



UNIVERSIDADE ESTADUAL PAULISTA
"JÚLIO DE MESQUITA FILHO"
Câmpus de São José do Rio Preto

Clarice Silva Cesário

**Aspectos parasitológicos e sanitários de tamanduás-bandeira
(*Myrmecophaga tridactyla*) de vida livre**

*Parasitological and sanitary aspects of free-living giant anteaters
(*Myrmecophaga tridactyla*)*

São José do Rio Preto
2021

Clarice Silva Cesário

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Tese apresentada como parte dos requisitos para obtenção do título de Doutora em Biologia Animal, junto ao Programa de Pós-Graduação em Biologia Animal do Instituto de Biociências, Letras e Ciências Exatas da Universidade Estadual Paulista “Júlio de Mesquita Filho”, Câmpus de São José do Rio Preto.

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Coorientador: Prof. Dr. Arnaldo Maldonado Jr.

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Comissão Examinadora

Prof^a. Dr^a. Rita de Cassia Bianchi
UNESP – Câmpus de Jaboticabal
Orientadora

Prof. Dr. Estevam Guilherme Lux Hoppe
UNESP – Câmpus de Jaboticabal

Prof^a. Dr^a. Natalie Olifiers
Universidade Veiga de Almeida - Rio de Janeiro

Prof^a. Dr^a. Karin Werther
UNESP – Câmpus de Jaboticabal

Prof^a. Dr^a. Raquel de Oliveira Simões
Universidade Federal Rural do Rio de Janeiro

São José do Rio Preto
17 de maio de 2021

À **fauna** e aos **tamanduás-bandeira**,
por despertarem meu encanto acadêmico.

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inspiradoras e símbolos de persistência.

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“O fascismo é a máscara mortuária do conhecimento.”
[MÁRCIA TIBURI, 2018 – em ‘Como conversar com um fascista’]

***“Para navegar contra a corrente são necessárias condições raras:
espírito de aventura, coragem, perseverança e paixão.”***
[NISE DA SILVEIRA - em ‘Nise: arqueóloga dos mares’ (Horta, 2008)]

RESUMO

O tamanduá-bandeira (*Myrmecophaga tridactyla*) é vulnerável à extinção em várias escalas e tem sido crescente alvo de pesquisas nas últimas décadas. Nossos objetivos gerais com este trabalho foram (1) discutir sobre as variações dos parâmetros fisiológicos de tamanduás-bandeira de vida livre anestesiados, (2) caracterizar seu estado de saúde a partir de exames sanguíneos e infestação de parasitas, e (3) estudar a taxonomia de nematódeos do gênero *Aspidodera* encontrados em um dos indivíduos. As capturas e imobilizações de nove tamanduás-bandeira ocorreram em uma área protegida de bioma Cerrado no estado de São Paulo, sudeste do Brasil, entre 2014 e 2015, com uso da associação de cetamina, midazolam e xilazina, além de atropina e ioimbina. Os animais apresentaram bradipneia, bom relaxamento muscular e estabilidade dos parâmetros fisiológicos ao longo do monitoramento anestésico. A temperatura do ar e a corporal tiveram queda durante o monitoramento e correlacionaram-se positivamente. Os exames sanguíneos demonstraram eritrocitose, trombocitopenia, hemácias microcíticas e hipocrômicas, leucopenia, eosinofilia, alta concentração de LDH, ALT, AST, ureia, ácido úrico e triglicérides, além de baixas taxas de lipase, amilase, creatinina e fosfatase alcalina. Das quatro espécies de carrapatos identificadas, *Amblyomma sculptum*, *A. nodosum* e *A. calcaratum* foram encontradas frequentemente nos indivíduos, enquanto *A. parvum*, ocorreu em apenas um. Dos morfotipos de ovos/oocistos de parasitas gastrointestinais encontrados nas fezes, os de maior prevalência foram Trychostrongylidae, *Aspidodera* spp. e Apicomplexa. Mas além desses, identificamos Ascaridoidea, *Trichuris* spp. e *Physaloptera* spp. Apenas um tamanduá-bandeira mostrou-se positivo para o hemoparasita *Mycoplasma* spp. Todos testaram negativo para os demais agentes investigados (*Ehrlichia canis*, *E. chaffeensis*, *Anaplasma* spp. e *Hepatozoon* spp.). Redescrevemos duas espécies de *Aspidodera*, *A. fasciata* e *A. scoleciformis*, a partir de estruturas de machos e fêmeas, as comparamos com as congêneras encontradas em Xenarthra e incluímos *A. fasciata* na filogenia molecular do grupo. Descrevemos uma nova espécie de *Aspidodera* a partir de dois espécimes fêmeas com uso de microscopia eletrônica e criamos uma nova chave de identificação para o gênero. Este estudo traz informações inéditas para a espécie e um alerta para o limitado conhecimento sobre saúde e parasitismo de tamanduás-bandeira de vida livre.

Palavras-chave: Medicina da Conservação. Tamanduá-bandeira. Parasitismo. Imobilização animal. Taxonomia integrativa.

ABSTRACT

The giant anteater (*Myrmecophaga tridactyla*) is vulnerable to extinction at various scales and has been a growing research target in recent decades. Our general objectives with this work were (1) to discuss about the variations in physiological parameters of anesthetized free-living giant anteaters, (2) characterize their health status from blood exams and parasite infestation, and (3) to study the taxonomy of nematodes from *Aspidodera* genus found in one of the individuals. The captures and immobilizations of nine giant anteaters occurred in a protected area of the Cerrado biome in São Paulo state, Southeastern Brazil, between 2014 and 2015, using the combination of ketamine, midazolam and xylazine, in addition to atropine and yohimbine. The animals presented bradypnea, good muscle relaxation and physiological parameters stability during anesthetic monitoring. Air and body temperature decreased during the monitoring and were positively correlated. Blood exams showed erythrocytosis, thrombocytopenia, microcytic and hypochromic red blood cells, leucopenia, eosinophilia, high concentration of LDH, ALT, AST, urea, uric acid and triglycerides, in addition to low rates of lipase, amylase, creatinine and alkaline phosphatase. Of the four tick species identified, *Amblyomma sculptum*, *A. nodosum* and *A. calcaratum* were frequently found on the individuals, while *A. parvum* occurred in only one. Of the egg/oocysts morphotypes of gastrointestinal parasites found in feces, the most prevalent were Trychostrongylidae, *Aspidodera* spp. and Apicomplexa. In addition to these, we identified Ascaridoidea, *Trichuris* spp. and *Physaloptera* spp. Only one giant anteater was positive for the hemoparasite *Mycoplasma* spp. All of them tested negative for the other agents investigated (*Ehrlichia canis*, *E. chaffeensis*, *Anaplasma* spp. and *Hepatozoon* spp.). We redescribed two species of *Aspidodera*, *A. fasciata* and *A. scoleciformis*, from male and female structures, compared them with the congeneric ones found in Xenarthra and included *A. fasciata* in the molecular phylogeny of the group. We described a new species of *Aspidodera* from two female specimens using electron microscopy and created a new identification key for the genus. This study brings unprecedented information for the species and an alert to the limited knowledge about health and parasitism of free-living giant anteaters.

Keywords: Conservation Medicine. Giant anteater. Parasitism. Animal immobilization. Integrative taxonomy.

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LISTA DE SÍMBOLOS E ABREVIACÕES /
LIST OF SYMBOLS AND ABBREVIATIONS

ha	hectare
%	percent
kg	kilogram
km²	square kilometers
°	degree
'	minute
”	second
° C	Celsius degree
®	Trademark
mg/kg	milligram per kilogram
mg/ml	milligram per milliliter
bpm	heart beats per minute
rpm	breathing movements per minute
±	more or less
min	minutes
GPS	Global Positioning System
µl	microliters
g/dL	gram per decilitre
fL	fentoliter
Pcg	Picogram
e.g.	<i>exempli gratia</i>
™	Trademark
ISIS	International Species Information System
n°	number
OPG	eggs per gram of feces
OoPG	oocytes per gram of feces
cm	centimeter
RBC	Red Boold Cels
MCV	Mean Corpuscular Volume
MCHC	Mean Corpuscular Hemoglobin Concentration
MCH	Mean Corpuscular Haemoglobin

WBC	White Blood Cels
TPP	Total Plasma Protein
A/G	Relation of Albumin and Globulin
mg/dL	milligram per deciliter
mmol/dL	millimoles per liter
ALT	Alanine Transaminase
IU/L	International Unit per Liter
AST	Aspartate Transaminase
AF	Alkaline Phosphatase
GGT	Gamma-Glutamyl Transferase
CK	Creatine Kinase
LDH	Lactate Dehydrogenase
µm	micrometers
SEM	Scanning Electron Microscopy
pH	potential of Hydrogen
M	Molar
O₅O₄	osmium tetroxide
kV	quilovolts
PCR	Polymerase Chain Reaction
DNA	Deoxyribonucleic Acid
rRNA	ribosomal Ribonucleic Acid
Na	sodium
ML	Maximum Likelihood
nm	nanometers
CO₂	carbon dioxide
BI	Bayesian Inference
AIC	Akaik Information Criterion
aLRT	approximate Likelihood-Ratio Test
ML-BP	nonparametric Bootstrap Percentages
BPPs	Bayesian Posterior Probabilities
n.sp.	new species

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1. INTRODUÇÃO GERAL

O tamanduá-bandeira (*Myrmecophaga tridactyla* Linnaeus, 1758) é um mamífero Xenarthra com origem há 13 milhões de anos (Delsuc & Douzery, 2008; Gibb *et al.*, 2015). Sua distribuição original compreende do norte da América Central, onde é o mamífero mais ameaçado (Superina *et al.*, 2010), ao sul da América do Sul (Miranda *et al.*, 2014a). Ele é considerado extinto em Belize, Costa Rica e Guatemala, regionalmente extinto no Uruguai, e pouco se sabe sobre seu estado de conservação em algumas áreas, incluindo em partes do Brasil (Miranda *et al.*, 2014a,b). No Brasil está presente nos seis biomas (Paglia *et al.*, 2012), mas possivelmente extinto nos Pampas, quase extinto na Caatinga (Miranda *et al.*, 2014a; Dias *et al.*, 2019), em baixas densidades na Mata Atlântica (Passos *et al.*, 2017) e sob drásticas reduções populacionais no Cerrado (Miranda *et al.*, 2014a; Diniz & Brito, 2015; Rocha *et al.*, 2018; Bertassoni *et al.*, 2019). A principal ameaça à espécie é a perda e fragmentação do habitat, que desencadeiam atropelamentos (Diniz & Brito, 2013; Freitas *et al.*, 2014; Desbiez *et al.*, 2020), perseguição por cães (Lacerda *et al.*, 2009), morte em incêndios (Silveira *et al.*, 1999; Diniz & Brito, 2013), caça (Koster, 2008; Ferreira *et al.*, 2013; Bertassoni *et al.*, 2017) e depressão gênica (Collevatti *et al.*, 2007; Clozato *et al.*, 2017). Áreas menores que 200 ha não suprem suas exigências ecológicas (Zanirato, 2017), explicando, em parte, a redução de 30% de sua população nos últimos 26 anos (Miranda *et al.*, 2014a). Estima-se que a espécie provavelmente desapareça do estado de São Paulo entre 20 e 30 anos (FAPESP, 2017).

Tamanduás-bandeira desenvolveram adaptações ao longo do seu processo evolutivo para conservarem energia, provavelmente como resultado de uma dieta pobre em calorias (formigas e cupins) (Fernandes & Young, 2007). Eles são homeotérmicos imperfeitos e possuem baixas temperatura corporal e taxa de metabolismo basal - cerca de 3° C a menos e o equivalente a 34%, respectivamente - quando comparados com mamíferos de massa corporal similar (McNab, 1984, 1985). Seus padrões de uso do habitat dependem da temperatura do ar, se estão ou não em atividade e da sazonalidade (Medri & Mourão, 2005; Camilo-Alves & Mourão, 2006; Di Blanco *et al.*, 2015, 2016). Embora seja uma espécie geralmente considerada de áreas abertas, porque as usam mais durante seus períodos de atividade, áreas florestais agem como um refúgio de temperatura e podem ser um fator chave para tamanduás-bandeira (Bertassoni *et al.*, 2019). Eles utilizam ambientes com maior cobertura vegetal para termorregular (Camilo-Alves & Mourão, 2006), repousar (Medri & Mourão, 2005) e se abrigar (Kreutz *et al.*, 2012).

Apesar de crescente o número de publicações envolvendo o tamanduá-bandeira nas duas últimas décadas (Diniz & Brito, 2012), há muitas lacunas sobre seu estado sanitário e

parasitismo, especialmente para indivíduos de vida livre. Essas lacunas se manifestam, inclusive, em áreas temáticas que subsidiam todo tipo de trabalho de conservação e manejo, como a captura e imobilização farmacológica. Os títulos que abordam o assunto são muito escassos, estão dispersos, são pouco aprofundados e, na maioria das vezes, permanece na experiência e rotina do pesquisador. A escolha de um protocolo anestésico seguro para um indivíduo depende do seu estado de saúde, do conhecimento sobre drogas, fisiologia e comportamento da espécie, e das possíveis influências ambientais. Estas variáveis, no entanto, são pouco conhecidas para a maioria das espécies silvestres, e não é diferente para tamanduás-bandeira.

Os maiores problemas clínicos em tamanduás de cativeiro são as doenças digestivas (26%) - principalmente enteroparasitoses (13,5%) -, as desordens nutricionais (20%), as injúrias (15,5%) e as doenças respiratórias (10%). Mas, cerca de 13% dos indivíduos tem afecções de causa inconclusa (Diniz *et al.*, 1995), sugerindo que nossa capacidade de monitoramento da saúde ainda é insuficiente, mesmo em condições mais controladas, como as de cativeiro. Por isso é importante estabelecer parâmetros sanitários que contribuam no diagnóstico precoce de doenças e em programas de conservação. O perfil hematológico de tamanduás-bandeira (Sanches *et al.*, 2013; Di Nucci *et al.*, 2014; Rojano-Bolaño *et al.*, 2014; Oliveira *et al.*, 2017) começou a ser mais investigado nos últimos anos. Ainda sabemos pouco sobre os limites de referência dos parâmetros, quais circunstâncias e como elas interferem nos resultados, o que dificulta as comparações entre os estudos já realizados. Sobre o perfil bioquímico, as referências para a espécie são ainda mais raras (Di Nucci *et al.*, 2014; Oliveira *et al.*, 2018) e este tipo de informação não tem sido bem explorada.

Considerando a crescente importância do parasitismo, não apenas na saúde das populações (Ebert *et al.*, 2000; Tompkins *et al.*, 2001; Watson, 2013), mas nas comunidades (Poulin, 1999; Telfer & Bown, 2012) e nos ecossistemas (Thomas *et al.*, 2005; Tompkins *et al.*, 2011), é urgente o entendimento sobre as espécies de parasitas que se relacionam com o tamanduá-bandeira. É esperado que (1) hospedeiros com grande massa corporal sustentem uma comunidade parasitária mais rica já que eles provêm maior área e, como consequência, é possível que espécies que possuem diferentes nichos possam coexistir (Bordes & Morand, 2008; Esser *et al.*, 2016); (2) seus longos e fartos pelos amplifiquem sua superfície de contato com o ambiente comparado com os demais mamíferos (Szabó *et al.*, 2019); (3) a mirmecofagia os predisponha ao consumo de hospedeiros intermediários e ovos/cistos por meio da ingestão acidental de partículas de solo contaminadas (Freitas *et al.*, 2006); (4) sua área de vida relativamente grande dentre hospedeiros vertebrados e sua distribuição em vários biomas os

tornem mais expostos a uma maior diversidade de espécies parasitas (Linardi & Avelar, 2014); e (5) conhecemos pouco a diversidade parasitária porque existem poucos levantamentos e descrições de espécies para este hospedeiro.

As parasitoses gastrointestinais mais frequentes em tamanduás-bandeira são causadas por protozoários dos gêneros *Cryptosporidium*, *Eimeria*, *Isospora*, *Entamoeba*, *Acanthamoeba* e *Giardia* (Pérez *et al.*, 2015; Rojano *et al.*, 2015; Marinho & Valdes, 2012; Marques & Ludwig, 2011; Freitas *et al.*, 2006; Coke *et al.*, 2002; Diniz *et al.*, 1995; Gardner *et al.*, 1991). Elas estão associadas a quadros diarreicos e debilidade física, bem caracterizados em animais de cativeiro (Diniz *et al.*, 1995; Cubas *et al.*, 2014). O encontro de ovos de *Strongyloides* spp. (Diniz *et al.*, 1995; Marinho & Valdes, 2012), *Ancylostoma* spp. (Marinho & Valdes, 2012), *Ascaris* spp. (Rojano-Bolaño *et al.*, 2014; Rojano *et al.*, 2015) e *Trichuris* spp. (Diniz *et al.*, 1995) nas fezes foi relatado principalmente em regiões onde a espécie convive mais proximamente ao ser humano e a animais domésticos, como os cães. Mas o estudo mais robusto sobre nematódeos brasileiros é da década de 90 (Vicente *et al.*, 1997), e nele há um pequeno número de trabalhos que envolvem tamanduás-bandeira. Desde então, pouco foi acrescentado ao grupo devido à escassez de especialistas em taxonomia e parasitologia de mamíferos silvestres de médio e grande porte. A impossibilidade da coleta destes hospedeiros para obtenção de amostras frescas também é uma das razões, visto que boa parte das espécies estão ameaçadas de extinção, como o tamanduá-bandeira. Além disso, amostras oriundas de animais atropelados muitas vezes se degradam até que sejam recuperadas (Griese, 2007) e os espécimes de coleções helmintológicas são muito antigos, pouco conservados ou pouco numerosos (comunicação pessoal). Existem pelo menos onze espécies de nematódeos descritos para tamanduás-bandeira pertencentes às famílias Aspidoderidae, Molineidae e Physalopteridae, os quais apresentam alta relação de especificidade com hospedeiros Xenarthra (Navone, 1990; Vicente *et al.*, 1997).

Tamanduás-bandeira são os principais hospedeiros das formas adultas dos carrapatos das espécies *Amblyomma calcaratum* e *A. nodosum*, enquanto as formas jovens preferem se alimentar em passeriformes (Nava *et al.*, 2017; Ogrzewalska & Pinter, 2016). *Amblyomma sculptum*, do complexo *A. cajennense* (Nava *et al.*, 2014), também é comum (Martins *et al.*, 2015; Witter *et al.*, 2016; Cançado *et al.*, 2017; Pascoal, 2017; Martins *et al.*, 2004), mas não apresenta relação de especificidade, portanto, parasita uma grande variedade de hospedeiros (Pereira *et al.*, 2000; Nava *et al.*, 2014). A alta prevalência e intensidade de infestações por carrapatos em tamanduás-bandeira é comum e há possibilidade de encontro de mais de uma espécie de carrapato num mesmo hospedeiro (Szabó *et al.*, 2019). Várias das cerca de 70 espécies de carrapatos do Brasil (Barros-Battesti *et al.*, 2015; Krawczak *et al.*, 2015; Wolf *et*

al., 2016) já foram infectadas por pelo menos oito espécies de rickettsias (Oliveira *et al.*, 2008; Almeida *et al.*, 2011; Labruna *et al.*, 2011). O carrapato *A. sculptum* é o mais comum em picadas a humanos no país e é o principal vetor da bactéria causadora de febre maculosa, *Rickettsia rickettsii* (Martins *et al.*, 2016). Em tamanduás-bandeira, estudos sobre identificação de bactérias desse gênero são escassos, mas há relatos de sua infecção por *R. parkeri* (Nieri-Bastos *et al.*, 2018; Szabó *et al.*, 2019), também encontrada em carrapato da espécie *A. nodosum* numa área endêmica de febre maculosa no Brasil (Moerbeck *et al.*, 2018). Infecções simultâneas com *A. nodosum* e *A. sculptum* nessas circunstâncias podem representar um risco de infecção em *A. sculptum* por *R. parkeri*, colocando o tamanduá-bandeira como possível fonte desta *Rickettsia* para humanos (Szabó *et al.*, 2019). Outra bactéria, *Anaplasma marginale*, causadora de anaplasmoze, provoca muitos danos à pecuária nacional e já foi identificada e isolada de um tamanduá-bandeira apresentando os sintomas da doença (Guillemi *et al.*, 2016). No entanto, permanecemos necessitando de esforços para conhecer quais espécies de ectoparasitas e hemoparasitas infectam tamanduás-bandeira, os possíveis danos à sua saúde e o verdadeiro papel deste hospedeiro nestas e em diversas outras doenças infecciosas.

A carência de informações sobre estado sanitário e parasitismo em tamanduás-bandeira, as dificuldades em obtê-las na vida livre e a oportunidade de se explorar estes aspectos em populações monitoradas, nos motivou a realizar o estudo registrado nesta tese. Ela se compõe de quatro capítulos originais em formato geral de artigo científico, escritos no idioma inglês. As porções textuais “Introdução geral”, “Objetivos” e “Considerações finais” estão em português. No Capítulo 1, apresento um estudo de caso sobre captura ativa, contenção físico-química e monitoramento anestésico de tamanduás-bandeira de vida livre. No Capítulo 2, realizo um levantamento sanitário da população com uso de análises hematológicas, bioquímica sérica, identificação e infestação de carrapatos e ovos/cistos de parasitas gastrintestinais e identificação de hemoparasitas. No Capítulo 3, caracterizo a morfologia de duas espécies de nematódeos, *Aspidodera scoleciformis* e *A. fasciata*, e incluo este último na árvore filogenética do gênero *Aspidodera*. No Capítulo 4, descrevo uma nova espécie de *Aspidodera* (*A. serrata* Cesário *et al.*, 2021) e apresento nova chave de identificação para o gênero.

Este estudo fez parte de um projeto (FAPESP nº 2013/04957-8) sobre a população de tamanduás-bandeira da Estação Ecológica de Santa Bárbara (EESB, -22°48'59", -49°14'12") e seu entorno. Ele envolveu também outras linhas de pesquisa: genética populacional (Ribeiro, 2016), ecologia espacial e populacional (Bertassoni, 2017), ocupação e padrão de atividade (Zanirato, 2017), toxicologia (Carvalho, 2018), dieta (Polli, 2018) e parasitologia – filo Acanthocephala (Gomes, 2019). A EESB é uma unidade de conservação localizada no estado

de São Paulo, sudeste do Brasil, que apresenta um mosaico de fitofisionomias típicas do bioma Cerrado, com destaque para as áreas campestres, raras no estado (Durigan & Ratter, 2006; Melo & Durigan, 2011). É o maior remanescente de área com vegetação nativa da região, imerso em uma paisagem constituída por diferentes usos do solo, como: plantios de cana-de-açúcar, fazendas de pecuária bovina, granjas de frango e suíno, brejos e a Floresta Estadual de Santa Bárbara - composta por silvicultura e malha urbana. Três estradas fragmentam a EESB, as rodovias SP-280, a SP-261 e a estrada de acesso “da Erva”, as últimas não pavimentadas (Figura 1). A EESB contém espécies exóticas como *Pinus* spp., *Eucalyptus* spp., *Brachiaria* spp. (Melo & Durigan, 2011) e fauna invasora, como javali (*Sus scrofa*), lebre europeia (*Lepus europaeus*), cães (*Canis familiaris*) e gatos (*Felis catus*).

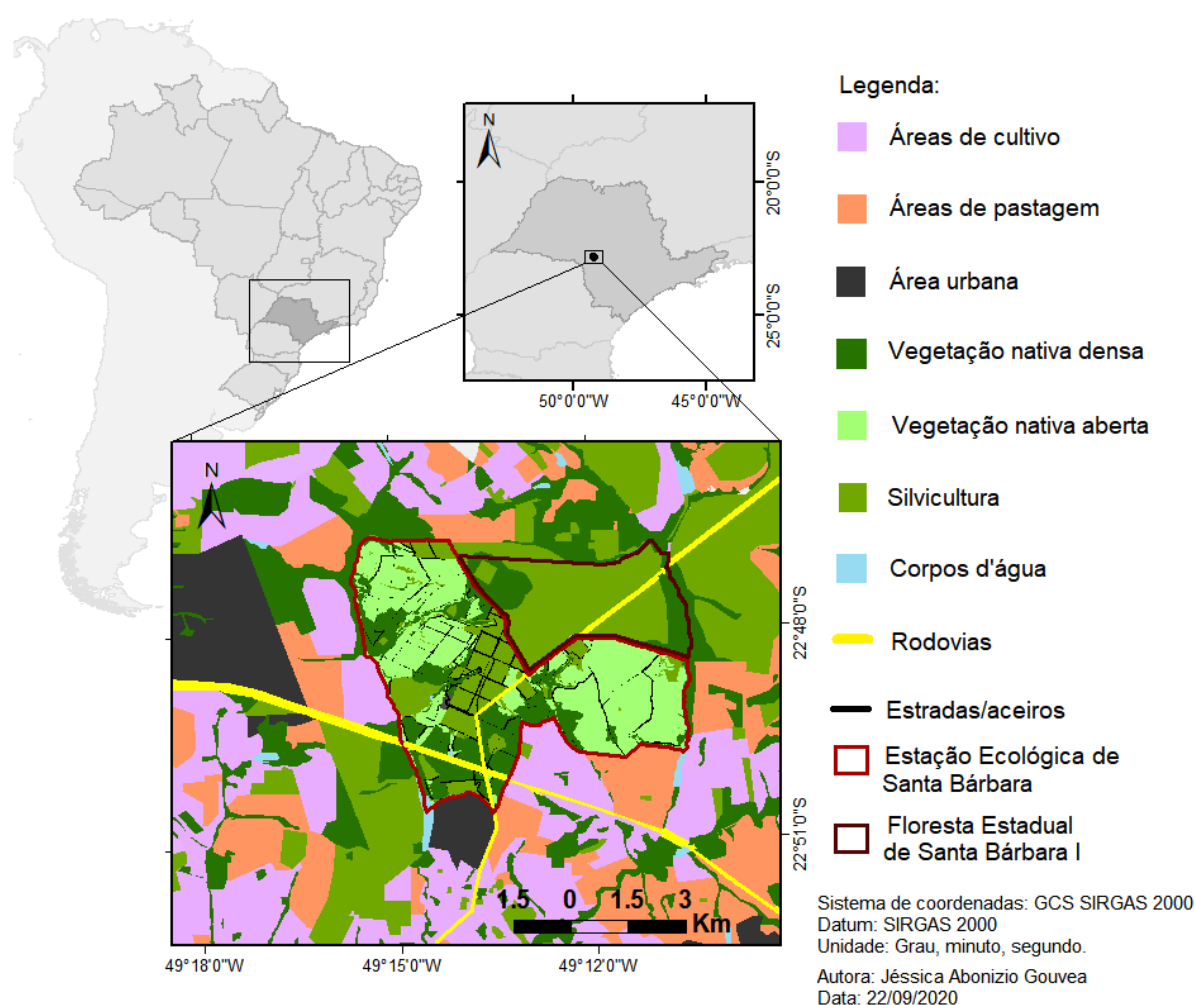


Figura 1. Ocupação do solo da Estação Ecológica de Santa Bárbara (delineado vermelho) e entorno, localizada no município de Águas de Santa Bárbara, estado de São Paulo, Brasil.

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2. OBJETIVOS

2.1. Objetivo geral

Analisar alguns parâmetros sanitários e parasitológicos de uma população de tamanduás-bandeira de vida livre do Cerrado do estado de São Paulo, Brasil.

2.2. Objetivos específicos

- Discutir sobre as variações dos parâmetros fisiológicos de tamanduás-bandeira de vida livre anestesiados em campo.
- Avaliar o estado sanitário de tamanduás-bandeira de vida livre através de hematologia, bioquímica sanguínea e levantamento de carrapatos, hemoparasitas e ovos/cistos de parasitos gastrintestinais.
- Caracterizar a filogenia molecular e a morfologia de nematódeos do gênero *Aspidodera* encontrados em um tamanduá-bandeira.
- Descrever nova espécie de nematódeo do gênero *Aspidodera* em um tamanduá-bandeira e propor nova chave taxonômica para o gênero.

3. MANUSCRITO 1:

Imobilização, monitoramento anestésico e influência da temperatura do ar em tamanduás-bandeira (*Myrmecophaga tridactyla*) de vida livre

Resumo

Capturamos, realizamos imobilização física e química e monitoramos a anestesia de nove tamanduás-bandeira (*Myrmecophaga tridactyla*) em uma área protegida de bioma Cerrado, no estado de São Paulo, sudeste do Brasil. Também calculamos o esforço e sucesso de captura e testamos a correlação da temperatura do ar com a do corpo de indivíduos anestesiados. As capturas ocorreram principalmente em maio e junho, temperaturas amenas ($24,0 \pm 3,5^\circ \text{C}$), áreas abertas e ao entardecer. O conhecimento sobre a área de estudo e população, a experiência da equipe e a possibilidade de tiros com pistola anestésica foram elementos importantes no sucesso de captura. Os animais receberam a associação de cetamina ($12,2 \pm 4,2 \text{ mg/kg}$), midazolam ($0,3 \pm 0,1 \text{ mg/kg}$) e xilazina ($0,9 \pm 0,4 \text{ mg/kg}$), e ainda, atropina ($0,04 \text{ mg/kg}$) e ioimbina ($0,3 \text{ mg/kg}$). Observamos pouca salivação, ausência de convulsões ou vocalizações, bom grau de relaxamento muscular e estabilidade de parâmetros fisiológicos. Embora dentro das faixas consideradas normais para a espécie, a taxa respiratória esteve baixa em todos os indivíduos e a temperatura corporal, comparativamente mais baixa em dois. A temperatura do ar caiu do início para o final do monitoramento e correlacionou-se positivamente com a temperatura corporal. O estresse de captura provavelmente afetou os tempos de indução ($11,8 \pm 8,9 \text{ min}$), imobilização ($66,3 \pm 21,4 \text{ min}$) e recuperação inicial ($11,4 \pm 5,0 \text{ min}$), superiores aos reportados em literatura. Estudos como este são necessários dada à escassez e dispersão de informações e por contribuir para estabelecermos estratégias de campo em trabalhos que envolvam captura e anestesia de tamanduás-bandeira.

Palavras-chaves: Busca ativa. Contenção físico-química. Drogas anestésicas. Homeotermia imperfeita. Xenarthra. Medicina da conservação.

Immobilization, anesthetic monitoring and air temperature influence on free-living giant anteaters (*Myrmecophaga tridactyla*)

Abstract

We captured, performed physical and chemical immobilization and monitored the anesthesia of nine giant anteaters (*Myrmecophaga tridactyla*) in a protected area of Cerrado biome from São Paulo state, Southeastern Brazil. We also calculated the capture effort and success and tested the correlation between air and body temperature in anesthetized individuals. Captures occurred mostly in May and June, mild temperatures ($24.0 \pm 3.5^\circ \text{C}$), open areas and at dusk. The knowledge about the study area and population, team experience and the possibility of anesthetic pistol shots were important elements in the capture success. The animals received the association of ketamine ($12.2 \pm 4.2 \text{ mg/kg}$), midazolam ($0.3 \pm 0.1 \text{ mg/kg}$) and xylazine ($0.9 \pm 0.4 \text{ mg/kg}$), and also atropine (0.04 mg/kg) and yohimbine (0.3 mg/kg). We observed little salivation, absence of convulsions or vocalizations, good degree of muscle relaxation and physiological parameters stability. Although within the ranges considered normal for the species, the respiratory rate was low in all individuals and body temperature was comparatively lower in two. Air temperature dropped from the beginning to the end of the monitoring and correlated positively with body temperature. Capture stress probably affected induction ($11.8 \pm 8.9 \text{ min}$), immobilization ($66.3 \pm 21.4 \text{ min}$) and initial recovery ($11.4 \pm 5.0 \text{ min}$) times, higher than those reported on literature. Studies like this are necessary given the scarcity and dispersion of information and because they contribute on establishing field strategies to works involving capture and anesthesia of giant anteaters.

Keywords: Active search. Physicochemical restraint. Anesthetic drugs. Imperfect homeothermia. Xenarthra. Conservation medicine.

3.1. Introduction

Much of research, conservation and management work in medium and large wild mammals require immobilization. It is one of the biggest challenges for the professionals involved because it can expose the animals to injury, morbidity and even death (Nicholson *et al.*, 2000; Arnemo *et al.*, 2006). However, it is surprising how little we know (or report) about capture and anesthesia of most species. This gap is even more relevant in free-living populations, where adverse environmental conditions impose additional concerns, the human and material infrastructure is less and the health and responses of individuals to stress and drugs are little or unknown. Therefore, we need to increase efforts to understand if, how and when physicochemical restraint can affect animal survival.

The few information available on capture and anesthesia of giant anteaters (*Myrmecophaga tridactyla*) is dispersed in some unpublished materials, gray literature or concentrated on researchers' experiences and routines. Aside from the difficulties imposed by the environment and the giant anteaters capture method - active via persecution - there are physiological, behavioral and anatomical particularities that make them anesthetic management quite complex. They feed on prey with low caloric content (McNab, 2000), have low body temperature and basal metabolism rate (McNab, 1984, 1985). As a result, fluctuations in air temperature can affect their body temperature (Camilo-Alves & Mourão, 2006; Fernandes & Young, 2007), as well as influence their activity pattern (Mourão & Medri, 2007; Di Blanco *et al.*, 2016; Bertassoni & Ribeiro, 2019) and habitat use (Mourão & Medri, 2007). Although giant anteaters, in general, escape when they feel threatened, they can behave defensively (Haddad *et al.*, 2014). This also requires caution considering its strength, size and body weight, from 26 to 41 kg in adulthood (Medri & Mourão, 2005).

Every Xenarthra, mammalian superorder to which the giant anteater belongs, can be immobilized with cyclohexamine drugs (ketamine, tiletamine) associated with diazepines (midazolam, diazepam, zolazepam) or alpha2-adrenergic agonists (xylazine, dexmedetomidine and medetomidine) (West *et al.*, 2007). Despite diazepines and "alpha2" have a synergistic effect if combined (Bol *et al.*, 2000), curiously, they are little reported in the same protocol on literature. The most cited associations for captive or free-living giant anteaters are ketamine/xylazine, ketamine/midazolam and tiletamine/zolazepam (Deem & Fiorello, 2002; Miranda *et al.*, 2006; West *et al.*, 2007; Rojano-Bolaño *et al.*, 2014). Few studies mention the use of atropine (Gillespie & Adams, 1985; Deem & Fiorello, 2002; Satake & Fagliari, 2006) and antagonists (Kreeger, 1999; Deem & Fiorello, 2002; West *et al.*, 2007). Only two present

detailed data on anesthetic monitoring (Carregaro *et al.*, 2009; Canelo, 2017), which is a concern, because such records indicate the effects of drugs, sedation degree and emergencies signs, in addition to enabling comparative studies.

It is notoriously tricky to find and capture giant anteaters in wildlife in most of their occurrence area (Bertassoni & Ribeiro, 2019). Data on capture effort and success are not available, and reports on xenarthrans physiological parameters under anesthesia are rare (Gasparotto *et al.*, 2017). Due to their imperfect homeothermia, probably their physiological parameters alter under anesthesia and air temperature will likely affect their body temperature. Anesthetic risks are prompt to happen (Miranda *et al.*, 2006), but the right drug's combination could minimize them. With this in mind, the need for improvements and dissemination of methods on capture and anesthesia of giant anteaters on field, we aim to: (1) calculate the capture effort and success; (2) assess variations in the physiological parameters of anesthetized individuals; (3) test the correlation between air and body temperature of anesthetized individuals; (4) classify the quality of anesthesia promoted by the association of drugs.

3.2. Material and methods

3.2.1. Study area

We carried out fieldwork at Santa Bárbara Ecological Station (SBES; - 22°48'59", - 49°14'12") and surroundings, an area of 67 km². The relief is of low altitudes (600 to 680 m), the climate is hot and humid with dry winter (Cwa) and the average temperatures vary between 17 and 24° C (Melo & Durigan, 2011). The SBES (27 km²) is a protected area of Cerrado biome in São Paulo state, Southeastern Brazil, of high relevance in the state for maintaining natural portions of open field and savanna vegetation (Melo & Durigan, 2011). It is composed by open habitats (41% - Cerrado grassland and humid grassland), exotic timber plantation (22% - plots of *Pinus* spp. and *Eucalyptus* spp.), savanna (21% - Cerrado *stricto sensu* and dense Cerrado), forest (13% - Cerradão, seasonal and alluvial forests, and its transition areas), and anthropogenic sites (3% - roads, built-up areas, and urban) (Bertassoni *et al.*, 2020). Two state highways, a municipal road and several internal dirt roads (firebreaks), fragment the SBES area. The surroundings are characterized by sugar cane culture, *Pinus* spp., *Eucalyptus* spp., pastures with *Brachiaria* sp., extensive cattle breeding and urban area (Águas de Santa Bárbara municipality).

3.2.2. Data collection

We conducted the fieldwork from 10/16 to 11/19/2014, 1/9 to 02/10/2015 and 05/26 to 07/02/2015. They occurred across the internal dirt roads and surroundings of SBES with the aid of a car at low speed (20 km/h). We concentrated the searches during the day and early night, except in the second campaign (January-February), when we also searched at all night and dawn and on foot too. We performed the immobilization by silent approach on foot, siege, application of drugs with blowpipe or CO₂ injection pistol and physical restraint with a net. We used records from camera traps that were arranged in a grid with a distance of 1.2 km in SBES, obtained for a parallel study (Bertassoni, 2017), to plan the searches on areas of better individuals' detection.

The anesthetic drugs we used were ketamine hydrochloride (Dopalen® [100mg / ml] Ceva Saúde Animal Ltda), midazolam maleate (Dormonid® [15mg / 3ml] Cenexi S.A.S.) and xylazine hydrochloride (Anasedan® 2% Ceva Saúde Animal Ltda), intramuscularly. We slightly adapted the anesthetic protocol to meet the objectives, field conditions and according to individual responses. In the first individual, the only who received the injection manually, the protocol was ketamine 10 mg/kg and midazolam 0.2 mg/kg. As we did not achieve the desired muscle relaxation, we started to use ketamine 10 mg/kg, midazolam 0.2 - 0.5 mg/kg and xylazine 0.5 - 0.8 mg/kg, from the second captured individual. We based the drug volume on estimated weight. We used atropine 0.04 mg/kg [0.5 mg/ml] from the fourth animal and yohimbine 0.3 mg/kg [2.0 mg/ml] at the end of the procedures (except for TB01 and TB07 individuals). We performed the following procedures with a team of veterinarians and biologists: physical examination, anesthetic monitoring, sample collection (blood, ectoparasites, hair and feces), microchip application, biometry, photograph, installation of telemetry equipment and data record.

We placed the anesthetized individuals in lateral decubitus with members and head stretched, and anterior claws tied. We lubricated the eyes with an ophthalmic ointment and covered them with a cotton band. We cleaned nostrils, mouth and tongue and humidified the tongue. We measured the following physiological parameters during anesthesia: heart rate (HR) by chest auscultation, respiratory rate (RR) by direct observation and body temperature (BT) using a digital thermometer placed in rectum. We constantly checked these parameters and recorded them after the induction, every 15 minutes (monitoring moments: 0, 15, 30, 45 and 60 min). We also monitored local air temperature (AT) every 15 minutes with a portable USB Tag-temp. We observed the effect of the anesthetic protocol on each individual and during all monitoring moments to classify the anesthetic quality based on: physiological parameters (HR,

RR and BT); muscle relaxation; drugs adverse effects; and duration of induction (IND), immobilization (IMO) and initial recovery (REC_i). We considered “initial recovery” as the time from the application of the antagonist or the appearance of the first voluntary movements (TB01 and TB07) until the first steps, and not the time taken for complete release.

3.2.3. Data analyses

We calculated capture effort for each campaign by the ratio between time in hours spent on active searches per captured individual (h/ind) and kilometers traveled by car per captured individual (km/ind). Capture success for each campaign was the ratio (%) between individuals captured and the total individuals (the sum of the sighted, not caught and captured) (Table 1).

We qualitatively assessed the physiological parameters variation among sampled individuals (HR_i, RR_i and BT_i, Table 4) and throughout the monitoring moments (HR_t, RR_t and BT_t, Table 3) by mean and standard deviation. We calculated the average, minimum and maximum air temperature over the three campaigns (Table 1). To test the correlation between air and body temperature of anesthetized individuals, we used the Spearman's correlation. We tested the stability of air temperature through monitoring moments by Shapiro-Wilk, ANOVA and Tukey's Test.

We subjectively classified the anesthetic quality on a scale ranging from good, satisfactory to unsatisfactory, taking into account the: values of physiological parameters and safe margin, vital signs stability, occurrence of adverse effects, steps duration and animal handling capacity provided by the drugs.

3.3. Results

The active searches lasted 779 hours distributed in 98 days and we traveled 12605 km by car (Bertassoni *et al.*, 2020). One capture occurred in January - the second campaign -, when the capture effort was 191 h/ind and 2564 km/ind, and capture success was 12.5%. In this period we sighted giant anteaters in difficult to reach places (e.g. covered vegetation, muddy areas, dense shrub areas) in four occasions and we were not able to approach them. We tried to capture, without success, in three opportunities (Table 1). The other captures (89%) were concentrated between May and June 2015 - the third campaign -, with capture effort of 37 h/ind and 564.9 km/ind, and capture success of 50%. In this period we sighted giant anteaters five times and tried to capture three times (Table 1). Capture events were mostly around 17:30 h (Figure 1)

with local air temperature of $24 \pm 3.5^\circ \text{C}$ (18.2 to 27.4°C). Average air temperatures during the three campaigns were $25.9 \pm 4^\circ \text{C}$, $27.1 \pm 3.6^\circ \text{C}$ and $19.7 \pm 3.6^\circ \text{C}$, respectively (Table 1). Three of the nine giant anteaters captured (TB01, TB06 and TB09) started to flee and were contained. We surrounded an animal in an area of forest and dirty field, but it managed to escape when hit by the anesthetic dart.

Table 1. Characteristics of free-living giant anteaters (*Myrmecophaga tridactyla*) capture campaigns at Santa Bárbara Ecological Station, Cerrado of São Paulo state, Brazil.

Characteristics	CAMPAIGN 1	CAMPAIGN 2	CAMPAIGN 3
Period of year	10/16 to 11/19/2014	1/9 to 02/10/2015	05/26 to 07/02/2015
Days on field	27 days	33 days	38 days
Period of day	morning, afternoon, early night	morning, afternoon, night, dawn	morning, afternoon, early night
Search time	292 hours	191 hours	296 hours
Traveled distance	5762 Km	2564 Km	4279 Km
Average air temperature	$25.9 \pm 4^\circ \text{C}$	$27.1 \pm 3.6^\circ \text{C}$	$19.7 \pm 3.6^\circ \text{C}$
Air temperature range	17.1 to 41.7°C	19.7 to 42.3°C	10.1 to 29.9°C
Sightings*	9	4	5
Attempts**	9	3	3
Captures	0	1	8
Total	18	8	16
Capture effort	0	191 h/ind	37 h/ind
Capture effort	0	2564 km/ind	534.9 km/ind
Capture success	0	12.5%	50%

*Sightings: number of times we saw giant anteaters in difficult to reach places and the approach did not belong.

**Attempts: number of times we saw giant anteaters and tried to capture them, but we were unsuccessful.

We captured and anesthetized four female (a juvenile and three adults) and five adult males with unknown health histories and an average of $34 \pm 5.1 \text{ kg}$ (Table 2). Upon visual inspection, all individuals were alone, without impairment of locomotion, physically healthy and with normal foraging, alert and escape behaviors. The animals received the association of ketamine ($12.2 \pm 4.2 \text{ mg/kg}$), midazolam ($0.3 \pm 0.1 \text{ mg/kg}$) and xylazine ($0.9 \pm 0.4 \text{ mg/kg}$), in addition to atropine (0.04 mg/kg) and yohimbine (0.3 mg/kg) (Table 2).

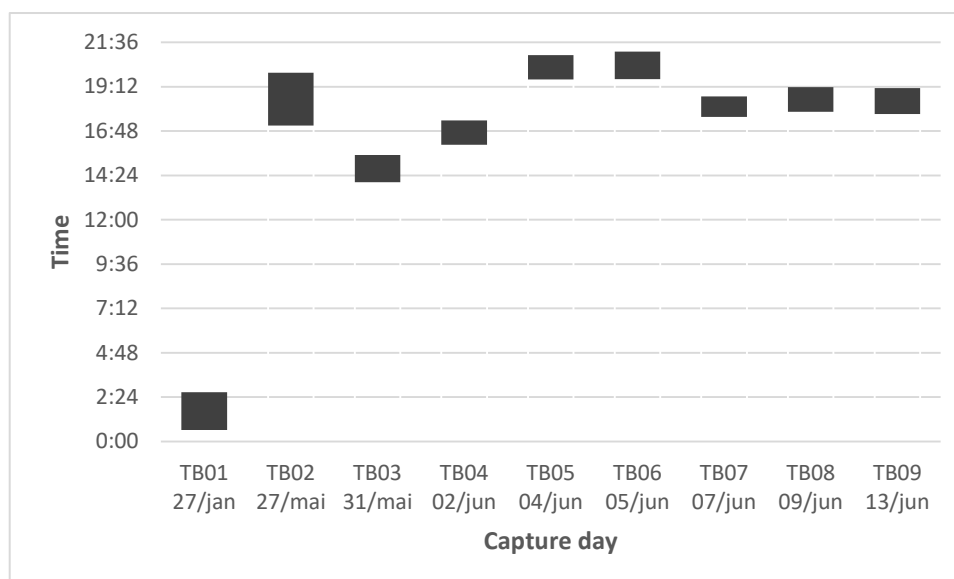


Figure 1. Day and time of captures and procedures duration of nine free-living giant anteaters (*Myrmecophaga tridactyla*) anesthetized with ketamine, midazolam and xylazine in 2015 at Santa Bárbara Ecological Station, Cerrado of São Paulo state, Brazil.

Table 2. Doses used to immobilize nine free-living giant anteaters (*Myrmecophaga tridactyla*) anesthetized with ketamine, midazolam and xylazine in 2015, at the Santa Bárbara Ecological Station, Cerrado of São Paulo state, Brazil.

ID	Sex	Age	Weight (kg)	Doses applied (mg/Kg)	ATR	YOH
TB01	F	A	32	Keta 10 + Mida 0.2	no	no
TB02	M	A	38.7	Keta 7.27 + Mida 0.36 + Xyla 0.36	no	yes
TB03	F	A	34.8	Keta 6.83 + Mida 0.34 + Xyla 0.34	no	yes
TB04	M	A	35.2	Keta 12.5 + Mida 0.63 + Xyla 0.63	no	yes
TB05	M	A	36.6	Keta 9.7 + Mida 0.19 + Xyla 0.77	yes	yes
TB06	M	A	38	* Keta 17.79 + Mida 0.36 + Xyla 1.42	yes	yes
TB07	F	A	33	Keta 12.2 + Mida 0.24 + Xyla 0.97	yes	no
TB08	M	A	36.2	Keta 13.8 + Mida 0.27 + Xyla 1.1	yes	yes
TB09	F	J	21.6	Keta 17.5 + Mida 0.35 + Xyla 1.4	yes	yes

ID: individual, **F**: female, **M**: male, **A**: adult, **J**: juvenile, **Keta**: ketamine hydrochloride, **Mida**: midazolam maleate, **Xyla**: xylazine hydrochloride, **ATR**: atropine application 0.04 mg/kg (0.5 mg/ml), **YOH**: yohimbine application 0.3 mg/kg (2.0 mg/ml), **no**: not applied, **yes**: applied, *A dart ricocheted and it must not have injected the entire volume, but in dose recording we disregarded this possibility.

The HRi average was 57.9 ± 4 bpm. The least stable HRi was from TB01 (85.3 ± 17.3 bpm) and the most stable was from TB07 (50.8 ± 1.8 bpm) (Figure 2). The lowest HRi recorded was 38 bpm in the monitoring moment 60' (TB03) and the highest was 116 bpm in the 0'

(TB01). The HRt presented an average value of 58 ± 4 bpm and was similar through all monitoring moments (Table 3). The RRI average was 7.3 ± 0.5 rpm. The least stable RRI was from TB02 (6.2 ± 2.2 rpm) and the most stable was from TB07 (7.6 ± 0.9 rpm) (Figure 2). The lowest RRI recorded was 3 rpm in the monitoring moment 0' (TB06) and the highest was 12 rpm in the 45' (TB01). The RRT presented an average value of 7.2 ± 0.4 bpm and was similar through all monitoring moments (Table 3). The BTi average was $34.5 \pm 0.5^\circ\text{C}$. The least stable BTi was from TB02 ($35.1 \pm 0.96^\circ\text{C}$) and the most stable was from TB03 ($32.5 \pm 0.17^\circ\text{C}$) (Figure 3). The lowest BTi recorded was 32.1°C in the monitoring moment 15' (TB08) and the highest was 36.7°C in the 0' (TB02). The BTt presented an average value of $34.6 \pm 0.3^\circ\text{C}$ and was similar through the monitoring moments (Table 3).

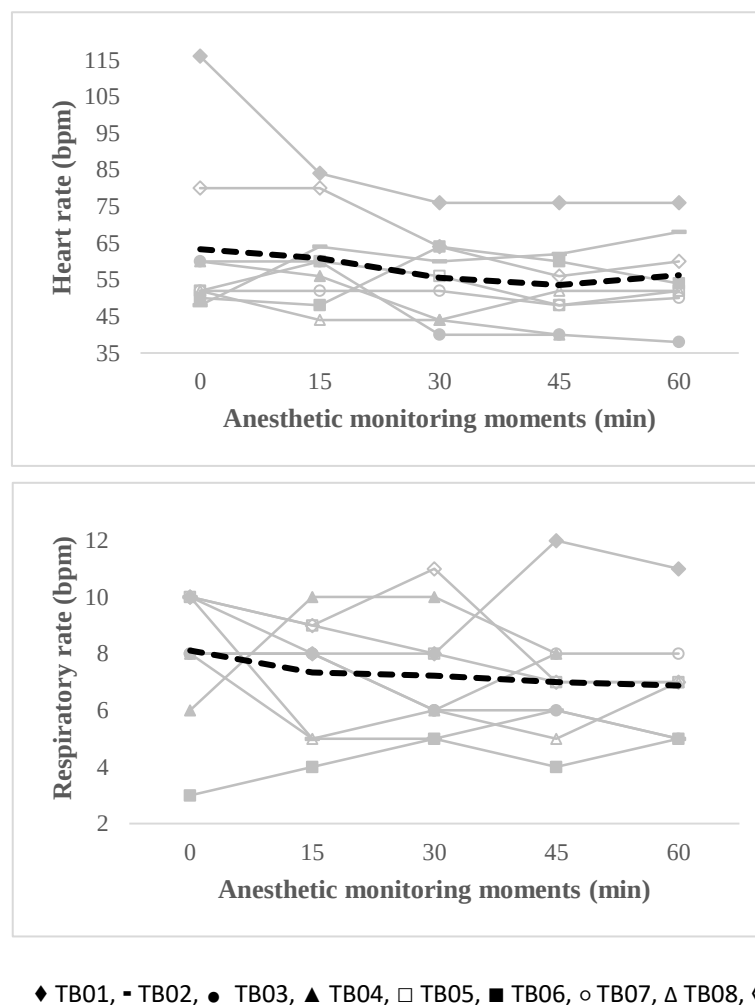


Figure 2. Variation in heart and respiratory rates of nine free-living giant anteaters (*Myrmecophaga tridactyla*) anesthetized with ketamine, midazolam and xylazine in 2015, at Santa Bárbara Ecological Station, Cerrado of São Paulo state, Brazil.

Table 3. Physiological parameters and air temperature during the monitoring of nine free-living giant anteaters (*Myrmecophaga tridactyla*) anesthetized with ketamine, midazolam and xylazine in 2015, at the Santa Bárbara Ecological Station, Cerrado of São Paulo state, Brazil.

Parameters	Anesthetic monitoring moments (min)						
	0	15	30	45	60	Average	SD
Heart rate (bpm)	63	61	56	54	56	58	3.8
Respiratory rate (rpm)	8	7	7	7	7	7,2	0.4
Body temperature (°C)	35	34.7	34.4	34.3	34.4	34.6	0.3
Air temperature* (°C)	24	22.9	20.5	18.4	18	20.8	2.7

min: minutes, **bpm:** beats per minute, **rpm:** respiratory movements per minute, **SD:** standard deviation.

*: air temperature dropped significantly during the monitoring.

Air temperature positively correlated to body temperature of anesthetized giant anteaters TB05 ($S = 4.4409e-15$, $p\text{-value} = 0.02$) and TB09 ($S = 4.4409e-15$, $p\text{-value} = 0.02$). It dropped from the beginning to the end of the monitoring and was significantly different into the moments 0' and 45' (diff = -5.0, lwr = -9.33, upr = -0.67, $P\text{.adj} = 0.02$), 0' and 60' (diff = -5.72, lwr = -10.05, upr = -1.39, $P\text{.adj} = 0.004$), 15' and 60' (diff = -4.67, lwr = -8.99, upr = -0.34, $P\text{.adj} = 0.03$) (Table 3). The biggest oscillation of AT was the drop of 10.6° C during the TB07 monitoring. This trend did not occur with TB03, the only procedure performed before 4 pm, in which the AT rose 2.4° C. The lowest AT occurred in the minute 60' with the individual TB06 (13.1° C), and the highest, in the 0' with TB02 (27.4° C) (Figure 3). The mean AT during monitoring moments was $20.8 \pm 2.7^\circ \text{C}$ (Table 3) and it showed a downward trend in the six hours after animal release, remaining on average at $19.4 \pm 2^\circ \text{C}$.

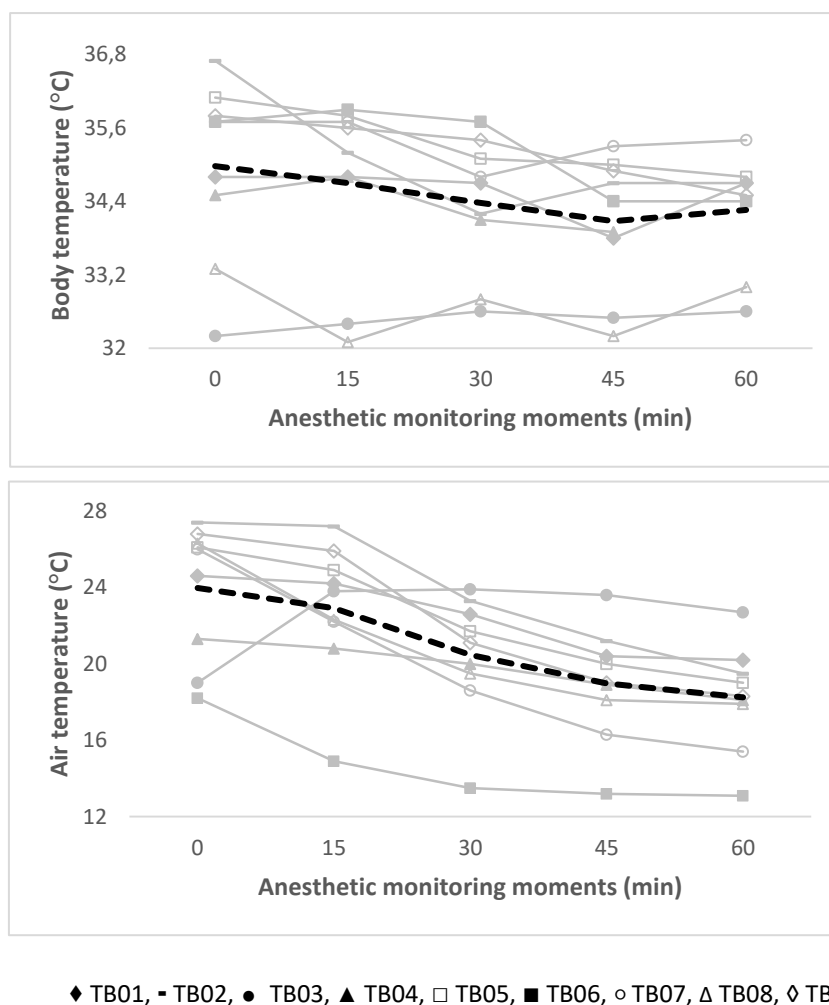


Figure 3. Variation of air temperature and body temperature of nine free-living giant anteaters (*Myrmecophaga tridactyla*) anesthetized with ketamine, midazolam and xylazine in 2015, at Santa Bárbara Ecological Station, Cerrado of São Paulo state, Brazil.

The quality of anesthesia promoted by drugs association was similar among individuals and classified as good to satisfactory, with the following considerations to be taken. Muscle relaxation was good in general, but with undesirable tone and reflex in some monitoring moments of some individuals (TB01, TB02, TB04 and TB06). The TB02 and TB06 also showed moderate salivation, and the last was the only with pale mucosa during physical examination and monitoring. We did not observe involuntary muscle activity, convulsions, vocalizations or regurgitation and, whenever verified, the arterial pulse was strong and regular in all animals. The induction lasted 11.8 ± 8.9 min, immobilization lasted 63.7 ± 16.2 min and the initial recovery, 8.2 ± 4.9 min. The animal with the longest induction time was TB06 (30 min). The shortest immobilization time was from TB07 (44 min), the reason he did not receive the reversing drug. The longest initial recovery time was from TB05 (17 min) (Table 4).

Table 4. Physiological parameters and duration of monitoring steps of nine free-living giant anteaters (*Myrmecophaga tridactyla*) anesthetized with ketamine, midazolam and xylazine in 2015, at Santa Bárbara Ecological Station, Cerrado of São Paulo state, Brazil.

ID	Parameters			Steps duration (min)		
	HR (bpm)	RR (rpm)	BT (°C)	IND	IMM	REC _i
TB01	85.6	9.8	34.6	6	87	14
TB02	60.4	6.2	35.1	12	89	6
TB03	47.6	6.6	32.5	8	60	10
TB04	50	8.5	34.3	23	53	3
TB05	53.6	8.2	35.4	3	57	17
TB06	55.2	4.2	35.2	30	49	4
TB07	50.8	7.6	35.4	9	44	13
TB08	48.8	6.2	32.7	7	59	6
TB09	68	8.8	35.2	8	75	6
Average				11.8	63.7	8.2
SD				8.9	16.2	4.9

ID: individual, **HR:** heart rate, **RR:** respiratory rate, **BT:** body temperature, **IND:** induction time, **IMM:** immobilization time, **REC_i:** initial recuperation time, **min:** minutes, **bpm:** beats per minute, **rpm:** respiratory movements per minute, **° C:** Celsius degree, **SD:** standard deviation.

3.4. Discussion

Active capture of giant anteaters is laborious because involves tracking, sighting, chasing and restraining, but also dealing with the species behaviour, anatomy and physiology. The small population size (Bertassoni, 2017) and landscape characteristics influenced on the difficulty of search and capture (Bertassoni & Ribeiro, 2019). We increased the success after we (1) accumulated knowledge about the population and the study area habitat features; (2) optimized the searches by establishing favorable regions, times and temperatures for encounters; and (3) reinforced the team with members that were more experienced and the possibility of shooting at greater distances with an anesthetic pistol (on May and June). Certain temporal and spatial conditions also favored giant anteater's activity allied to good opportunities of immobilizations. On hot season, air temperature reached up to 41 - 42° C and we saw few individuals during the day, suggesting that at high temperatures they avoid exposure to sun and overheating (Mourão & Medri, 2007). Camera trap records have indicated a predominantly nocturnal activity and we have performed the first capture at 00:37 h on hot season. However, we gave up the nocturnal searches due to the poor visibility. Nonetheless, as the days became colder and temperatures

less variable, we started to see them more often during the day and managed to capture in milder temperatures ($24.0 \pm 3.5^\circ \text{C}$). In colder days, giant anteaters tend to start and end their activity earlier and prefer resting in open areas (Rodrigues *et al.*, 2008), using several shallow torpors as a mean of saving energy (Fernandes & Young, 2007). The species peak activity in different study areas is around 18:00 h (Shaw *et al.*, 1987; Camilo-Alves & Mourão, 2006; Mourão & Medri, 2007; Di Blanco *et al.*, 2015; Di Blanco *et al.*, 2016), similar to the times we were most successful on capture (around 17:30 h). The use of open habitats during activity periods (Camilo-Alves & Mourão, 2006; Mourão & Medri, 2007; Macedo *et al.*, 2010; Di Blanco *et al.*, 2016; Bertassoni *et al.*, 2020) contributed to animal visualization (Desbiez & Medri, 2010), quieter approaches, siege space, free targeting and less chance of escape, as also observed by other authors (Luba *et al.*, 2015).

The drugs combination we used gathered desirable characteristics in wild mammal's field anesthesia: suites the species and objective, easy to apply, promotes quick and smooth induction and recovery, good safety margin, partial reversible (xylazine by yohimbine and midazolam by flumazenil), cost-effective and available on most countries (Hernandez *et al.*, 2006; Chinnadurai *et al.*, 2016). The major disadvantage was the high volume, since a single 5 ml dart was not sufficient in some cases. In free-living giant anteaters, ketamine with xylazine are used at the doses of 12 mg/kg and 0.5 mg/kg (Rojano-Bolaño *et al.*, 2014), 9.6 mg/kg and 1.6 mg/kg (Shaw *et al.*, 1987; Collevatti *et al.*, 2007), and 10 mg/kg and 1 mg/kg (Deem & Fiorello, 2002; Miranda *et al.*, 2006; Sataki & Fagliari, 2006), respectively. There are several reports of immobilization in captivity with ketamine and midazolam associated or not with isoflurane (Miranda *et al.*, 2006; Satake & Fagliari, 2006; Sanches *et al.*, 2013; Di Nucci *et al.*, 2014; Borges *et al.*, 2017; Oliveira *et al.*, 2018). Only one study with free-living giant anteaters combined ketamine 8 mg/kg, midazolam 0.5 mg/kg and xylazine 0.5 mg/kg (Luba *et al.*, 2015), a protocol similar to ours. These three drugs were also successfully used in sloth handling (Lescano *et al.*, 2014) and armadillo surgery (Rojas *et al.*, 2013; Gasparotto *et al.*, 2017). The Tamandua Project in Pantanal has been using this combination for years (personal communication). However, sedative effects are achieved with lower doses than the one reported here. We cannot state the cause of this finding, but it is important to note that the anthropic impacts on SBES, as well as the history of agrochemicals use for forest management, are not observed in the Pantanal regions of capture.

Some researchers opt for tiletamine and zolazepam (Mourão & Medri, 2007; Perez *et al.*, 2015) on giant anteater's chemical containment, but the limitations are prolonged recovery, absence of antagonistic drugs, excessive salivation, lesser degree of sedation and analgesia

(West *et al.*, 2007; Orozco, 2011) and contraindication to supplementary doses (Murray *et al.*, 2000). A recent combination, of butorphanol, detomidine and midazolam (BDM), all at a dose of 0.1 mg/kg and supplementation of 0.03 mg/kg (Kluyber *et al.*, 2021), seems to be promising. Although some of these drugs are not readily available on some countries and it is a high cost protocol, it is fully reversible and provided good results considering body temperature (34.5 ± 2.52 °C), heart rate (43.47 ± 7.39 bpm), respiratory rate (8.49 ± 2.79 rpm) and oxygen saturation (90.1 ± 4.38 %). In addition, the inductions was rapid and smooth, with a wide margin of safety and 12 ± 5.39 min of recovery time (Kluyber *et al.*, 2021).

The comparative higher and unstable heart rate of TB01 (85.6 ± 17.3 bpm), although within the normal range for the species, between 32 and 140 bpm (Vinci & Miranda, 2012), was credited to the absence of xylazine and atropine. Xylazine reduces ketamine negative effects (Wright, 1982), such as heart rate increase, in addition to reducing ketamine volume and potentiating its sedative and analgesic effects (Waterman, 1983). Atropine maintains cardiovascular parameters more stable (Flôres *et al.*, 2008) and is fundamental for sialorrhea control, highly recommended to *Xenarthra* (Miranda & Costa, 2007; Gasparotto *et al.*, 2017). The HRi (57.9 ± 4 bpm) was similar to the obtained by the two studies that detailed the anesthetic monitoring, of 58 ± 4 bpm (Carregaro *et al.*, 2009) and 45 to 70 bpm (Canelo, 2017), and the one that used BDM, 43.47 ± 7.39 bpm (Kluyber *et al.*, 2021). However, it was far below from 110 to 160 bpm, also considered normal (Reece, 1977; Deem & Fiorello, 2002). This wide range could reflect different immobilization approaches, environmental conditions, anesthetic drugs and management. However, we know very little about anesthesia in this species and are unable to refute if that is a result of physiological stress, for example.

The respiratory rates were constantly low but within the normal range for the species, between 3 and 38 rpm (Vinci & Miranda, 2012). This tendency in giant anteaters was seen when submitted to other anesthetic protocols, of 5.9 ± 1.3 rpm, 3.2 ± 1.2 rpm (Canelo, 2017) and 5 ± 2 rpm (Carregaro *et al.*, 2009), differing from 10 to 24 rpm (Reece, 1977; Deem & Fiorello, 2002), also considered normal. Xylazine can cause cardiorespiratory depression, a side effect not always present, especially if ketamine is associated (Thurmon *et al.*, 1996). Transient apnea in a giant anteater anesthetized with ketamine and xylazine was reversed after doxapram hydrochloride administration (Strom, 2003) and has been associated with high doses of medetomidine and dexmedetomidine (Rojas-Moreno, 2014). Tiletamine and zolazepam caused depressive respiratory effects in other *Xenarthra* (Orozco, 2011; Rojas-Moreno, 2011), but it is unknown if anteaters respond well to momentary hypoxemia, as the fossorial species of the superorder. The information available are insufficient to assist on the choice of immobilization

protocols and cannot answer if this apparent plasticity in cardiorespiratory rates prevent damage to organs.

Body temperature of giant anteaters covers from 32.1 to 38.8° C (Vinci & Miranda, 2012) and could reach 30° C (Deem & Fiorello, 2002), a wide range that include our results and all literature citations (Wislocki, 1933; Wislocki & Enders, 1935; Fernandes & Young, 2007; Carregaro *et al.*, 2009; Mendonça, 2010; Canelo, 2017; Kluyber *et al.*, 2021). The giant anteaters thermoneutrality is between 15 and 36° C and air temperatures below 15° C can result in drops of up to 2° C in their body temperature (MacNab, 1984). Only in TB06 procedure the air temperature was below this zone, but abrupt drops (between 4 and 11° C) during anesthesia also resulted in falls on body temperature of similar magnitude, up to 1.9° C in TB05. In the first case, we believe that pre-existing liver damage (ongoing investigation) may have affected the body's ability to absorb the drugs used, resulting in TB06 long induction time. In the second, it is likely that the air temperature interference on body temperature reduced the body's ability to metabolize drugs, resulting in TB05 long initial recovery time. Drugs and activity can also cause fluctuations on body temperature, but with our field study, we were unable to discriminate its cause. Xylazine can depress the brain thermoregulatory center (West *et al.*, 2007) and the anteater's body temperature can reduce between 4 and 6.5° C while sleeping, increasing during activity (Fernandes & Young, 2007). Although body temperature is not a useful indicator of anesthetic depth in the species (Deem & Fiorello, 2002), its monitoring is essential to anticipate interventions in emergencies. We recommend to avoid captures on days of abrupt changes in air temperatures, both to prevent hypo or hyperthermia (Miranda & Costa, 2007). We suggest that the choice of season and day time of captures are based on air temperatures within the species' thermoneutrality zone.

The duration of induction, immobilization and initial recovery we obtained were longer than those reported for giant anteaters anesthetized with similar protocols, from about 4 to 7 min, 30 to 45 min and 4 ± 1 min, respectively (Shaw *et al.*, 1987; Collevatti *et al.*, 2007; Mendonça, 2010; Rojas-Moreno, 2014). The agitation caused by the dart impact, attempts to escape and chase probably caused stress. Stress was observed on giant anteater captures by other authors (West *et al.*, 2007). For this reason and based on the team's experience, we suggest that, whenever possible, prioritize only remote chemical immobilization, much less stressful than that performed by adding physical restraint. Stress affects drugs absorption, can results in sedation failure (Sinclair, 2003), affects vital parameters and survival (Arnemo *et al.*, 2006). Some drugs should be used with caution in liver disease (Tiburcio *et al.*, 2014) and critical patients (Hodgson & Dunlop, 1990; Guedes & Natalini, 2002), due to their impaired ability to

deal with stress (Fletcher, 2014). Negative anesthesia effects in giant anteaters were reported with the exclusive use of ketamine, due to catatonia and involuntary movements (Wallach & Boever, 1983; Gillespie & Adams, 1985; Nowak, 1991), and with the use of phencyclidine with promazine, due to muscle fasciculation, salivation and lacrimation (Reece, 1977). Part of what prevents progress in the area is the lack of detailed reports and a reluctance to report “obvious” or with any negative impact results. In lesser anteaters (*Tamandua tetradactyla*), the use of ketamine 20 mg/kg with xylazine 1 mg/kg had better effects on immobilization time, anesthetic depth, muscle relaxation and heart rate compared with ketamine 10 mg/kg with xylazine 1 mg/kg, without affecting the induction and recovery times and without negative respiratory effects (Fournier-Chambrillon *et al.*, 1997).

We strongly recommend that researchers report their capture efforts and success, as well as provide the anesthetic monitoring parameters. This data has the potential to provide if and how much field procedures involving capture and anesthesia can affect the fitness of individuals. We need more empirical, responsible and legally supported evidence, investment in staff training and science, development and adaptation of biomedical techniques and commitment to reporting and monitoring. We need to stimulate publications related to immobilization techniques, better understand the effects of drugs commonly used in captivity and wildlife routines and evaluate modern drugs.

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4. MANUSCRITO 2:

Estado de saúde do ameaçado tamanduá-bandeira (*Myrmecophaga tridactyla*) no Cerrado brasileiro

Resumo

Relacionar múltiplas ferramentas para acessar a saúde de mamíferos de grande porte de vida livre é desafiador em muitos aspectos, incluindo os técnicos, custos financeiros, amostragem e dificuldades no trabalho de campo. Nós avaliamos a saúde de nove tamanduás-bandeira (*Myrmecophaga tridactyla*) em uma área protegida do bioma Cerrado brasileiro através de condição física, hematologia, bioquímica sérica, infestação de carrapatos, alguns hemoparasitas e parasitas gastrintestinais. Os animais estavam em sua maioria em boa condição física e normais à inspeção visual, exceto dois indivíduos. Um deles mostrou comportamento anormal à aproximação na recaptura, não ofereceu resistência à contenção física e perdeu peso. Um outro apresentou características de senilidade, como descamação e pintas de pigmentação no focinho, pele seca, abaulamento abdominal e perda de massa muscular. A hematologia e a bioquímica sérica mostraram um grande número de alterações na maioria dos indivíduos, sinalizando dano em órgãos como fígado e rins, possivelmente induzidos por doenças infecciosas, doenças crônicas e estresse. Apenas um indivíduo testou positivo para *Mycoplasma* spp. Todos os indivíduos estavam parasitados por carrapatos e parasitas gastrintestinais. Coinfecções foram frequentes em ambos os grupos de parasitas. Nós identificamos quatro espécies de carrapatos do gênero *Amblyomma*, comumente encontrados em tamanduás. Dos oito morfotipos de ovos/oocistos identificados a partir das fezes frescas, Apicomplexa, Trichostrongylidae e *Aspidodera* spp. foram os mais frequentes e abundantes. Comparando os resultados dos tamanduás-bandeira que capturamos, os machos tiveram maior intensidade de infestação parasitária que as fêmeas, e as cargas parasitárias individuais foram, em sua maioria, baixas. Determinar o estado de saúde de uma população é um processo complexo mas, utilizando de achados combinados, como os apresentados neste estudo, é possível contribuir para a sinalização de possíveis acometimentos e para desenvolver estratégias de conservação.

Palavras-chave: Medicina da conservação. Parasitismo. Doença. Parâmetros sanguíneos. Carga parasitária. Xenarthra.

Health status of the endangered giant anteater (*Myrmecophaga tridactyla*) in the Brazilian Cerrado

Abstract

Linking multiple tools to assess large-sized free-living mammal's health is challenging in many aspects, including technical, financial costs, sampling size and fieldwork difficulties. We assessed the health of nine giant anteaters (*Myrmecophaga tridactyla*) in a protected area of the Brazilian Cerrado biome by physical condition, hematology, serum biochemistry, infestation of ticks, some hemoparasites and gastrointestinal parasites. The animals were mostly in good physical condition and normal on visual inspection, except two individuals. One of them showed abnormal behavior when approaching on recapture, did not offer resistance to physical restraint and lost weight. Another presented senility characteristics, as flaking and pigmentation spots on the snout, dry skin, abdominal bulging, and muscle mass loss. Hematology and serum biochemistry showed a large number of alterations in most individuals, signaling damage to organs such as liver and kidneys, possible induced by infectious diseases, chronic diseases and stress. Only one individual tested positive for *Mycoplasma* spp. All individuals were parasitized by ticks and gastrointestinal parasites. Coinfections were frequent by both parasite groups. We identified four tick species of the genus *Amblyomma*, commonly found in anteaters. Of the eight eggs/oocysts morphotypes identified from fresh feces, Apicomplexa, Trichostrongylidae and *Aspidodera* spp. were the most frequent and abundant. Comparing the results of the giant anteaters we captured, males had higher parasite infestation intensity than females, and individual parasite loads were mostly low. Determining the health status of a population is a complex process but, using combined findings, such as those presented in this study, can contribute to signal possible impairments and contribute to the development of conservation strategies.

Keywords: Conservation medicine. Parasitism. Disease. Blood parameters. Parasitic load. Xenarthra.

4.1. Introduction

Researchers and conservationists constantly use tools to assess wildlife health. It is necessary, however, a wide knowledge about species physiology and a robust database to make possible the interpretation of phenomena and comparison of scenarios (Whilde *et al.*, 2017). This is particularly challenging in large-sized free-living mammals, in which sampling biological data requires high financial costs, long-term research and chemical immobilization (Caughlan & Oakley, 2001). The true burden of parasites on wildlife is also laborious to assess, because of host properties, its response to parasites and diseases, parasite properties and sampling sizes, which are possible reasons why this field is under-researched (Scott, 1988). Other limitations in determining health status to conservation purposes refers to the data origin (the vast majority from captive animals), influences of capture stress, environment, life history and our still present difficulties on linking multiple physiological and non-physiological tools (Madliger *et al.*, 2018). The paucity information about health may be a major obstacle of planning effective strategies to improve wildlife population's survival and viability in the long term (Mangini *et al.*, 2012; Medici & Desbiez, 2012). Information about mortality and morbidity is essential to protect species from present and future threats (Desbiez *et al.*, 2020).

The giant anteater (*Myrmecophaga tridactyla*) is a large-sized insectivorous mammal with low basal metabolic rate and reduced thermoregulation capacity (McNab, 1985). It is vulnerable to extinction global and regionally (Bressan *et al.*, 2009; Miranda *et al.*, 2014) and is distributed from Central America to South America (Miranda *et al.*, 2014). Its vulnerability and status as a flagship in Brazil encouraged long-term researches focusing on the species conservation, such as those developed by the 'Instituto de Conservação de Animais Silvestres' and the 'Instituto Tamanduá'. The expressive increase on specialists' efforts and scientific publications since the 2000's resulted in the approval of the National Action Plan for the Conservation of the species on July 2019 (MMA/ICMBio, 2019). It specifies eight objectives and places the expansion on health knowledge as strategic point to minimize the main impacts on the species. Indeed, giant anteater health and parasitology are not yet well explored by science (Diniz & Brito, 2013; Amaral *et al.*, 2020), particularly the free-living populations who live under great anthropic impact, such as those in the Brazilian Cerrado (savanna). The Cerrado biome in São Paulo state is highly degraded and fragmented, occupies 1% of the total area, and of this, only 18% is protected by few legal reserves (Durigan *et al.*, 2011). The state presents, in general, very low values of habitat suitability to the giant anteater, mainly because of land-use changes and the expansion of biofuel crops (Bertassoni *et al.*, 2019).

It is extremely urgent to explore the combination of ecologic and health data in wildlife in order to build a better understanding of ecosystem health as a whole and to guarantee the survival of threatened species (Cunningham *et al.*, 2017). Therefore, we aimed to assess the health status of a giant anteater population in a São Paulo state protected area of Cerrado. To this, we evaluated the physical condition, hematology, serum biochemistry, infestation of ticks, some hemoparasites and gastrointestinal parasites.

4.2. Material and methods

4.2.1. Study area

Santa Bárbara Ecological Station (SBES, -22°48'59", -49°14'12") is a 2.712 hectare Cerrado remaining in São Paulo state, Southeastern Brazil, of high relevance for the maintenance of natural portions of field and savanna (Melo & Durigan, 2011). There are at least 59 mammal species, corresponding to approximately 30% of the total known vertebrate species for the biome (Marinho-Filho *et al.*, 2002). Seven mammal species in SBES are under some degree of threat, including the giant anteater, whose population was estimated in 16 individuals (Bertassoni, 2017). This site is under anthropic impact because of exotic fauna (e.g. *Sus scrofa*, *Lepus europaeus*, *Canis familiaris* and *Felis catus*) and flora (e.g. *Pinus* spp., *Eucalyptus* spp. and *Brachiaria* spp.), illegal hunting, use of agrochemicals, fragmentation by roads and for bordering the urban area and livestock farms (Bertassoni *et al.*, 2017).

Data collection occurred between October 2014 and June 2015. We searched for giant anteaters on SBES roads, firebreaks and on the surrounding roads with the aid of vehicle in low speed. We captured the giant anteaters by perimeter enclosure, darting and physical restraint with netting. The drug association was ketamine (12.2 ± 4.2 mg/kg), midazolam (0.3 ± 0.1 mg/kg) and xylazine (0.9 ± 0.4 mg/kg) - except the TB01 that did not receive xylazine -, in addition to atropine (0.04 mg/kg) and yohimbine (0.3 mg/kg). The procedures carried out during immobilization included the placement of a very high frequency-global positioning system (GPS) telemetry waistcoat on adults, subcutaneous insertion of a microchip, morphometric measurements, sex and age class determination, physical examination and collection of blood, feces, hair and ectoparasites. We recaptured the individuals TB02 and TB03 to replace the damaged waistcoat with the GPS equipment.

4.2.2. Data collection and analyses

4.2.2.1 *Physical condition*

Physical condition included visual inspection before capture (locomotion, physical appearance and behavior) and physical examination. Physical examination included body, skin and fur condition, assessment of anatomic cavities and reproductive structures, palpation, thoracic and abdominal auscultation, evaluation of musculoskeletal integrity and condition of nails and footpads. We calculated two body scores to help the understanding of body proportionality: the ratio between body length (from snout to tip of tail) and scapular height (B/H), and the ratio between weight and scapular height (W/H). We categorized individuals into two age classes (juvenile and adult) based on morphometry. Physiological parameters (heart rate, respiratory rate and body temperature) were registered every 15 min during the anesthesia. All procedures performed had the approval of The Ethical Animal Use Committee of São Paulo State University (CEUA- Unesp FCAV n° 003414/13) and of regional (COTEC n° 429/2014 D23/2013 PGH) and Brazilian (ICMBio n° 38326-5/2015) environmental agencies.

4.2.2.2. *Hematology and serum biochemistry*

We collected up to 10 ml of blood through venipuncture of cephalic or saphenous veins with disposable syringe and 25 x 8 gauge needles. Then, we transferred to standard collection tubes, refrigerated (2 - 6° C) and sent the samples to a commercial veterinary laboratory (Labor Care, Bauru/ SP, Brazil) within 24 hours. Veterinary technicians analyzed them with automated equipment for hematological (ABCTM Vet, scil® animal care company) and biochemical (Cobas c111, Roche®) tests. It was not possible to collect blood samples for hematology and serum biochemistry from two individuals (TB03 and TB07).

The hematological parameters investigated were complete blood count, leucocyte formula and hematimetric indices. Biochemical profiling involved measurement of: protein, albumin, globulin, urea, creatinine, sodium, potassium, calcium, phosphorus, alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (AF), gamma-glutamyl transferase (GGT), creatine kinase (CK), lactate dehydrogenase (LDH), bilirubin (total, indirect and direct), total cholesterol, triglycerides, amylase, lipase, uric acid, and glycohaemoglobin. We presented all blood parameters by mean and standard deviation, minimum and maximum values in parentheses when appropriate, and qualitatively compared

them to other similar studies about the species (Satake, 2002; ISIS, 2013; Sanches *et al.*, 2013; Di Nucci *et al.*, 2014; Rojano-Bolaño *et al.*, 2014; Buitrago, 2015; Oliveira *et al.*, 2017, 2018).

4.2.2.3. Ticks assessment

In the field, we performed tick infestation intensity by counting specimens from all development stages found on the host body during three minutes. We then collected some ticks directly from the skin and stored them in 70% ethanol. We took the specimens to the Laboratory of Biology and Parasitology of Wild Mammal Reservoirs of Oswaldo Cruz Institute (LABPMR - IOC/Fiocruz), Rio de Janeiro RJ, Brazil. We carried out the identification using a stereoscopic microscope with the aid of taxonomic keys (Aragão & Fonseca, 1961; Barros-Battesti *et al.*, 2006; Martins *et al.*, 2010; Teixeira & Miranda, 2012). We also performed tick species frequency (the number of giant anteaters with at least one tick divided by the total number of giant anteaters) and tick species richness (total of tick species identified).

4.2.2.4. Hemoparasites assessment

We carried out the molecular analyses at the Laboratory of Imonoparasitology from Unesp/Jaboticabal. We performed the DNA extraction of blood samples using the QIAamp DNA Blood Mini Kit (Qiagen, Valencia, CA, USA), according to the manufacturer's recommendations. We measured the DNA sample concentration and ratios (260/280 and 260/230) in a spectrophotometer (Nanodrop, Thermo Scientific®) and stored them at -20 ° C until we performed the PCR.

We eliminated false negative results due to the presence of inhibitors by submitting the DNA samples to a conventional PCR (cPCR). To amplify the endogenous *gapdh* gene, we used the primers GAPDH-F (5'-CCTTCATTGACCTC AACTACAT-3') and GAPDH-R (5'-CCAAAGTTGTCATGGATGACC -3') (Birkenheuer *et al.*, 2003). The final total volume of amplification reaction was 25 µL, containing a mixture of: 5 µL of the DNA-sample, 0.2 mM of each desoxynucleotide, 0.4 µM of each initiator oligonucleotide, 3.0 mM of Magnesium Chloride, 1.25 U of Taq Platinum DNA Polymerase (Life Technologies®, Carlsbad, California, United States), PCR buffer and sterile ultrapure water qsp. The thermal sequence and amplification time were initial denaturation at 95 ° C for 5 minutes, 35 cycles composed by denaturation at 95 ° C for 15 seconds, annealing at 50 ° C for 30 seconds, extension at 72 ° C for 30 seconds and final extension at 72 ° C for 5 minutes.

We used nPCR and cPCR assays based on the 16S rRNA gene to detect the agents Anaplasmataceae (*Anaplasma* spp. and *Ehrlichia* spp.) and *Mycoplasma* spp., respectively, in addition to cPCR assays based on the 18S rRNA gene to *Hepatozoon* spp. (Table 1). As positive controls, we used DNA from *E. canis* (Jaboticabal strain), *E. chaffeensis* and *A. phagocytophilum* (kindly provided by Prof. Dr. John Stephen Dumler, Uniformed Services University of the Health Sciences, Bethesda, Maryland, United States), ‘Candidatus’ *M. haemominutum* (André *et al.*, 2014) and *H. caimani* (Bouer *et al.*, 2017). We used sterile ultrapure water (Nuclease-Free Water, Promega Corporation) as negative control.

We performed all cPCR assays using 5 µL of the DNA samples in a mixture containing: 1.25 U of Taq DNA Platinum Polymerase (Invitrogen, Carlsbad, California, USA), PCR buffer (PCR buffer 10 X - 100 mM Tris-HCl, pH 9.0, 500 mM KCl), 0.2 mM deoxynucleotides (dATP, dTTP, dCTP, and dGTP) (Invitrogen), 1.5 mM Magnesium Chloride (Invitrogen), 0.5 µM of each initiator oligonucleotide (Invitrogen) and sterile ultrapure water (Invitrogen) qsp 25 µL. We used 1 µL of the amplified product from the first PCR reaction in the nPCR assays.

We subjected the products amplified in the PCR assays to horizontal electrophoresis in 1.0% agarose gel stained with Ethidium Bromide (0.5 µL / mL) in TEB running buffer pH 8.0 (44.58 M Tris- base; 0.44 M boric acid; 12.49 mM EDTA). We performed it at 90 V/150mA for 60 minutes and used a 100 base pair molecular weight marker (Life Technologies®) for the determination of the amplified products. We visualized and analyzed the results using an ultraviolet light transilluminator (ChemiDoc MP Imaging System, BIO RAD®).

4.2.2.5. Coproparasitological assessment

We collected fresh feces from eight individuals after physical restraint that had no direct contact with the soil and stored them in formaldehyde 10%. We performed centrifugal-fluctuation in hypersaturated sugar solution and sedimentation in ether-formalin on the LABPMR - IOC/Fiocruz. We analyzed the slides of each sample under an Axion Scope A1 Light Microscope (LM) (Zeiss, Göttingen, Germany) and performed the OPG and OoPG tests based on counting eggs or oocysts present in a gram of animal feces. We classified the gastrointestinal parasites at maximum gender level, based on descriptions and findings from literature (Anderson *et al.*, 1974; Khalil *et al.*, 1994; Freitas *et al.*, 2006; Taylor *et al.*, 2017). We classified the parasites as “morphotypes” (M1 and M2) when it was not possible to identify them at any taxonomic level.

We performed egg/oocysts morphotype richness (total of egg/cysts identified) and frequency of infested hosts (the number of giant anteaters with at least one egg/cysts morphotype divided by the total number of giant anteaters). We also calculated the prevalence of parasites (the number of hosts parasitized by a specific parasite divided by the total number of giant anteaters) and infestation intensity (individual load: total number of eggs/oocysts per gram of feces in each individual; and parasite load: total number of eggs/oocysts per gram of feces of each parasite).

We elaborated a scale about the infestation intensity in males and females to verify whether the parasitism has a sexual tendency. For this purpose, we subdivided the abundance values of ticks and eggs/oocysts into three categories (low, medium and high), considering the sampled population data, and used pictograms to represent the individual position on the scale.

Table 1. Oligonucleotide sequences and thermal conditions used in conventional PCR assays.

Agent	Initiators oligonucleotide sequences	Size (bp)	Thermal sequences	References
<i>Ehrlichia</i> spp. - ECC - ECB	5'- GAACGAACGCTGGCGGCAAGC-3' 5'- CGTATTACCGCGGCTGCTGGCA -3'	478	94°C por 5 min 40 ciclos: 94°C por 1 min, 60°C por 1 min e 72°C por 1 min 72°C por 5 min	Murphy <i>et al.</i> , 1998
Nested <i>E. canis</i> - ECAN-5 - HE3	5'-CAATTATTATAGCCTCTGGCTATAGGA -3' 5'-TATAGGTACCGTCATTATCTTCCTAT-3'	358	94°C por 5 min 40 ciclos: 94°C por 1 min, 55°C por 1 min e 72°C por 1 min 72°C por 5 min	Murphy <i>et al.</i> , 1998
Nested <i>E. chaffeensis</i> - CHAF - GAIUR	5'-CAATTGCTTATAACCTTTTGGTTATAAAT-3' 5'-GACTTGCCGGGACTTC TCT - 3'	410	94°C por 5 min 40 ciclos: 94°C por 1 min, 55°C por 1 min e 72°C por 1 min 72°C por 5 min	Persing, 1996
<i>Anaplasma</i> spp. - gE3a - gE10R - gE2 - gE9f	5'- CACATGCAAGTCGAACGGATTATTC-3' 5'- TTCCGTTAAGAAGGATCTAATCTCC'-3' 5'- GGCAGTATTAAGCAGCTCCAGG-3' 5'-AACGGATTATTCTTTATAGCTTGCT-3'	932 546	94°C por 5 min 40 ciclos: 94°C por 30s, 55°C por 30s e 72°C por 1 min 72°C por 5 min	Massung <i>et al.</i> , 1997
<i>Mycoplasma</i> spp. HemMycop16S-41s HemMyco16S-938as	5'- GYATGCMATAAYACATGCAAGTCGARCG-3'' 5'- CTCCACCACTTGTTTCAGGTCCCGTC-3'	800	94°C por 2 min 55 ciclos: 94°C por 15s, 68°C por 15s and 72°C por 18s 72°C por 1 min	Maggi <i>et al.</i> , 2013
<i>Hepatozoon</i> spp. Hemo1 Hemo2	5'TATTGGTTTTAAGAACTAAT TTTATGATTG 3' 5' CTTCTCCTTCCTT AAGTGATAAGGTTACAC 3'	800	94°C por 3 min 35 ciclos: 94°C por 45s, 48°C por 1min e 72°C por 1min 72°C por 7 min	Perkins & Keller, 2001
<i>Hepatozoon</i> spp. HepF300 Hep900	5'GTTTCTGACCTATCAGCTTT CGACG 3' 5'CAAATCTAAGAATT TCACCTCTGAC 3'	600	94°C por 3 min 35 ciclos: 94°C por 30s, 60°C por 30s e 72°C por 1min 72°C por 7 min	Ujvari <i>et al.</i> , 2004

4.3. Results

4.3.1. Physical condition

We captured nine giant anteaters (four females and five males), being one juvenile (female, TB09) and eight adults, with an average weight of 34 ± 5.1 kg. They were mostly in good physical condition and normal on visual inspection, that is, without impairment of locomotion, with an aspect of complete physical health and normal foraging, alert and escape behaviors. The exception was for two individuals. The TB03 did not exhibit normal escape behavior on recapture, 45 days after capture, and had lost 1.4 kilograms. While we approached and surrounded, it remained immobile, although in alert position, and did not react with the expected resistance to physical restraint. On the first capture and monitoring, it presented the lowest heart rate (47.6 ± 11.3 bpm) and body temperature ($32.5 \pm 0.2^\circ$ C). The TB06 had senility characteristics: many scars on snout, dry skin, flaking, pigmentation spots on the snout, mild alopecia abdominal and medial on members (Figure 1), abdominal bulging and muscle mass loss (projections of the coxal bone and some vertebrae palpable). It also presented the lowest respiratory rate (4.2 ± 0.8 rpm), pale mucosa membranes, poor muscle relaxation and long anesthesia induction (Table 2). The body score B/H remained between 3.0 and 3.3 in the population, while in TB06 it was 2.7. The W/H remained between 0.51 and 0.58, but in TB06 and TB07 it was 0.48, while in the juvenile TB09, it was 0.38. We observed scars on snout and small abrasions in almost all individuals, but no injury appeared to be life threatening. There were rounded shape cysts, 0.5 cm in diameter, located in the axillary regions of two individuals (TB03 and TB06), soft and with mobility in touch (Figure 1D).

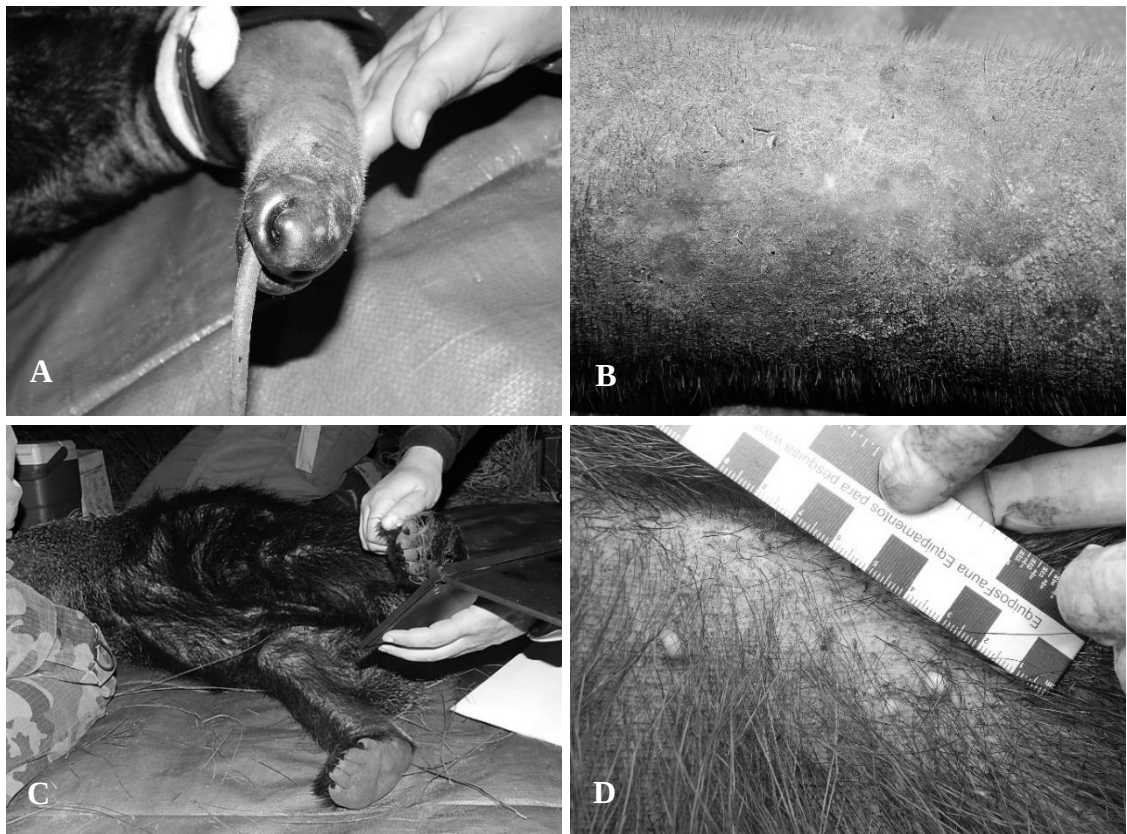


Figure 1. Observations on physical examination of an anesthetized senile giant anteater (*Myrmecophaga tridactyla*) from Santa Bárbara Ecological Station, Cerrado of São Paulo state, Brazil. **A.** Scars on snout. **B.** Dry skin, flaking and pigmentation spots on the snout. **C.** Mild alopecia abdominal and medial on members. **D.** Subcutaneous cysts on axilla (0.5 cm).

Table 2. Evaluation of visual inspection, physical exam, observations on anesthetic monitoring and general condition of nine giant anteaters (*Myrmecophaga tridactyla*) captured on Santa Bárbara Ecological Station, Cerrado of São Paulo state, Southeastern Brazil.

ID	Sex	Age	Weight (kg)	B/H	W/H	Visual inspection*	Observations on physical exam	Observations on anesthetic monitoring	General condition
TB01	F	A	32	3.3	0.51	Normal	Scar on snout, abrasion on hand pads.	Reasonable muscle relaxation.	Good
TB02	M	A	38.7	3.3	0.56	Normal	Abrasion on right hand pad, rectal prolapse.	n.n.	Good
TB03	F	A	34.8/ 33.4**	3.2	0.54	Normal/ Anormal **	Subcutaneous cysts on axillary regions (0.5 cm), cut on tongue, loss of 1.4 kg on recapture.	Lowest heart rate (47.6 ± 11.3 bpm), lowest body temperature ($32.5 \pm 0.2^\circ$ C).	Good/ Average**
TB04	M	A	35.2	3.2	0.57	Normal	Scar on snout, gas in gastrointestinal tract, tunguiasis on hand pads.	n.n.	Good
TB05	M	A	36.6	3.0	0.58	Normal	Scar on snout and right footpad, abrasion in left foot finger.	n.n.	Good
TB06	M	A	38	2.7	0.48	Normal	Scars, flaking and pigmentation spots on the snout, scar on left ear, dry skin, subcutaneous cysts on axillary regions (0.5 cm), abdominal bulging, mild alopecia abdominal and medial on members, pale mucosa membranes, muscle mass loss.	Lowest respiratory rate (4.2 ± 0.8 rpm), poor muscle relaxation, long induction.	Average
TB07	F	A	33	3.0	0.48	Normal	Scar on right ear, tunguiasis on left hand pad.	n.n.	Good
TB08	M	A	36.2	3.3	0.54	Normal	Scar on snout, alopecia and pigmentation on snout tip, tail tip with consolidated fracture, dry skin, tunguiasis on hand pads, abrasion on right foot and left finger.	n.n.	Good
TB09	F	J	21.6	3.2	0.38	Normal	Bulge with bone consistency around the trachea in the thyroid region.	n.n.	Good

ID: individual, F: female, M: male, A: adult, J: juvenile, n.n.: nothing noteworthy.

B/H: ratio between body length (from snout to tip of tail) and scapular height, W/H: ratio between weight and scapular height.

* Visual inspection performed at distance before capture. We considered as "normal": without impairment of locomotion, with an aspect of complete physical health and with normal foraging, alert and escape behaviors; ** Observations on recapture.

4.3.2. Hematology and serum biochemistry

Comparing to other researches from captive and free-living giant anteaters (Table 3), the hematology results for all individuals, except TB01, registered marked erythrocytosis (4.31 ± 1.07 , 2.54 - 6), leucopenia (6963 ± 2406 , 4200 - 11300), low means corpuscular volume (MCV, 100 ± 34.77 , 61 - 173), low corpuscular hemoglobin (MCH, 36.43 ± 14.39 , 20 - 61) and eosinophilia (9.71 ± 2.21 , 7 - 14). We observed thrombocytopenia (102 ± 25.28 , 64 - 132) in almost all individuals (except in TB05). The individual TB01 presented leucocytosis with neutrophilia and the TB06, leucopenia with neutropenia (Table 4). Except for TB01, the serum biochemistry showed high concentration of ALT (91.43 ± 17.06 , 57 - 107) and AST (65.43 ± 23.35 , 39 - 105). All individuals presented high values of LDH (1012.67 ± 638.69 , 609 - 2277), urea (57.3 ± 6.5 , 48.6 - 67), uric acid (2.56 ± 1.06 , 1.07 - 3.67) and triglycerides (30.71 ± 13.11 , 10 - 46). Creatinine (0.54 ± 0.05 , 0.5 - 0.6), AF (9.71 ± 6.45 , 4 - 22) and lipase (19.29 ± 8.06 , 11 - 36), were in low concentration in all giant anteaters. Low Amylase concentration (481.96 ± 250.07 , 105.8 - 809.9) only did not occur in TB01 and TB05 (Table 5). The TB01 was the only individual with almost none alterations in biochemical analyses. The TB06 and TB09 were the ones with the greatest alterations, both in hematology and serum biochemistry. This was the first time that glycohaemoglobin has been quantified in two giant anteaters (Table 6). The serum of five samples showed hemolysis into a lesser or medium degree.

Table 3. Comparison of hematological data (means and standard deviation) between the present study and other researches from literature about captive and free-living giant anteaters (*Myrmecophaga tridactyla*).

PARAMETER	Present study	Oliveira <i>et al.</i> , 2017	Buitrago, 2015	Rojano-Bolaño <i>et al.</i> , 2014	Di Nucci <i>et al.</i> , 2014	ISIS, 2013	Sanches <i>et al.</i> , 2013	Satake, 2002	Satake, 2002
	FL, n=7, Bra	C, n=12, Bra	C, n=10 to 37, Col	FL, n=11, Col	C, n=30, Arg	C, FL, n=?, Wor	C, n= 13, Bra	FL, n=30, Bra	C, n=30, Bra
RBC ($\times 10^6/\mu\text{L}$)	4.31 $\pm$ 1.07	2.07 $\pm$ 0.4	2.39 $\pm$ 0.75	1.97 $\pm$ 0.3	2.38 $\pm$ 0.38	2.36	2.36 $\pm$ 0.14	2.19 $\pm$ 0.07	2.46 $\pm$ 0.13
Hematocrit (%)	40.43 $\pm$ 3.15	38.08 $\pm$ 5.93	30.47 $\pm$ 12.81	26 $\pm$ 5.26	34.9 $\pm$ 5.45	35.6	37.7 $\pm$ 1.06	33.93 $\pm$ 0.75	38.17 $\pm$ 1.12
Haemoglobin (g/dL)	14.76 $\pm$ 3	11.33 $\pm$ 2.15	12.7 $\pm$ 5.71	11.86 $\pm$ 1.56	13.8 $\pm$ 1.69	13.1	11.8 $\pm$ 0.52	10.91 $\pm$ 0.47	12.46 $\pm$ 0.43
MCV (fL)	100 $\pm$ 34.77	186.52 $\pm$ 21.72	133.92 $\pm$ 22.2	120.55 $\pm$ 16.67	147.9 $\pm$ 7.22	150.3	165.12 $\pm$ 8.71	153.86 $\pm$ 20.32	165.63 $\pm$ 29.36
MCHC (g/dL)	36.14 $\pm$ 6.2	29.68 $\pm$ 2.56	37.53 $\pm$ 8.67	48.6 $\pm$ 6.92	38.4 $\pm$ 1.25	36.1	31.26 $\pm$ 0.96	32.79 $\pm$ 2.45	32.83 $\pm$ 2.14
MCH (Pcg)	36.43 $\pm$ 14.39	55.08 $\pm$ 5.94	50.84 $\pm$ 10.44	60.36 $\pm$ 5.98	56.6 $\pm$ 3.2	54.5	51.07 $\pm$ 2.27	51.48 $\pm$ 5.96	53.1 $\pm$ 6.39
WBC (/μL)	6963 $\pm$ 2406	8142 $\pm$ 2441	9420 $\pm$ 4530	10620 $\pm$ 5230	8550 $\pm$ 2690	8420	11870 $\pm$ 2880	9730 $\pm$ 760	8690 $\pm$ 570
Bastonet (%)	0	0	0	0	0	-	0	0.45	0.04
Neutrophils (%)	71.71 $\pm$ 7.8	71.50 $\pm$ 10.34	63.1 $\pm$ 23.79	64.8 $\pm$ 16.05	59.6 $\pm$ 9.9	65.87	72.62 $\pm$ 3.67	82.59	68.62
Lymphocytes (%)	16.71 $\pm$ 5.61	18.50 $\pm$ 8.25	29.7 $\pm$ 21.45	23.1 $\pm$ 7.63	35 $\pm$ 9.12	20.76	18.77 $\pm$ 3.17	9	17.12
Eosinophils (%)	9.71 $\pm$ 2.21	6.67 $\pm$ 4.7	5.7 $\pm$ 6.25	9.2 $\pm$ 7.78	2.5 $\pm$ 2.3	8.09	6.92 $\pm$ 1.67	3.32	9.36
Basophils (%)	0	0	1.4 $\pm$ 3.27	0	0	1.35	0	0.16	0.16
Monocytes (%)	1.86 $\pm$ 2.12	3.33 $\pm$ 2.57	1.24 $\pm$ 2.31	1.98 $\pm$ 2.95	3.2 $\pm$ 1.74	3.45	1.69 $\pm$ 0.04	4.46	4.68
Thrombocytes (/μL)	102 $\pm$ 25.28	123.46 $\pm$ 31.36	140.88 $\pm$ 58.74	129.08 $\pm$ 51.57	93.33 $\pm$ 41.13	124	-	117.25 $\pm$ 7.16	168.32 $\pm$ 8.77
TPP (g/dL)	7.03 $\pm$ 0.29	6.23 $\pm$ 0.49	-	-	-	-	8.1 $\pm$ 0.15	-	-

FL: free-living giant anteaters, C: captive giant anteaters, RBC: Red Blood Cell count, MCV: Mean Corpuscular Volume, MCHC: Mean Corpuscular Hemoglobin Concentration, MCH: Mean Corpuscular Haemoglobin, WBC: White Blood Cell count, TPP: Total Plasma Protein, n: sample size, Bra: Brazil, Col: Colombia, Wor: World, Arg: Argentina.

Table 4. Hematological data from seven free-living giant anteaters (*Myrmecophaga tridactyla*) from the Santa Bárbara Ecological Station, Cerrado of São Paulo state, Southeastern Brazil.

PARAMETER	TB01	TB02	TB04	TB05	TB06	TB08	TB09	Min.	Max.	Mean	SD
RBC ($\times 10^6/\mu\text{L}$)	2.54	4.65	4.5	4.5	3.5	4.5	6	2.54	6	4.3	1.07
Hematocrit (%)	44	40	42	44	36	40	37	36	44	40.4	3.15
Haemoglobin (g/dL)	15.6	11.6	15.2	20.4	15.5	13	12	11.6	20.4	14.8	3
MCV (fL)	173	86	93	97	102	88	61	61	173	100	34.77
MCHC (g/dL)	35	29	36	46	43	32	32	29	46	36.1	6.2
MCH (Pcg)	61	24	33	45	44	28	20	20	61	36.4	14.39
WBC (/μL)	11300	5400	8000	7800	4200	6300	5040	4200	11300	6862.9	2405.89
Bastonet (%)	0	0	0	0	0	0	0	0	0	0	0
Neutrophils (%)	81	75	68	64	60	76	78	60	81	71.7	7.8
Lymphocytes (%)	11	13	22	25	20	15	11	11	25	16.7	5.62
Eosinophils (%)	7	9	9	9	14	9	11	7	14	9.7	2.21
Basophils (%)	0	0	0	0	0	0	0	0	0	0	0
Monocytes (%)	1	3	1	2	6	0	0	0	6	1.9	2.12
Thrombocytes (/μL)	113	64	107	132	118	70	110	64	132	102	25.28
TPP (g/dL)	7.6	6.8	7.2	6.8	7	7	6.8	6.8	7.6	7	0.29

RBC: Red Blood Cell count, **MCV:** Mean Corpuscular Volume, **MCHC:** Mean Corpuscular Hemoglobin Concentration, **MCH:** Mean Corpuscular Haemoglobin, **WBC:** White Blood Cell count, **TPP:** Total Plasma Protein.

Table 5. Comparison of biochemical data (means and standard deviation) between the present study and other researches from literature about captive and free-living giant anteaters (*Myrmecophaga tridactyla*).

PARAMETER	Present study FL, n=7, Bra	Oliveira <i>et al.</i> , 2018 C, n=12, Bra	Buitrago, 2015 C, n=4 to 15, Col	Di Nucci <i>et al.</i> , 2014 C, n=30, Arg	ISIS, 2013 C, FL, n=?, Wor	Satake, 2002 FL, n=30, Bra	Satake, 2002 C, n=30, Bra
Protein (g/dL)	5.87 ± 0.91	6.05 ± 0.56	6.6 ± 1.2	7.66 ± 1.08	6.2	6.87 ± 0.14	6.98 ± 0.12
Albumin (g/dL)	2.43 ± 0.1	3.29 ± 0.33	2.4 ± 30.3	3.62 ± 0.51	2.5	1.53 ± 0.03	1.48 ± 0.03
Globulin (g/dL)	3.44 ± 0.86	2.76 ± 0.36	4.4 ± 0.9	-	-	-	-
Relation A/G	0.76 ± 0.25	1.19	0.54	0.95 ± 0.21	-	-	-
Urea (mg/dL)	57.3 ± 6.5	53.46 ± 18.28	18.2 ± 16.5	40.3 ± 15.95	16.53	41.24 ± 1.87	36.17 ± 1.87
Creatinine (mg/dL)	0.54 ± 0.05	1.05 ± 0.37	0.9 ± 0.4	1.17 ± 0.63	1.18	0.74 ± 0.03	1.13 ± 0.05
Sodium (mmol/L)	134 ± 6.83	-	-	129.1 ± 10.53	139	-	-
Potassium (mmol/L)	7.14 ± 2.09	-	-	10.6 ± 5.46	4.9	-	-
Calcium (mg/dL)	7.74 ± 0.98	-	-	9.84 ± 1.26	9.32	9.86 ± 0.19	9.06 ± 0.14
Phosphorus (mg/dL)	4.34 ± 1.12	-	-	4 ± 1.48	5.79	4.49 ± 0.19	5.16 ± 0.21
ALT (IU/L)	91.43 ± 17.06	15.49 ± 7.98	76.4 ± 60.8	42 ± 15.19	46	72.23 ± 3.32	45.62 ± 3.5
AST (IU/L)	65.43 ± 23.35	21.12 ± 7.5	54.8 ± 41.3	35.8 ± 10.79	31	43.7 ± 3.48	30.58 ± 3.48
AF (IU/L)	9.71 ± 6.45	-	108.6 ± 84.5	22.8 ± 13.27	20	30.95 ± 4.81	26.7 ± 3.16
GGT (IU/L)	24.29 ± 9.59	65.18 ± 54.57	27.4 ± 16.3	24.3 ± 21.56	22	23.41 ± 3.34	74.51 ± 14.31
Bilirubin total (mg/dL)	0.08 ± 0.04	-	-	0.28 ± 0.11	0.13	0.13 ± 0.02	0.11 ± 0.01
Bilirubin indirect (mg/dL)	0.07 ± 0.03	-	-	0.27 ± 0.11	0.13	0.1 ± 0.09	0.08 ± 0.04
Bilirubin direct (mg/dL)	0.02 ± 0.04	-	-	0.01	0.06	0.03 ± 0.01	0.03
Total cholesterol (mg/dL)	71.14 ± 27	62.79 ± 20.08	74.1 ± 11.6	93.1 ± 19.7	88.03	91.04 ± 4.4	83.94 ± 4.58
Triglycerides (mg/dL)	30.71 ± 13.11	10.71 ± 5.29	10.3 ± 0.6	16.3 ± 10.12	21.24	-	-
Amylase (IU/L)	481.96 ± 250.07	1037.92 ± 149.04	-	1018 ± 309.6	635	681.26 ± 31.34	717.24 ± 36.35
Lipase (IU/L)	19.29 ± 8.06	28.80 ± 5.11	-	35 ± 19.5	20	-	-
CK (IU/L)	146.82 ± 51.2	111.61 ± 70.16	-	171.7 ± 67.26	131	242.97 ± 46.40	122.28 ± 11.34
Uric acid (mg/dL)	2.56 ± 1.06	-	-	0.91 ± 0.65	-	0.49 ± 0.04	0.4 ± 0.04
Glycohaemoglobin (%)	4.26 ± 0.82	-	-	-	-	-	-
LDH (U/L)	1012.67 ± 638.69	-	-	158.7 ± 73	169	194.27 ± 20.76	200.53 ± 25.24

FL: free-living giant anteater, C: captive giant anteater, ALT: Alanine Transaminase, AST: Aspartate Transaminase, AF: Alkaline Phosphatase, GGT: Gamma-Glutamyl Transferase, CK: Creatine Kinase, LDH: Lactate Dehydrogenase, n: sample size, Bra: Brazil, Col: Colombia, Wor: World, Arg: Argentina.

Table 6. Serum biochemistry data from seven free-living giant anteaters (*Myrmecophaga tridactyla*) from the Santa Bárbara Ecological Station, Cerrado of São Paulo state, Southeastern Brazil.

PARAMETER	TB01	TB02	TB04	TB05	TB06	TB08	TB09	Min.	Max.	Mean	SD
Protein (g/dL)	6.9	5.9	6.1	6	4.2	5.3	6.7	4.2	6.9	5.87	0.91
Albumin (g/dL)	2.6	2.5	2.4	2.4	2.4	2.3	2.4	2.3	2.6	2.43	0.1
Globulin (g/dL)	4.3	3.4	3.7	3.6	1.8	3	4.3	1.8	4.3	3.44	0.86
Relation A/G	0.6	0.7	0.6	0.7	1.3	0.8	0.6	0.6	1.3	0.76	0.25
Urea (mg/dL)	48.6	60	59.5	61.1	50.2	54.7	67	48.6	67	57.3	6.5
Creatinine (mg/dL)	0.6	0.6	0.6	0.5	0.5	0.5	0.5	0.5	0.6	0.54	0.05
Sodium (mmol/L)	145	137	134	130	137	132	123	123	145	134	6.83
Potassium (mmol/L)	4.7	7.2	5.8	6	7.9	7.2	11.2	4.7	11.2	7.14	2.09
Calcium (mg/dL)	7.98	5.63	8.27	8.47	7.52	8.19	8.1	5.63	8.47	7.74	0.98
Phosphorus (mg/dL)	6.1	4.7	3.7	4.3	4.1	2.5	5	2.5	6.1	4.34	1.12
ALT (IU/L)	57	91	93	107	100	86	106	57	107	91.43	17.06
AST (IU/L)	39	56	105	69	83	41	65	39	105	65.43	23.35
AF (IU/L)	14	5	4	5	22	8	10	4	22	9.71	6.45
GGT (IU/L)	32	20	25	37	22	27	7	7	37	24.29	9.59
Bilirubin total (mg/dL)	0.07	0.08	0.04	0.05	0.13	0.07	0.13	0.04	0.13	0.08	0.04
Bilirubin indirect (mg/dL)	0.06	0.08	0.04	0.05	0.03	0.07	0.13	0.03	0.13	0.07	0.03
Bilirubin direct (mg/dL)	0.01	0	0	0	0.1	0	0	0	0.1	0.02	0.04
Total cholesterol (mg/dL)	114.5	64.1	43.9	53.5	95.5	44.9	81.6	43.9	114.5	71.14	27
Triglycerides (mg/dL)	42	29	10	37	46	17	34	10	46	30.71	13.11
Amylase (IU/L)	778.5	531.2	809.9	105.8	440	380.3	328	105.8	809.9	481.96	250.07
Lipase (IU/L)	20	20	16	18	14	11	36	11	36	19.29	8.06
CK (IU/L)	66.8	-	156.5	160.9	170.8	111.4	214.5	66.8	214.5	146.82	51.2
Uric acid (mg/dL)	1.07	-	1.58	2.53	2.92	3.67	3.6	1.07	3.67	2.56	1.06
Glycohaemoglobin (%)	3.68	-	-	-	4.84	-	-	3.68	4.84	4.26	0.82
LDH (U/L)	609	-	664	965	628	933	2277	609	2277	1012.67	638.69

ALT: Alanine Transaminase, **AST:** Aspartate transaminase, **AF:** Alkaline Phosphatase, **GGT:** Gamma-Glutamyl Transferase, **CK:** Creatine Kinase, **LDH:** Lactate Dehydrogenase.

4.3.3. Ticks

Tick infestation intensity was between 10 (TB07) and 108 (TB08) ticks per host in three minutes counting (Table 7). The frequency of tick infestation was 100% (all individuals were parasitized) and ticks richness was four. All species were of the *Amblyomma* genus: *A. nodosum*, *A. sculptum*, *A. calcaratum* and *A. parvum*, listed from the most to the least prevalent (Table 7). We found the *A. nodosum* in almost all individuals (89%, [8/9]) and it was present in co-infections with all other tick species. The combination between two different species of ticks in the same host (55.6%, [5/9]) was slightly more common than the presence of a single species of tick per host (44.4%, [4/9]). We found *A. parvum* in only one individual (TB09).

Table 7. Diversity and infestation of ticks on nine free-living giant anteaters (*Myrmecophaga tridactyla*) from the Santa Bárbara Ecological Station, Cerrado of São Paulo state, Southeastern Brazil.

ID	<i>Amblyomma</i> species				Ticks evaluated (N)			Infestation (N/3 min)
	<i>A. sculptum</i>	<i>A. nodosum</i>	<i>A. calcaratum</i>	<i>A. parvum</i>	Total	Male	Female	
TB01	x				7	0	7	78
TB02	x	x			9	5	4	22
TB03		x			6	4	2	21
TB04	x	x			6	1	5	61
TB05		x			5	4	2	30
TB06		x	x		5	3	2	88
TB07		x			4	3	1	10
TB08		x	x		7	6	1	108
TB09		x		x	6	2	2	30

ID: individual, N: number of ticks identified, N/3 min: number of ticks counted in three minutes.

4.3.4. Hemoparasites

All samples were positive for the endogenous gapdh gene. The average DNA concentration was 44.6 ng/μL. Only one giant anteater (TB02) was positive for *Mycoplasma* spp., however, as the band presented in the agarose gel electrophoresis was weak, it was not possible to sequence the product. All giant anteaters were negative for the other agents tested.

4.3.5. Coproparasitology

We obtained a gastrointestinal parasite richness of eight morphotypes, which were, from largest to smallest size: M1, M2, Ascaridoidea, Trychostrongylidae, *Trichuris* spp., *Aspidodera* spp., *Physaloptera* spp. (Figure 2) and Apicomplexa (Figure 3). The frequency of parasitism was 100% and each giant anteater was parasitized by at least four parasite morphotypes. The least prevalent parasite was *Trichuris* spp. (11.1%, [1/9]), followed by M1 (33.4%, [3/9]). The most prevalent and that occurred in all individuals were Apicomplexa, Trychostrongylidae and *Aspidodera* spp., being the maximum parasite infestation intensities of 7245.3, 1842 and 1172 eggs or oocysts/gram of feces, respectively. The highest infestation intensities occurred in the individuals TB04 (10486.4 eggs or oocysts/gram of feces) and TB06 (7803.9 eggs or oocysts/gram of feces). *Physaloptera* spp. was also present in the entire population, but presented low egg count per gram of feces (Table 8).

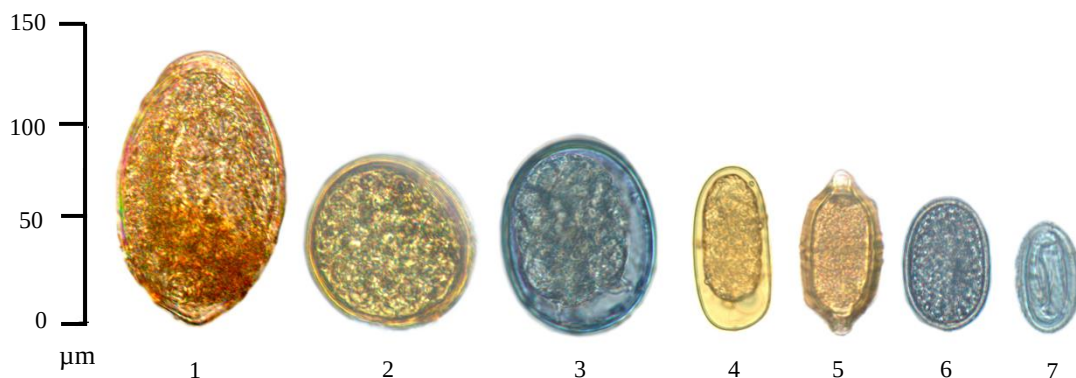


Figure 2. Helminth eggs from free-living giant anteaters (*Myrmecophaga tridactyla*) from Santa Bárbara Ecological Station, Cerrado of São Paulo state, Southeastern Brazil.

1. M1, 2. M2, 3. Ascaridoidea, 4. Trychostrongylidae, 5. *Trichuris* spp., 6. *Aspidodera* spp., 7. *Physaloptera* spp.



Figure 3. Apicomplexa from free-living giant anteaters (*Myrmecophaga tridactyla*) from Santa Bárbara Ecological Station, Cerrado of São Paulo state, Southeastern Brazil.

Table 8. Parasitic load obtained by OPG and OoPG from free-living giant anteaters (*Myrmecophaga tridactyla*) from the Santa Bárbara Ecological Station, Cerrado of São Paulo state, Southeastern Brazil.

Parasites	TB01	TB02	TB03	TB04	TB06	TB07	TB08	TB09	PARAS. LOAD	MEAN	SD
M1	31.6	5.5	1.4	0	0	0	0	0	38.5	4.8	11
M2	63.3	18.3	27.2	129.2	146	26.7	105.9	0	516.6	64.6	55.6
Ascaridoidea	0	0.9	40.8	10.6	0	0	28.9	0	81.2	10.2	16
Trychostrongylidae	209	9.1	267	1842	569	122	597.5	218	3833.6	479.2	587.3
Trichuris spp.	0	0	0.7	0	0	0	0	0	0.7	0.1	0.2
Aspidodera spp.	162	22	194	1172	358	127	135.9	28.3	2199.2	274.9	377.4
Physaloptera spp.	31.6	3.3	13.6	87.3	48.9	20.8	19.3	1.3	226.1	28.3	28.3
Aplicompelxa	876	280.4	1926	7245.3	6682	2309	1633.7	4076	25028.4	3128.6	2618.9
IND. LOAD	1373.5	339.5	2470.7	10486.4	7803.9	2605.5	2521.2	4323.6			
MEAN	171.7	42.4	308.8	1310.8	975.5	3275	315.2	540.5			
SD	294.6	96.5	660.9	2494.0	2314.9	803.1	568.5	1430.6			

OPG: number of eggs per gram of feces, **OoPG:** number of oocytes per gram of feces, **IND. LOAD:** individual parasitic load, **PARAS. LOAD:** parasite load, **SD:** standard deviation.

Male giant anteaters had infestation intensity predominantly high (50%, [4/8]), while in females it was low (75%, [6/8]) (Figure 4). High intensity infestations occurred mainly involving ticks (60%, [3/5]). High intensity infestations caused by gastrointestinal parasites occurred only in males. The TB06 male individual was the only one with infestation by both categories of parasites at a high level, while TB02 (male), TB03 and TB07 were always in the low degree. In general, infestations with intensities considered low were the most frequent in the studied population (56.25%, [9/16]) (Figure 4).

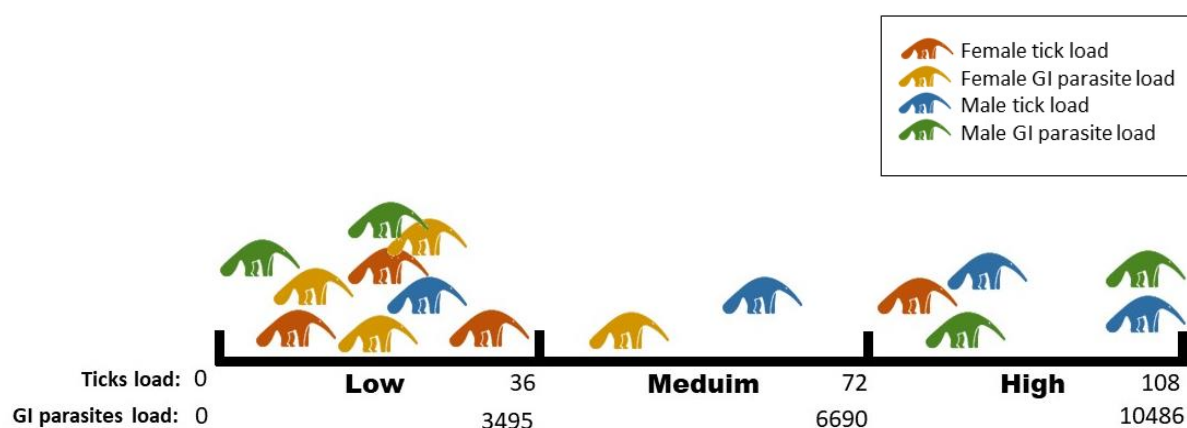


Figure 4. Scale of parasitism intensity based on tick and gastrointestinal load of eight giant anteaters (*Myrmecophaga tridactyla*) (four male and four female) from the Santa Bárbara Ecological Station, Cerrado of São Paulo state, Southeastern Brazil.

Three of the nine giant anteaters died during the study period. Hunters probably killed the female TB01 (Bertassoni *et al.*, 2017), the female TB03 died during the anesthesia induction when recaptured and we found the senile male TB06 dead a day after its capture. We investigated possible death causes of the last two considering the findings on the present study, but also the histopathological and toxicological assessments, which will be published in the near future.

4.4. Discussion

4.4.1. Physical condition

Behavior and physical inspection of free-living giant anteaters, prior to capture and without them noticing our presence can help veterinarians to assess health status of individuals with unknown life history. Wild animals under threat can mask disease symptoms and perform a fight or flight if the escape is an impossibility or a higher energy expended decision. That is why physically restrained animals expressing excessive calm, apathy or paralysis, instead of the expected agitation, anxiety, irritability and even aggression (Carramenha & Carregaro, 2012), set up an alert regarding its healthiness. Exhaustion by stress can cause a decrease in action and reaction capacity, energy reserves depletion, loss of the immune system's ability to respond (Oliveira, 2006) and even death (Bondan & Orsini, 2006). Therefore, we believe the stress by captures, the incident of waistcoat damage and its uncomfortable loading, could negatively affect the TB03, considering its pre-existing pathological conditions, reducing its ability of maintaining homeostasis, culminating in its death. The most common causes of death due to physical restraint are acidosis, ventricular fibrillation, cholinergic bradycardia and capture myopathy (Pachaly *et al.*, 1993), which we cannot discard as death cause of both TB03 and TB06 individuals. With that in mind, we recommend a careful inspection prior to capture and strategies that inflict the least stress on animals. Considering apparently healthy giant anteaters living on environments favorable to management, we suggest whenever possible, only the use of chemical restraint to perform more invasive procedures. It reduces the chances of accidents and injuries during the handling, besides being much less stressful.

Chronic lacerations and scars were quite common on the giant anteaters, probably due to the behavior of territory defense, as seen in other studies (Superina, 2012; Arenales *et al.*, 2020). Tongue, snout and mouth are very important on the performance of several behaviors

(Bertassoni & Costa, 2010), but they are delicate on anteaters, easily injured during physical restraint with nets. We reinforce the recommendation, therefore, of searching for them, as well as abrasions on hands and feet, during the physical evaluation (Miranda *et al.*, 2006), so that they can be properly cleaned and, if necessary, treated with topical medications. Tungiasis and rectal prolapse have already been observed in anteaters (Arenales *et al.*, 2020), but it is the first time cysts are reported. They resembled sebaceous cysts or epidermoid cysts; however, we could not confirm since we did not collect the material to anatomic-pathologic diagnosis.

Tunguíasis caused by *Tunga penetrans* in giant anteaters has been identified in Brazil (Frank *et al.*, 2012) and in Argentina (Di Nucci *et al.*, 2017), expanding the host and distribution range of this zoonotic flea species. The importance of these records is because this is one of the pathogenic species for humans, in addition to *T. trimamillata* (Heukelbach, 2005), and it also infests several other species of domestic and wild animals (Linardi & De Avelar, 2014). Although this parasite can evolve into serious complications in humans, its impact on giant anteaters or the importance of this host in the epidemiological cycle is unknown. In spite of the broad spectrum and category of hosts, the pig is considered the most important animal reservoir for *T. penetrans* (Linardi & De Avelar, 2014). Another five species of this genus are found in Brazil (De Avelar *et al.*, 2013; Widmer & Azevedo, 2011; Pampiglione *et al.*, 2002), including *T. bondari*, which parasitizes the *Tamandua tetradactyla* (Pampiglione *et al.*, 2009). In our study, it was not possible to identify the species of this flea in the individuals where it occurred (TB04, TB07 and TB08).

4.4.2. Hematology and serum biochemistry

The TB01, the only individual that did not receive xylazine, showed the least alterations in blood exams. Sedative agents such as alpha2-adrenergic agonists, which include xylazine, can alter hematology and serum biochemistry, affecting homeostasis and causing deleterious effects to the patient (Lang *et al.*, 1979). The reduction in hematocrit (Ambrósio *et al.*, 2012) and the hyperglycemic effect (Gülanber *et al.*, 2001; Chauhan & Pandey, 2006; Ismail *et al.*, 2010), have already been observed in some animal species after administration of xylazine (Custer *et al.*, 1977). However, the literature on these interferences is very scarce and there is no consistency about drugs final effect on hematological parameters (Seal *et al.*, 1987; Kiliç, 2008; Topal *et al.*, 2010; Çamkerten *et al.*, 2013; Piccoli *et al.*, 2013). It is particular unknown to wildlife and when the drugs are combined with others on a protocol. In any case, we cannot

assume any effect of the drugs used on the giant anteaters' blood exams, since the most usual mentioned changes did not occur in our studied population. This is an open research field considering wildlife and its development would contribute a lot in better choices of anesthetic protocols.

It is reasonable to expect, however, that the use of drugs potentially harmful to certain organs in animals with previous lesioned tissues potentiate negative results in blood exams. The TB06 showed the greatest alterations that contributed to understand the circumstances of the death, which occur about 24 hours after immobilization. Aside from its abnormal body score (which is difficult to observe in the species prior to restraint because of the large fur volume, and in this case, enhanced by a nighttime capture where visibility is less), we did not observe, at visual inspection, any sign that could suggest health impairment. Upon physical evaluation and anesthetic monitoring we observed some problems, but they did not indicate, at that point, its serious health condition (as observed by blood tests and histopathology, ongoing investigation). Adding this event to our experience, we believe that wild animals, especially the free-living, can mask signs of illness when they feel threatened. Given the TB09 was the only juvenile, we cannot ignore that some of its alterations may have been caused by physiological influences. In some species, mainly domestics, the hematological and biochemical evaluations must take into account factors such as age and sex in determining the reference interval and clinical interpretation of results (Nabi *et al.*, 2019; Connolly *et al.*, 2020).

Erythrocytosis is an absolute or a relative increase in erythrocyte indices (Thrall *et al.*, 2014). In the relative, there is no real increase in the global red blood cell count, hematocrit and hemoglobin concentration, but hemoconcentration from dehydration or splenic contraction (Soto *et al.*, 2008). Splenic contraction because of stress or pain (Thrall *et al.*, 2014) is likely to occur during free-living giant anteater's capture. Another result that points to stress is thrombocytopenia (in TB02, TB06, TB08 and TB09), which may be a reflection of the platelets transient sequestration by the spleen (Ferreira-Neto *et al.*, 1981). It also occurs in renal failure and chronic liver disease by disorders in the platelets production, as well as in infectious diseases like ehrlichiosis, babesiosis, leptospirosis and salmonellosis, by platelets destruction (Garcia-Navarro & Pachaly, 1994). Our results can both indicate relative as well as absolute erythrocytosis, since we observed high levels of renal enzymes and urea concentration, suggesting renal failure. The low values of MCV and MCH constitute hypochromic microcytic red blood cells, a possible consequence of uremia and chronic infections (Thrall *et al.*, 2014). The only published study about hematological profile of free-living giant anteaters is from

Colombia, with an anesthetic protocol similar to ours. In this study, the very low erythrocyte count was due to the destruction caused by parasites (Rojano-Bolaño *et al.*, 2014).

Leucopenia present in the studied population was between 14.5 and 41.3% lower than the values obtained to the species from other researches. It indicates destruction of immune defenses or an activity suppression of the bone marrow and lymphatic organs (Bush, 2004). Some parasites that usually cause leucopenia have already been identified on giant anteaters: *Entamoeba* spp., *Acanthamoeba* spp. (Coke *et al.*, 2002), *Eimeria* spp. (Freitas *et al.*, 2006), *Coccidia* spp., *Giardia lamblia*, *Isospora* spp. (Rojano *et al.*, 2015), *Toxoplasma gondii* (Richini-Pereira *et al.*, 2016; Marujo *et al.*, 2017), *Babesia* spp., *Anaplasma* spp., *Sarcocystis* spp. (Arenales *et al.*, 2020), *Erlichia* spp. (Calchi *et al.*, 2020), *Leishmania* spp. (Richini-Pereira *et al.*, 2014), *Tetratrichomonas* spp. (Ibañez-Escribano *et al.*, 2013), *Leptospira interrogans* (Lenharo *et al.*, 2012; Miranda *et al.*, 2014), *Brucella abortus* (Miranda *et al.*, 2014), H1N1 (Nofs *et al.*, 2009), picobirnavirus (Haga *et al.*, 1999) and orthopoxvirus (Ashpole *et al.*, 2020). Hemoparasites from the genus we found on TB02, the *Mycoplasma*, have been associated with leucopenia in serval (*Felis serval*, Johnsrude *et al.*, 1996) and domestic cats (Do *et al.*, 2021), dogs (Soto *et al.*, 2017) and sheeps (Marinaro *et al.*, 2015).

Leucopenia with lymphopenia (TB09) occurs in several situations, like in the acute inflammation and severe infectious processes (Garcia-Navarro & Pachaly, 1994). Leucopenia with neutropenia (TB06) is often a sign of clinical worsening and could be an unfavorable prognosis. The possible causes are immune-mediated, toxin ingestion, serious and chronic infections (Garcia-Navarro & Pachaly, 1994). The TB06 lived in an area with invasive vegetation managed with glyphosate. There probably were other agrochemicals since it had portions of pasture, extensive cattle and forestry (*Pinnus* spp.). A study about the concentration of some possibly toxic substances on this giant anteater population is underway (Carvalho, 2018). Therefore, we cannot rule out that leucopenia with neutropenia has been caused by the close contact with such substances. The leucocytosis with neutrophilia (TB01) can be due to a sudden release of neutrophils from the marginal pool in situations of intense exercise or stress, but also in the acute inflammation and pyogenic bacterial infections (Garcia -Navarro & Pachaly, 1994; Meyer *et al.*, 1995).

The eosinophilia corroborate the intense endo and ectoparasitosis observed. Similar hematologic result occurred in Colombia's free-living anteaters, parasitized by *Ascaris*, *Taenia*, *Coccidia*, *Babesia* and ticks of the *Amblyomma* complex (Rojano-Bolaño *et al.*, 2014). The TB06 had the highest parasitic load in coproparasitological exams and the highest egg load of

Physaloptera spp. and M2. In both groups there are species capable of causing extensive tissue damage by fixing themselves deeply on the mucosa membranes of the organs they occur (Lértora *et al.*, 2016; Arenales *et al.*, 2020), stimulating the production and attraction of eosinophils for inflammatory focus. Damage to tissues that contain a large number of mast cells, which are the most in contact with the external environment (skin, lungs, gastrointestinal tract and uterus), results in the release of histamine and other factors in the mast cell, attracting more eosinophils than usual to the affected area (Bush, 2004).

Not every parasitism relationship causes changes in hematology or negatively affects the survival, fertility and abundance of hosts. There are a number of environmental, parasite and host attributes that will influence, for example, the abundance, diversity and cost of parasitism (Krasnov *et al.*, 2004; Bordes & Morand, 2008; O'Connor & Bernhardt, 2018). In free-living coatis (*Nasua nasua*), gastrointestinal parasites were not associated with decreased in hematological parameters or body condition (Olifiers *et al.*, 2015). However, in the nine-banded armadillo (*Dasypus novemcinctus*), the body condition was inversely proportional to the number of *Aspidodera sogandaresi* in the infected animals (Varela-Stokes *et al.*, 2008). Similarly, we also know very little about the effect of coinfections on hosts' health. The role of simultaneous infection by multiple parasites on host health is usually overlooked even though coinfection is the rule (Lello *et al.*, 2005; Bordes & Morand, 2008; Telfer *et al.*, 2010). Other aspect more explored by science is the sexual bias of parasitism in mammals (Schalk & Forbes, 1997). We observed that male giant anteaters had a higher parasitic load and more pronounced eosinophilia than females. This result is because estrogen seems to play an important role in resistance to many parasites (Prado *et al.*, 1998; Klein, 2000), whereas testosterone depresses the immune system of males (Schuurs & Verheul, 1990; Zuk & McKean, 1995).

Decrease in one or more biochemical parameters level can gives important information about the functioning of the organs (Alvarez & Whitemore, 2009; Contreras-Zentella & Hernández-Muñoz, 2016). The increase indicates some dysfunctions or damage in the organ that produce, store or eliminate them. Hemolysis can also cause an increase in LDH, ALT, AST, AF, potassium, glucose and hemoglobin, and therefore mask those (Freitas *et al.*, 2018). We observed an increase in the first three enzymes but not in potassium, AF (which was even below the values found in the literature) and not always in the concentration of free hemoglobin.

Liver profile indicated the possibility of hepatocellular lesions in some individuals, especially TB06. The ALT enzyme is hepatospecific and its increase is directly proportional to the number of damaged cells (Thrall *et al.*, 2014). Its slight increase is usually associated with

liver congestion and steatosis. Pancreatitis can eventually induce mechanical damage to the liver, as they are close, promoting an increase in ALT (Thrall *et al.*, 2014). Low levels of amylase and lipase may indicate chronic pancreatitis caused by permanent damage to the cells responsible for their production. One of the possible causes is high levels of triglycerides (Zhang *et al.*, 2019), also observed in part of the population. The increase in AST activity may be related to the severity of liver injury, even without symptoms (Moreira *et al.*, 2012), but muscle and cardiac injuries should be excluded. In intense exercise and muscle necrosis, the CK will be high, so we can exclude these suspicions since the values we obtained were low. The increase in AST occurs in infectious and toxic hepatitis, cirrhosis, biliary obstruction and fatty liver (González, 2003). LDH, like AST, is present in several tissues and needs to be evaluated in combination with other findings to address its clinical significance. In domestic cattle and horses, it increases in cholelithiasis, liver failure and fasciolosis (Smith, 1993). Three individuals (TB01, TB02 and TB03) contained a gastrointestinal parasitic morphotype (M1) that resemble eggs from *Fasciola* spp., which alert us about the great contact of these giant anteaters with cattle (raised in an extensive system in most of the properties that border the protected area) and dogs (in great abundance on area - Cesário *et al.*, 2017). High levels of uric acid in blood can also point to a reduction in liver function, due to reduction of its transformation into allantoin (eliminated by urine) mediated by uricase, an enzyme produced in the liver (Hill *et al.*, 2011). In TB06 there were low total proteins concentration, mainly caused by severe malnutrition, liver and kidney disease (Thrall *et al.*, 2014).

Elevated levels of uric acid can be found in renal failure and indicate a risk factor for chronic disease (Johnson *et al.*, 2013), but other tests would be necessary to support this suspicion. When there is liver disease concomitant with renal failure, the diagnosis becomes unclear, as there is not enough urea production for abnormal accumulation to occur in the presence of low glomerular filtration rate (Stockham & Scott, 2002; Fettman & Rebar, 2004). However, the two individuals who died presented lesions in both organs on the histopathology (ongoing study). Blood assessments seem to point to a non-renal azotemia, in which there is an urea increase with normal creatinine levels or below the range (as in our case) (Thrall *et al.*, 2014). This indicates that renal function is probably preserved and the greatest protein degradation is by diet or gastrointestinal tract bleeding, in which there are reabsorption of blood proteins (Stockham & Scott, 2002; Fettman & Rebar, 2004). The vast majority of blood studies are about captive animals that may not receive the same protein supply obtained from ants and termites on free-living. Intestinal bleeding is also a possibility, considering the high parasitic

load and the presence of parasites that live connected to the mucosa membranes. However, we did not observed erythropenia or assessed iron deficiency.

4.4.3. Ticks and Hemoparasites

The tick species most found in the Myrmecophagidae family, to which the giant anteaters belong, are *Amblyomma nodosum*, *A. sculptum* and *A. calcaratum* (Muñoz-Garcia *et al.*, 2019), all registered in the present study. The coinfections we found are also known for the host, but infestations with only one species of tick, different from what we observed, seem to be more reported (Muñoz-Garcia *et al.*, 2019). The frequent combination between *A. nodosum* and *A. sculptum* in hosts raises the recent issue about the role of anteaters in the transmission of *Rickettsia* spp. to humans and domestic animals. Strains with zoonotic potential were isolated from those species of ticks found in anteaters (Szabó *et al.*, 2019; Muñoz-Garcia *et al.*, 2019), including in spotted fever endemic regions (Moerbeck *et al.*, 2018). It is possible that *A. sculptum* is also involved in the transmission of *Anaplasma marginale* between giant anteaters (Guillemi *et al.*, 2020).

Recently, a high rate of *Anaplasma* spp. and *Ehrlichia* spp. was detected in Brazilian xenarthrans mammals, including in the giant anteater. However, the host epidemiological role and the *Amblyomma* genus vectorial capacity for Anaplasmataceae agents is still unknown (Calchi *et al.*, 2020). In adulthood, *A. calcaratum* has a high affinity with the Myrmecophagidae family (Bloemer *et al.*, 1987), but it can also be found in humans and several bird species (Costa-Pinheiro *et al.*, 2015). A strain of *Rickettsia* spp. found in *A. calcaratum* nymphs collected from Brazilian tanagers (*Ramphocelus carbo*) (Ogrzewalska *et al.*, 2013) was also present in *A. nodosum* of anteaters (Moerbeck *et al.*, 2018). Although the isolation of *R. rickettsii* from *A. parvum* in Mexico, its importance as disease vector is also unknown (Dzul-Rosado *et al.*, 2013). Of the agents that can involve ticks in their transmission cycles that we investigated in giant anteaters' blood, only *Mycoplasma* spp. was weakly positive in an individual. To our knowledge, this is the first record of this hemoparasite in giant anteater, but it is reported to carnivores (Koneval *et al.*, 2017; Millan *et al.*, 2018), primates (Bonato *et al.*, 2015), deer (André *et al.*, 2020) and bats (Mascarelli *et al.*, 2014).

4.4.4. Coproparasitology

The study of gastrointestinal parasites diversity in wildlife contributes greatly to the species conservation. It reveals ecosystem health condition through the symbiotic relationship between parasite and host and allows monitoring of environmental changes that threaten the survival of these animals (Hudson *et al.*, 2006). Coproparasitology is also an important tool in captivity sanitary control and needs to be included as a routine, among other management actions, to improve animal health and welfare (Barbosa *et al.*, 2019). Despite revealing a lot of information, investigations on gastrointestinal parasites of free-living alive giant anteaters are very scarce, because although the methodologies for the analysis are quite simple, obtaining fresh feces samples is extremely difficult. For this reason, the study of adult parasites from running-over animals has been the main source of knowledge on helminth fauna in most wild species, including the giant anteater (Griese, 2007; Rojano *et al.*, 2015; Oliveira *et al.*, 2020).

We know very little about the pathogenic characteristics of the parasites and their impact on wildlife. Small populations of species vulnerable to extinction, such as giant anteaters, should receive special attention when it comes to generalist infectious agents that are dispersed by other species, including domestic ones (Woodroffe, 1999). In this sense, we draw attention to our findings of *Trichuris* spp. and Ascaridoidea. These parasites can circulate in domestic and/or human populations (García-Sanches *et al.*, 2019; Garcia *et al.*, 2020), which have shared infectious agents with wild species due to the physical approach resulting from habitat loss (Deem *et al.*, 2001). Tricurids have already been reported to the host (Diniz *et al.*, 1995), like the *Trichuris discolor* (Zanetti *et al.*, 2005) and *Ascaris lumbricoides* (Rojano *et al.*, 2015).

In anteaters, the most frequently reported gastrointestinal parasites are from Protozoa, especially coccidia, giardia and amoeba (Lainson & Shaw, 1982; Gardener *et al.*, 1991; Freitas *et al.*, 2006; Miranda & Paula, 2013; Rojano *et al.*, 2015). We could not identify at a higher taxonomic level the parasite belonging to this group in our study, but Apicomplexa was the most frequent and had the highest infestation intensity in giant anteaters. We found a large number of eggs/oocysts per gram of feces, pointed out by the marked eosinophilia, although without any other common changes provoked by parasitism, such as erythropenia. *Physaloptera magnipapilla* has been linked to anemia and gastritis in a free-living giant anteater from Argentina (Lértora *et al.*, 2016). Although we found parasite eggs of this genus in the samples, in addition to adult individuals in TB03 and TB06 (personal communication), anemia did not occur in any of them. About 13 species of this genus have been reported in Brazil, with *P.*

magnipapilla, *P. papillotruncata*, *P. semilanceolata* and *P. praeputialis* present in giant anteater and collared anteater (*Tamandua tetradactyla*) stomachs (Vicente *et al.*, 1997; Giese, 2007; Ramos *et al.*, 2016). We found a great parasite richness, but there were at least two other helminths species in the population. We obtained *Gigantorhynchus echinodiscus* adults from TB03 (Gomes *et al.*, 2019) and *Mathevotaenia* spp. from TB03 and TB06 (personal communication). However, we did not recover eggs from them in any sample. There are six valid species of acanthocephalans from *Gigantorhynchus* genus that parasitize marsupials and anteaters in South America, with reports of *G. echinodiscus* on anteaters in Brazil (Travassos, 1917; Machado-Filho, 1941; Gomes *et al.*, 2019), Venezuela (Días-Ungria, 1958), Panama (Dunn, 1934) and Trinidad Island (Camerón, 1939). Also in South America, there are 15 species of *Mathevotaenia* spp. in the small intestine of several animals, with *M. tetragonocephala* in giant anteater and collared anteater (Schmidt & Martin, 1978).

Determining the health status of a population is a complex process that should not count on limited sampling. It requires caution especially considering free-living animals, in which the individual health history is generally unknown, as well as the evolution of clinical parameters and symptoms. The comparative analytical approach, often used when the data set available for the species is small, also has limitations. This is because season, biome, drugs, nutrition, habitat and stress can influence the indexes (Kuttner & Wissner, 1987; Rietkerk *et al.*, 1994; Vogel *et al.*, 1999; Kusak *et al.*, 2005). Combined findings on wildlife health, such as those presented in this study, can, however, signal possible impairments and give robustness to inferences. In addition, they can promote a better understanding of some physiological particularities in the species (Almeida *et al.*, 2011). Knowing these parameters well and conducting strategic health surveys of wild populations can contribute to the prevention of epidemics and assessment of anthropic impact level (Miranda *et al.*, 2007; Barnes *et al.*, 2008; Superina & Mera-Sierra, 2008; Superina *et al.*, 2010).

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5. MANUSCRITO 3:

Novos dados morfológicos e genéticos de duas espécies de *Aspidodera* (Nematoda: Heterakoidea) em tamanduá-bandeira *Myrmecophaga tridactyla* Linnaeus, 1758 (Mammalia: Myrmecophagidae)

Resumo

Aspidodera fasciata (Schneider, 1866) Railliet & Henry, 1913 e *A. scoleciformis* (Diesing, 1851) Railliet & Henry, 1912 são nematódeos relativamente comuns na América do Sul. No entanto, eles não têm recebido muita atenção há quase trinta anos, por isso ainda faltam informações taxonômicas e filogenéticas a respeito dessas espécies. Nós redescrevemos *A. fasciata* e *A. scoleciformis* coletados de um tamanduá-bandeira, *Myrmecophaga tridactyla* Linnaeus, 1758, do Cerrado brasileiro (Savana), estado de São Paulo, por microscopia de luz e eletrônica de varredura. Também inferimos a filogenia de *A. fasciata*. Fornecemos pela primeira vez detalhes da coifa, lábios, cordões cefálicos, estruturas sexuais e extremidade posterior, em machos e fêmeas. Isso contribuiu para o diagnóstico e evitou erros de identificação, devido à simpatria e semelhança dos congêneres. Análises filogenéticas moleculares mostraram agrupamento de *A. fasciata* com *A. sogandaresi* Jimenez-Ruiz, Gardner e Varela-Stokes, 2006, congruente com estudos morfológicos que os separam em duas espécies diferentes. Também fornecemos uma comparação entre as duas espécies estudadas e demais *Aspidodera* que ocorrem em outros hospedeiros da mesma superordem. Enfatizamos a importância da adoção de taxonomia integrativa e investigação de hospedeiros ameaçados, a fim de aumentar nosso conhecimento sobre a biodiversidade parasitária.

Palavras-chaves: Aspidoderidae. Coinfecção. Sistemática filogenética. Parasitismo. Xenarthra. Conservação de mamíferos silvestres.

New morphological and genetic data of two *Aspidodera* species (Nematoda: Heterakoidea) in the giant anteater *Myrmecophaga tridactyla* Linnaeus, 1758 (Mammalia: Myrmecophagidae)

Abstract

Aspidodera fasciata (Schneider, 1866) Railliet & Henry, 1913 and *A. scoleciformis* (Diesing, 1851) Railliet & Henry, 1912 are nematodes relatively common in South America. However, they have not received much attention for almost thirty years, so there is still a lack of taxonomic and phylogenetic information regarding these species. We redescribed *A. fasciata* and *A. scoleciformis* collected from a giant anteater, *Myrmecophaga tridactyla* Linnaeus, 1758, from the Brazilian Cerrado (Savanna), state of São Paulo, by light and scanning electronmicroscopy. We also inferred phylogeny from *A. fasciata*. We provided for the first time details of the hood, lips, cephalic cordons, sexual structures and posterior end, in both males and females. This contributed to the diagnosis and prevented misidentifications, due to the sympatry and similarity of the congeners. Molecular phylogenetic analyses showed *A. fasciata* grouping with *A. sogandaresi* Jimenez-Ruiz, Gardner and Varela-Stokes, 2006, which was congruent with morphological studies that separate them into two different species. We also provided a comparison between the two species studied and the other *Aspidodera* that occur in other hosts from the same superorder. We emphasize the importance of adopting integrative taxonomy and investigating threatened hosts, in order to increase our knowledge on the parasite biodiversity.

Keywords: Aspidoderidae. Coinfection. Phylogenetic systematics. Parasitism. Xenarthra. Wild mammal conservation.

5.1. Introduction

The genus *Aspidodera* Railliet & Henry, 1912 (Nematoda: Heterakoidea) comprises 12 known species (Santos *et al.*, 1990; Vicente *et al.*, 1997; Jiménez-Ruiz *et al.*, 2006; Jiménez *et al.*, 2013; Chagas-Moutinho *et al.*, 2014; Cesário *et al.*, 2021). They are distributed in the southern Nearctic and Neotropic, diagnosed by the expanded cephalic end adorned with cordons (Inglis, 1957; Santos *et al.*, 1990; Anderson *et al.*, 2009). *Aspidodera fasciata* (Schneider, 1866) and *Aspidodera scoleciformis* (Diesing, 1851) Railliet & Henry, 1912 are the most recorded species of the genus, widely found in Brazil and Paraguay with a southern distribution reaching Argentina (Navone, 1990; Ezquiaga *et al.*, 2019).

Aspidodera fasciata was originally described as *Heterakis fasciata* Schneider, 1866 from the nine banded armadillo, *Dasypus novemcinctus* Linnaeus, 1758, collected in Brazil (Vicente, 1966). It also parasitize *Cabassous unicinctus* Linnaeus, 1758, *Tolypeutes tricinctus* Linnaeus, 1758, *T. matacus* Desmarest, 1804, *Euphractus sexcinctus* Linnaeus, 1758 and *Priodontes maximus* Kerr, 1792, occurring in Bolivia, USA, Paraguay and Trinidad too (Travassos, 1913; Proença, 1937; Cameron, 1939; Pallares & Usher, 1971; Santos *et al.*, 1990; Vicente *et al.*, 1997). *Aspidodera scoleciformis* is the type species of the genus, described as *Aspidocephalus scoleciformis* Diesing, 1851 from *T. tricinctus*, collected in Brazil (Santos *et al.*, 1990). It also parasitize *D. novemcinctus*, *E. sexcinctus*, *Chaetophractus villosus* Desmarest, 1804, *C. unicinctus*, *Marmosa murina* Linnaeus, 1758, *M. cinerea* Temminck, 1824 and *Monodelphis domestica* Wagner, 1842, occurring in Paraguay too (Travassos, 1913; Proença, 1937; Pallares & Usher, 1971; Santos *et al.*, 1990; Vicente *et al.*, 1997).

Currently, studies that involve *A. fasciata* and *A. scoleciformis* are based on morphometry with scarce morphological representations (Travassos, 1913; Proença, 1937; Cameron, 1939; Vicente *et al.*, 1966; Pallares & Usher, 1971; Santos *et al.*, 1990; Vicente *et al.*, 1997). There are no findings in scientific literature about both species since the genus review (Santos *et al.*, 1990), lacking the ultrastructure and molecular data. Therefore, we redescribed *A. fasciata* and *A. scoleciformis* from the giant anteater, *Myrmecophaga tridactyla* Linnaeus, 1758 (Mammalia: Myrmecophagidae), and contributed with molecular data and phylogenetic approach to the genus *Aspidodera*. We also aimed to compile data on morphology and morphometry of all *Aspidodera* species that parasitize Xenarthra, for comparison purposes and to facilitate future identification, especially in coinfections.

5.2. Material and methods

5.2.1. Specimens collection

The specimens were collected from the large intestine of one adult female giant anteater *M. tridactyla* that died in Santa Bárbara Ecological Station (22°48'59" S, 49°14'12" W), a Cerrado (Savanna) remaining in São Paulo state, Southeastern Brazil. We realized the necropsy on the Laboratory of Pathology of UNESP - Jaboticabal/SP a day after the obit, on 16 July 2015, and stored the entire gastrointestinal tract in 70% ethanol. The screening of the material was performed on January 2016 at the Laboratory of Biology and Parasitology of Wild Mammal Reservoirs of Oswaldo Cruz Institute (LABPMR - IOC/Fiocruz) - Rio de Janeiro/RJ. The nematodes were washed in saline solution (NaCl 0.85%) and stored in 70% ethanol.

Morphological and morphometric characterization were performed using the Axion Scope A1 Light Microscope (LM) (Zeiss, Göttingen, Germany) and drawing were made with the aid of a camera lucida attached to a Nikon Eclipse E200MVR light microscope (Nikon Corporation, Tokyo, Japan). Measurements were in micrometers (µm) and presented as range followed by mean in parentheses. Proportions between hood/body length, esophagus/body length, spicule/body length and distance of vulva to anterior end/body length were expressed as percentage. We provided morphological and morphometric data of *Aspidodera* species from original descriptions and other case reports from xenarthrans hosts (Pallares & Usher, 1971; Diaz-Ungria, 1979; Fugita *et al.*, 1995; Santos *et al.*, 1990; Vicente *et al.*, 1997). Data from the present study were represented in the second row of the tables, in bold (Tables 2, 3 and 4). Specimens were deposited in the Helminthological Collection of the Oswaldo Cruz Institute (Coleção Helmintológica do Instituto Oswaldo Cruz - CHIOC), Rio de Janeiro/RJ, Brazil.

Specimens prepared for Scanning Electron Microscopy (SEM) was fixed for 1 hour at room temperature in 2.5% glutaraldehyde in 0.1 M Na-cacodylate buffer and washed in the same buffer (pH 7.2), post-fixed in 2% OsO₄ in 0.1 M Na-cacodylate buffer and then washed 3 times in the same buffer. They were subsequently dehydrated in a graded series of ethanol (30–100%) in 1 hour steps, and then critical point-dried in CO₂, sputter-coated with gold thick of a layer about 20 nm, and examined using a JEOL JSM-6390 LV microscope (JEOL, Akishima, Tokyo, Japan) with an accelerating voltage of 15 kV at the Electron Microscopy Platform of the Oswaldo Cruz Institute.

5.2.2. Molecular analyses

Total genomic DNA was isolated using the QIAamp DNA mini kit according to the manufacturer's protocol (Qiagen, Venlo, Netherlands). DNA amplifications by polymerase chain reaction (PCR) were conducted using the primers and cycling conditions to amplify mitochondrial 16S rDNA (Jiménez *et al.*, 2012). PCR amplifications were performed using Promega PCR Master Mix (Promega Corporation, Wisconsin, USA). Reactions were 25 µL following the manufacturer's protocol. The thermalcycling profile was programmed on an Eppendorf Mastercycler Ep System (Eppendorf, Hamburg, Germany). Successful amplifications were purified using the QIAquick PCR purification kit (Qiagen Ltd., Hilden, Germany) following the manufacturer's protocol. Sequencing reactions using Big Dye Terminator v3.1 cycle sequencing kit (Applied Biosystems, California, USA) were performed using the same primers mentioned above in a Gene Amp (Applied Biosystems) thermocycler, analyzed using an ABI 3730 DNA analyzer (Applied Biosystems). Both procedures and cycle-sequenced product precipitations were conducted at the subunit RPT01A- DNA sequencing platform of the IOC PDTIS/Fiocruz. Fragments were assembled into contigs and manually edited for ambiguities using the software package Geneious 9.1.8 (<http://www.geneious.com>; Kearse *et al.*, 2012), and the consensus sequence was extracted and saved in FASTA.

The 16S dataset included representative sequences of the Aspidoderidae family retrieved from GenBank dataset. The accession numbers and references of specimens used in this study are listed (Table 1). We aligned all sequences using the CLUSTAL program under default parameters in the Geneious package, followed by manual edition of the sequences, removing the non-complementary regions. The resulting aligned matrix was manually trimmed of poorly aligned regions using the Mesquite 3.51 software package (Maddison & Maddison, 2018). Phylogenetic relationships based on partial mitochondrial 16S gene sequence were inferred using maximum likelihood (ML) and Bayesian inference (BI). ML was carried out using the online PhyML 3.0 software (Guindon *et al.*, 2010). The model of nucleotide evolution was selected by Smart Model Selection (SMS), run in PhyML (Lefort *et al.*, 2017), using the Akaike Information Criterion (AIC). Node support was computed by the Approximate Likelihood-Ratio Test for Branches (aLRT) (Anisimova & Gascuel, 2006) and by nonparametric bootstrap percentages (ML-BP) after 1000 pseudoreplications. Bayesian phylogenetic inferences (BI) were obtained in MrBayes version 3.2.6 (Ronquist *et al.*, 2012) on XSEDE, using the CIPRES Science Gateway V.3.1 (Miller *et al.*, 2010). Node support in

the Bayesian trees was computed using the Bayesian Posterior Probabilities (BPPs) of the tree samples after the removal of a “burn-in” fraction of 25%.

Table 1. Accession numbers of GenBank sequences used in our phylogenetic analyzes with 16S rRNA gene.

Species	Accession number	Host	Locality	Reference
<i>Aspidodera scoleciformis</i>	JN852756	<i>Chaetophractus villosus</i>	Argentina	Jiménez et al., 2012
<i>Aspidodera scoleciformis</i>	JN852757	<i>Euphractus sexcinctus</i>	Paraguay	Jiménez et al., 2012
<i>Aspidodera raillieti</i>	JN852768	<i>Didelphis pernigra</i>	Bolivia	Jiménez et al., 2012
<i>Aspidodera raillieti</i>	JN852769	<i>Didelphis marsupialis</i>	Bolivia	Jiménez et al., 2012
<i>Aspidodera raillieti</i>	JN852770	<i>Didelphis pernigra</i>	Bolivia	Jiménez et al., 2012
<i>Aspidodera raillieti</i>	KX954131	<i>Didelphis albiventris</i>	Brazil	Santos et al., 2017 (unpublished)
<i>Aspidodera raillieti</i>	JN852767	<i>Didelphis marsupialis</i>	Guatemala	Jiménez et al., 2012
<i>Aspidodera binansata</i>	JN852758	<i>Dasypus novemcinctus</i>	Peru	Jiménez et al., 2012
<i>Aspidodera sogandaresi</i>	JN852771	<i>Dasypus novemcinctus</i>	USA	Jiménez et al., 2012
<i>Aspidodera sogandaresi</i>	JN852772	<i>Dasypus novemcinctus</i>	Mexico	Jiménez et al., 2012
<i>Aspidodera</i> sp.	JN852773	<i>Dasypus novemcinctus</i>	Mexico	Jiménez et al., 2012
<i>Aspidodera</i> sp.	JN852774	<i>Dasypus novemcinctus</i>	Mexico	Jiménez et al., 2012
<i>Aspidodera</i> sp.	JN852775	<i>Dasypus novemcinctus</i>	Mexico	Jiménez et al., 2012
<i>Aspidodera fasciata</i>	present study	<i>Myrmecophaga tridactyla</i>	Brazil	present study
<i>Lauroia bolivari</i>	JN852776	<i>Dasypus novemcinctus</i>	Bolivia	Jiménez et al., 2012
<i>Lauroia trinidadensis</i>	JN852777	<i>Dasypus novemcinctus</i>	Mexico	Jiménez et al., 2012
<i>Lauroia trinidadensis</i>	JN852778	<i>Dasypus novemcinctus</i>	Mexico	Jiménez et al., 2012

5.3. Results

5.3.1. Taxonomic summary

Host: *Myrmecophaga tridactyla* Linnaeus, 1758.

Site: Large intestine.

Locality: Santa Bárbara Ecological Station (22°48'59"S, 49°14'12"W)

Santa Bárbara, São Paulo, Brazil.

Specimens deposited: *Aspidodera fasciata* CHIOC n° 38751 (paratypes: 4 males and 4 females), *Aspidodera scoleciformis* CHIOC n° 38750 (paratypes: 1 male and 1 female).

5.3.2. Redescription of *Aspidodera fasciata* (Schneider, 1866) Railliet & Henry, 1913 (Figures 1A-1J, 3A-3L)

General: Sexually dimorphic. Females wider than males (body width ratio 1.51:1; Tables 2 and 3). Body fusiform, cuticle thick with fine transverse striations. Thick neck-like cuticle posterior to hood (or cephalic cap) with small papillae (Figures 1B, 1C and 3D). Maximum width of body between first third to mid-length of body. Medium hood with anastomosing cordons (Figures 1B, 1C and 3A), six anteriad and six posteriad loops in cephalic cordons with smooth margins, and cephalic cordons reaching the base of the hood (Figures 1B, 1C, 3A and 3D). Three lips with two posterior slender projections (Figure 3A). Dorsal lip with two digitiform projections, lateroventral lips with small projections (Figure 3B). Dextroventral lip projection with marked undulation that fits on sinistroventral lip sockets (Figures 3B and 3C). Lateroventral lips with an amphid, a papilla and a papillary nodule (Figures 1B, 1C and 3C). Interlabium long and slender, labial-root trapezoidal shaped (Figures 1B, 1C and 3A). Pore-like papilla absent. Short vestibulum in the first third of the hood with well developed cuticular lamellae (Figures 3B and 3C). Esophagus with a terminal pyriform bulb and posterior conspicuous valve (Figure 1A) followed by the long intestine. Nerve ring surrounding the esophagus at the end of its first third and excretory pore in middle portion of esophagus (Figures 1A, 3A and 3E). Lateral alae absent (Figures 3A and 3K). Tail of both sexes conical, with pointed tip (Figures 1F, 1G, 3H and 3L).

Male (20 specimens, Table 2): Body 9500- 12210 (10950) long and 363- 593 (453) width. Hood 210- 312 (266) long, 2.5% of body length. Esophagus with posterior bulb 1449- 1964 (1758) long, 16.5% of body length. Bulb of esophagus 244- 300 (279) long and 202- 267 (242) width. Nerve ring and excretory pore at 536- 618 (536) and 830- 1009 (972), respectively, from anterior end. Posterior end slightly curved ventrally with a precloacal genital sucker 118- 143 (135) long (Figures 1G, 1H, 3H and 3I), anterior to cloaca (Figures 3H, 3I and 3J). A pair of spicules equal (Figure 1G), short and robust, with a larger alate in the median portion, 358- 457 (376) long, representing 4.0% of body length (Figures 1J and 3J). Gubernaculum slightly curved, bevel-shaped with a groove from the middle portion to the tapering tip, 146- 168 (154) length (Figures 1I and 3J). Caudal end with spine projection measuring 52- 64 (57) long (Figures 3H and 3L). Posterior end with 57 pairs caudal papillae, variable between individuals: 20 pairs precloacal (6 anterior, 8 lateral and 5 posterior to the genital sucker, and a single pedunculate papilla connected on genital sucker), 5 pairs adcloacal (4 median and 1 marginal);

and 32 pairs post cloacal (18 submedian – the last pair being double - and 14 marginal) (Figures 1H, 3H, 3I and 3L).

Female (20 specimens; Table 3): Body 10052- 11605 (10539) long and 568- 815 (630) width. Hood 276- 321 long (296), 2.8% of body length. Esophagus with posterior bulb 1861- 2361 (2073) long, 19.7% of body length. Bulb 250- 347 (295) long and 221- 268 (246) width. Nerve ring and excretory pore 553- 670 (605) and 800- 1061 (958), respectively, from anterior end. Vulva slit-like, located at 4026- 5210 (4467) from anterior end, 42.4% of body length (Figures 1D and 3F). Anus opening 648- 776 (707) to posterior end (Figures 1F and 3G). Ellipsoid eggs not embrionated in uterus with thick and smooth shell, 62- 69 (65) long and 45- 47 (46) width (Figures 1D and 1E).

5.3.3. Redescription of *Aspidodera scoleciformis* (Diesing, 1851) Railliet & Henry, 1912 (Figures 2A-2I, 4A-4K)

General: Sexually dimorphic. Females wider than males (body width ratio 1.27:1; Tables 2 and 3). Body fusiform, cuticle thick with transverse striations and small lined papillae posterior to hood (or cephalic cap) (Figures 2B, 2C and 4D). Neck-like cuticle absent. Maximum width of body between first third to mid-length of body. Short to medium hood with anastomosing cordons (Figures 2B, 2C and 4B), six anteriad and six posteriad loops in cephalic cordons with smooth margins, and cephalic cordons touching the base of the hood (Figures 2B, 2C and 4D). Three lips with two posterior slender projections (Figure 4A). Dorsal lip with two digitiform projections, lateroventral lips with small projections (Figure 4B). Dextroventral lip projection with undulation that fits on sinistroventral lip sockets (Figure 4B). Lateroventral lips with an amphid, a papilla and a papillary nodule (Figures 2B, 2C and 4C). Interlabium wide, labial-root square shaped (Figures 2B, 2C and 4A) with a pore-like papilla on apex (Figures 2A, 2B, 4B and 4C). Long vestibulum with well developed cuticular lamellae (Figures 4B and 4C). Long esophagus with a terminal pyriform bulb and posterior conspicuous valve (Figure 2A). Nerve ring surrounding the esophagus at the end of its first fifth and excretory pore anterior to the middle portion of esophagus (Figures 2A and 4E). Lateral alae absent (Figures 4A and 4I). Tail of both sexes conical, with pointed tip (Figures 2F, 2G, 4G and 4J).

Male (seven specimens; Table 2): Body 8368- 11190 (10150) long and 507- 589 (555) width. Hood 116- 148 long (136), 1.4% of body length. Esophagus with bulb 2119- 2307 (2226) long, representing 22.2% of body length. Bulb of esophagus 313- 352 (336) long and 223- 326

(294) width. Nerve ring and excretory pore at 489- 535 (512) and 801- 914 (849), respectively, from anterior end. Posterior end of body slightly curved ventrally with a precloacal genital sucker 96- 114 (107) long (Figures 2G, 4H and 4K). Spicules subequal, long and slender (Figure 2G and 2I), measuring 1208- 1361 (1296) long, 13.0% of body length. Gubernaculum bevel-shaped with a groove from the middle portion to the tapering tip, 143- 201 (164) long (Figure 2H). Caudal spine short 32- 45 (36) long (Figures 4H and 4J). Caudal papillae 38 pairs, variable between individuals: 10 pairs precloacal (4 anterior, 3 lateral and 3 posterior to genital sucker, and a single pedunculate papilla connected on genital sucker); 6 pairs adcloacal (1 principal, 3 central and 2 lateral); and 22 pairs post cloacal (11 submedian and 11 marginal) (Figures 4H and 4K).

Female (12 specimens; Table 3): Body 8973- 10631 (9752) long and 565- 752 (661) width. Hood 142- 176 long (163), 1.7% of body length. Esophagus with bulb 1919- 2382 (2158) long, 22.1% of body length. Bulb of esophagus 308- 412 (360) long and 284- 338 (313) width. Nerve ring and excretory pore at 489- 649 (586) and 755- 1127 (905), respectively, from anterior end. Vulva slit-like, 4105- 4421 (4284) from anterior end, 44.4% of body length (Figures 2D and 4F). Anus opening 606- 882 (692) to posterior end (Figures 2F and 4G). Ellipsoid eggs not embrionated in uterus with thick and smooth shell, 54- 61 (58) long and 40- 44 (42) width (Figures 2D and 2E).

Table 2. Morphometric data for males of *Aspidodera* nematodes that parasitize xenarthrans.

	<i>A. fasciata</i>	<i>A. soganderesi</i>	<i>A. binansata</i>	<i>A. scoleciformis</i>	<i>A. lacombeae</i>	<i>A. kinsellai</i>	<i>A. vazi</i>	<i>A. ansirupta</i>
Bo ^a	4620- 9200 x 150- 610 9500- 12210 x 363- 539	4400- 7000 x 160- 337	5150- 7000 x 300- 500	6930- 11000 x 330- 630 8368- 11190 x 507- 589	10850- 13050 x 360- 640	4538- 7312 x 287- 425	4120- 8160 x 300- 493	3500- 5300 x 220- 460
H	170- 310 210- 312	191- 264	130- 180	110- 148 116- 148	190- 260	325- 389	219- 480	56- 100
Es	1325- 1820 1449- 1964	1052- 1502	1000- 1580	1700- 2600 2119- 2307	2220- 2470	1287- 1736	1060- 1720	620- 1120
Bu ^a	190- 360 x 150- 330 244- 300 x 202- 267	167- 229 x 148- 209	178- 230 x 150- 220	217- 430 x 235- 350 313- 352 x 223- 326	320- 390 x 250- 340	169- 229 x 159- 230	162- 260 129- 240	160- 270 x 160- 250
NR	429- 890 536- 618	390- 548	440- 670	410- 920 489- 535	570- 720	437- 640	760- 790	270- 586
EP	697- 950 830- 1009	552- 775	605- 720	490- 1000 801- 914	860- 1210	675- 955	575- 1070	415- 660
S	202- 400 358- 457	220- 370	290- 405	890- 1900 1208- 1361	1150- 1410	675- 850	760- 1100	230- 300
CS	30- 50 52- 64	25- 46	120- 160	10- 43 32- 45	09- 14	125- 185	100- 280	110- 260

Bo: Body; **H:** Hood; **Es:** Esophagus; **Bu:** Bulb; **NR:** Nervous Ring to anterior end; **EP:** Excretory Pore to anterior end; **S:** Spicules; **CS:** Caudal spine.

^a Length is followed by the width.

Range in bold: present study. Other ranges: compiled morphometry range from literature.

Table 3. Morphometric data for females of *Aspidodera* nematodes that parasitize xenarthrans.

	<i>A. fasciata</i>	<i>A. soganderesi</i>	<i>A. binansata</i>	<i>A. scoleciformis</i>	<i>A. lacombeae</i>	<i>A. kinsellai</i>	<i>A. vazi</i>	<i>A. serrata</i>	<i>A. ansirupta</i>
Bo^a	5400- 10000 x 270- 660 10052-11605 x 568- 815	4711- 6886 x 219- 385	5540- 7490 x 320- 480	6770- 1020 x 400- 690 8973- 10631 x 565- 752	10470- 12000 x 320- 690	5307- 6906 x 346- 557	5000- 9200 x 380- 570	6105- 7157 x 152- 184	4690- 6000 x 320- 460
H	200- 304 276- 321	213- 312	130- 200	121- 170 142- 176	230- 280	362- 440	344- 530	319- 361	60- 99
Es	1200- 1850 1861- 2361	1038- 1504	1100- 1480	1800- 2630 1919- 2382	2320- 2550	1324- 1640	1060- 1650	1244- 1340	1100- 1320
Bu^a	190- 330 x 150- 263 250- 347 x 221- 268	158- 221 x 123- 238	180- 370 x 180- 250	217- 380 x 235- 357 308- 412 x 284- 338	340- 410 x 300- 350	166- 244 x 172- 235	186- 260 x 180- 240	186- 205 x 155- 171	190- 230 x 170- 280
NR	470- 900 553- 670	361- 523	330- 690	460- 920 489- 649	620- 830	504- 611	780- 1100	542- 659	290- 614
EP	660- 10000 800- 10061	549- 757	610- 720	620- 1000 755- 1127	950- 1160	700- 955	736- 1100	659- 787	530- 604
V	2350- 3760 4026- 5210	1811- 2807	2380- 4620	2380- 4650 4105- 4421	4990- 5990	2431- 3112	2550- 3367	2815- 3157	1980- 2750
A	480- 540 648- 776	n.i.	850- 959	550- 660 606- 882	520- 600	n.i.	526- 757	521- 574	580- 700
E^a	52- 72 x 40- 48 62- 69 x 45- 47	60- 92 x 40- 70	50- 78 x 40- 50	40- 67 x 27- 42 54- 61 x 40- 44	40- 54 x 30- 39	57- 78 x 37- 61	50- 72 x 36- 54	54- 66 x 41- 50	58- 94 x 35- 43

Bo: Body; **H:** Hood; **Es:** Esophagus; **Bu:** Bulb; **NR:** Nervous Ring to anterior end; **EP:** Excretory Pore to anterior end; **V:** Vulva to anterior end; **A:** Anus to posterior end; **E:** Egg. ^a Length is followed by the width.

n.i. nonexistent information.

Range in bold: present study. Other ranges: compiled morphometry range from literature.

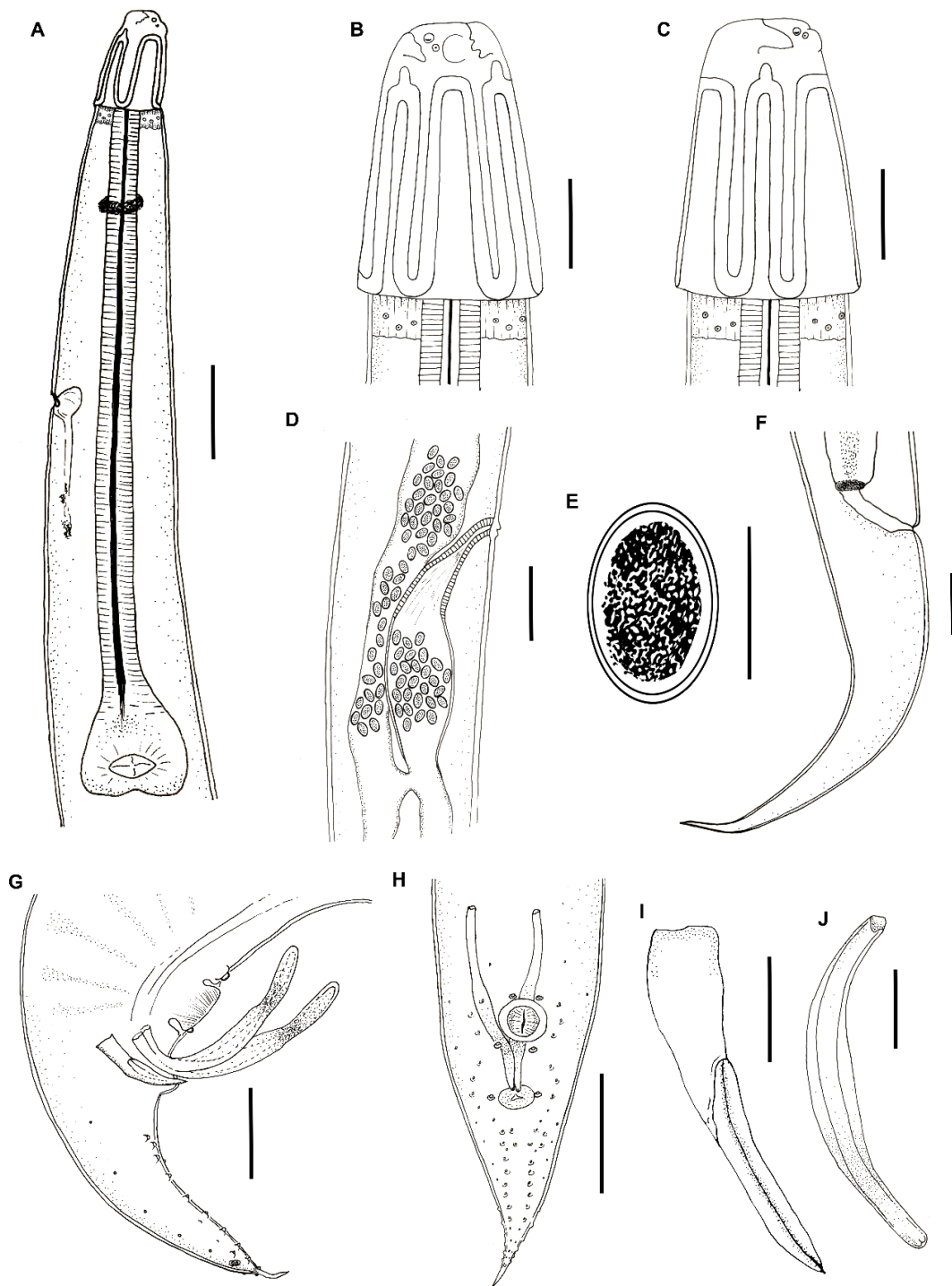


Figure 1A-1J. Line drawing of *Aspidodera fasciata* from *Myrmecophaga tridactyla*. 1A. Anterior end with hood and cephalic cordons, nerve ring, excretory pore and esophagus with terminal bulb; 1B (dextroventral region), 1C (dorsal-dextroventral region). Hood showing amphid, papilla, papillary nodule, interlabium, labial-root and neck-like cuticle with papillae; 1D. Genital system of female showing vulva and uterus with eggs; 1E. Egg; 1F. Posterior end of female showing rectum and anus; 1G (lateral view), 1H (ventral view). Posterior end of male showing genital sucker, spicules, gubernaculum, caudal spine and caudal papillae; 1I. Gubernaculum; 1J. Spicule.

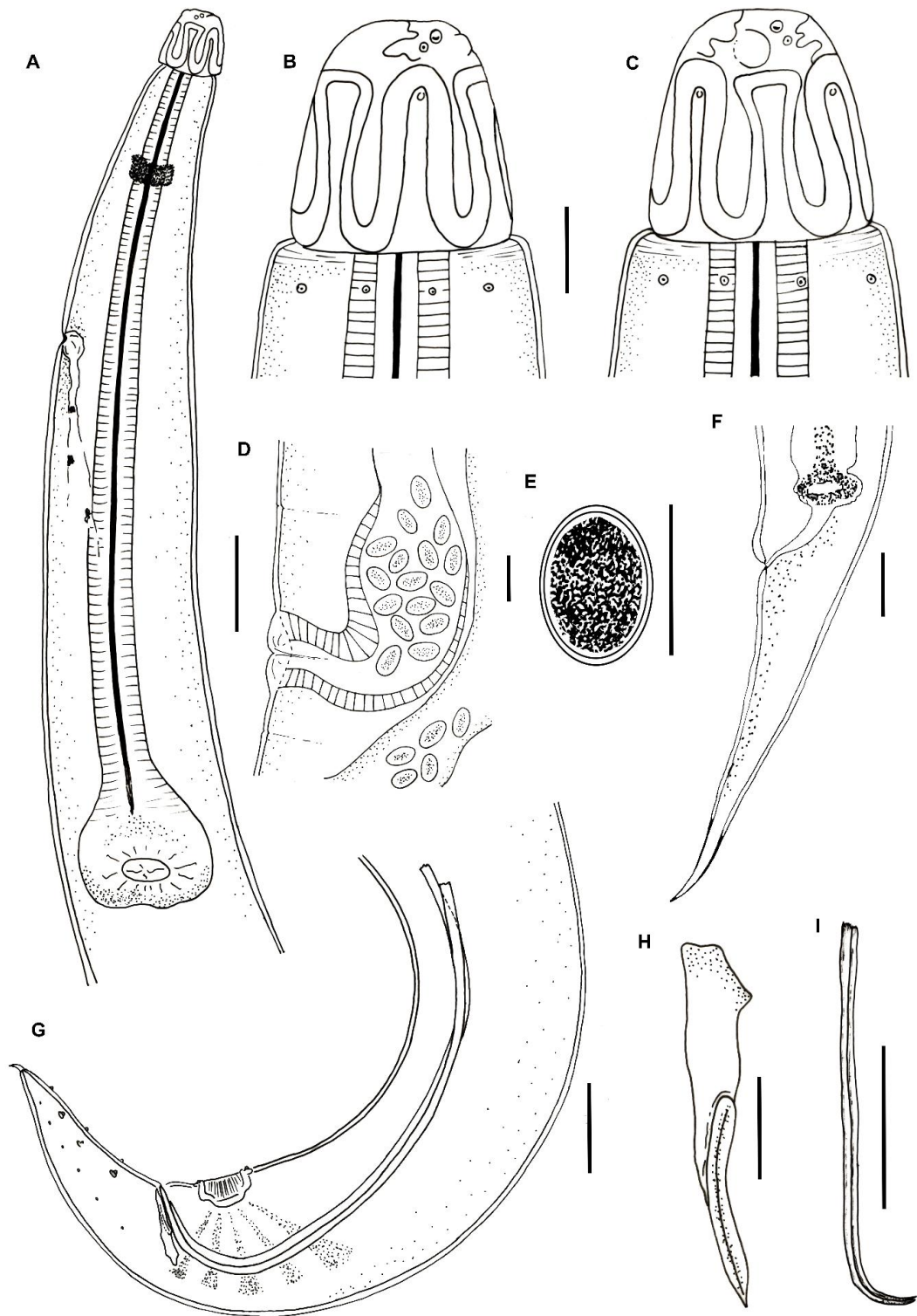


Figure 2A-2I. Line drawing of *Aspidodera scoleciformis* from *Myrmecophaga tridactyla*. 2A. Anterior end with hood and cephalic cordons, nerve ring, excretory pore and esophagus with terminal bulb; 2B (dorsal-dextroventral region), 2C (sinistroventral region). Hood showing amphid, papilla, papillary nodule, interlabium with pore-like papilla on apex, labial-root and a cordon of papillae posterior to hood; 2D. Genital system of female showing vulva and uterus with eggs; 2E. Egg; 2F. Posterior end of female showing rectum and anus; 2G Posterior end of male showing genital sucker, spicules, gubernaculum, caudal spine and caudal papillae; 2H. Gubernaculum; 2I. Spicule.

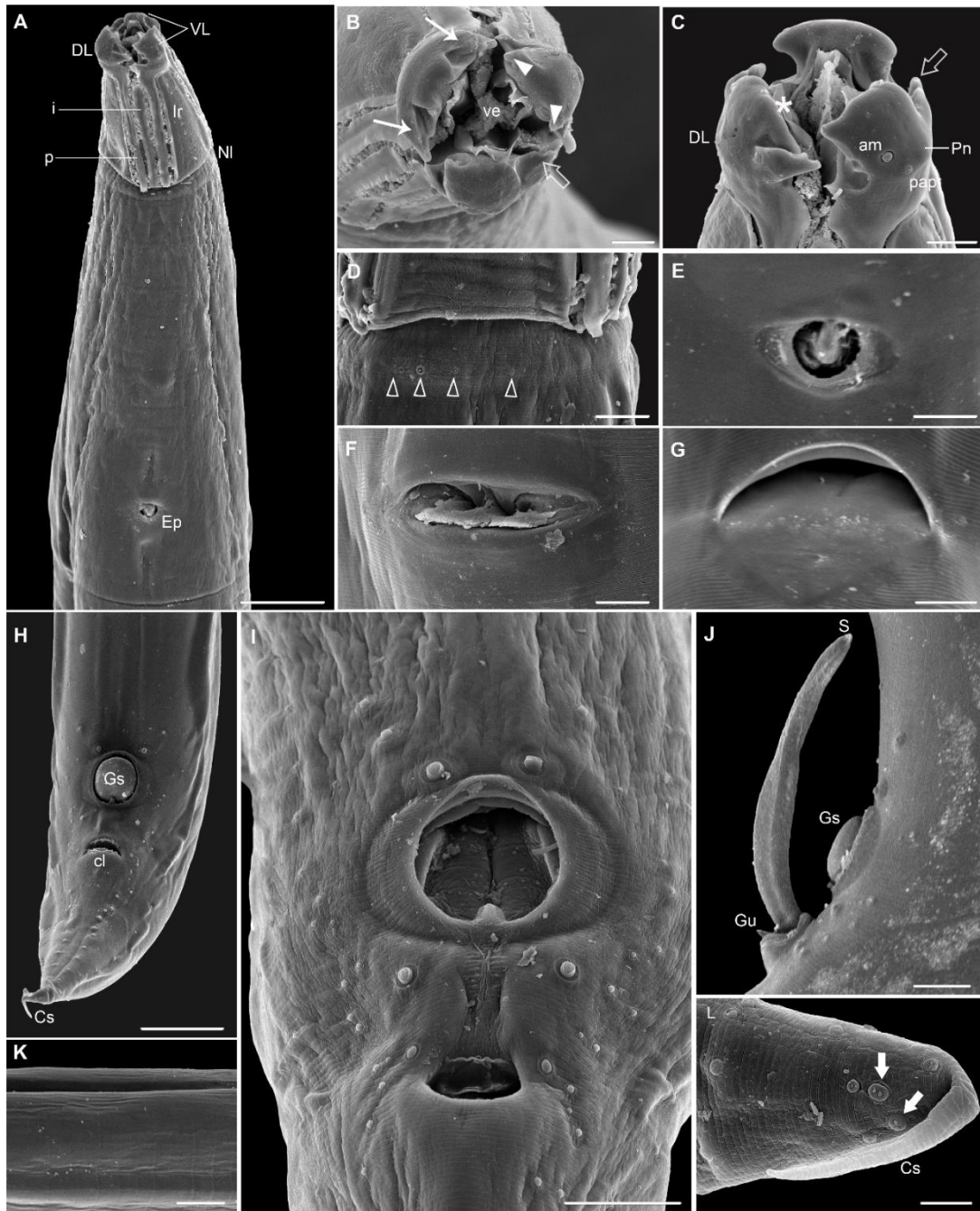


Figure 3A-3L. Scanning electron micrographs of adult *Aspidodera fasciata* from *Myrmecophaga tridactyla*. 3A. Anterior end with hood and cephalic cordons, dorsal lip (DL), ventral lips (VL), interlabium (i), slender projection of lip (p), inter-root expansion (Ir), neck-like cuticle (NI), excretory pore (Ep); 3B. Oral aperture with lips showing digitiform projections (thin arrow), small projections (full arrowhead), dextroventral lip projection marked undulated (empty large arrow) and vestibulum (ve); 3C. Oral aperture with dorsal lip (DL), amphid (am), papilla (pap), papillary nodule (Pn), dextroventral lip projection marked undulated (empty large arrow) and the oral vestibulum lined with cuticular lamellae (asterisk); 3D. Neck-like cuticle with papillae (empty arrowhead); 3E. Excretory pore; 3F. Vulva; 3G. Anus; 3H. Posterior end of male showing genital sucker (Gs), cloaca (cl), caudal spine (Cs) and distribution of caudal papillae; 3I. Genital sucker, cloaca and some papillae in detail; 3J. Exposed spicule (S), genital sucker (Gs) and the tapering tip of gubernaculum (Gu); 3K. Lateral alae absent; 3L. Tale of male showing double papillae (full large arrow) and caudal spine (Cs).

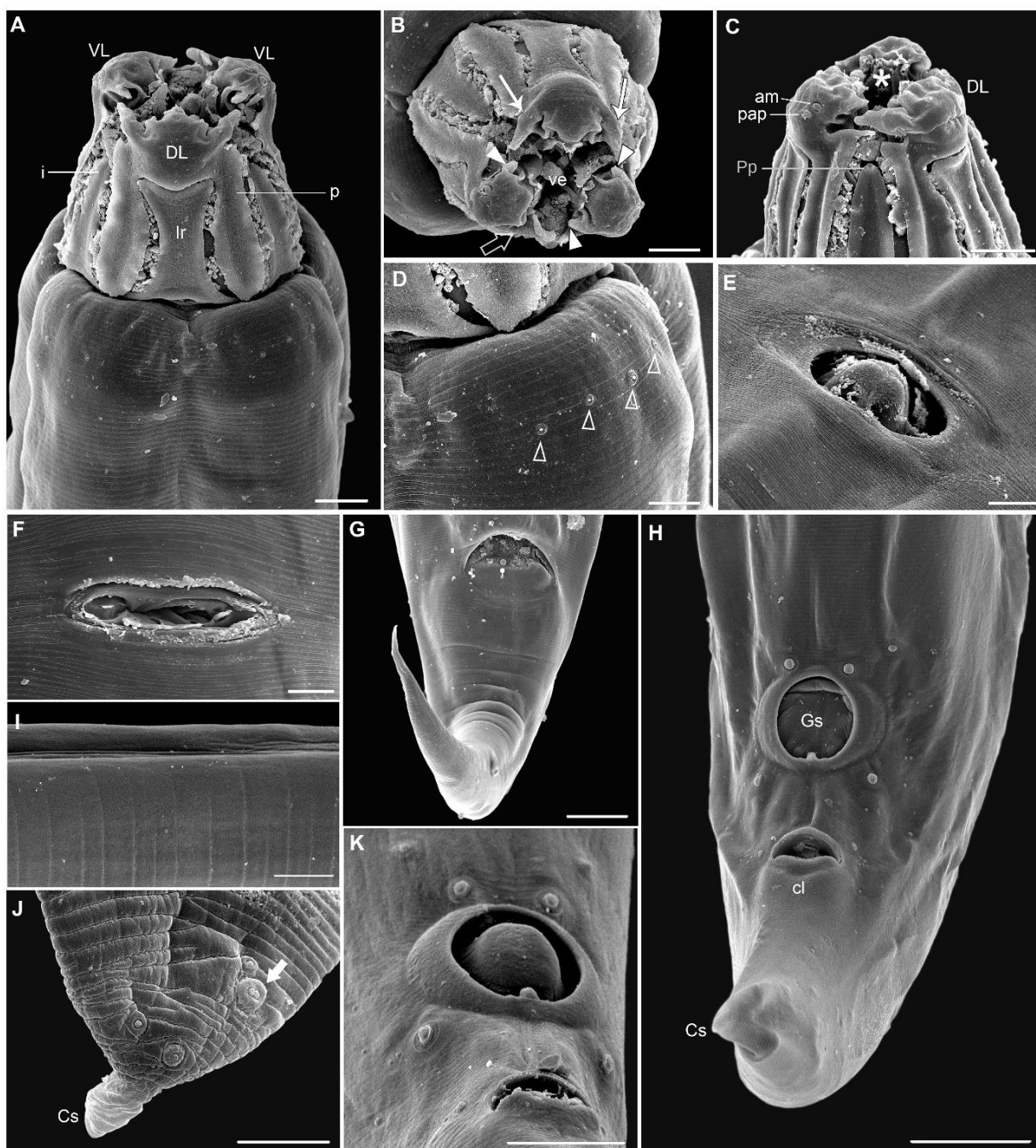


Figure 4A-4k. Scanning electron micrographs of adult *Aspidodera scoleciformis* from *Myrmecophaga tridactyla*. 4A. Anterior end with hood and cephalic cordons, dorsal lip (DL), ventral lips (VL), interlabium (i), slender projection of lip (p), inter-root expansion (Ir); 4B. Oral aperture with lips showing digitiform projections (thin arrow), small projections (full arrowhead), dextroventral lip projection (empty large arrow) and vestibulum (ve); 4C. Oral aperture with dorsal lip (DL), amphid (am), papilla (pap), pore-like papilla (Pp) on the apex of interlabium and the oral vestibulum lined with cuticular lamellae (asterisk); 4D. Lined papillae posterior to hood (empty arrowhead); 4E. Excretory pore; 4F. Vulva; 4G. Anus; 4H. Posterior end of male showing genital sucker (Gs), cloaca (cl), caudal spine (Cs) and distribution of caudal papillae; 4I. Lateral alae absent; 4J. Tale of male showing simple papilla (full large arrow) and caudal spine (Cs); 4K. Genital sucker, cloaca and some papillae in detail.

5.3.4. Molecular analyses

The 16S rRNA gene sequence from *A. scoleciformis* was unable to obtain due to the genomic DNA degradation and few samples available for the procedure. However, a consensus sequence of 16S gene was generated from *Aspidodera fasciata*. The 16S matrix resulted from the alignment of the *A. fasciata* present study sequences and other sequences from *Aspidodera* species, retrieved from GenBank (Table 1). They totalized 17 taxa and 800 characters, of which 480 were constant, 89 variable parsimony uninformative and 231 parsimony informative.

The ML best-fit model chosen by SMS in PhyML under AIC was GTR+G, with four substitution rate categories and gamma shape parameter of 0.462. It resulted in a tree with a score $\ln L = -3639, 88514$. The ML and BI showed similar topologies with little variation in nodes and support values (Figure 5).

The phylogenetic analysis of genus *Aspidodera* showed a well-supported monophyletic group (aLRT=1.0, BP-ML=1000, BPP=1.0). *Aspidodera fasciata* sequence formed a well-supported monophyletic group with *A. sogandaresi* Jimenez-Ruiz, Gardner and Varela-Stokes, 2006 (aLRT=1.0, BP-ML=783, BPP=0.95) and a strongly supported clade (aLRT=1.0, BP-ML=919, BPP=0.99). *Aspidodera fasciata* and *A. sogandaresi* formed a sister group to the one composed by the other *Aspidodera* species sequences. The *A. raillieti* Travassos, 1913 sequences from different localities and hosts formed a monophyletic group with moderate to strong support and nested with the clade formed by *A. binansata* Railliet & Henry, 1913, *A. scoleciformis* and *Aspidodera* sp. (aLRT=1.0, BP-ML=1000, BPP=0.77).

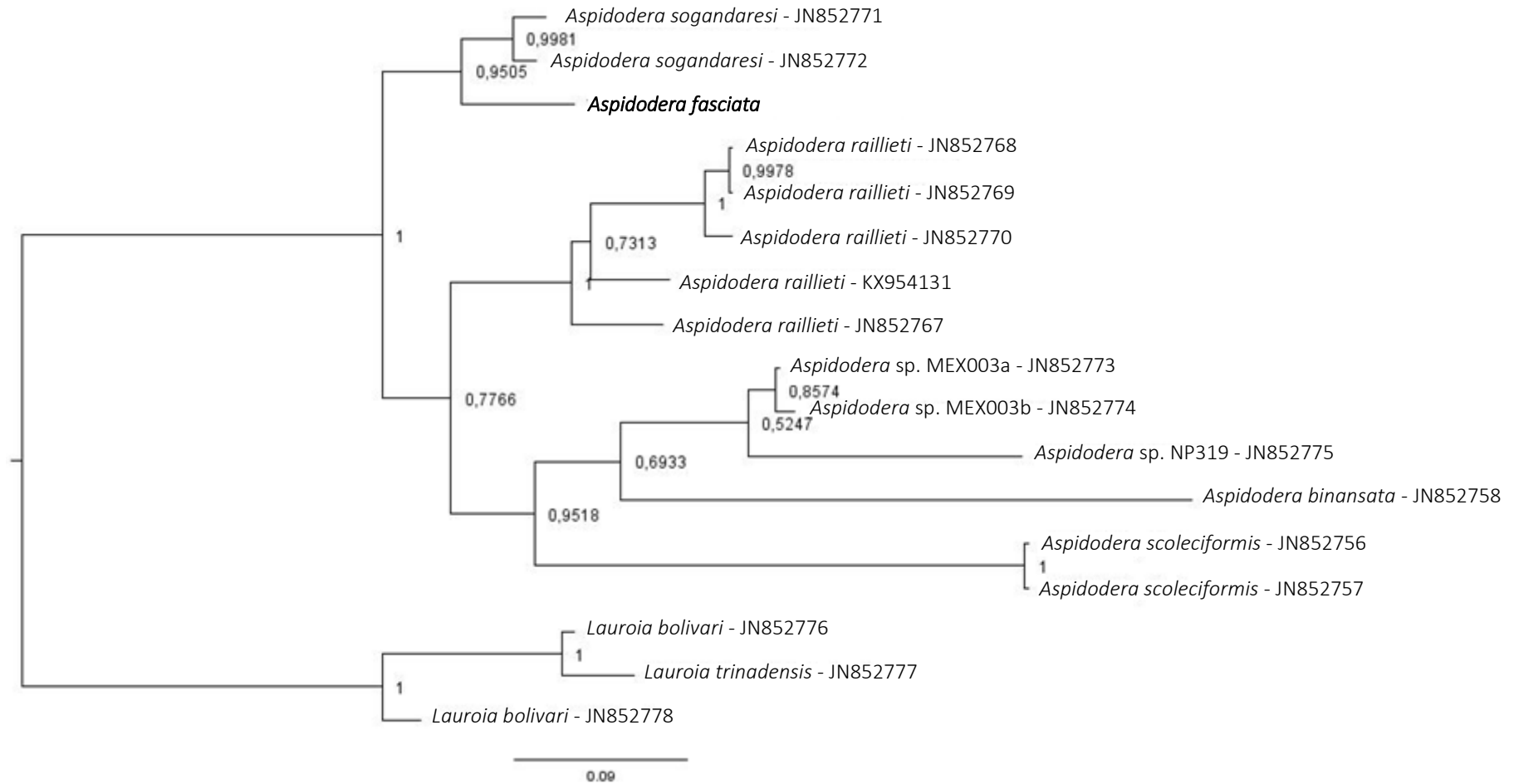


Figure 5. Bayesian inference phylogenetic reconstruction tree of 16S rRNA gene sequences of *Aspidodera fasciata* in the present study (consensus sequence) and *Aspidodera* sequences from GenBank. The species *Lauroia bolivar* and *L. trinadensis* are outgroups. Node values are BP-ML.

5.3.5. Remarks

The species most similar to *A. fasciata* is *A. sogandaresi*. They have in common some morphometric aspects (Tables 2 and 3), trapezoidal cephalic cordon, medium size hood, same number and arrangement of amphid, papilla and papillary nodule, short and robust spicules, small caudal spine and absence of lateral alae (Table 4). The morphological differences between them are mostly in details of lips on hood. In *A. fasciata* the digitiform projection of dextroventral lip is conspicuous with marked undulations, while in *A. sogandaresi* it is blunt with shallow undulations. The *A. kinsellai* Jiménez, Carreno and Gardner, 2013 and *A. vazi* Proença, 1937 also present trapezoidal cephalic cordon, but their hoods are large, spicules are medium and caudal spine are long, differing from *A. fasciata* (tables 2, 3 and 4). The trapezoidal cordon shape of *A. fasciata* differs from the rounded cordon of *A. lacombeae* Vicente, 1964 (homogeneous loops) and from the single aspect cordon of *A. ansirupta* Proença, 1937 (interrupted anteriad loops), *A. serrata* Cesário *et al.*, 2021 (serrated aspect) and *A. scoleciformis* (square shaped) (Table 4). *Aspidodera fasciata* have some morphometric similarities to *A. binansata* but this one differs by the rounded aspect of cephalic cordons with three posteriad loops on dorsal lip, by the presence of the lateral alae, long female tail and long caudal spine (Table 4).

There is no report about misidentifications regarding *A. scoleciformis*. This is probably due to the combination of easily observable characteristics, even at light microscopy, that makes it unique in the genre. We believe this is the reason of absolute concordance between morphometric parameters obtained by us and available in the literature compilation (Tables 2 and 3). *Aspidodera scoleciformis* can be differentiated between the congeners mainly by the square shaped groove on cephalic cordons. It also presents short hood (only bigger than *A. ansirupta*, which also has an unmistakable non-anastomosed cephalic cordon) and long body (only smaller than *A. lacombeae*, which has uniform rounded cephalic cordon and a bigger hood). Its long spicules are similar to those on *A. lacombeae* and, the medium caudal spine is similar to those on *A. fasciata* and *A. sogandaresi* (Table 4).

Table 4. Morphological data of *Aspidodera* nematodes that parasitize xenarthrans.

	<i>A. fasciata</i>	<i>A. soganderesi</i>	<i>A. binansata</i>	<i>A. scoleciformis</i>	<i>A. lacombeae</i>	<i>A. kinsellai</i>	<i>A. vazi</i>	<i>A. serrata</i>	<i>A. ansirupta</i>
Anastomose on cephalic cordon	yes	yes	yes	Yes	yes	yes	yes	Yes	no
Cephalic cordon	trapezoidal	trapezoidal	rounded (three posteriad loops on dorsal lip)	Square	rounded (homogeneous loops)	trapezoidal	trapezoidal	Serrated	rounded posteriad (interrupted anteriad loops)
Hood	medium	medium	short to medium	short to medium	medium	long	medium to long	Long	short
Spicules	short and robust	short and robust	short and robust	long and slender	long and slender	medium	medium	n.i.	short
Caudal spine	short	short	long	Short	short	long	long	n.i.	long
Lateral alae	absent	absent	present	Absent	absent	absent	present	Present	present

n.i. nonexistent information.

5.4. Discussion

Our *A. fasciata* specimens presented marked undulated projection on dextroventral lip, cephalic cordons trapezoidal shaped and short-robust spicules, which confirmed the species identification by SEM (Jiménez-Ruiz *et al.*, 2006). They were larger in lengths than the ones from literature (Tables 2 and 3), but the ratios hood/body and spicules/body lengths were similar to those from armadillos of Paraguay (Pallares & Usher, 1971) - us: 2.4% and 3.8% vs. them: 2.4% and 3.4%, respectively. Griesse (2007) and Moutinho (2009) obtained similar morphometry of *A. fasciata* to ours. However, the few specimens sampled, absence of some features measurement and the data not validated by scientific sieves, led us not to include them in the review tables. We consider the great morphometric range, well characterized in all species of *Aspidodera* that parasitize xenarthrans (Tables 2 and 3), as possible intraspecific variability. It is probably a result of different infection intensity or due to other host-parasite-environment relationships, that can affect the parasites size, but also the diversity they are found in the host (Poulin & George-Nascimento, 2007; Bordes & Morand, 2008). Further study will be necessary to evaluate these influences, although they seem to be quite common in plant parasitic nematodes (Salalia *et al.*, 2017; Sumita *et al.*, 2018).

The improvement of techniques and microscopes allowed elucidating some taxonomic mistakes throughout history, like those that have already occurred with *A. fasciata*. Yorke & Maplestone (1926) recorded it, but later, Proença (1937) described it as *A. ansirupta*. Sprehn (1932 *apud* Vicente *et al.*, 1997) referred to *A. fasciata* as *A. binansata*, which also was mistakenly identified as *A. fasciata* by Travassos (1913). Chandler and Chapin reported *A. fasciata* from USA armadillos in late 40's (*apud* Jiménez-Ruiz *et al.*, 2006). Both considered them different from the South American specimens, by being smaller, but none found sufficient to warrant description of a new species. Only in 2006, Jiménez-Ruiz and collaborators analyzed additional specimens and concluded it was actually a new species, *A. sogandaresi*. In fact, *A. fasciata* and *A. sogandaresi* are very similar and difficult to differentiate using only morphology and morphometry. Our molecular phylogenetic analyses suggested that *A. fasciata* from *M. tridactyla* is closely related to *A. sogandaresi* by forming a well-supported monophyletic group, consistent with morphological data.

The present work provide the first ultrastructural observation of *A. scoleciformis* and details the following structures of *A. fasciata*: amphid, cephalic papilla, excretory pore, vulva, anus, caudal papillae, spicule, cloaca and caudal spine. It also presented *Aspidodera* phylogeny

including new molecular data about *A. fasciata*, showing its grouping with *A. sogandaresi*. It provided a taxonomic comparative approach on *Aspidodera* from xenarthrans hosts, very useful in differentiating species with high similarity and likelihood of meeting in sympatry. We recommend the priority use of morphology and SEM during identification of dubious specimens and morphometry on extremes differentiation, considering the significant overlap between the *Aspidodera*.

5.5. References

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6. MANUSCRITO 4:

Uma nova espécie de *Aspidodera* (Nematoda: Heterakoidea) parasitando o tamanduá-bandeira *Myrmecophaga tridactyla* (Pilosa: Myrmecophagidae) no Brasil e nova chave para as espécies

Resumo

O aumento relativamente recente nas descrições de espécies de *Aspidodera* sugere que há várias espécies que ainda não foram documentadas e descritas de maneira adequada. Descrevemos *Aspidodera serrata* n. sp. do tamanduá-bandeira *Myrmecophaga tridactyla* do bioma Cerrado (Cerrado), Brasil. A nova espécie é distinta no gênero devido aos atributos dos cordões cefálicos, posição das papilas em forma de poros e espessamento da cutícula do corpo. Como nenhum espécime macho foi recuperado do hospedeiro, características morfológicas idênticas em ambos os sexos e específicas para o gênero foram fundamentais durante a identificação. Enfatizamos a relevância das características da coifa e da microscopia eletrônica de varredura para basear as descrições em *Aspidodera*. Fornecemos uma nova chave para o gênero para auxiliar nos esforços de identificação das espécies presentes em infecções múltiplas. Este é o primeiro relato de uma coinfecção com 3 congêneres no mesmo hospedeiro individual envolvendo *Aspidodera*. Incitamos os pesquisadores a preservarem e documentarem os parasitas metazoários de espécies de tetrápodes consideradas vulneráveis, uma vez que podem ser fontes valiosas de informações sobre a distribuição de parasitas na natureza.

Palavras-chaves: Aspidoderidae. Nematoda. Coinfecção. Microscopia eletrônica de varredura. Microscopia de luz. Chave taxonômica.

CESÁRIO, C. S.; GOMES, A. P. N.; MALDONADO JR, A.; OLIFIERS, N.; JIMENEZ, F. A.; BIANCHI, R. C. A new species of *Aspidodera* (Nematoda: Heterakoidea) parasitizing the giant anteater *Myrmecophaga tridactyla* (Pilosa: Myrmecophagidae) in Brazil and new key to species. *Comparative Parasitology*, NewYork, v. 88, n. 1, p. 7-14, JAN 2021. DOI: 10.1654/1525-2647-88.1.7 Disponível em: <https://bv.fapesp.br/pt/publicacao/198047/a-new-species-of-aspidodera-nematoda-heterakoidea-parasit/>. Acesso em: 25/10/2021.

A new species of *Aspidodera* (Nematoda: Heterakoidea) parasitizing the giant anteater *Myrmecophaga tridactyla* (Pilosa: Myrmecophagidae) in Brazil and new key to species

Abstract

The relatively recent surge in *Aspidodera* species descriptions suggests there are several species that are yet to be documented and properly described. We describe *Aspidodera serrata* n. sp. from the giant anteater *Myrmecophaga tridactyla* from the Cerrado biome (Savanna), Brazil. The new species is distinctive in the genus because of the attributes of the cephalic cordons, position of pore-like papillae and a thickening of body cuticle. Since no male specimens were recovered from the host, morphological characteristics that are identical in both sexes and specific for the genus were fundamental during the identification. We emphasize the relevance of hood features and scanning electron microscopy to base descriptions in *Aspidodera*. We provide a new key for the genus to assist in the efforts to identify the species present in multiple infections. This is the first report of a coinfection with 3 congeners in the same individual host involving *Aspidodera*. We urge researchers to preserve and document the metazoan parasites from tetrapod species considered vulnerable, since they can be valuable sources of information on the distribution of parasites in the wild.

Keywords: Aspidoderidae. Nematoda. Coinfection. Scanning electron microscopy. Light microscopy. Taxonomic key.

6.1. Introduction

The family Aspidoderidae Freitas, 1956 is a group of nematodes that parasitize the cecum and large intestine of xenarthrans, didelphiomorphs, and hystricognath and sigmodontine rodents (Santos *et al.*, 1990) distributed in the southern Nearctic and Neotropical regions (Jiménez-Ruiz *et al.*, 2008). The genus *Aspidodera* Railliet and Henry, 1912 has 11 known species, which can be diagnosed primarily by the expansion of the anterior end and a hood adorned with cordons (Inglis, 1957). The presence of *Aspidodera* in a number of different mammal taxa indicates the occurrence of multiple events of host switching and continental dispersion (Jiménez-Ruiz *et al.*, 2008). However, the recent surge in species descriptions (Jiménez *et al.*, 2013; Chagas-Moutinho *et al.*, 2014) suggests that many more *Aspidodera* have yet to be discovered and described formally.

The giant anteater *Myrmecophaga tridactyla* Linnaeus, 1758 is a vulnerable Neotropical xenarthran mammal with an ample distribution in Central and South America (Miranda *et al.*, 2014). Little is known about the helminth fauna of *M. tridactyla*, and most of the published data are found in articles dealing with the description of its helminth species (Vicente *et al.*, 1997). Giant anteaters may be exposed to helminths through the accidental consumption of soil particles contaminated with eggs, such as those of aspidoderids.

Three *Aspidodera* species have been reported parasitizing two anteater species in Brazil. In *M. tridactyla*, the nematode *A. fasciata* (Schneider, 1866) Railliet and Henry, 1913 was collected from giant anteaters in Mato Grosso state (Vicente, 1966), while *A. scoleciformis* (Diesing, 1851) Railliet and Henry, 1912 was collected from giant anteaters examined in São Paulo state (Vicente, 1966). In the case of the southern tamandua, *Tamandua tetradactyla*, *A. lacombeae* Vicente, 1964 was collected in Amazonas state. In the present study, we describe a new species, *A. serrata* n. sp., infecting a giant anteater in the savanna biome (Cerrado) of Southeastern Brazil and provide a new taxonomic key for the genus *Aspidodera*, based on 2 female specimens.

6.2. Material and methods

The carcasses of two adult giant anteaters (*M. tridactyla*), a male and a female, were found in the Santa Bárbara Ecological Station (SBES, 22°48' S, 49°14' W, state of São Paulo, Southeastern Brazil), and necropsied in June and July 2015, respectively. The SBES

encompasses 2712 hectares of protected Brazilian savanna biome, characterized by a vegetation mosaic, which includes Cerrado *sensu lato*, gallery forest, patches of semideciduous forest, and tracts of exotic *Pinus* and *Eucalyptus* trees (Melo & Durigan, 2011).

Nematodes were collected from the large intestine, washed in saline solution and stored in 70% ethanol. They were separated, cleared in lactophenol and analyzed under an Axion Scope A1 Light Microscope (Zeiss, Göttingen, Germany) for morphological characterization. Drawings were obtained with the aid of a camera lucida attached to a standard Zeiss microscope. The morphological features used for general identification of the specimens were obtained from the diagnoses of Proença (1937), Vicente (1966), Santos *et al.* (1990), and Vicente *et al.* (1997). All measurements were recorded in micrometers. Proportions between hood length/body length, esophagus length/body length, distance of nerve ring to anterior end/esophagus length and distance of excretory pore to anterior end/esophagus length, were expressed as percentage. For egg length and width, the range was given first, followed by sample mean, sample size and the coefficient of variation (as a percent value), the last two between parentheses. Egg measurements were taken only from the holotype, since the paratype was prepared to perform the SEM technique.

The morphometric data on the *Aspidodera* species most similar to the new species were included for comparative purposes, and were obtained from the literature (Travassos, 1913; Proença, 1937; Pallares & Usher, 1971; Diaz-Ungria, 1979; Santos *et al.*, 1990; Vicente *et al.*, 1997; Jiménez-Ruiz *et al.*, 2006; Jiménez *et al.*, 2013). The relative abundance of each species was calculated as its percentage of the total number of congener specimens collected from an individual host. The specimen of the new species was deposited in the Helminthological Collection of the Oswaldo Cruz Institute (CHIOC nº 38749) in Rio de Janeiro, Brazil.

The specimen prepared for Scanning Electron Microscopy (SEM) was fixed for 1 hour at room temperature in 2.5% glutaraldehyde in 0.1 M Na-cacodylate buffer and washed in the same buffer (pH 7.2), post-fixed in 2% OsO₄ in 0.1 M Na-cacodylate buffer and then washed 3 times in the same buffer. This specimen was subsequently dehydrated in a graded series of ethanol (30–100%) in 1 hour steps, and then critical point-dried in CO₂, sputter-coated with gold thick of a layer about 20 nm, and examined using a JEOL JSM-6390 LV microscope (JEOL, Akishima, Tokyo, Japan) with an accelerating voltage of 15 kV at the Electron Microscopy Platform of the Oswaldo Cruz Institute.

6.3. Results

Nematoda Rudolphi, 1808
Secernentea Linstow, 1905
Ascaridida Skrjabin and Schulz, 1940
Heterakoidea Railliet and Henry, 1912
Aspidoderidae Freitas, 1956
***Aspidodera* Railliet and Henry, 1912**
***Aspidodera serrata* n. sp.**
(Figures 1-12)

6.3.1. Generic diagnosis

Aspidodera. Expanded cephalic end adorned with cordons, lips with papillae, simple mouth leading into a vestibule and esophagus with a terminal bulb (Inglis, 1957; Santos *et al.*, 1990; Anderson *et al.*, 2009).

6.3.2. Description (based on 2 female specimens)

Body fusiform and tail sharp (Figure 1). Long hood (or cephalic cap) with anastomosing cordons (Figures 2, 6, 7). Cephalic cordons with 6 anteriad and 6 posteriad loops, and smooth anterior and serrated posterior margins (Figures 2, 6, 7). Oral opening with 3 lips (Figure 8), each presenting 2 slender posterior projections (Figure 7). Dorsal lip with 2 digitiform projections, lateroventral lips with small, acute projection. Dextroventral lip projection presenting shallow double undulation that fits in sinistroventral lip sockets (Figure 8). Lateroventral lips with an amphid, a papilla and a papillary nodule (Figures 2, 8). Cephalic cordons touching base of hood (Figures 2, 6, 7). Serrated margin enclosing cuticular expansions from body to hood, named interlabium and labial-root. Interlabium long and slender, labial-root trapezoidal in shape (Figures 6, 7). Pore-like papilla on labial-root of lateroventral lips at mid-dorsal portion (Figures 2, 7). Fine transversal cuticular striations along body, thick neck-like cuticle posterior to hood (Figures 1, 2, 7). Short vestibulum does not exceed half-length of the hood. Long esophagus with terminal pyriform bulb with valve, followed by long intestine. Nerve ring surrounding esophagus, placed on the anterior half of the entire esophagus (including vestibulum and bulb). Excretory pore located on the posterior half of the entire esophagus (Figure 1).

Female. Oviparous, didelphas, amphidelphas, with ventral vulva and lightly protuberant lips (Figure 10) opening in mid-anterior portion of body. A relatively short and muscular ovojector goes from the vulvar opening to the posterior region of body. Ovojector connects to the uteri through vulvar vestibules. Opposite uteri, a posterior and an anterior (Figure 3). Elliptical eggs not embrionated in uterus (Figure 3) with thick and smooth shell (Figure 5). Anus with a small papilla on each side and posterior protuberance (Figures 4, 11). Larger papilla and phasmid on both side of tail, in final third of length (Figure 12). Lateral alae present (Figure 9), starting posterior to the thick neck-like cuticle to the anus.

Holotype: Body length 7157, width at level of vulva 152, hood 319 long and 175 wide. Ratio between hood length and body length, 4.5%. Esophagus length with bulb 1340 (including vestibulum), bulb 205 long and 155 wide. Ratio between esophageal length (from the oral opening to the bulb) and body length, 18.7%. Nerve ring to anterior end 659, excretory pore to anterior end 787. Ratio between nerve ring-anterior end and length of the esophagus, 49.2%. Ratio between excretory pore-anterior end and length of the esophagus, 58.7. Vulva to anterior end 3157, anus to posterior end 521, elliptical eggs range 54–66, 62.1 (n = 10, 6.2%) long and 41–50, 46.3 (n = 10, 5.6%) wide.

Paratype: Body length 6105, width at level of vulva 184, hood 361 long and 244 wide. Ratio between hood length and body length, 5.9%. Esophagus length with bulb 1244 (including vestibulum), bulb 186 long and 171 wide. Ratio between esophageal length (from the oral opening to the bulb) and body length, 20.4%. Nerve ring to anterior end 542, excretory pore to anterior end 659. Ratio between nerve ring-anterior end and length of the esophagus, 43.6%. Ratio between excretory pore-anterior end and length of the esophagus, 53.0%. Vulva to anterior end 2815, anus to posterior end 574.

6.3.3. Taxonomic summary

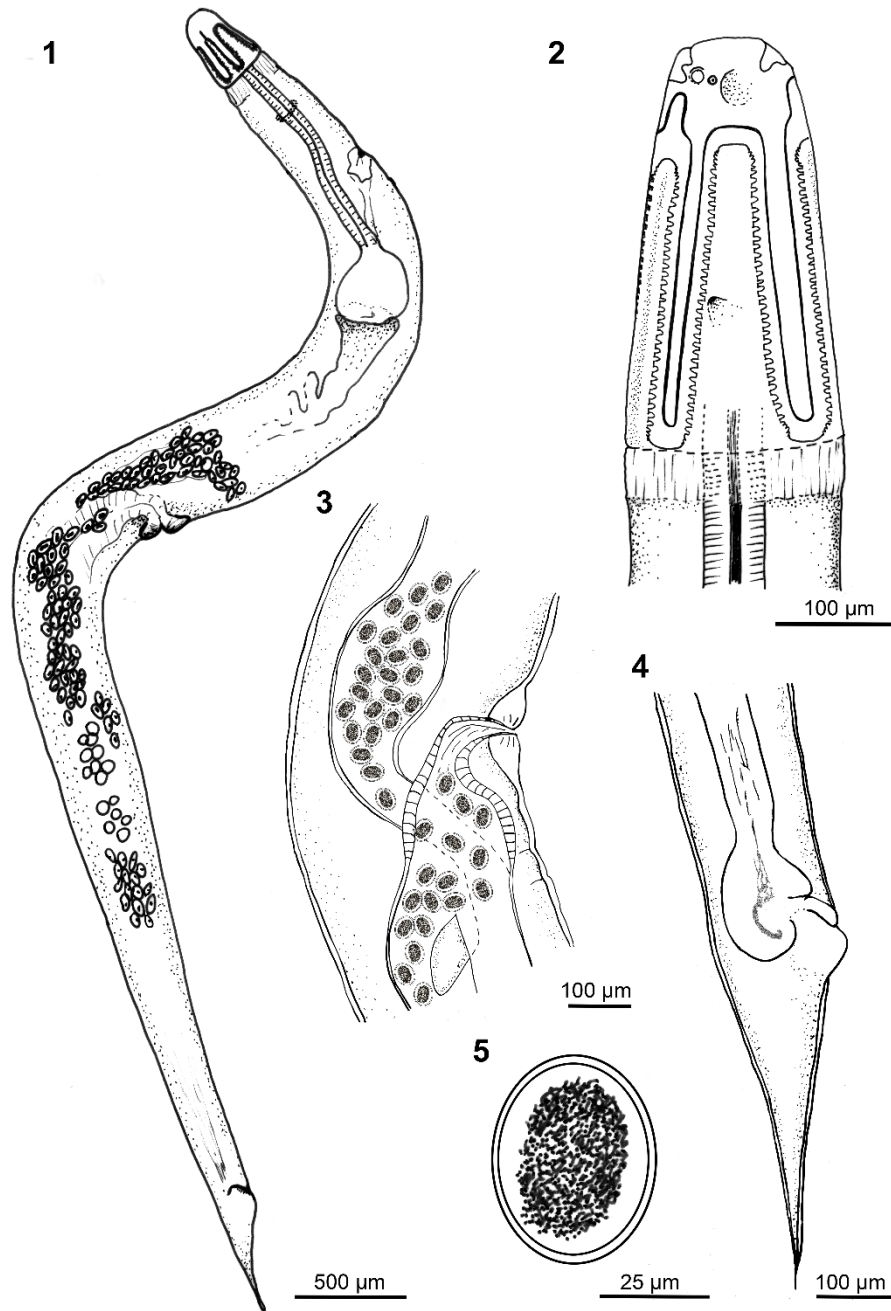
Type host. Giant anteater, *Myrmecophaga tridactyla* Linnaeus, 1758.

Type locality. Santa Bárbara Ecological Station, municipality of Águas de Santa Bárbara, state of São Paulo, Brazil (22°48'59" S, 49°14'12" W).

Site of infection. Large intestine.

Specimen deposited. Holotype CHIOC n° 38749 (female).

Etymology. The specific epithet (*serrata*) refers to the most characteristic morphological structure of the new species, the serrated posterior margin of the velum in the cephalic cordons.



Figures 1-5. Line drawings of *Aspidodera serrata* n. sp., female. 1. Entire nematode, lateral view. 2. Hood showing dextroventral lip, amphid, papilla, papillary nodule, interlabium, labial-root with pore-like papilla, serrated cephalic cordons and neck-like cuticle. 3. Genital system showing vulva, muscular ovojector, vulvar vestibules and posterior and anterior uterus. 4. Terminal portion of the gastrointestinal system, showing rectum and anus. 5. Unembryonated egg with thick and smooth shell.

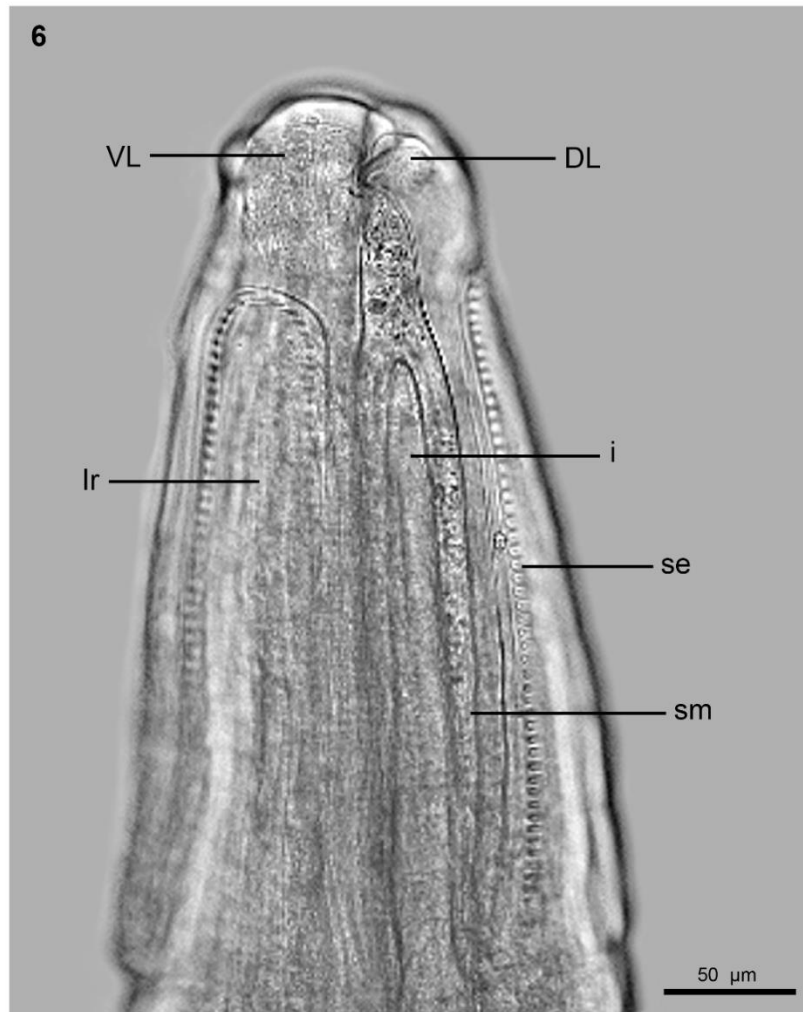
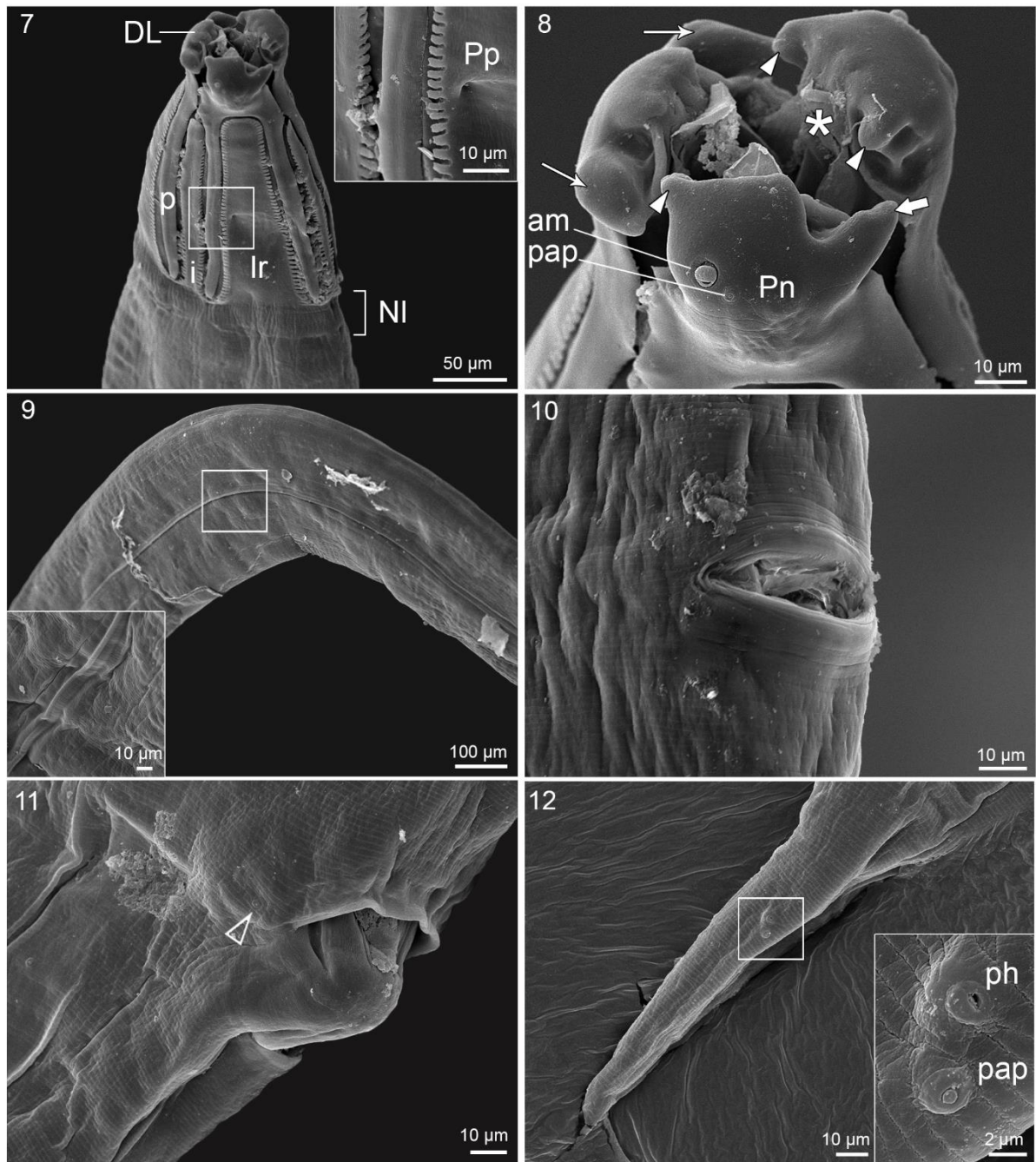


Figure 6. Light micrograph of *Aspidodera serrata* n. sp., female. Hood showing sinistroventral lip (VL), dorsal lip (DL), cephalic cordons with anterior smooth cordons (sm) and posterior serrated cordons (se), interlabium (i) and inter-root expansions (Ir).



Figures 7-12. Scanning electron micrographs of *Aspidodera serrata* n.sp., female. 7. Hood showing dorsal lip (DL), lateroventral lips, serrated cephalic cordons, slender projections of lips (p), interlabium (i), inter-root expansions (Ir) and neck-like cuticle (NI). In detail, pore-like papilla (Pp) and serrated cephalic cordons. 8. Oral aperture with lips showing digitiform projections (thin arrow), small acute projections (full arrowhead), dextroventral lip projection shallow undulated (large arrow), amphid (am), papilla (pap), papillary nodule (Pn). Cephalic end showing the oral vestibulum lined with cuticular membranes with an external smooth edge (asterisk). 9. Lateral alae. 10. Vulva. 11. Anus with small papillae (empty arrowhead). 12. Posterior end. In detail, phasmid (ph) and papilla (pap) on tail.

6.3.4. Remarks

The nematodes recovered from the giant anteater (*M. tridactyla*) in Cerrado biome of São Paulo state, Brazil, presents 3 putative autoapomorphies: (i) cephalic cordons with serrated posterior margins, (ii) pore-like papillae in the mid-dorsal portion of the labial-root in the lateroventral lips, and (iii) a neck-like cuticle posterior to the hood. Serrated cordons and neck-like cuticles had not been reported previously in the genus. A pore-like papilla has been observed at the interlabium apex in *A. raillieti* Travassos, 1913 (Chagas-Moutinho *et al.*, 2007), *A. lanfredi* Chagas-Moutinho, Sant'Anna, Oliveira-Mendes and De Souza, 2014, and *A. scoleciformis* (Sant'Anna, personal communication *apud* Chagas-Moutinho *et al.*, 2014), but not in the labial-root.

Aspidodera serrata n. sp. possesses anastomosed cephalic cordons, distinguishing it from *A. ansirupta* Proença, 1937, in which the cephalic cordons are not anastomosed. The shape of the cephalic cordons also distinguishes the new species from *A. binansata* Railliet and Henry, 1913, *A. lacombeae*, *A. scoleciformis*, and *A. subulata* (Molin, 1860) Railliet and Henry, 1912. The number and position of the amphids, papillae and papillary nodules, and also the trapezoidal labial-root and slender interlabium of *A. serrata* n. sp. are all similar to the configuration found in *A. kinsellai* Jiménez, Carreno and Gardner, 2013, *A. sogandaresi* Jiménez-Ruiz, Gardner and Varela-Stokes, 2006, *A. vazi* Proença, 1937, and *A. fasciata*. The digitiform projection of the dextroventral lip of *A. serrata* n. sp. is similar to that found in *A. kinsellai* and *A. sogandaresi*, but contrasts with that found in *A. fasciata*, which has distinctly undulating projections. A lateral alae is present in *A. serrata* n. sp., *A. binansata*, *A. ansirupta* and *A. raillieti*, although it is absent in *A. kinsellai*, *A. sogandaresi*, *A. fasciata*, *A. scoleciformis*, *A. subulata*, *A. lacombeae*, and *A. lanfredi*.

There is considerable overlap in morphometric features between *A. serrata* n. sp. and its most similar congeners (*A. kinsellai*, *A. sogandaresi*, *A. vazi*, and *A. fasciata*), due primarily to the considerable amplitude of the parameters. Even so, *A. kinsellai* appears to be the species most similar to *A. serrata* n. sp., especially in body length and hood length and width (Table 1). The most divergent values were found in *A. vazi* (hood and bulb) and *A. sogandaresi* (nerve ring to anterior end and vulva to anterior end).

Table 1. Comparative measurements for female specimens of *Aspidodera serrata* n. sp. (this study) and the most similar *Aspidodera* species (literature compilation). Ranges of body, hood, bulb and egg are for length and width (separated by a “x”).

SPECIES*	BODY	HOOD	ESOPHAGUS	BULB	EXCRETORY PORE†	NERVE RING†	VULVA†	EGG
<i>A. serrata</i> n. sp.	6105-7157 x 152-184	319-361 x 175-244	1244-1340	186-205 x 155-171	659-787	542-659	2815-3157	54-66 x 41-50
<i>A. kinsellai</i>	5307-6906 x 346-557	362-440 x 175-258	1324-1640	166-244 x 172-235	700-955	504-611	2431-3112	57-78 x 37-61
<i>A. sogandaresi</i>	4711-6886 x 219-385	213-312 x 114-258	1038-1504	158-221 x 123-238	549-757	361-523	1811-2807	60-92 x 40-70
<i>A. vazi</i>	5000-9200 x 380-570	344-530 x ---	1060-1650	186-260 x 180-240	736-1100	780-1100	2550-3367	50-72 x 36-54
<i>A. fasciata</i>	5400-10000 x 270-660	200-304 x ---	1200-1850	190-330 x 150-263	660-1000	480-900	2350-3760	52-60 x 40-48

* All measurements are reported as micrometer values.

† Distance to anterior end.

6.3.5. Key for the species

1. Cephalic cordons non-anastomosed anteriorly in labial-root *Aspidodera ansirupta*
 Cephalic cordons anastomosed anteriorly in labial-root 2
2. Dorsal lip with 2 anterior loops and 3 posterior loops *A. binansata*
 Dorsal lip with 1 anterior loop and 2 posterior loops 3
3. Cephalic cordons with serrated aspect *A. serrata* n. sp.
 Cephalic cordons with smooth aspect 4
4. Cephalic cordons square shaped in labial-root *A. scoleciformis*
 Cephalic cordons trapezoidal or rounded shaped in labial-root 5
5. Long caudal spine, above 0.1 mm length 6
 Short to medium caudal spine, up to 0.07 mm length 7
6. Constriction between caudal spine and tail *A. vazi*
 Without constriction between caudal spine and tail *A. kinsellai*
7. Cephalic cordons surpass the level of vestibulum, always touching the base of the hood 8
 Cephalic cordons do not surpass vestibulum and do not touch the base of the hood *A. subulata*
8. Hosts are marsupials and hood is up to 0.19 mm length 9
 Hosts are xenarthrans and hood is longer than 0.17 mm 10
9. Small acute projection in sinistroventral lip, base of interlabium wider than the apex, papillary nodule present on lateroventral lips *A. lanfredi*
 Digitiform projection in sinistroventral lip, slender interlabium, papillary nodule absent on lateroventral lips *A. raillieti*
10. Short caudal spine up to 0.014 mm length *A. lacombeae*
 Medium caudal spine above 0.025 mm length 11
11. Conspicuous digitiform projection of dextroventral lip with marked undulations *A. fasciata*
 Blunt projection of dextroventral lip with shallow undulations *A. sogandaresi*

6.3.6. *Aspidodera* species recovered from the host

We recovered 3 species of *Aspidodera* from the adult female *M. tridactyla* host, including the new species described here. The adult male *M. tridactyla* host did not contain any *Aspidodera*. *Aspidodera fasciata* was the most abundant, with 86.9% of the specimens collected (139/160) and a sex ratio of 1.2: 1 (76 males vs. 63 females). *Aspidodera scoleciformis* provided a further 11.9% of the specimens, with a sex ratio of 0.58: 1 (7 males vs. 12 females). *Aspidodera serrata* n. sp. was represented by only 2 females (1.2%).

6.4. Discussion

The aspidoderid hood is an important diagnostic character for the detection of morphological variability and conservatism (Inglis, 1957; Jiménez-Ruiz *et al.*, 2008). This is illustrated perfectly in *A. serrata* n. sp., whose unique characteristics provide important insights into the variability in the cephalic structures that are nevertheless typical of the genus. This permitted the description of the new species based only on female specimens, with the use of the SEM. It is important to note, however, that other well-established morphological and morphometric features were fundamental to the diagnosis of the new species and its insertion in the taxonomic key.

The 2 known species we found in the giant anteater, *A. fasciata* and *A. scoleciformis*, as well as *A. raillieti*, occur in the greatest diversity of host species among the congeners. However, while *A. scoleciformis* and *A. raillieti* seem to be more generalist, as they occur in xenarthrans and marsupials (in the first case) and in marsupials and rodents (in the second case), *A. fasciata* has only been reported, so far, in xenarthrans (armadillos and giant anteater). Other hosts for *A. fasciata* are *Cabassous unicinctus* Linnaeus, 1758, *Dasypus novemcinctus* Linnaeus, 1758, *Euphractus sexcinctus* Linnaeus, 1758, *Priodontes maximus* Kerr, 1792, *Tolypeutes matacus* Desmarest, 1804 and *T. tricinctus* Linnaeus, 1758 (Santos *et al.*, 1990; Vicente *et al.*, 1997). *Aspidodera scoleciformis* also parasitize *C. unicinctus*, *Chaetophractus villosus* Desmarest, 1804, *D. novemcinctus*, *E. sexcinctus*, *Marmosa murina* Linnaeus, 1758, *Micoureus paraguayanus* Tate, 1931, *Monodelphis domestica* Wagner, 1842 and *T. tricinctus* (Santos *et al.*, 1990; Vicente *et al.*, 1997).

The coinfection of a host by multiple parasite species is a common pattern in natural populations (Petney & Andrews, 1998; Griffiths *et al.*, 2011). Associations have been reported between a number of pairs of *Aspidodera* species, including *A. fasciata* and *A. scoleciformis* (Travassos, 1913; Vicente, 1966; Fugita *et al.*, 1995), *A. fasciata* and *A. binansata* (Cameron, 1939), *A. raillieti* and *A. subulata* (Vicente, 1966; Santos *et al.*, 1990), and *A. kinsellai* and *A. sogandaresi* (Jiménez *et al.*, 2013). A coinfection of the giant anteater (*M. tridactyla*) by *A. fasciata* and *A. scoleciformis* was already recorded (Vicente, 1966), and in the present study, both these species were found in sympatry with *A. serrata* n. sp. As in all previous cases of sympatry, *A. fasciata* was the most abundant species, reflecting its dominance in the different hosts it occurs.

This study presents the description of the 12th *Aspidodera* species known to science, *A. serrata* n. sp., distinguished based on the serrated pattern of its cephalic cordons. The study also updates the taxonomic key for the species of the genus, including features that can only be discerned by electron microscopy. The study also documents the first reported case of triple coinfection by *Aspidodera* species in a single host specimen. This discovery of a new *Aspidodera* species in the giant anteater (*M. tridactyla*) reinforces the need for the more systematic sampling of the biodiversity of the Neotropical region and underscores the importance of examining every single individual parasite found in an infected organism. This practice will help scientists to detect rare species as well as providing information on associations among the species found within the same ecosystem. The very low intensity of *A. serrata* n. sp. found in the giant anteater (*M. tridactyla*) specimen may suggest that this mammal is not the principal host, although *Aspidodera* seems to be poorly host specific in general.

6.5. References

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7. CONSIDERAÇÕES FINAIS

As Unidades de Conservação da Natureza (UCs) existem como medida estratégica para enfrentar a intensa conversão do uso da terra e manter a diversidade biológica. São espaços territoriais e marinhos detentores de atributos naturais e/ou culturais de especial relevância para a conservação, preservação e uso sustentável de seus recursos. Entretanto, sua distribuição irregular no tempo e no espaço, adicionado a pressões imobiliárias, turísticas, do agronegócio e de outras fontes antrópicas (caça, invasão biológica, atropelamentos, incêndios, agrotóxicos), ameaçam sua eficiência, de forma que nem sempre se garante a manutenção e conexão dos ecossistemas. A avaliação de áreas prioritárias para criação de UCs é baseada numa série de indicadores que resumidamente avaliam seu valor biológico adicionado ao seu grau de ameaça. Por isso é de praxe se realizar o monitoramento de fauna, onde geralmente se avalia o número, a diversidade e a composição de espécies. Entretanto, recentemente tem-se incorporado a saúde dos animais silvestres como abordagem complementar, e muitas vezes indispensável, na proteção da biodiversidade e concretização da saúde única. O contexto atual de pandemia reforça essa necessidade de se identificar determinantes ambientais de saúde para preservá-la em todas as espécies, a fim de se desenvolver medidas de controle e prevenção.

Os resultados com o atual trabalho e demais originados de uma pesquisa com os tamanduás-bandeira da Estação Ecológica de Santa Bárbara/SP sugerem o quão fragilizadas podem estar essa e outras populações que vivem em condições semelhantes. Pudemos perceber que os métodos combinados de avaliação da saúde, envolvendo exame físico, monitoramento anestésico, exames sanguíneos, coproparasitológicos, métodos moleculares e estudo de parasitos adultos são muito esclarecedores em retratar o estado dos indivíduos e da população, mesmo com uma amostragem pequena. Realizamos um esforço de campo expressivo na busca desses indivíduos e verificamos que as estratégias e os métodos de imobilização física e química, comumente usados para a espécie em diferentes locais, foram eficientes. Destacamos que, em locais com provável baixa densidade de tamanduás-bandeira, onde não se conhece o histórico de saúde da população e com paisagem desfavorável à captura, é preciso redobrar o planejamento, ter acesso a técnicas e equipamentos que garantam segurança e bem-estar e incorporar pessoas experientes na equipe. Obtivemos uma grande riqueza de informações, ainda não esgotadas, sobre os parasitas adultos de tamanduás-bandeira a partir de um único hospedeiro, resultando na descrição de uma nova espécie e redescritção de outras duas. Isso demonstra o quanto ainda desconhecemos sobre muitos aspectos básicos das espécies da fauna

brasileira, já que mesmo para uma espécie de mamífero de grande porte e carismática, como o tamanduá-bandeira, sua helmintofauna ainda é tão pouco conhecida.

Os desafios em busca da conservação da biodiversidade perpassam questões técnicas, éticas, financeiras, de amostragem, de recursos humanos e começam desde antes do campo, passando pelas bancadas, publicações e culminam na transformação do resultado em ação. Apesar de o atual momento político do Brasil ser provavelmente o de maior retrocesso na área ambiental, pudemos acompanhar alguns avanços. Das 20 metas de Aichi estabelecidas na COP-10 para a detenção da perda da diversidade biológica, e que deveriam ser cumpridas até 2020, o maior avanço do Brasil foi na meta 19. Ela trata do conhecimento científico e tecnológico sobre a biodiversidade e seu registro e compartilhamento em plataformas abertas. Os Planos de Ação Nacionais de conservação de espécies ameaçadas, como o PAN do tamanduá-bandeira e do tatu-canastra (2019-2024, 1º ciclo), foram frutos do esforço de grupos de especialistas que transcendem à qualidade técnica e deslocam do eixo os entraves políticos ao desenvolvimento ambiental, carregando consigo o enorme afeto pelo ofício de se conservar espécies. Um dos oito objetivos deste PAN é ampliar o conhecimento sobre história natural, ecologia e saúde do tamanduá-bandeira, visto a enorme defasagem que ainda temos, mesmo diante de tantas informações reunidas nos últimos 15 anos para a espécie. E é claro que uma abordagem tão ampla é extremamente desafiadora, porque envolve grandes equipes multidisciplinares e imersões longas e de alto custo financeiro em campo, análises complexas e, mais que isso, transpor os vazios entre as ilhas da ciência, educação e política. Este é o grande desafio: superar o academicismo, criar soluções, traduzir o conteúdo, sensibilizar e democratizar a ciência.

ANEXO A

Parecer de aprovação do projeto na Comissão de Ética no Uso de Animais (CEUA) da Faculdade de Ciências Agrárias e Veterinárias da Universidade Estadual Paulista.

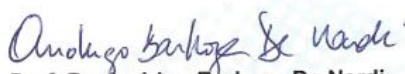


UNIVERSIDADE ESTADUAL PAULISTA
"JÚLIO DE MESQUITA FILHO"
Câmpus de Jaboticabal

**CEUA – COMISSÃO DE ÉTICA NO USO DE ANIMAIS****CERTIFICADO**

Certificamos que o Protocolo nº 003414/13 do trabalho de pesquisa intitulado **"Telemetria via satélite como ferramenta para avaliar o uso da paisagem por tamanduás-bandeira (*Myrmecophaga tridactyla*) no Parque Estadual das Furnas do Bom Jesus e áreas antropizadas do entorno, São Paulo, Brasil"**, sob a responsabilidade da Profª Drª Rita de Cássia Bianchi, de acordo com os Princípios Éticos na Experimentação Animal, adotado pelo Colégio Brasileiro de Experimentação (COBEA) foi aprovado pela COMISSÃO DE ÉTICA NO USO DE ANIMAIS (CEUA), em reunião ordinária de 13 de março de 2013.

Jaboticabal, 22 de março de 2013.


Prof. Dr. Andrigo Barboza De Nardi
Coordenador - CEUA

ANEXO B

Autorização para execução do projeto concedida pelo Instituto Brasileiro do Meio Ambiente e dos Recursos Naturais (IBAMA) e Instituto Chico Mendes de Conservação da Biodiversidade (ICMBio), Brasil.



Ministério do Meio Ambiente - MMA
Instituto Chico Mendes de Conservação da Biodiversidade - ICMBio
Sistema de Autorização e Informação em Biodiversidade - SISBIO

Autorização para atividades com finalidade científica

Número: 38326-5	Data da Emissão: 16/06/2015 09:10	Data para Revalidação*: 15/07/2016
* De acordo com o art. 28 da IN 03/2014, esta autorização tem prazo de validade equivalente ao previsto no cronograma de atividades do projeto, mas deverá ser revalidada anualmente mediante a apresentação do relatório de atividades a ser enviado por meio do Sisbio no prazo de até 30 dias a contar da data do aniversário de sua emissão.		

Dados do titular

Nome: Alessandra Bertassoni da Silva	CPF: 043.845.829-00
Título do Projeto: USO DA PAISAGEM POR TAMANDUÁS-BANDEIRA (<i>Myrmecophaga tridactyla</i>) COM USO DE TELEMETRIA VIA SATÉLITE NA ESTAÇÃO ECOLÓGICA DE SANTA BÁRBARA E SUAS ÁREAS ANTROPIZADAS DO ENTORNO, SÃO PAULO, BRASIL	
Nome da Instituição : UNESP CAMPUS S.J. R. PRETO	CNPJ: 48.031.918/0011-04

Cronograma de atividades

#	Descrição da atividade	Início (mês/ano)	Fim (mês/ano)
1	Caracterização da paisagem	06/2013	07/2013
2	Compra e importação de equipamentos	06/2013	09/2013
3	Visitas prévias à área de estudo	06/2013	11/2013
4	Capturas de tamanduas-bandeira	12/2013	03/2014
5	Monitoramento via satélite e campo	12/2013	03/2015
6	Retirada dos coletores equipados	12/2014	03/2016
7	Análise de dados	02/2015	10/2017

Observações e ressalvas

1	As atividades de campo exercidas por pessoa natural ou jurídica estrangeira, em todo o território nacional, que impliquem o deslocamento de recursos humanos e materiais, tendo por objeto coletar dados, materiais, espécimes biológicos e minerais, peças integrantes da cultura nativa e cultura popular, presente e passada, obtidos por meio de recursos e técnicas que se destinem ao estudo, à difusão ou à pesquisa, estão sujeitas a autorização do Ministério de Ciência e Tecnologia.
2	Esta autorização NÃO exige o pesquisador titular e os membros de sua equipe da necessidade de obter as anuências previstas em outros instrumentos legais, bem como do consentimento do responsável pela área, pública ou privada, onde será realizada a atividade, inclusive do órgão gestor de terra indígena (FUNAI), da unidade de conservação estadual, distrital ou municipal, ou do proprietário, arrendatário, posseiro ou morador de área dentro dos limites de unidade de conservação federal cujo processo de regularização fundiária encontra-se em curso.
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6	O titular de autorização ou de licença permanente, assim como os membros de sua equipe, quando da violação da legislação vigente, ou quando da inadequação, omissão ou falsa descrição de informações relevantes que subsidiaram a expedição do ato, poderá, mediante decisão motivada, ter a autorização ou licença suspensa ou revogada pelo ICMBio, nos termos da legislação brasileira em vigor.
7	Este documento não dispensa o cumprimento da legislação que dispõe sobre acesso a componente do patrimônio genético existente no território nacional, na plataforma continental e na zona econômica exclusiva, ou ao conhecimento tradicional associado ao patrimônio genético, para fins de pesquisa científica, bioprospecção e desenvolvimento tecnológico. Veja maiores informações em www.mma.gov.br/cgen .
8	Em caso de pesquisa em UNIDADE DE CONSERVAÇÃO, o pesquisador titular desta autorização deverá contactar a administração da unidade a fim de CONFIRMAR AS DATAS das expedições, as condições para realização das coletas e de uso da infra-estrutura da unidade.

Outras ressalvas

1	Todos os procedimentos nos animais deverão ser realizados por médico veterinário.
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Equipe

#	Nome	Função	CPF	Doc. Identidade	Nacionalidade
1	GUILHERME DE MIRANDA MOURAO	Pesquisador e colaborador	488.045.506-78	418653 SSP-MS	Brasileira
2	Rita de Cassia Bianchi	Orientadora e coordenação geral	019.855.387-09	1031222 SSP-ES	Brasileira

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