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Estrutura da comunidade de helmintos parasitos de *Bothrops atrox* (Linnaeus, 1758) (Serpentes: Viperidae) da Amazônia Oriental Brasileira

Belém,

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Dissertação apresentada ao Programa de Pós-Graduação em Zoologia, do convênio da Universidade Federal do Pará e Museu Paraense Emílio Goeldi, como requisito parcial para obtenção do título de Mestre em Zoologia.

Área de concentração: Biodiversidade e Conservação
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FRED GABREL HAICK DE MOURA

ESTRUTURA DA COMUNIDADE DE HELMINTOS PARASITOS DE *Bothrops atrox* (SERPENTES: VIPERIDAE)

Dissertação apresentada ao Programa de Pós-Graduação em Zoologia, do convênio da Universidade Federal do Pará e Museu Paraense Emílio Goeldi, como requisito parcial para obtenção do título de Mestre em Zoologia, sendo a COMISSÃO JULGADORA composta pelos seguintes membros:

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“É preciso sentir a necessidade da experiência, da observação,
ou seja, a necessidade de sair de nós próprios para aceder à
escola das coisas, se as queremos conhecer e compreender.”

Émile Durkheim

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SUMÁRIO

RESUMO

As serpentes desempenham um papel importante nos ciclos de vida de uma ampla variedade de helmintos parasitos, atuando tanto como hospedeiras definitivas quanto intermediárias. Diversos fatores podem influenciar a diversidade, a composição e a estrutura das comunidades parasitárias associadas a esses répteis. *Bothrops atrox,* uma serpente peçonhenta pertencente à família Viperidae com ampla distribuição pela Amazônia, é de grande relevância médica e, apesar de possuir uma helmintofauna relativamente bem conhecida, ainda há lacunas quanto à dinâmica das relações parasito-hospedeiro envolvendo essa espécie. Diante disso, nosso objetivo nesse estudo foi analisar a diversidade e estrutura da comunidade parasitária de *B. atrox* de duas localidades da Amazônia Brasileira. Estruturamos essa dissertação em dois capítulos, sendo que no primeiro apresentamos os resultados obtidos a partir de uma análise comparativa da estrutura da comunidade de helmintos parasitos de *B. atrox* de dois ambientes contrastantes da região amazônica: floresta ombrófila e campos naturais. Nossas análises revelam uma maior abundância de parasitos em hospedeiros proveniente de áreas florestadas, além de mostrar uma correlação positiva entre o tamanho dos hospedeiros e a abundância parasitária. Este é o primeiro estudo dedicado a investigar a diversidade e a estrutura das comunidades de helmintos de *B. atrox* em diferentes ecossistemas. No segundo capítulo apresentamos, com base em características morfológicas e dados moleculares, uma nova espécie do gênero *Kalicephalus*, que apresentou os maiores índices de abundância, dominância e prevalência nas comunidades florestais. Esse capítulo também tráz novas perspectivas sobre a história evolutiva do gênero, contribuindo para o entendimento da diversidade e da evolução desse grupo de parasitos. Assim, nosso estudo fornece dados inéditos para a compreensão da diversidade e ecologia de helmintos parasitos de serpentes na Amazônia.

Palavras-chave: Diversidade; Helmintos; Parasitos; *Bothrops atrox;* Amazônia

ABSTRACT

Snakes play an important role in the life cycles of a wide variety of parasitic helminths, acting as both definitive and intermediate hosts. Several factors can influence the diversity, composition, and structure of parasite communities associated with these reptiles. *Bothrops atrox*, a venomous snake belonging to the family Viperidae and widely distributed throughout the Amazon, is of great medical relevance. Despite having a relatively well-documented helminth fauna, gaps remain in our understanding of the parasite-host dynamics involving this species. Therefore, the objective of this study was to analyze the diversity and structure of the helminth community of *B. atrox* in two localities within the Brazilian Amazon. This dissertation is structured into two chapters. In the first, we present results from a comparative analysis of the helminth community structure in *B. atrox* from two contrasting Amazonian environments: ombrophilous forest and natural open fields. Our analyses revealed a higher abundance of parasites in hosts from forested areas, and a positive correlation between host body size and parasite abundance. This is the first study specifically dedicated to investigating the diversity and structure of helminth communities in *B. atrox* across different ecosystems. In the second chapter, we describe a new species of the genus *Kalicephalus*, based on morphological characteristics and molecular data. This species showed the highest levels of abundance, dominance, and prevalence in forest communities. The chapter also brings new insights into the evolutionary history of the genus, contributing to a better understanding of its diversity and evolution. Thus, our study provides new data for understanding the diversity and ecology of helminth parasites of snakes in the Amazon.

Keywords: Diversity: Helminth; Parasite; *Bothrops atrox*; Amazon

INTRODUÇÃO GERAL

As serpentes são vertebrados pertencentes à Ordem Squamata Oppel, 1811, com ampla distribuição geográfica e grande diversidade de hábitos, ocupando desde planícies desérticas até ambientes oceânicos (Wallach et al., 2014). A região Neotropical abriga uma notável diversidade de serpentes, distribuídas em 12 famílias (Guedes et al., 2017); no Brasil são registradas mais de 430 espécies, das quais aproximadamente 190 ocorrem no Bioma Amazônico (Nogueira et al., 2019; Uetz et al., 2024).

Entre essas, destacam-se as serpentes pertencentes à família Viperidae Oppel, 1811, caracterizada por apresentar uma dentição especializada para a inoculação de peçonha. Inserido nessa família, o gênero *Bothrops* Wagler, 1824 compreende serpentes neotropicais com ampla distribuição na América do Sul, e grande diversidade de traços ecológicos. Atualmente são descritas 48 espécies para esse gênero (Carrasco et al. 2012; Wallach et al., 2014; Uetz et al., 2024).

Das 29 espécies de *Bothrops* registradas no Brasil, sete ocorrem na região Amazônica: *Bothrops atrox* (Linnaeus, 1758); *B. bilineatus* (Wied, 1821); *B. brazili* Hoge, 1954; *B. marajoensis* Hoge, 1966; *B. mattogrossensis* Amaral, 1925; *B. oligobalius* Dal Vechio, Prates, Grazziotin, Graboski & Rodrigues, 2021; e *B. taeniatus* Wagler, 1824 (Nogueira et al., 2019; Costa et al., 2021).

Bothrops atrox (Fig. 1), popularmente conhecida como jararaca-da-amazônia, é uma serpente amplamente distribuída pelo Bioma Amazônico, ocorrendo em grande parte da região Norte da América do Sul (Cunha e Nascimento, 1993; Maschio, 2008; Nogueira et al., 2019). Os indivíduos dessa espécie são predominantemente noturnos e apresentam uma dieta ontogenética - que varia conforme seu estágio de desenvolvimento - e generalista, incluindo centopeias peixes, anuros, lagartos, outras serpentes, e pequenos mamíferos (Martins e Oliveira, 1999; Martins et al., 2003; Maschio, 2008; Bisneto e Kaefer, 2019).

Figura 1. Visão geral da Jararaca-da-Amazônia (*Bothrops atrox)* em seu ambiente natural. (Fotografia de Valdir Hobus)

O uso de habitat por *B. athrox* também varia conforme o estágio de desenvolvimento. Indivíduos juvenis são mais frequentemente encontrados em arbustos, enquanto os adultos tendem a ocupar o solo, especialmente em florestas de várzea. Além disso espécimes podem ser frequentemente observados nas proximidades de áreas habitadas, onde são responsáveis pela maioria dos acidentes ofídicos registrados na região (Cunha e Nascimento, 1975; Oliveira e Martins, 2001; Martins et al., 2003; Fraga et al., 2013).

Aspectos gerais do parasitismo em serpentes

Parasitos e patógenos desempenham um papel central nos ecossistemas, infuenciando a ecologia, a evolução e o crescimento populacional de seus hospedeiros. Além disso, atuam como importantes reguladores da biodiversidade nas comunidades naturais (Brooks & Hoberg, 2007). A análise dos padrões de abundância dos parasitas em seus hospedeiros, bem como do seu ciclo de transmissão, é considerada fundamental para o entendimento das interações parasito-hospedeiro (Bonsall, 2004). Os parasitos também fornecem informações valiosas sobre a ecologia, o comportamento e as interações tróficas dos hospedeiros (Brooks e Hoberg, 2000).

Segundo Aho (1990), a fauna parasitária associada aos répteis era, até então, caracterizada pouco diversa e composta majoritariamente por espécies isolacionistas e não interativas. No entanto, essa perspectiva tem mudado nas últimas décadas, especialmente após a realização de novos inventários. Esses estudos vem revelando que a comunidade de helmintos associada a répteis é, na verdade, rica e diversa, com o número de espécies aumentando à medida que novos hospedeiros são investigados e diferentes áreas geográficas são amostradas (Vicente et al., 1993; Ávila e Silva, 2010; Lacerda et al., 2023). Esses estudos não apenas ampliam o conhecimento sobre essa biodiversidade muitas vezes negligenciada, como também contribuem para a compreensão de padrões biogeográficos e de processos coevolutivos entre parasitos e hospedeiros (Platt, 1992).

Os répteis participam de uma ampla variedade de ciclos de vida de metazoários, atuando como hospedeiros definitivos ou intermediários (Aho, 1990: Zelmer e Platt, 2008; Kuśmierek et al. 2019). Entre eles, as serpentes destacam-se por hospedarem endoparasitos pertencentes a três principais grupos: Nematoda (Diesing, 1861), Acantocephala e Platyhelminthes Gegenbaur, 1859, da Classe Trematoda Rudophi, 1808, e da Classe Cestoda Van Beneden, 1849.

O Filo Nematoda é um dos grupos mais diversos entre os metazoários, composto por organismos de vida livre e parasitária que podem infectar tanto plantas como animais. Esses organimso colonizam uma ampla gama de ambientes - marinhos, dulcícolas e terrestres - e podem apresentar ciclos de vida simples ou complexos, podendo ser monoxenos, heteroxênicos ou vetoriais (Goater et al. 2014).

Morfologicamente, os nematódeos possuem corpo cilíndrico, recoberto por uma cutícula e são dióicos, apresentando dismorfismos sexual evidente (Fig. 2). Os machos possuem estruturas reprodutivas como testículos, vesícula seminal, espículos e gubernáculo, enquanto as fêmeas apresentam vulva, vagina, ovojector, útero e ovários (Goater et al., 2014). O sistema digestório é completo, composto por boca, esôfago, intestino, reto, ânus ou cloaca (Taylor et al. 2007; Schmidt-Rhaesa et al., 2013). esses parasitos apresentam ainda diversas estruturas sensoriais distribuídas pelo corpo, como deirídeos, fasmídeos, papilas e anfídeos, além de estruturas especializadas para fixação e penetração nos tecidos dos hospedeiros, como dentes e estiletes (Basyoni e Rizk, 2016).

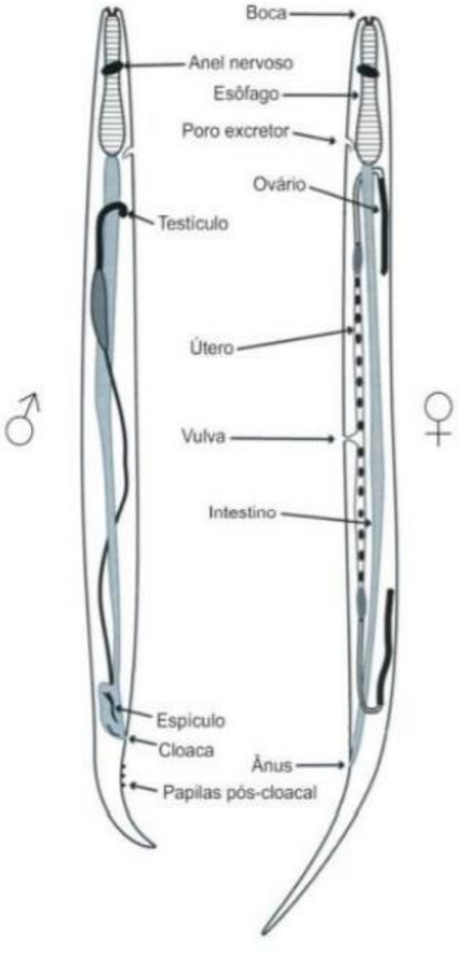

Figura 2. Morfologia geral de representantes do filo Nematoda.
Adaptado de Adaptado de Goater et al. (2014)

A Subclasse Acantocephala compreende um grupo relativamente pequeno de espécies de organismos parasitas obrigatórios, atualmente incluídos no filo Rotífera (Near, 2002). Esses organismos são caracterizados pela presença de uma probóscide eversível, revestida por ganchos (Fig. 3), que utilizam para fixação aos tecidos dos hospedeiros. O corpo apresenta forma cilíndrica ou achatada e, não possui sistema digestivo completo. São dioicos e possuem o ciclo de vida complexo, envolvendo ao menos dois hospedeiros. Os hospedeiros intermediários são, geralmente, artrópodes ou peixes, enquanto os hospedeiros definitivos incluem peixes, répteis, aves e mamíferos (Brusca et al., 2022).

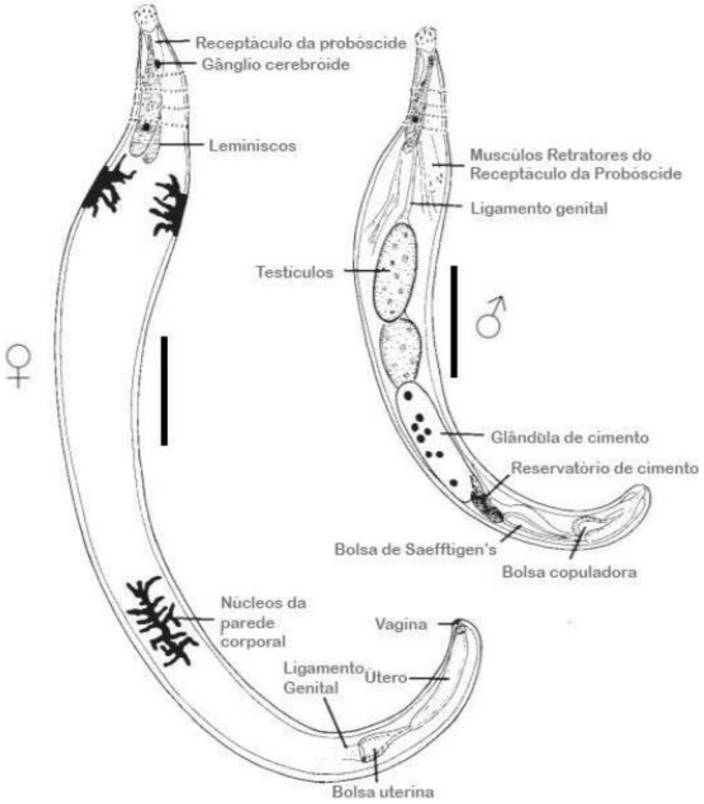

Figura 3. Morfologia Geral de representantes da subclasse Acantocephala: fêmea (♀) e macho (♂).Adaptado de Roberts e Janovy (2009).

Os platelmintos da Classe Trematoda possuem o corpo em forma de folha e são caracterizados pela presença de duas ventosas: uma ventosa oral, que circunda a boca, e a outra localizada na região ventral do corpo, chamada acetábulo, cuja posição pode variar conforme o táxon (Gibson, 2002). Esses helmintos apresentam sistema digestório incompleto e, em sua maioria, são hermafroditas. Seu ciclo de vida é complexo e envolve, obrigatoriamente, um molusco como hospedeiro intermediário, sendo necessário para que atinja a maturidade nos hospedeiros vertebrados definitivos (Zemmer et al 2020).

Os platelmintos da classe Cestoda são parasitos obrigatórios do sistema digestivo de vertebrados. Esses organismos não possuem sistema digestivo, e a absorção de nutrientes ocorre diretamente por meio do tegumento que reveste eu corpo (Goater et al., 2014). O corpo dos cestódeos é dividido em três regiões principais: o escólex, que abriga estruturas de fixação como ganchos e ventosas; o pescoço, que conecta o corpo aos segmentos finais do corpo; e o estróbilo, composto por múltiplas proglotes -unidades segmentadas que contém os sistemas reprodutivos (Roberts et al., 2012). O ciclo de vida é heteroxênico, exigindo um ou mais hospedeiros intermediários para completar seu desenvolvimento (Goater et al., 2014), ou seja, os ovos geralmente liberados nas fezes do hospedeiro definitivo são ingeridos por hospedeiros intermediários, onde se desenvolvem em forma larvais que, ao serem ingeridas por um novo hospedeiro vertebrado, completam o ciclo parasitário.

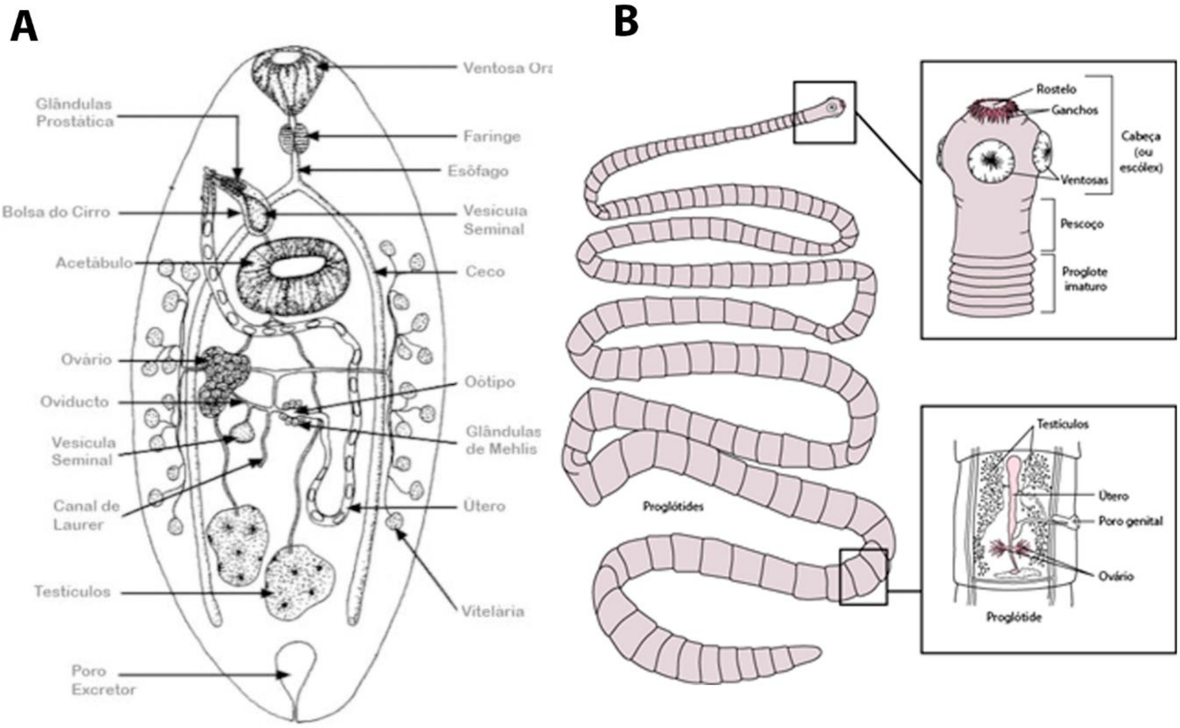

Figura 4. Morfologia Geral de representantes do filo Platyhelminthes. A: Morfologia geral de trematoda. B. Morfologia geral de Cestoda. Fonte: Adaptado de Smyth e Wakelin (1994); Manual MSD.

Diversidade de helmintos parasitas de *bothrops*

A helmintofauna das serpentes do gênero *Bothrops,* no território brasileiro, é composta por 42 táxons de helmintos registrados em 14 espécies de jararacas, a saber: *Bothrops alcatraz* Marques, Martins & Sazima, 2002; *Bothrops alternatus* Duméril, Bibron & Duméril, 1854; *Bothrops atrox; Bothrops cotiara* (Gomes, 1913); *Bothrops diporus* Cope, 1862; *Bothrops erythromelas* Amaral, 1923; *Bothrops fonsecai* Hoge e Belluomini, 1959; *Bothrops insularis* (Amaral, 1922); *Bothrops jararaca* (Wied-Neuwied, 1824); *Bothrops jararacussu* Lacerda, 1884; *Bothrops lutzi* (Miranda-Ribeiro, 1915); *Bothrops mattogrossensis* Amaral, 1925; *Bothrops moojeni* Hoge, 1966; e *Bothrops neuwiedi* Wagler, 1824 (Quadro 1).

No que diz respeito a diversidade de helmintos que parasitam serpentes do gênero *Bothrops*, destacam-se representantes de diferentes filos e classes. Entre os acantocéfalos, há registros da família Centrorhynchidae Van Cleave, 1916. Entre os platelmintos, destacam-se os da classe Cestoda, pertencentes à família Proteocephalidae La Rue, 1911, e os da classe Trematoda, distribuídos nas famílias Dicrocoeliidae Odhner, 1910; Diplodiscidae Cohn, 1904; Opisthogonimidae Travassos, 1928; Plagiorchiidae Lühe, 1901; Proterodiplostomidae Dubois, 1936; e Reniferidae Pratt, 1902. Já entre os nematódeos, estão representadas as famílias Ascarididae Baird, 1853; Camallanidae Railliet & Henry, 1915; Diaphanocephalidae Travassos, 1920; Dioctophymatidae Railliet, 1915; Onchocercidae Leiper, 1911; Physalopteridae Railliet, 1893; e Rhabdiasidae Railliet, 1916.

Quadro 1. Registros de helmintos associados à serpentes do gênero *Bothrops*

Hospedeiro	Família	Helminto	Localidade	Referência
Bothrops alcatraz	**Cestoda** Proteocephalidae	*Ophiotaenia* sp.	Brasil	Araoz et al. (2018)
Bothrops alternatus	**Cestoda** Proteocephalidae	*Crepidobothrium garzonii* de Chambrier, 1998	Paraguai	de Chambrier (1998)
		Crepidobothrium viperis (Beddard, 1913) Meggitt, 1927	América do Sul	Woodland (1925)
		Ophiotaenia jarara Fuhrmann, 1927	Brasil	de Chambrier et al. 1991
	Digenea Proterodiplostomidae	*Heterodiplostomum lanceolatum* Dubois, 1936	Argentina	Poumarau (1968); Lunaschi e Sutton (1985)
	Diplodiscidae	*Catadiscus freitaslenti* Ruiz, 1943	Argentina	Poumarau (1968)
	Opisthogonimidae	*Opisthogonimus fonsecai* Ruiz & Leão, 1942	Brasil	Pinto et al. (2012)
	Plagiochiirdae	*Styphlodora condita* Faria, 1911	Argentina	Poumarau (1968)
		Styphlodora gili Mane-Garzon & Holcman-Spector, 1967	Uruguai	Mañé-Garzón e Holcman-Spector (1967)

		Travtrema stenocotyle (Cohn, 1902) Goodman, 1951	Argentina	Poumarau (1968)
	Nematoda Rhabdiasidae	*Acanthorhabdias acanthorhabdias* Pereira, 1927	Brasil	Siqueira et al. (2009)
	Diaphanocephalidae	*Kalicephalus costatus costatus* (Rudolphi, 1819)	Brasil	Vicente et al. (1993)
		Kalicephalus inermis inermis Molin, 1861	Brasil	Vicente et al. (1993)
Bothrops atrox	**Acanthocephala** Centrorhynchidae	*Centrorhynchus* sp.	Brasil	Conga et al. (2024)
	Cestoda Proteocephalidae	*Ophiotaenia* calmetti (Barrois, 1898) La Rue, 1911	Brasil	Santos e Rolas (1973)
		Ophiotaenia jarara	Guiana	McAllister et al. (2010a)
	Digenea Plagiochiirdae	*Sticholecitha serpentis* (Prudhoe, 1949)	Brasil	Conga et al. (2024)
	Opisthogonimidae	*Opisthogonimus lecithonotus* Lühe, 1900	Brasil	Pereira (1929a)
	Reniferidae	*Renifer monstruosum* (Braun, 1901)	Venezuela	Caballero e Vogelsang (1947); Diaz-Ungria (1967)
	Nematoda Camallanidae	*Camallanus* sp.	Brasil	Conga et al. (2024)
	Ascarididae	*Ophidascaris arndti* Sprehn, 1929	Brasil	Vicente et al. (1993)
	Diaphanocephalidae	*Kalicephalus inermis*	Brasil e Guiana	Vicente et al. (1993); McAllister

				et al. (2010b)
	Dioctophymatidae	*Eustrongylides* sp	Brasil	Conga et al. (2024)
	Rhabdiasidae	*Serpentirhabdias atroxi* Kuzmin, Giese, Melo, Costa, Maschio & Santos, 2016	Brasil	Kuzmin et al. (2016)
Bothrops cotiara	**Digenea** Reniferidae	*Renifer heterocoelium* (Travassos, 1921)	Brasil	Correa et al. (1990)
	Nematoda Diaphanocephalidae	*Kalicephalus costatus costatus* (Rudolph, 1819)	Brasil	Vicente et al. (1993
		Kalicephalus inermis inermis Molin, 1861	Brasil	Vicente et al. (1993
Bothrops diporus	**Digenea** Dicrocoeliidae	*Infidum infidum* (Faria, 1910) Travassos, 1916	Argentina	Martínez et al. (1996)
	Diplodiscidae	*Catadiscus freitaslenti*	Argentina	Poumarau (1968)
		Catadiscus longicoecalis Poumarau, 1965	Argentina	Poumarau (1965)
	Opisthogonimidae	*Opisthogonimus lecithonotus*	Argentina	Lunaschi e Drago (2007)
	Plagiochiirdae	*Styphlodora condita* Faria, 1911	Argentina	Boero et al. (1972)
		Travtrema stenocotyle (Cohn, 1902) Goodman, 1951	Argentina	Poumarau, (1968); Lunaschi e Sutton (1985)
	Nematoda Diaphanocephalidae	*Kalicephalus* sp.	Brasil	Marques et al. (2020)
Bothrops erythromelas	**Nematoda** Physalopteridae	*Physaloptera* sp.	Brasil	Oliveira et al. (2018)
Bothrops fonsecai	**Nematoda** Ascarididae	*Ophidascaris arndti* Sprehn, 1929	Brasil	Simões et al. (2022)

Bothrops insularis (Amaral, 1922)	**Digenea** Reniferidae	*Renifer heterocoelium* (Travassos, 1921)	Brasil	Correa et al. (1990)
Bothrops jararaca	**Cestoda** Proteocephalidae	*Crepidobothrium gerrardii* Baird, 1860	Brasil	Travassos (1965)
		Ophiotaenia jarara Fuhrmann, 1927	Brasil	Zehnder e Mariaux (1999);
		Ophiotaenia azevedoi (de Chambrier, Vaucher and Renaud, 1992)	Brasil	de Chambrier et al. (1992)
	Digenea Dicrocoeliidae	*Paradistomum parvisimum* (Travassos, 1918) Travassos, 1919	Brasil Brasil	Travassos (1944b)
		Infidum similis Travassos, 1916	Brasil	Travassos (1944b)
	Opisthogonimidae	*Opisthogonimus fonsecai* Ruiz & Leão, 1942	Brasil	Pinto et al. (2012)
		Opisthogonimus interrogativus (Nicoll, 1914) Pereira, 1929	Brasil	Pereira (1929a)
	Reniferidae	*Renifer heterocoelium* (Travassos, 1921)	Brasil	Correa et al. (1990)
	Plagiochiirdae	*Styphlodora gili* Mane-Garzon & Holcman-Spector, 1967	Brasil	Fabio (1979)
	Nematoda Diaphanocephalidae	*Kalicephalus costatus costatus* (Rudolph, 1819)	Brasil	Vicente et al. (1993)
		Kalicephalus inermis inermis Molin, 1861	Brasil	Vicente et al. (1993)

		Kalicephalus subulatus Molin, 1861	Brasil	Vicente et al. (1993)
	Physalopteridae	*Physaloptera obtusíssima* Molin, 1860	Brasil	Vicente et al. (1993)
	Ascarididae	*Ophidascaris travassossi* Vaz, 1938	Brasil	Siqueira et al. (2009)
		Ophidascaris tuberculatum Siqueira, Panizzutti, Muniz-Pereira & Pinto, 2005	Brasil	Siqueira et al. (2005)
		Travassosascaris araujoi Sprent, 1978		Desiderio et al. (1996)
	Rhabdiasidae	*Serpentirhabdias vellardi* (Pereira, 1928)		Grego et al. (2004)
Bothrops jararacussu	**Cestoda**			
	Proteocephalidae	*Ophiotaenia jarara*	Brasil	Santos e Rolas (1973)
	Digenea			
	Opisthogonimidae	*Opisthogonimus lecithonotus*	Brasil	Pereira (1929)
	Nematoda			
	Ascarididae	*Ophidascaris trichuriformis* Vaz, 1935	Brasil	Mendoza-Rodan e Fiorillo (2016)
	Diaphanocephalidae	*Kalicephalus appendiculatus* Molin, 1861	Brasil	Vicente et al. (1993)
		Kalicephalus costatus costatus	Brasil	Vicente et al. (1993)
		Kalicephalus inermis inermis	Brasil	Vicente et al. (1993)

Bothrops lutzi	**Nematoda** Ascarididae	*Ophidascaris* sp	Brasil	Oliveira et al. (2023)
	Onchocercidae	*Oswaldofilaria* sp.	Brasil	Oliveira et al. (2023)
	Physalopteridae	*Physaloptera* sp.	Brasil	Oliveira et al. (2023)
Bothrops mattogrossensis	**Digenea** Reniferidae	*Renifer heterocoelium*	Brasil	Correa et al. (1990)
Bothrops moojeni	**Cestoda** Proteocephalidae	*Crepidobothrium gerrardii*	Brasil	Silva (2014)
	Digenea Dicrocoeliidae	*Infidum infidum*	Brasil	Barrella e Silva (2003)
	Opisthogonimidae	*Opisthogonimus artigasi* Ruiz & Leão, 1942	Brasil	Barrella e Silva (2003)
		Opisthogonimus fonsecai	Brasil	Barrella e Silva (2003);
		Opisthogonimus lecithonotus	Argentina e Brasil	Barrella e Silva (2003); Silva (2004)
	Plagiochiirdae	*Sticholecitha serpentis*	Brasi	Barrella e Silva (2003); Silva et al. (2005);
		Styphlodora condita	Brasil	Barrella e Silva (2003)
		Travtrema stenocotyle	Brasil	Barrella e Silva, (2003); Pinto et al. (2012)
	Reniferidae	*Renifer heterocoelium*	Brasil	Correa et al. (1990)
	Nematoda Rhabdiasidae	*Serpentirhabdias viperidicus*	Brasil	Morais et al. (2017)

		Morais, Aguiar, Müller, Narciso, Silva & Silva, 2017		
Bothrops neuwiedi	**Digenea** Diplodiscidae	*Catadiscus freitaslenti*	Argentina	Poumarau, 1968
		Catadiscus longicoecalis	Argentina	Poumarau, 1968
	Opisthogonimidae	*Opisthogonimus afranioi* Pereira, 1929	Brasil	Pereira (1929^{a})
		Opisthogonimus fonsecai	Brasil	Pinto et al. (2012)
		Opisthogonimus lecithonotus	Argentina	Poumarau (1968)
	Plagiorchiidae	*Styphlodora condita*	Argentina	Poumarau (1968)
		Travtrema stenocotyle	Argentina e Brasil	Poumarau (1968); Pinto et al. (2012)
	Reniferidae	*Renifer heterocoelium*	Brasil	Travassos (1921a)

Fatores que influenciam na estrutura das comunidades parasitárias

Diversos fatores podem influenciar a abundância de helmintos numa população de hospedeiros, afetando tanto a proporção de indivíduos infectados quanto a carga parasitária por hospedeiro (Fontes et al. 2003; Salkeld e Schwarzkopf 2005). Entre os fatores intrínsecos do hospedeiro, destacam-se a morfologia, tamanho, fisiologia, sexo, dieta, idade, história ontogenética, filogenia, distribuição geográfica, densidade populacional e a resposta imunológica (Kennedy et al., 1996; Morand e Harvey 2000; Krasnov et al. 2005). Por outro lado, fatores extrínsecos, como alterações no hábitat, aumento da pressão de predação, variabilidade ambiental e sazonalidade, podem modificar as condições biológicas dos hospedeiros, tornando-os mais suscetíveis à infecção parasitária (Oppliger et al. 1998).

Além disso, os traços ecológicos dos próprios parasitos também desempenham um papel fundamental na estruturação das comunidades parasitárias. Entre eles, destacam-se o tipo de ciclo de vida, o modo de infecção, a especificidade quanto ao hospedeiro, o sítio de infecção, a razão sexual e a capacidade reprodutiva (Agosta et al., 2010; Wells e Clark, 2019; Euclydes et al., 2021).

Hospedeiros com maior tamanho corporal tendem a apresentar níveis mais elevados de parasitismo. Isso ocorre porque, à medida que o organismo cresce, aumenta a probabilidade de exposição a fontes potenciais de infecção. Além disso, um corpo maior oferece uma maior variedade de nichos ecológicos passíveis de serem explorados pelos parasitos, bem como uma maior superfície de contato com o ambiente, o que potencializa as chances de infecção (Connor e McCoy, 1979; Kuris et. al, 1980; Aho, 1990; Kamiya et al., 2014; Morand, 2015).

A densidade populacional dos hospedeiros também exerce forte influência sobre a diversidade das comunidades parasitárias. De acordo com a teoria epidemiológica, modelos matemáticos demonstram que a densidade populacional é um parâmetro crucial para a permanência dos parasitos em uma população de hospedeiros. Hospedeiros com altas densidades populacionais tendem a ser mais suscetíveis à colonização por uma maior variedade de parasitos do que aquelas com densidades mais baixas, uma vez que o aumento do contato entre indivíduos vorece a transmissão e manutenção dos ciclos parasitários (Anderson e May, 1978; Morand e Poulin, 1998).

O sexo do hospedeiro tem sido amplamente investigado como fator preditivo nas infecções parasitárias de serpentes e lagartos (Jiménez-Ruiz et al., 2002; Ibrahim e Soliman, 2005; Adeoye e Ogunbanwo, 2007; Brown e Symondson, 2014; Knapp et al., 2018; Mégia-Palma et al., 2024). Sabe-se que diferenças biológicas entre os sexos dos hospedeiros podem tornar um deles mais suscetível ao parasitismo (Poulin, 1996). Nesse contexto, estudos indicam que fêmeas tendem a investir mais recursos na resposta imunológica, o que pode resultar em menor carga parasitária em comparação aos machos, os quais, por sua vez, geralmente apresentam níveis mais elevados de infecção (Roved et al., 2017; Álvarez-Ruizet al., 2018). Entretanto, durante o período reprodutivo, as fêmeas podem alocar uma quantidade significativa de recursos para produção de ovos e manutenção da gestação, o que pode compromoter sua resposta imune e torná-la mais suscetíveis a infecções (Luppi, 2003; Amo et al., 2005; Weiss et al., 2011). Além disso, um fator amplamente reconhecido para explicar a diferença dos níveis de infecção entre machos e fêmeas é o efeito imunossupressor da testosterona (Olsson et al., 2000; Roberts et al., 2004). Durante a estação reprodutiva, o aumento da atividade dos machos em busca de parceiras e na defesa de territórios tambpem contribui para usa mior exposioção a parasitos (Zielinski e Vanderbergh 1993; Adeoye e Ogunbanwo, 2007).

As características dos habitats ocupados pelos hospedeiros também influenciam significativamente a composição da fauna parasitárias das serpentes. Serpentes semiaquáticas, por exemplo, são mais frequentemente infectadas por trematódeos e cestódeos, cujos ciclo de vida estão fortemente associados ao ambiente aquático. Por outro lado, serpentes terrestres e arborícolas tendem a ser parasitadas principalmente por nematódeos de ciclo de vida direto (Rau e Gordon, 1980;

Fontenot e Font, 1996; Jiménez-Ruiz et al., 2002; Santos et al., 2006; Ávila et al., 2013; Matias et al., 2018).

Apesar de contar com uma helmintofauna relativamente bem documentada, incluindo registros de helmintos pertencentes aos filos Nematoda, Platyhelminthes e à subclasse Acantocephala (Vicente et al., 1993; Kuzmin et al., 2016; Conga et al., 2024), ainda se conhece pouco sobre a dinâmica da relação parasito-hospedeiro envolvendo essa serpente. Diante disso, nosso principal objetivo nesse estudo foi analisar a diversidade e a estrutura da comunidade parasitária de *Bothrops atrox* de duas localidades da Amazônia Brasileira, buscando elucidar aspectos ecológicos relacionados a eesa interação.

Dessa forma, buscamos, nesse estudo, responder as seguintes perguntas: (1) O tamanho do hospedeiro é um fator preditor da composição da comunidade de helmintos parasitos? e (2) A estrutura da comunidade de helmintos parasitos de *Bothrops atrox* varia entre ambientes de floresta e campos naturais?

Para tanto, propomos as seguintes hipóteses:

(Hi) Espécimes hospedeiros de maior tamanho corporal apresentarão maiores níveis de parasitismo. Essa hipótese baseia-se na premissa de que indivíduos de maior tamanho corporal oferecem uma maior variedade de nichos ecológicos para exploração por diferentes parasitos, comportam comunidades mais numerosas e estão mais expostos a potencias fontes de infecção devido à maior área de contato com o ambiente (Kuris et al., 1980; Shine, 1989; Krasnov et al., 2005).

(Hii) A diversidade e abundância de helmintos parasitas em *Bothrops atrox* são influenciadas pelas condições ambientais das localidades amostradas, afetando diretamente tanto as infracomunidades quanto as comunidades componentes.

OBJETIVOS

Objetivo geral

- Caracterizar a estrutura e diversidade da comunidade de helmintos parasitos de *Bothrops atrox* de duas localidades da Amazônia Brasileira.

Objetivos específicos

- Identificar, até o menor nível taxonômico possível, os helmintos parasitos de *Bothrops atrox*;
- Avaliar se a composição das comunidades parasitárias de *Bothrops atrox* varia entre as localidades amostradas;
- Determinar a riqueza e abundância das espécies que compõem a comunidade parasitária de *Bothrops atrox*;
- Descrever uma nova espécie de nematódeo parasito de *B. atrox*;

Analisar se fatores bióticos relacionados aos hospedeiros influenciam na diversidade e abundância dos parasitos.

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Capítulo 1

Influência do ambiente na diversidade de helmintos parasitos de *Bothrops atrox* em dois ecossistemas amazônicos

O Capítulo I desta Dissertação foi elaborado e formatado conforme as normas da revista científica Biologia, as quais se encontram em anexo (Anexo 1)

Influência do ambiente na diversidade de helmintos parasitos de *Bothrops atrox* em dois ecossistemas amazônicos

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RESUMO

Bothrops atrox é a serpente peçonhenta de maior relevância médica na região Norte do Brasil. Até o momento,,sua helmintofauna, é composta por 11 táxons registrados. Neste estudo, analisamos espécimes provenientes de dois habitats amazônicos ecologicamente contrastantes — floresta ombrófila e campos naturais — com o objetivo de investigar a composição das comunidades de helmintos associadas a essa espécie, bem como as interações parasito-hospedeiro. Dos 18 espécimes examinados, 17 estavam parasitados, resultando em uma prevalência de 94,4%. Identificamos 11 táxons, sendo sete nematódeos, três cestódeos e um acantodéfalo. Observamos uma relação positiva entre o tamanho corporal e a abundância de parasitos nas serpentes da Floresta, assim como uma correlação entre o comprimento rostro-cloacal dos hospedeiros e a sua carga parasitária. Embora a riqueza e diversidade não tenham diferido significativamete entre os habitats, a equitabilidade foi maior nos campos naturais. O estudo também revelou oito novos registros de helmintos para *B. atrox*, incluindo os primeiros relatos de *Crepidobothrium* sp., *Kalicephalus costatus costatus*, *Hastospiculum nordestinum*, *Physaloptera* sp., *Ortleppascaris* sp. e *Pachysentis* sp. parasitando essa espécie. Nossos achados ampliam o conhecimento sobre a diversidade de parasitos associados a *B. atrox*, destacando seu papel ecológico como hospedeiro definitivo e paratênico em ciclos de vida parasitários complexos. Nossos resultados indicam que a composição e abundância das comunidades parasitárias estão relacionadas tanto às características dos hospedeiros, quanto aos traços ecológicos dos parasitos e estrutura do habitat. Este é o primeiro estudo a comparar as comunidades componentes de helmintos parasitos de *B. atrox* entre dois ecossistermas amazônicos contrastantes: floresta ombrófila e campos naturais.

Palavras-chave: Diversidade; Helmintos; Parasitos; *Bothrops;* Amazônia

INTRODUÇÃO

A Jararaca-do-Norte, *Bothrops atrox* (Viperidae) (Linnaeus, 1758) é a serpente peçonhenta de maior importância médica na região Norte do Brasil. Com ampla distribuição pelo bioma Amazônia, essa serpente possui hábitos predominantemente terrestres e uma dieta generalista composta por pequenos mamíferos, lagartos e anuros (Santos-Costa et al. 2015; Bisneto e Kaefer 2019).

A helmintofauna de *B. atrox* é composta por 11 táxons registrados no Brasil, Guiana e Venezuela (Pereira, 1929; Caballero e Vogelsang 1947; Diaz-Ungria 1967; Santos e Rolas 1973; Vicente et al. 1993; McAllister et al. 2010a; 2010b; Kuzmin et al. 2016; Conga et al. 2024). Apesar de sua ampla distribuição e reconhecida importância ecológica e médica, os estudos parasitológicos envolvendo essa espécie ainda são escassos e pontuais, com maior ênfase em aspectos toxicológicos e da história natural (Oliveira e Martins 2001; Núñes et al. 2009; Fraga et al. 2013; Silva et al. 2019; Hatakeyama et al. 2020).Diante desse cenário, o presente estudo teve como objetivos: (i) caracterizar a estrutura e a composição da comunidade de helmintos parasitas de *Bothrops atrox* provenientes de dois habitats distintos da Amazônia Brasileira (floresta e campos naturais); (ii) avaliar a influência de fatores relacionados ao hospedeiro (tamanho corporal e sexo) e ao ambiente (tipo de habitat) sobre os padrões de infecção e diversidade parasitária; e (iii) verificar se o tipo de habitat influencia a abundância de parasitos e a riqueza de espécies da comunidade.

MATERIAL E MÉTODOS

Área de estudo e obtenção dos hospedeiros

Os espécimes utilizados para esse estudo foram obtidos em duas localidades da Amzônia Brasileira com características ecológicas contrastantes. A primeira área corresponde à Reserva Extrativista Beija–Flor Brilho de Fogo, situada ao sul do munícipio de Pedra Branca do Amapari, estado do Amapá (0º47’30.6’’N, 51º58’42.1’’O). Essa unidade de conservação abrange uma área de 68.524 mil hectares, com vegetação predominante de floresta ombrófila densa submontana (Drumond et al. 2008). De acordo com a classificação climática de Köppen–Geiger, o clima da região é do tipo equatorial (Af), com temperatura média anual de 27,6°C, variando entre 25,8 a 29,0°C, influenciada pela morfologia local e pelo microclima. A precipitação média anual é de aproximadamente 2.850 mmm, com picos mensais que se aproximam de 400 mm (Alvares et al. 2013).

A segunda localidade de estudo foi a Fazenda Nossa Senhora do Amparo, com 112 hectares de extensão, situada no município de Soure, estado do Pará (0º40’01.83”S, 48º32’11.06”O), na região da Ilha de Marajó. A área é circundada pelo rio Paracauari e caracterizada por vegetação de campos naturais, com presença de formações secundárias, como matas ciliares, várzeas, manguezais, restingas e capoeiras. O clima local, também classificado como equatorial, apresenta temperaturas médias que variam entre 25 °C e 29 °C, influenciadas pelos regimes hidrológicos e pela maritimidade da ilha (Lima et al., 2005) (Figura 1).

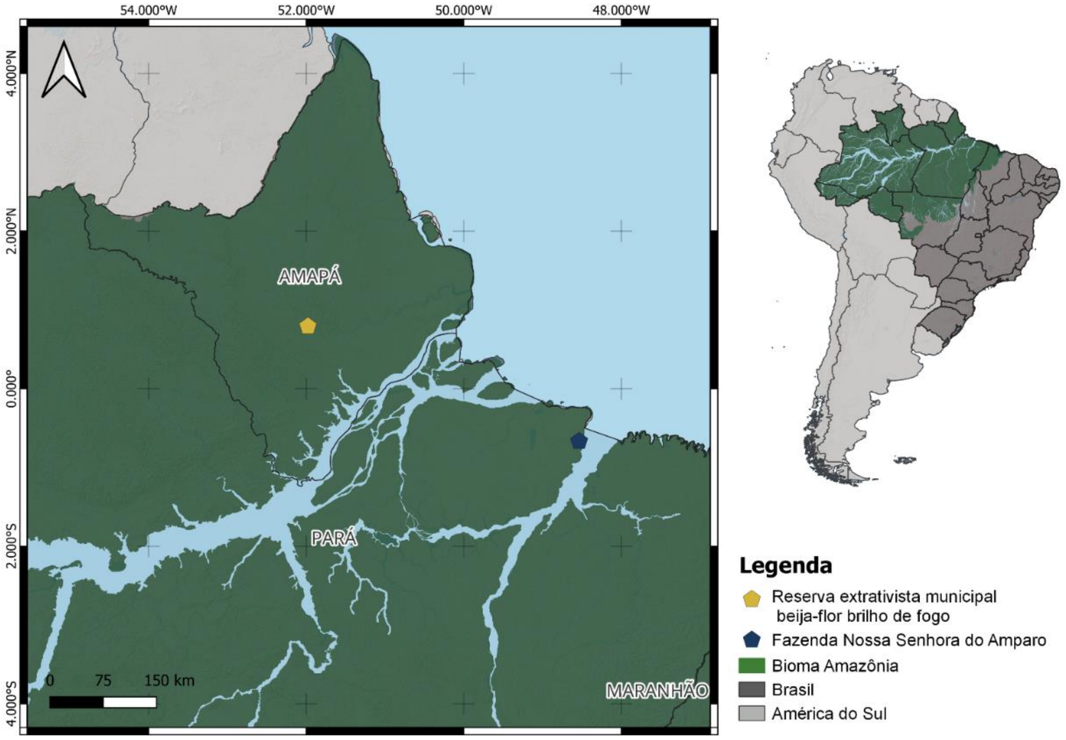

Figura 1. Mapa das áreas de estudo amostradas: Reserva Extrativista Municipal Beija-Flor Brilho de Fogo, localizado na parte sul do município Pedra Branca do Amapari, estado do Amapá, e Fazenda Nossa Senhora do Amparo, no município de Soure, estado do Pará.

Foram realizadas três expedições na Fazenda Nossa Senhora do Amparo, os meses de agosto de 2022 e janeiro de 2023. Na reserva extrativista Beija-Flor Brilho de Fogo, ocorreram cinco coletas nos períodos de setembro de 2020, junho e setembro de 2021, março de 2023 e julho de 2024. As autorizações foram concedidas pelo Instituto Brasileiro do Meio Ambiente e dos Recursos Naturais Renováveis – IBAMA/ICMBio e pelo Comitê de Ética no Uso de Animais (CEUA ID 8341260821; SISBio 53557; SISBio: 48102-2).

A captura dos espécimes foi realizada por meio de busca ativa e visual, encontros ocasionais e por coletas realizadas por terceiros. Após a captura, os espécimes foram anestesiados e eutanasiadas com injeção de lidocaína a 2%. Em seguida, foram medidos, pesados, sexados e necropsiadas. Os órgãos internos foram removidos e dissecados sob estereomicroscópio. Os helmintos parasitos encontrados foram lavados em solução salina (NaCl 0,9%), fixados em etanol 70% aquecido e armazenados na mesma solução, à temperatura ambiente.

Identificação de helmintos

Após a fixação, os parasitos foram acondicionados em tubos e transportados para o laboratório. Os exemplares pertencentes ao filo Nematoda foram hidratados em água destilada e clarificados em Lactofenol de Aman a 20% e, posteriormente, armazenados em Etanol 70%. Para os helmintos do filo Platyhelminthes e da subclasse Acanthocephala, seguimos o protocolo de Amato et al. (1991), onde os helmintos são corados com Carmim Acético e montados em Goma de Damar em lâminas permanentes.

Para realização da análise morfológica e identificação dos caracteres taxonômicos, levamos os exemplares ao Microscópio Olympus BX 41 equipado com câmara clara (sem ajuste de Zoom), onde realizamos os desenhos em profundidade dos exemplares. Realizamos a fotomicrografias em microscópio Olympus BX 53 com sistema de captura de imagem. Para a identificação dos helmintos utilizamos catálogos, chaves de identificação e artigos científicos com descrição das espécies.

Também realizamos uma busca bibliográfica sistemática para compilar os registros de helmintos parasitando *Bothrops atrox*; Para isso, utilizamos sete bases de dados eletrônicas: Google, Google Scholar, PubMed, Scielo, Science Direct, Scopus e Web of Science. Os termos de busca aplicados foram: Tópico: [(“*Bothrops atrox*") e (“helminth" or "parasites" or "nematodes" or "platyhelminthes" or "acanthocephalan")]. Com base nessa busca, elaboramos uma tabela contendo todos os registros compilados, incluindo dados provenientes da literatura publicada, informações disponíveis online e os novos registros obtidos no presente estudo.

Análises estatísticas

Calculamos a prevalência, intensidade e abundância da infecção seguindo Bush et al. (1997) e Reiczigel et al. (2019). Os descritores ecológicos de riqueza e diversidade da comunidade parasitária incluiram riqueza de espécies, dada como o número de espécies de helmintos, índice de diversidade de Shannon (H’), uniformidade (E’) com H’/H’ máximo. Utilizamos o índice de dominância de Berger-Parker (d) para determinar as espécies mais abundantes, com auxílio do programa Diversity (Pisces Conservation Lrd., Reino Unido) (Rohde et al., 1995; Magurran, 2004).

Examinamos a estrutura da comunidade de helmintos conforme a metodologia descrita por Thul et al. (1985), onde as espécies de helmintos são classificadas em quatro grupos (dominante, codominante, subordinado e malsucedido) levando-se em consideração a prevalência, intensidade e fator de maturação (igual a 1,0 se for encontrado pelo menos um espécime maduro das espécies e igual a 0 caso contrário), estando relacionado ao grau de especificidade do hospedeiro.

Para avaliar os efeitos das características do hospedeiro, CRC e sexo na diversidade e abundância de parasitos construímos Modelos Lineares Generalizados (GLMs), utilizando o programa estatístico R (Nelder e Wedderburn 1972). Para testar qual a família de distribuição de erros foi a mais adequada para nossa variável resposta utilizamos a função fitdist, presente no pacote ―fitdistrplus (Delignette-Muller e Dutang 2015), ambos os testes foram realizados programa estatístico R (R CORE TEAM 2019). Também verificamos se o tamanho das serpentes variou entre as localidades, para tanto realizamos um teste de Kruskall-Wallis.

Para verificar se a diversidade e a abundância de parasitas diferem em resposta aos diferentes habitats (campos naturais e floresta), utilizamos a Análise de Variância de acordo com a normalidade dos dados (Shapiro–Wilk < 0,05). Para testar a diversidade de parasitos, utilizamos o teste de Kruskal–Wallis, e para testar o número de parasitas individuais, utilizamos teste t de Welch. Para a análise, utilizamos os pacotes "dunn.test" e as figuras foram geradas com "ggplot2" (Wickham et al. 2020) implementados no software R (R Core Team 2023).

RESULTADOS

Neste estudo, examinamos 18 espécimes de *Bothrops atrox*, sendo nove provenientes de cada uma das localidades amostradas. Desses, 17 apresentaram infecção por pelo menos um táxon de helmintos, resultando em uma prevalência total de 94,4%. No total, coletamos 260 helmintos, com uma intensidade média de infecção de 15,29 e uma abundância média geral de 14,44. As serpentes estavam parasitadas por 11 táxons de endoparasitas, pertencentes a três grupos principais: Acanthocephala, Nematoda e Platyhelminthes (Tabela 1; Figs. 2, 3, 4).

Os nematódeos representaram o grupo mas diverso, com sete táxons representados: *Kalicephalus atroxi*, *Kalicephalus costatus costatus* (Rudolphi, 1819)*, Serpentirhabdias atroxi* Kuzmin, Giese, Vasconcelos Melo, da Costa, Maschio, dos Santos, 2016*, Hastospiculum nordestinum* Ferreira-Silva, Alcantara, Ávila, Silva, 2020, *Camallanus* sp., larvas de *Physaloptera* sp. e larvas de *Ortleppascaris* sp.; seguidos de três táxons da classe Cestoda: *Ophiotaenia* sp.1, *Ophiotaenia* sp.2 e *Crepidobothrium* sp.; e, por fim, um táxon do grupo Acanthocephala: *Pachysentis* sp.

Tabela 1. Helmintos de *B. atrox* dos ambientes de Floresta e Campos Naturais. N: número de helmintos, prevalência (%) com intervalo de confiança de 95%, intensidade média com intervalos, abundância média com intervalo de confiança de 95%, valor de importância (I) e frequência de dominância. Classificação dos helmintos: I ≥ 1,0, espécies dominantes; I =

0, espécies não detectadas; 0,01 ≤ I < 1,0, espécies codominantes. Abreviações: P = Prevalência; IC = Intervalo de Confiança; IM = Intensidade Média; AM = Abundância Média; FD = Frequência de Dominância; TR = Traqueia, ESO = Esôfago; CC = Cavidade Celomática; ID = Intestino Delgado; PUL = Pulmão; EST = Estômago.

Helminto	N	P (%) ± IC	IM	AM ± IC	Sítio de Infecção	I	Classificação	FD (%)
Floresta								
Camallanus sp.	1	11,1 ± 0,6–44,4	1	0,111 ± 0–0,33	TR	0,05	Codominante	0,51
Pachysentis sp.	20	22,2 ± 4,1–55,8	10	2,22 ± 0–7,89	CC	2,26	Dominante	10,2
Crepidobothrium sp.	2	11,1 ± 0,57–44,3	2	0,22 ± 0–0,67	ID	0,11	Codominante	1,02
Hastospiculum nordestinum	4	11,1 ± 0,57–44,3	4	0,44 ± 0–1,33	CC	0,22	Codominante	2,04
Kalicephalus atroxi	144	88,9 ± 55,6–99,4	18	16 ± 7,56–26	ESO, EST, ID	65,3	Dominante	73,47
Ophiotaenia sp. 1	3	33,3 ± 9,8–67,6	1	0,33 ± 0–0,56	ID	0,51	Codominante	1,53
Serpentirhabdias atroxi	22	77,8 ± 44,2–95,9	3,1	2,44 ± 1,22–4,44	PUL	8,73	Dominante	11,22
Campos naturais								
Pachysentis sp.	4	22,2 ± 0,41–55,8	2	0,44 ± 0–1,33	CC	1,53	Dominante	6,25
Hastospiculum nordestinum	5	22,2 ± 0,41–55,8	2,5	0,44 ± 0–1,33	CC	1,95	Dominante	7,81
Kalicephalus costatus costatus	13	33,3 ± 0,97–67,6	4,3	1,44 ± 0,11–5	ESO, EST, ID	7,61	Dominante	20,31
Ophiotaenia sp. 2	4	44,4 ± 16,9–74,8	1	0,44 ± 0,11–0,67	ID	3,12	Dominante	6,25
Ortleppascaris sp. (larva)	27	44,4 ± 16,9–74,9	6,7	3 ± 0,78–6	CC	0	Mal-sucedido	42,19
Physaloptera sp. (larva)	7	11,1 ± 0,05–44,3	7	0,78 ± 0–1,56	EST	0	Mal-sucedido	10,94
Serpentirhabdias atroxi	4	22,2 ± 0,41–55,8	2	0,44 ± 0–1,33	PUL	1,56	Dominante	6,25

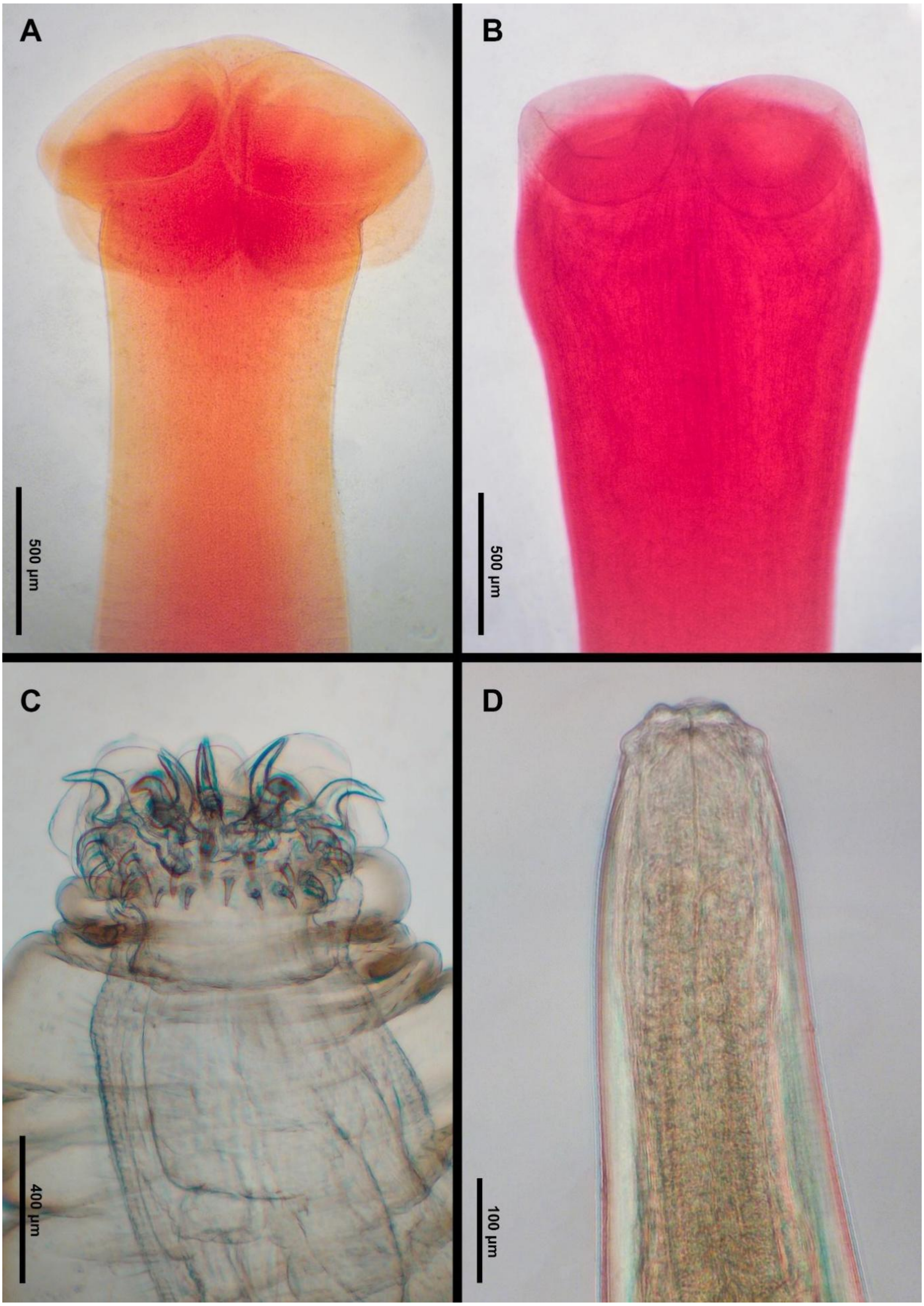

Figura 2. Fotomicrografias de helmintos associados a *B. atrox* da Amazônia brasileira. A) Escólex e pescoço de *Crepidobothrium* sp. B) Escólex e pescoço de *Ophiotaenia* sp2. C) Probóscide com espinhos de *Pachysentis* sp. D) Larva de *Ortleppascaris*, região anterior.

Figura 3. Fotomicrografias de helmintos associados *B. atrox* da Amazônia Brasileira. A) Região anterior de *Kalicephalus costatus costatus*. B) Bursa copulatória de *K. costatus costatus.* C) Cauda da fêmea de *K. costatus costatus.* D) Região anterior de *Kalicephalus atroxi.* E) Bursa copulatória de *K. atroxi.* F) cauda da fêmea de *K. atroxi.*

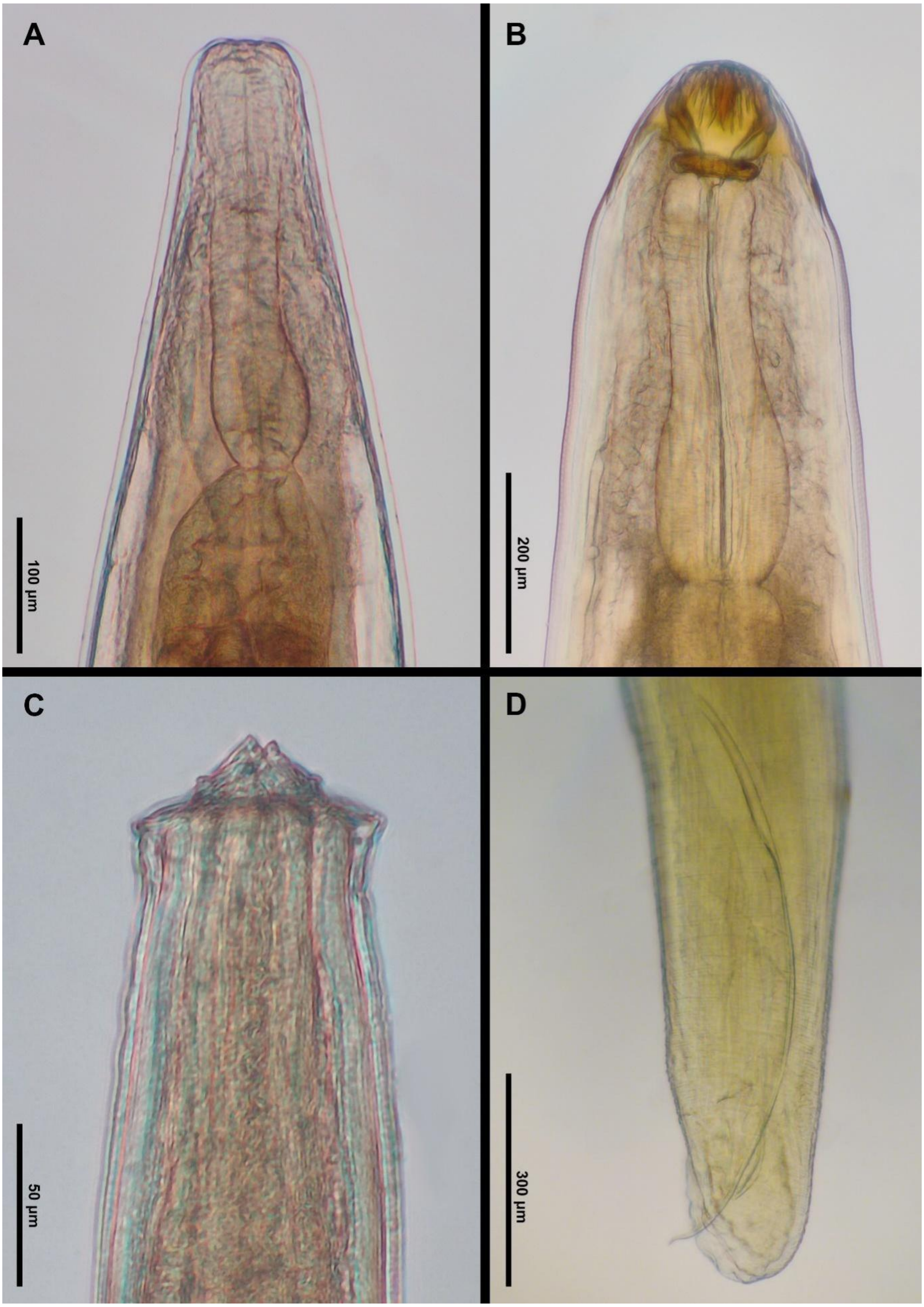

Figura 4. Fotomicrografias de helmintos parasitos de *B. atrox* da Amazônia Brasileira. A) região anterior de *Serpentirhabdias atroxi.* B) região anterior de *Camallanus* sp. C) região anterior de *Physaloptera* sp. (larva). D) região posterior de *Hastospiculum nordestinum.*

Em áreas de florestas, registramos 196 indivíduos de sete espécies de helmintos (prevalência de 100%, intensidade média de 21,78 e abundância média de 21,78)> Nos camps naturais, registramos 64 espécimes de sete espécies de endoparasitas (prevalência de 88,9%, intensidade média de 8 e abundância média de 7,11). As populações de *B. atrox* provenientes de ambientes florestais estavam parasitadas por sete táxons de helmintos: *Kalicephalus atroxi* (88,9%)*, Serpentirhabdias atroxi* (77,8%)*, Ophiotaenia* sp. 1 (33,3%), *Pachysentis* sp. (22,2%), *Hastospiculum nordestinum* (11,1%)*, Camalannus* sp. (11,1%) e *Crepidobothrium* sp. (11,1%). dentre esses, classificamos três táxons como dominantes, quatro como codominantes (Tabela 1).

Nos campos naturais, as comunidades apresentaram uma riqueza de sete táxons de helmintos: *Ophiotaenia* sp.2 (44,4%), *Kalicephalus costatus costatus* (33,3%)*, Serpentirhabdias atroxi* (22,2%)*, Hastospiculum nordestinum* (22,2%)*, Pachysentis* sp. (22,2%), larvas de *Physaloptera* sp. (11,1%) e larvas de *Ortleppascaris* sp. (44,4%). Dentre esses*,* classificamos cinco táxons como dominantes e duas como mal-sucedidos (Tabela 1).

A comunidade parasitária de *B. atrox* nos ambientes de floresta apresentou menor diversidade, maior dominância e baixa equitabilidade, tanto nas infracomunidades quanto na comunidade componente (Tabela 2). Em contraste, nos campos naturais, observamos uma maior diversidade e um maior equilíbrio na distribuição das espécies parasitárias, com menor dominância de táxons em relação ao ambientre florestal (Tabela 3).

Tabela 2. Características da comunidade parasitária das infracomunidades de helmintos e comunidades componentes de *B. atrox* no estado do Amapá, Brasil. Abreviação: DP = Desvio Padrão.

Características da comunidade (Floresta)	**Valores**
Infracomunidades	
Média do índice de diversidade de Brillouin (HB) (± DP)	0,39 ± 0,27
Equitabilidade média (E) (± DP)	0,26 ± 0,17
Comunidade componente	
Riqueza de táxons	7
Índice de diversidade de Shannon-Wiener (H)	0,91
Equitabilidade (J)	0,47
Índice de dominância de Berger-Parker (d)	0.73

O teste de Kruskal-Wallis revelou uma diferença significativa na abundância de parasitos entre os ambientes de campo e floresta (χ^2 = 7,06, p = 0,0079), com uma maior abundância de parasitos observada nas serpentes provenientes do ambiente florestado (Fig. 5). O teste t de Welch indicou que não houve diferença significativa na diversidade de parasitos entre os dois amvientes (p = 0,5988) (Fig. 6). O tamanho das serpentes variou significativamente entre os ambientes analisados, conforme evidenciado pelo teste de Kruskall-Wallis (p = 0, 00218) (Fig. 7).

Tabela 3. Características da comunidade parasitária das infracomunidades de helmintos e comunidades componentes de *B. atrox* no estado do Pará, Brasil. Abreviação: DP, desvio padrão.

Características da comunidade (Campos naturais)	**Valores**
Infracomunidades	
Média do índice de diversidade de Brillouin (HB) (± DP)	0,35 ± 0,33
Equitabilidade média (E) (± DP)	0,24 ± 0,21
Comunidade componente	
Riqueza de táxons	7
Índice de diversidade de Shannon-Wiener (H)	1,64
Equitabilidade (J)	0,84
Índice de dominância de Berger-Parker (d)	0,42

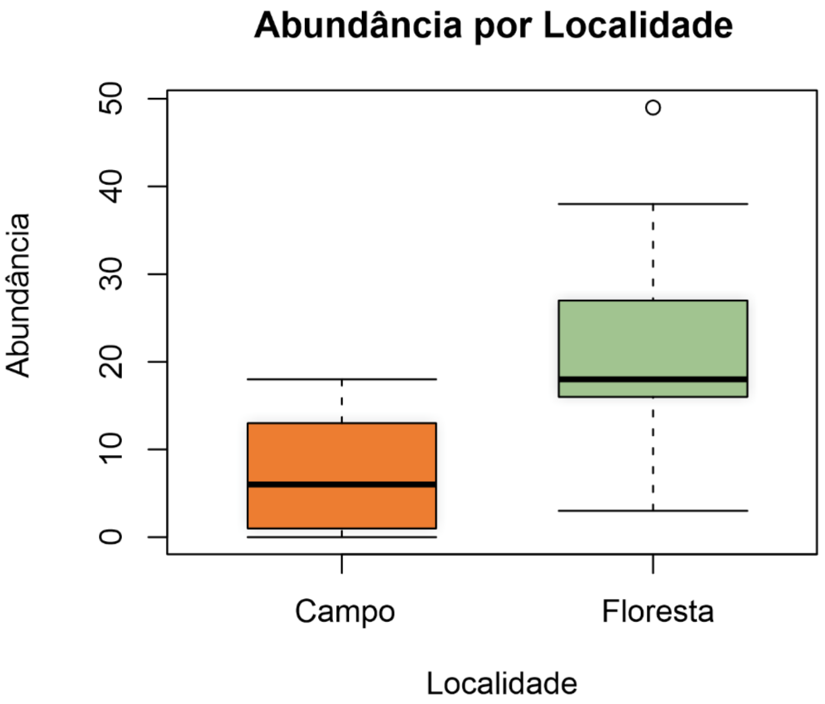

Figura 5. Abundância de helmintos em *Bothrops atrox* capturados em floresta e campos naturais. A floresta apresentou abundância significativamente maior (teste de Kruskall-Wallis, p < 0,01).

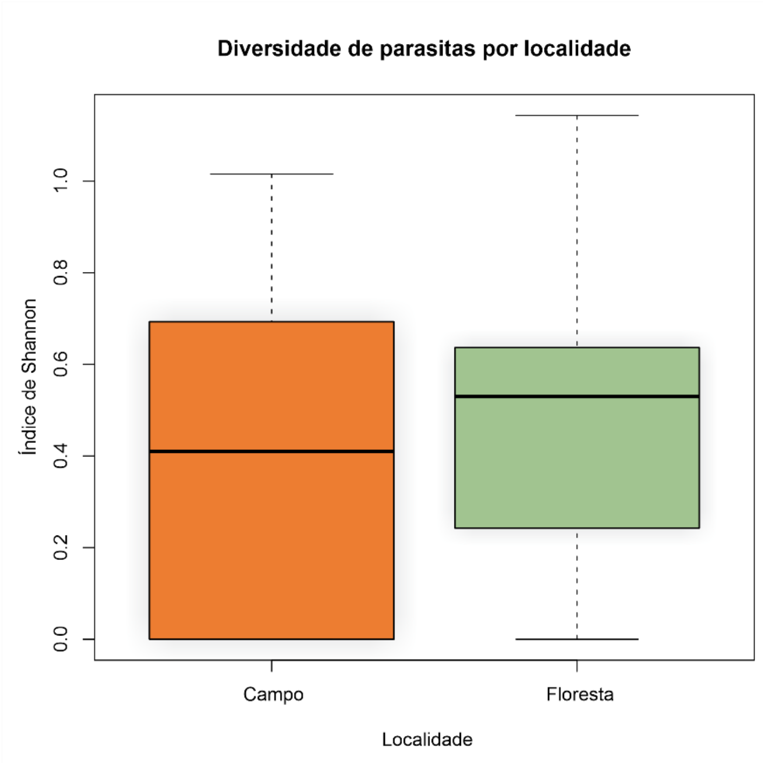

Figura 6. Diversidade de helmintos em *Bothrops atrox* de floresta e campos naturais. Apesar da tendência de maior diversidade nos campos naturais, não houve diferença estatisticamente significativa entre os ambientes (teste t de Welch, p > 0,05).

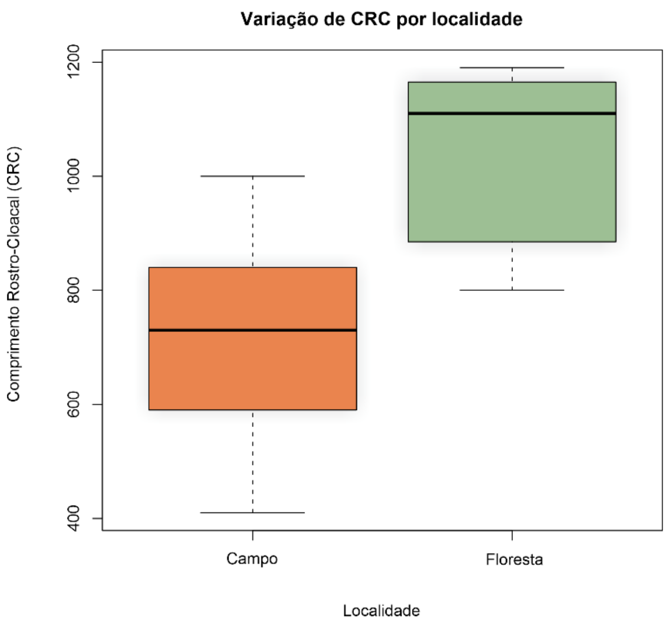

Figura 7. Tamanho corporal (CRC) de *Bothrops atrox* capturados em floresta e campos naturais. Indivíduos da floresta foram significativamente maiores do que aqueles dos campos naturais (p < 0,01).

Os modelos lineares generalizados não indicaram haver relações significativas entre o

CRC e o sexo dos hospedeiros com a diversidade de parasitos. No entanto, observamos uma associação significativa entre o CRC e a abundância de parasitos (p = 0,00038).

Em nossa revisão bibliográfica, encontramos 11 táxons de helmintos previamente registrados como parasitando *Bothrops atrox* (Tabela 4). Com os novos registros obtidos no presente estudo, esse número aumenta para 18 táxons de helmintos associados a *B. atrox* na região Norte do Brasil, ampliando assim o conhecimento sobre a diversidade parasitária dessa espécie de serpente.

Tabela 4. Registros de helmintos parasitos de *Bothrops atrox*. NH = Novo registro de hospedeiro. NL = Nova localidade para o estado do Pará ou Amapá.

Helminto	Família	País	NH	NL	Referência
Acanthocephala					
Pachysentis sp.	Oligacanthorynchidae	Brasil	●	●	**Presente estudo**
Centrorhynchus sp.	Centrorhynchidae	Brasil			Conga et al. (2024)
Cestoda					
Crepidobothrium sp.	Proteocephalidae	Brasil	●	●	**Presente estudo**
Ophiotaenia calmetti		Brasil			Santos e Rolas (1973)
Ophiotaenia jarara		Guiana			
Ophiotaenia sp.1		Brasil		●	**Presente estudo**
Digenea					
Opisthogonimus lecithonotus	Opisthogonimidae	Brasil			Pereira (1929)
Renifer monstruosum	Reniferidae	Venezuela			Caballero e Vogelsang (1947), Diaz-Ungria (1967)
Sticholecitha serpentis	Plagiochiirdae	Brasil			Conga et al. (2024)
Nematoda					
Camallanus sp.	Camallanidae	Brasil			Conga et al. (2024), **presente estudo**
Eustrongylides sp.	Dioctophymatidae	Brasil			Conga et al. (2024)
Hastospiculum nordestinum	Diplotriaenidea	Brasil	●	●	**Presente estudo**
Kalicephalus atroxi	Diaphanocephalidae	Brasil	●	●	**Presente estudo**
Kalicephalus costatus costatus		Brasil	●	●	**Presente estudo**
Kalicephalus inermis inermis		Brasil e Guiana			Vicente et al. (1993), McAllister et al. (2010)
Ophidascaris arndti	Ascarididae	Brasil			Vicente et al. (1993)
Ortleppascaris sp.	Heterocheilidae	Brasil	●	●	**Presente estudo**
Physaloptera sp.	Physalopteridae	Brasil	●	●	**Presente estudo**
Serpentirhabdias atroxi	Rhabdiasidae	Brasil			Kuzmin et al. (2016), **presente estudo**

Discussão

No presente estudo, as comunidades parasitárias de *B. atrox* apresentaram alta riqueza e prevalência geral, quando comparadas a estudos parasitológicos anteriores do hospedeiro na América do Sul (Conga et al. 2024). De maneira geral, identificamos sete novos registros, aumentando a riqueza parasitária conhecida para essa espécie de serpente. A composição dos nas comunidades parasitárias variou entre os habitats, com apenas três táxons de endoparasitos *(Hastospiculum nordestinum*, *Pachysentis* sp., e *Serpentirhabdias atroxi*) sendo comuns entre as localidades, apesar da riqueza semelhante observada em ambos os ambientes (sete táxons).

A estrutura das comunidades parasitárias também apresentou variação tanto no nível de infracomunidades quanto nas comunidades componentes. Nos campos naturais observamos uma maior diversidade, maior equitabilidade de espécies e uma baixa dominância, sugerindo uma distribuição mais equilibrada entre as espécies parasitárias e menor predominância de um único táxon, refletido pelas cinco espécies dominantes no índice de importância. Por outro lado, no ambiente florestado, a comunidade foi caracterizada por baixa diversidade, baixa equitabilidade e alta dominância, indicando a predominância de uma ou poucas espécies sobre as demais. Esse padrão foi refletido pela dominância de dois táxons no índice de importância. A diversidade nas infracomunidades também foi baixa, indicando uma distribuição desigual dos parasitas entre os indivíduos.

Embora nossas análises estatísticas não tenham revelado diferenças significativas na diversidade de helmintos entre os ambientes florestais e campos naturais, observamos uma maior diversidade nas comunidades componentes dos campos naturais. em contrapartida, encontramos diferenças significativas na abundância de parasitos, com indivíduos provenientes das áreas de floresta apresentando uma abundância significativamente maior, corroborando estudos que demonstram que os ambientes florestais tendem a apresentar maior abundância e biomassa de nematódeos (Kergunteuil et al. 2016; Shao et al. 2013).

As análises ecológicas evidenciaram a importância da dinâmica de transmissão na determinação das infracomunidades de helmintos em cada localidade. Encontramos estágios larvais exclusivamente em hospedeiros de ambientes de campos naturais, sugerindo que, nessas áreas, *B. atrox* pode atuar mais como hospedeiro paratênico ou acidental, em comparação aos hospedeiros provenientes de ambientes florestais. Dos dois táxons registrados, os nematódeos do gênero *Physaloptera* possuem ciclo de vida heteroxênico, utilizando insetos como hospedeiros intermediários, os quais compõem a dieta de anuros (Oliveira et al. 2014; Teles et al. 2018), que, por sua vez, são predados por *B. atrox* (Bisneto e Kaefer, 2019). Assim, a presença desses parasitos em *B. atrox* pode estar diretamente associada aos hábitos alimentares generalistas dessa serpente. Este é o primeiro registro de *Physaloptera* sp. parasitando *B. atrox*.

O ciclo de vida de *Ortleppascaris* envolve o desenvolvimento de larvas em anuros que atuam como hospedeiros intermediários ou paratênicos (Silva et al. 2013), enquanto os crocodilianos são considerados seus hospedeiros definitivos (Zhao et al. 2016). Este estudo fornece o primeiro registro desse táxon parasitando serpentes, ampliando o entendimento do seu ciclo de vida ao indicar um novo grupo de vertebrados atuando como hospedeiro paratênico. Adicionalmente, observamos larvas de *Ortleppascaris* sp. em *Leptodactylus macrosternum* Miranda-Ribeiro, 1926, em áreas de campos abertos (dados não publicados), o que reforça a hipótese de que a infecção em *B. atrox* seja mediada por sua dieta, se forma semelhante ao sugerido para *Physaloptera* sp.

Serpentirhabdias atroxi apresentou maior prevalência, intensidade, abundância nos ambientes de floresta (77,8%, 3,1 e 2,44, respectivamente) em comparação aos campos naturais (22,2%, 2 e 0,44). Esse padrão pode ser atribuído às condições ambientais mais favoráveis das areas de floresta para o desenvolvimento das formas larvais do parasito, como elevada umidade do solo e temperaturas amenas, que favorecem a sobevivência das larvas e aumentam as chances de infecção dos hospedeiros definitivos (Baker 1979; Langford e Janovy Jr. 2009; Kuzmin 2013). Essa espécie foi originalmente descrita parasitando *B. atrox* na Floresta Nacional de Caxiuanã, no Estado do Pará (Kuzmin et al. 2016). Assim, os registrados apresnetados neste ampliam sua distribuição geográfica para o estado do Amapá.

Também registramos a ocorrência do nematódeo *Hastospiculum nordestinum* em ambos os ambientes amostrados. Essa espécie foi originalmente descrita na cavidade corporal da serpente *Xenodon merremii* (Wagler, 1824), no Nordeste do Brasil (Ferreira-Silva et al. 2020). O presente achado representa o primeiro registro de *H. nordestinum* parasitando, *Bothrops atrox*, ampliando tanto sua distribuição geográfica quanto o espectro de famílias hospedeiras conhecidas. Além disso, o novo registro corrobora o padrão biogeográfico previamente observado para espécies do gênero *Hastospiculum*, cujos representantes são majoritariamente associados a serpentes nas Américas, enquanto nas regiões da Ásia, África, Europa e Oceania são mais frequeentemente encontrados parasitando lagartos (Ashour 1994; Bolette 1998).

Kalicephalus atroxi foi o táxon mais prevalente, dominante e abundante nas populações de *B. atrox* provenientes dos ambientes de floresta, com prevalência de 88,9%. Essa espécie foi originalmente descrita a partir de exempares de *B. atrox* analisados neste estudo (ver Capítulo 2). Os nematódeos do gênero *Kalicephalus* são parasitos gastrointestinais amplamente distribuídos em serpentes ao redor do mundo. Caracterizam-se por sua baixa especificidade parasitária e por apresentarem ciclo de vida direto (Anderson 2000). A infecção ocorre por meio da penetração ativa de larvas infectantes nas mucosas do hospedeiro ou pela ingestão de presas contendo larvas em terceiro estágio de desenvolvimento (Schad 1956).

Nos campos, encontramos *Kalicephalus costatus costatus*, que por sua vez, é frequentemente encontrado parasitando diversas famílias de serpentes da América do Sul (Vicente et al. 1993; Dias et al. 2004; Pinto et al. 2010). Nosso estudo representa um novo registro de hospedeiro (*Bothrops atrox*) e uma nova localidade de ocorrência (estado do Pará) para *K. costatus costatus*.

Nematódeos da família Camallanidae são amplamente registrados parasitando anfíbios, répteis e peixes (Moravec, 1998). Na região Neotropical, o gênero *Camallanus* á havia sido reportado em *Bothrops atrox* na região do Marajó, mesmo hospedeiro relatado no presente estudo (Conga et al. 2024). A ocorrência de *Camallanus* em serpentes é considerada rara, possivelmente em razão de seu ciclo de vida heteroxênico, que requer um hospedeiro intermediário para o desenvolvimento das larvas infectantes (Anderson, 2000).

Indivíduos de cestódeos foram registrados parasitando *Bothrops atrox* em ambas as localidades analisadas. Os espécimes pertencem aos gêneros *Ophiotaenia* e *Crepidobothrium*, ambos comumente reportados em serpentes no Brasil (de Chambrier et al. 2015). O exemplar de *Ophiotaenia* sp. encontrado em espécimes de *Bothrops atrox* do Marajó apresentou um escólex mais largo e ausência de órgão apical em comparação com o espécime de *Ophiotaenia* sp. proveniente de Brilho de Fogo. Essas diferenças morfológicas indicam que os dois exemplares podem representar táxons distintos, possivelmente correspondendo a novas espécies. Os espécimes apresentavam desenvolvimento imaturo, o que pode indicar uma infecção recente. Supõe-se que os gêneros *Ophiotaenia* e *Crepidobothrium* sejam transmitidos ao longo da cadeia trófica, com ciclos de vida que envolvem dois ou três hospedeiros (Rees 1963; Freze 1965). Em ciclos mais complexos, os hospedeiros intermediários podem incluir peixes girinos e outros anfíbios (Freeman 1973). Assim presume-se que *Bothrops atrox* adquire esses parasitos por meio da alimentação, ao predar esses organismos durante suas atividades de forrageio em ambientes alagados.

Até o momento, há poucos estudos sobre o registro, ecologia e taxonomia de proteocefalídeos, sendo este o primeiro a identificar *Bothrops atrox* como novo hospedeiro dos gêneros *Ophiotaenia* e *Crepidobothrium* na região amazônica. Santos e Rolas (1973) relataram a presença de *Ophiotaenia calmettei* (Barrois, 1898) em *B. atrox*; no entanto, os espécimes analisados provinham dos estados do Rio de Janeiro e da Bahia. Com base em nossas análises morfológicas e na distribuição geográfica dos espécimes de *Ophiotaenia* provenientes de Brilho de Fogo e Soure, na Ilha do Marajó, consideramos que esses indivíduos podem representar novos táxons.

A identificação de *Pachysentis* sp. amplia a diversidade de acantocéfalos associados a serpentes na Amazônia. Frequentemente registrado em hospedeiros carnívoros da família Canidae (Amin et al. 2021; Aleuy et al. 2024), este helminto passa a integrar a comunidade parasitária de serpentes, ugerindo plasticidade ecológica ou ampliação de nicho parasitário. Sua ocorrência em ambas as localidades reforça a hipótese de que *B. atrox* possa atuaa como hospedeiro definitivo alternativo, adquirindo esses parasitos por meio de uma dieta que inclui possíveis hospedeiros intermediários, como anfíbios e lagartos (Bolette 1997).

Investigamos se o tamanho do corpo dos hospedeiros influencia a diversidade e a carga parasitária. Nossos modelos lineares generalizados indicaram uma relação significativa entre o CRC e a abundância dos helmintos parasitos. Esses achados são consistentes com a hipótese de que animais maiores oferecem maior quantidade de recursos e espaço para os parasitas,uma maior variedade de nichos a serem ocupados, alem de abrigar comunidades parastárias mais numerosas e apresentarem maior exposição a fontes potenciais de infecção, devido à maior área de contato com o ambiente (Kuris et al. 1980; Korallo et al. 2007; Kamiya et al. 2014; Morand 2015). O maior tamanho corporal das jararacas em ambientes de floresta pode ser um dos fatores determinantes para a maior carga parasitária observada, uma vez que o macrohabitat também tende a influenciar o tamanho das serpentes do gênero *Bothrops*, em função da disponibilidade de presas e da pressão de predação (Martins et al. 2001).

O presente estudo contribui significativamente para o conhecimento da helmintofauna associada a *Bothrops atrox* na região Norte do Brasil, elevando para 18 o número de táxons parasitarios registrados para essa serpente e acrescentando sete novos registros de ocorrência parasitária nos estados do Pará e Amapá. Nossos resultados reforçam que áreas ecologicamente diversas e historicamente subamostradas, como a Amazônia oriental, podem abrigar uma diversidade oculta e subestimada de helmintos parasitos (Poulin 1997; Poulin 2010). Ademais, evidencia-se a relevância de estudos parasitológicos para uma compreensão mais aprofundada das interações parasito-hospedeiro.

Este é o primeiro estudo a investigar e comparar a estrutura da comunidade de helmintos parasitas em populações de *B. atrox* provenientes de dois ambientes contrastantes da Amazônia brasileira: floresta ombrófila e campos naturais.

Agradecimentos

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J.K.S. Neves, A.J.S. Félix e G.L. Rebêlo ajudaram com observações de espécimes e análises por microscopia de luz, L.F.F. Trindade e C.E. Costa-Campos realizaram a estatística. G.F. Maschio, F.G. Haick e F.T.V Melo escreveram o manuscrito, revisaram e prepararam as pranchas de Luz. Todos os autores revisaram o manuscrito.

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Capítulo 2

A new species of *Kalicephalus* (Nematoda: Diaphanocephalidae), parasite of *Bothrops atrox* (Serpentes: Viperidae) from Brazilian Amazon

O Capítulo II desta Dissertação foi elaborado e formatado conforme as normas da revista científica Journal of Helminthology, as quais se encontram em anexo (Anexo II)

A new species of *Kalicephalus* (Nematoda: Diaphanocephalidae), a parasite of *Bothrops atrox* (Serpentes: Viperidae) from the Brazilian Amazon

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Abstract

Kalicephalus (Molin, 1861) comprises 33 species of gastrointestinal snake and lizard parasites with a wide distribution, with seven taxa occurring in the Neotropical realm. In the present study, we describe *Kalicephalus atroxi* n. sp., found parasitizing the snake *Bothrops atrox*, from the Eastern Amazon in the State of Amapá, North of Brazil, using an integrative approach involving light microscopy and scanning and sequencing of the internal transcribed spacer 1 region (ITS1). The new species have a buccal capsule characteristic of the genus, a slight cuticular inflation in the cephalic region, an amphidelphic female reproductive system, a vulva with prominent lips, and a long tail, tapering posteriorly. The males have spicules long and alate and lobed copulatory bursa with dorsal rays distinct from their congeners. Molecular analyses and phylogenetic reconstructions cluster the new species into a well-supported clade with *K. costatus costatus*, from *Chironius fuscus*, from northern Brazil. *Kalicephalus atroxi* n. sp. is the eighth taxon of the genus in the Neotropics, the seventh in Brazil, the second described parasitizing *B. atrox* in Brazil, and the first species of snake nematode described in the State of Amapá.

Keywords: Amazon, Viperidae, Nematoda, Taxonomy, Molecular.

Introdução

Kalicephalus (Molin, 1861) are gastrointestinal parasites of snakes and lizards (Schad, 1956; Anderson, 2009). To date, we know 33 species for the genus worldwide, with seven reported in the Neotropical region: *Kalicephalus inermis inermis* Molin, 1861; *Kalicephalus inermis macrovulvus* Caballero, 1954; *Kalicephalus subulatus* Molin, 1861; *Kalicephalus inermis coronellae* Ortlepp, 1923; *Kalicephalus costatus costatus* (Rudolph, 1819); *Kalicephalus appendiculatus* Molin, 1861; and *Kalicephalus rectiphilus neoctiphilus* Schad, 1962.

Despite the diversity among *Kalicephalus* species, most taxa have been identified based only on morphological features (Pereira et al. 2018; Larki et al. 2023). The current genetic database for this genus remains very limited, with sequences for only five species. These include *Kalicephalus branchycephalus* Maplestone, 1931; *Kalicephalus indicus* Ortlepp, 1923; and *Kalicephalus bungari* (MacCallum, 1918), all collected in snakes from Hunan Province, China. Additionally, Chilton et al. (2006), also conducted a molecular characterisation of *Kalicephalus cristatus* from Australia.

The common lancehead, *Bothrops atrox* (Linnaeus, 1758), is a venomous Viperidae family snake widely distributed throughout northern South America (Nogueira et al., 2019). This species is primarily terrestrial and nocturnal, mainly inhabiting rainforests, although can also be found near urban areas (Cunha & Nascimento, 1975; Martins & Oliveira, 1998; Oliveira & Martins, 2001; Fraga et al., 2013). In Brazil, *B. atrox* is responsible for most snakebite accidents in the Amazon region, highlighting its significant medical importance (Pardal et al., 2004; Silva et al., 2019).

In the present study, we describe a new species of the *Kalicephalus* parasitising *B. atrox* from Amapá state, Brazil. Also, we added the sequences of *K. costatus costatus*, parasites of the snake *Chironius fuscus* (Linnaeus, 1758) from the same locality. For the first time, we provide data of internal transcribed spacer 1 (ITS1) for *Kalicephalus* species in the Neotropical region.

Material and Methods

Host collection and morphological analyses

During a helminthological survey between September 2020 and September 2021, we collected six specimens of *Bothrops atrox* and one *Chironius fuscus* in the 'Beija-flor Brilho de Fogo' Extractive Reserve, Pedra Branca do Amapari municipality, state of Amapá, Brazil.

The snakes were anaesthetised and euthanised by injection of sodium thiopental; then measured, weighed, and necropsied. The internal organs were removed and dissected under a stereomicroscope. The nematodes found were collected, washed in saline solution (0.9% NaCl), fixed in heated 70% ethanol, and preserved in the same solution at room temperature.

For morphological analyses, the helminths were cleared in Amann's lactophenol, mounted on temporary slides, and examined in an Olympus BX41 light microscope equipped with a drawing tube. The illustrations were prepared using the CorelDraw 2021 software and processed using Adobe Photoshop Version 21.0.2 software.

The nematodes were post-fixed in 1% OsO_4, dehydrated through a graded ethanol series, critical-point dried in carbon dioxide (CO_2), mounted on stubs, coated with a thin layer of gold, and analysed using a Vega3 Scanning Electron Microscope (TESCAN) at the Structural Biology Laboratory of the Federal University of Pará (LBE-UFPA)

All morphometric data are provided in micrometers (unless otherwise indicated) as holotype/allotype values, followed by the mean of the paratypes and the minimum and maximum values in parentheses. The holotype, allotype, and type series were deposited in the

Invertebrate Collection of the Museu Paraense Emílio Goeldi (MPEG) (Belém, state of Pará, Brazil).

Molecular analyses and phylogenetic study

For molecular analyses, a single specimen of *K. atroxi* n. sp. and *K. costatus costatus* were transferred to microtubes containing 100% ethanol and preserved in a freezer at -20 ºC. We cut the anterior and posterior portions and stored them separately to confirm each sample's identity. The middle portion of the nematode body was then kept in absolute ethanol for later molecular characterization.

Genomic DNA was extracted using Chelex® Molecular Biology Grade Resin according to the manufacturer's instructions. The polymerase chain reaction (PCR) was performed to amplify the internal transcribed spacer 1 (ITS-1) region using the protocol of Liu *et al.* (2019). The PCR products were visualized on 1% agarose gel electrophoresis, and purified using a Qiagen® QIAquick PCR Purifcation kit. The fragments were sequenced according to the protocols for the Big Dye® terminator v.3.1 Cycle Sequencing kit in an ABI 3730 DNA Analyzer at the Human Genome and Stem Cell Research Center, Biosciences Institute, University of São Paulo.

Contiguous sequences were assembled and edited using Geneious 7.1.3 (Kearse et al., 2012) and subsequently deposited in the National Center for Biotechnology Information (NCBI). The ITS-1 datasets were aligned and trimmed using Muscle (Edgar, 2004) in Geneious 7.1.3 software (Kearse et al., 2012). The most suitable evolutionary nucleotide substitution model was TPM3+G, as determined by the Akaike Information Criterion (AIC) in the jModelTest software (Posada, 2008).

We conducted phylogenetic reconstructions using maximum likelihood (ML) and Bayesian inference (BI). The ML analysis was performed in RAxML, and we considered only nodes with a bootstrap greater than 70% to be well-supported (Guindon and Gascuel 2003). For BI analysis, we used MrBayes and considered nodes with Bayesian posterior probabilities above 90% as well-supported. (Ronquist and Huelsenbeck 2003). Both analyses were conducted in CIPRES Science Gateway (Miller et al. 2010).

The phylogenetic analyses included three genera: *Kalicephalu*s (*K. costatus costatus, K. brachycephalus,* and *K. indicus*); *Diaphanocephalus* Travassos, 1920 (*D. galeatus* (Rudolphi, 1819) and *Ancylostoma* Dubini, 1843 (*A. caninum* (Ercolani, 1859) Hall, 1913, *A. ceylanicum* Looss, 1911*, A. duodenale* Dubini, 1843 and *A. braziliense* Faria, 1910). We used *Oswaldocruzia filiformis* (Goeze, 1782) (access number: XXX) as an outgroup in the phylogenies. The trees were visualized in the software FigTree v1.3.3 (Rambaut, 2009) and edited in Adobe Photoshop 21.0.2 software.

Results

Taxonomic Summary

Kalicephalus atroxi **n. sp. Haick, Tavares-Costa e Melo, 2023**

Type host: *Bothrops atrox* (Linnaeus, 1758) (Squamata: Viperidae: Crotalinae)

Type locality: “Beija-Flor Brilho de Fogo” Extractive Reserve, Pedra Branca do Amapari municipality, state of Amapá, Brazil (0º47'30,6"N, 51º58'42,1' 'W)

Site of infection: Oesophagus, Stomach, and Small Intestine.

Parasitological descriptors: Prevalence 88,9% (8 of 9 analysed); mean intensity 18 (2–39);

Zoobank register: It will be inserted after the article's acceptance

Genbank Accession number: It will be inserted after the article's acceptance

Etymology: The species was named in reference to the specific epithet of the type host.

General. Body cylindrical, both extremities gradually attenuated. Cuticle finely transversely striated. Cephalic inflation present. Corona radiata absent. Buccal capsule with two valves, formed by numerous chitinous pieces, each valve with an anterior transverse ridge and three parenchymatous bands (Fig. 1A), ending in sensory papillae associated with amphids (Fig. 2A). Buccal capsule with rectangular anterior plates. Dorsal gutter well-developed, extending from oesophagus to mid-region of buccal capsule. Oesophagus claviform, muscular, wider in bulbar region. Nerve ring located in mid-region of oesophagus and excretory pore at oesophageal bulb (Fig. 1A). Intestine straight, with anal glands at its posterior extremity (Fig. 1C).

Females. (Based on the allotype and 10 paratypes, all gravid specimens): Total length 12.13; 10.26 (7.52–12.53) mm, maximum width 487; 430 (267–553). Buccal capsule 213; 206 (181–229) deep, head diameter 272; 261 (235–304). Oesophagus 477; 432 (392–477) long. Maximum width at bulb region 237; 231 (171–272). Nerve ring and excretory pore located at 381; 370 (323–419) and 536; 468 (344–563) from the anterior end. Amphidelphic, vulva with prominent lips situated at 4.13; 3.55 (2.43–4.25) mm from posterior extremity (Fig. 1B; 2C), with a vulva-body ratio of 1.93; 1.90 (1.54–2.20). Vagina short, bifurcating into oviducts and short, muscular sphincters (Fig. 1B). Eggs at morula stage in uterus and vagina, with a smooth, thin and elliptical shell 65; 61 (50.5–68) long and 37; 36 (30–41) wide. Anal glands at final portion of intestine. Anus with posterior lip more prominent. Tail elongated, 427; 417 (344–517) long (Fig. 2C), tapering posteriorly, with a terminal spine (Fig. 1C).

Males. (Based on the holotype and 10 paratypes, all adult specimens): Total length 9.7; 8.57 (6.72–11.24) mm, maximum width 333; 291 (200–340). Buccal capsule 168; 166 (159–179) deep, head diameter 239; 217 (189–239). Oesophagus 421; 390 (349–421) long, maximum width at bulb region 235; 212 (152–251). Nerve ring and excretory pore situated at 330; 317 (291–333) and 544; 439 (301–544) from anterior end. Testis single, tubular, flexing posteriorly in anterior region. Caudal bursa wide and oblique, typical of the genus (Fig. 1E; 2B). Ray 1 indistinct, rays 2 and 3 originating from a common trunk. Rays 4, 5, and 6 arise from a common trunk and run parallel. Rays 7 and 8 separate; ray 7 reduced, not reaching bursal edge. Dorsal ray type IV (according to Schad, 1962), bifurcating innermost terminal ray (Fig. 1D, E). Spicules 720; 692 (629–733) long and equal in size, covered with sheath with fine transversal striations (Fig. 1F). Gubernaculum 162; 163 (143–196) long, navicular in shape (Fig. 1G). Telamon with crests in lateral view (Fig. 1D), genital cone well-developed (Fig. 1E; 2B).

Remarks

Kalicephalus atroxi n. sp. was assigned to the genus *Kalicephalus* based on the following morphological traits: presence of complex buccal capsule bivalved (each valve with an internal transversal ridge), corona radiata absent, the males have copulatory bursa divided in lobes, and spicules are alate.

Our specimens belong to the subgenus *Inermiformes* Lichtenfels, 1980, based on equal spicules, a amphidelphic reproductive system and parasiting snakes from the new world. Thus, we will compare and differentiate the new species from those included in this subgenus: *Kalicephalus inermis inermis* (Molin, 1861); *Kalicephalus inermis macrovulvus* (Caballero, 1954); *Kalicephalus subulatus* (Molin, 1861) and *Kalicephalus coronellae* (Ortlepp, 1923).

The new species resembles *Kalicephalus inermis inermis* by having a type IV copulatory bursa; however, in *K. atroxi* n. sp. the internal branches of the dorsal rays are bifurcated (simple in *K. inermis inermis*). *Kalicephalus atroxi* n. sp. is smaller than *Kalicephalus inermis inermis* in body length (9.56–15.67 mm × 530–820 in *K. inermis inermis vs* 7.52–12.53 mm x 267-553 in *K. atroxi* n. sp.); oesophagus total length (580–730 × 260–360 in *K. inermis inermis vs* 392–477 × 171–272 in *K. atroxi* n. sp.), and spicules lenght (680–1000 in *K. inermis inermis vs* 629–733 in *K. atroxi* n. sp.). Also, the females of the new species has an elongated and tapered tail, while in *K. inermis inermis* it is short and conical.

Kalicephalus atroxi n. sp. can be easily distinguished from *Kalicephalus inermis macrovulvus* by several characteristics. Firstly, the new species features a type IV copulatory bursa, whereas *K. inermis macrovulvus* has a type V. Additionally, they differentiate by having smaller dimensions of spicules (629–733 in *K. atroxi* n. sp. *vs* 700–900 in *K. inermis macrovulvus*), and vulva not prominent, while in *K. inermis macrovulvus* vulvar lips are very protruded. Lastly, the tail of the new species has a tapered end, in contrast to the rounded tip end in *Kalicephalus inermis macrovulvus*.

Although, both the new species and *Kalicephalus subulatus* are found in the Amazon region, the females of *K. atroxi* n. sp. have a terminal spine at the end of the tail, which is absent in *K. subulatus*. Addiionally, the new species features a type IV copulatory bursa, while *K. subulatus* has a type III. Furthermore, *K. atroxi* n. sp. has larger spicules (630–730 n *K. atroxi* n. sp. *vs* 510–650 in *K. subulatus*) and longer tail (344–517 *K. atroxi* n. sp. *vs* 240–390 em *K. subulatus*).

Kalicephalus atroxi n. sp. resembles *Kalicephalus inermis coronellae* by the presence of slight cephalic cuticular inflation. However, the new species differs in the type IV copulatory bursa (type III in *Kalicephalus inermis coronellae*), the gretaer dimensions of buccal capsule (181–229 × 235–304 in *K. atroxi* n. sp. *vs* 120–150 × 210–240 in *K. inermis coronellae*), longer oesophagus (392–477 in *K. atroxi* n. sp. *vs* 300–360 in *K. inermis coronellae*) and larger spicules (630–730 in *K. atroxi* n. sp. *vs* 480–560 in *K. inermis coronellae*). In addition, while *Kalicephalus inermis coronellae* is distributed from the northeast in the USA to southern Mexico, the new species has been exclusively found in Brazil.

Currently, only three species of the genus are registered from Brazil: *Kalicephalus costatus costatus* (Rudolph, 1819) and *Kalicephalus appendiculatus* Molin, 1861 (subgenus

Kalicephalus) and *Kalicephalus rectiphilus neorectiphilus* Schad, 1962 (subgenus *Rectiphiloides* Lichtenfels, 1980).

We already differentiated *K. costatus costatus* from the new species. Thus, species in the subgenus *Kalicephalus* can be easily distinguished from *K. atroxi* n. sp. by their female reproductive system, which is prodelphic. In these species, the vulva is located closer to the anus than to the middle of the body. In addition, in all species found in Brazil have the terminal branches of the dorsal ray classified as the type III.

Kalicephalus rectiphilus neorectiphilus, a parasite of *Palusophis bifossatus* (Raddi, 1820), can be distinguisehd from the new species by its dorsal ray, which is of type II and is exclusive to species allocated to the subgenus *Rectiphiloides*. In contrast, *Kalicephalus atroxi* n. sp. belongs to the subgenus *Inermiformes,* in which the dorsal ray is of the type IV. Furthermore, the members of the subgenus *Rectiphiloides* typically infect the rectum of snakes.

Molecular analyses and phylogenetic study

The ITS1 region sequencing of K*alicephalus atroxi* n. sp. resulted in 568bp, while a BLASTn search revealed no identical match with any other Diaphanocephalidae available in the NCBI database. The sequencing of the ITS1 region of *Kalicephalus costatus costatus* resulted in a sequence of 627 base pairs.

A pairwise genetic comparison between congeners of the new species revealed the lowest genetic distance to *Kalicephalus costatus costatus* with a nucleotide divergence of 7–8 %. This was followed by *K. indicus*, which showed a difference of 54-55% (see Suplementary 1). The alignment of our sequences with those available in GenBank generated a matrix of 379 base pairs.

Maximum likelihood and Bayesian inference phylogenetic analysis based on 11 taxa resulted similar topologies, forming two main clades. The sequences of *K. atroxi* n. sp. formed a well-supported monophyletic group with *Kalicephalus costatus costatu* parasites of *Chironius* *fuscus* (Linnaeus, 1758) (Serpentes: Colubridae), from the same locality of the new species.

Clade B3, consisting of *K. atroxi* n. sp. and *Kalicephalus costatus costatus* showed a low support with a sister group fomed by *Kalicephalus brachycephalus* (MK685982) + *Kalicephalus indicu*s (MK630251), both of which were found parasitizing the snake *Elaphe carinata* (Günther, 1864) (Serpentes: Colubridae) from Hunan Province, China (Clade B2). The *Kalicephalus* sequences formed a highly supported monophyletic grouping (clade B), which incluced three sequences of *Diaphanocephalus galeatus* (MN589649, MH134561, MN589648) from *Dracaena paraguayensis*, Amaral, 1950 (Sauria: Teiidae) from State of Mato Grosso do Sul, Brasil.

Finally, the clade formed by nematodes belonging to the family Diaphanocephalidae (*Kalicephalus* and *Diaphanocephalus*) constitutes a sister group distinct from the other members of the internal group, which belong to the family Ancylostomatidae (Clade A) (Fig. 3).

Discussion

Kalicephalus atroxi n. sp. is the eighth species of the genus described in the Neotropical region, the sixth identified in Brazil, and the first recorded from the state of Amapá. The main morphological characters distinguishing the new taxon from its congeners includes cuticular inflation in the cephalic region, the morphology of the female reproductive system, tail shape, type of dorsal ray (which has a single bifurcation in the deepest rays) and the length of spicules.

Observations using Scanning Electron Microscopy (SEM) have been madefor the genus *Kalicephalus* including *Kalicephalus subulatus* from *Xenodon merremii* (Wagler, 1824) in Argentina (González et al., 2018), *Kalicephalus guangdongensis* Zhang, Zhang & Zhang, 2011 and *Kalicephalus schadius fotedari* Kalia & Nayital, 1989 from *Varanus salvator* (Laurenti, 1768) in China (Zhang et al. 2011), and *Kalicephalus viperae viperae* (Lark et al., 2023). The

SEM analysis allowed us to observe the ultrastructural aspects of the buccal capsule of *Kalicephalus atroxi* n. sp., the vulva opening, the arrangement of the phasmids on the female tail, and details of the copulatory bursa in males. However, using this technique, we do not observe the excretory pore or the presence of cervical papillae (deirids).

The ITS1 sequence of the ribosomal gene from *Kalicephalus atroxi* n. sp. forms a well-supported monophyletic clade with *Kalicephalus costatus costatus*, a species found distributed across the Neotropical region. This group also showed a low genetic diference (ony 7% nucleotide divergence), besides these species being very different morphologically, particularly on the copulatory bursa and the female reproductive system. The dorsal rays of types III and IV are the most common trait shared among *Kalicephalus* species (Schad, 1962). The morphology of the male copulatory bursa and the female reproductive system in *Kalicephalus* spp. does not necessarily reflect the phylogenetic relationships within the group, as these characters appear to be more related to functional adaptations than to evolutionary lineage. Thus, based on this and our findings, we suggest that these traits can be useful for species-level differentiation; however, they may have evolved independently and might not carry a true phylogenetic signal.

In contrast, the genetic divergence between the new species and those from the Oriental region is significant, showing 55% of nucleotide divergence between *K. atroxi* n. sp. and *K. indicus* and 54% between *K. atroxi* n. sp. and *K. brachycephalus*. These differences indicate a significant separation between *Kalicephalus* species that parasitise snakes in the Neotropical and Oriental regions. However, because of low nodal support, the addition of new sequences to the phylogeny of *Kalicephalus* spp. may alter the phylogenetic relationshios found here for these taxa.

Our phylogenetic analyses and the high nucleotide divergence between *K. costatus* and *K. indicus* (52% of divergence) indicate that those should be considered as independent and valid species, rather than subspecies as proposed by Schad (1962). We also should highlight that the division of *Kalicephalus* in subgenera is an artificial classification, since we clearly observed the paraphyly of the gropu (separation of clade B2 and B3). Additionally reinforcing that the morphology of the female reproductive system do not reflect the evolutionary relationships among this species.

The phylogenetic reconstruction from this study shows groupings similar to those found by Liu et al. (2019). Notably, we found the formation of a sister group within the clade that encaompasses Diaphanocephalidae and Ancylostomatidae. However, it should be noted that the authors did not use sequences from *D. galeatus*. Overall, this study supports the cloase relationship between the *Diaphanocephalus* and *Kalicephalus* lineages, which form a monophyletic group with high nodal support, thereby reinforcing the monophyly of the Diaphanocephalidae family.

The clades grouping Diaphanocephalidae and Ancylostomatidae are well-spported in ML analysis (87% of bootstrap). According to Chilton et al. (2006), Diaphanocephaloidea and Ancylostomatoidea are more closely related to each other groups within Strongylida.

Finally, *Kalicephalus atroxi* n. sp. is the first species of *Kalicephalus* from the Neotropical region to be characterized using molecular data; and the second species of *Kalicephalus* found parasitising the snake *Bothrops atrox*. Thus our study contributes to the knowledge of the parasitic fauna of this important snake species from the Amazon region and adds data on these parasites' geographical distribution.

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AUTHOR CONTRIBUTIONS: F. Haick wrote the main manuscript and prepared the figures. L.F.S. Tavares-Costa performed the phylogenetic analyses. M.I. Müller carried out the PCR and sequencing. G.F. Maschio Santos and F.T.V. Melo contributed to the writing of the manuscript, reviewed the content, and prepared the line drawings. All authors reviewed the final manuscript.

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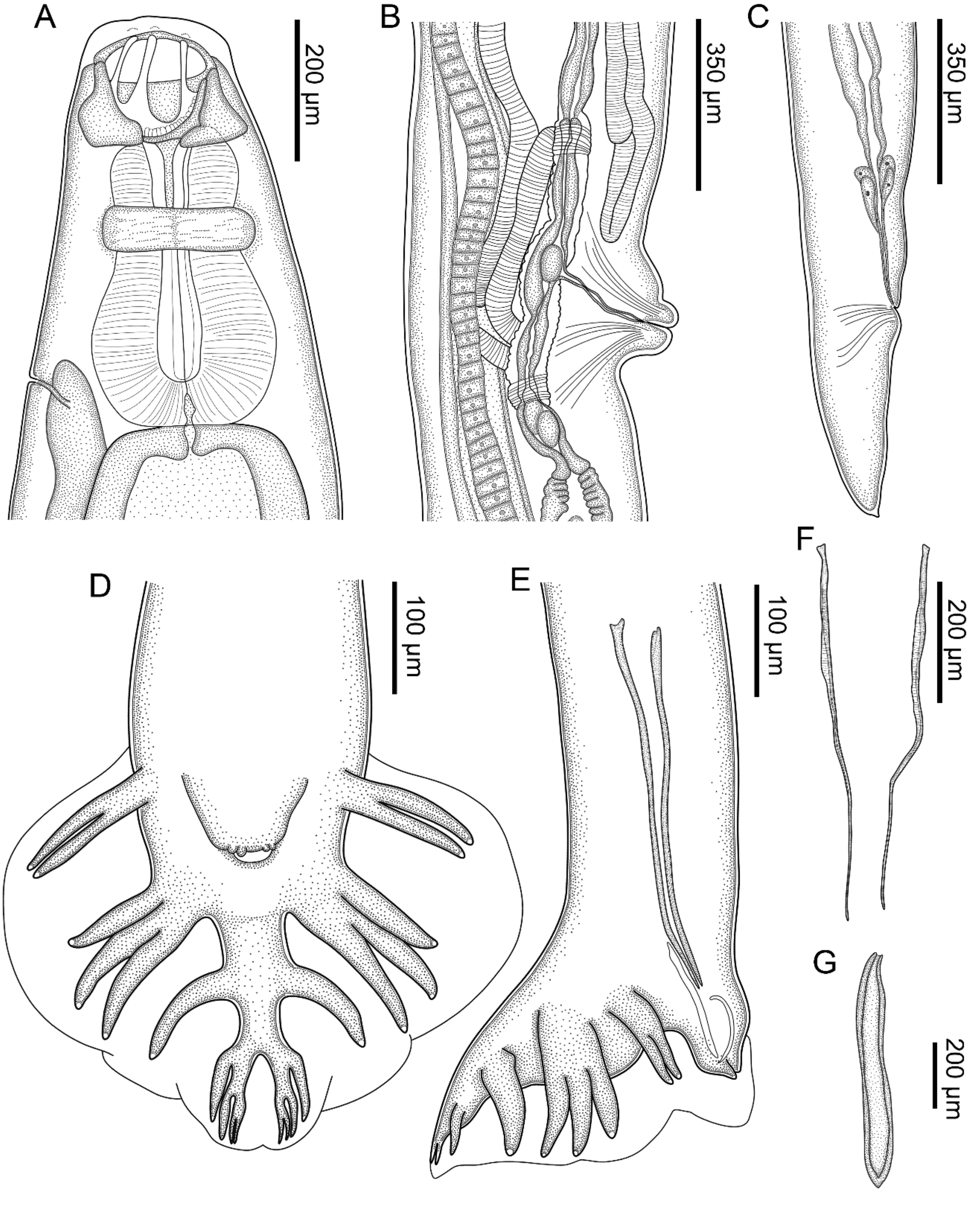
LEGENDAS DAS FIGURAS

Fig. 1 Line drawings of *Kalicephalus atroxi* n. sp. from *Bothrops atrox*. (A) Anterior end of the body, lateral view; (B) Vulvar region, lateral view; (C) Posterior end of the female, lateral view; (D) Copulatory bursa, ventral view; (E) Posterior end of the male body, lateral view) (F) Spicules, ventral view; (G) Gubernaculum, ventral view;. Scale bar: A, D, F = 200 µm; B, C, E = 350 µm; G = 100 µm.

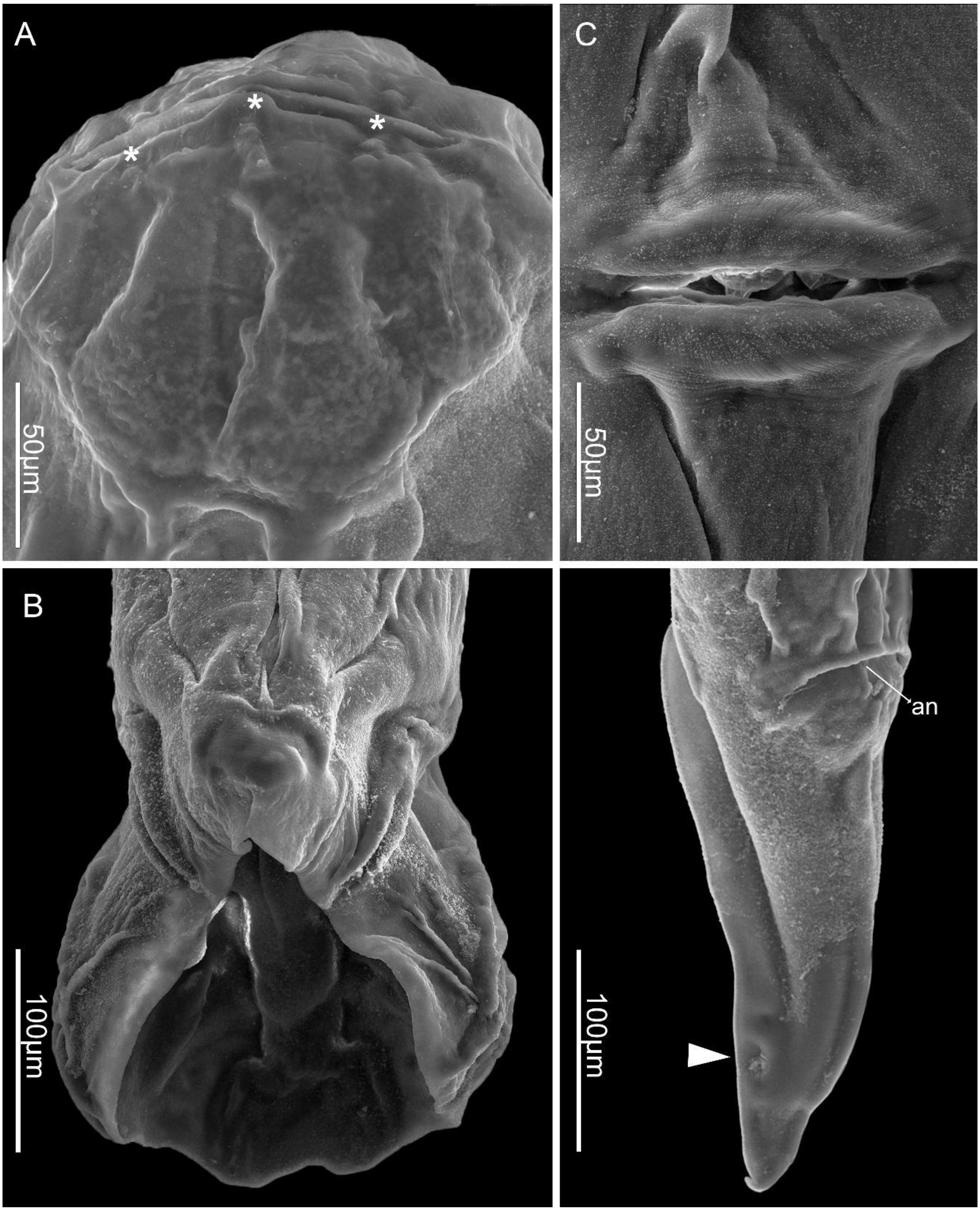
Fig. 2 Scanning Electron Microscopy of *Kalicephalus atroxi* n. sp. from *B. atrox*: (A) Details of the buccal capsule (asterisks: papillae associated with amphids) (50 µm); (B) Details of the copulatory bursa, genital cone, and dorsal rays (100 µm); (C) Vulvar region (50 µm); (D) Posterior end of the female (arrow:– phasmids) (100 µm). Scale bar: A, C = 50 µm; , D = 100 µm;

Fig. 3 Maximum Likelihood phylogenetic tree of Ancylostomatidae and Diaphanocephalidae based on ITS1 gene sequences using *Oswaldocruzia filiformis* as outgroup and indicating the position of *Kalicephalus atroxi* n. sp. (shown in bold and italics). GenBank accession numbers follow each taxon. Support values above or below the nodes: posterior probabilities <0.90 and bootstrap values <70 are not shown.

1



457 **2**



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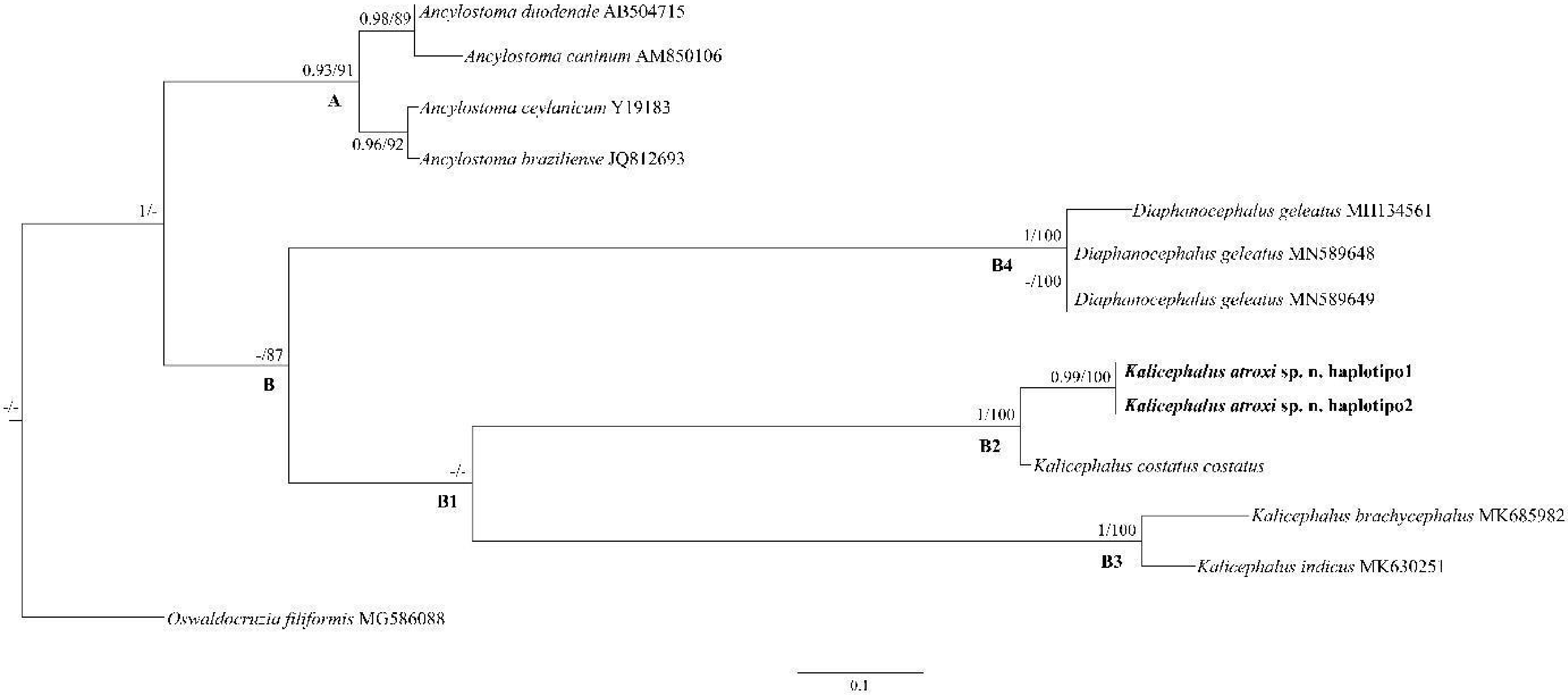
3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Ancylostoma caninum AM850106
0.93/91
A
Ancylostoma ceylanicum Y19183
0.96/92
Ancylostoma braziliense JQ812693
1/-
Diaphanocephalus geleatus MH134561
1/100
Diaphanocephalus geleatus MN589648
B4
-/100
Diaphanocephalus geleatus MN589649
-/87
B
0.99/100
Kalicephalus atroxi sp. n. haplotipo1
Kalicephalus atroxi sp. n. haplotipo2
1/100
B2
-/-
Kalicephalus costatus costatus
-/-
B1
Kalicephalus brachycephalus MK685982
1/100
B3
Kalicephalus indicus MK630251
Oswaldocruzia filiformis MG586088
0.1

Anexo 1

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Negotiation research spans many disciplines (Thompson 1990).

This result was later contradicted by Becker and Seligman (1996).

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Book chapter

Brown B, Aaron M (2001) The politics of nature. In: Smith J (ed) The rise of modern genomics, 3rd edn. Wiley, New York, pp 230-257

Online document

Cartwright J (2007) Big stars have weather too. IOP Publishing PhysicsWeb. http://physicsweb.org/articles/news/11/6/16/1. Accessed 26 June 2007

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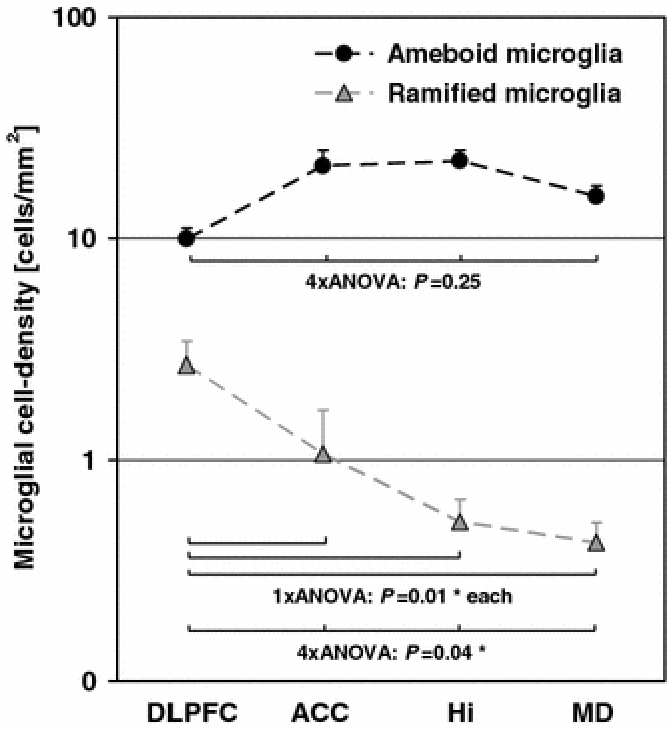

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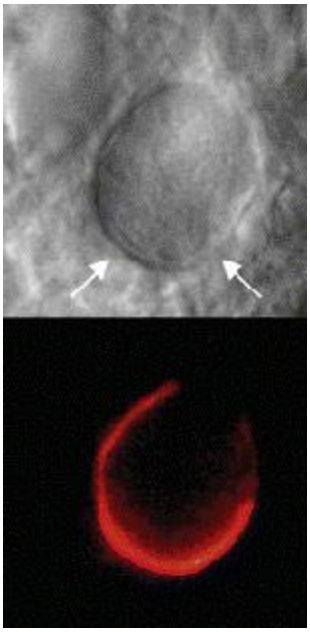

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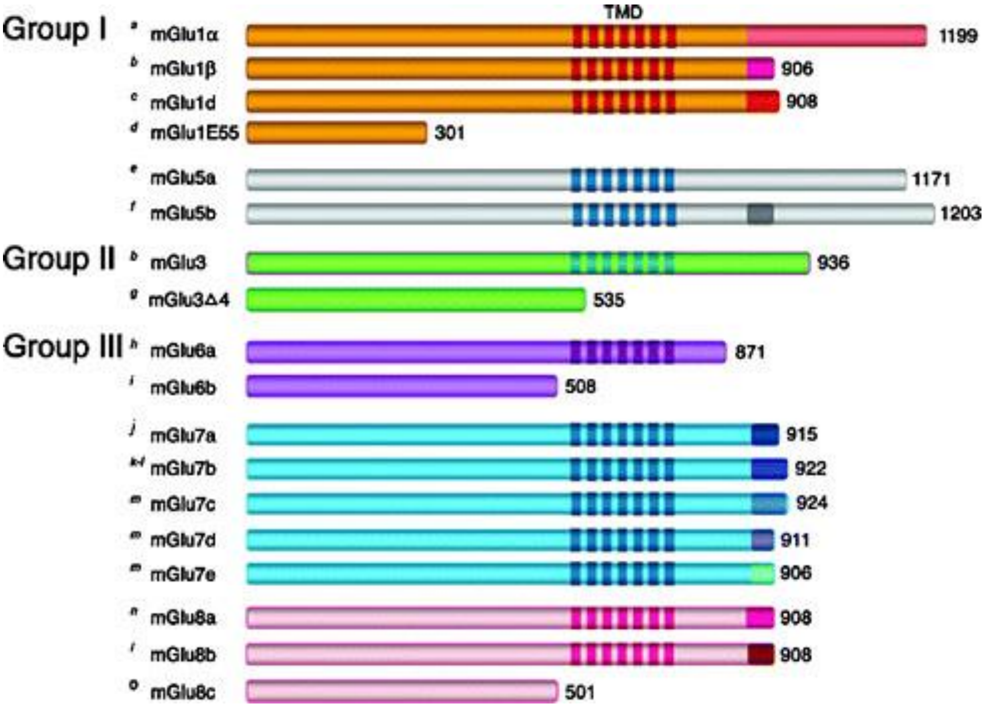

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Research involving human participants, their data or biological material

Ethics approval

When reporting a study that involved human participants, their data or biological material, authors should include a statement that confirms that the study was approved (or granted exemption) by the appropriate institutional and/or national research ethics committee (including the name of the ethics committee) and certify that the study was performed in accordance with the ethical standards as laid down in the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards.

If doubt exists whether the research was conducted in accordance with the 1964 Helsinki Declaration or comparable standards, the authors must explain the reasons for their approach, and demonstrate that an independent ethics committee or institutional review board explicitly approved the doubtful aspects of the study. If a study was granted exemption from requiring ethics approval, this should also be detailed in the manuscript (including the reasons for the exemption).

Retrospective ethics approval

If a study has not been granted ethics committee approval prior to commencing, retrospective ethics approval usually cannot be obtained and it may not be possible to consider the manuscript for peer review. The decision on whether to proceed to peer review in such cases is at the Editor's discretion.

Ethics approval for retrospective studies

Although retrospective studies are conducted on already available data or biological material (for which formal consent may not be needed or is difficult to obtain) ethics approval may be required dependent on the law and the national ethical guidelines of a country. Authors should check with their institution to make sure they are complying with the specific requirements of their country.

Ethics approval for case studies

Case reports require ethics approval. Most institutions will have specific policies on this subject. Authors should check with their institution to make sure they are complying with the specific requirements of their institution and seek ethics approval where needed. Authors should be aware to secure informed consent from the individual (or parent or guardian if the participant is a minor or incapable) See also section on Informed Consent.

Cell lines

If human cells are used, authors must declare in the manuscript: what cell lines were used by describing the source of the cell line, including when and from where it was obtained, whether the cell line has recently been authenticated and by what method. If cells were bought from a life science company the following need to be given in the manuscript: name of company (that provided the cells), cell type, number of cell line, and batch of cells.

It is recommended that authors check the NCBI database for misidentification and contamination of human cell lines. This step will alert authors to possible problems with the cell line and may save considerable time and effort.

Further information is available from the International Cell Line Authentication Committee (ICLAC).

Authors should include a statement that confirms that an institutional or independent ethics committee (including the name of the ethics committee) approved the study and that informed consent was obtained from the donor or next of kin.

Research Resource Identifiers (RRID)

Research Resource Identifiers (RRID) are persistent unique identifiers (effectively similar to a DOI) for research resources. This journal encourages authors to adopt RRIDs when reporting key biological resources (antibodies, cell lines, model organisms and tools) in their manuscripts.

Examples:

Organism: $Filip1^{tm1a(KOMP)Wtsi}$ RRID:MMRRC_055641-UCD

Cell Line: RST307 cell line RRID:CVCL_C321

Antibody: Luciferase antibody DSHB Cat# LUC-3, RRID:AB_2722109

Plasmid: mRuby3 plasmid RRID:Addgene_104005

Software: ImageJ Version 1.2.4 RRID:SCR_003070

RRIDs are provided by the Resource Identification Portal. Many commonly used research resources already have designated RRIDs. The portal also provides authors links so that they can quickly register a new resource and obtain an RRID.

Clinical Trial Registration

The World Health Organization (WHO) definition of a clinical trial is "any research study that prospectively assigns human participants or groups of humans to one or more health-related interventions to evaluate the effects on health outcomes". The WHO defines health interventions as “A health intervention is an act performed for, with or on behalf of a person or population whose purpose is to assess, improve, maintain, promote or modify health, functioning or health conditions” and a health-related outcome is generally defined as a change in the health of a person or population as a result of an intervention.

To ensure the integrity of the reporting of patient-centered trials, authors must register prospective clinical trials (phase II to IV trials) in suitable publicly available repositories. For example www.clinicaltrials.gov or any of the primary registries that participate in the WHO International Clinical Trials Registry Platform.

The trial registration number (TRN) and date of registration should be included as the last line of the manuscript abstract.

For clinical trials that have not been registered prospectively, authors are encouraged to register retrospectively to ensure the complete publication of all results. The trial registration number (TRN), date of registration and the words 'retrospectively registered’ should be included as the last line of the manuscript abstract.

Standards of reporting

Springer Nature advocates complete and transparent reporting of biomedical and biological research and research with biological applications. Authors are recommended to adhere to the minimum reporting guidelines hosted by the EQUATOR Network when preparing their manuscript.

Exact requirements may vary depending on the journal; please refer to the journal’s Instructions for Authors.

Checklists are available for a number of study designs, including:

Randomised trials (CONSORT) and Study protocols (SPIRIT)

Observational studies (STROBE)

Systematic reviews and meta-analyses (PRISMA) and protocols (Prisma-P)

Diagnostic/prognostic studies (STARD) and (TRIPOD)

Case reports (CARE)

Clinical practice guidelines (AGREE) and (RIGHT)

Qualitative research (SRQR) and (COREQ)

Animal pre-clinical studies (ARRIVE)

Quality improvement studies (SQUIRE)

Economic evaluations (CHEERS)

Summary of requirements

The above should be summarized in a statement and placed in a ‘Declarations’ section before the reference list under a heading of ‘Ethics approval’.

Examples of statements to be used when ethics approval has been obtained:

- All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. The study was approved by the Bioethics Committee of the Medical University of A (No. ...).

- This study was performed in line with the principles of the Declaration of Helsinki. Approval was granted by the Ethics Committee of University B (Date.../No. ...).

- Approval was obtained from the ethics committee of University C. The procedures used in this study adhere to the tenets of the Declaration of Helsinki.

- The questionnaire and methodology for this study was approved by the Human Research Ethics committee of the University of D (Ethics approval number: ...).

Examples of statements to be used for a retrospective study:

- Ethical approval was waived by the local Ethics Committee of University A in view of the retrospective nature of the study and all the procedures being performed were part of the routine care.

- This research study was conducted retrospectively from data obtained for clinical purposes. We consulted extensively with the IRB of XYZ who determined that our study did not need ethical approval. An IRB official waiver of ethical approval was granted from the IRB of XYZ.

- This retrospective chart review study involving human participants was in accordance with the ethical standards of the institutional and national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. The Human Investigation Committee (IRB) of University B approved this study.

Examples of statements to be used when no ethical approval is required/exemption granted:

- This is an observational study. The XYZ Research Ethics Committee has confirmed that no ethical approval is required.

- The data reproduced from Article X utilized human tissue that was procured via our Biobank AB, which provides de-identified samples. This study was reviewed and deemed exempt by our XYZ Institutional Review Board. The BioBank protocols are in accordance with the ethical standards of our institution and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Authors are responsible for correctness of the statements provided in the manuscript. See also Authorship Principles. The Editor-in-Chief reserves the right to reject submissions that do not meet the guidelines described in this section.

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Informed consent

All individuals have individual rights that are not to be infringed. Individual participants in studies have, for example, the right to decide what happens to the (identifiable) personal data gathered, to what they have said during a study or an interview, as well as to any photograph that was taken. This is especially true concerning images of vulnerable people (e.g. minors, patients, refugees, etc) or the use of images in sensitive contexts. In many instances authors will need to secure written consent before including images.

Identifying details (names, dates of birth, identity numbers, biometrical characteristics (such as facial features, fingerprint, writing style, voice pattern, DNA or other distinguishing characteristic) and other information) of the participants that were studied should not be published in written descriptions, photographs, and genetic profiles unless the information is essential for scholarly purposes and the participant (or parent/guardian if the participant is a minor or incapable or legal representative) gave written informed consent for publication. Complete anonymity is difficult to achieve in some cases. Detailed descriptions of individual participants, whether of their whole bodies or of body sections, may lead to disclosure of their identity. Under certain circumstances consent is not required as long as information is anonymized and the submission does not include images that may identify the person.

Informed consent for publication should be obtained if there is any doubt. For example, masking the eye region in photographs of participants is inadequate protection of anonymity. If identifying characteristics are altered to protect anonymity, such as in genetic profiles, authors should provide assurance that alterations do not distort meaning.

Exceptions where it is not necessary to obtain consent:

- Images such as x rays, laparoscopic images, ultrasound images, brain scans, pathology slides unless there is a concern about identifying information in which case, authors should ensure that consent is obtained.

- Reuse of images: If images are being reused from prior publications, the Publisher will assume that the prior publication obtained the relevant information regarding consent. Authors should provide the appropriate attribution for republished images.

Consent and already available data and/or biologic material

Regardless of whether material is collected from living or dead patients, they (family or guardian if the deceased has not made a pre-mortem decision) must have given prior written consent. The aspect of confidentiality as well as any wishes from the deceased should be respected.

Data protection, confidentiality and privacy

When biological material is donated for or data is generated as part of a research project authors should ensure, as part of the informed consent procedure, that the participants are made aware what kind of (personal) data will be processed, how it will be used and for what purpose. In case of data acquired via a biobank/biorepository, it is possible they apply a broad consent which allows research participants to consent to a broad range of uses of their data and samples which is regarded by research ethics committees as specific enough to be considered “informed”. However, authors should always check the specific biobank/biorepository policies or any other type of data provider policies (in case of non-bio research) to be sure that this is the case.

Consent to Participate

For all research involving human subjects, freely-given, informed consent to participate in the study must be obtained from participants (or their parent or legal guardian in the case of children under 16) and a statement to this effect should appear in the manuscript. In the case of articles describing human transplantation studies, authors must include a statement declaring that no organs/tissues were obtained from prisoners and must also name the institution(s)/clinic(s)/department(s) via which organs/tissues were obtained. For manuscripts reporting studies involving vulnerable groups where there is the potential for coercion or where consent may not have been fully informed, extra care will be taken by the editor and may be referred to the Springer Nature Research Integrity Group.

Consent to Publish

Individuals may consent to participate in a study, but object to having their data published in a journal article. Authors should make sure to also seek consent from individuals to publish their data prior to submitting their paper to a journal. This is in particular applicable to case studies. A consent to publish form can be found

here. (Download docx, 36 kB)

Summary of requirements

The above should be summarized in a statement and placed in a ‘Declarations’ section before the reference list under a heading of ‘Consent to participate’ and/or ‘Consent to publish’. Other declarations include Funding, Competing interests, Ethics approval, Consent, Data and/or Code availability and Authors’ contribution statements.

Please see the various examples of wording below and revise/customize the sample statements according to your own needs.

Sample statements for "Consent to participate":

Informed consent was obtained from all individual participants included in the study.

Informed consent was obtained from legal guardians.

Written informed consent was obtained from the parents.

Verbal informed consent was obtained prior to the interview.

Sample statements for “Consent to publish”:

The authors affirm that human research participants provided informed consent for publication of the images in Figure(s) 1a, 1b and 1c.

The participant has consented to the submission of the case report to the journal.

Patients signed informed consent regarding publishing their data and photographs.

Sample statements if identifying information about participants is available in the article:

Additional informed consent was obtained from all individual participants for whom identifying information is included in this article.

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Images will be removed from publication if authors have not obtained informed consent or the paper may be removed and replaced with a notice explaining the reason for removal.

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Research involving animals, their data or biological material

The welfare of animals (vertebrate and higher invertebrate) used for research, education and testing must be respected. Authors should supply detailed information on the ethical treatment of their animals in their submission. For that purpose they may use the ARRIVE checklist which is designed to be used when submitting manuscripts describing animal research.

For studies involving client-owned animals, authors must also document informed consent from the client or owner and adherence to a high standard (best practice) of veterinary care.

Authors are recommended to comply with:

- The International Union for Conservation of Nature (IUCN) Policy Statement on Research Involving Species at Risk of Extinction and consult the IUCN red list index of threatened species.

- Convention on the Trade in Endangered Species of Wild Fauna and Flora

When reporting results authors should indicate:

- … that the studies have been approved by a research ethics committee at the institution or practice at which the studies were conducted. Please provide the name of ethics committee and relevant permit number;

- … whether the legal requirements or guidelines in the country and/or state or province for the care and use of animals have been followed.

Researchers from countries without any legal requirements or guidelines voluntarily should refer to the following sites for guidance:

– The Basel Declaration describes fundamental principles of using animals in biomedical research

– The International Council for Laboratory Animal Science (ICLAS) provides ethical guidelines for researchers as well as editors and reviewers

– The Association for the study of Animal Behaviour describes ethical guidelines for the treatment of animals in research and teaching

– The International Association of Veterinary Editors' Consensus Author Guidelines on Animal Ethics provide guidelines for authors on animal ethics and welfare

Researchers may wish to consult the most recent (ethical) guidelines available from relevant taxon-oriented professional societies.

If a study was granted exemption or did not require ethics approval, this should also be detailed in the manuscript.

Summary of requirements

The above should be summarized in a statement and placed in a ‘Declarations’ section before the reference list under a heading of ‘Ethics approval’.

Please see the various examples of wording below and revise/customize the sample statements according to your own needs.

Examples of statements to be used when ethics approval has been obtained:

- All procedures involving animals were in compliance with the European Community Council Directive of 24 November 1986, and ethical approval was granted by the Kocaeli University Ethics Committee (No. 29 12 2014, Kocaeli, Turkey).

- All procedures performed in the study were in accordance with the ARVO Statement for Use of Animals in Ophthalmic Vision and Research. The ethical principles established by the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 8523, revised 2011) were followed. The research protocol was approved by the Ethics Committee on Animal Use (Protocol No. 06174/14) of FCAV/Unesp, Jaboticabal.

- This study involved a questionnaire-based survey of farmers as well as blood sampling from their animals. The study protocol was assessed and approved by Haramaya University, research and extension office. Participants provided their verbal informed consent for animal blood sampling as well as for the related survey questions. Collection of blood samples was carried out by veterinarians adhering to the regulations and guidelines on animal husbandry and welfare.

- All brown bear captures and handling were approved by the Ethical Committee on Animal Experiments, Uppsala, Sweden (Application C18/15) and the Swedish Environmental Protection Agency in compliance with Swedish laws and regulations.

- The ethics governing the use and conduct of experiments on animals were strictly observed, and the experimental protocol was approved by the University of Maiduguri Senate committee on Medical Research ethics. Proper permit and consent were obtained from the Maiduguri abattoir management, before the faecal samples of the cattle and camels slaughtered in this abattoir were used for this experiment.

Examples of statements to be used when no ethical approval is required/exemption granted:

- No approval of research ethics committees was required to accomplish the goals of this study because experimental work was conducted with an unregulated invertebrate species.

- As the trappings of small mammals were conducted as part of regular pest control measures in accordance with the NATO Standardized Agreement 2048 "Deployment Pest and Vector Surveillance and Control ", no approval by an ethics committee was required.

- All experiments have been conducted as per the guidelines of the Institutional Animal Ethics Committee, Department of Zoology, Utkal University, Bhubaneswar, Odisha, India. However, the insect species used in this study is reared for commercial production of raw silk materials, as a part of agro-based industry. Therefore, use of this animal in research does not require ethical clearance. We have obtained permission from the office of Research officer sericulture, Baripada, Orissa, India for the provision of infrastructure and support for rearing of silkworm both in indoor and outdoor conditions related to our study to promote sericulture practices.

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Research Data Policy

This journal operates a type 1 research data policy. The journal encourages authors, where possible and applicable, to deposit data that support the findings of their research in a public repository. Authors and editors who do not have a preferred repository should consult Springer Nature's list of repositories and research data policy.

List of Repositories

Research Data Policy

General repositories - for all types of research data - such as figshare and Dryad may also be used.

Datasets that are assigned digital object identifiers (DOIs) by a data repository may be cited in the reference list. Data citations should include the minimum information recommended by DataCite: authors, title, publisher (repository name), identifier.

DataCite

If the journal that you're submitting to uses double-blind peer review and you are providing reviewers with access to your data (for example via a repository link, supplementary information or data on request), it is strongly suggested that the authorship in the data is also blinded. There are data repositories that can assist with this and/or will create a link to mask the authorship of your data.

Authors who need help understanding our data sharing policies, help finding a suitable data repository, or help organising and sharing research data can access our Author Support portal for additional guidance.

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ANEXO 2

Policy on prior publication

When authors submit manuscripts to this journal, these manuscripts should not be under consideration, accepted for publication or in press within a different journal, book or similar entity, unless explicit permission or agreement has been sought from all entities involved. However, deposition of a preprint on the author's personal website, in an institutional repository, or in a preprint archive shall not be viewed as prior or duplicate publication. Authors should follow the Cambridge University Press Preprint Policy regarding preprint archives and maintaining the version of record.

Scope

Journal of Helminthology publishes original papers, review articles and short communications on all aspects of pure and applied helminthology, particularly those helminth parasites of environmental health, medical or veterinary importance. As of June 1st 2024, the journal will no longer consider papers focused on free-living helminth taxa, unless the latter are directly relevant to the study of parasitic helminths. Research papers on the ecology and evolution of helminths in wildlife hosts, including plant and insect parasites, are also published along with taxonomic papers contributing to the systematics of a group. The journal will be of interest to academics and researchers involved in the fields of human and veterinary parasitology, public health, microbiology, ecology, epidemiology and biochemistry.

Categories of papers

The Editor welcomes original, creative, high-quality contributions suitable for the journal's international readership. The novelty and importance of submitted papers should be made clear in the Introduction.

All empirical studies must include clear and appropriate statistical analyses of the data, following best-practice for conducting analyses and reporting results in the field of study.

In addition, for taxonomic papers describing new helminth species, we now require both morphological and genetic data, in accordance with the principles of integrative taxonomy. For papers investigating the anthelmintic properties of plant extracts or other novel compounds, we now require evidence beyond simple *in vitro* effects on worm or egg survival, such as chemical characterisation of the active compounds, elucidation of their mode of action, and/or *in vivo* tests of their efficacy and toxicity.

There are no page charges for papers published in *Journal of Helminthology*.

The journal accepts the following contributions:

Research Articles

This category is intended for full-scale studies of an appropriate length.

Reviews

Journal of Helminthology will publish scholarly, comprehensive reviews that summarise and critically evaluate research in the field, addressing and identifying future implications. Reviews may be invited by the Editor but may also be submitted. Authors wishing to submit papers in this category are advised to contact the Editor before doing so.

Short Communications

This category is for short, definitive reports of exciting developments with the potential for wider application and further exploration. Manuscripts should be formatted as for full length papers but should keep figures and tables preferably to one of each maximum. References should be restricted to the essential only and the article should take up no more than four pages of the journal.

Commentaries

Papers in this section provide readers of *Journal of Helminthology* with focused, view-point coverage of topical issues which are of high current interest. Articles of this type will be invited but may also be submitted. Authors wishing to submit articles for this section are asked to consult with the Editor.

Acceptance or rejection of the commentary is at the discretion of the Editor, and commentaries will be peer reviewed. Also, the Editor may request or allow a response to the commentary.

Online submission

All manuscripts should be submitted online at:

http://www.editorialmanager.com/joh

After submitting your manuscript, you will receive an email acknowledging receipt of the manuscript and providing the manuscript reference number. You should quote the reference number of your manuscript in all correspondence relating to your manuscript.

Please ensure that your manuscript is uploaded in the correct file formats and using the correct journal styles. You should particularly note the following instructions:

- *The uploaded manuscript must be saved as a DOC file (not DOCX) or an RTF file.*

- *The manuscript file should include title, authors, email address of corresponding author, abstract (250 words), main text, references and captions for tables and figures.*
- *All figures should be uploaded in TIF format as separate files, and saved at final size and at appropriate resolution. Colour figures must be saved as CMYK (not RGB). Large files can be uploaded as ZIP files.*
- *Tables must be inserted at the end of the main document, not supplied as separate files.*
- *Line numbering and page numbering of the manuscript file are required*
- *A cover letter must be supplied at initial submission. This can be uploaded as a separate file.*
- *Appended to the Summary should be 3-10 relevant* ***key words*** *suitable for indexing.* ***Nothing else should appear on the summary page.***

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ORCID IDs

Journal of Helminthology now requires that all corresponding authors identify themselves using ORCID when submitting a manuscript to the journal. Joining ORCID is fast, free and you do not need to have a current affiliation. ORCID provides a unique identifier for researchers and, through integration in key research workflows such as publication and grant applications, provides the following benefits:

- Discoverability: ORCID increases the discoverability of your publications, by enabling smarter publisher systems and by helping readers to reliably find work that you've authored.
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- Keeping track: Your ORCID profile is a neat place to record and display (if you choose) validated information about your research activities.

If you don't already have an ID, you'll need to create one if you decide to submit a manuscript to *Journal of Helminthology*. You can register for one directly from your user account on ScholarOne or Editorial Manager or via https://orcid.org/register. If you already have an ID, please use this when submitting by linking it to your ScholarOne user account. Simply log in to your account using your normal username and password. Edit your account by clicking on your name at the top right of the screen and from the dropdown menu, select 'E-Mail / Name'. Follow the instructions at the top of the screen to update your account.

For more information on ORCID please visit: https://www.cambridge.org/using-ORCID

NOTE: Registering new species of helminths with ZooBank

Since *Journal of Helminthology* has now moved to electronic-only publication, authors of papers where new species are described should register these details, including new zoological names or other nomenclaturally relevant information, with ZooBank (zoobank.org) before FirstView online publication. Zoobank is an open-access, community-generated registry for zoological nomenclature.

Originality and copyright

To be published in *Journal of Helminthology*, a manuscript cannot have been published previously, nor can it be under review for publication elsewhere. Papers with multiple authors are reviewed in the assumption that all authors have contributed materially to the research report, have approved the submitted manuscript and concur with its submission to *Journal of Helminthology*.

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All contributions must be written in English. Papers should be as concise as clarity permits, and figures should be restricted to the minimum number required for a clear explanation. You should ensure that text, figures, citations and references adhere to the journal styles described in this document. An EndNote style for the journal can be downloaded here.

Please note that line numbering and page numbering are required for all submitted manuscripts.

The title page should include:

- The **title** of the article, which **should be short** (preferably up to 12 words) but informative and accurately reflect the content.
- Authors' names and contact details: please list a brief affiliation for each author including country (assigned with superscript numbers) below the author names, and in addition, indicate the corresponding author with an asterisk and in this case provide an email address
- Word count, including all text but excluding tables, figures and references.

In *Short Communications*, add the text 'Short Communication: ' at the start of the title of your paper.

The ***Abstract*** should be an unstructured abstract. It must not exceed 250 words, but it must provide the reader with a self-contained summary of the paper. It should include a brief introduction to the paper, the method, the key findings and the conclusions.

The ***body of the manuscript*** should be broken into sections, such as the Introduction, Material and Methods, Results, Discussion, Acknowledgements, References.

In *Short Communications*, the results and the discussion should be described and discussed under one heading 'Results and Discussion'.

Please ensure that all headings conform to journal style:

- Main headings typed in bold with an initial capital only: '**Summary**', '**Materials and methods**'
- Subheadings: typed in italic (except for those words and symbols which would be italicized in the text), e.g. '*Sample collection*'.

You should identify within the text where figures and tables should be inserted. Use standard abbreviations (e.g. fig. and figs.), use metric units, use British rather than American spellings, and use 'z' not 's' spellings in words with 'ize'. Use italics for taxonomic nomenclature.

You should cite all figures, tables and Supplementary files within the text. Figures/tables should be numbered sequentially as they appear in the text.

For research citations, use '&' for two authors and '*et al.*' for three or more authors. Use a, b etc. as defined by the alphabetical order in the Reference list if citing more than one paper by the same author. Arrange chronologically by year of publication in the citation. For example, '(Blackman *et al.*, 1994; Roberts & Kumar, 1995)'.

Use the styles 'Polaszek (1996)', 'Zhang & Hewitt (1997)' and 'Harry *et al.* (1998)' at the start of or within a sentence. Otherwise use '(Polaszek, 1996)', '(Taylor & Davies, 1989)' or '(Hu *et al.*, 1996)'.

Acknowledgements

You may acknowledge individuals or organisations that provided advice, support (non-financial). Formal financial support and funding should be listed in the following section.

Financial support

Please provide details of the sources of financial support for all authors, including grant numbers.

For example, "This work was supported by the Medical research Council (grant number XXXXXXX)". Multiple grant numbers should be separated by a comma and space, and where research was funded by more than one agency the different agencies should be separated by a semi-colon, with "and" before the final funder. Grants held by different authors should be identified as belonging to individual authors by the authors' initials. For example, "This work was supported by the Wellcome Trust (A.B., grant numbers XXXX, YYYY), (C.D., grant number ZZZZ); the Natural Environment Research Council (E.F., grant number FFFF); and the National Institutes of Health (A.B., grant number GGGG), (E.F., grant number HHHH)". Where no specific funding has been provided for research, please provide the following statement: "This research received no specific grant from any funding agency, commercial or not-for-profit sectors."

Conflict of interest declaration

All authors must include a conflict of interest declaration in their manuscript. This declaration will be subject to editorial review and may be published in the article.

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If the manuscript has multiple authors, the author submitting must include conflict of interest declarations relevant to all contributing authors. Example wording for a declaration is as follows: "Conflict of interest: Author A is employed at company B. Author C owns shares in company D, is on the Board of company E and is a member of organisation F. Author G has received grants from company H." If no competing interests exist, the declaration should state "Conflict of interest: The author(s) declare none".

Ethical standards

Where research involves human and/or animal experimentation, the following statements should be included (as applicable): “The authors assert that all procedures contributing to this work comply with the ethical standards of the relevant national and institutional committees on human experimentation and with the Helsinki Declaration of 1975, as revised in 2008.” and “The authors assert that all procedures contributing to this work comply with the ethical standards of the relevant national and institutional guides on the care and use of laboratory animals.”

Publication Ethics

Please visit this page for information on our ethical guidelines.

References

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Molloy, S, Holland C, and O'Regan, M. (1995) Population biology of *Pomphorhynchus laevis* in brown trout from two lakes in the west or Ireland. *Journal of Helminthology* **69**, 229–23.

Online publications: If a paper is published online, but has not yet been published in a printed issue, please list the article’s DOI. Once the paper has been published in print, the full reference should be given in the usual way.

Book

Bailey NTJ (1995) *Statistical Methods in Biology*. 3rd edn. Cambridge: Cambridge University Press.

Chapter in a Book

Sanderson CJ (1993) Cytokines active in eosinophils. In Makino S. and Fukuda T. (eds), *Eosinophils: Biological and Clinical Aspects*. Boca Raton: CRC Press, pp. 274–284.

Websites

World Health Organization (2011) *World Report on Disability.* Geneva: WHJO. Available at https://www.who.int/disabilities/world_report/2011/en (accessed January 1, 2023).

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