the sister group of Parasitiformes (Figure 107). The Acariformes–Solifugae relationship was implied in former studies that pointed out similarities in the morphology of Solifugae and Oribatida (GRANDJEAN, 1936; 1954). Other studies showed the similarities of the tracheal systems in solifugids and Prostigmata mites (REUTER, 1909; CODDINGTON, 2004). On the other hand, the relationship between Parasitiformes and Pseudoscorpionida is not shown or demonstrated on

studies based on morphological characters. However, this relationship has been recovered before as Pseudoscorpionida being sister group of for Opilioacariformes and Parasitiformes in other molecular analysis 18S rDNA (GIRIBET et al., 2002; SHULTZ, 2007).

Figure 107 – Current phylogeny of Acari



Source: based on (DABERT et al, 2016), adapted from (wikipedia.org/wiki/Acari)

1.2 Molecular markers used in Acari Phylogeny and Barcoding

1.2.1 18S ribosomal RNA (rDNAs)

These markers are the most used due to the conservation of many regions of their nucleotide sequences. Moreover, eucaryotes nuclear rRNA genes have identical or almost identical sequences, thus making it easier to amplify and sequence these fragments. Due to its size, and slow rate of evolution, the 18S rRNA gene is the most frequently sequenced for molecular phylogeny studies (VAN DE PEER et al. 2000; OTTO; WILSON, 2001; KLOMPEN et al., 2007; DABERT et al., 2010; PEPATO et al., 2010). Also, the whole sequence of 18S rDNA is easy to amplify by PCR and some regions are conserved, which allows to align them easily. These sequences have been used mainly to reconstruct the phylogeny of tick groups and allowed to infer recent

Acariformes phylogenies (BLACK et al., 1997; DOBSON; BARKER, 1999). The variable region V4 has been used to infer family phylogenies. Also, it is well conserved to be easily aligned and provide accurate phylogenetic information within super families and cohorts (OTTO; WILSON, 2001).

1.2.2 Mitochondrial DNA (mtDNA)

These types of molecular markers have a higher rate of base substitution than most nuclear genes, thus they are useful for studying clades that have diverged relatively recently, and lower categories such as genera and species (CUROLE; KOCHER, 1999; DABERT, 2006). The high variability in mites amino acids makes it difficult to use universal primers for amplifying specific regions in mtDNA of the Acari. The 16S rDNA has been used to resolve the phylogeny of ticks (Ixodidae and Argasidae), and some fewer studies on mite phylogeny (BLACK; PIESMAN, 1994; MANGOLD et al., 1998; BARKER; MURRELL, 2003). On the other hand, the cytochrome oxidase subunit I (COI) is used for mite phylogeny due to its strong sequence conservation among taxa. Nonetheless, COI has a faster rate of nucleotide divergence, yet the rate of substitutions within the gene could allow comparisons among lower taxonomic levels (species and genera) (OTTO; WILSON, 2001; DABERT et al., 2010; PEPATO et al., 2010).

2 OBJECTIVES

- Asses the phylogenetic relationships of the mites and ticks associated to ectothermic hosts applying molecular phylogeny of selected molecular markers (18s rRNA, COI and 16s rRNA).
- Use the different molecular markers (18s rRNA, COI, and 16s rRNA) for endogenous control of mites and ticks.
- Evaluate the usefulness of the 18s rRNA, COI, for barcoding and phylogeny and of mites (Trombidiformes and Mesostigmata).
- Use 16s rRNA for barcoding and phylogeny of species of ticks (Ixodida).

3 MATERIAL AND METHODS

3.1 DNA extraction of mites and ticks

The standardization of a reliable DNA extraction method for different genera of Trombidiformes based on lysis with guanidine isothiocyanate protocol (GT) (CHOMKZYNSKI, 1993), was established, which allowed the preservation of a voucher (MENDOZA-ROLDAN, 2015). Later, this technique was applied successfully in Mesostigmata, and Ixodida from reptiles (MENDOZA-ROLDAN et al., 2019). Thus, in the present study the same protocol was used for the collected mites in the IBSP laboratories of field trips. Extractions were performed of one specimen or in pools. Mites and ticks were placed in a sterile microtube (Eppendorf), and each individual was punctured in the idiosoma with a sterile needle (1.20 * 40 – 18G). After, 30 µl of sterile TE buffer were added and the Acari were gently crushed, avoiding destruction. 120 µl sterile TE buffer was added to obtain a final volume of 150 µl, and then homogenized for 15 seconds. 450 µl of GT were added and the sample was homogenized for 15 seconds. Microtubes were homogenized every 2.5 minutes, for 10 minutes. Later, chloroform was added, microtubes were homogenized for 15 seconds, and left to rest for 2 minutes. Then they were centrifuged at 12000 rpm for 5 minutes and the supernatant was recovered and placed on a steril microtube (1.5 mL). The lower layer containing the mites and ticks was recovered to create the vouchers (mounted on slides). 600 µl of isopropyl alcohol were added to the supernatant and the microtubes were incubated for 24 hours at -20° C. after incubation, samples were centrifuged at 4° C (12000 rpm for 15 minutes), then the supernatant was discarded and 800 µl of ethanol 70% were added. Samples were then centrifugated at 4° C for 5 minutes and then the supernatant was discarded. The “pellet” was maintained at 56° C for 10 minutes or dried at room temperature. When samples were totally dry, they were resuspended in 25 – 50 µl of sterile TE buffer and homogenized. Finally, samples were incubated for 15 minutes at 56° C and after freezed at -20° C.

3.2 Quantification of extracted DNA

To assess the quantity and quality of the obtained DNA, quantification of the samples was performed using a spectrophotometer with wavelength of 260 nm (Nanodrop 2000 Spectrophotometer® UV-Vis, ThermoScientific, USA).

3.3 Polymerase chain reaction (PCR)

PCRs of mites of the orders Trombidiformes and Mesostigmata were performed for endogenous control, barcoding and molecular phylogeny. Primers of the gene 18S rRNA (18S+ and 18S-), that amplify a fragment of the V4 region were used of 480 bp (OTTO; WILSON, 2001). These primers were used in a previous study, and it showed to be informative to recover relationships between families, cohorts and genera (MENDOZA-ROLDAN et al.,, 2017). Also, primers of the mitochondrial cytochrome oxidase of the subunits I of the UEA5/UEA8 region (COI-2F e COI-2R) (OTTO; WILSON, 2001), and (COI 1: CI-J-1571 e CI-N-2191) (SIMON et al., 1999) were used. For all the reactions negative controls (autoclaved and DNA-free Milli-Q water), and positive controls (Trombiculidae mites pools) were used. The PCR cycle conditions were described by Otto & Wilson (2001). Reactions were performed in thermocycler Mastercycler Gradient (Eppendorf® California, USA), with the following cycles: initial denaturation at 94 ° C for 1 minute, followed by 30 cycles of 20 seconds at 94 ° C, 50 ° C for 30 seconds and 72 ° C for 1minute and 30 seconds, with a final cycle lowering the temperature to 25 ° C (Table 34).

PCRs of ticks were performed for endogenous control, barcoding and molecular phylogeny of the genus *Ornithodoros*. Primers of the gene 16S+ e 16S-, which amplify a 460 bp fragment of the mitochondrial 16S rRNA gene from practically all tick species (MANGOLD et al., 1998). The conditions of the PCR cycles were: initial denaturation at 94 ° C for 3 minutes, followed by 11 cycles of 30 seconds at 94 ° C, 30 seconds at 48 ° C, initial extension at 72 ° C for 40 seconds and final extension at 94 ° C for 30 seconds (Table 35).

Table 35 – List of primers used for molecular phylogeny of Acari

Gene/ primers	Agent	Sequence (5' - 3')	Reference
18s rRNA	Mites		

Mite18S-1F		ATATTGGAGGGCAAGTCTGG	(OTTO;
Mite18S-1R		TGGCATCGTTTATGGTTAG	WILSON, 2001)
COI-1	Mites		
CI-J-175I		GGWGCWCCWGAYATRGCWTTYCC	(SIMON et. al,
CI-N-219I		GGWARAATTAAAATATAWACTTC	1999)
COI-2	Mites		
Mite COI-2F		TTYGAYCCIDYIGGRGGAGGAGATCC	(OTTO;
Mite COI-2R		GGRTARTCWGARTAWCGNCGWGGTAT	WILSON, 2001)
16S	Ticks		
16S +		F- CCGGTCTGAACTCAGATCAAGT	MANGOLD et
16S -		R- GCTCAATGATTTTTTAAATTGCTGT	al., 1998

Source: (MENDOZA-ROLDAN, J. A., 2019)

3.4 Reading and analysis of PCR products

All PCR products (5 µL amplified DNA) were subjected to 1.5% agarose gel horizontal electrophoresis [1.5 mg Ultra-Pure Agarose Invitrogen® Carlsbad, CA; 100 mL of 1X TAE (121g Tris Base, 28.5 mL glacial acetic acid, 50 mL of 0.5 M EDTA pH 8.0 H2O milli-Q qsp)] plus SYBR® Safe DNA Gel Stain (0.1 µl / mL) and 1X TAE running buffer pH 8.0 at 100V / 80mA. The gel was visualized with ultraviolet light (UV) in a darkroom (Alphalmager®). The samples that revealed DNA bands same level as the positive control, confirming the nucleotide amplification, were considered positive for the PCR reaction used.

3.5 Purification and Sequencing of Nucleotides

Samples of amplified products of the PCRs that had concentrations above 20 ng / µl were selected. The amplified products were then subjected to DNA purification through the commercial product ExoSap-IT (USB Corporation). Part of the purified samples were sequenced at the Center for Human Genome and Stem Cell Research at the Institute of Biosciences- USP, and others at the Bacteriology Laboratory - Unit 2, of the Instituto Butantan. Sanger sequencing was performed, which is for DNA from PCR products and plasmids, using the ABI 3730 DNA Analyzer. This is a 48-capillary DNA analysis system with Life Technologies-Applied Biosystems technology. Sequencing reactions were performed by the BigDye Terminator v3.1 Cycle Sequencing Kit. The runs were done in 36 cm capillaries using the POP7 polymer.

3.6 Sequence analysis

The sequences obtained were edited using the SeqMan program (Lasergene, DNAstar, Madison, Wis.) and also analyzed using Geneious version 11.1.4 software and submitted to identify similarities to known sequences using the Basic Local Alignment Search Tool (BLAST) (ALTSCHUL et al., 1990) to verify homology with corresponding sequences available from GenBank.

3.7 Phylogenetic analyses

Phylogenetic trees were inferred by the Maximum Likelihood (ML) method, Maximum Parsimony (MP), and Bayesian analysis for the 18S rRNA gene from mite families. ML trees were constructed using the MEGA 7 program (KUMAR, et al., 2016). Alignments were performed with the ClustalW program (HUNG; WENG et al., 2016) and was manually adjusted in the GeneDoc v. 2.6.01 (NICHOLAS et al., 1997). The alignments were also submitted to ML analysis using the MEGA 7 program (KUMAR, et al., 2016), among the 56 available sequence evolution models, the one that best explains the sequence lineage obtained was used. This method allows estimating the relative probability of the data obtained fit a given tree and a model that describes the evolution process. The probability is calculated for all possible topologies by varying the size of the branches. Thus, the tree with the highest likelihood (relative probability) is considered the best estimate of the phylogeny. In order to determine the values that support each arm of the phylogenetic tree we use the statistical method "bootstrap" (FELSENSTEIN, 1985). Thus, the greater the number of times a given arm occurs in the estimate, the greater the confidence of the arm's existence. Maximum Parsimony (MP) was also performed in Mega7 and due to the number of taxa, the analysis was made using heuristic algorithms to search for the most parsimonious tree. Parsimony methods look for the tree that minimizes the number of steps (nucleotide or amino acid substitution) to explain the patterns observed in the data. Additionally, Bayesian analyses were performed with the program MrBayes v3.1.2 (HUELSENBECK; RONQUIST, 2001) with 2,000,000 generations. The first 25% of these trees represented the "Burn in" and the rest of the trees were used to calculate Bayesian analyses.

ML and Bayesian analyses were also performed to confirm the morphological identification of argasid tick *Ornithodoros (Alectorobius)* sp. Amplified sequences were aligned using ClustalW and with the corresponding mitochondrial 16S rDNA sequences of *Ornithodoros.* In addition, sequences of other Argasidae species available in the GenBank database were included and some *Ixodes* sequences were used as outgroups. Phylogenetic analyses were carried out using the maximum likelihood (ML) method with the program MEGA 7. Support was tested with 2000 bootstrap pseudoreplicates and Bayesian analyses were performed with the program MrBayes v3.1.2 (HUELSENBECK; RONQUIST, 2001) with 2,000,000 generations. The first 25% of these trees represented the "Burn in" and the rest of the trees were used to calculate Bayesian analyses.

4 RESULTS

4.1 DNA Extraction and Endogenous Control

The method of DNA extraction of mites and ticks with lysis protocol with guanidine isothiocyanate (GT) showed good results (amount of DNA in ng / μL) and allowed to obtain a satisfactory amount of DNA, even when extracted from individuals, and at the same time, preserving the voucher specimen. 139 samples of different mite and tick species (Tombidiformes, Mesostigmata, Ixodida) were used, analyzed individually or in pools of three or five specimens. Of these orders 12 species of Trombidiformes, four species of Mesostigmata and five species of Ixodida were analyzed. From the Trombidiformes, the number of samples were: one *A. longisetosus* (Oribatida), 18 *B. jimenezi*, 18 *E. alfreddugesi*, four *E. ophidica*, two *E. tropica*, one *F. anguina*, nine *G. harrisi*, nine *G. hemidactyli*, 20 *H. achalai*, six *H. hepatica*, two *O. parkeri*, and one *O. ekans.* The number of samples used for Mesostigmata were: six *O. natricis*, three *Z. oudemansi*, two *O. rotundus*, and one *Chironobius* sp. n. Finally, the number of samples used for ticks were: one *A. dissimile*, two *A. humerale*, 31 *O. rotundatum*, one *A. sculptum*, and two *Ornithodoros (Alectorobius)* (Table 35).

Furthermore, 74 samples (21 of 22 species) were amplified for the MITE 18S V4 gene; 31 samples (10 of 22 species) for COI 1 gene and eight samples (three of 22 species) for COI 2; and 35 samples of the 16S mRNA gene (all of the tick species) (Table 36).

Table 36 – Species of Acari, DNA quantification and results of genes used for mites and ticks

			Genes			
IBSP	**Species**	**GT (ng/ul)**	**18S V4**	**COI 1**	**COI 2**	**16S**
12911	*G. hemidactyli*	65.9	A	NA	A	-
12912	*G. hemidactyli*	25.7	A	NA	NA	-
12913	*G. hemidactyli*	25.5	A	A	A	-
12916	*G. hemidactyli*	84.4	A	NA	A	-
12930	*G. hemidactyli*	26.1	A	NA	NA	-
12931	*G. hemidactyli*	14.5	A	NA	A	-
12933	*G. hemidactyli*	14	A	NA	A	-
12940	*G. hemidactyli*	34	A	A	A	-
12908	*O. parkeri*	45.1	A	NA	A	-
12950	*E. alfreddugesi*	276.3	A	NA	NA	-
12951	*E. alfreddugesi*	94.4	A	NA	NA	-
12952	*E. alfreddugesi*	155	A	NA	NA	-
12917	*E. alfreddugesi*	73.6	NA	NA	NA	-
12955	*E. ophidica*	96	NA	NA	NA	-
12956	*E. ophidica*	65	A	NA	NA	-
12906	*E. tropica*	68.5	A	NA	A	-
12918	*H. achalai*	177.5	A	NA	NA	-
12919	*H. achalai*	212.2	NA	NA	NA	-
12920	*H. achalai*	270.6	NA	NA	NA	-
12921	*H. achalai*	160.9	NA	NA	NA	-
12922	*H. achalai*	56.9	NA	NA	NA	-
12923	*H. achalai*	76.6	NA	NA	NA	-
12924	*H. achalai*	124	NA	NA	NA	-
12925	*H. achalai*	51.6	A	NA	NA	-
12926	*H. achalai*	265.6	A	NA	NA	-
12927	*H. achalai*	84.5	NA	NA	NA	-
12928	*H. achalai*	221.5	NA	NA	NA	-
12929	*H. achalai*	316.7	NA	NA	NA	-
12934	*H. hepatica*	40.1	NA	NA	NA	-
12935	*H. hepatica*	51.6	NA	NA	NA	-

(Continues)

IBSP	Species	Genes				
		GT (ng/ul)	18S V4	COI 1	COI 2	16S
12957	*H. hepatica*	268	A	NA	NA	-
12932	*A. dissimile*	202.9	NA	NA	-	A
12910	*A. humerale*	127.7	A	A	-	A
12909	*A. rotundatum*	95.2	A	A	-	A
12915	*A. rotundatum*	103.3	A	A	-	A
12936	*A. rotundatum*	426.0	A	A	-	A
12937	*A. rotundatum*	289	A	A	-	A
12938	*A. rotundatum*	280	A	A	-	A
12939	*A. rotundatum*	90.6	A	A	NA	A
12954	*A. rotundatum*	93	A	A	NA	A
12978	*A. rotundatum*	63	A	A	NA	A
12907	*O. natricis*	126.4	A	NA	NA	-
12983	*O. natricis*	69.1	A	NA	NA	-
12586	*O. natricis*	45.3	A	NA	NA	-
12953	*H. achalai*	31.6	NA	NA	NA	-
12978	*A. rotundatum*	96	-	-	-	A
12992	*A. longisetosus*	92.7	A	A	NA	-
12990	*A. rotundatum*	143.6	-	NA	NA	A
12910	*A. humerale*	144	-	NA	NA	A
12908	*O. parkeri*	192.3	A	NA	NA	-
12907	*O. natricis*	486	A	NA	NA	-
12953	*Z. oudemansi*	59.4	A	NA	NA	-
12950	*E. alfreddugesi*	128.4	A	NA	NA	-
12951	*E. alfreddugesi*	231.7	A	NA	NA	-
12952	*E. alfreddugesi*	155.2	A	NA	NA	-
12925	*H. achalai*	121.1	A	NA	NA	-
12926	*H. achalai*	172.9	NA	NA	NA	-
12927	*H. achalai*	128.8	NA	NA	NA	-
12928	*H. achalai*	163.8	A	A	NA	-
12929	*H. achalai*	150.8	A	NA	NA	-
12934	*H. hepatica*	270.6	NA	NA	NA	-
12935	*H. hepatica*	160.5	NA	NA	NA	-
12918	*H. achalai*	162.5	NA	NA	NA	-
12919	*H. achalai*	98.5	NA	NA	NA	-
12983	*O. natricis*	342.4	NA	NA	NA	-
12955	*E. ophidica*	118.4	A	NA	NA	-
12956	*E. ophidica*	58.1	A	NA	NA	-

(Continues)

IBSP	Species	Genes				
		GT (ng/ul)	18S V4	COI 1	COI 2	16S
12906	*E. tropica*	182.7	A	A	NA	-
12921	*H. achalai*	156.9	A	NA	NA	-
14907	*O. ekans*	149.2	A	NA	NA	-
13660	*O. rotundus*	133.5	A	NA	NA	-
13766	*A. rotundatum*	122	-	-	-	A
13767	*A. rotundatum*	120	-	-	-	A
13768	*A. rotundatum*	110	-	-	-	A
14828	*E. alfreddugesi*	148.1	A	NA	NA	-
14829	*E. alfreddugesi*	85.8	A	A	NA	-
14830	*A. rotundatum*	84.2	-	-	-	A
14831	*E. alfreddugesi*	89	A	A	NA	-
14832	*A. sculptum*	554	-	-	-	A
14833	*E. alfreddugesi*	141.1	A	NA	NA	-
14834	*E. alfreddugesi*	126.1	A	NA	NA	-
14835	*E. alfreddugesi*	99.7	A	NA	NA	-
14836	*E. alfreddugesi*	208.6	A	NA	NA	-
14837	*G. hemidactyli*	125.8	A	A	NA	-
14838	*Ornithodoros (Alectorobius)*	90	A	NA	NA	-
14839	*E. alfreddugesi*	97.7	A	A	NA	-
14840	*E. alfreddugesi*	133.8	A	NA	NA	-
14845	*A. rotundatum*	266.5	-	-	-	A
14846	*B. jimenezi*	121.4	NA	NA	NA	-
14847	*B. jimenezi*	158.1	NA	NA	NA	-
14848	*B. jimenezi*	168.8	NA	NA	NA	-
14849	*B. jimenezi*	108.5	NA	NA	NA	-
14850	*B. jimenezi*	80.3	NA	NA	NA	-
14851	*B. jimenezi*	154.5	NA	NA	NA	-
14852	*B. jimenezi*	87.4	NA	NA	NA	-
14853	*B. jimenezi*	86.7	NA	NA	NA	-
14854	*B. jimenezi*	94.7	NA	NA	NA	-
14855	*B. jimenezi*	66.4	NA	NA	NA	-
14856	*B. jimenezi*	-	NA	NA	NA	-
14857	*B. jimenezi*	75.7	NA	NA	NA	-
14858	*B. jimenezi*	78	NA	NA	NA	-
14859	*B. jimenezi*	295.2	NA	NA	NA	-
14860	*B. jimenezi*	112.2	NA	NA	NA	-

(Continues)

IBSP	Species	Genes				
		GT (ng/ul)	18S V4	COI 1	COI 2	16S
14861	*B. jimenezi*	105.7	NA	NA	NA	-
14862	*B. jimenezi*	54.6	NA	NA	NA	-
14864	*A. rotundatum*	131.5	-	-	-	A
14865	*A. rotundatum*	83.9	-	-	-	A
14866	*A. rotundatum*	183.2	-	-	-	A
14867	*G. harrisi*	345.8	A	A	NA	-
14868	*O. rotundus*	88.2	A	NA	NA	-
14869	*A. rotundatum*	286.3	-	-	-	A
14870	*A. rotundatum*	158.7	-	-	-	A
14871	*A. rotundatum*	206.7	-	-	-	A
14873	*A. rotundatum*	154.6	-	-	-	A
14874	*O. natricis*	79.4	A	A	NA	-
14875	*A. rotundatum*	1444.2	-	-	-	A
14876	*E. alfreddugesi*	279.9	A	NA	NA	-
14878	*Chironobius* sp. n.	113.6	A	-	-	A
14879	*A. rotundatum*	1963.8	-	-	-	A
14880	*A. rotundatum*	86.1	-	-	-	A
14881	*E. alfreddugesi*	136.6	A	A	NA	-
14882	*A. rotundatum*	312.7	-	-	-	A
14883	*A. rotundatum*	1052	-	-	-	A
14884	*Z. oudemansi*	109.9	A	A	NA	-
14885	*A. rotundatum*	88.4	-	-	-	A
14886	*F. anguina*	158	A	NA	NA	-
14887	*G. harrisi*	117.4	A	A	NA	-
14888	*G. harrisi*	37.1	A	A	NA	-
14889	*G. harrisi*	286.9	A	A	NA	-
14890	*G. harrisi*	106.2	A	A	NA	-
14891	*G. harrisi*	187.4	A	A	NA	-
14892	*G. harrisi*	217.2	A	A	NA	-
14893	*G. harrisi*	121.6	A	A	NA	-
14894	*G. harrisi*	127	A	A	NA	-
14895	*A. rotundatum*	88	-	-	-	A
14896	*H. hepatica*	67	A	NA	NA	-
14897	*B. jimenezi*	122	A	NA	NA	-
14898	*A. rotundatum*	98	-	-	-	A
14899	*A. rotundatum*	99	-	-	-	A

Source: (MENDOZA-ROLDAN, J. A., 2019)

Legend: A: Amplified, NA: Not Amplified, -: not applied.

Of the total amount of amplified samples, sequences were generated for the following species and genes: MITE 18S V4 gene *A. longisetosus*, *E. alfreddugesi*, *E. ophidica*, *E. tropica*, *F. anguina*, *B. jimenezi*, *G. harrisi*, *G. hemidactyli*, *H. achalai*, *H. hepatica*, *O. parkeri*, *O. ekans*, , *Z. oudemansi*, *O. rotundus*, *Chironobius* sp., *A. humerale*, *O. rotundatum*, and *Ornithodoros (Alectorobius)*; COI 1 gene *E. alfreddugesi*, *E. tropica*, *H. achalai*, *G. harrisi*, and *O. natricis;* 16S mRNA *A. rotundatum*, *Ornithodoros (Alectorobius)* sp.

Due to the larger number of species sequenced, the MITE 18S V4 gene was used to infer phylogenies of the Acari. On the other hand, 16S mRNA of *Ornithodoros (Alectorobius)* sp. was used to confirm the morphological identification and phylogenetic analyses were carried out using the maximum likelihood (ML) and Bayesian method.

4.2 Phylogeny of Acari using the MITE 18S V4 gene

For the phylogenetic analyzes, sequences of the 18S V4 gene obtained in the present study were used (20 sequences), as well as sequences of other mites and some arachnids (46 sequences), which are deposited in GenBank (Table 37).

Table 37 – Sequences of the gene 18S rRNA V4 region of species of Chelicerata used for phylogenetic analyses

Chelicerata				**Genbank**
Acari	**Suborder**	**Family**	**Species**	
		Demodicidae	*Demodex brevis*	HQ727999
		Demodicidae	*Demodex canis*	HQ727998
Trombidiformes	Prostigmata	Eriorhynchidae	*Eriorhynchus*	AF142116
		Erythraeidae	*Eryhrites*	AF142105
		Erythraeidae	*Erytrhoides*	AF142106
		Harpirhynchidae	*O. ekans*	KU891263
		Harpirhynchidae	***O. ekans***	**This study**
		Harpirhynchidae	***O. parkeri***	**This study**
		Harpirhynchidae	*H. charadrius*	KY922182.1
		Harpirhynchidae	*Harpypalpus holopus*	KY922185.1

(Continues)

Chelicerata				Genbank
Acari	**Suborder**	**Family**	**Species**	
		Harpirhynchidae	*Harpyrhynchoides zumpti*	KY922181.1
		Leeuwenhoekiidae	*H. hepatica*	KU891269
		Leeuwenhoekiidae	*H. yungicola*	KU891272
		Leeuwenhoekiidae	***Hannemania achalai***	**This study**
		Leeuwenhoekiidae	***Hannemania hepatica***	**This study**
		Pterygosomatidae	*Cyclurobia* sp.	KY922190
		Pterygosomatidae	*Geckobia* A	AF142113
		Pterygosomatidae	*Geckobia* B	AF142114
		Pterygosomatidae	*G. hemidactyli* 1	KU891266
		Pterygosomatidae	***G. hemidactyli***	**This study**
		Pterygosomatidae	***B. jimenezi***	**This study**
		Pterygosomatidae	***G. harrisi***	**This study**
		Trombiculidae	*F. ewingi* 1	KU891275
		Trombiculidae	***F. anguina***	**This study**
		Trombiculidae	*Eutrombicula daemoni*	MG707783.1
		Trombiculidae	*E. goeldii*	MG817639.1
		Trombiculidae	*E. splendens*	KP325057.1
		Trombiculidae	***E. alfreddugesi***	**This study**
		Trombiculidae	***E. ophidica***	**This study**
		Trombiculidae	***E. tropica***	**This study**
Sarcoptiformes	Astigmata	Psoroptidae	*Psoroptes cuniculi*	EU152574
		Psoroptidae	*Psoroptes ovis*	JQ000241
		Psoroptidae	*Chorioptes bovis*	KF891892.1
	Oribatida	Oribatulidae	*Oribatula tibialis*	EU433990
		Trhypochthoniidae	*Archegozetes longisetosus*	HQ661379.1
		Trhypochthoniidae	***Archegozetes longisetosus***	**This study**
Mesostigmata		Heterozerconidae	*Narceoheterozercon ohioensi*	AY620928
		Heterozerconidae	***Z. oudemansi***	**This study**
		Macronyssidae	*O. natricis*	FJ911853
		Macronyssidae	***O. natricis***	**This study**
		Macronyssidae	*Ornithonyssus bursa*	FJ911854.1
		Ixodorhynchidae	***Chironobius* sp. n.**	**This study**
		Ixodorhynchidae	***O. rotundus***	**This study**
Ixodida		Ixodidae	*Rhipicephalus sanguineus*	L76342
		Ixodidae	*Amblyomma maculatum*	L76344

Conclusion

Chelicerata				Genbank
Acari	**Suborder**	**Family**	**Species**	
		Ixodidae	*Amblyomma tuberculatum*	L76345
			Amblyomma rotundatum	KJ584369.1
		Ixodidae	*A. variegatum*	L76346
		Ixodidae	***A. rotundatum* 1**	**This study**
		Ixodidae	***A. rotundatum* 2**	**This study**
		Ixodidae	***A. rotundatum* 3**	**This study**
		Ixodidae	*Ixodes affinis*	L76350
		Ixodidae	***A. humerale***	**This study**
		Ixodidae	*Dermacentor andersoni*	L76340
		Ixodidae	*Ixodes ricinus*	GU074707.1
		Argasidae	*Argas persicus*	L76353
		Argasidae	*Argas lahorensis*	L76354
		Argasidae	*Ornithodoros moubata*	L76355
		Argasidae	*Carios puertoricensis*	L76357.1
		Argasidae	*Otobius megnini*	L76356
		Argasidae	***Ornithodoros* snake**	**This study**
			Carios mimon	KC769599.1
Arachnida				
Scorpiones		Buthidae	*Androctonus australis*	X74761
Solifugae		Daesiidae	*Gluvia dorsalis*	AF007103.1
Pseudoscorpiones		Pseudogarypidae	*Pseudogarypus bicornis*	EU559368.1
Merostomata				
Xiphosura		Limulidae	*Limulus polyphenus*	X90467

Source: (MENDOZA-ROLDAN, J. A., 2019).

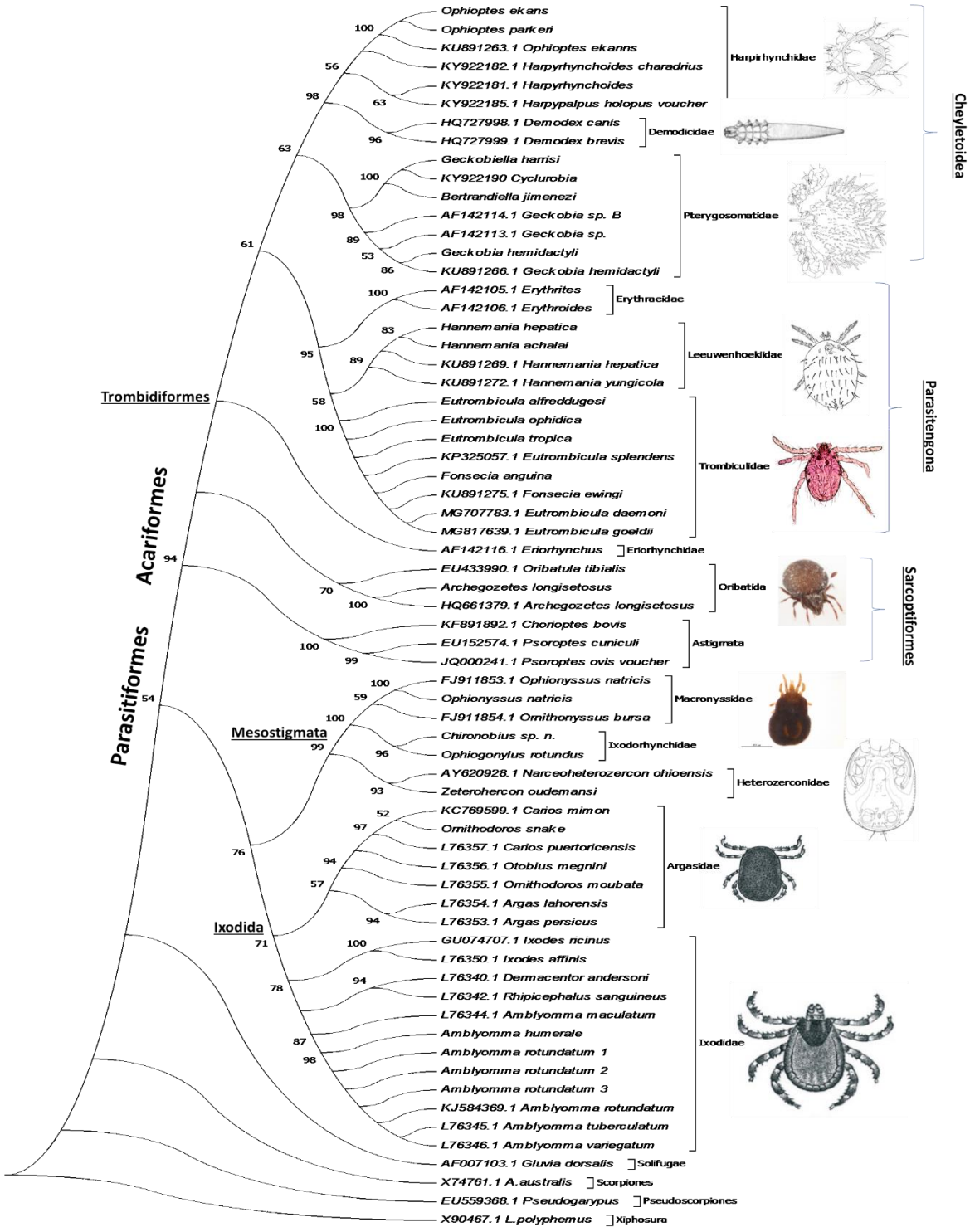
Maximum likelihood (ML), maximum parsimony (MP) and Bayesian (BA) analyses were performed with the abovementioned sequences. The crustacean *Limulus polyphenus* was used as outgroup. The ML tree was generated based on the General Time Reversible model, using a discrete Gamma distribution to model evolutionary rate differences among sites (5 categories (+*G*, parameter = 0.6862). The rate variation model allowed for some sites to be evolutionarily invariable ([+*I*], 11.52% sites). The best-fitting substitution models were determined with the

Bayesian Information Criterion using the ML model test, Support was tested with 2000 bootstrap pseudoreplicates. The analysis involved 66 nucleotide sequences.

The resulting ML tree showed a unsuported monophyletic Acari, that divided in two major clades: Parasitiformes, which had a low bootstrap value (54%), and Acariformes that had hihgh values of boostrap (94%), thus confirming it is monopyletic. The groups recovered inside this clade were divided in three major groups: The topologies obtained by the ML method evidenced the order Trombidiformes as a poliphyletic group and with two major clades (Cheyletoidea with 61% of bootstrap value, and Parasitengona with 94%). In Cheyletoidea, the three families were recovered (Demodecidae, Harpirhynchidae, and Pterygosomatidae). In Parasitengona, three families were recovered (Erthaeidae, Leeuwenhoekiidae, and Tombiculidae) yet one (Eriorhynchidae), did not grouped this clade. The Tcombiculida had a high boostraop value (100%), yet it did not divide the genus *Eutrombicula* from *Fonsecia*. The order Sarcoptiformes also was inferred as polyphyletic with both major groups with no suporting values and separated (Oribatida and Astigmata). The relation with the sister group Solifugae was not recovered with this method. On the other hand, Parasitiformes was divided in Mesostigmata and Ixodida with fair confidence values (76). Mesostigmata showed high bootstrap values (99%), and the families were recovered also with fair to high bootstraps (Macronyssidae 59%, Ixodorhynchidae 96%, both related; and Heterozerconidae 93%). Finally, Ixodida was recovered with both families having fair confidence levels (Argasidae 57% and Ixodidae 78%). The *ornithodoros* from *Philodryas nattereri* snake grouped with *Ornithodoros mimon* (*Carios mimon*), and *Ornithodoros puertoricencis* (*Carios puertoricensis*) (boostrap 97%). Additionally, polytomy was seen on the *Amblyomma* genus. This method did not show Mesostigmata being sister group of Pseudoscorpiones (Figure 108).

The evolutionary history was inferred using the Maximum Parsimony method (MP), as wel. The most parsimonious tree with length = 524 is shown. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (2000 replicates) are shown next to the branches. The MP tree was obtained using the Subtree-Pruning-Regrafting (SPR) algorithm with search level 1 in which the initial trees were obtained by the random addition of sequences (10 replicates). The analysis involved 66 nucleotide sequences. All positions containing gaps and missing data were eliminated. There were a total of 248 positions in the final dataset.

Figure 108 – Phylogenetic tree of Acari based on the partial sequences of the ribosomal 18S rRNA V4 gene, using maximum likelihood (ML)



Source: (MENDOZA-ROLDAN, J. A., 2019)

Legend: Phylogenetic tree based on the 18S rRNA V4 gene, by maximum likelihood (ML) method using the General Time Reversible evolutionary model with Gamma distribution and invariable sites, of 66 Chelicerata sequences, using *L. polyhpemus* as outgroup. Numbers of nodes correspond to the Bootstrap value of 2000 pseudoreplicates.

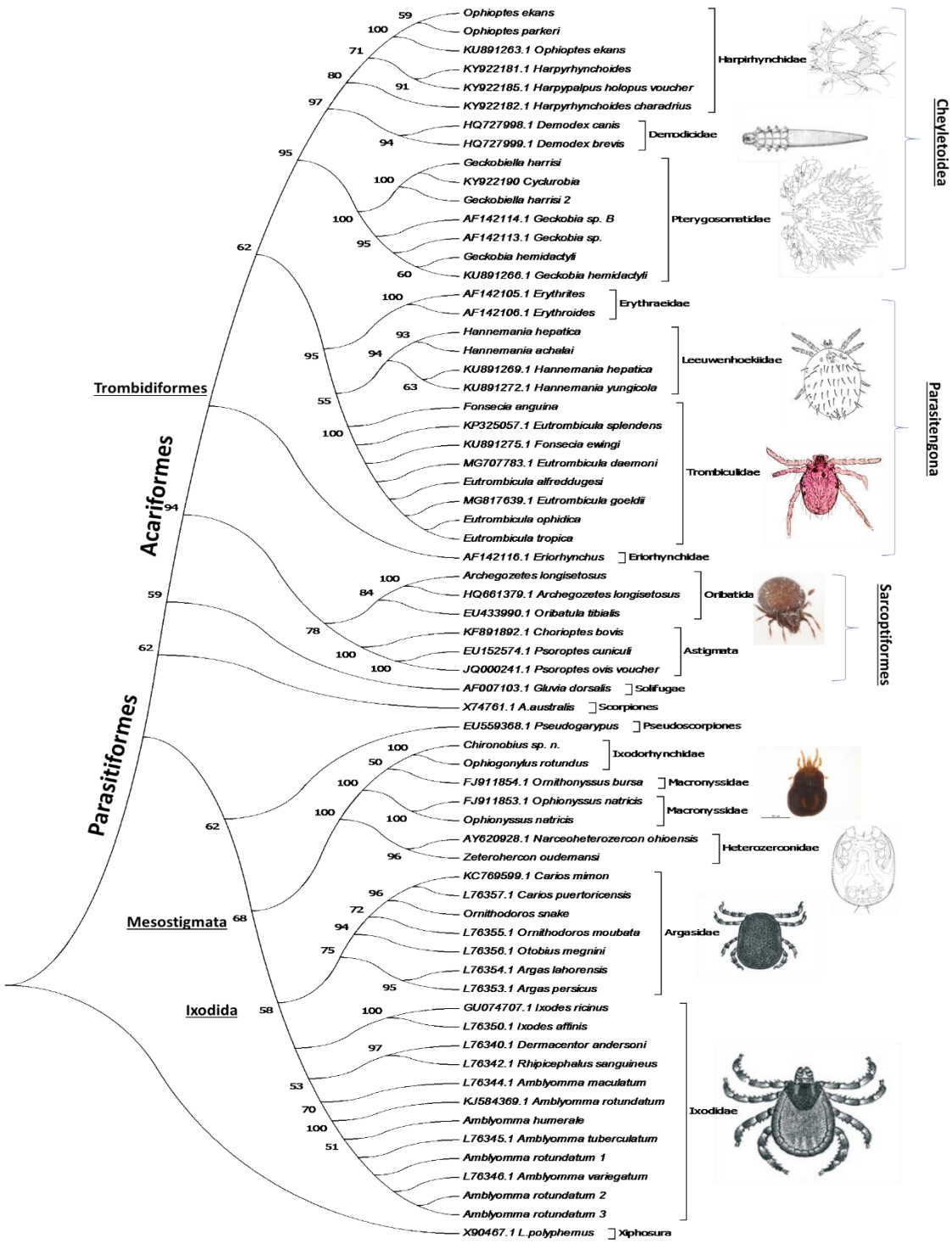
In MP analysis, the order acari was recovered as polyphyletic, having Arachnid sequences grouped inside. Acari was divided in Parasitiformes with no boostrap support, and Scorpiones 62%, Solifugae 59%, and Acariformes 94% group together. Thus none of the major groups whoed monophyly. Acariformes was inferred with Sarcoptiformes recoverd with fair confidence values (78%), and trombidiformes shown as polyphyletic with no bootstrap. The clades inside this order grouped similar to that seen in the ML tree. On the other hand Oribatida and Astigmata were sister groups inside Sarcoptiformes (78%).

Regarding Parasitiformes, this group included divided into Pseudoscorpiones (62%), Mesositgmata (68%), and Ixodida. MP methos did show Pseudoscorpiones as sister group of Mesostigmata. Differently from ML, MP did not recovered Macronyssidae as a group, but rather grouped Ixodorynchidae and Macronyssidae (100%). Ixodida divided in Argasidae (58%) and Ixodidae (with no boostrap support). All the Ornithodoros sequences were grouped together (72%) (Figure 109).

Bayesian analyses were performed with the General Time Reversible model, using a discrete Gamma distribution to model evolutionary rate differences among sites (+*G*). The rate variation model allowed for some sites to be evolutionarily invariable ([+*I*]), with 2,000,000 generations. The first 25% of these trees represented the "Burn in" and the rest of the trees were used to calculate Bayesian analyses.

Bayesian analysis recovered Acari as Polyphyletic with polytomy grouping Acariformes, Parasitiformes with Scorpiones and Pseudoscorpiones. It also showed the monophyly of the order Thrombidiformes, with strong supports of branches (98%). However Acariformes also was recovered monophyletic (100%). The clades Parasitengona and Cheyletoidea had a high probability of branches (99% and 97%, respectively). The family Demodicidae was recovered inside the family Harpyrhinchidae. Sarcoptiformes was also recovered as monophyletic (99%). Parasitiformes was inferred as monophyletic with high boostrap support for Mesostigmats (100%) and Ixodida (93%). Relationships of Mesostigmata groups were similar to those of the ML tree. Ixodida was also similar to the ML tree, with the exception of *Ixodes* being closer to Argasidae (61%) than Ixodidae. In general the Bayesian method recovered higher and more reliable bootstraps values (Figure 110).

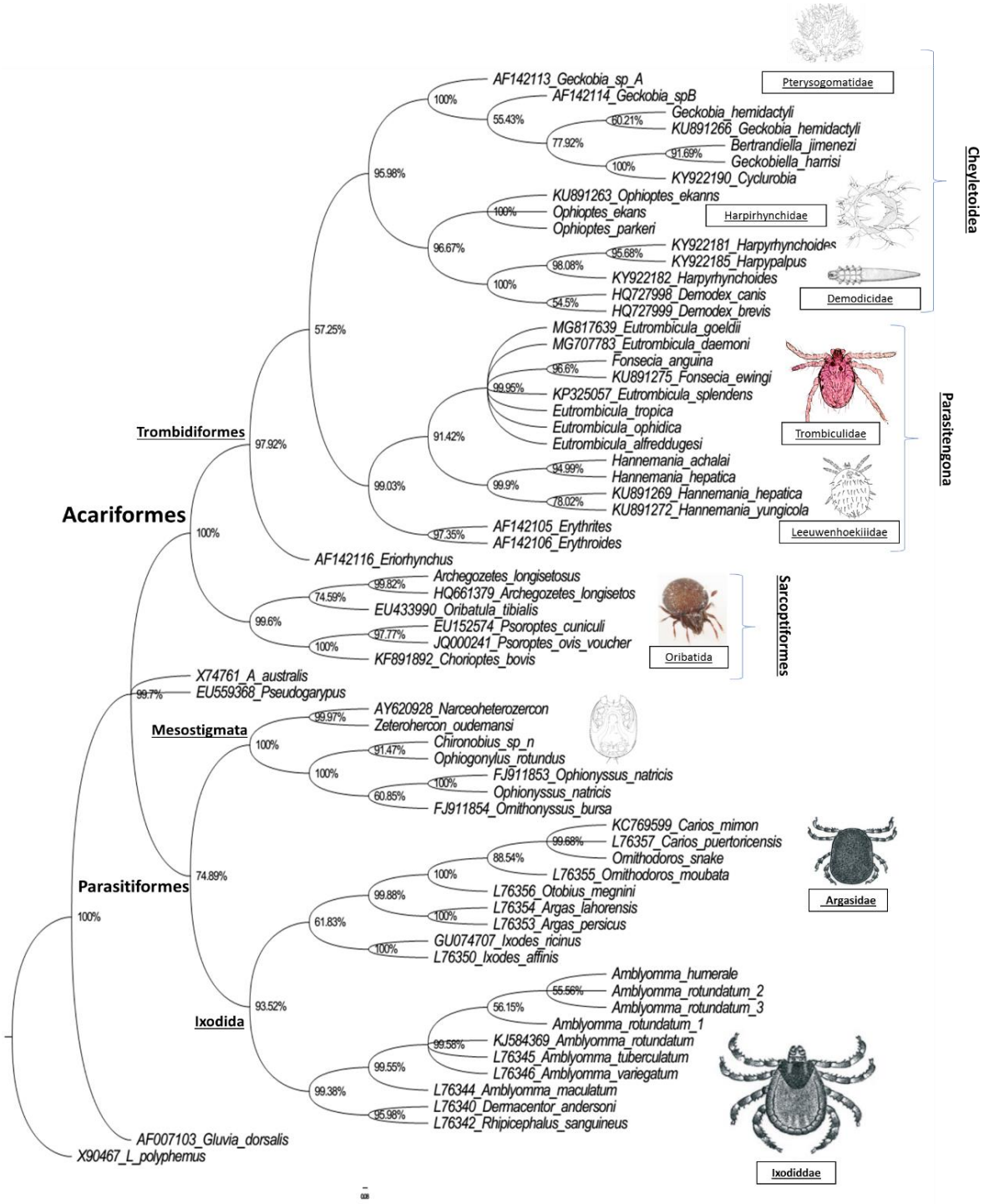
Figure 109 – Phylogenetic tree of Acari based on the partial sequences of the ribosomal 18S rRNA V4 gene, using maximum parsimony (MP)



Source: (MENDOZA-ROLDAN, J. A., 2019)

Legend: Phylogenetic tree based on the 18S rRNA V4 gene, using the maximum parsimony (MP) method, of 66 Chelicerata sequences, using *L. polyhpemus* as outgroup. Numbers of nodes correspond to the Bootstrap value of 2000.

Figure 110 – Phylogenetic tree of Acari based on the partial sequences of the ribosomal 18S rRNA V4 gene, using Bayesian analysis (BA)



Source: (MENDOZA-ROLDAN, J. A., 2019)

Legend: Phylogenetic tree based on the 18S rRNA V4 gene, using the Bayesian (BA) method, of 66 Chelicerata sequences, using *L. polyhpemus* as outgroup. Numbers of nodes correspond to the Bootstrap value of trees with 2,000,000 generations.

4.3 Phylogeny of *Ornithodoros* using the tick 16S gene

It was used 16S mRNA of *Ornithodoros* (*Alectorobius*) sp. n. to confirm the morphological identification and phylogenetic analyses were carried out using the maximum likelihood (ML) and Bayesian method. The sequences generated for this species were submitted to to identify similarities to known sequences using the Basic Local Alignment Search Tool (BLAST). The BLAST showed 98% identity with *Ornithodoros* sp. CECAP26 (GenBank accession number MH061499.1), from a gray short-tailed opossum (*Monodelphis domestica*), from Bahia State. It also had 91% similarity with *Ornithodoros puertoricensis* (GenBank accession number AF113932.1), and 89% with *Ornithodoros capensis* ((GenBank accession number KY825215.1) (Table 38).

Given that no high similarities were found to a described species, a phylogeny was inferred using ML and BA with sequences of three genera (*Argas*, *Ixodes* and *Ornithodoros*), that were formerly used in Muñoz-Leal et al. (2017), to infer the position of *Ornithodoros saraivai* (Table 39).

Table 38 – Sequences similarities (BLAST) with *Ornithodoros* (*Alectorobius*) sp. n.

Sample	Identities	Genbank	Reference	Locality
IBSP 14838	414/422(98%)	*Ornithodoros* sp. CECAP26 MH061499.1	MAIA et al., 2018	Bahia state
	429/474(91%)	*Ornithodoros puertoricensis* AF113932.1	KLOMPEN et al., 1998	-
	425/475(89%)	*Ornithodoros capensis* KY825215.1	KIM et al., 2017	South Korea

Source: (MENDOZA-ROLDAN, J. A., 2019).

Table 39 – Sequences of the gene 16S mRNA of species of Argasidae used for phylogenetic analyses

Genus	**Species**	**Genbank**
Argas	*Argas keiransi*	DQ295778
	Argas monachus	EU283344
	Argas monolakensis	L34305
	Argas neghmei	DQ295781
	Argas persicus	AF001402
	Argas polonicus	AF001403
	Argas reflexus	AF001401
	Argas robertsi	AY436768
	Argas vulgaris	AF001404
Ixodes	*Ixodes holocyclus*	AB051084
	Ixodes uriae	AB030017
Onithodoros	*Onithodoros capensis*	AB076082
	Ornithodoros	**This study**
	Ornithodoros atacamensis	KT894587
	Ornithodoros braziliensis	GU198363
	Ornithodoros cavernicolous	JF14963
	Ornithodoros cavernicolous 2	JF714964
	Ornithodoros coriaceus	AY668970
	Ornithodoros dyeri	KU551919
	Ornithodoros faccinii	KP961242
	Ornithodoros fonsecai	GQ120967
	Ornithodoros guaporensis	KC493652
	Ornithodoros gurneyi	AY436767
	Ornithodoros hasei	KX099896
	Ornithodoros kohlsi	KX130783
	Ornithodoros lahillei	KP403288
	Ornithodoros marinkellei	HM582438
	Ornithodoros marinkellei 2	HM582439

(Conclusion)

Genus	Species	Genbank
	Ornithodoros microlophi	JX455899
	Ornithodoros mimon	KC677675
	Ornithodoros mimon 2	KC677676
	Ornithodoros mimon 3	GU198362
	Ornithodoros moubata	L34328
	Ornithodoros parkeri	EU00925
	Ornithodoros peruvianus	HQ111351
	Ornithodoros porcinus	L34329
	Ornithodoros puertoricensis	AF113932
	Ornithodoros quilinensis	JN255575
	Ornithodoros rietcorreai	KX130782
	Ornithodoros rietcorreai 2	KX130781
	Ornithodoros rioplatensis	EU283343
	Ornithodoros rondoniensis	EU90907
	Ornithodoros rostratus	DQ295780
	Ornithodoros saraivai	KX812526
	Ornithodoros sawaii	AB2424430
	Ornithodoros sonrai	DQ234726
	Ornithodoros sonrai 2	DQ250441
	Ornithodoros sp.	JF895756
	Ornithodoros sp. CECAP26	MH061499.1
	Ornithodoros turicata	L34327
	Ornithodoros vespertilionis	HM75184
	Ornithodoros viguerasi	JQ397632
	Ornithodoros xerophylus	KP040287

Source: (MENDOZA-ROLDAN, J. A., 2019).

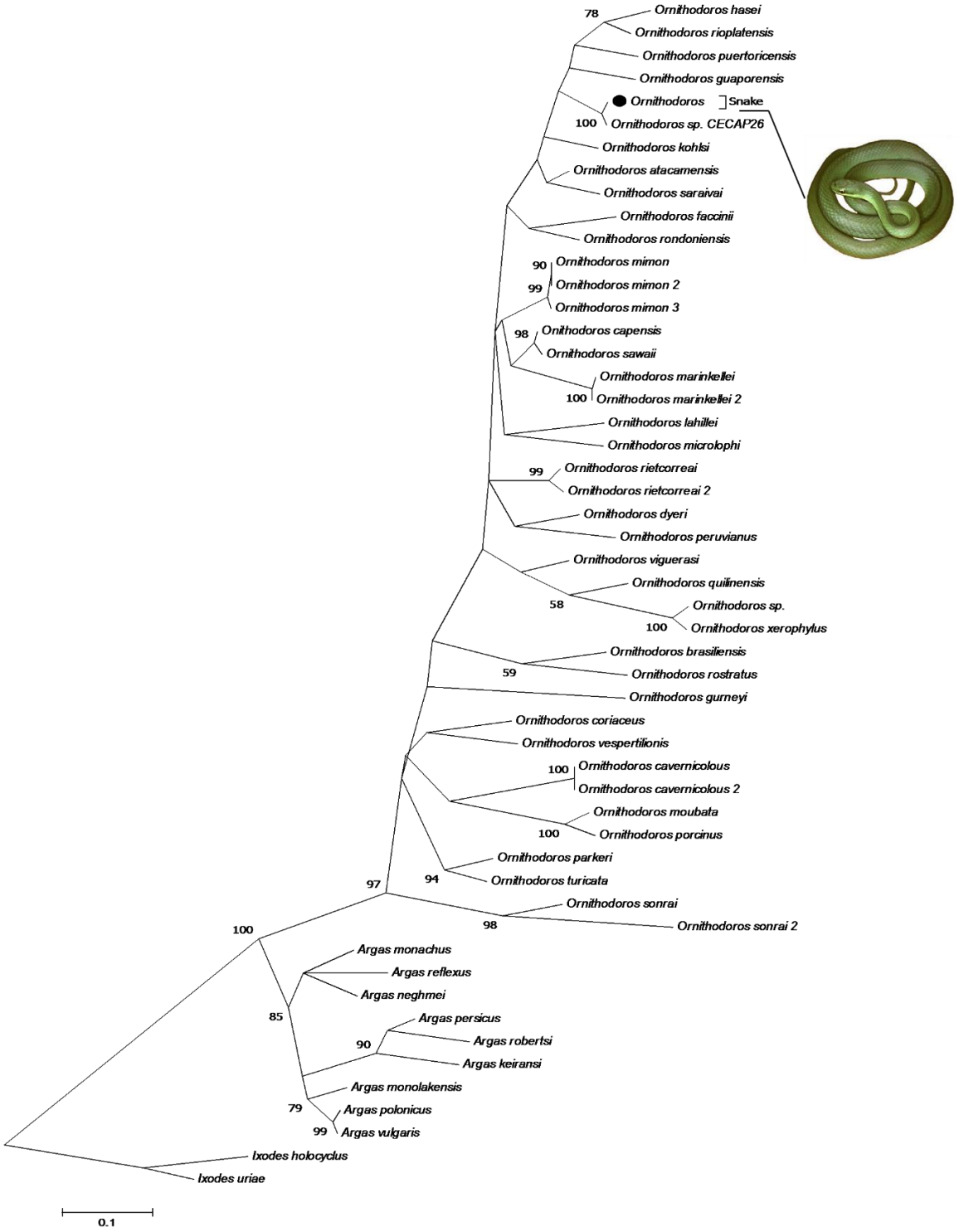
Maximum likelihood (ML), and Bayesian (BA) analyses were performed with the abovementioned sequences. The Ixodidae ticks *Ixodes holocyclus* and *Ixodes uriae* were used as outgroups. The evolutionary history was inferred by using the Maximum Likelihood method based on the General Time Reversible model. The tree with the highest log likelihood is shown. A discrete Gamma distribution was used to model evolutionary rate differences among sites (5 categories (+*G*, parameter = 0.5056)). The rate variation model allowed for some sites to be evolutionarily invariable ([+*I*], 22.16% site). The best-fitting substitution models were determined with the Bayesian Information Criterion using the ML model test, Support was tested with 2000 bootstrap pseudoreplicates. The analysis involved 52 nucleotide sequences.

The resulting ML tree showed recovered the two genera separated with high bootstrap supports (*Argas* 100% and *Ornithodoros* 97%). Nonetheless, relationships between *Ornithodoros* were not well recovered, and polytomy ocurred. In addition, the consensus sequence of *Ornithodoros* generated in this study formed am unsupported clade with: *Ornithodoros rioplatensis*, *Ornithodoros hasei* [from great fruit-eating bat (*Artibeus lituratus*)], *Ornithodoros puertoricensis* and *Ornithodoros guaporensis*. Moreover, it was closely related with *Ornithodoros sp.* CECAP26 (100%) (Figure 111).

Furthermore, Bayesian analyses were performed with the General Time Reversible model, using a discrete Gamma distribution to model evolutionary rate differences among sites (+*G*). The rate variation model allowed for some sites to be evolutionarily invariable ([+*I*]), with 2,000,000 generations. The first 25% of these trees represented the "Burn in" and the rest of the trees were used to calculate Bayesian analyses.

Bayesian analysis recovered higher bootstrap levels for the genera, yet showed a polytomy for most of the sequences of *Ornithodoros*. Morover, the consensus sequence of *Ornithodoros* generated in this study formed am supported clade with the same sequences mentioned for the ML tree, and also with *Ornithodoros atacamensis* (from the lizard *Liolaemus nigromaculatus*), *Ornithodoros kohlsi* and *Ornithodoros saraivai* (from the frog *Cycloramphus boraceiensis*). It was also related to *Ornithodoros sp.* CECAP26 (100%) (Figure 112).

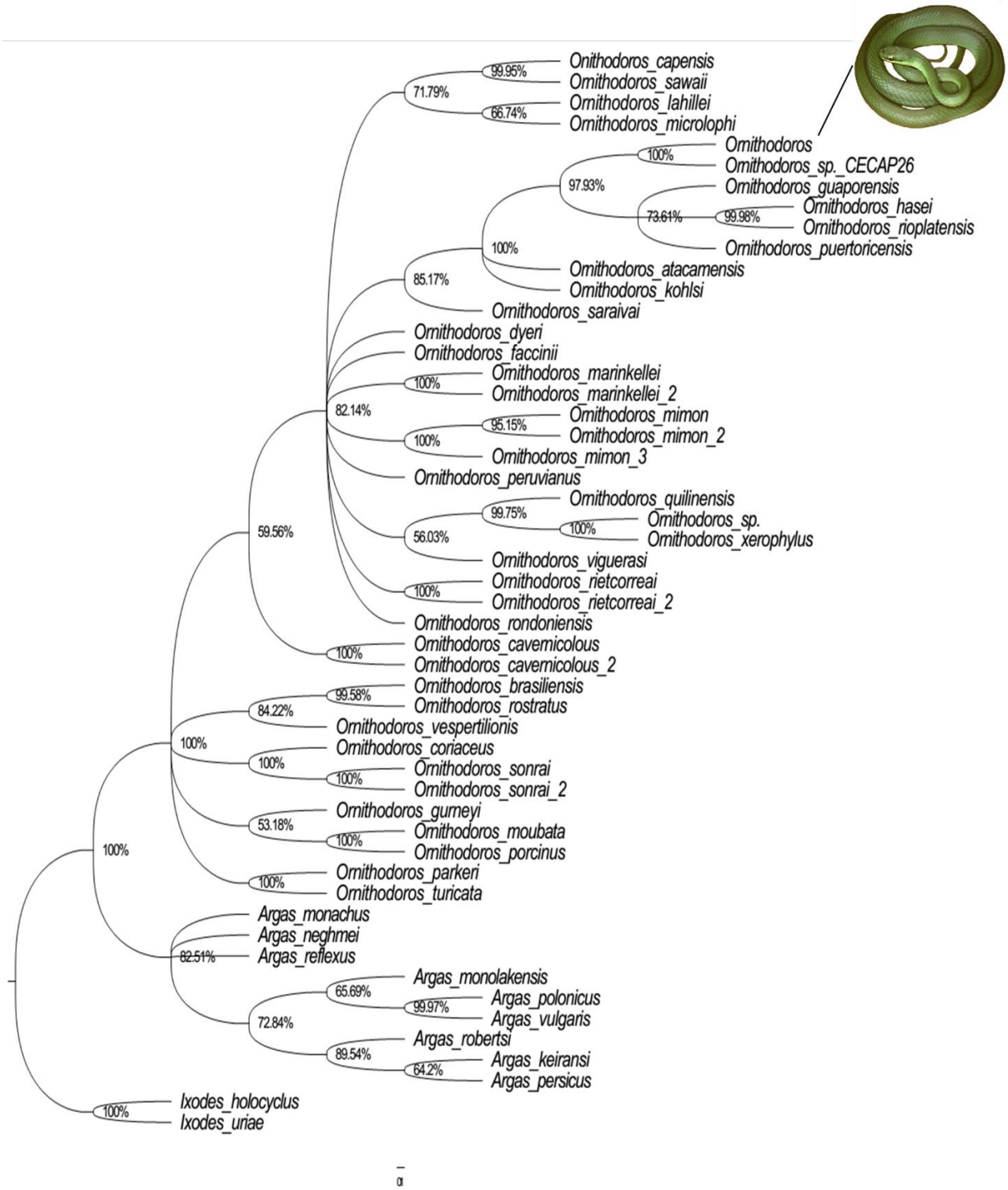
Figure 111 – Phylogenetic tree of Argasidae based on the partial sequences of the 16S gene, maximum likelihood (ML)



Source: (MENDOZA-ROLDAN, J. A., 2019)

Legend: Phylogenetic tree based on the 16S gene, by maximum likelihood (ML) method using the General Time Reversible evolutionary model with Gamma distribution and invariable sites, of 52 Argasidae sequences, using *Ixodes holocyclus* and *Ixodes uriae* as outgroup. Numbers of nodes correspond to the Bootstrap value of 2000 pseudoreplicates.

Figure 112 – Phylogenetic tree of Acari based on the partial sequences of the 16S gene, using Bayesian analysis (BA)



Source: (MENDOZA-ROLDAN, J. A., 2019)

Legend: Phylogenetic tree based on the 16S gene, using the Bayesian (BA) method, of 52 Argasidae sequences using *Ixodes holocyclus* and *Ixodes uriae* as outgroup. Numbers of nodes correspond to the Bootstrap value of trees with 2,000,000 generations.

5 DISCUSSION

In this study, the method used for DNA extraction of mites and ticks was lysis protocol with Guanidine Isothiocyanate (GT). As in former studies, this method showed to be useful for the three different orders (Trombidiformes, Mesotigmata and Ixodida) (MENDOZA-ROLDAN, 2015; MENDOZA-ROLDAN et al., 2019). This method even worked for highly quitinized mites as the Oribatida, allowing to extract a fair amount of DNA (92.7ng/ul), and preserving the mite intact, thus later mounting a voucher. Therefore, this method of DNA extraction is highly recommended when performing morphological and molecular studies together, because it allows to extract DNA of fair quality and quantity and preserve the voucher of the animal, that can be later morphologically identified (ROWLEY et al, 2007). This way, it is possible to match both morphological and molecular identifications. On the other hand, this method did not work for some individuals of some species (*B. jimenezi, H. hepatica, H. achala, H. achalai* and *A. dissimile*). This probably occurred due to the low quality of the samples. Depending on the preservation method, antiquity of the sample, and concentration and type of preservative reagent (Alcohol, formalin, RNAlater etc), the DNA can degrade rapidly, thus extraction is not possible (DESLOIRE et al., 2006). Some of these samples beforementioned, were collected from animals that were already preserved in formalin or in alcohol, therefore hindering the extraction of DNA. To ensure best extraction results, fresh and properly conserved samples should be used.

Furthermore, of the 139 samples of different mite and tick species (Tombidiformes, Mesostigmata, Ixodida) 74 samples (21 of 22 species) were amplified for the MITE 18S V4 gene; 31 samples (10 of 22 species) for COI 1 gene and eight samples (three of 22 species) for COI 2; and 35 samples of the 16S mRNA gene (all of the tick species). Of the total amount of amplified samples, sequences were generated for the following species and genes: MITE 18S V4 gene *A. longisetosus*, ***E. alfreddugesi, E. ophidica, E. tropica, F. anguina, B. jimenezi, G. harrisi****, G. hemidactyli,* ***H. achalai****, H. hepatica,* ***O. parkeri,*** *O. ekans,* , ***Z. oudemansi, O. rotundus, Chironobius*** **sp**., *A. humerale, O. rotundatum,* and ***Ornithodoros (Alectorobius)*****;** COI 1 gene *A. longisetosus, G. hemidactyli****, E. alfreddugesi, E. tropica, H. achalai, G. harrisi,*** *O. natricis* and ***Z. oudemansi;*** 16S mRNA *A. rotundatum,* ***Ornithodoros (Alectorobius)*** **sp.** The species highlighted in bold represent generated sequences of species that do not have sequences deposited in GenBank. Furthermore, COI 2 amplified the least number of species and was not used for

sequencing. Additionally, COI 1 gene amplified half of the species studied. This also occurred in a former study that tested the usefulness of this marker (OTTO; WILSON, 2001). Of these 10 species, only eight mite species were sequenced. Thus, this marker showed fragments that proved to be phylogenetically uninformative. This could be due to a number of factors, the most important one being the lack of enough sequences generated and deposited in the Genbank, also the number of variable nucleotides was very high, even for members of the same genus, which translates in a high rate fo nucleotide substitution. These factors prevented the construction of phylogenetic trees mainly because of homoplasy due to saturation of substitutions which makes even very similar sequences very divergent (WAKELEY,1996).

On the other hand, although the COI gene was not suitable for phylogenetic analyses, it can be useful for barcoding. DNA barcoding is based on amplifying and sequencing DNA regions that are informative at species level. For most animal groups studied, the COI subunit 1 (648-bp region) has shown to be a useful tool for barcoding (HEBERT et al., 2003; HAJIBABAEI et al., 2007). In this order of ideas, the sequences generated for the COI 1 were analyzed through BLAST and *A. longisetosus* was 99% identical to the *A. longisetosus* sequence number HQ711372; *G. hemidactyli* was 98% identical to *Geckobia* sp. B sequence number AF142139; and *O. natricis* was 100% identical to *O. natricis* sequence number MG414305. The sequences that did not have previous sequences deposited in GenBank had various similariry indexes depending on the family. ***E. alfreddugesi, E. tropica*** had less than 80% of identity with *Leptotrombidium pallidum* sequence number AB180098 (Trombiculidae); *H. achalai* of the family Leeuwenhoekiidae, had 77% of similarity with *Hybalicus* sp. sequence number KY922354, which is a Trombidiformes mites from the family Lordalychidae; ***G. harrisi*** had 82% of similarity with *Tetranychus urticae* sequence number HM486506, which is a Trombidiformes mite of the family Tetranychidae; and ***Z. oudemansi*** had 80% identity with *Narceoheterozercon ohioensis*, sequence number AY624001. These results showed the nucleotide variability within the same family of mites. Thus, to improve the usefulness of this gene, it is important to create a barcode library for each family with an adequate number of species, and design COI primers specific for each targeted family or genus. The sequences of the COI 1 generated in this study will increase the number of available sequences and therefore aid the molecular identification of mite species, as it is currently performed with ticks using 16s mRNA.

Furthermore, due to the larger number of species sequenced (21 of 22), the MITE 18S V4 gene was used to infer phylogenies of the Acari. The results of this study showed that the 18S V4 gene amplified for most of the species and could be used for phylogenetic analyses. The 18S V4 rRNA gene generates a product of ~480 base pairs (bp), and in general it is a constant and conserved fragment. The alignment of the generated sequences showed that most parts are identical in mite, ticks and other arachnids, which restricted the informative sites to a few regions. These results are similar to that seen in tick phylogeny and Prostigmata mites (BLACK; PIESMAN, 1994; OTTO; WILSON, 2001).

Additionally, the alignment was used to infer phylogenetic trees for the Acari subclass using maximum likelihood (ML), maximum parsimony (MP) and Bayesian (BA) analyses. The generated trees for each analysis had similar results with slight differences on clade grouping. All the analyses inferred a polyphyletic Acari, with different bootstrap values for the monophyly of Acariformes and Parasitiformes. This constant result recovered in many of the recent studies, has led to the hypothesis that the term mite is, from a systematic point of view, descriptive and an artificial term that does not have biological meaning, which translates that the set of traits that define the term mite have evolutionary independent origin, also known as homoplasies (when a character has been gained of lost independently in separate lineages over the course of evolution) (WYBOUW, 2019). Also, the polyphyly of Trombidiformes was demonstrated. Nonetheless, the relationship with the sister group Solifugae was not recovered in any of the analyses. Former studies revovered this Solifugae + Acariformes with high values of bootstrap support (DABERT et al., 2010; PEPATO et al., 2010; KLIMOV et al., 2018). Possibly this clade was not recovered in the present study due to the number of sequences used and the region V4 which is very conserved among Chelicerata (OTTO; WILSON, 2001). This was also observed in the clade formed by the Trombiculidae family, which generated polytomy among the sequences of two genera (*Eutrombicula* and *Foncesia*). Therefore, the 18S gene is not useful for species barcoding as sequences are highly similar. Nonetheless, as this fragment amplifies all the families if Acari and other Chelicerata, it is important to generate more and more sequences to which compare with. Moreover, all the trees recovered the two major clades of Trombidiformes (Cheyletoidea and Parasitengona), with high similar bootstrap values. However, in the Bayesian analyses Demodicidae was recovered as sister group of Harpirhynchinae subfamily (family Harpirhynchidae), which is associated to passerine birds. Cladistic analyses demonstrated this

relationship of Cheyletoidea mites of mammals and birds, thus it is presumed that the common ancestor of this clade could have occurred on the common ancestor of birds and mammals (BOCHKOV 2002). Furthermore, Ophioptinae (subfamily of snake mites), could be a basal group could be probably explained by peculiarities of the skin and ecdysis in reptiles. However, Cheyletoidea parasitic origin on snakes could have originated much later after Demodicidae and Harpirhynchinae divergence, thus explaining its molecular distance (BOCHKOV, 2008).

Furthermore, although the 18S V4 fragment could not separate the species among Trombiculidae (except in the Bayesian tree, were *Foncesia* was separated from *Eutrombicula*), it separated Leewhenhoekiidae and Trombiculidae, thererofe making this marker useful for phylogenetic studies at families and superfamilies levels. Moreover, the sarcoptiformes clade was recovered properly in the parsimony and Bayesian trees (in the ML tree it eas recovered but not supported), which is a well-established phylogenetic hypothesis (DOMES et al., 2007).

Additionally, all the trees recovered Parasitiformes with fair bootstrap supports. Only the MP tree inferred Parasitiformes as the sister group of Pseudoscorpiones, which characters such as the fusion of the labrum to the epistome, and a ventrally placed cheliceral apotele support this hypothesis (PEPATO et al., 2010). Furthermore, Mesostigmata was recovered placing Heterozeconidae as a basal group and Ixodorhynchidae and Macronyssidae related, which is in accordance to the superfamilies division of the primitive Heterozerconoidea and the diverse Dermanyssoidea (KLOMPEN et al., 2007). Finally, Ixodida was recovered also by all the trees with both families being divided with fair branch supports. However, BA tree placed *Ixodes* closer to Argasidae, but due to the low bootsrap value, it could be considered part of Ixodidae. Additionally, like that observed in Trombiculidae, the 18S V4 fragment could not separate the different species of *Amblyomma*, and some species of *Ornithodoros.* Thus, these findings have the same implications as in other species. This gene can be used to study phylogenetic relationships to a family and in some cases genera level. For species, the variable regions are limited, which avoids differentiating properly closely related or recently diverged species and subspecies.

Finally, BA tree showed an inferred phylogeny closer to what has been hypothesized before (DABERT et al., 2010; PEPATO et al., 2010; KLIMOV et al., 2018). The MP tree was the less informative. MP analysis looks for the shortest possible tree that explains the data. This can produce biased results differently from ML that is based on the probability of the observed data occur according to the parameters of a statistical model. Moreover, the Bayesian inference derives

from probability two antecedents: a prior probability and a "likelihood function" derived from a statistical model for the observed data. Thus, Bayesian analyses give a best and robust perspective of the relationships among clades. In other words, using the 18S V4 for Acari is more reliable if Bayesian is used, and less reliable when MP trees are inferred (VAN DAM et al., 2019).

It is important to note that the V4 region, although very small and conserved, must continue to be used because it is informative. The phylogenetic analyzes carried out in the present study, although using a very conserved fragment of the V4 region, allowed to group the mites and ticks studied in the groups corresponding to those already positioned by the morphological cladistics, with high bootstraps supports high Bayesian probabilities. Thus, in order to better differentiate these intraordinal relationships, it would be necessary to compare a larger number of sequences of less conserved fragments, and to use the taxonomic and biological knowledge of the different groups to construct a more concise phylogeny.

Furthermore, 16S mRNA of *Ornithodoros (Alectorobius)* sp. was used to confirm the morphological identification and phylogenetic analyses were carried out using the maximum likelihood (ML) and Bayesian method (BA). Morphological analyses showed that *Ornithodoros (Alectorobius)* sp. was Similar to *Ornithodoros (Alectorobius) rioplatensis* n. sp. Venzal, Estrada Peña & Mangold ,2008 and *Ornithodoros (Alectorobius) puertoricensis* (Fox, 1947). However, *Ornithodoros (Alectorobius)* sp. larvae are larger and hypertricous. These differences prevented to further identified the collected larvae. Thus, molecular analyses were performed. The BLAST showed 98% identity with *Ornithodoros* sp. CECAP26 (GenBank accession number MH061499.1), from a gray short-tailed opossum (*Monodelphis domestica*), from Bahia State. Given that no higher similarities were found to a described species, a phylogeny was inferred using ML and BA with sequences of three genera (*Argas, Ixodes* and *Ornithodoros*), that were formely used in Muñoz-Leal et al. (2017), to infer the position of *Ornithodoros saraivai*. The generated trees (ML and BA) showed similar results. However, BA tree showed the clade *Ornithodoros rioplatensis, Ornithodoros hasei* [from great fruit-eating bat (*Artibeus lituratus*)], *Ornithodoros puertoricensis* and *Ornithodoros guaporensi*; and other related sequences *Ornithodoros atacamensis* (from the lizard *Liolaemus nigromaculatus*), *Ornithodoros kohlsi* and *Ornithodoros saraivai* (from the frog *Cycloramphus boraceiensis*) and *Ornithodoros sp.* CECAP26, related to *Ornithodoros (Alectorobius)* sp. with high bootstrap values. These results imply that the species found in this study from the snake *P. nattereri* is highly identical to *Ornithodoros sp.* CECAP26,

thus both sequences belong to the same species or they are both new species highly related, and both belong to the *Alectorobius* group. The findings of this study seem to indicate it is a new species. However, further studies are needed to determine if this is in fact a new species parasitic of snakes.

6 CONCLUSIONS

1. The method lysis protocol with Guanidine Isothiocyanate (GT) used for DNA extraction of mites and ticks was showed to be useful for the three different orders (Trombidiformes, Mesotigmata and Ixodida) as in former studies. Therefore, this method of DNA extraction is highly recommended when performing morphological and molecular studies together, because it allows to extract DNA of fair quality and quantity and preserve the voucher of the animal, that can be later morphologically identified
2. To ensure best extraction results, fresh and properly conserved samples should be used.
3. Of the 139 samples of different mite and tick species (Tombidiformes, Mesostigmata, Ixodida) 74 samples (21 of 22 species) were amplified for the MITE 18S V4 gene; 31 samples (10 of 22 species) for COI 1 gene and eight samples (three of 22 species) for COI 2; and 35 samples of the 16S mRNA gene (all of the tick species).
4. Sequences were generated for the genes: MITE 18S V4, COI 1 gene, 16S mRNA, with new sequences generated for most species of mites and ticks.
5. COI 2 amplified the least number of species and was not used for sequencing, and COI 1 marker for mRNA, amplified half of the species studied.
6. COI 1 showed fragments that proved to be phylogenetically uninformative and prevented the construction of phylogenetic trees due to homoplasy due to saturation of substitutions which makes even very similar sequences very divergent.
7. COI 1 can be useful for barcoding, but to improve the usefulness of this gene, it is important to create a barcode library for each family with an adequate number of species which can aid the molecular identification of mite species.
8. The MITE 18S V4 gene was used to infer phylogenies of the Acari because it amplified for most of the species. The alignment of the generated sequences showed that most parts

are identical in mite, ticks and other arachnids, which restricted the informative sites to a few regions.

9. The maximum likelihood (ML), maximum parsimony (MP) and Bayesian (BA) analyses inferred a polyphyletic Acari, with different bootstrap values for the monophyly of Acariformes and Parasitiformes.
10. The polyphyly of Trombidiformes was demonstrated. Nonetheless, the relationship with the sister group Solifugae was not recovered in any of the analyses.
11. The Trombiculidae family had polytomy among the sequences of two genera (*Eutrombicula* and *Foncesia*). Therefore, the 18S gene is not useful for species barcoding as sequences are highly similar.
12. The maximum likelihood (ML), maximum parsimony (MP) and Bayesian (BA) analyses recovered the two major clades of Trombidiformes (Cheyletoidea and Parasitengona), with high similar bootstrap values.
13. The Bayesian analyses recovered Demodicidae as sister group of Harpirhynchinae subfamily.
14. The maximum likelihood (ML), maximum parsimony (MP) and Bayesian (BA) analyses separated Leewhenhoekiidae and Trombiculidae, therefore making 18S V4 marker useful for phylogenetic studies at families and superfamilies level.
15. The sarcoptiformes clade was recovered properly in the parsimony and Bayesian trees, which is a well-established phylogenetic hypothesis.
16. The maximum likelihood (ML), maximum parsimony (MP) and Bayesian (BA) analyses recovered Parasitiformes with fair bootstrap supports. Only the MP tree inferred Parasitiformes as the sister group of Pseudoscorpiones.
17. Mesostigmata was recovered placing Heterozeconidae as a basal group and Ixodorhynchidae and Macronyssidae related, which is in accordance to the superfamilies division of the primitive Heterozerconoidea and the diverse Dermanyssoidea.
18. Ixodida was recovered also by all the trees with both families being divided with fair branch supports. However, the 18S V4 fragment could not separate the different species of *Amblyomma*, and some species of *Ornithodoros.*
19. 18S V4 fragment can be used to study phylogenetic relationships to a family and in some cases genera level.

20. Bayesian (BA) analyses showed an inferred phylogeny closer to what has been hypothesized before. Thus, using the 18S V4 for Acari is more reliable if BA is used, and less reliable when MP trees are inferred.
21. The maximum likelihood (ML), and Bayesian (BA) trees imply that the species found in this study from the snake *P. nattereri* is highly identical to *Ornithodoros sp.* CECAP26, thus both sequences belong to the same species or they are both new species highly related, and both belong to the *Alectorobius* group.

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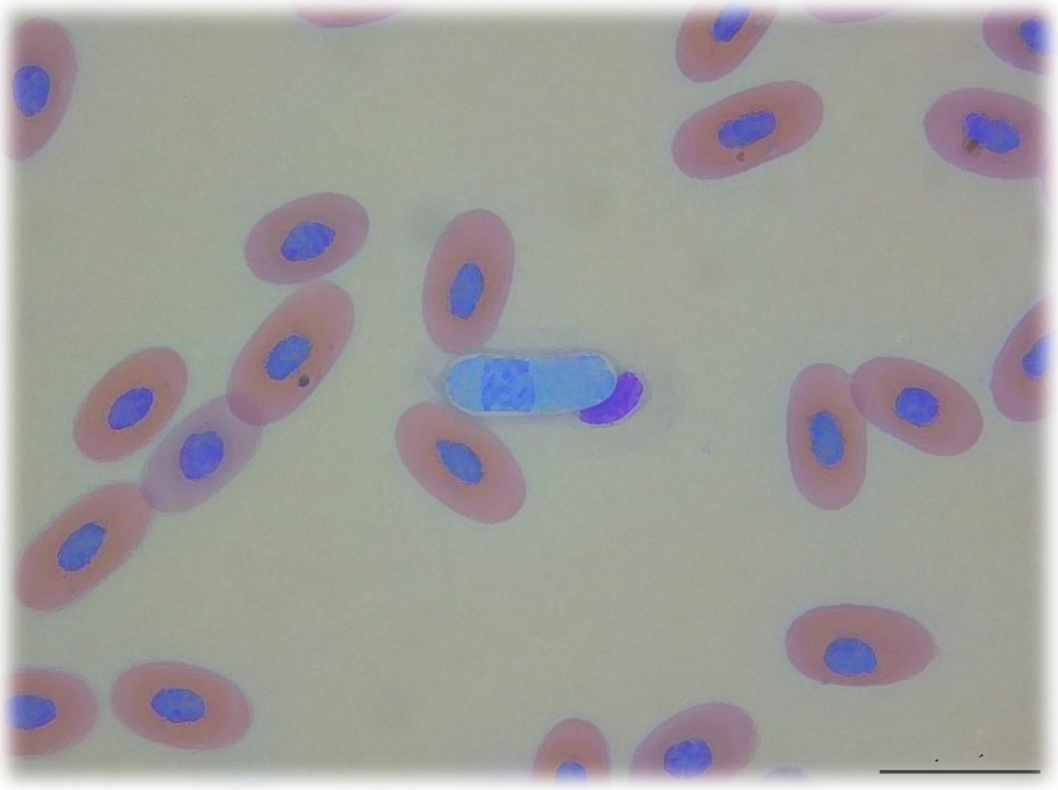


(Mendoza-Roldan, 2018)

CHAPTER VI: Molecular detection of associated pathogens

1 INTRODUCTION

Mites and ticks (Macronyssidae, Trombiculidae, Pterygosomatidae, Ixodidae and Argasidae) of reptiles and amphibians have been pointed as suitable vectors of pathogenic agents. These mites have been indicated as vectors of bacterial, viral, protozoal, and even helminthic diseases (NADCHATRAM, 1970; BURRIDGE, 2001; FRANCES, 2005; VÁCLAV et a., 2011; BOWER et al., 2018). From the Tromidiformes order, the family Pterygosomatidae has been pointed as vectors and intermediate hosts of protozoa. The genus *Hirstiella* has been recorded as vector of hemogregarines and *Plasmodium* sp. (NEWELL; RYCKMAN, 1964). On the other hand, the species *Geckobiella texana* was found naturally infected with *schellackia occidentalis* (BONORRIS; BALL, 1955), though its vectorial capacity has not been proven. Also, this family has been proven a vector of *Hepatozoon* spp. The transmission occurs by passive pathway when the host eats the mite. (WALTER; PROCTOR, 2013).

Regarding the Mesostigmata order, the most studied species is the macronissid mite *O. natricis.* This mite has been suggested as vector of pathogens such as: *Arenavirus*, etiological agent of the inclusion bodies disease (IBD) in boid snakes (BECK et al., 2005; CHANG; JACOBSON, 2010; DIVERS; STAHL, 2018); it is also the mechanical vector of *Aeromonas,* the casuative agent of hemorrhagic disease in reptiles. Also, the species *Ophionyssus galloticolus*, is a known vector of the *Karyolysus* sp. protozoa, that infects lacertid lizards (BANNERT et al., 2000). However, it is not well known if this or other protozoa cause lesions to their ectothermic host.

Regarding ticks, the epidemiological role of the Argasidae and Ixodidae families in the transmission of diseases is better understood. Concerning Argasid ticks, the species *Ornithodoros turicata,* parasitizes mainly tortoises, among other hosts. This tick is the vector of *Borrelia turicatae,* bacteria that belong to the relapsing fever clade, of which tortoises are natural reservoirs. Other borrelial diseases are associated with ixodid ticks and reptiles (mainly lacertid lizards) and are one of the most widespread vector-borne diseases in the northern hemisphere. Additionally, A disease related to the presence of ticks is the “viper plague” in Viperidae snakes, which causative agent is *Erlichia ruminatum.* This disease was introduced to the United States with the importation of a *Bitis gabonica* snake, from Ghana (KIEL et al., 2008). Other importation events are the

introduction of exotic species of ticks and mites to Florida, USA. Where four species of *Amblyomma* ticks, parasites of lizards and tortoises, were found infected with *E. ruminantium* or "Heartwater" disease and *Coxiella burnetti*, which produces Q fever (BURRIDGE et al., 2000). Finally, other rickettsial agents of the spotted fever group have been detected in ticks that infest reptiles.

Nonetheless, the relation between ectoparasites, ectothermic hosts and the circulation of pathogenic agents is not fully known and understood, as well of the implications of these infections to the public human health. Furthermore, four groups of pathogens may be present in the mites and ticks that parasitize the herpetofauna: *Borrelia* spp, *Coxiella* spp, *Hepatozoon* spp, and *Rickettsia* spp.

1.1 *Borrelia* genus

Borrelia are spirochete bacteria divided in the relapsing fever, the reptilian *Borrelia*, monotreme associated *Borrelia*, and the Lyme borreliosis groups. This last group includes around 20 species within the *Borrelia burgdorferi* (sensu lato) complex, nine of which can be pathogenic to animals and humans (MENDOZA-ROLDAN et al., 2019). The *Borrelia burgdorferi* sensu lato group, which causes Lyme disease and other borreliosis, includes species such as *Borrelia lusitaniae* (pathogenic in humans), that use reptiles as natural reservoirs. Ticks of the genus *Ixodes* (*Ixodes ricinus, Ixodes scapularis, I. persulcatus* and *Ixodes pacificus*) are vectors and reservoirs of *Borrelia burgdorferi* sensu lato (LANE, 1990; LEVIN et al., 1996; KUO et al., 2000; SZEKERES et al., 2016; MACDONALD et al., 2017; MENDOZA-ROLDAN et al., 2019). There is also a clade of reptile-associated *Borrelia*, with no demonstrated pathogenicity. This clade has been identified in species of ixodid ticks specialized in reptiles, such as the goanna tick (*Bothriocroton undatum*) (PANETTA et al., 2017). In South America, several studies have revealed the presence of borrelial species in this region of the continent. However, no studies have shown the association of reptiles as reservoirs in the neotropical region (NEED; ESCAMILLA, 1991; DALL'AGNOL et al., 2017; IVANOVA et a., 2014; MUÑOZ-LEAL et al., 2019).

1.2 *Coxiella* genus

Coxiella is a genus of obligatory intracellular gram-negative bacteria belonging to the order Rickettsiales, family Rickettsiaceae, considered to be the causative agent of the zoonotic disease known as Q fever (SCOLA, 2002; MAURIN; RAOULT, 1999). The only species described is *Coxiella burnetii,* widely distributed, with the exception of Antarctica (KAZAR, 2005). Reptiles are reservoirs for these bacteria. The *Hyalomma aegyptium* tick which parasitizes Mediterranean chelonians, is a potential vector (ŠIROKÝ, 2010). Other ticks were recorded as vectors of *C. burnetii*, such as *Amblyomma nuttalli* Dönitz, 1909 from Guinea Bissau (ARTHUR, 1962) and *A. variegatum* in Africa (GIROUD, 1951). However, studies show that there is no scientific evidence of the involvement of these ticks in the transmission of *Coxiella* to reptiles (BURRIDGE, 2001). Furthermore, Mesostigmata mites may have an important role in the transmission of *Coxiella* (*Dermanyssus gallinae, D. passerines, Ornithonyssus bacoti,* and *Steatonyssus viator* associated with birds, *Allodermanyssus sanguineus Hirstionyssus ericen,* and *Androlaelaps (Haemolaelaps) casalis* associated with rodents) (MORO et al., 2005; REEVES et al., 2007). Finally, *Coxiella* has been found to be a common symbiont of ticks (MACHADO-FERREIRA et al., 2011; ŠPITALSKÁ, et al., 2018).

1.3 *Hepatozoon* genus

The genus *Hepatozoon* comprises more than 300 species of protozoa belonging to the Apicomplexa phylum, affecting a great variety of domestic and wild animals (O'DWYER, 2003). Members of this genus are common intracellular protozoa in reptiles and amphibians (TELFORD, 1984; 2008). Mites and ticks, as well as hematophagous Diptera participate in the transmission of *Hepatozoon*. The transmission mechanism is the ingestion of an infected vertebrate intermediate host (for example, a snake may feed on a gecko that has *Hepatozoon*) or the ingestion of infected arthropod vectors (WOZNIAK; TELFORD, 1991). The species of *Hepatozoon* that affect reptiles and amphibians seem well adapted to their hosts, since few pathological changes (TELFORD, 2008). However, under captive conditions, transmission is facilitated (HULL; CAMIN, 1960; ÚNGARI et al., 2018). Inflammatory symptoms are more evident in hosts that are not natural, and in cases of high parasitemia, hemolytic can anemia occurs (WOZNIAK et al., 1996; 1998).

Pterygosomatidae mites of the genus *Hirstiella* are vecttors of *Hepatozoon sauromali* and they have been found naturally infected (LEWIS; WAGNER, 1964). Infection caused by *Hepatozoon lygosomarum* in a lizard of the species *Oligosoma nigriplantare* was also confirmed. This host acquired the protozoan by ingesting the *Ophionyssus scincorum* mite which was infected (ALLISON; DESSER, 1981). *A. rotundatum* and *A. dissimile* ticks are known vectors of Hepatozoon in Brazil (FACCINI, LUZ, 2013).

1.4 *Rickettsia* genus

The Rickettsiaceae family is composed of obligate gram-negative, aerobic and intracellular bacteria (OLANO, 2005; SAHNI; RYDKINA, 2009), which multiply by binary fission and are associated with invertebrate vectors (BIBERSTEIN; HIRSH, 2003; RAOULT et al., 2005). The species *Rickettsia* are distributed throughout the world, infecting vertebrates, being kept in the wild through arthropod vectors (ticks, lice, fleas and mites) (PAROLA et al., 2005). Reptiles and amphibians participate directly in the epidemiology of some pathogens of both the Rickettsiales order and the Rickettsiaceae family (ANDOH et al., 2015; NOVAKOVA et al., 2015). A rickettsial disease in humans, known as African Fever, is caused by *Rickettsia africae* and transmitted by *A. variegatum* (PAROLA et al., 1999). This rickettsial disease, originally from Africa, has been reported from ticks imported into North America, infesting reptiles (BURRIDGE; SIMMONS, 2003). Another *Rickettsi*a discovered in reptiles, which has as a vector the Australian tick *Bothriocroton hydrosauri* is *Rickettsia honei*. This bacterium was isolated from both the saurian hosts of the Scincidae family as well as from the tick, and causes the disease called Flinder island spotted fever, in Australia (STENOS et al., 2003; UNSWORTH et al., 2007). A similar *Rickettsia* to *R. anan* was detected in ticks from the species *Amblyomma exornatum* Koch, 1844, in varanid lizards imported to the USA (REEVES, 2006).

Like ticks, mites can also participate as vectors of some Rickettsial agents. *Rickettsia akari* that produces "Rickettsialpox", a disease transmitted to humans by the mite *Liponyssoides sanguineus*, which is an ectoparasite of the common mouse (*Mus musculus*) (RADULOVIC, 1996; PADDOCK et al., 2006). In Japan, larvae of some species of *Leptotrombidium* (Trombiculidae) disseminate tsutsugamushi disease in humans (Scrub typhus) caused by the bacterium *Orientia tsusugamushi* (TAKAHASHI et al. 2004; PHASOMKUSOLSIL et al., 2009).

In South America different species of *Rickettsia* have been detected linked to ticks that were infesting reptiles. For example, in the Colombian Caribbean, *Rickettsia* sp. strain Colombianensi was detected in *Amblyommma dissimile* ticks on Iguanas and other reptiles, as well as *Rickettsia belli* (MIRANDA et al., 2012; SANTODOMINGO et al., 2018). In brazil, studies show the correlation between *R. belli* and species of ectothermic host-related ticks (*A. dissimile* and *Amblyomma rotundatum*), which may be a symbiont of these parasites. Moreover, recent research found also R. *bellii* in both species of ticks, and *Rickettsia* sp. strain Colombianensi, *Hepatozoon*, and *Anaplasma* in *A. dissimile*, all these ticks from snakes of southeastern Brazil, and *Rickettsia* sp. strain Colombianensi in ticks from toads in the Brazilian amazon. (OGRZEWALSKA, et al., 2018; LUZ et al., 2018).

2 OBJECTIVE

- Detect the presence of selected pathogens (*Borrelia*, *Coxiella*, *Hepatozoon*, and *Rickettsia*) in the studied mites and ticks, and in the collected hosts (blood and tissue) using molecular biology.

3 MATERIAL AND METHODS

3.1 DNA extraction

3.1.1 Mites and ticks

DNA extraction was performed using a lysis with guanidine isothiocyanate protocol (GT) (CHOMKZYNSKI, 1993), which allowed the preservation of a voucher (MENDOZA-ROLDAN, 2015). Later, this technique was applied successfully in Mesostigmata, and Ixodida from reptiles (MENDOZA-ROLDAN et al., 2019). Thus, in the present study the same protocol was used for the collected mites in the IBSP laboratories and of field trips.

3.1.2 Blood and tissues

Eventually, some tissue samples (blood or liver) were obtained (techniques for blood draw detailed in chapter 4) from parasitized hosts in the laboratories of the Instituto Butantan or in field trips. These blood and tissue samples were used for pathogen detection. When animals were euthanized or brought dead to the laboratories, liver tissue of parasitized animals was extracted (~25mg). Tissues were collected with approval of the Ethics Committee of Animal Use (Comissão de Ética no Uso de Animais - CEUA) of the Faculty of Veterinary Medicine of the University of de São Paulo (Faculdade de Medicina Veterinária e Zootecnia da Universidade de São Paulo - FMVZ/USP), protocol nº 7491300715.

DNA was extracted of liver tissue (25 mg) from reptiles, by using a PureLink™ Genomic DNA Mini Kit (Thermo Fisher Scientific, São Paulo, Brazil). Additionally, DNA was extracted from reptile blood (~20 µl) by using a PureLink™ Genomic DNA Mini Kit (Thermo Fisher Scientific, São Paulo, Brazil).

3.2 Polymerase chain reaction (PCR) for pathogens

Borrelia **-** Nested PCR was performed in. The primary reaction contained 2.5µl of DNA as the template, 12.5µl DreamTaq Green PCR Master Mix, 8µl Nuclease-free water, and a 1.0 M concentration (each) of primers FlaLL and FlaRL. The nested reaction mixture contained 1µl of the primary PCR product as the template, plus a 1.0 M concentration (each) of primers FlaLS and FlaRS. Cycling conditions for both reactions involved an initial 3-min denaturation at 95°C and then 40 cycles, with each cycle consisting of a 1-min denaturation at 95°C, a 1-min annealing at 55°C, and a 1-min extension at 75°C. The positive control for both assays was Borrelia *anserina*. Each PCR set included at least one negative control, with water substituted for the DNA template (STROMDAHL et al., 2003; MUÑOZ-LEAL et al., 2019) (Table 39).

Coxiella - All samples were tested for the presence of Coxiella using a primer pair (CAPI-844-F and CAPI-844-R), which amplified a 601 bp fragment of the CAPI gene, (REEVES et al., 2006). The amplification reaction was performed in 200 µl microtubes by adding 2.5 µl of extracted DNA plus 22.5 µl of Mix [22.5µl of DNA as the template, 12.5µl DreamTaq Green PCR Master Mix,

8µl Nuclease-free water, and a 1.0 M concentration (each) of primers] totalizing a volume of 25 µl of Mix per microtube. For each reaction, positive control (*Coxiella burnetti cell* culture - COX Atg 5p) and negative control (Milli-Q water) were used. The conditions of the PCR cycles were: initial denaturation at 95 ° C for 5 minutes, followed by 40 cycles of 1 minute at 95 ° C, 1 minute at 55 ° C, initial extension at 72 ° C for 40 seconds and final extension at 72 ° C for 10 minutes (Table 39).

Hepatozoon - For DNA detection of *Hepatozoon* spp. of PCR (primer HEP 2) was performed with the pairs of primers called HEP2-144-169 F and HEP2-743-718 R, that amplify a fragment of approximately 574 -pb of the 18S rRNA gene (ALMEIDA et al., 2013). *Hepatozoon canis* was used as positive control. For this PCR (Hep2), an initial denaturation was used for 5 minutes at 95 ° C, 30 seconds at 50 ° C, 1 minute at 72 ° C, followed by 7 minutes of final extension at 72 ° C. (Table 39).

Rickettsia – Each DNA sample was PCR-tested using a pair of primers (CS-78F and CS-323R), which amplify a fragment of 401 base pairs (bp) from the gene citrate synthase (*gltA*), present in all species of *Rickettsia* (LABRUNA et al., 2004). Negative controls (DNA free water) and positive (*Rickettsia vini*) were used for each reaction. The PCR temperature conditions performed in the Mastercycler Gradient (Eppendorf California) thermocycler for the gltA gene were: 1 cycle at 95 ° C for 5 minutes, followed by 40 cycles of 30 seconds at 95 ° C, 30 seconds at 58 ° C ° C, 40 seconds at 72 ° C and 7 minutes at 72 ° C.

Positive samples for this gene were tested by a second PCR using a pair of primers (Rr190.70F and Rr190.701R) that amplify a 632 bp fragment of the ompA gene, present only in Spotted fever group (SFG) rickettsiae, as previously described (REGNERY et al., 1991; PACHECO et al., 2007). Negative controls were used for each reaction (Milli-Q water) and positive (*Rickettsia vini*). Cycle conditions for the ompA gene: 1 cycle at 95 ° C for 5 minutes, followed by 35 cycles of 40 seconds at 95 ° C, 30 seconds at 58 ° C, 45 seconds at 72 ° C, with final extension for 10 minutes at 72 ° C (Table 40).

Table 40 - List of primers used in Polymerase Chain Reactions (PCR) for pathogen screening

Gene/ primers	Agent	Primer sequence (5' - 3')	Reference
Fla	*Borrelia*		
FLA LL		ACATATTCAGATGCAG ACAGAGG	(STROMDAHL et al., 2003)
FLA RL		GCAATCATAGCCATTG CAGATTGT	
FLA LS		AACAGCTGAAGAGCTT GGAATG	
FLA RS		CTTTGATCACTTATCAT TCTAATAGC	
cap	*Coxiella*		
CAPI-844F		ATTTAGTGGGTTTCGCGCAT	(REEVES et al., 2006)
CAPI-844R		CATCAGCATACGTTTCGGGAA	
18S rRNA	*Hepatozoon*		
HEP2 144-196 F		GGTAATTCTAGAGCTAATACATGAGC	(ALMEIDA et al., 2013)
HEP2 743-718		ACAATAAAGTAAAAAACAYTTCAAAG	
gltA	*Rickettsia*		
CS-62F		GCAAGTATCGGTGAGGATGTAAT	(LABRUNA et al., 2004)
CS-462R		GCTTCCTTAAAATTCAATAAATCAGGAT	
OmpA	SFG *Rickettsia*		
Rr 190.70		ATGGCGAATATTTCTCCAAAA	(REGNERY et al.,1991)
Rr 190.701		GTTCCGTTAATGGCAGCATCT	

Source: (MENDOZA-ROLDAN, J. A., 2019)

3.3 Reading and analysis of PCR products

All PCR products (5 µL amplified DNA) were subjected to 1.5% agarose gel horizontal electrophoresis [1.5 mg Ultra-Pure Agarose Invitrogen® Carlsbad, CA; 100 mL of 1X TAE (121g Tris Base, 28.5 mL glacial acetic acid, 50 mL of 0.5 M EDTA pH 8.0 H2O milli-Q qsp)] plus SYBR® Safe DNA Gel Stain (0.1 µl / mL) and 1X TAE running buffer pH 8.0 at 100V / 80mA. The gel was visualized with ultraviolet light (UV) in a darkroom (Alphalmager®). The samples that revealed DNA bands same level as the positive control, confirming the nucleotide amplification, were considered positive for the PCR reaction used.

3.4 Purification and Sequencing of Nucleotides

Samples of amplified products of the PCRs that had concentrations above 20 ng / µl were selected. The amplified products were then subjected to DNA purification through the commercial product ExoSa-IT (USB Corporation). Part of the purified samples were sequenced at the Center for Human Genome and Stem Cell Research at the Institute of Biosciences- USP, others at the Bacteriology Laboratory - Unit 2, of the Instituto Butantan, and a large portion of the samples were

sequenced at the Laboratório de Biologia Molecular Aplicada e Sorologia (LABMAS), of the FMVZ-USP. Sanger sequencing was performed, which is for DNA from PCR products and plasmids, using the ABI 3730 DNA Analyzer. This is a 48-capillary DNA analysis system with Life Technologies-Applied Biosystems technology. Sequencing reactions were performed by the BigDye Terminator v3.1 Cycle Sequencing Kit. The runs were done in 36 cm capillaries using the POP7 polymer.

3.5 Sequence analyses

The sequences obtained were edited using the SeqMan program (Lasergene, DNAstar, Madison, Wis.) and also analyzed using Geneious version 11.1.4 software and submitted to to identify similarities to known sequences using the Basic Local Alignment Search Tool (BLAST) (ALTSCHUL et al., 1990) to verify homology with corresponding sequences available from GenBank.

3.6 Distribution of detected pathogens

Distribution maps were generated using QGIS version 3.4.4-Madeira, to illustrate the origin of the detected pathogens (QGIS DEVELOPMENT TEAM, 2015).

4 RESULTS

4.1 Pathogens detected from mites and ticks

A total of 139 samples of different mite and tick species (Tombidiformes, Mesostigmata, Ixodida) were screened for the selected pathogens *Borrelia*, *Coxiella*, *Hepatozoon*, and *Rickettsia*. Of these, two samples of *A. rotundatum* of *C. hortullanus* and *O. melanogenys*, from Acre state, amplified for *Borrelia*. No samples amplified for *Coxiella*. Furthermore, seven samples amplified *Hepatozoon* (*G. hemidactyli, Ornithodoros (Alectorobius), E. alfreddugesi* from São Paulo state; and four *A. rotundatum* from snakes of Acre state). *gltA* gene of *Rickettsia* amplified in 19 samples (*O. rotundus, A. sculptum, E. alfreddugesi*, *O. natricis,* five *G. harrisi* and two *A. rotundatum* from

São Paulo state; one *A. rotundatum* from Minas Gerais state four *A. rotundatum*, two from Mato Grosso state and Two from Espirito Santo state; *Chironobius* sp. n, and three *A. rotundatum* from Acre state. Of these 19 samples, eight amplified for OmpA gene for Spotted fever group (SFG) *Rickettsia* (*O. natricis, O. rotundus,* and four *G. harrisi* from São Paulo state; and *Chironobius* sp. n, and *A. rotundatum*, from Acre state) (Table 41).

Table 41 – species of Acari, results of pathogens detected

IBSP	Species	Pathogens genes				
		Fla* *Borrelia	***Cap* Coxiella**	**18S *Hepatozoon***	***gltA* *Rickettsia***	**OmpA SFG *Rickettsia***
12911	*G. hemidactyli*	NA	NA	NA	NA	NA
12912	*G. hemidactyli*	NA	NA	NA	NA	NA
12913	*G. hemidactyli*	NA	NA	A	NA	NA
12916	*G. hemidactyli*	NA	NA	NA	NA	NA
12930	*G. hemidactyli*	NA	NA	NA	NA	NA
12931	*G. hemidactyli*	NA	NA	NA	NA	NA
12933	*G. hemidactyli*	NA	NA	NA	NA	NA
12940	*G. hemidactyli*	NA	NA	A	NA	NA
12908	*O. parkeri*	NA	NA	NA	NA	NA
12950	*E. alfreddugesi*	NA	NA	NA	NA	NA
12951	*E. alfreddugesi*	NA	NA	NA	NA	NA
12952	*E. alfreddugesi*	NA	NA	NA	NA	NA
12917	*E. alfreddugesi*	NA	NA	NA	NA	NA
12955	*E. ophidica*	NA	NA	NA	NA	NA
12956	*E. ophidica*	NA	NA	NA	NA	NA
12906	*E. tropica*	NA	NA	NA	NA	NA
12918	*H. achalai*	NA	NA	NA	NA	NA
12919	*H. achalai*	NA	NA	NA	NA	NA
12920	*H. achalai*	NA	NA	NA	NA	NA
12921	*H. achalai*	NA	NA	NA	NA	NA
12922	*H. achalai*	NA	NA	NA	NA	NA
12923	*H. achalai*	NA	NA	NA	NA	NA
12924	*H. achalai*	NA	NA	NA	NA	NA
12925	*H. achalai*	NA	NA	NA	NA	NA
12926	*H. achalai*	NA	NA	NA	NA	NA
12927	*H. achalai*	NA	NA	NA	NA	NA
12928	*H. achalai*	NA	NA	NA	NA	NA
12929	*H. achalai*	NA	NA	NA	NA	NA
12934	*H. hepatica*	NA	NA	NA	NA	NA
12935	*H. hepatica*	NA	NA	NA	NA	NA
12957	*H. hepatica*	NA	NA	NA	NA	NA
12932	*A. dissimile*	NA	NA	NA	NA	NA
12910	*A. humerale*	NA	NA	NA	NA	NA
12909	*A. rotundatum*	NA	NA	NA	NA	NA
12915	*A. rotundatum*	NA	NA	NA	NA	NA
12936	*A. rotundatum*	NA	NA	NA	A	NA
12937	*A. rotundatum*	NA	NA	NA	A	NA

(Continues)

IBSP	Species	Pathogens genes				
		Fla* *Borrelia	***Cap* Coxiella**	**18S *Hepatozoon***	***gltA* *Rickettsia***	**OmpA SFG *Rickettsia***
12938	*A. rotundatum*	NA	NA	NA	NA	NA
12939	*A. rotundatum*	NA	NA	NA	NA	NA
12954	*A. rotundatum*	NA	NA	NA	NA	NA
12978	*A. rotundatum*	NA	NA	NA	A	NA
12907	*O. natricis*	NA	NA	NA	NA	NA
12983	*O. natricis*	NA	NA	NA	NA	NA
12586	*O. natricis*	NA	NA	NA	NA	NA
12953	*H. achalai*	NA	NA	NA	NA	NA
12978	*A. rotundatum*	NA	NA	NA	N	NA
12992	*A. longisetosus*	NA	NA	NA	NA	NA
12990	*A. rotundatum*	NA	NA	NA	NA	NA
12910	*A. humerale*	NA	NA	NA	NA	NA
12908	*O. parkeri*	NA	NA	NA	NA	NA
12907	*O. natricis*	NA	NA	NA	NA	NA
12953	*Z. oudemansi*	NA	NA	NA	NA	NA
12950	*E. alfreddugesi*	NA	NA	NA	NA	NA
12951	*E. alfreddugesi*	NA	NA	NA	NA	NA
12952	*E. alfreddugesi*	NA	NA	NA	NA	NA
12925	*H. achalai*	NA	NA	NA	NA	NA
12926	*H. achalai*	NA	NA	NA	NA	NA
12927	*H. achalai*	NA	NA	NA	NA	NA
12928	*H. achalai*	NA	NA	NA	NA	NA
12929	*H. achalai*	NA	NA	NA	NA	NA
12934	*H. hepatica*	NA	NA	NA	NA	NA
12935	*H. hepatica*	NA	NA	NA	NA	NA
12918	*H. achalai*	NA	NA	NA	NA	NA
12919	*H. achalai*	NA	NA	NA	NA	NA
12983	*O. natricis*	NA	NA	NA	NA	NA
12955	*E. ophidica*	NA	NA	NA	NA	NA
12956	*E. ophidica*	NA	NA	NA	NA	NA
12906	*E. tropica*	NA	NA	NA	NA	NA
12921	*H. achalai*	NA	NA	NA	NA	NA
14907	*O. ekans*	NA	NA	NA	NA	NA
13660	*O. rotundus*	NA	NA	NA	A	A
13766	*A. rotundatum*	NA	NA	NA	NA	NA
13767	*A. rotundatum*	NA	NA	NA	NA	NA
13768	*A. rotundatum*	NA	NA	NA	NA	NA

(Continues)

IBSP	Species	Pathogens genes				
		Fla* *Borrelia	***Cap* Coxiella**	**18S *Hepatozoon***	***gltA* *Rickettsia***	**OmpA SFG *Rickettsia***
14828	*E. alfreddugesi*	NA	NA	NA	NA	NA
14829	*E. alfreddugesi*	NA	NA	NA	NA	NA
14830	*A. rotundatum*	NA	NA	NA	NA	NA
14831	*E. alfreddugesi*	NA	NA	NA	NA	NA
14832	*A. sculptum*	NA	NA	NA	A	NA
14833	*E. alfreddugesi*	NA	NA	NA	NA	NA
14834	*E. alfreddugesi*	NA	NA	NA	NA	NA
14835	*E. alfreddugesi*	NA	NA	NA	NA	NA
14836	*E. alfreddugesi*	NA	NA	NA	NA	NA
14837	*G. hemidactyli*	NA	NA	NA	NA	NA
14838	*Ornithodoros (Alectorobius)*	NA	NA	A	NA	NA
14839	*E. alfreddugesi*	NA	NA	A	A	NA
14840	*E. alfreddugesi*	NA	NA	NA	NA	NA
14845	*A. rotundatum*	NA	NA	NA	NA	NA
14846	*B. jimenezi*	NA	NA	NA	NA	NA
14847	*B. jimenezi*	NA	NA	NA	NA	NA
14848	*B. jimenezi*	NA	NA	NA	NA	NA
14849	*B. jimenezi*	NA	NA	NA	NA	NA
14850	*B. jimenezi*	NA	NA	NA	NA	NA
14851	*B. jimenezi*	NA	NA	NA	NA	NA
14852	*B. jimenezi*	NA	NA	NA	NA	NA
14853	*B. jimenezi*	NA	NA	NA	NA	NA
14854	*B. jimenezi*	NA	NA	NA	NA	NA
14855	*B. jimenezi*	NA	NA	NA	NA	NA
14856	*B. jimenezi*	NA	NA	NA	NA	NA
14857	*B. jimenezi*	NA	NA	NA	NA	NA
14858	*B. jimenezi*	NA	NA	NA	NA	NA
14859	*B. jimenezi*	NA	NA	NA	NA	NA
14860	*B. jimenezi*	NA	NA	NA	NA	NA
14861	*B. jimenezi*	NA	NA	NA	NA	NA
14862	*B. jimenezi*	NA	NA	NA	NA	NA
14864	*A. rotundatum*	NA	NA	NA	NA	NA
14865	*A. rotundatum*	NA	NA	NA	NA	NA
14866	*A. rotundatum*	NA	NA	NA	NA	NA
14867	*G. harrisi*	NA	NA	NA	NA	NA
14868	*O. rotundus*	NA	NA	NA	NA	NA
14869	*A. rotundatum*	NA	NA	NA	A	NA

(Conclusion)

IBSP	Species	Pathogens genes				
		Fla* *Borrelia	***Cap* Coxiella**	**18S *Hepatozoon***	***gltA* *Rickettsia***	**OmpA SFG *Rickettsia***
14870	*A. rotundatum*	NA	NA	NA	A	NA
14871	*A. rotundatum*	NA	NA	NA	A	NA
14873	*A. rotundatum*	NA	NA	NA	A	NA
14874	*O. natricis*	NA	NA	NA	A	A
14875	*A. rotundatum*	NA	NA	A	NA	NA
14876	*E. alfreddugesi*	NA	NA	NA	NA	NA
14878	*Chironobius* sp. n.	NA	NA	NA	A	A
14879	*A. rotundatum*	NA	NA	A	NA	NA
14880	*A. rotundatum*	NA	NA	NA	A	NA
14881	*E. alfreddugesi*	NA	NA	NA	NA	NA
14882	*A. rotundatum*	A	NA	A	A	A
14883	*A. rotundatum*	A	NA	A	NA	NA
14884	*Z. oudemansi*	NA	NA	NA	NA	NA
14885	*A. rotundatum*	NA	NA	NA	A	NA
14886	*F. anguina*	NA	NA	NA	NA	NA
14887	*G. harrisi*	NA	NA	NA	NA	NA
14888	*G. harrisi*	NA	NA	NA	NA	NA
14889	*G. harrisi*	NA	NA	NA	A	NA
14890	*G. harrisi*	NA	NA	NA	NA	NA
14891	*G. harrisi*	NA	NA	NA	A	A
14892	*G. harrisi*	NA	NA	NA	A	A
14893	*G. harrisi*	NA	NA	NA	A	A
14894	*G. harrisi*	NA	NA	NA	A	A
14895	*A. rotundatum*	NA	NA	NA	NA	NA
14896	*H. hepatica*	NA	NA	NA	NA	NA
14897	*B. jimenezi*	NA	NA	NA	NA	NA
14898	*A. rotundatum*	NA	NA	NA	NA	NA
14899	*A. rotundatum*	NA	NA	NA	NA	NA

Source: (MENDOZA-ROLDAN, J. A., 2019)

Legend: A: Amplified, NA: Not Amplified.

4.2 Pathogens detected from hosts' blood and tissue

Fortyeight samples of blood from reptiles that were parasitized, were screened for the selected pathogens *Borrelia*, *Coxiella*, *Hepatozoon*, and *Rickettsia*. Of these seven samples of snakes amplified for *Hepatozoon* (*P. nattererii* co-infested with *E. alfreddugesi* and *Ornithodoros (Alectorobius)* sp. from São Paulo state; and *C. multiventris* infested with *E. alfreddugesi*, *Chironobius* sp. n, *A. rotundatum* and another *C. multiventris* infested only with *A. rotundatum*, *C. scurrulus* infested with *E. alfreddugesi* and *A. rotundatum*, *C. hortullanus* infested with *A. rotundatum*, *O. melanogenys* infested with *Z. oudemansi* and *A. rotundatum*, and *P. viridissima* infested with *A. rotundatum*, all from Acre state), and one sample of a lizard *P. vitticeps* infested with *O. natricis* from São Paulo state (Table 42).

Table 42 – Species of hosts and their Acari, results of pathogens detected on blood

IBSP of Acari	Host	Species of Acari	Pathogens genes				
			Fla *Borrelia*	*Cap* Coxiella	18S *Hepatozoon*	*gltA* *Rickettsia*	OmpA SFG *Rickettsia*
12907	*C. durissus terrificus*	*O. natricis*	NA	NA	NA	NA	NA
12908	*C. bicarinatus*	*O. parkeri*	NA	NA	NA	NA	NA
12909	*C. durissus terrificus*	*A. rotundatum*	NA	NA	NA	NA	NA
12911	*H. mabouia*	*G. hemidactyli*	NA	NA	NA	NA	NA
12912	*H. mabouia*	*G. hemidactyli*	NA	NA	NA	NA	NA
12913	*H. mabouia*	*G. hemidactyli*	NA	NA	NA	NA	NA
12915	*C. durissus terrificus*	*A. rotundatum*	NA	NA	NA	NA	NA
12916	*H. mabouia*	*G. hemidactyli*	NA	NA	NA	NA	NA
12917	*S. pullatus*	*E. alfreddugesi*	NA	NA	NA	NA	NA
12930	*H. mabouia*	*G. hemidactyli*	NA	NA	NA	NA	NA
12940	*H. mabouia*	*G. hemidactyli*	NA	NA	NA	NA	NA
12950	*A. reticulata*	*E. alfreduggesi*	NA	NA	NA	NA	NA
12951	*K. calcarata*	*E. alfreduggesi*	NA	NA	NA	NA	NA
12952	*K. calcarata*	*E. alfreduggesi*	NA	NA	NA	NA	NA
12954	*B. jararaca*	*A. rotundatum*	NA	NA	NA	NA	NA
12955	*K. calcarata*	*E. ophidica*	NA	NA	NA	NA	NA
12956	*K. calcarata*	*E. ophidica*	NA	NA	NA	NA	NA
12933	*H. mabouia*	*G. hemidactyli*	NA	NA	NA	NA	NA

(Continues)

IBSP of Acari	Host	Species of Acari	Pathogens genes				
			Fla Borrelia	*Cap* Coxiella	18S *Hepatozoon*	*gltA Rickettsia*	OmpA SFG *Rickettsia*
12940	*H. mabouia*	*G. hemidactyli*	NA	NA	NA	NA	NA
12978	*C. durissus terrificus*	*A. rotundatum*	NA	NA	NA	NA	NA
14829	*C. nigropunctatum*	*E. alfreduggesi*	NA	NA	NA	NA	NA
14831	*A. dorsivittatum*	*E. alfreduggesi*	NA	NA	NA	NA	NA
14832	*S. merianae*	*A. sculptum*	NA	NA	NA	NA	NA
14833	*A. dorsivittatum*	*E. alfreduggesi*	NA	NA	NA	NA	NA
14834	*A. meridionalis*	*E. alfreduggesi*	NA	NA	NA	NA	NA
14835	*C. nigropunctatum*	*E. alfreduggesi*	NA	NA	NA	NA	NA
14836	*C. nigropunctatum*	*E. alfreduggesi*	NA	NA	NA	NA	NA
14837	*H. mabouia*	*G. hemidactyli*	NA	NA	NA	NA	NA
14838	*P. nattererii*	*E. alfreddugesi* *Ornithodoros (Alectorobius)* sp.	NA	NA	A	NA	NA
			NA	NA	NA	NA	NA
14871	*D. neuwiedi*	*A. rotundatum*	NA	NA	NA	NA	NA
14873	*B. leucurus*	*A. rotundatum*	NA	NA	NA	NA	NA
14874	*P. vitticeps*	*O. natricis*	NA	NA	A	NA	NA
14874	*P. vitticeps*	*O. natricis*	NA	NA	NA	NA	NA
14875	*C. multiventris*	*E. alfreddugesi* *Chironobius* sp. n *A. rotundatum*	NA	NA	A	NA	NA
14879	*C. multiventris*	*A. rotundatum*	NA	NA	A	NA	NA
14880	*C. scurrulus*	*E. alfreddugesi* *A. rotundatum*	NA	NA	A	NA	NA
14882	*C. hortullanus*	*A. rotundatum*	NA	NA	A	NA	NA
14883	*O. melanogenys*	*Z. oudemansi* *A. rotundatum*	NA	NA	A	NA	NA
14885	*P. viridissima*	*A. rotundatum*	NA	NA	A	NA	NA
14886	*E. typhlus*	*E. alfreddugesi*	NA	NA	NA	NA	NA
14887	*T. catalanensis*	*G. harrisi*	NA	NA	NA	NA	NA
14888	*T. catalanensis*	*G. harrisi*	NA	NA	NA	NA	NA

(Conclusion)

IBSP of Acari	Host	Species of Acari	Pathogens genes				
			Fla Borrelia	*Cap* Coxiella	18S *Hepatozoon*	*gltA Rickettsia*	OmpA SFG *Rickettsia*
14889	*T. catalanensis*	*G. harrisi*	NA	NA	NA	NA	NA
14890	*T. catalanensis*	*G. harrisi*	NA	NA	NA	NA	NA
14891	*T. catalanensis*	*G. harrisi*	NA	NA	NA	NA	NA
14892	*T. catalanensis*	*G. harrisi*	NA	NA	NA	NA	NA
14893	*T. catalanensis*	*G. harrisi*	NA	NA	NA	NA	NA
14894	*T. catalanensis*	*G. harrisi*	NA	NA	NA	NA	NA

Source: (MENDOZA-ROLDAN, J. A., 2019)

Legend: A: Amplified, NA: Not Amplified.

Furthermore, 12 samples of liver from reptiles that were parasitized, were screened for the selected pathogens *Borrelia*, *Coxiella*, *Hepatozoon*, and *Rickettsia*. Of these three samples amplified for *Borrelia* (two *K. calcarata* infested with *E. alfreddugesi* from Pará state and one *A. dorsivittatum* infested with *E. alfreddugesi* from São Paulo state (Table 43).

Table 43 – Species of hosts and their Acari, results of pathogens detected on liver tissue

IBSP of Acari	Host	Species of Acari	Pathogens genes				
			Fla Borrelia	*Cap* Coxiella	18S *Hepatozoon*	*gltA Rickettsia*	OmpA SFG *Rickettsia*
12933	*H. mabouia*	*G. hemidactyli*	NA	NA	NA	NA	NA
13660	*X. neuwiedii*	*O. parkeri*	NA	NA	NA	NA	NA
14867	*T. torquatus*	*G. harrisi*	NA	NA	NA	NA	NA
14829	*C. nigropunctatum*	*E. alfreddugesi*	NA	NA	NA	NA	NA
14830	*B. insularis*	*A. rotundatum*	NA	NA	NA	NA	NA
12958	*H. mabouia*	*G. hemidactyli*	NA	NA	NA	NA	NA
12951	*K. calcarata*	*E. alfreddugesi*	A	NA	NA	NA	NA
12952	*K. calcarata*	*E. alfreddugesi*	A	NA	NA	NA	NA
12913	*H. mabouia*	*G. hemidactyli*	NA	NA	NA	NA	NA
14837	*H. mabouia*	*G. hemidactyli*	NA	NA	NA	NA	NA
12930	*H. mabouia*	*G. hemidactyli*	NA	NA	NA	NA	NA
14831	*A. dorsivittatum*	*E. alfreddugesi*	A	NA	NA	NA	NA

Source: (MENDOZA-ROLDAN, J. A., 2019)

Legend: A: Amplified, NA: Not Amplified.

4.3 Sequences identity (BLAST)

The amplified samples were sequenced and submitted to BLAST analyses for sequence similarity in the GenBank database. The two sequences generated from ticks and the three sequences generated from lizards of *Borrelia* had no significant similarities found. Moreover, the *Hepatozoon* sequences had similarities with three different sequences: *Hepatozoon* sp. BT-2016, *Hepatozoon ayorgbor*, and *Hepatozoon* sp. CCS-2010. *Hepatozoon* sp. BT-2016 was identified in samples from Trombiculidae, Pterygosomatidae and Argasidae of gecko lizard and snake. *Hepatozoon ayorgbor* was identified from Ixodidae and from snakes, and *Hepatozoon* sp. CCS-2010 was identified from a lizard host (Table 44).

Table 44 – Identification of the sequenced samples and the BLAST results of *Hepatozoon*

Sample	Identity	GenBank	Reference	Locality
IBSP 12940 T	(97.70%)	*Hepatozoon* sp. BT-2016 KU680466	Tom A et al., 2016	Morocco
IBSP 14838 I	(98.57%)	*Hepatozoon* sp. BT-2016 KU680466	Tom A et al., 2016	Morocco
IBSP 14839 T	(98.96%)	*Hepatozoon* sp. BT-2016 KU680466	Tom A et al., 2016	Morocco
IBSP 14875 I IBSP 14879 I IBSP 14882 I IBSP 14883 I	(98.97%)	*Hepatozoon ayorgbor* EF157822	Sloboda et al., 2007	Ghana
IBSP 14838 H	(96%)	*Hepatozoon* sp. BT-2016 KU680466	Tom A et al., 2016	Morocco
IBSP 14874 H	(99.80%)	*Hepatozoon* sp. CCS-2010 HM585212	Salakij et al., 2010	Thailand

(Conclusion)

Sample	Identity	GenBank	Reference	Locality
IBSP 14875 H IBSP 14879 H IBSP 14882 H IBSP 14883 H IBSP 14885 H	(99%)	*Hepatozoon ayorgbor* EF157822	Sloboda et al., 2007	Ghana

Source: (MENDOZA-ROLDAN, J. A., 2019)

Legend: T: Trombidiformes; I: Ixodidae; H: host.

On the other hand, *gltA* gene of *Rickettsia*, had similarities with *Rickettsia amblyommatis Rickettsia bellii*, and *Rickettsia rhipicephali*. Most ticks' samples, and one Trombiculidae sample (*E. alfreddugesi*) were identical to *Rickettsia bellii* (*A. sculptum* and *A. rotundatum*). One sample of *A. rotundatum* from Acre state had 99.75% identity with *Rickettsia amblyommatis* (KY273545). Moreover, samples from Mesostigmata mites (*Chironobius* sp. n, *O. natricis*, and *O. rotundus*), and Pterygosomatidae (*G. harrisi*) mites had >99% identity with *Rickettsia rhipicephali* (Table 45).

Table 45 – Identification of the sequenced samples and the BLAST results of *gltA* gene of *Rickettsia*

Sample	Identity	GenBank	Reference	Locality
IBSP 12936 I IBSP 12937 I	(99.80%)	*Rickettsia bellii* JQ664297	Barbieri, A.,et al, 2012	El Salvador
IBSP 12978 I	(99.20%)	*Rickettsia bellii* EU826511	Tomassone L. et al., 2008	Argentina
IBSP 13660 M	(99.40%)	*Rickettsia rhipicephali* CP013133	Felsheim R. et al., 2015	Ribeirão Grande, São Paulo, Brazil
IBSP 14832 I	(99.70%)	*Rickettsia bellii* KU557517	Oliveira H. et al., 2016	Nova Iguacu, Rio de Janeiro, Brazil
IBSP 14839 T	(100%)	*Rickettsia bellii* KU557517	Oliveira H. et al., 2016	Nova Iguacu, Rio de Janeiro, Brazil

(Conclusion)

Sample	Identity	GenBank	Reference	Locality
IBSP 14870 I IBSP 14871 I IBSP 14873 I	(100%)	*Rickettsia bellii* KU557517	Oliveira H. et al., 2016	Nova Iguacu, Rio de Janeiro, Brazil
IBSP 14874 M IBSP 14878 M	(99.01%)	*Rickettsia rhipicephali* CP013133	Felsheim R. et al., 2015	Ribeirão Grande, São Paulo, Brazil
IBSP 14880 I	(98.76%)	*Rickettsia bellii* KU557517	Oliveira H. et al., 2016	Nova Iguacu, Rio de Janeiro, Brazil
IBSP 14882 I	(99.75%)	*Rickettsia amblyommatis* KY273545	Bitencourth K. et al., 2017	Cerrado biome, Brazil
IBSP 14885 I	(99.75%)	*Rickettsia bellii* KU557517	Oliveira H. et al., 2016	Nova Iguacu, Rio de Janeiro, Brazil
IBSP 14889 T IBSP 14891 T IBSP 14892 T IBSP 14893 T IBSP 14894 T	(99.50%)	*Rickettsia rhipicephali* KX434745	Krawczak, 2016	Derrubadas, Rio Grande do Sul, Brazil

Source: (MENDOZA-ROLDAN, J. A., 2019)

Legend: T: Trombidiformes; Mesostigmata; I: Ixodidae.

Finally, of the 19 positive sequences for *gltA* gene of *Rickettsia*, eight amplified for *OmpA* gene for Spotted fever group (SFG) *Rickettsia.* Of these amplicons, seven were sequenced (*Chironobius* sp. n did not generate a high-quality sequence). Furthermore, four species of SFG *Rickettsia* were identified. Mesostigmata mites *O. rotundus* sample was similar with *Rickettsia rhipicephali* and *O. natricis* sample with *Rickettsia aeschlimannii.* A sample from *A. rotundatum* from Acre state had 99.62% identity with *Rickettsia amblyommatis*. Finally, Pterygosomatidae mites *G. harrisi* from São Paulo state were 98.02% similar with *Rickettsia rickettsia* (Table 46).

Table 46 – Identification of the sequenced samples and the BLAST results of *OmpA* gene for SFG *Rickettsia*

Sample	Identity	GenBank	Reference	Locality
IBSP 13660 M	(94.70%)	*Rickettsia rhipicephali* CP013133	Felsheim R. et al., 2015	Ribeirão Grande, São Paulo, Brazil
IBSP 14874 M	(97.11%)	*Rickettsia aeschlimannii* MF002555	Guo, L. et al., 2017	China
IBSP 14882 I	(99.62%)	*Rickettsia amblyommatis* KY053885	Ogrzewalska, M. et al., 2016	Acre, Brazil
IBSP 14891 T IBSP 14892 T IBSP 14893 T IBSP 14894 T	(98.02%)	*Rickettsia rickettsii* MF988095	Faccini-Martinez et al., 2018	Espirito Santo, Brazil

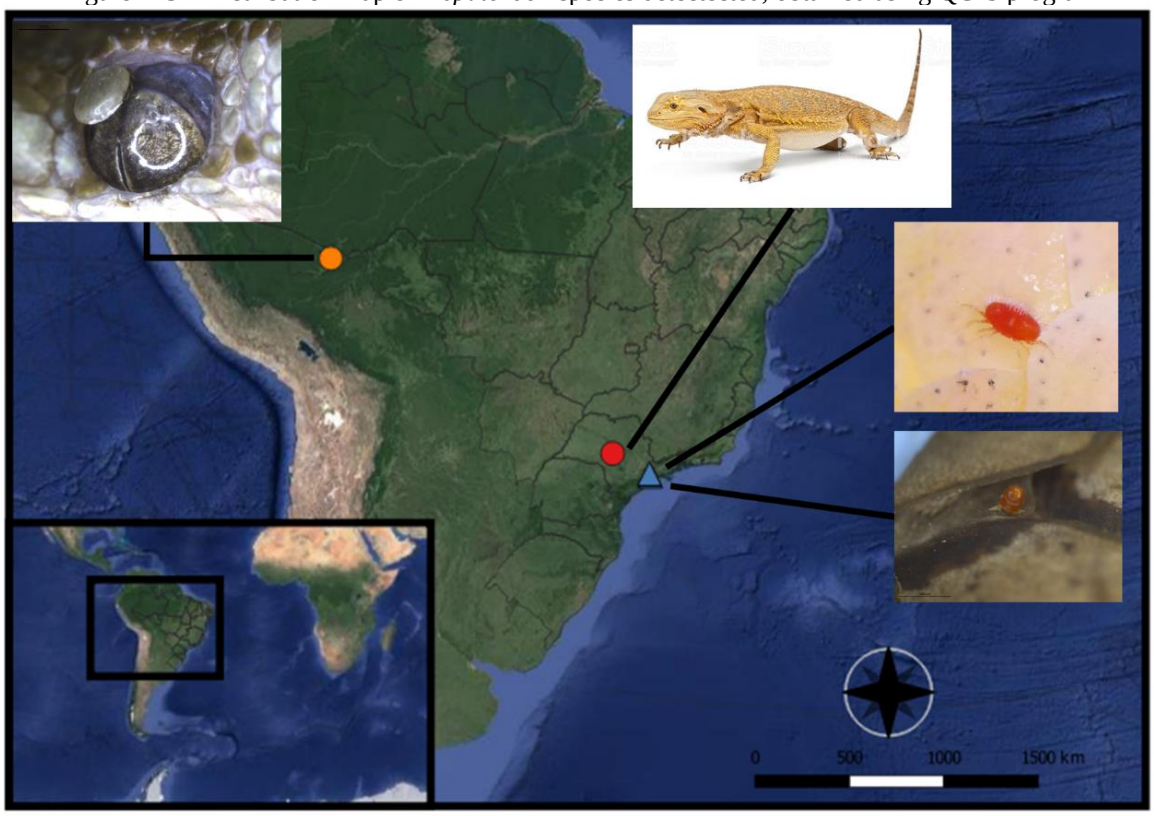
Source: (MENDOZA-ROLDAN, J. A., 2019)

Legend: T: Trombidiformes; Mesostigmata; I: Ixodidae.

4.4 Distribution of detected pathogens

Of the four studied pathogens, two (*Hepatozoon* and *Rickettsia*) were detected from the DNA extracted from the samples of ectoparasites and hosts. *Hepatozoon* was detected from Trombidiformes and Ixodida Acari and hosts (lizards and snakes' blood). The different species of *Hepatozoon* found in this study were *Hepatozoon* sp. BT-2016 from São Paulo, SP (*Geckobia hemidactyli* from *H. mabouia*), and São Bernardo do Campo, SP [*Eutrombicula alfreddugesi* and *Ornithodoros (Alectorobius)* sp. from *Philodryas nattererii* (both parasites and host positives)]; *Hepatozoon ayorgbor* from ticks and snakes from Iracema, AC (*Amblyomma rotundatum* fron *C. multiventris, C. scurrulus, C. hortullanus, O. melanogenys,* and *P. viridissima*); and *Hepatozoon* sp. CCS-2010 from a *Pogona vitticeps* lizards from Zoo Bauru, SP (Figure 113).

Figure 113 – Distribution map of *Hepatozoon* species detectected, obtained using QGIS program



Source: (MENDOZA-ROLDAN, J. A., 2019).

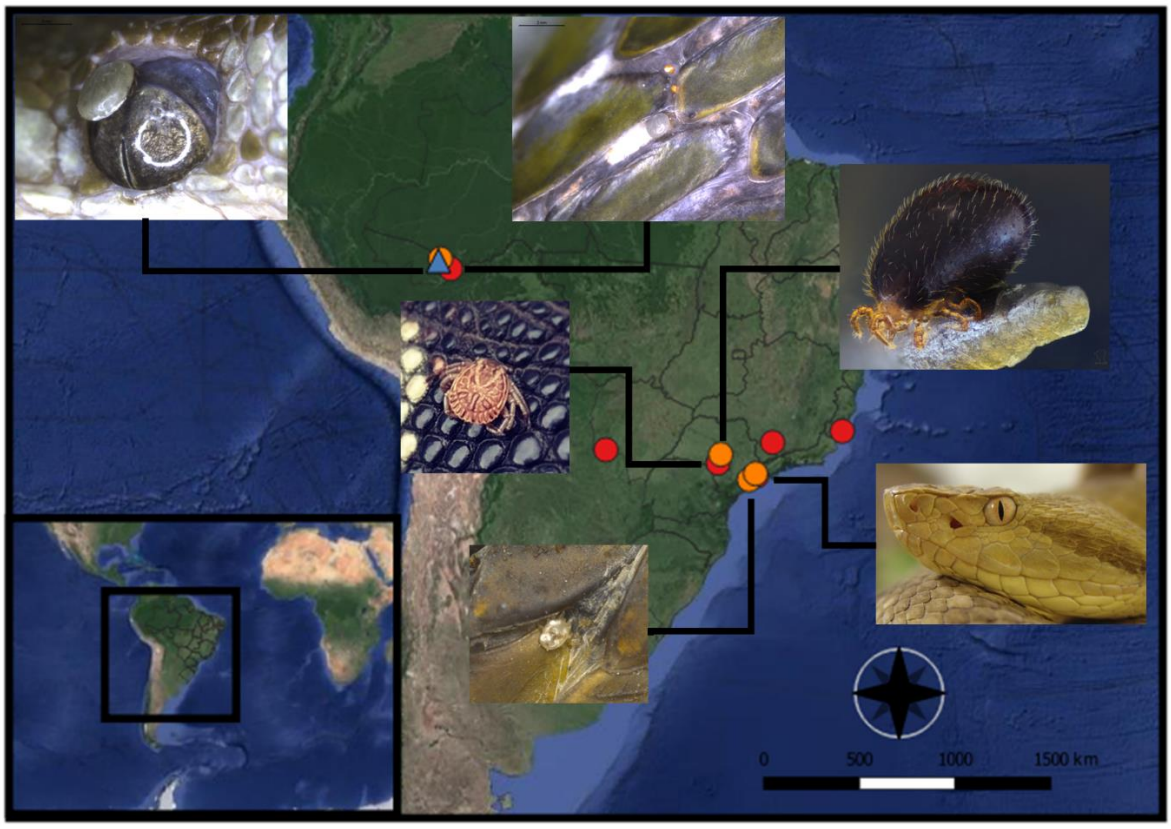
Legend: (red circle) Hepatozoon sp. CCS-2010 from a *Pogona vitticeps*, (blue triangles) *Hepatozoon* sp. BT-2016 from (*Geckobia hemidactyli* and *Eutrombicula alfreddugesi* and *Ornithodoros (Alectorobius)* sp. and *Philodryas nattererii*, (orange circle) *Hepatozoon ayorgbor* from *Amblyomma rotundatum* fron *C. multiventris, C. scurrulus, C. hortullanus, O. melanogenys,* and *P. viridissima.*

.

On the other hand, *Rickettsia* species were detected from only ectoparasites of the three main orders (Trombidiformes, Mesostigmata and Ixodida). *Rickettsia bellii* was identified from *A. rotundatum* ticks from *Bothrops insularis* from Ilha da Queimada Grande, SP; *A. rotundatum* from Varginna, MG from *C. durissus terrificus*; *A.sculptum* from Santa Bárbara, SP, on, *Salvator merianae; Eutrombicula alfreddugesi* São Bernardo do Campo, SP on *Philodryas nattererii*; *A. rotundatum* from Caracol, MS from *Dipsas turgidus; A. rotundatum* from Anchieta, ES from *Dipsas neuwiedi* and *Bothrops leucurus; A. rotundatum* from Iracema, AC on *Chironius multiventris* and *Philodryas viridissima. Rickettsia rhipicephali* was identified from *Ophiogongylus rotundus* from *X. neuwiedii* from Juquitiba, SP; *Ophionyssus natricis* on *Pogona vitticeps* from Zoo Bauru, SP; *Chironobius* sp.n. from Iracema, AC on *Chironius multiventris;* and from *Geckobiella harrisi* from São Paulo, SP on *Tropidurus catalanensis.* Finally, *Rickettsia*

amblyommatis was identified from *A. rotundatum* from Iracema, AC on *Corallus hortulanus* (Figure 114).

Figure 114 – Distribution map of *gltA* gene of *Rickettsia* species detectected, obtained using QGIS program

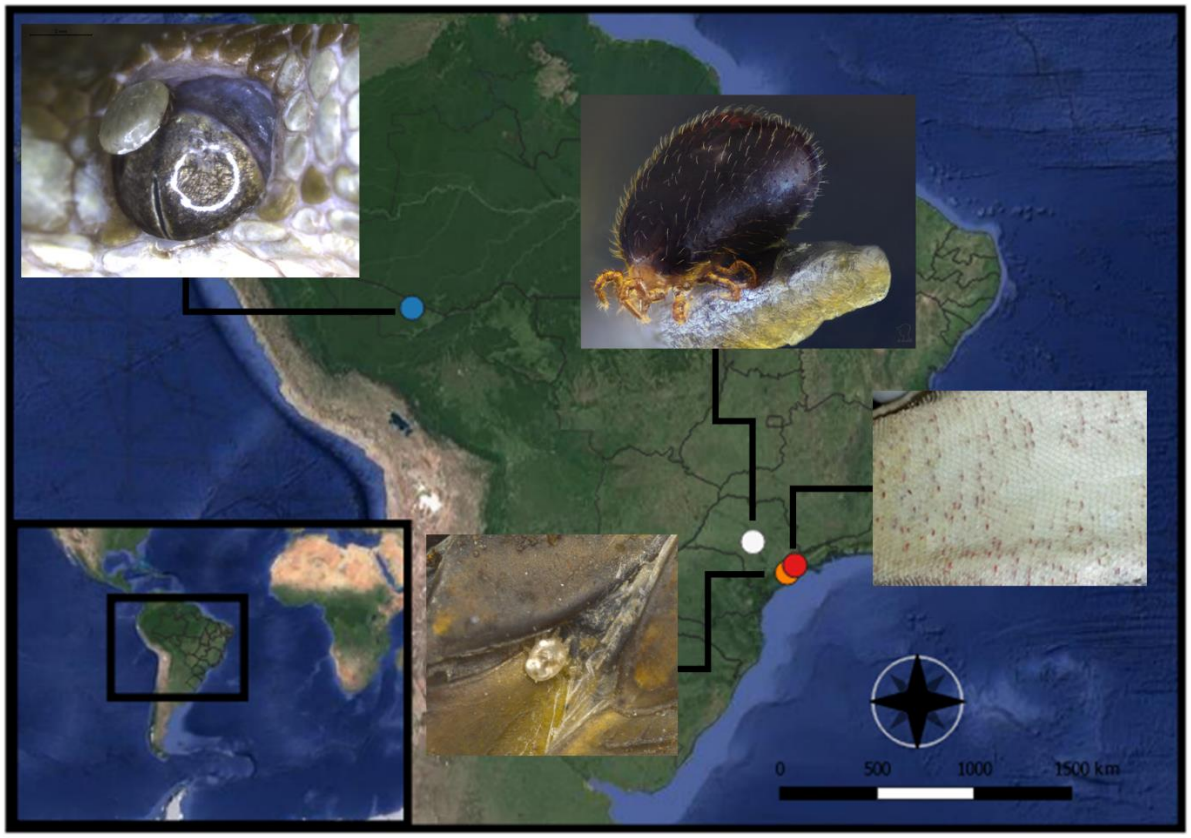


Source: (MENDOZA-ROLDAN, J. A., 2019).

Legend: (red circle) *Rickettsia bellii* from *A. rotundatum*, *A. sculptum* (blue triangle) *Rickettsia amblyommatis* from *A. rotundatum* from Iracema, AC on *Corallus hortulanus*, (orange circles) *Rickettsia rhipicephali* from *Ophiogongylus rotundus*, *Ophionyssus natricis*, *Chironobius* sp.n. and *Geckobiella harrisi.*

Finally, the identified species of Spotted fever group (SFG) *Rickettsia*, were distributed as follows: *Rickettsia rhipicephali* from *Ophiogongylus rotundus* from *X. neuwiedii* from Juquitiba, SP; *Rickettsia aeschlimannii* from *Ophionyssus natricis* on *Pogona vitticeps* from Zoo Bauru, SP; *Rickettsia amblyommatis* was identified from *A. rotundatum* from Iracema, AC on *Corallus hortulanus;* and *Rickettsia rickettsi* from *Geckobiella harrisi* from São Paulo, SP on *Tropidurus catalanensis* (Figure 115).

Figure 115 –Distribution map of *Spotted fever group (SFG) Rickettsia* species detectected, obtained using QGIS program



Source: (MENDOZA-ROLDAN, J. A., 2019).

Legend: (red circle) *Rickettsia rickettsi from Geckobiella harrisi from São Paulo, SP on Tropidurus catalanensis*, (white circle) *Rickettsia aeschlimannii* from *Ophionyssus natricis* on *Pogona vitticeps* from Zoo Bauru, SP, (orange circle) *Rickettsia rhipicephali* from *Ophiogongylus rotundus*, from *X. neuwiedii* from Juquitiba, SP, (blue circle) *Rickettsia amblyommatis* from *A. rotundatum* from Iracema, AC on *Corallus hortulanus.*

5 DISCUSSION

In this study, three of the four pathogens studied were amplified through PCR (*Borrelia*, *Hepatozoon*, and *Rickettsia*). From these three, sequences were generated and identified through BLAST for two pathogens (*Hepatozoon*, and *Rickettsia*). The two sequences generated from ticks and the three sequences generated from lizards for *Borrelia* had no significant similarities found on BLAST. This lack of similarities could be due to non-specific amplification of the samples, defective sequencing or even due to nested PCR sensitivity, and although nested PCR is superior in both sensitivity and specificity to a standard PCR, the technique is much more prone to

contamination (SCHMIDT, 1997; WODECKA et al., 2010). Nonetheless, further attempts should be held where reptile and ectoparasites samples from the neotropical region are screened for *Borrelia*, as these spirochetes use reptiles (mainly lacertid lizards) as their natural reservoirs, and immature stages of ticks as their main vector (ATTACHEMENT 3) (MARGOS et al., 2018; CUTLER et al, 2019; MENDOZA-ROLDAN et al., 2019; MUÑOZ-LEAL et al., 2019). This is reinforced by the results of this study, where the positive samples for *Borrelia* were from lizards (Lacertidae and Scincidae), and nymphs of *A. rotundatum* ticks. However, as shown in chapter 4, the infestation rates of ticks in lizards from Brazil are lower than those seen in other regions (North America and Europe). Thus, it is also important to screen samples from other reptiles that could be potential reservoirs, such as snakes, which are associated to relapsing-fever *Borrelia* in other regions (Asia) (TRINACHARTVANI et al., 2016; PANETTA et al., 2017).

Regarding *Hepatozoon*, A total of 15 samples amplified for *Hepatozoon* (Seven of mites and ticks, and eight of reptile hosts). The sequences generate matched three main species with host and geographical delimitations. Furthermore, *Hepatozoon* sp. BT-2016 (accession number KU680466), described in the European common gecko *Tarentola mauritanica* (Linnaeus, 1758), from Morroco. Sequences that had a high similarity with this species were from a Pterygosomatidae mite *G. hemidactyli* that infest the tropical house gecko *H. mabouia* from São Paulo municipality (mite and lizards are exotic introduced species in Brazil), and from a Paraguay Green Racer snake *P. nattereri*, infested with trombiculid larvae *E. alfreddugesi,* and Argasidae larvae *Ornithodoros (Alectorobius)* sp. from São Bernardo do Campo, São Paulo. In Brazil, *Hepatozoon* sequences have been generated from Gekkonidae lizards (*G. hemidactyli* and *Phyllopezus pollicaris*) from the North, Northeast and Southeast regions (HARRIS, 2015). The sequences generated in that study (accession numbers KM234612 - KM234618), are highly similar with *Hepatozoon* sp. BT-2016, which means these sequences are probably lineages from *Hepatozoon* specific of geckoes, some of them with African origin (*G. hemidactyli*). On the other hand, the sequences of the infected *P. nattereri* snake, and its ectoparasites (*E. alfreddugesi,* and *Ornithodoros (Alectorobius)* sp.), are also highly similar with *Hepatozoon* sp. BT-2016. Borges-nojosa et al. (2017), described a species of *Hepatozoon* (*Hepatozoon musa*) from *P. nattereri* in the northeast region that is molecularly highly similar to *Hepatozoon cuestensis* described from rattlesnakes (O'DWYER et al., 2013). These snake-related species of *Hepatozoon* are slightly distant from the gecko-related species. In this case, the infection of a gecko-related *Hepatozoon*

on a snake could be explained by a possible infection of an unnatural reptilian host. This theory is supported by the reports of *P. nattereri* predating *H. mabouia* (GODINHO et al., 2014), and the pathologic sings seen on the snake that match an infection of an unnatural host (severe lethargy and anorexia, and multifocal random hepatocellular necrosis) (WOZNIAK et al., 1996; 1998). Moreover, the vectorial capacity of pterygosomatid mites of lizards is well understood. The infection occurs by the direct ingestion of the infected invertebrates. However, in snakes the infection could occur by predation on infected fist vertebrate hosts, direct ingestion of the infected invertebrates, or by salivary transmission through mosquito bites (*Culex, Aedes*, *Anopheles, Lutzomyia,* and *Phlebotomus*) (TELFORD, 2008). Thus, the significance of the ectoparasites *E. alfreddugesi*, and *Ornithodoros* (*Alectorobius*) being infected needs further studies because it could only mean that the parasites ingested gamonts of *Hepatozoon*, and they may not have a role in the sporogonic phase of the protozoa. Additional studies where Acari are dissected and their gut is observed through optical microscopy, should be held. This would allow to observe if these parasites harbor oocysts containing many sporocysts within which sporozoites form. Henceforth, screening of *Hepatozoon* from reptiles and their ectoparasites is important for proper introduction of new animals to a site where captive reptiles are kept. Also, the presence of mosquitos should be monitored, as well as the health conditions of the prey. This would avoid introducing *Hepatozoon* and spreading of the protozoa through ingestion of the infected invertebrates or salivary transmission through mosquito bites, that can also favor infections of unnatural hosts, thus producing clinically ill animals.

Furthermore, *Hepatozoon* sp. CCS-2010 (accession number HM585212) was identified in a blood sample from a bearded dragon *P. vitticeps* that was infested with Mesostigmata mite *O. natricis* (mites not infected with *Hepatozoon*) from the Bauru Zoo, SP. *Hepatozoon* sp. CCS-2010, was described in Asian water monitor *Varanus salvator* (Laurenti, 1768) from Thailand. This sequence of *Hepatozoon*, is highly similar with the beforementioned Gecko-related sequences of *Hepatozoon*. These findings suggest that this can be an exotic species of *Hepatozoon* of Asian origin. The bearded dragon *P. vitticeps* belongs to the Agamidae family and it is native to Australia, though it is popularly kept as a pet and exhibited in zoos (PASMANS et al., 2008). Moreover, this exotic lizard was infested with a macronyssid mite that is also exotic (*O. natricis*). Although this mite is a known vector or a myriad of pathogens to reptiles, it was observed that it is not a good vector for *Hepatozoon*, thus probably preventing transmission (BALL et al., 1969). In the Bauru

Zoo, two *P. vitticeps* were infected with *O. natricis* mites, yet only one was infected by the *Hepatozoon* sp. CCS-2010. Hence, this study showed the importance of monitoring exotic species kept in captivity conditions.

Additionally, *Hepatozoon ayorgbor* (accession number EF157822) was identified from ticks *A. rotundatum* and infested snakes (*C. multiventris, C. scurrulus, C. hortullanus*, *O. melanogenys*), and one snake which ticks were negative (*P. viridissima*) from Iracema, AC. *Hepatozoon ayorgbor* was described from ball python *Python regius* (Shaw, 1802), from Ghana. Its sporogonic phase was described in mosquitoes (species non specified) (SLOBODA et al., 2007). *H. ayorgbor* sequences are related to lizard and rodent species of *Hepatozoon*, possibly due to the diet of *P. regius* includes rodents, thus, rodents could be an important first intermediate host. However, role of rodents in the life cycle well as the possibility of inoculative transmission of *Hepatozoon* by mosquito vectors, is still poorly understood (SLOBODA, et al., 2007; 2008). Moreover, *H. ayorgbor* is distant from other snake-related *Hepatozoon* species (*H. musa* and *H. cuestensis*). The findings of this study also imply that this species has a low hosts specificity among snake species. Nonetheless, *H. ayorgbor* has a high specificity for snakes as definitive hosts, and infected ticks could imply also infection through passive transmission. Moreover, the infected snakes came from the north region to the laboratories of the Instituto Butantan (southeast region). Pathogenicity in infected snakes is generally low, with some animals presenting slight anemia, and hypertrophy of erythrocytes (TELFORD, 2008). Still, animals infected with *Hepatozoon ayorgbor* in general, have no visible changes in their health status. Nevertheless, it is still important to assess the presence of hemoparasites in newly introduced reptiles due to possible pathogenicity in new unnatural hosts in another geographical region.

Regarding *Rickettsia*, three species were identified for the *gltA* gene, and four species were identified for the *OmpA* gene for the Spotted Fever Group *Rickettsia.* These species were identified from ixodid ticks, trombiculid, pterygosomatid, and Mesostigmata mites. None of the hosts tissue samples tested yielded positive for *Rickettsia*, even with the presence of ectoparasites. The detection of *Rickettsia* in vertebrates is an infrequent event, since once infected, animals have a short ricketsemia (only for a few days or weeks), and after that the bacteria is no longer found (BURGDORFER et al., 1989).

Morover, for the *gltA* gene, most of the sequences (10 of 19), were highly similar to *Rickettsia bellii*. This species of *Rickettsia* was identified from mainly *A. rotundatum* infesting

snakes from the southeast region (*Bothrops insularis* from Ilha da Queimada Grande, SP; *C. durissus terrificus* from Varginha, MG; *Dipsas turgidus* from Caracol, MS; *Dipsas neuwiedi* and *Bothrops leucurus* from Anchieta, ES), and north region (*Chironius multiventris* and *Philodryas viridissima* from.Iracema, AC). It was also identified from *A. sculptum* from Santa Bárbara, SP, on, a tegu lizard *Salvator merianae*, and a trombiculid mite *Eutrombicula alfreddugesi* São Bernardo do Campo, SP on *Philodryas nattererii* snake. Furthermore, *R. bellii* is considered the most primitive species of the genus and it has been detected in 28 species of ticks (Mainly the *Amblyomma* genus) (PAROLA et al., 2013; KRAWCZAK et al., 2018; SANTODOMINGO et al., 2018). Though there have been several previous records of *R. bellii* infecting *A. rotundatum*, reports on snakes are mostly from Vipers (OGRZEWALSKA et al., 2018). Hence here, the results from *A. rotundatum* of most snakes (Colubridae, Boidae and Viperidae from north and southeastern regions) are new. Moreover, infection of *A. sculptum* from *Salvator merianae,* to our knowledge, this would be the first report of *R. bellii i*n *A. sculptum* associated to reptiles. Furthermore, *R. bellii* is historically associated to Ixodida Acari, thus the presence of this bacteria in the Trombiculidae mite *Eutrombicula alfreddugesi* from São Bernardo do Campo, SP on *Philodryas nattererii* snake, is unprecedented. The transmission of this bacterium is linked to coevolution with their specific tick species host, generating possible symbiotic associations. Nonetheless, horizontal transmission among ticks via vertebrate host cannot be discarded (KRAWCZAK et al., 2018). This last mechanism could explain the infection of the Trombiculid mite. Additionally, the epidemiological significance of *R. bellii* is low given that it has an unknown pathogenicity to humans and has never been detected in vertebrate hosts (PAROLA et al., 2013).

In addition, *Rickettsia rhipicephali* was identified from three Mesostigmata mite species (*Ophiogongylus rotundus* from *X. neuwiedii* snake from Juquitiba, SP; *Ophionyssus natricis* on *Pogona vitticeps* lizard from Zoo Bauru, SP; and *Chironobius* sp.n. from Iracema, AC on *Chironius multiventris* snake) and from Pterygosomatidae mites *Geckobiella harrisi* from São Paulo, SP on *Tropidurus catalanensis* lizard. This is the first time *R. rhipicephali* has been detected on Mesostigmata mites and Pterygosomatidae mites. Possible vectors of rickettsiae include ticks, lice, fleas and mites. From the Trombidiformes order, Trombiculid mites are known vectors of Scrub typhus (Asia, Indian Subcontinent and Australia), Mesostigmata mites have been pointed as vectors of rickettsial bacteria (mainly *Rickettsia akari*) rickettsial pox (MORO et al., 2005;

PAROLA et al. 2013), and Tombiculidae and Laelapidae mites of rodents have been detected infected with *Rickettsia helvetica* and *R. monacensis*, both SFG rickettsiae (MIŤKOVÁ et al., 2015). However, the findings of the present study are the first to detect *Rickettsia* from Mesostigmata mites from two reptile-specific families (Ixodirhynchidae and *Ophionyssus* from Macronyssidae), and from and from Pterygosomatidae mites. *R. rhipicephali* is a SFG *Rickettsia* which pathogenicity is unknown (PAROLA et al., 2013). This *Rickettsia* was described in *Rhipicephalus sanguineus* and *Dermacentor* ticks from mammals in the United States (HAYES et al.,1979; ZERINGÓTA et al., 2017). This species has also been detected in *Rhipicephalus* ticks Africa and Europe (PAROLA et al.,2013). In Brazil, it was detected in *Haemaphysalis juxtakochi* ticks from Rondônia, São Paulo, and Mato Grosso states (LABRUNA et al., 2005; LABRUNA et al., 2007; SOARES et al., 2015). It was also detected on *Amblyomma* sp. haplotype Nazaré ticks from birds from the Atlantic forest of Minas Gerais state (ZERINGÓTA et al., 2017). Furthermore, there is still no consensus of the risk to human or animal health of *R. rhipicephali* or if in fact it is a symbiotic association, given that the pathogenicity of *R. rhipicephali* has not been determined, though experimental infections in mammals showed scrotal swelling and splenomegaly, and even death (BURGDORFER et al., 1975). Of the identified *Rickettsia rhipicephali* sequences, only one also matched the identification for *OmpA* gene for SFG *Rickettsia* (*Ophiogongylus rotundus* from *X. neuwiedii* snake from Juquitiba).

The other species of *Rickettsia* identified from the *gltA* gene was *Rickettsia amblyommatis* from *A. rotundatum* from Iracema, AC on *Corallus hortulanus*. This sample also matched the identification with also matched the identification for *OmpA* gene for SFG *Rickettsia* and was highly identical with sequence KY053885. This *Rickettsia amblyommatis OmpA* gene was detected from *Amblyomma humerale* infesting capybaras *Hydrochoerus hydrochaeris*, from Acre state as well. This bacterium belongs to the SFG Rickettsia species however, the pathogenic potential remains unclear for humans and animals. The following species of ticks have been detected infected with this agent: *Amblyomma americanum*, *Amblyomma longirostre* in Brazil, *Amblyomma neumannii* and *Amblyomma hadanii* in Argentina, *Amblyomma cajennense* in Mexico, Costa Rica and Colombia, *Amblyomma mixtum* and *Haemaphysalis juxtakochi* in *Amblyomma coelebs* in French Guyanae, and *Dermacentor variabilis* in the United States, and more recently infecting *Amblyomma pseudoconcolor* in northeast Brazil (KARPATHY et a., 2016;

COSTA et al., 2017; SILVA et al., 2018). *A. humerale* and *A. rotundatum* are both reptile-associated ticks, and this is the first report of *A. rotundatum* infected with *R. amblyommatis*.

Finally, two samples identified as *R. rhipicephali* with the *gltA* gene (Macronyssidae mite *Ophionyssus natricis* on *Pogona vitticeps* lizard from Zoo Bauru, SP; Pterygosomatidae mites *Geckobiella harrisi* from São Paulo), were identified with *OmpA* gene for SFG *Rickettsia* as *Rickettsia aeschlimannii* and *Rickettsia rickettsii*, respectively. *Rickettsia aeschlimannii* sequence (accession number MF002555) was detected from *Haemaphysalis punctate* from China, was fairly similar with the sequence generated from *O. natricis*. *R. aeschlimannii* is an SFG *Rickettsia* species described from *Hyalomma marginatum* in Morocco, and in Portugal, Zimbabwe, and Niger (BEATI et al. 1997; PAROLA, et a., 2001). It has also been detected *Rhipicephalus* ticks in South Africa (PRETORIUS; BIRTLES, 2002). Furthermore, this bacterium has been detected in the tortoise tick *Hyalomma aegyptium* from Algeria (BITAM et al., 2009). To our knowledge, the detection of *R. aeschlimannii* from a macronyssid mite (*O. natricis*), is unprecedented. As stated earlier, *O. natricis* is a hematophagous mite with worldwide distribution. This mite prefers snake hosts but can bite other animals and even humans (SCHULTZ, 1975; AMANATFARD et al., 2014). Moreover, *R. aeschlimannii* produces clinical manifestations in humans similar to the Mediterranean spotted fever (MSF) that is caused by *R. conorii* (multiple eschars, fever, and a maculopapular rash) (PAROLA et al., 2005; KOKA et al., 2017). Hence, further studies should focus on assessing the epidemiological role and importance of *O. natricis* for *R. aeschlimannii* and its distribution in Brazil.

The other samples identified as *R. rhipicephali* with the *gltA* gene, was highly similar with *OmpA* gene for SFG *Rickettsia*, to *Rickettsia rickettsii* (accession number MF988095) detected on humans from Espirito Santo state. This result, to our knowledge, is the first record of *Rickettsia* in Pterygosomatidae mites (*Geckobiella harrisi* from São Paulo, on *Tropidurus catalanensis* lizard). Moreover, *R, rickettsii* is the causative agent of Brazilian spotted fever (BSF), which is a highly lethal rickettsial disease that has been reported mainly in the Southeastern region of Brazil (FACCINI-MARTÍNEZet al., 2018). The tick *Amblyomma cajennense* sensu lato (*A. sculptum*) is the main vector of BSF, and the capybara is the main natural reservoir of the bacteria (SOUZA et a., 2009; POLO et al., 2017). Furthermore, the state of São Paulo is an endemic area for BSF (HORTA et al., 2007), and capybaras and their ticks *A. sculptum*, are distributed throughout the state (ROCHA et a., 2017). Reptiles have been pointed as possible reservoirs for other species of

SFG *Rickettsia* in Asia and Australia (UNSWORTH et al., 2007; VILCINS et a., 2009; SUMRANDEE et a., 2014; KHO et a., 2015). However, whether reptiles may act as reservoirs for any Rickettsia species is still unknown (STENOS et al. 2003). Nevertheless, the high prevalence of infected mites from different hosts (4 of 8), could suggest that Tropiduridae lizards are natural reservoirs for *Rickettsia*. Still, the epidemiological significance of *Geckobiella harrisi* infested mites is low because these ectoparasites are highly specific and associated to lizards, thus chances of transmission to other hosts are null. However, the detection of SFG *Rickettsia* species on reptile mites (Mesostigmata and Pterygosomatidae) should be highlighted and show the importance of an integrative assessment of ectoparasites of reptiles.

6 CONCLUSIONS

1. Three of the four pathogens studied were amplified through PCR (*Borrelia*, *Hepatozoon*, and *Rickettsia*), and sequences were generated and identified through BLAST for two pathogens (*Hepatozoon*, and *Rickettsia*).
2. The two sequences generated from ticks and the three sequences generated from lizards for *Borrelia* had no significant similarities found on BLAST. Nonetheless, further attempts should be held where reptile and ectoparasites samples from the neotropical region are screened for *Borrelia*.
3. It was amplified 15 samples for *Hepatozoon* (Seven of mites and ticks, and eight of reptile hosts). The sequences generate matched three main species with host and geographical delimitations. *Hepatozoon* sp. BT-2016, *Hepatozoon* sp. CCS-2010 and *Hepatozoon ayorgbor*.
4. *Hepatozoon* sp. BT-2016 was identified in an exotic Pterygosomatidae mite, and from a Paraguay Green Racer snake, infested with trombiculid and Argasidae larvae, both from São Paulo state.
5. *Hepatozoon* sp. BT-2016 is highly similar with *Hepatozoon* sequences from Gekkonidae lizards (*G. hemidactyli* and *Phyllopezus pollicaris*) from the North, Northeaste and Southeast regions, which could imply these sequences are probably lineages from *Hepatozoon* specific of geckoes, some of them with African origin.
6. *Hepatozoon* sp. BT-2016 in the snake *P. nattereri* could be explained by a possible infection of an unnatural reptilian host.

7. the significance of the ectoparasites *E. alfreddugesi*, and *Ornithodoros* (*Alectorobius*) being infected needs further studies because it could only mean that the parasites ingested gamonts of *Hepatozoon*, and they may not have a role in the sporogonic phase of the protozoa.

8. Additional studies where Acari are dissected and their gut is observed through optical microscopy, should be held. This would allow to observe if these parasites harbor oocysts containing many sporocysts within which sporozoites form.

9. *Hepatozoon* sp. CCS-2010 was detected in a bearded dragon *P. vitticeps* that was infested with Mesostigmata mite *O. natricis* (mites not infected with *Hepatozoon*) from the Bauru Zoo, SP; and this could be an exotic species of *Hepatozoon* of Asian origin.

10. *O. natricis* is probably not a good vector for *Hepatozoon,* thus possibly preventing transmission.

11. *Hepatozoon ayorgbor* was identified from ticks *A. rotundatum* and infested snakes, and one snake which ticks were negative, from the state of Acre. This species related to lizard and rodent species of *Hepatozoon* and has a low hosts specificity among snake species.

12. Animals infected with *Hepatozoon ayorgbor* in general, have no visible changes in their health status. Nevertheless, it is still important to assess the presence of hemoparasites in newly introduced reptiles due to possible pathogenicity in new unnatural hosts in another geographical region.

13. Three species were identified for the *gltA* gene, and four species were identified for the *OmpA* gene for the Spotted Fever Group *Rickettsia* from ixodid ticks, trombiculid, pterygosomatid, and Mesostigmata mites. None of the hosts tissue samples tested yielded positive

14. Most of the sequences were highly similar to *Rickettsia bellii*. This species of *Rickettsia* was identified from mainly *A. rotundatum* infesting snakes from the southeast and north regions.

15. *Rickettsia bellii* was also identified from *A. sculptum* from Santa Bárbara, SP, on, a tegu lizard *Salvator merianae*, and a trombiculid mite *Eutrombicula alfreddugesi* São Bernardo do Campo, SP on *Philodryas nattererii* snake.

16. *R. bellii* in *A. sculptum* associated to reptiles is a new report and presence of this agent in the Trombiculidae mite *Eutrombicula alfreddugesi* from São Bernardo do Campo, SP on *Philodryas nattererii* snake, is unprecedented.

17. *Rickettsia rhipicephali* was identified from three Mesostigmata mite species (to from the southeastern region and one from the north region) and from one species of Pterygosomatidae mite from the southeastern region. This is the first time *R. rhipicephali* has been detected on Mesostigmata and Pterygosomatidae mites. Still, the pathogenic potential remains unclear for humans and animals

18. *Rickettsia amblyommatis* was identified from the *gltA* gene and *OmpA* gene *A. rotundatum* from Iracema, AC on *Corallus hortulanus*. This is the first report of *A. rotundatum* infected with *R. amblyommatis*. Still, the pathogenic potential remains unclear for humans and animals.

19. Two samples identified as *R. rhipicephali* with the *gltA* gene (Macronyssidae mite *Ophionyssus natricis* on *Pogona vitticeps* lizard from Zoo Bauru, SP; Pterygosomatidae mites *Geckobiella harrisi* from São Paulo), were identified with *OmpA* gene for SFG *Rickettsia* as *Rickettsia aeschlimannii* and *Rickettsia rickettsii*, respectively.

20. The detection of *R. aeschlimannii* from a macronyssid mite (*O. natricis*), is unprecedented, and further studies should focus on assessing the epidemiological role and importance of *O. natricis* for *R. aeschlimannii* and its distribution in Brazil.

21. *R. rickettsii* in Pterygosomatidae mites (*Geckobiella harrisi* from São Paulo, on *Tropidurus catalanensis* lizard), is also a new report. Nevertheless, the epidemiological significance of infested mites is low because these highly specific and associated to lizards, thus chances of transmission to other hosts are null.

22. The detection of SFG *Rickettsia* species on reptile mites (Mesostigmata and Pterygosomatidae) highlights the importance of an integrative assessment of ectoparasites of reptiles.

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ATTACHMENT 3 - *Borrelia burgdorferi* (sensu lato) in ectoparasites and reptiles in southern Italy[4]

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Borrelia burgdorferi (*sensu lato*) in ectoparasites and reptiles in southern Italy

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Abstract

Background: *Borrelia burgdorferi* (*sensu lato*) is a complex containing pathogenic bacteria of which some species, such as *Borrelia lusitaniae*, use birds, small mammals and reptiles as reservoirs. In Italy, the bacteria have been detected in reptilian and avian reservoirs in the northern and central regions.

Results: Here, 211 reptiles from three orders [Squamata (Sauria with seven species in five families and Ophidia with 11 species in three families), Crocodylia (one family and two species), and Testudines (two families and two species)] were examined for ectoparasites and molecular detection of *B. burgdorferi* (*s.l.*) in three different sites of southern Italy, an area for which no information was previously available on the occurrence of borreliosis in animals and humans. *Borrelia lusitaniae* was molecularly detected in larvae and nymphs (11.6%) of *Ixodes ricinus* infesting lizards (i.e. *Podarcis muralis*, *Podarcis siculus* and *Lacerta bilineata*) and in 12.3% blood samples of *P. siculus*. Finally, *B. lusitaniae* and *Borrelia garinii* were detected in 5.1% (32/630) of questing *I. ricinus*.

Conclusions: These results show the circulation of *B. lusitaniae* in southern Italy and suggest that *P. siculus* could play a role as a reservoir, representing a potential medical threat to humans living in or visiting these localities.

Keywords: Reptiles, Ectoparasites, *Borrelia lusitaniae*, *Borrelia garinii*, *Ixodes ricinus*, *Podarcis siculus*

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GENERAL CONCLUSIONS

This study totalizes 56 species of Acari from reptile and amphibians that occur in Brazil, increasing nine new species to the Brazilian territory. New records of hosts and localities are reported for mites and ticks, most ot them new for the North region, which historically has none to scarce records. Furthermore, through an extensive effort to describe, catalogue and revise new and known species of Acari, integrating morphology, taxonomy, and molecular biology, information regarding hosts and localities of the Acari that parasitize herpetofauna was updated, and generated distribution information, host-parasite associations and keys of identification for specific studied groups. This also allowed to describe two new species (*Chironobius* sp. n. and *Ornithodoros* (*Alectorobius*) sp. n.). Finally, of the four pathogens selected (*Borrelia*, *Coxiella*, *Hepatozoon*, and *Rickettsia*) two pathogens (*Hepatozoon*, and *Rickettsia*) were detected from hosts (*Hepatozoon*) and ixodid ticks, trombiculid, pterygosomatid, and Mesostigmata mites (*Hepatozoon*, and *Rickettsia*) (detection on mites is unprecedented in most cases). The detection of SFG *Rickettsia* species on reptile mites (Mesostigmata and Pterygosomatidae) highlights the importance of an integrative assessment of ectoparasites of reptiles. Thus, this study helped updating the knowledge of the Acari fauna in reptiles and amphibians, and their associated pathogens.