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Diversidade de helmintos parasitos de uma população de *Osteocephalus cabrerai* (Anura: Hylidae) na Amazônia brasileira

Belém,
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“Por milhões de anos
A Humanidade viveu como os animais
Então aconteceu algo
Que libertou o poder da nossa imaginação
Nós aprendemos a falar

Com a tecnologia à nossa disposição
As possibilidades são ilimitadas
Tudo o que precisamos fazer, é garantir que
continuemos falando”

Stephen Hawking
Pink Floyd – Keep Talking/Talkin' Hawkin'

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SUMÁRIO

Diversidade de helmintos parasitos de uma população de *Osteocephalus cabrerai* (Anura: Hylidae) na Amazônia brasileira

RESUMO

Os anfíbios são bons modelos de estudo para a diversidade de parasitos devido sua diversidade de estratégias de vida, se destacando como hospedeiros de diversos grupos de nematódeos, trematódeos, cestódeos, monogêneos e acantocéfalos. A estrutura da comunidade parasitária de anuros pode ser influenciada por diversos fatores bióticos ou abióticos e, apesar de terem sido muito estudadas, os táxons encontrados raramente são todos identificados ao nível de espécie. Até o momento, não existe nenhum estudo parasitológico para representantes de *Osteocephalus cabrerai*, sendo a fauna parasitária desse anuro totalmente desconhecida. O objetivo do presente estudo é caracterizar a diversidade de helmintos parasitos de *O. cabrerai*, da Reserva Extrativista Beija-Flor Brilho de Fogo. Esta dissertação é composta por dois capítulos. O primeiro consiste em um artigo já publicado que apresenta os primeiros registros de parasitos para *O. cabrerai*, analisando a composição e as características da comunidade parasitária, os padrões de distribuição dos parasitos e a correlação entre os fatores bióticos dos hospedeiros e a intensidade parasitária. Encontramos predominantemente nematódeos de ciclo de vida monoxênico, com alta prevalência e infecção abundante. A maioria dos espécimes encontrados representam o primeiro registro de parasitos para o gênero *Osteocephalus*. Além disso, nas nossas análises, os indivíduos com maior massa corporal tiveram uma tendência maior a infecção por helmintos parasitos. O segundo capítulo apresenta uma nota científica com as primeiras análises moleculares e filogenéticas que incluem *Aplectana pella*, chegando à conclusão de que o gênero *Aplectana* não é monofilético. Nós fornecemos o primeiro estudo sobre a estrutura da comunidade de helmintos de *O. cabrerai*, e adicionamos a primeira sequência de *Aplectana* da região Neotropical. Dessa forma, contribuímos para a compreensão da diversidade e ecologia de helmintos parasitos de anuros, além das relações filogenéticas de espécies da família Cosmocercidae.

Palavras-chave: Diversidade; Helmintos; Parasitos; *Osteocephalus*; Amazônia.

Diversity of parasitic helminths from a population of *Osteocephalus cabrerai* (Anura: Hylidae) in the Brazilian Amazon

ABSTRACT

Amphibians are good models for studies of parasite diversity due to their wide range of life strategies, standing out as hosts for various groups of nematodes, trematodes, cestodes, monogeneans, and acanthocephalans. The structure of parasitic communities in anurans can be influenced by various biotic or abiotic factors. Despite being extensively studied, the taxa found are rarely identified to the species level. To date, no parasitological studies have been conducted on representatives of *Osteocephalus cabrerai*, leaving the parasitic fauna of these anurans completely unknown. This study aims to characterize the diversity of parasitic helminths of *O. cabrerai* from the Beija-Flor Brilho de Fogo Extractive Reserve. This thesis comprises two chapters. The first chapter consists of a published Research Article that presents the first records of parasites for *O. cabrerai*, analyzing the composition and characteristics of the parasitic community, patterns of parasite distribution, and the correlation between host biotic factors and the characteristics of the community. We predominantly found nematodes with a monoxenous life cycle, high prevalence, and abundant infection. Most specimens represent the first record of parasites for the genus *Osteocephalus*. Additionally, in our analyses, individuals with greater body mass tended to have higher susceptibility to parasitic helminth infections. The second chapter presents a Short Communication with the first molecular and phylogenetic analyses involving *A. pella*, concluding that the genus *Aplectana* is not monophyletic. We provide the first study on the helminth community structure of *O. cabrerai* and add the first sequence of *Aplectana* from the Neotropical region. Thus, we enhance the understanding of the diversity and ecology of parasitic helminths in anurans, as well as the phylogenetic relationships of species within the family Cosmocercidae.

Keywords: Diversity; Helminths; Parasites; *Osteocephalus*; Amazon.

INTRODUÇÃO GERAL

O parasitismo é uma interação entre seres vivos de espécies diferentes na qual os benefícios são unilaterais e uma das envolvidas sofrendo consequências negativas pela interação (Neves, 2011; Méthot e Alizon, 2014). O estilo de vida parasitário é encontrado nos mais diversos grupos de seres vivos, entre eles estão os vírus, bactérias, protozoários, fungos, plantas e animais. Nos metazoários, o parasitismo surgiu diversas vezes de forma independente, especialmente entre os filos Nematoda Cobb, 1919 e Platyhelminthes Gegenbaur, 1859 (Weinstein e Kuris, 2016; Lucius et al., 2017).

Os anfíbios são bons modelos de estudo para a diversidade de parasitos devido sua diversidade de estratégias de vida (comportamentos, hábitats, dieta, modo de forrageio), se destacando como hospedeiros de diversos helmintos. Até 2014, foram registrados aproximadamente 290 táxons de helmintos parasitos de espécies de anuros da América do Sul (Campião et al., 2014). Além de serem os hospedeiros definitivos para uma grande diversidade de parasitos, os anfíbios também podem desempenhar papéis cruciais como hospedeiros intermediários ou paratênicos em ciclos de vida de diversos parasitos (Campião et al., 2015).

A fauna de helmintos associada a anuros do Brasil é a mais estudada dentre os países da América do Sul, no entanto, somente cerca de 8% do total das espécies de sapos brasileiros apresentam algum estudo helmintológico (Campião et al., 2014; Martins-Sobrinho et al., 2017). Essa lacuna ressalta a necessidade de realizar mais estudos em parasitologia, que podem fornecer informações tanto sobre os parasitos quanto dos hospedeiros, como dieta, hábitat e modo reprodutivo, sendo úteis para entender a estrutura das teias alimentares, biodiversidade, estresse ambiental e podendo ser, também, utilizados como ferramentas de conservação (Marcogliese, 2004).

Os helmintos parasitos de anuros estão classificados em três grupos: Platyhelminthes, Nematoda e Acanthocephala Kohlreuther, 1771.

O filo Platyhelminthes é composto por dois grupos (Catenulida e Rhabditophora) de vermes acelomados e achatados dorsoventralmente de vida livre ou parasitos, sendo os primeiros animais bilaterais e triploblásticos (Brusca, Giribet e Moore, 2022). No grupo Rhabditophora, destacamos o clado Neodermata, que inclui os grupos Trematoda, Monogenea e Cestoda, parasitos de vertebrados (Fig. 1).

Os platelmintos da classe Trematoda são caracterizados por apresentarem duas ventosas, uma próxima a boca e outra na região ventral do corpo (Gibson, 2002). Seu sistema digestório é incompleto, com pré-faringe, faringe, esôfago e cecos, com uma vesícula excretora geralmente terminal (Gibson, 2002). O sistema reprodutor masculino é composto geralmente por dois testículos, vesícula seminal, ducto ejaculatório e cirro (Neves et al., 2005). Já o sistema reprodutor feminino possui um ovário ligado a um oviduto, receptáculo seminal, oótipo, vitelário, glândulas de Mehlis, útero, vagina e canal de Laurer (Neves et al., 2005).

Trematódeos adultos se reproduzem em seus hospedeiros definitivos, que liberam ovos nas fezes e eclodem em larvas que infectam moluscos aquáticos, que são hospedeiros intermediários obrigatórios. Nos moluscos, essas larvas se transformam em cercárias (podendo também passar por outros estágios como rédeas e esporocistos), que saem dos moluscos e algumas formam cistos em outros animais ou plantas aquáticas que serão consumidos pelo hospedeiro definitivo (Zemmer et al., 2020). Em algumas espécies, as cercárias podem infectar diretamente o hospedeiro definitivo através da penetração ativa de pele e mucosas (Poulin e Cribb, 2002).

Os Monogenea são frequentemente encontrados parasitando brânquias, superfície corporal, cavidade nasal, ureteres e ductos intestinais de vertebrados aquáticos. Porém, também são encontrados parasitando a boca, bexiga urinária e cloaca de anfíbios e répteis e órbita ocular de quelônios e mamíferos (Loker e Hofkin, 2022). O corpo de indivíduos adultos é dividido em prohaptor, onde está localizada a boca, a qual é geralmente rodeada por ventosa e glândulas adesivas, tronco e opistohaptor, característica responsável pela fixação do corpo e também diagnóstica do grupo (Kearn, 1994; Ruppert, Fox e Barnes, 2004). Seu ciclo de vida é monoxênico, onde os adultos depositam ovos que eclodem ao entrar em contato com o ambiente externo e liberam um oncomiracídio que procura um novo hospedeiro para se aderir (Whittington et al., 1999; Whittington e Kearn, 2011).

Os cestódeos adultos são parasitos intestinais de vertebrados, com o corpo dividido em três partes: escólex, onde estão localizadas estruturas de fixação como ganchos e ventosas na região anterior do corpo; pescoço, que dá origem aos segmentos que formam a última parte do corpo; e estróbilo, segmentado em várias partes chamadas proglotes, que podem apresentar diferentes estágios de maturação (Roberts et al., 2012). Diferente de todos os outros platelmintos, os cestódeos não possuem sistema digestório, absorvendo recursos pelo tegumento (Goater et al., 2014). Apresentam um ciclo de vida heteroxênico, podendo envolver um ou mais hospedeiros intermediários (onde indivíduos no estágio larval se desenvolvem), e também ambientes terrestres e aquáticos (Benesh et al., 2017).

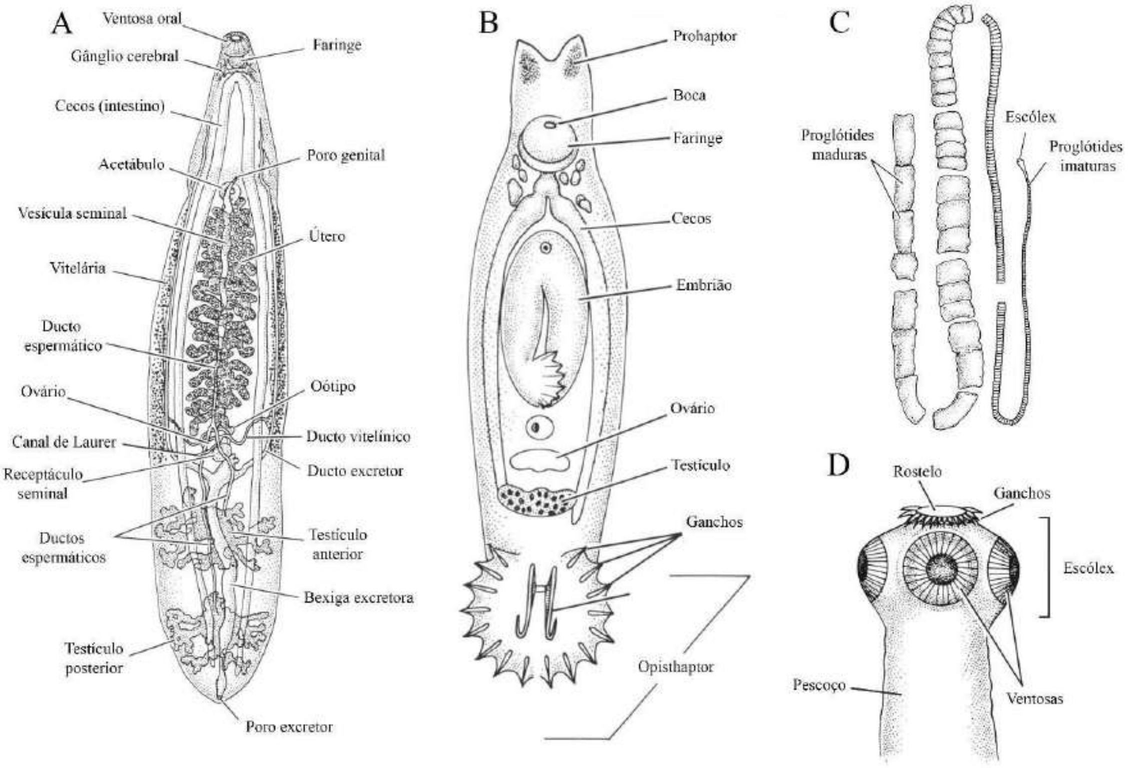

Figura 1. Morfologia interna e externa de representantes do filo Platyhelminthes. A: Morfologia interna do Trematoda *Clonorchis sinensis* (Looss, 1907) adulto, vista ventral. B: Morfologia interna de um Monogenea do gênero *Gyrodactylus* von Nordmann, 1832 adulto, vista ventral. C: Representação geral do Cestoda *Taenia saginata* Goeze, 1872 com destaque ao estróbilo composto de proglotes imaturas e maduras, vista ventral D: Representação de um pescoço e escólex com rostelo, ganchos e ventosas do Cestoda *Taenia solium* Linnaeus, 1758, vista ventral. Adaptado de Brusca, Giribet e Moore (2022).

O filo Nematoda é composto por animais de vida livre ou parasitos de corpo cilíndrico revestido por uma cutícula fina que serve de suporte para o esqueleto hidrostático e para a musculatura (Fig. 2) (Basyoni e Rizk, 2016). O sistema digestivo é completo, com boca, esôfago, intestino, reto e ânus ou cloaca (Schmidt-Rhaesa et al., 2013). Os nematódeos podem apresentar diversas estruturas sensoriais ao longo do corpo, como deirídeos, fasmídeos, papilas, anfídeos, ou até estruturas para penetração nos órgãos, como dentes e estiletes (Basyoni e Rizk, 2016).

Os representantes de Nematoda, em sua maioria, são dióicos e os machos têm como estruturas reprodutoras os testículos, ducto deferente, vesículas seminais, ducto ejaculatório, e estruturas que podem estar presentes, como espículos e gubernáculo (Schmidt-Rhaesa et al., 2013). As fêmeas, por sua vez, apresentam ovário, oviduto, útero, ovojetor, vagina e vulva (Goater et al., 2014). São um dos grupos mais diversos, apresentando também uma grande variedade de ciclos de vida, podendo ter ciclos monoxenos e heteroxênicos em ambiente terrestre, aquático ou por transmissão vetorial (Goater et al., 2014).

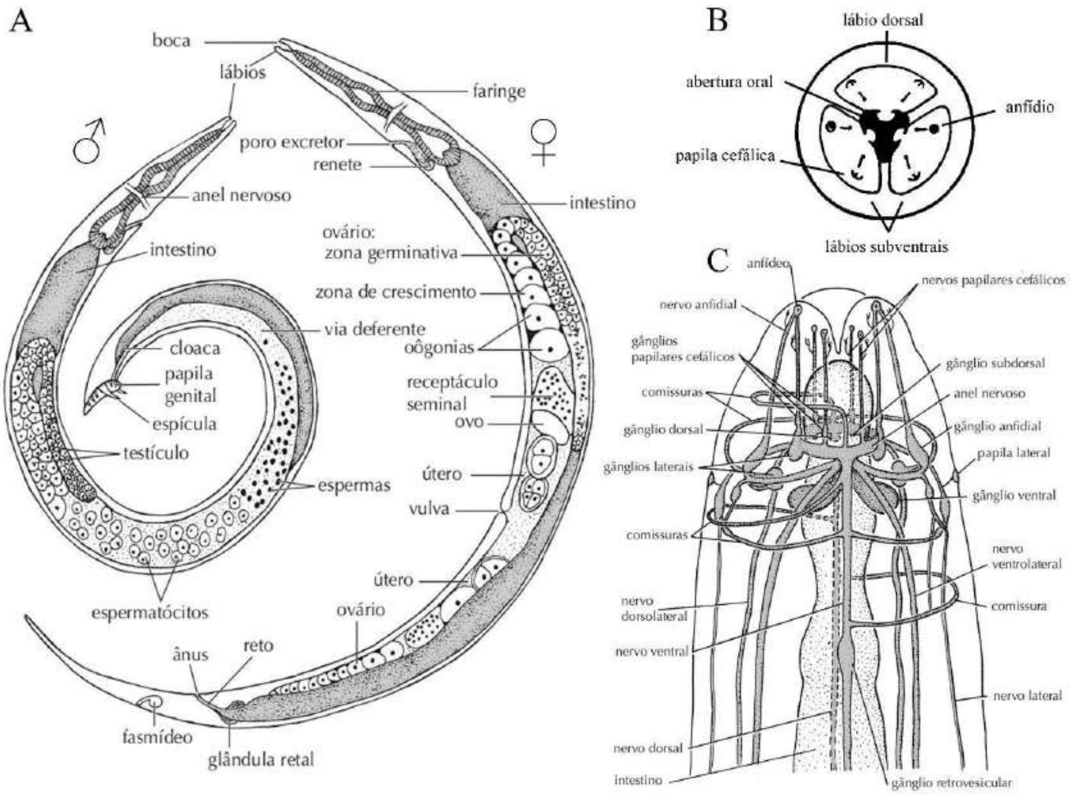

Figura 2. Morfologia geral de representantes do filo Nematoda. A: Morfologia interna geral de machos e fêmeas, representando aspectos do sistema digestivo, nervoso, excretor e reprodutor, vista lateral. B: Região cefálica do nematódeo *Enterobius vermicularis* (Linnaeus, 1758), destacando a abertura oral rodeada por três lábios contendo papilas cefálicas e anfídios, vista apical. C: Representação ilustrativa do sistema nervoso de nematódeo destacando o gânglio cerebral, cordões nervosos lateral, dorsal e ventral e inervação dos anfídeos e papilas, vista dorsal. Adaptado de Chitwood e Chitwood (1950) e Pechenik (2016).

O grupo Acanthocephala está atualmente classificado em um clado de organismos endoparasitos obrigatórios, sendo considerado uma subclasse do filo Rotifera (Near, 2002). Os acantocéfalos têm como uma das suas principais características morfológicas a presença de uma probóscide eversível com ganchos com a função de adesão/fixação nos tecidos dos órgãos de seus hospedeiros definitivos (Fig. 3) (Crompton e Nickol, 1985; Kennedy, 2006). Os parasitos desse grupo não possuem sistema digestivo, obtendo nutrientes por meio da superfície corporal a partir do conteúdo intestinal do hospedeiro (Crompton e Nickol, 1985; Goater, et al., 2014). O sistema reprodutor dos machos é constituído por dois testículos, glândula de cemento, bolsa copulatória, canal ejaculatório, bolsa de Saefftigen e cirro (Gomes, 2019). Já as fêmeas apresentam um ovário, ligamentos musculares, sino uterino, útero, vagina e poro genital (Gomes, 2019). Esses animais podem se desenvolver tanto em ambientes aquáticos quanto terrestres, apresentando um ciclo de vida heteroxênico, que depende de um ou mais hospedeiros intermediários, que geralmente são artrópodes, anfíbios, répteis e mamíferos (Kennedy, 2006).

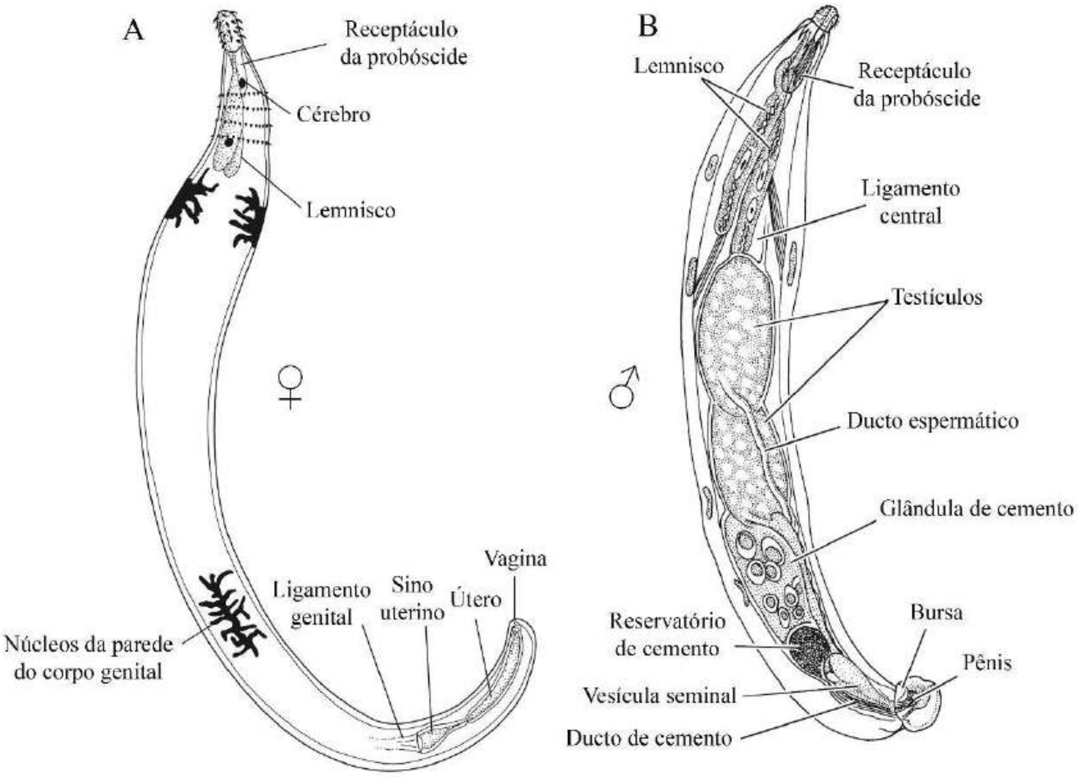

Figura 3. Representações de representantes da subclasse Acanthocephala. A: Aspectos gerais de fêmeas de *Quadrigyrus nickolii* Schmidt e Hugghins, 1973 representando a probóscide eversível e aspectos do sistema reprodutor e nervoso, vista lateral. B: Macho adulto de *Paulisentis fractus* Van Cleave e Bangham, 1949, com representação de sua probóscide eversível e aspectos do sistema reprodutor, vista lateral. Adaptado de Pechenik (2016) e Brusca, Giribet e Moore (2022).

Diversos fatores bióticos e abióticos influenciam a estrutura da comunidade parasitária. Os fatores bióticos podem ser o ciclo de vida, estágio de infecção, potencial reprodutivo, modo e sítio de infecção do parasito e características do hospedeiro, como tamanho, dieta, sexo, massa, resposta imune e comportamento. Já os fatores abióticos, como altitude, heterogeneidade ambiental, mudanças sazonais, clima, precipitação, temperatura e umidade (Aho, 1990; Bolek e Coggins, 2003; Chandra e Gupta, 2007; Hamann et al., 2012; de Oliveira et al., 2023). Além disso, outros fatores como o esforço amostral e mudanças ambientais causadas por ação antrópica também devem ser considerados ao avaliar a riqueza de espécies de parasitos (McKenzie, 2007; Campião et al., 2015)

Atualmente, duas teorias são utilizadas para explicar como esses fatores bióticos influenciam sobre as comunidades parasitárias: A primeira considera que os recursos para serem explorados variam conforme o tamanho e massa dos hospedeiros (Fig. 4A). A segunda teoria postula, por meio de modelos matemáticos, que a alta densidade populacional de hospedeiros facilita a colonização por várias espécies de parasitos e apresentam uma maior riqueza parasitária em relação a hospedeiros que vivem em baixas densidades populacionais (Fig. 4B). Além disso, outros fatores como o esforço amostral, mudanças ambientais causadas por ação antrópica, (Campião et al., 2015)

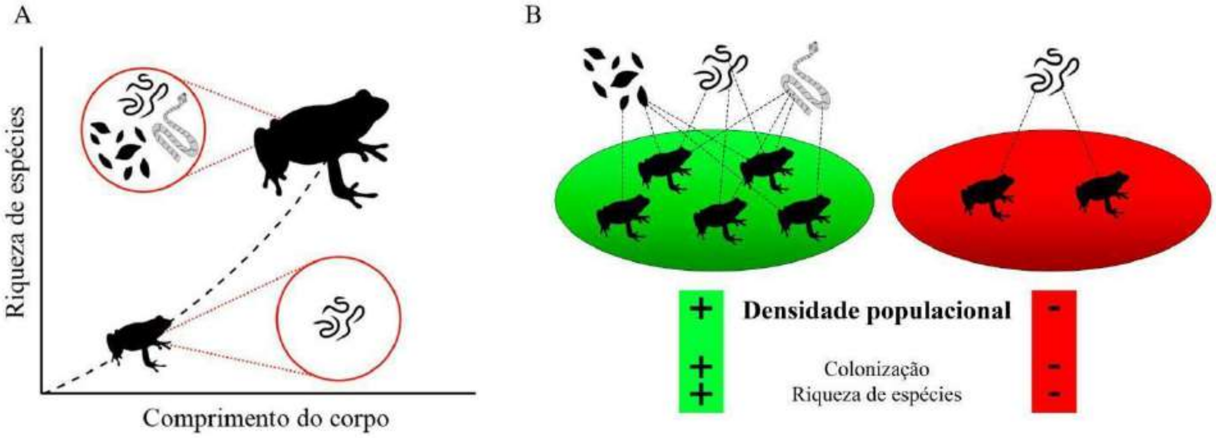

Figura 4. Representações de duas teorias que visam explicar o impacto de fatores bióticos sobre a riqueza de espécies de parasitos. A: Tamanho do corpo do hospedeiro e a influência na riqueza de espécies de parasitos. B: Comparação de populações de hospedeiros que vivem em maior e menor densidade, resultando em uma riqueza de espécies de parasitos distinta nos dois grupos.

Devido à grande variedade de comportamentos, hábitats, dieta, modo de forrageio, tamanho e distribuição geográfica entre os anuros, muitos estudos vêm sendo conduzidos utilizando-os como modelo para entender a estrutura das comunidades parasitárias de helmintos.

Em relação ao hábito dos hospedeiros anuros, estudos como de Draghi et al. (2020), Luque et al. (2005), Ibrahim (2008) e Hamann et al. (2013a) observaram que anfíbios de hábito terrestre são mais frequentemente parasitados por nematódeos de ciclo de vida direto (Fig. 5). Por outro lado, anfíbios de hábito aquático ou arborícola costumam ser infectados com maior frequência por trematódeos de ciclo de vida heteroxênico, que na maioria das vezes têm seu ciclo relacionado à água (Fig. 5) (McAlpine e Burt, 1998; Muzzall et al., 2001; Hamann et al., 2013b). Porém, anuros de hábitos arborícolas também podem ser parasitados majoritariamente por nematódeos de ciclo de vida direto (Martins-Sobrinho et al., 2017; González et al., 2021).

Diversos estudos foram conduzidos com anuros de hábitos arborícolas, porém não há um padrão conhecido sobre a influência positiva ou negativa do hábito arborícola na riqueza parasitária em comparação a anuros de hábitos terrestres e aquáticos. Alguns estudos encontraram uma menor diversidade de helmintos em anuros arborícolas (Fig. 6) (Aho, 1990; Goldberg e Bursey, 2008; González e Hamann, 2008; Draghi et al., 2020; González et al., 2021), enquanto outros destacam que a riqueza de parasitos é similar ou maior quando comparado a outros hábitos (Koller e Gaudin, 1977; Bursey et al., 2001; Yoder e Coggins 2007; Hamann et al., 2010; Campião et al., 2016; Silveira et al., 2022; de Oliveira et al., 2022). Essa falta de conhecimento sobre um padrão mostra que ainda são necessários mais estudos para estabelecer padrões gerais para essas relações, considerando a diversidade de modos de vida de anuros e seus helmintos parasitos (Martins-Sobrinho, 2017).

Figura 5. Ilustração da suscetibilidade do hospedeiro ao parasito em relação ao hábito terrestre, aquático ou arborícola.

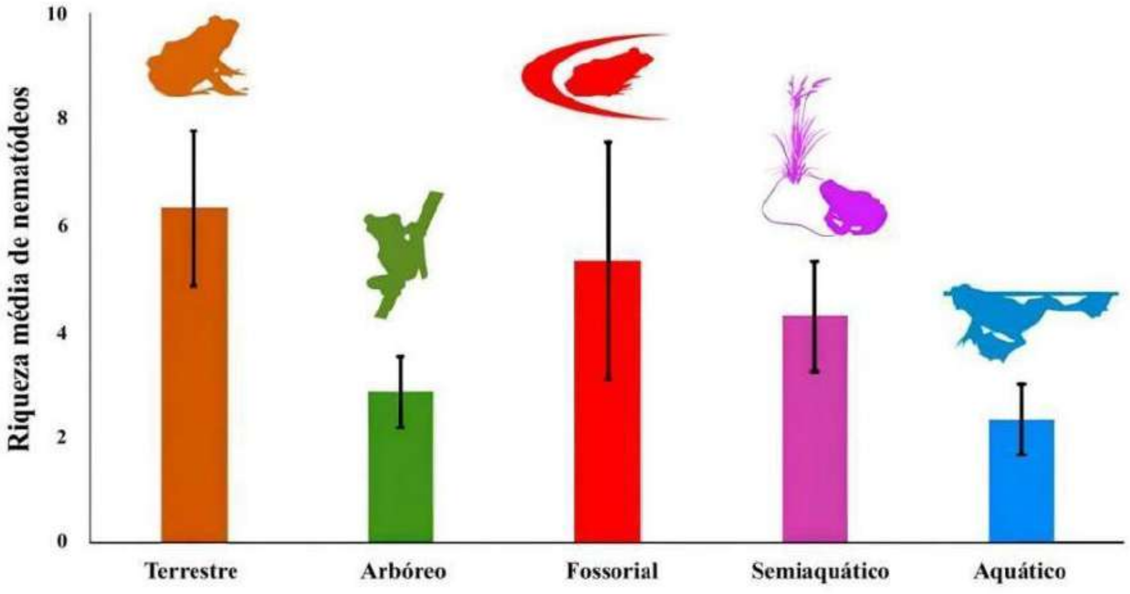

Figura 6. Gráfico de riqueza média de nematódeos conforme o hábitat dos hospedeiros. Adaptado de González et al. (2021).

A dieta e o modo de forrageio dos hospedeiros são outros fatores que influenciam na riqueza de espécies de helmintos parasitos de anuros (Toledo et al., 2018; González et al., 2021). Sobre a dieta, os anuros podem ser classificados como especialistas, intermediários (quando se alimentam de diferentes animais, mas alguns ítens são consumidos em maior quantidade) ou generalistas (Fig. 7A) (Hamann et al., 2012; González et al., 2021). Grande parte das infecções por helmintos parasitos em anuros ocorre pela rota trófica, atuando como hospedeiros paratênicos ou hospedeiros definitivos. Quanto ao modo de forrageio, os anuros podem ser agrupados em ativos, intermediários e “senta e espera” (Fig. 7B) (Hamann et al., 2012; González et al., 2021). Anuros que apresentam uma dieta especialista e modo de forrageio “senta e espera” geralmente apresentam uma menor riqueza de espécies e uma menor frequência de infecção por parasitos (Fig. 7) (Hamann et al., 2012; González, 2021).

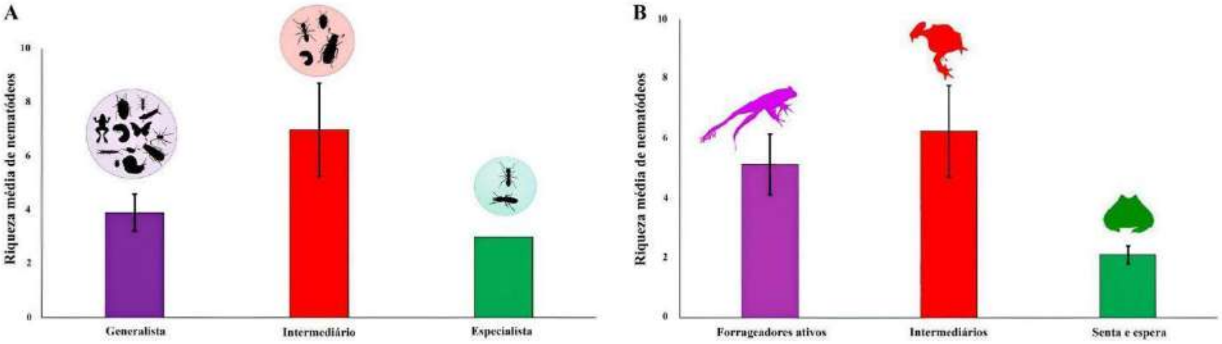

Figura 7. Gráficos de riqueza média de nematódeos de acordo com características da alimentação do hospedeiro. A: Dieta. B: Modo de forrageio. Adaptado de González et al. (2021).

A identificação de espécies de parasitos pode ser dificultada devido a diversos fatores, dentre eles: a similaridade morfológica entre grupos de parasitos, principalmente entre fêmeas, a ocorrência de estágios larvais, raridade de espécimes machos recuperados em amostras de alguns grupos (Fig. 8D), sendo que alguns apresentam apenas fêmeas hermafroditas infectantes (Fig. 8) (Poulin e Leung, 2010).

Todas essas características interferem na delimitação de espécies e relações de ancestralidade entre os diversos grupos de parasitos. Por exemplo, alguns nematódeos da família Atractidae são morfologicamente semelhantes a Cosmocercidae e, portanto, compartilham de um histórico taxonômico complexo e impreciso (Fig. 8C). Além desses, os filarídeos da família Onchocercidae, que algumas vezes foram descritas espécies apenas com indivíduos fêmeas e, também, as microfilárias, que apresentam uniformidade morfológica, dificultando sua identificação (Fig. 8A) (Souza Lima et al., 2012; Cavalcante et al., 2016).

Os representantes das famílias Rhabdiasidae e Physalopteridae também são bons exemplos em que há dificuldade da diagnose de espécies apenas através da morfologia. Em Rhabdiasidae, os estágios adultos, comumente encontrados nos pulmões dos hospedeiros, são fêmeas hermafroditas que apresentam uma grande uniformidade morfológica e poucos caracteres para auxiliar na diferenciação das espécies (Fig. 8B). Trabalhos mais recentes demonstram que para o gênero *Rhabdias*, existe uma grande quantidade de espécies crípticas (Müller et al., 2018). Além disso, Physalopteridae frequentemente não são identificados ao nível de espécie por serem geralmente encontrados em estágio larval parasitando anuros (Fig. 8E).

Nesses casos, a biologia molecular tornou-se uma ferramenta importante para auxiliar na resolução de problemas taxonômicos, na descrição de novas espécies e revelar a diversidade críptica de parasitos (Tkach et al., 2014; Poulin et al., 2019). Atualmente, marcadores moleculares mitocondriais (COI, 12S e 16S) e ribossomais (18S, 28S e ITS) são os mais comumente utilizados para a classificação de nematódeos parasitos (Müller et al., 2018; Santos et al., 2022).

O marcador COI é utilizado para elucidar relações de espécies com relações próximas de ancestralidade e diversidade críptica devido sua rápida evolução. Esse marcador vem sendo utilizado ainda de forma concatenada a outros genes mitocondriais para potencializar sua capacidade de identificação de variabilidade genética em complexos de espécies (Müller et al., 2018; Santos et al., 2022; Rebêlo et al., 2022).

Os genes ribossomais são mais utilizados para inferir relações de ancestralidade entre gêneros de nematódeos, podendo ser trabalhados a um escopo comparativo maior em relação a genes mitocondriais de rápida mutação, porém não apresentam uma boa resolução para a delimitação de linhagens de espécies crípticas (Müller et al., 2018; Santos et al., 2022).

Devido à escassez de estudos sobre a diversidade de helmintos parasitos de *Osteocephalus* spp., abordagens utilizando técnicas de biologia molecular e informações sobre a composição e estrutura de suas comunidades parasitárias possibilitarão identificar e descrever novas espécies de parasitos, revelar a diversidade críptica de alguns grupos e elucidar as relações filogenéticas entre eles. Além disso, podem fornecer informações que servirão de base para estudos posteriores, bem como na compreensão da evolução e da coevolução desses organismos.

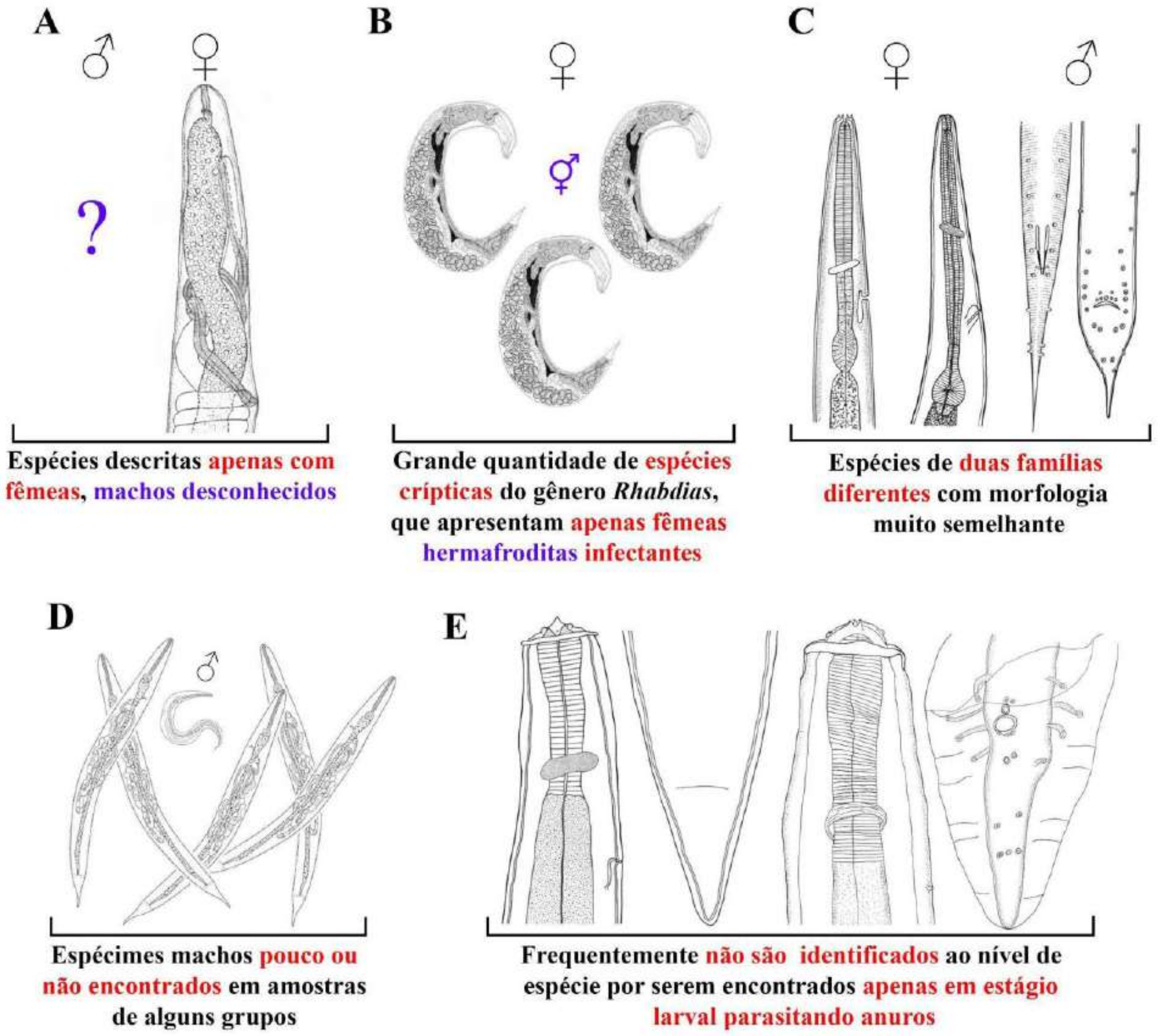

Figura 8. Representação de problemas taxonômicos de alguns grupos de nematódeos e como a biologia molecular pode auxiliar em sua resolução. A: Nematódeo *Ochoterenella albareti* (Bain, 1979) descrito apenas com espécimes fêmeas, tendo seus machos desconhecidos. B: Nematódeos do grupo *Rhabdias breviensis* com uma grande diversidade críptica, além de apresentarem apenas fêmeas hermafroditas infectantes. C: Morfologia muito similar de machos e fêmeas de *Raillietnema nanus* Bursey, Goldberg e Kraus, 2006 e *Labeonema longispiculatum* Moravec e Jirků, 2017 das famílias Cosmocercidae e Atractidae, respectivamente. D: Raridade de indivíduos machos nas amostras de alguns grupos, como os Pharyngodonidae. E: Nematódeos do gênero *Physaloptera* Rudolphi, 1819 em estágio larval e em estágio adulto, ilustrando a dificuldade de identificação de nematódeos da família, que são geralmente encontrados em estágio larval parasitando anuros. Adaptado de Bain (1979), Bursey, Goldberg e Kraus (2006), Pereira et al. (2012), Nascimento et al. (2013), Moravec e Jirků (2017), Pereira et al. (2017) e Felix-Nascimento et al. (2021).

OBJETIVOS

OBJETIVO GERAL

- Caracterizar a diversidade de helmintos parasitos de *Osteocephalus cabrerai*, da Reserva Extrativista Beija-Flor Brilho de Fogo.

OBJETIVOS ESPECÍFICOS

- Identificar até o menor nível taxonômico possível, os helmintos parasitos de *Osteocephalus cabrerai*;
- Caracterizar geneticamente *Aplectana pella*;
- Analisar se a adição de *Aplectana pella* na filogenia de Cosmocercidae tornaria o gênero *Aplectana* monofilético;
- Inferir a riqueza, abundância e intensidade das espécies que compõem a comunidade parasitária de *Osteocephalus cabrerai*;
- Analisar se fatores bióticos relacionados aos hospedeiros influenciam na prevalência, intensidade e abundância de infecção dos parasitos.

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Capítulo 1

Filling the gaps on parasites of *Osteocephalus*: Helminth community structure of *Osteocephalus cabrerai* (Anura: Hylidae) from the Brazilian Amazon

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Filling the gaps on parasites of *Osteocephalus*: Helminth community structure of *Osteocephalus cabrerai* (Anura: Hylidae) from the Brazilian Amazon

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ABSTRACT

Osteocephalus cabrerai is an arboreal anuran widely distributed in South America. However, there are no parasitological studies conducted on the species, resulting in a parasite fauna completely unknown. Thus, this study aims to characterize the parasitic community structure of *O. cabrerai* in the municipality of Pedra Branca do Amapari, Amapá state, Amazon region, Brazil. We collected and necropsied 84 specimens of *O. cabrerai* to search for helminths. Parasite community structure was analyzed using helminth parasite richness, diversity, and abundance. The helminth component community of *O. cabrerai* comprises six nematode species: *Cosmocerca parva*, *Cosmocerca podicipinus*, *Oxyascaris oxyascaris*, *Oswaldocruzia chabaudi*, and *Physaloptera* sp. (larvae). Most helminth species represent the first record for the genus *Osteocephalus*, except *Physaloptera* sp. The helminth infections in the host showed a typical aggregated distribution pattern for parasites. We only found a positive correlation between the host weight and total intensity. Our bibliography revision reinforces the need for further studies on the helminth fauna of *Osteocephalus* spp.

1. Introduction

The hylids are one of South America's most studied anuran. However, most records are for species of the genera *Boana* Gray, 1825, *Dendropsophus* Fitzinger, 1843; and *Phyllomedusa* Wagler, 1830; Campião et al., (2014, 2015). Currently, the genus *Osteocephalus* Steindachner, 1862 is composed of approximately 28 species, and the parasitic fauna is known only for *Osteocephalus taurinus* Steindachner, 1892, and *Osteocephalus leprieurii* (Duméril and Bibron, 1841) (Campião et al., 2014; Frost, 2024). The helminth fauna of those species is composed of 13 helminth taxa recorded in the states of Amapá, Amazonas, Pará, and Mato Grosso in Brazil, the city of Cusco, Peru, and Ecuador (Vaucher, 1981, 1987; Bursey et al., 2001; Smales, 2007; Campião et al., 2014; Feitosa et al., 2015; Tavares-Costa et al., 2018; Pedroso-Santos et al., 2019; Santos et al., 2019, 2022; Anjos et al., 2021).

Several studies on parasites of *Osteocephalus* spp. focused on descriptions of new species and isolated records of new hosts and localities (e.g. Feitosa et al., 2015; Tavares-Costa et al., 2018; Pedroso-Santos et al., 2019; Anjos et al., 2021; Santos et al., 2022). In this context, characterizing the parasite community of *Osteocephalus* spp. in the Amazon region is essential for understanding factors that affect parasite distribution and supporting future ecological studies.

To date, no parasitological studies have been conducted on the species *Osteocephalus cabrerai* (Cochran and Goin, 1970), resulting in a parasite fauna completely unknown. Thus, this study aims to characterize the parasite community of *O. cabrerai* as well as understand factors that influence in parasite distribution on this host in the Brazilian Amazon Region.

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2. Materials and methods

2.1. Host sampling and collection of parasites

During helminthological surveys, 84 specimens of *O. cabrerai* (Fig. 1) were collected between May 2019 and March 2022 in the "Beija-Flor Brilho de Fogo" Extractive Reserve (0°47 ‘30.6′ N, 51°58 ‘42.1′ W), located in the municipality of Pedra Branca do Amapari, Amapá state, Brazil. The amphibian hosts were identified according to Pedroso-Santos et al. (2019) and Frost (2024).

The hosts were anesthetized, measured, weighed, and necropsied for helminthological examination. All internal organs were placed in Petri dishes with saline solution (NaCl 0.9%), dissected, and examined under a LEICA EZ4 stereomicroscope. The helminths found were cleaned in saline solution, killed with heated 70% alcohol, and preserved in the same solution at room temperature.

For morphological analysis, the nematodes were hydrated in distilled water, cleared in Amann’s lactophenol 20%, mounted on temporary slides, and examined under an Olympus BX41 microscope (Olympus, Tokyo, Japan) coupled with a drawing tube (without zoom adjustment), and Olympus BX53 microscope (Olympus America, Center Valley, Pennsylvania) equipped with differential interference contrast optics and a digital imaging system.

We also conducted a bibliographic reference search to compile the records of helminth parasitics in *Osteocephalus* spp., using seven electronic databases (Google, Google Scholar, PubMed, Scielo, Science Direct, Scopus and Web of Science). We used such search strings: Topic: [(“*Osteocephalus*") and (“helminth" or "parasites" or "nematodes" or "platyhelminthes" or "acanthocephalan")]. We prepared a table with all this compilation, including published records, available data, and information from the present study.

2.2. Data analysis

The infection prevalence, mean intensity, and mean abundance were calculated according to Bush et al. (1997). We calculate confidence intervals in 95% for prevalence with Sterne’s method and mean abundance with Bootstrap BCa using Quantitative Parasitology 3.0 software.

We classified the helminth communities at the infracommunity (all helminth populations of a single specimen of *O. cabrerai*) and component community levels (all helminth infracommunities within the *O. cabrerai* population). The total number of helminth species (= richness), Shannon index (H′), and Pielou evenness index (J′) as H′/H’ maximum represents the community richness and diversity (Zar, 2010). The Brillouin diversity index (HB) and Pielou evenness index (E) were used to describe the diversity and uniformity of helminth infracommunities. The Berger–Parker index of dominance (d) was used to determine the most abundant species in the component community (Magurran, 2004). The frequency of dominance (FD) was used to determine the percentage of infra communities in which a parasite species is numerically dominant. All these parameters were calculated using the PAST 4.11 software (Hammer et al., 2001; Magurran, 2004).

Fig. 1. Dorsolateral view of an adult specimen of *Osteocephalus cabrerai* from Amapá state, Brazil.

The variance-to-mean ratio (ID) and the index of discrepancy of Poulin (D) were calculated for species with prevalence >10% using the Quantitative Parasitology 3.0 software to determine the distribution pattern of parasite infra communities (Rózsa et al., 2000). The significance of ID for each parasite species was tested using d-statistics, where d < 1.96 represents a random distribution and d > 1.96, an aggregate distribution (Ludwig and Reynolds, 1988).

We used the method proposed by Thul et al. (1985) to calculate the importance of each parasite in the community. Thus, the helminth species were classified into four groups (dominant, codominant, subordinate, and unsuccessful) based on their prevalence, intensity, and maturity factor (equal to 1.0 if at least one mature specimen of species is found and equal to 0 if otherwise), which is related to the degree of host specificity.

The Kendall Correlation (τ) was used to test the correlation between morphological characters of the host (Snout-vent length [SVL] and body weight), and helminth mean species richness, intensity, and abundance at the infra community level and abundance of helminth species at the component community level. These analyses were performed using the R 4.4.0 software. We did not test the association between host sex and parasite community characteristics due to our samples’ low number of females (males = 60; females n = 24).

Richness was estimated using the species accumulation curve, which analyzes the number of observed species as a function of the sampling effort. We used the non-parametric richness estimator Chao 2 to check if the observed richness approached the estimated richness. These statistical analyses were performed using the Vegan (Oksanen, 2010) package in R software.

3. Results

The overall parasite prevalence was 64.28% (55 specimens infected out of 84 collected). We obtained 292 specimens of nematodes (mean abundance, 3.47; mean intensity, 5.4) assigned to six different taxa, *Aplectana pella* Borges, Willkens, Santos, Costa-Campos and Melo, 2023, *Cosmocerca parva* Travassos, 1925, *Cosmocerca podicipinus* Baker and Vaucher, 1984, *Oswaldocruzia chabaudi* Ben Slimane and Durette-Desset, 1996, *Oxyascaris oxyascaris* Travassos, 1920, and *Physaloptera* sp. (larvae) (Table 1, Fig. 2). The curve of the accumulated species observed did reach an asymptote (Fig. 3), indicating that the observed helminth species richness (6 species) was equal to the expected (Chao 2 richness estimator = 6) (Fig. 4).

The component helminth community of *Osteocephlus cabrerai* presented low diversity, the predominance of certain parasite species, and high uniformity (Table 2). The large intestine was the most infected organ (=4 spp.), followed by the small intestine (=1 spp.) and stomach (=1 spp.) (Table 1). The most common helminth in *O. cabrerai* is *C. parva* (41.67%). Less frequently, frogs are parasitized by *A. pella* (27.38%) and *O. chabaudi* (14.29%). All of them showed aggregated distribution (Table 3). Other taxa of helminths presented a prevalence below 10%: *C. podicipinus* (3.57%), *O. oxyascaris* (5.95%) and *Physaloptera* sp. (1.19%). Hosts were predominantly infected by one species of helminths (Fig. 5).

Table 1

Helminth parasites record of *O. cabrerai* from Amapá state, Brazil. N: number of helminths, prevalence (%) with 95% confidence interval, mean intensity with ranges, mean abundance with 95% confidence interval, site of infection of helminth parasites of *O. cabrerai,* importance value (I), and frequency of dominance. Classification of helminths: $I \geq 1.0$, dominant species; $I = 0$, unsuccessful species; $0.01 \leq I < 1.0$, codominant species. Abbreviations: P, prevalence; CI, confidence interval; MI, mean intensity; MA, mean abundance; FD, frequency of dominance; Small intestine, SI; Large intestine, LI; Stomach, S.

Helminth	N	P (%) ± CI	MI	MA ± CI	Site of Infection	I	Classification	FD (%)
Aplectana pella	99	27.38 ± 0.188–0.380	4.3; range 1–15	1.18 ± 0,69–1,9	LI	14.441	Dominant	33.90
Cosmocerca parva	161	41.67 ± 0.315–0.524	4.6; range 1–27	1.92 ± 1.27–3.17	LI	35.737	Dominant	55.13
Cosmocerca podicipinus	9	3.57 ± 0.010–0.100	3; range 2–4	0.107 ± 0.023–0.303	LI	0.171	Codominant	3.08
Oswaldocruzia chabaudi	16	14.29 ± 0.082–0.237	1.33; range 1–3	0.19 ± 0.095–0.321	SI	1.218	Dominant	5.47
Oxyascaris oxyascaris	6	5.95 ± 0.024–0.135	1.2; range 1–2	0.071 ± 0.011–0.143	LI	0.190	Codominant	2.05
Physaloptera sp. (larvae)	1	1.19 ± 0.001–0.064	1; range 1–1	0.011 ± 0.001–0.064	S	0	Unsuccessful	0.34

We found three dominant species of nematodes (widespread species in the host population), two codominant species (contributed to a lesser degree in the helminth community), and one unsuccessful species (accidental infection), which was able to enter the host but did not reach maturity therein (Table 1).

The mean SVL of the anurans was 53.2 ± 7.5 mm (29–80.3), and the body weight was 7.07 ± 3.3 g (2–18.9). We observed a positive correlation between the host body weight and the total intensity of infection (total intensity vs. SVL: $\tau = 0.16$; $p = 0.10$; total intensity vs. weight: $\tau = 0.31$; $p = 0.00$). In contrast, the total abundance was not correlated with characters of the host (total abundance vs. SVL: $\tau = 0.02$; $p = 0.73$; total abundance vs. weight: $\tau = 0.04$; $p = 0.62$). There was no correlation between host variables and mean helminth richness (mean helminth richness vs. SVL: $\tau = - 10$; $p = 0.35$; mean helminth richness vs. weight: $\tau = - 2$; $p = 0.84$). Also, there were no significant correlations between helminth abundance species and the host body size or weight (*A. pella* vs. SVL: $\tau = 0.02$; $p = 0.75$, *A. pella* vs. weight: $\tau = 0.02$; $p = 0.78$; *C. parva* vs. SVL: $\tau = 0.10$, $p = 0.22$, *C. parva* vs. weight: $\tau = 0.11$, $p = 0.15$; *O. chabaudi* vs. SVL: $\tau = - 0.6$, $p = 0.49$, *O. chabaudi* vs. weight: $\tau = - 0.13$, $p = 0.13$).

In our bibliography analysis, we found 13 helminth taxa parasitizing two species of *Osteocephalus*. Additionally, if we include the records of the present work, at least 18 helminths taxa are associated with the *Osteocephalus* spp. in South America. The highest helminth richness was found in *O. taurinus* ($n = 13$ spp.), followed by *O. cabrerai* ($n = 6$ spp.) and *O. leprieurii* ($n = 1$ spp.). Brazil harbors the richest helminth fauna registered for *Osteocephalus* spp., with 11 species, followed by Peru ($n = 6$ spp.) and Ecuador ($n = 3$ spp.). Cosmocercidae was the most common parasite ($n = 6$ spp.), followed by Physalopteridae ($n = 3$ spp.), Polystomatidae ($n = 3$ spp.), Pharyngodonidae ($n = 3$ spp.), Molineidae ($n = 2$ spp.), Onchocercidae ($n = 1$ spp.), Rhabdiasidae ($n = 1$ spp.) and Oligacanthorhynchidae ($n = 1$ spp.) (see Table 4).

4. Discussion

The component helminth community of *O. cabrerai* comprised six nematode species. The diversity indices, the helminth population's dominance, and the parasite community's evenness indicate that the sampling effort was sufficient to characterize the helminth species in the studied area and the host. The Chao estimate and the observed data in the species accumulation curve also indicate that. The parasites found in the present study are also the most common among all anuran families from South America, including *Osteocephalus* spp. (Campião et al., 2014; see Table 4).

In our analysis, nematodes of the Cosmocercidae family showed the highest prevalence, mean intensity, and mean abundance in the helminth community. The most prevalent, abundant, and dominant species was *C. parva*, while *C. podicipinus* was codominant in the helminth community. Species of the *Cosmocerca* Diesing, 1816 are distributed worldwide (Ni et al., 2020), and both species found in the present study are considered generalists. Additionally, at least one of them was registered parasitizing anurans from the families Aromobatidae, Bufonidae, Craugastoridae, Dendrobatidae, Eleutherodactylidae, Hylidae, Hylodidae, Leptodactylidae, Microhylidae, Odontophrynidae, Ranidae and Strabomantidae (Goldberg and Bursey, 2008; Santos and Amato, 2013; Campião et al., 2014).

In the present study, *Aplectana pella* was the second most dominant species among the cosmocercids. Recently, Santos et al. (2023) described *A. pella* as parasitizing *Boana boans* (Linnaeus, 1758) collected from the same locality as *O. cabrerai* in this study. Both host species exhibit arboreal habits and occupy equivalent ecological niches. Our findings also support the hypothesis that hosts exposed to similar environmental conditions share helminth taxa (Aho, 1990; Krasnov et al., 2011; Poulin et al., 2011; Toledo et al., 2018; Draghi et al., 2020).

We also found that *Oxyascaris oxyascaris* was codominant and had low prevalence in the component community. The type-host for these nematode species is *Mastigodryas bifossatus* Raddi, 1820(= *Drymobius bifossatus*) (snake); however, they are commonly found infecting Leptodactylidae species (Campião et al., 2014; Lins et al., 2017; Silva et al., 2018). Some previous studies registered the species in other tree frogs: *Boana raniceps* (Cope, 1862), *Boana faber* (Wied-Neuwied, 1821) and *Trachycephalus typhonius* (Linnaeus, 1758) (Campião et al., 2016, 2017; Euclydes et al., 2022; Euclydes and Campião, 2024).

Oswaldocruzia chabaudi was one of the dominant nematodes found in the community of *O. cabrerai*. This species also infects other arboreal hylids, such as *B. boans*, *Boana fasciata* (Günther, 1858), *Boana geographica* (Spix, 1824), and *Boana wavrini* (Parker, 1936; Campião et al., 2014; Willkens et al., 2021). These results reinforce that *O. chabaudi* is mainly associated with arboreal amphibians, which occupy similar ecological niches and have overlapping geographical distributions, as proposed by Willkens et al. (2021).

Our study indicates that *O. cabrerai* represents the definitive host for most helminth species. Also, we conclude that this frog had a minor role as a paratenic host, as the only nematode larvae found were *Physaloptera* sp. (larvae) in the gastric mucosa. In the Neotropical region, nematodes of the genus *Physaloptera* are commonly found as non-encysted larvae in anurans (González and Hamann, 2012; Madelaire et al., 2012; Campião et al., 2014). These nematodes also showed the lowest values of importance (unsuccessful pioneer), prevalence, intensity, and abundance, indicating that this might represent an unsuccessful host or an accidental host species. The heteroxenic life cycle of these species involves arthropods as intermediate hosts and vertebrates as definitive or paratenic hosts (Hamann et al., 2015; Martins-Sobrinho et al., 2017; Alcantara et al., 2018; Coimbra et al., 2023).

We found predominantly generalist nematodes with monoxenous life cycles. In this context, the contact of *O. cabrerai* with terrestrial environments, mainly during the reproductive period (Menin et al., 2011) may promote the transmission and infection of direct life cycle helminths. Additionally, the absence of digenean's and cystacanths indicates that *O. cabrerai* interacts less with helminths of complex life cycles and encounters fewer species of potential intermediate hosts. Thus, we reinforce the importance of understanding the host's diet for new insights into its interactions with intermediate hosts and their parasites.

The distribution of helminths in *O. cabrerai* showed a typical aggregated pattern for parasites, which could be influenced by specific

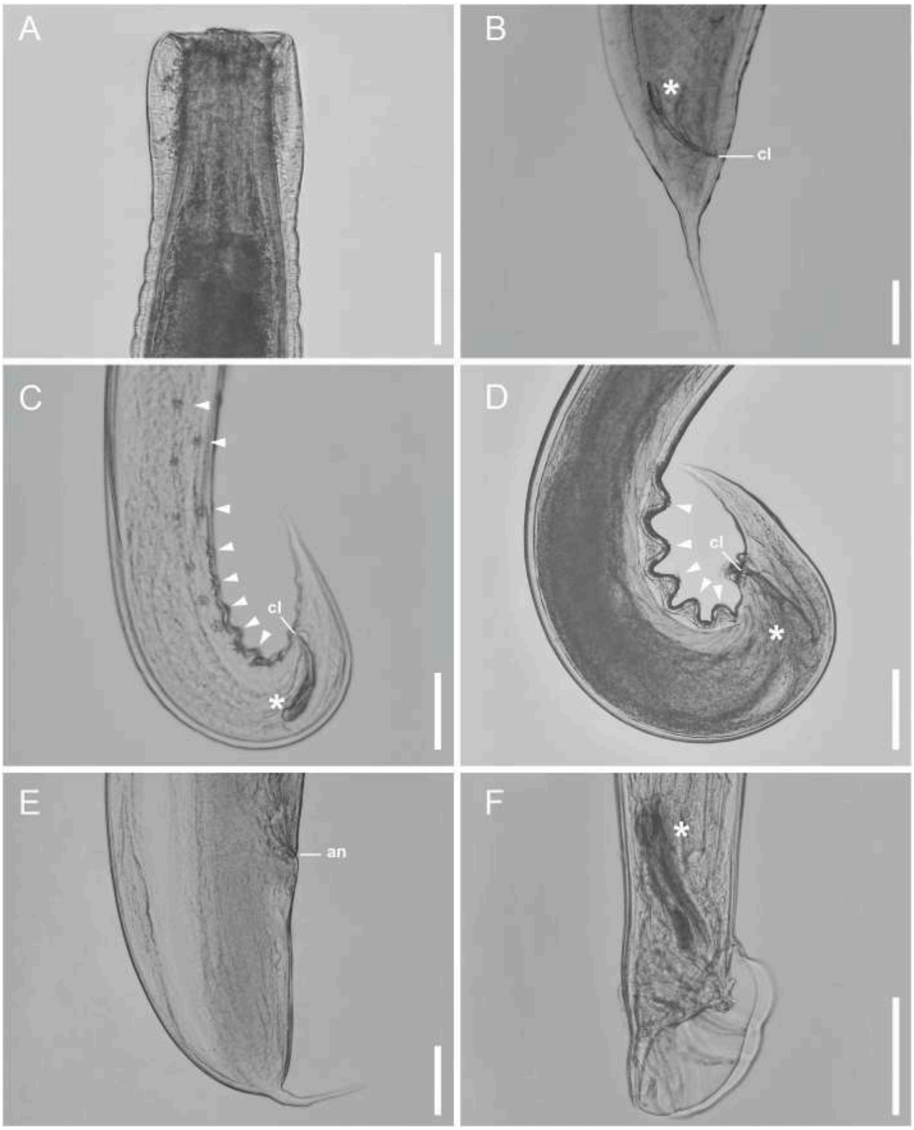

Fig. 2. Photomicrographs of the helminth species associated with *Osteocephalus cabrerai* from Amapá state, Brazil. A, *Physaloptera* sp. (larvae) anterior region. B, *Aplectana pella* male caudal region, spicules (asterisk). C, *Cosmocerca parva* male caudal region, precloacal papillae (arrowheads) and spicules (asterisk). D, *Cosmocerca podicipinus* male caudal region, preclocal papillae (arrowheads) and spicules (asterisk). E, *Oxyascaris oxyascaris* female caudal region. F, *Oswaldocruzia chabaudi* male caudal region, spicules (asterisk). Abbreviations: Cloaca, cl; Anus, an. *Scale–bars:* A 50 μm; B, C, D, E, F 100 μm.

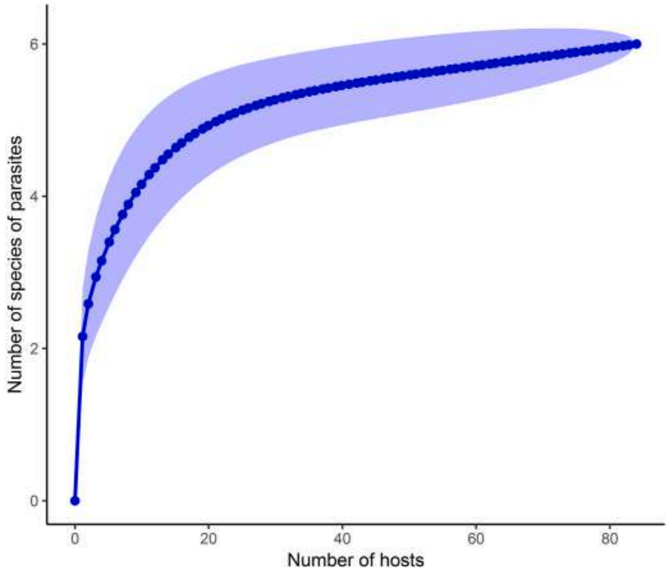

Fig. 3. Parasite species richness distributed in *Osteocephalus cabrerai* specimens from Amapá state, Brazil.

Table 2
Parasite community characteristics of the helminth infracommunities and component communities of *O. cabrerai* from Amapá state, Brazil. Abbreviation: SD, standard deviation.

Community characteristics	Values
Infracommunities	
Mean Brillouin diversity index (HB) (±SD)	0.16 ± 0.20
Mean evenness (E) (±SD)	0.14 ± 0.17
Component community	
Species richness	6
Shannon-Wiener diversity index (H)	1.06
Evenness (J)	0.59
Berger-Parker (d)	0.55

Table 3
Dispersion index (DI) and d-statistic (*d*), discrepancy index (D) and distribution for the parasite infracommunities (prevalence >10%) of *O. cabrerai* from the Brazilian Amazon. Distribution classification: d < 1.96, random distribution; d > 1.96, aggregate distribution.

Helminth	DI	*d*	D	Distribution
Aplectana pella	6.81	20.699	0.842	Aggregate
Cosmocerca parva	8.2	23.972	0.778	Aggregate
Oswaldocruzia chabaudi	1.45	2.592	0.875	Aggregate

aspects of the parasite (life cycle and transmission methods), host susceptibility to the parasite, infection, and immune response (Anderson and Gordon, 1982; Cardoso et al., 2021; Rubenina et al., 2021). In the aggregated pattern, most infected hosts have a small number of parasites, while a few hosts have many parasites (Anderson and Gordon, 1982; Draghi et al., 2020).

In general, parasite community characteristics of *O. cabrerai* were not correlated to host size (SVL and weight), except for a positive correlation between total intensity and host weight. The positive correlation observed in the present study is also observed in previous studies like Hamann et al. (2012, 2013) and Toledo et al. (2018). This data supports the hypothesis that larger hosts are capable of providing more habitats and a greater diversity of resources, such as different niches, compared to smaller hosts as proposed by other authors (Poulin, 1997; Duré and Kehr, 1999; Bolek and Coggins, 2003; Hamann et al., 2006, 2012, 2013; Hamann and González 2015; Kamiya et al., 2014; Morand, 2015; Čeirāns et al., 2021). Additionally, hosts with larger body sizes have a greater surface area, increasing the likelihood of contact with direct life-cycle nematodes in the soil (Aho, 1990; Morand, 2015).

However, the uncorrelated factors (total abundance vs. SVL, total abundance vs. weight, mean helminth richness vs. SVL, mean helminth richness vs. weight, helminth abundance species vs. SVL) indicate that other aspects of the host like physiology, behavior, and seasonality can also explain variations in the mean helminth richness, intensity, and species abundance (Poulin, 1997; Oliveira et al., 2019).

Although *Osteocephalus* genus is widely distributed in South America with 28 species, most do not have any information about their helminth fauna. In our bibliographic revision, we found parasitological studies for only two *Osteocephalus* species, in which *O. taurinus* was the most widely parasitized species (n = 13 spp.). In contrast, we found just one helminth record for *O. leprieurii*. Our study reinforces the need to add new data on the helminth fauna of this host group.

We provide the first study of the helminth community structure of *O. cabrerai*. The component community was composed predominantly of nematodes with a monoxenous life cycle, high prevalence, and abundant infection. Most parasite species here represent the first record for the genus *Osteocephalus*, except for *Physaloptera* sp. The helminth infections

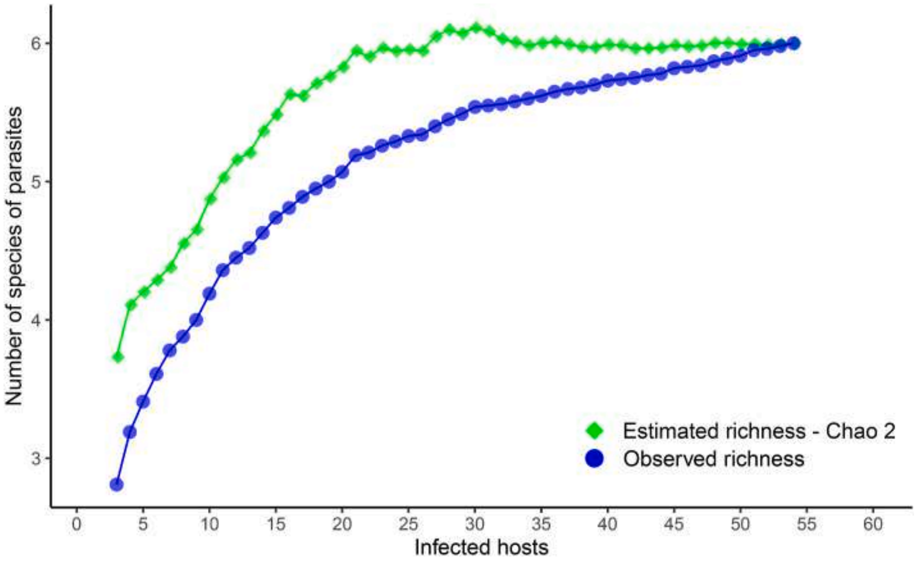

Fig. 4. Species accumulation curve with the 95% error bars showing the accumulation of helminth taxa found in *Osteocephalus cabrerai* from Amapá state, Brazil.

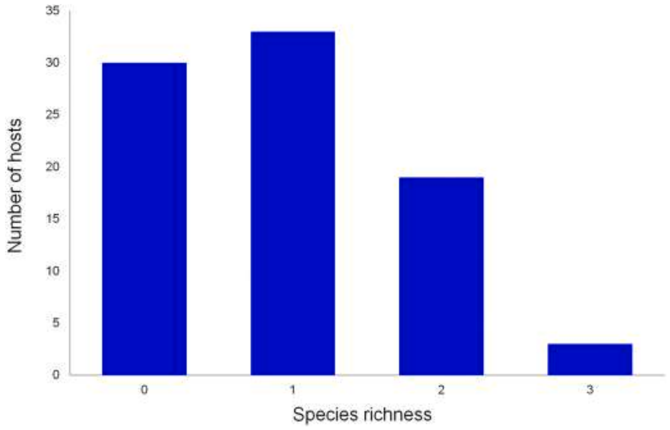

Fig. 5. Comparison between the observed and the estimated richness for helminth parasites of *Osteocephalus cabrerai* using Chao 2 richness estimator.

in the host showed a typical aggregated distribution pattern for parasites.

Our findings contribute to anurans helminth fauna and provide new insights into helminth communities' distribution patterns. *Osteocephalus cabrerai* body weight positively influences the total intensity of parasite infection. The bibliography revision reinforces that we still have little information on the helminth fauna of *Osteocephalus* spp. and that additional and future studies will be necessary to bring new data on amphibian parasites and their ecological relationships.

Ethics approval

All procedures contributing to this work comply with all applicable institutional, national, and international guidelines for animal care and use Animal Research Ethics Committee, Federal University of Pará, under license N8341260821CEUA/UFPa. The present study was approved by Instituto Chico Mendes de Conservação da Biodiversidade (ICMBio), Brazil, and host specimens were collected under license number SISBIO: 48,102–2.

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CRediT authorship contribution statement

Jorge Kevin Silva Neves: Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Evelyn Lebrego Cardoso:** Writing – review & editing, Methodology, Formal analysis, Data curation. **Gabriel Lima Rebêlo:** Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis. **Adriano José Silva Félix:** Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Data curation. **Soraya Almeida Machado:** Writing – review & editing, Methodology, Investigation, Formal analysis, Data curation. **Carlos Eduardo Costa-Campos:** Writing – review & editing, Resources, Project administration, Investigation, Funding acquisition, Formal analysis, Conceptualization. **Jeannie Nascimento Santos:** Writing – review & editing, Resources, Project administration, Funding acquisition, Formal analysis. **Francisco Tiago Vasconcelos Melo:**

Table 4
Records of parasitic helminth in *Osteocephalus* spp., including new reports for *O. cabrerai* from the present study.

Helminth	Helminth Family	Country	Reference
Host: *Osteocephalus cabrerai*			
Nematoda			
Aplectana pella Santos, Borges, Willkens, Santos, Costa-Campos and Melo, (2023)	Cosmocercidae	Brazil	**Present study**
Cosmocerca parva Travassos, 1925		Brazil	**Present study**
Cosmocerca podicipinus Baker and Vaucher, 1984		Brazil	**Present study**
Oxyascaris oxyascaris Travassos, 1920		Brazil	**Present study**
Oswaldocruzia chabaudi Ben Slimane and Durette-Desset, 1996	Molineidae	Brazil	**Present study**
Physaloptera sp. (larvae)	Physalopteridae	Brazil	**Present study**
Host: *Osteocephalus taurinus* Steindachner, 1862			
Monogenoidea			
Polystoma naponensis Vaucher (1987)	Polystomatidae	Ecuador	Vaucher (1987)
Mesopolystoma samiriensis Vaucher (1981)		Peru	Vaucher (1981)
Nematoda			
Cosmocerca brasiliense Travassos, 1925	Cosmocercidae	Peru	Bursey et al. (2001)
Cosmocercoides meridionalis Anjos, Oda, Campião, Ávila, Santos, Santos, Almeida, Melo and Rodrigues, 2021		Brazil	Anjos et al. (2021)
Kentropyxia hylae Feitosa, Furtado, Santos and Melo, 2015	Molineidae	Brazil	Feitosa et al. (2015)
Ochoterenella vellardi (Travassos, 1929) Esslinger, 1986	Onchocercidae	Peru	Bursey et al. (2001)
Physaloptera sp. (larvae)	Physalopteridae	Peru	Bursey et al. (2001)
Physalopteroides venancioi (Lent, Freitas and Proença, 1946) Sobolev, 1949		Peru	Bursey et al. (2001)
Batracholandros spectatus (Freitas and Ibañez, 1962) Freitas and Ibañez, 1965	Pharyngodonidae	Peru	Bursey et al. (2001)
Parapharyngodon politoedi Santos, Argôlo, Santos, Rodrigues, Gonzaléz, Santos and Melo, (2019)		Brazil	Santos et al. (2019)
Parapharyngodon curupira Santos et al., (2022)		Brazil	Santos et al. (2022)
Rhabdias sp.	Rhabdiasidae	Brazil	Tavares-Costa et al. (2018)
Acanthocephala			
Oligacanthorhynchus sp. (cystacanth)	Oligacanthorhynchidae	Ecuador	Smales (2007)
Host: *Osteocephalus leprieurii* (Duméril and Bibron, 1841)			
Monogenoidea			
Polystoma naponensis	Polystomatidae	Ecuador	Vaucher (1987)

Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

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

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Capítulo 2

ADDING A NEW PIECE TO THE PUZZLE OF COSMOCERCIDAE EVOLUTIONARY RELATIONSHIPS: GENETIC CHARACTERIZATION OF *Aplectana pella* PARASITIC IN *Osteocephalus cabrerai* FROM AMAZON REGION

O Capítulo II desta Dissertação foi elaborado, formatado e submetido para publicação em 24/09/2024, conforme as normas da revista científica *Revista Brasileira de Parasitologia Veterinária*, as quais se encontram em anexo (Anexo II). O manuscrito encontra-se atualmente em revisão. Para facilitar a leitura, as imagens estão apresentadas de acordo com a ordem de aparecimento no texto.

SHORT TITLE: GENETIC CHARACTERIZATION OF *APLECTANA PELLA*

ADDING A NEW PIECE TO THE PUZZLE OF COSMOCERCIDAE EVOLUTIONARY RELATIONSHIPS: GENETIC CHARACTERIZATION OF *Aplectana pella* PARASITIC IN *Osteocephalus cabrerai* FROM AMAZON REGION

ADICIONANDO UMA NOVA PEÇA AO QUEBRA-CABEÇA DAS RELAÇÕES EVOLUTIVAS DE COSMOCERCIDAE: CARACTERIZAÇÃO GENÉTICA DE *Aplectana pella* PARASITA EM *Osteocephalus cabrerai* DA REGIÃO AMAZÔNICA

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ABSTRACT

Aplectana comprises species of gastrointestinal helminths commonly found parasitizing amphibians and reptiles worldwide. However, most species of the genus are described based only on morphological traits. In the present study, we provided the first nucleotide sequence of ribosomal gene 18S rDNA in *Aplectana pella* and established the species' phylogenetic position between representatives of Cosmocercidae. During helminthological surveys, we found nematodes identified as *Aplectana pella* parasitising *Osteocephalus cabrerai* from the same locality as the original description. Pairwise genetic comparison between *A. pella* and its congeners revealed a low genetic divergence. We found that our sequences clustered with species of *Cosmocerca*, reinforcing the hypothesis that representatives of the genus *Aplectana* do not form a monophyletic group.

Keywords: Helminths, Nematodes, Cosmocercidae, *Aplectana*, Phylogenetic.

RESUMO

O gênero *Aplectana* inclui espécies de helmintos gastrointestinais comumente encontrados parasitando anfíbios e répteis mundialmente. No entanto, a maioria das espécies do gênero foi descrita com base apenas em caracteres morfológicos. No presente estudo, nós fornecemos a primeira sequência de nucleotídeos do gene ribossomal 18S rDNA de *Aplectana pella* e estabelecemos a posição filogenética da espécie em relação aos representantes de Cosmocercidae. Durante expedições helmintológicas, nós encontramos nematódeos identificados como *A. pella* parasitando *Osteocephalus cabrerai* na mesma localidade da descrição original. Comparações genéticas entre *A. pella* e seus congêneres revelaram uma baixa divergência genética. Nós encontramos que as nossas sequências agrupam com espécies de *Cosmocerca*, reforçando a hipótese que representantes do gênero *Aplectana* não formam um grupo monofilético.

Palavras-chave: Helmintos, Nematódeos, Cosmocercidae, *Aplectana*, Filogenia

Introduction

Nematodes of the genus *Aplectana* Railliet & Henry, 1916 (Nemata: Cosmocercidae) are common parasites of the gastrointestinal tracts of amphibians and reptiles globally. To date, 58 species have been described, and most occurring in anurans from the families Bufonidae and Leptodactylidae (Campião et al., 2014; Vieira et al., 2020; Santos et al., 2023).

In recent years, molecular approaches including DNA sequencing of specific genes have been shown to be useful in estimating phylogenetic relationships among species in the family Cosmocercidae (Alcantara et al. 2022; Rebêlo et al. 2023). However, the identification of species of *Aplectana* from the Neotropical region continues to be based on morphological traits (Chen et al., 2021a). Currently, available genetic data of *Aplectana* spp. include only *Aplectana chamaeleonis* (Baylis, 1929) from África, *Aplectana xishuangbannaensis* Chen, Gu, Ni & Li, 2021, *Aplectana dayaoshanensis* Chen, Ni, Gu, Sinsch & Li, 2021 from Asia (Chen et al., 2021a, 2021b).

During a long-term study of the parasites of vertebrates of the Neotropics, we collected specimens of *Aplectan*a from the large intestine of *Osteocephalus cabrerai* (Cochran and Goin, 1970) from the Brazilian Amazon. An initial morphological analysis of the nematodes from these frogs suggested that it represented a new species, from which we successfully obtained molecular data. However, further morphological analysis using scanning electron microscopy allowed us to identify it as a previously described species called *Aplectana pella* Santos, Borges and Melo, 2023.

Aplectana pella was described by Santos et al. (2023) from the intestines of the rusty tree-frog, *Boana boans* (Linnaeus, 1758). However, at the time, the authors used only light microscopy and scanning electron microscopy to characterize this species. Further investigation using the nucleotide sequence of the 18S rDNA (ribosomal gene) from individuals of *Aplectana pella* enabled us to establish the phylogenetic position of A. pella among representatives of the nematode family Cosmocercidae.

Material and Methods

During a helminthological survey in the Amazon basin, 84 specimens of *Osteocephalus cabrerai* were collected between May 2019 and March 2022 in the Beija-Flor Brilho de Fogo Extractive Reserve (0°47 '30.6' N; 51°58 '42.1' W), located in the municipality of Pedra Branca do Amapari, Amapá state, Brazil. The amphibian hosts were identified following Pedroso-Santos et al. (2019).

After capture, frogs were euthanized and standard field based morphological measurements were taken, and all specimens were necropsied for helminthological examination. All internal organs were removed and placed in Petri dishes with saline solution (NaCl 0.9%), dissected separately, and the contents of the organs and the organs themselves were examined with a LEICA EZ4 stereomicroscope. All helminths found were rinsed in saline solution, killed with heated 70% alcohol, and preserved in the same solution at room temperature; a collection tag with the host collecting number was placed in each vials. Methods generally followed Gardner et al. (2012).

For morphological analysis, nematodes were hydrated in distilled water, cleared in Amann's lactophenol 20%, mounted on temporary slides, and examined with an Olympus BX41 microscope (Olympus Corp., Tokyo, Japan) coupled with a drawing tube (without zoom adjustment). Two male specimens were post-fixed in 1% Osmium tetroxide (OsO4), dehydrated in an increasing ethanol series and critical-point dried in Carbon dioxide (CO2). Specimens were mounted on metallic stubs, coated with gold-palladium and examined with a scanning electron microscope Vega3 (TESCAN, Brno, Czech Republic) in the Laboratory of Structural Biology, Biological Sciences Institute, Federal University of Pará (UFPA), Brazil.

For molecular analysis, a single male was transferred to microtubes containing 100% ethanol and stored in a freezer at -20 °C. Genomic DNA was extracted using NucleoSpin Tissue (Macherey-Nagel, Düren, Germany) according to the manufacturer's instructions. The SSU rDNA gene (18S) was amplified using the protocol and primers described in Gomes et al. (2015).

The resulting amplicons were visualized on 1.5% agarose gel electrophoresis with GelRed Nucleic Acid Stain (Biotium, Hayward, California, USA) on an ultraviolet light transilluminator. PCR products were purified through Illustra GFX PCR DNA and Gel Band kit (GE Healthcare, Chicago, Illinois, USA) according to the manufacturer’s instructions and sequenced using the BigDye Terminator v3.1 Cycle Sequencing kit (Applied Biosystems, USA). Amplicons were sequenced on an Applied Biosystems™ 3730 DNA Analyzer at the DNA Sequencing Platform of the Oswaldo Cruz Foundation (RPT01A/PDTIS/FIOCRUZ).

Contiguous sequences were assembled in Geneious 7.1.3 and deposited in the National Center for Biotechnology Information (NCBI) (htttp://www.ncbi.nml.nih.gov). The 18S rDNA datasets were aligned and trimmed using Muscle in Geneious 7.1.3 software. We obtained the saturation-substitutions index of each aligned matrix using the software DAMBE 5 (Xia, 2013). Levels of genetic divergence were estimated using the MEGA11.0 software package. The most appropriate evolutionary nucleotide substitution model was determined using the Akaike Information Criterion (AIC) via the jModelTest program. Sequence alignments were then subjected to Maximum Likelihood (ML) and Bayesian Inference (BI) analyses in RAxML 8.2.12 and MrBayes 3.2.7a software, respectively. Both analyses were carried out in CIPRES Science Gateway. Only nodes with posterior probabilities greater than 90% were considered well-supported. Maximum likelihood inference (ML) was implemented and estimates of level of robustness of the tree estimations were done using bootstrap analysis through 1,000 repetitions, and only nodes with bootstrap values greater than 70% were considered well-supported.

The trees were visualized and edited in FigTree v1.4.4 software. We used *Ichtyobronema hamulatum* (Moulton, 1931) (access number: KY476351) and *Cucullanus grandistomis* (Ferraz & Thatcher, 1988) (access number: KX752094) as two separate outgroups. Detailed information on the nematode species included in the molecular analyses is provided in Table S1.

Results and Discussion

Prevalence, mean intensity, and mean abundance of parasite infections in the frogs studied here were 27.38%, 4.3 (range 1–15) and 1.18, respectively. All parasites were adults and were found in the large intestine. The morphology of the specimens analyzed here are identical to the original description and all measurements overlap the range of variation reported for *A. pella* (See Santos et al. 2023) (Table 1). We observed that the number and distribution of caudal papillae (two ventral pre cloacal papillae pairs near anterior cloacal lip; one ad-cloacal pair; five post cloacal pairs; one single unpaired papilla situated on anterior cloacal lip), spicule lengths and gubernaculum absent (see Fig. 1) are the same as indicated by Santos et al. (2023).

Aplectana pella was originally described in the hylid frog *Boana boans* from Amapá state, Brazil (Santos et al., 2023). Our specimens were discovered as parasites of another arboreal hylid, *O. cabrerai* from the same locality, with similar parasitological descriptors (prevalence: 27.38% in *O. cabrerai* vs. 25% in *B. boans*; mean intensity: 4.3 in *O. cabrerai* vs. 6.5 in *B. boans*; mean abundance: 1.18 in *O. cabrerai* vs. 1.63 in *B. boans*), indicating that both hosts occupy similar ecological niches, and supporting the hypothesis that hosts exposed to similar environmental conditions share helminth taxa (Aho, 1990; Krasnov et al., 2011; Poulin et al., 2011; Draghi et al., 2020; Neves et al., 2024) through a process known as Ecological Fitting (Janzen, 1985).

We obtained a fragment of 786pb long for the 18S rDNA gene from *A. pella*. The alignment of the gene upon trimming to the shortest sequence length resulted in 751bp and included 15 species distributed across 3 genera, including: *Aplectana* (four sequences), *Cosmocerca* Diesing, 1861 (four sequences), *Cosmocercoides* Wilkie, 1930 (four sequences), *Nemhelix* Morand & Petter, 1986 (one sequence) and the outgroup. The best-fitting nucleotide substitution model identified was TIM3 + G (gamma shape parameter a = 0.0340; lnL = -1761.1172). Xia's test provided no evidence for substitution saturation in the data matrix.

Pairwise genetic comparison between congeners of *A. pella* revealed the lowest genetic distance from *A. chamaeleonis* (1.08%), followed by *A. xishuangbannaensis* (3.56%), and *A. dayaoshanensis* (3.70%) (see Supplementary Table 2). This molecular marker is a well-conserved gene that evolves slowly (Koubková et al. 2008). Thus, our study reinforces the general idea that the

18S rDNA region is a good marker for discriminating among genera and is also a good candidate for phylogenetic studies.

Table 1. Morphometric data from *A. pella* of *O. cabrerai* and *A. pella* from the original description.

Characters	*A. pella* Host: *Osteocephalus cabrerai* Present study		*A. pella* Host: *Boana boans* Santos et al. (2023)	
	Males (n =10)	Females (n =10)	Males (n =7)	Females (n =10)
Total length (mm)	2.58 (2.10–3.13)	4.29 (2.86–5.52)	2.63 (2.3–2.9)	3.5 (2.3–4.3)
Maximum width	300 (253.3–386.6)	378.9 (263.1–512)	-	361.5 (289–436)
Body width at oesophago-intestinal junction	236 (208–280)	288.9 (192–350)	229.5 (205–251)	262.6 (178–306)
Body width at nerve ring	119.1 (96–138.6)	146.7 (120–180)	-	-
Body width at excretory pore	184.3 (160–216)	228.5 (162.6–269.3)	-	-
Lateral alae to anterior extremity	139.2 (93.3–186.6)	215.9 (173.3–280)	-	-
Lateral alae to posterior extremity	275.7 (226.6–317.3)	284.2 (250.6–325.3)	-	-
Oesophagus total length	468 (417–530.6)	593.7 (482.6–680)	464.3 (416–502)	565.5 (529–594)
Oesophagus in % of body length	18.2 (16.5–20.5)	14.3 (11.6–18)	17.6 (16.6-19.3)	16.5 (13.3–25.9)
Pharynx length	33.7 (26.6–40)	39.8 (32–50)	35 (27–42)	43.8 (32–50)
Pharynx width	27.7 (24–37.3)	34.4 (26.6–40)	28 (26–32)	32.3 (26–37)
Corpus length	320.5 (280–360)	414.4 (328–480)	302.7 (269–330)	377.1 (336–413)
Corpus width	44.2 (37.3–53.3)	50 (40–60)	-	-
Isthmus length	20.5 (10.6–29.3)	18.7 (13.3–25)	39 (32–45)	39.2 (32–50)
Isthmus width	29 (27–37)	37.3 (29.3–50)	-	-
Bulb length	93 (80–104)	120.7 (93.3–150)	87.5 (77–98)	105.5 (96–117)
Bulb width	113 (96–133)	145.9 (109.3–181.3)	109.2 (101–122)	129.5 (114–144)
Nerve ring from anterior end	184.2 (154.6–213.3)	218.7 (192–285)	194.5 (173–226.5)	213.9 (178–245)
Excretory pore	334.6 (266.6–392)	393.3 (277.3–490.6)	328.6 (312–344)	404.1 (349–453)
Tail length	327.6 (285.3–354.6)	373.2 (306.6–415)	307.7 (256–344)	358.1 (321–394)
Tail length in % of body length	12.8 (10.5–14.6)	9 (7–12)	11.7 (9.4–13.3)	10.3 (8.2–14.5)
Tail width	107.1 (74.6–149.3)	135.4 (90.6–213.3)	-	-
Spicules	110 (80.5–142.8)	-	106.5 (104–111)	-
Vulva to anterior end (mm)	-	2.29 (1.72–2.72)	-	1.63 (0.6–2)
Vulva in % of body length	-	54.5 (48.5–63.9)	-	45.3 (25.4–53.1)
Egg length	-	63.9 (45.7–78)	-	59.6 (54–67)
Egg width	-	40.9 (30.1–51.9)	-	36.5 (33–43)

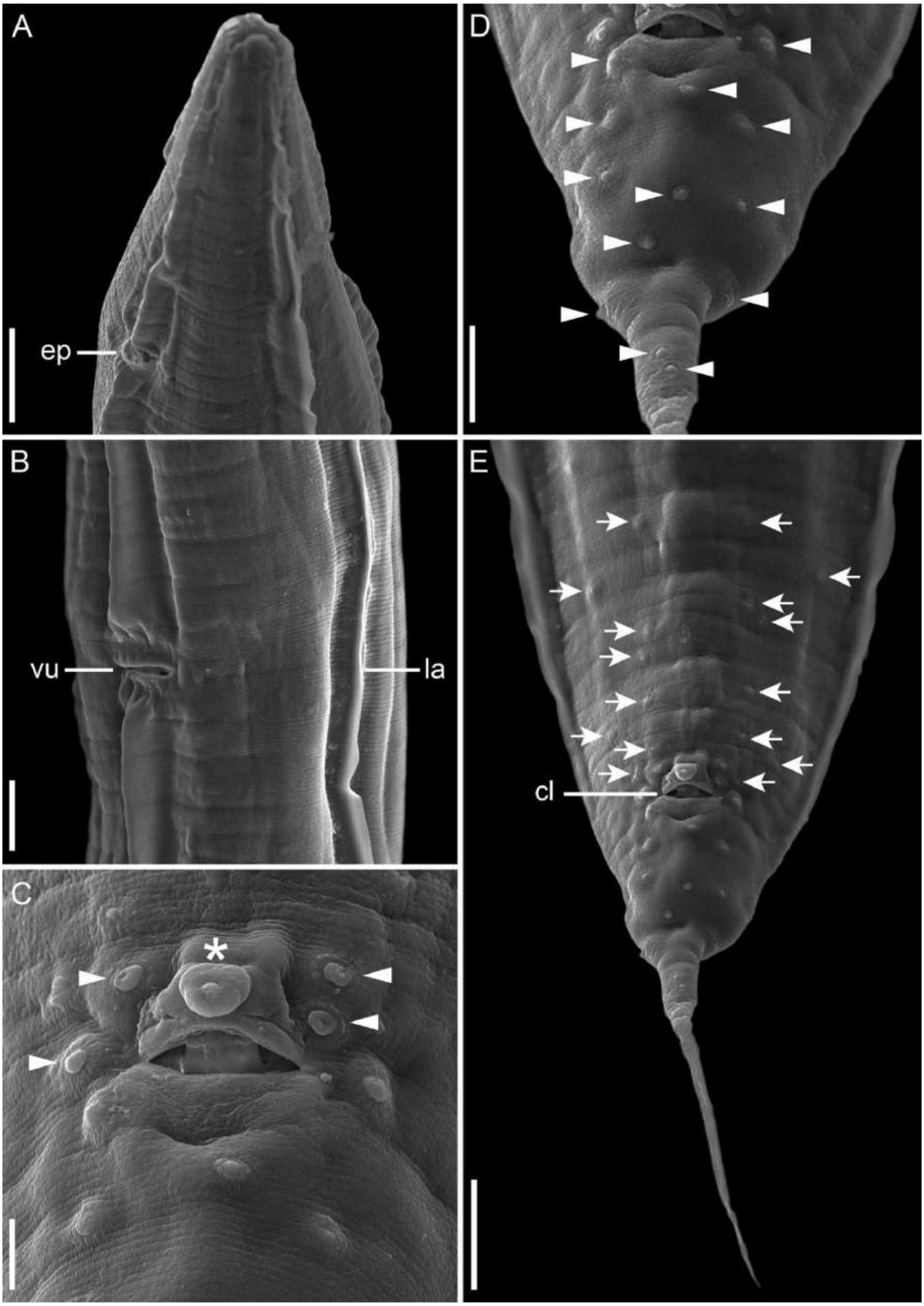

Fig. 1 Scanning electron micrographs of *Aplectana pella* from *Osteocephalus cabrerai*. (A) Male, anterior end, lateral view; (B) Female, vulva view; (C) Male, details of papillae near to cloaca; (D) Male, details of post-cloacal papillae; (E) Posterior end. Arrows: somatic papillae; arrowheads: cloacal papillae; asterisk: unpaired papillae. Abbreviations: cl, cloaca; ep, excretory pore; vu, vulva; la, lateral alae. Scale bars: A= 100µm; B, E= 50µm; C= 10µm; D= 25µm.

Our phylogenetic trees obtained using Maximum Likelihood (ML) and Bayesian Inference (BI) revealed similar topologies. The sequences of Cosmocercidae (100 bootstrap and 100 posterior probability) formed two large groups (Fig. 2). The first was composed of *Cosmocercoides* spp., *Cosmocerca longicauda* (Linstow, 1885) and *Nemhelix bakeri* Morand & Petter, 1986 (50 bootstrap and 96 posterior probability) and the second clade included *A. pella* that formed a separate branch with the paraphyletic group that includes species of *Aplectana* and *Cosmocerca* (93 bootstrap and 99 posterior probability). Our results reinforce that *Aplectana* is a non-monophyletic genus, consistent with findings from previous studies (Chen et al., 2021a, 2021b; Svitin et al., 2023).

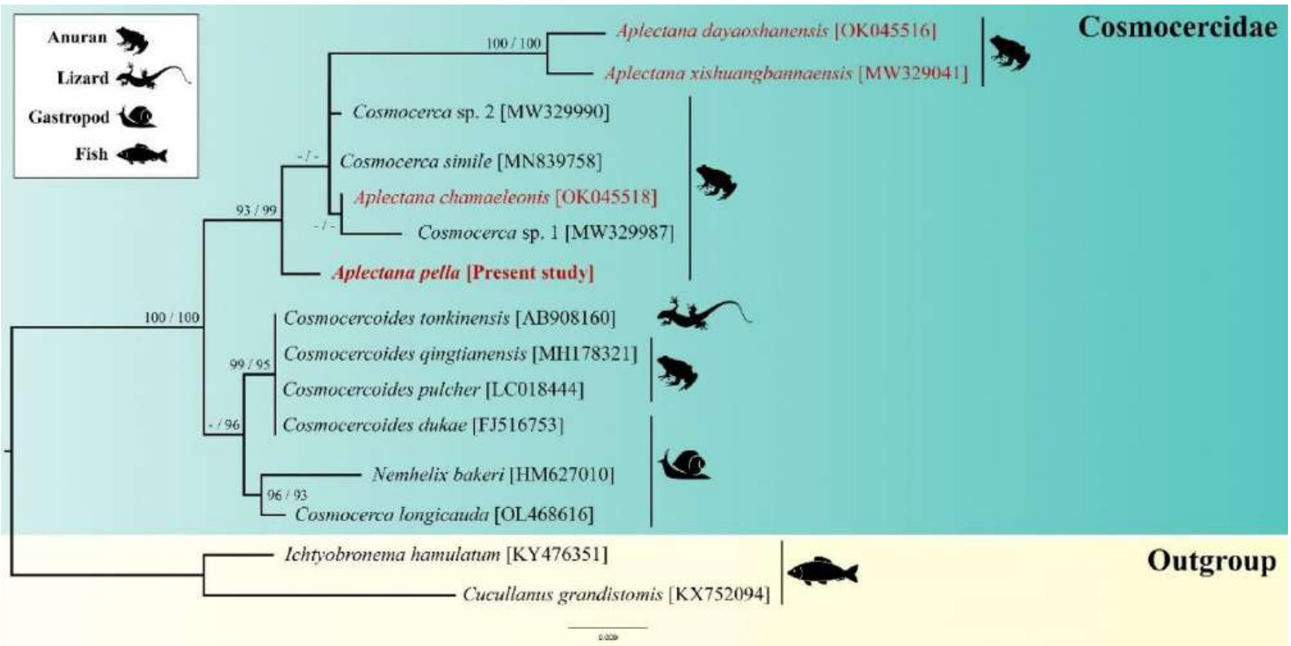

Fig. 2 Maximum likelihood topology based on 18S rDNA using *Ichtyobronema hamulatum* and *Cucullanus grandistomis* as outgroup. GenBank accession numbers are indicated next to species names. Numbers beside the nodes represent support value by bootstrap for maximum likelihood analyses and posterior probabilities for Bayesian analyses, respectively (bootstrap scores >70 and posterior probabilities >90). Branch-length scale bar indicates number of substitutions per site.

Traditional systematic studies have historically supported the evolutionary hypothesis that *Cosmocerca* is closely related to *Cosmocercoides,* mainly because those two genera share the presence of ornamented papillae in male caudal region (Wilkie, 1930; Chabaud, 1978). However, our results support recent phylogenetic studies that found *Cosmocerca* to be a non-monophyletic genus, closely related to *Aplectana* (Fig. 2) (Chen et al., 2021a, b; Harnoster et al. 2022; Ni et al. 2022; Svitin et al., 2023; Tuschida et al. 2023).

Cosmocercoides spp. did appear in our tree as monophyletic and clustered as a sister group to *N. bakeri* + *Cosmocerca longicauda* (Fig. 2). This result has also been observed in previous studies (Saito et al., 2021; Harnoster et al., 2022; Ni et al., 2022; Svitin et al., 2023; Tuschida et al., 2023).

However, some authors suggest that the authors who deposited the sequence of *C. longicauda* misidentified the species (Svitin et al., 2023; Félix et al., 2024). Thus, until now, only *Cosmocercoides* have been found parasitizing snails, and due to the high genetic divergence observed among *C. longicauda* and other *Cosmocerca* spp. we also reinforce that this sequence should be considered a representative of the genus *Cosmocercoides.*

This study presents the first molecular and phylogenetic analyses that include the species *A. pella* in an analysis that clearly shows that species in the genus *Aplectana* are not monophyletic in their origin Therefore, further molecular-phylogenetic studies are necessary to better understand the evolutionary relationships of species of *Aplectana*, particularly in the Neotropical region where significant gaps in the genetic database exist among the species. We also emphasize the importance of combined detailed morphological and molecular studies with more representatives of the genus to deepen our knowledge about the diversity, phylogenetic relationships, and ecology of Cosmocercidae.

Furthermore, detailed morphological and molecular studies with more representatives of the genus are essential to deepening our knowledge about the diversity, phylogenetic relationships, and ecology of Cosmocercids.

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Data and materials availability - Not applicable.

Authors contributions – J.K.S. Neves analysed the material and drafted the main manuscript, as well as preparing the images. G.L. Rebêlo, A.J.S. Félix, S.L. Gardner, A. Maldonado-Júnior, C.E. Costa-Campos, and F.T.V. Melo contributed to the writing and review of the article. All authors reviewed the manuscript.

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Table S1. Nematode species, hosts, localities, GenBank accession numbers, and references used in phylogenetic analyses.

Families	Species	Host	Locality	Accession numbers	Reference
Cosmocercidae	*Aplectana pella* Santos, Borges and Melo, 2020	*Osteocephalus cabrerai* (Cochran and Goin, 1970)	Brazil	PQ570691	**Present study**
	Aplectana dayaoshanensis Chen, Ni, Gu, Sinsch and Li, 2021	*Hylarana spinulosa* (Smith, 1923)	China	OK045516	Chen *et al.* (2021a)
	Aplectana chamaeleonis (Baylis, 1929)	*Hyperolius kivuensis* Ahl, 1931	Rwanda	OK045518	Chen *et al.* (2021a)
	Aplectana xishuangbannaensis Chen, Ni, Gu and Li, 2021	*Polypedates megacephalus* Hallowell, 1861	China	MW329041	Chen *et al.* (2021b)
	Cosmocerca longicauda (Linstow, 1885)	Snail	-	OL468616	Unpublished
	Cosmocerca simile Chen, Zhang, Feng and Li, 2020	*Bufo gargarizans* Cantor, 1842	China	MN839758	Chen *et al.* (2020)
	Cosmocerca sp. 1	*Hoplobatrachus chinensis* (Osbeck, 1765)	China	MW329987	Chen *et al.* (2021b)
	Cosmocerca sp. 2	*Bufo melanostictus* (Schneider, 1799)	China	MW329990	Chen *et al.* (2021b)
	Cosmocercoides dukae (Holl, 1928)	*Deroceras panormitanum* Lessona, and Pollonera, 1882	USA	FJ516753	Ross *et al.* (2010)
	Cosmocercoides pulcher Wilkie, 1930	*Bufo formosus* Boulenger, 1883	Japan	LC018444	Tran *et al.* (2015)
	Cosmocercoides qingtianensis Chen, Zhang, Nakao, and Li, 2018	*B. gargarizans*	China	MH178321	Chen *et al.* (2018)
	Cosmocercoides tonkinensis Tran, Sato, and Luc, 2015	*Acanthosaura lepidogaster* Cuvier, 1829	Vietnam	AB908160	Tran *et al.* (2015)
	Nemhelix bakeri Morand and Petter, 1986	Snail	-	HM627010	Saito *et al.* (2021)
Quimperiidae (Outgroup)	*Ichtyobronema hamulatum* (Moulton, 1931)	*Lota lota* (Linnaeus, 1758)	Russia	KY476351	Sokolov and Malysheva (2017)
Cucullanidae (Outgroup)	*Cucullanus grandistomis* (Ferraz & Thatcher, 1988)	*Oxydoras niger* (Valenciennes, 1821)	Brazil	KX752094	Pereira and Luque (2017)

Table S2. Pairwise genetic divergence levels (%) of the 18S gene among sequences of nematodes of the family Cosmocercidae. Names, GenBank accession, and values numbers are provided in the table.

	Cucullanus grandistomis (KX752094)	*Ichtyobronema hamulatum* (KY476351)	*Aplectana dayaoshanensis* (OK045516)	*Aplectana xishuangbannaensis* (MW329041)	*Aplectana pella* (PQ570691)	*Cosmocerca* sp. 1 (MW329987)	*Cosmocerca* sp. 2 (MW329990)	*Aplectana chamaeleonis* (OK045518)
Cucullanus grandistomis (KX752094)								
Ichtyobronema hamulatum (KY476351)	5,85%							
Aplectana dayaoshanensis (OK045516)	9,89%	6,87%						
Aplectana xishuangbannaensis (MW329041)	10,35%	6,58%	1,08%					
Aplectana pella (PQ570691)	8,82%	5,42%	3,70%	3,56%				
Cosmocerca sp. 1 (MW329987)	8,67%	5,28%	3,28%	3,14%	1,76%			
Cosmocerca sp. 2 (MW329990)	8,82%	5,71%	2,87%	2,73%	1,08%	0,94%		
Aplectana chamaeleonis (OK045518)	8,82%	5,42%	2,87%	2,73%	1,08%	0,67%	0,27%	
Cosmocerca simile (MN839758)	8,97%	5,56%	2,73%	2,59%	0,94%	0,81%	0,13%	0,13%
Nemhelix bakeri (HM627010)	9,73%	6,00%	5,12%	5,12%	2,59%	3,14%	3,00%	2,73%
Cosmocerca longicauda (OL468616)	8,82%	5,28%	4,41%	4,41%	2,04%	2,31%	2,17%	1,90%
Cosmocercoides dukae (FJ516753)	8,97%	4,84%	4,27%	4,27%	1,90%	2,17%	2,04%	1,76%
Cosmocercoides pulcher (LC018444)	8,97%	4,84%	4,27%	4,27%	1,90%	2,17%	2,04%	1,76%
Cosmocercoides qingtianensis (MH178321)	8,97%	4,84%	4,27%	4,27%	1,90%	2,17%	2,04%	1,76%
Cosmocercoides tonkinensis (AB908160)	8,97%	4,84%	4,27%	4,27%	1,90%	2,17%	2,04%	1,76%

Table S2. Continued.

	Cosmocerca simile (MN839758)	*Nemhelix bakeri* (HM627010)	*Cosmocerca longicauda* (OL468616)	*Cosmocercoides dukae* (FJ516753)	*Cosmocercoides pulcher* (LC018444)	*Cosmocercoides qingtianensis* (MH178321)	*Cosmocercoides tonkinensis* (AB908160)
Cucullanus grandistomis (KX752094)							
Ichtyobronema hamulatum (KY476351)							
Aplectana dayaoshanensis (OK045516)							
Aplectana xishuangbannaensis (MW329041)							
Aplectana pella (XXXXX)							
Cosmocerca sp. 1 (MW329987)							
Cosmocerca sp. 2 (MW329990)							
Aplectana chamaeleonis (OK045518)							
Cosmocerca simile (MN839758)							
Nemhelix bakeri (HM627010)	2,87%						
Cosmocerca longicauda (OL468616)	2,04%	1,35%					
Cosmocercoides dukae (FJ516753)	1,90%	1,62%	0,81%				
Cosmocercoides pulcher (LC018444)	1,90%	1,62%	0,81%	0,00%			
Cosmocercoides qingtianensis (MH178321)	1,90%	1,62%	0,81%	0,00%	0,00%		
Cosmocercoides tonkinensis (AB908160)	1,90%	1,62%	0,81%	0,00%	0,00%	0,00%	

CONCLUSÕES GERAIS

Fornecemos o primeiro estudo sobre a estrutura da comunidade de helmintos de *O. cabrerai*. Nossos achados contribuem para a compreensão da fauna de helmintos dos anuros e oferecem novas percepções sobre os padrões de distribuição das comunidades de helmintos.

A comunidade componente foi predominantemente composta por nematódeos de ciclo de vida monoxênico, apresentando alta prevalência e abundância elevada. A maioria dos espécimes de parasitos encontradas representa o primeiro registro para o gênero *Osteocephalus*, exceto *Physaloptera* sp. Nas nossas análises, o peso corporal de *Osteocephalus cabrerai* influenciou positivamente a intensidade total de infecção.

Este estudo também apresenta as primeiras análises moleculares e filogenéticas que incluem *A. pella*, reforçando que as espécies do gênero *Aplectana* não são monofiléticas. Portanto, novos estudos moleculares e filogenéticos são necessários para entender melhor as relações evolutivas de *Aplectana*, especialmente na região Neotropical, onde existem lacunas significativas na base de dados genética entre as espécies.

Enfatizamos a importância de estudos morfológicos e moleculares combinados, com mais representantes do gênero, para aprofundar nosso conhecimento sobre a diversidade, as relações filogenéticas e a ecologia da família Cosmocercidae.

ANEXO I

International Journal for Parasitology: Parasites and Wildlife

Introduction

The International Journal for Parasitology: Parasites and Wildlife (IJP:PAW) publishes the results of original research on parasites of all wildlife, invertebrate and vertebrate. This includes free-ranging, wild populations, as well as captive wildlife, semi-domesticated species (e.g. reindeer) and farmed populations of recently domesticated or wild-captured species (e.g. cultured fishes). Articles on all aspects of wildlife parasitology are welcomed including taxonomy, biodiversity and distribution, ecology and epidemiology, population biology and host-parasite relationships. The impact of parasites on the health and conservation of wildlife is seen as an important area covered by the Journal especially the potential role of environmental factors, for example climate. Also important to the journal is 'one health' and the nature of interactions between wildlife, people and domestic animals, including disease emergence and zoonoses.

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Most Elsevier journals have their reference template available in many of the most popular reference management software products. These include all products that support Citation Style Language styles, such as Mendeley. Using citation plug-ins from these products, authors only need to select the appropriate journal template when preparing their article, after which citations and bibliographies will be automatically formatted in the journal's style. If no template is yet available for this journal, please follow the format of the sample references and citations as shown in this Guide. If you use reference management software, please ensure that you remove all field codes before submitting the electronic manuscript. More information on how to remove field codes from different reference management software.

Journal abbreviations source

Journal names should be abbreviated according to the NLM catalogue: http://www.nlm.nih.gov/tsd/serials/lji.html.

New Taxa

It is a requirement of the International Commission on Zoological Nomenclature (ICZN) that the Author must obtain a Zoobank registration for any named new species for it to be considered valid, if it is to be published in an online only journal. This number must then be included in the published work. For more information please visit this site: https://www.iczn.org/the-code/electronic-publication-made-available-with-amendment-to-the-code/

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Elsevier accepts video material and animation sequences to support and enhance your scientific research. Authors who have video or animation files that they wish to submit with their article are strongly encouraged to include these within the body of the article. This can be done in the same way as a figure or table by referring to the video or animation content and noting in the body text where it should be placed. All submitted files should be properly labeled so that they directly relate to the video file's content. In order to ensure that your video or animation material is directly usable, please provide the files in one of our recommended file formats with a preferred maximum size of 50 MB. Video and animation files supplied will be published online in Elsevier Web products, including ScienceDirect: https://www.sciencedirect.com Please supply 'stills' with your files: you can choose any frame from the video or animation or make a separate image. These will be used instead of standard icons and will personalize the link to your video data. For more detailed instructions please visit our video instruction pages at https://www.elsevier.com/artworkinstructions.

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Include interactive data visualizations in your publication and let your readers interact and engage more closely with your research. Follow the instructions here to find out about available data visualization options and how to include them with your article.

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For non-integrated supplementary files, a footnote should be typed on the title page of the manuscript: ′ Note: Supplementary data associated with this article. A copy of supplementary material should be submitted at the same time as the manuscript. Preferred formats are Microsoft Office for text or graphics and AVI for movie files. Maximum size of files is 10 MB. If files cannot be reduced to

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This journal encourages and enables you to share data that supports your research publication where appropriate, and enables you to interlink the data with your published articles. Research data refers to the results of observations or experimentation that validate research findings, which may also include software, code, models, algorithms, protocols, methods and other useful materials related to the project.

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To foster transparency, we encourage you to state the availability of your data in your submission. This may be a requirement of your funding body or institution. If your data is unavailable to access or unsuitable to post, you will have the opportunity to indicate why during the submission process, for example by stating that the research data is confidential. The statement will appear with your published article on ScienceDirect. For more information, visit the Data Statement page.

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The following list will be useful during the final checking of an article prior to sending it to the journal for review. Please consult this Guide for Authors for further details of any item.

Ensure that the following items are present:

One Author designated as corresponding Author:

- E-mail address

- Full postal address

- Telephone and fax numbers

- Keywords

- All figure captions

- All tables (including title, description, footnotes)

Further considerations

- Manuscript has been "spellchecked" and "grammar-checked"

- References are in the correct format for this journal

- All references mentioned in the Reference list are cited in the text, and vice versa

- Permission has been obtained for use of copyrighted material from other sources (including the

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After acceptance

Use of the Digital Object Identifier

The Digital Object Identifier (DOI) may be used to cite and link to electronic documents. The DOI consists of a unique alpha-numeric character string which is assigned to a document by the publisher upon the initial electronic publication. The assigned DOI never changes. Therefore, it is an ideal medium for citing a document, particularly 'Articles in press' because they have not yet received their full bibliographic information. The correct format for citing a DOI is shown as follows (example taken from a document in the journal Physics Letters B):

https://doi.org/10.1016/j.physletb.2010.09.059

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To ensure a fast publication process of the article, we kindly ask authors to provide us with their proof corrections within two days. Corresponding authors will receive an e-mail with a link to our online proofing system, allowing annotation and correction of proofs online. The environment is similar to MS Word: in addition to editing text, you can also comment on figures/tables and answer questions from the Copy Editor. Web-based proofing provides a faster and less error-prone process by allowing you to directly type your corrections, eliminating the potential introduction of errors.

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Additional information

This section describes additional information for this journal.

Submission of sequence data to databases

Novel nucleotide or protein sequence data must be deposited in the GenBank™ , EMBL or DDBJ databases and an accession number obtained before the paper can be accepted for publication. Submission to any one of the collaborating databanks is sufficient to ensure entry in all. The accession number should be included as a footnote on the title page of the manuscript: 'Note: Nucleotide sequence data reported in this paper are available in the GenBank™ , EMBL and DDBJ databases under the accession number(s)'. If requested the database will withhold release of data until publication. The usual method for submitting sequence data is by the World Wide Web to either GenBank (via BankIt: http://www.ncbi.nlm.nih.gov/BankIt/), EMBL (via WebIn: http://www.ebi.ac.uk/subs/allsubs.html) or to DDBJ (via SAKURA: http://sakura.ddbj.nig.ac.jp/). Special types of submissions, such as genomes, bulk submissions, segmented sets, and population/phylogenetic/mutation studies, can be more easily prepared with the Sequin programme (available from the above Web sites). Authors are encouraged by the databases to update their entries as the need arises.

GenBank/DNA sequence linking. In order for automatic links to be made between papers and GenBank, authors should type the accession number in bold, underlined text. Letters in the accession number should always be capitalised. (See the example). When published they will appear in normal type.

Example: ′ GenBank accession nos. AI631510, AI631511, AI632198, and BF223228), a B-cell tumor from a chronic lymphatic leukemia (GenBank accession no. BE675048), and a T-cell lymphoma (GenBank accession no. AA361117)′ .

Additionally, any multiple alignments of nucleotide or protein data must be submitted to a recognised database and must also receive a unique accession number. The accession number can appear in the text in the relevant section of the Results, as: ′ Alignment files are available by anonymous FTP from

FTP.EBI.AC.UK in directory/pub/databases/embl/align or via the EMBLALIGN database via SRS at http://srs.ebi.ac.uk; under accession(s)′ . The usual method for submitting alignments is by the World Wide Web to the European Bioinformatics Institute (via Webin-Align: http://www.ebi.ac.uk). Microarray data, in MIAME-compliant format, should be submitted to ArrayExpress (http://www.ebi.ac.uk/arrayexpress/) or GEO (http://www.ncbi.nlm.nih.gov/geo/). Accession identifiers relating to the data should be provided in the manuscript text.

Policy on bioinformatics papers. In silico analysis: The following guidelines apply to papers that exclusively use in silico analysis or rely heavily on this approach for analysis and conclusions. Such papers should address a significant biological issue or issues. Bioinformatic data should be supported by novel or published biological data. Work would typically use information from a number of databases and even from a number of parasite or host species and use a number of analytical methods. Types of ′ metaanalysis′ are encouraged either across a wide range of parasites or, say, at a number of points in a metabolic or signalling pathway or an immune cascade. In silico analysis may be especially suitable for review articles.

Guidelines for reporting of protein identifications using ma

The following information should be provided for protein or peptide identifications using mass spectrometry:

1. The program, and version number, used to create peak lists and the parameters used in the creation of the list.

2. The program, and version number, of the program used for database searching. Parameters used for searching should be specified, including, but not limited to, precursor-ion mass tolerance, fragment-ion mass tolerance, modifications allowed for, missed cleavages and enzymes used in protein cleavage.

3. The name and version number of the sequence database used in searches. If a custom-made database is used then complete information on the origin of the sequences and database size should be disclosed. Given the dependence of scoring on database size, the use of a small database, or one excluding contaminants, should be justified. 4. A short description of the methods use to interpret the significance of search results, including any statistical analysis, confidence thresholds and other values specific to judging the certainty of the identification.

5. For large-scale experiments a false-positive determination should be reported. This may be the result of randomized database searches or other approaches.

6. Each protein identification should include the accession number, score generated by the search algorithm used, sequence coverage and the number of unique peptide sequences assigned in the protein identification.

7. Single peptide identifications should include an annotated MS/MS spectrum showing fragment assignments together with the peptide sequence, precursor mass, charge and error.

8. Identifications arising from peptide mass fingerprinting should include an annotated mass spectrum. The number of matched peaks, the number of unmatched peaks and the sequence coverage should also be reported along with all parameters and thresholds used to analyse the data. This includes mass accuracy, resolution, calibration methods, contaminant exclusions along with the scoring scheme used and measure of the false-positive rate.

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ANEXO II

Revista Brasileira de Parasitologia Veterinária

Políticas Editoriais
Política da seção

Os artigos submetidos à Revista Brasileira de Parasitologia Veterinária deverão caracterizar-se como científicos e originais, essencialmente sobre parasitas de animais em geral, não devendo ser publicados anteriormente nem estar sob consideração para publicação em outro periódico.
A RBPV atribui a seus artigos as categorias de:

Artigo Original: deve relatar dados de pesquisa original, não tendo sido publicado em nenhum outro periódico.

Deve ser organizado obedecendo à seguinte sequência: Título Original (inglês), Título Traduzido (português), Título resumido (inglês), Autor(es)/Filiação Institucional, Abstract (inglês), Keywords, Resumo (português), Palavras-chave (português), Introdução, Material e Métodos, Resultados, Discussão, Conclusões (ou combinação destes três últimos), Agradecimentos, Declaração de Ética, Declaração de Conflito de Interesse e Referências Bibliográficas. As tabelas e ilustrações deverão ser apresentadas separadas do texto e anexadas ao final do trabalho, sem legendas. As respectivas legendas deverão vir no texto logo após as referências bibliográficas.

Comunicação Científica: para essa categoria, o artigo submetido só será aceito desde que possua alto grau de ineditismo e originalidade, trazendo resultados novos de importância evidente, atribuindo ao Editor-chefe a continuidade da submissão ou não.
Pode ser organizado seguindo a mesma sequência de um Artigo Original, porém sem a necessidade de se destacar os tópicos, sendo escritas em texto corrido, conciso e limitado a 4.000 palavras, podendo incluir até 3 figuras ou tabelas, combinadas. Não mais que 25 referências devem ser citadas.

Artigo de Revisão: condicionado a submissão e a solicitação por convite do editor-chefe. Revisões não solicitadas serão avaliadas pelo editor-chefe ou editores científicos assistentes, desde que sejam escritas por pesquisadores especialistas no tópico escolhido. O texto principal dos artigos de revisão deve ter no mínimo 4.000 palavras.

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A submissão de trabalhos na RBPV é online pelo sistema ScholarOne. O autor correspondente deve fornecer um ID ORCID (Open Researcher and Contributor ID, http://orcid.org/) no momento da submissão inserindo-o no perfil do usuário no sistema de submissão. Recomendamos que seja feito o mesmo para os coautores.

O(s) autor(res) deverá(ão) anexar uma carta assinada por todos, responsabilizando-se por todo o processo de tramitação e originalidade do artigo, salvo resumo(s) apresentado(s) em eventos científicos, não submetidos à publicação em outros periódicos.

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R$ 1.000,00 (não-associados do CBPV).
US$ 300,00 (autores estrangeiros)

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Para autores estrangeiros:
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Endereço: Via de acesso Prof. Paulo Donato Castellane, s/n, Zona Rural. CEP: 14884-900. Jaboticabal – SP, Brasil.

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Os pesquisadores deverão assumir os custos da revisão. Caso um dos coautores seja estrangeiro nativo da língua inglesa, este deverá revisar o inglês do trabalho. O autor correspondente deverá enviar correspondência para revista confirmando que essa revisão foi feita por um dos autores nativos da língua inglesa.

Autoria

Trabalhos com número excessivo de autores deverão ser avaliados pelos editores científicos assistentes, em relação ao protocolo experimental.

É necessária a colaboração substancial de todos os autores no planejamento do estudo, obtenção, análise e interpretação de resultados, confecção do artigo e aprovação da versão final submetida e aceita.

Alterações de autoria, como exclusão ou inclusão de autores, devem ser feitas mediante um ofício ao Editor-chefe, com a justificativa, a qual será analisada dentro das políticas e submissão e aprovação. O autor excluído ou incluído, também deverá concordar, enviando um comunicado ao Editor-chefe.

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O processo de avaliação do trabalho dependerá da observância das Normas Editoriais, dos Pareceres do Corpo Editorial e/ou do Relator ad-hoc. Nesse processo, o editor-chefe e os editores científicos assistentes poderão sugerir ou solicitar as modificações necessárias, apesar de ser de responsabilidade dos autores os conceitos emitidos. Os artigos submetidos serão avaliados por, no mínimo, 2 revisores anônimos, selecionados pelo editor-chefe. Em caso de um parecer conflitante, o artigo será enviado a um terceiro revisor.

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Ética

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Experimentos que utilizam animais deverão ser conduzidos obedecendo às normas aprovadas pela Comissão de Ética e Bem-Estar Animal da instituição na qual o estudo foi desenvolvido, devendo os autores apresentarem o número de protocolo de submissão e aprovação dos trabalhos. Para autores estrangeiros, experimentos com animal também são previstos no "International Guiding Principles for Biomedical Research Involving Animals" emitido pelo "Council for the International Organizations of Medical Sciences" (https://grants.nih.gov/grants/olaw/guiding_principles_2012.pdf), devendo os autores apresentarem o número de protocolo de submissão e aprovação dos trabalhos.

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O arquivo principal não deve conter os nomes dos autores, filiações, agradecimentos, declaração de ética, declaração de conflito de interesse, etc., para facilitar a revisão às cegas pelos pares. Inserir estas informações em uma Página de Título, em arquivo separado.

Características dos elementos de um trabalho científico

Título Original/Título Traduzido

O título “cheio” e o subtítulo (se houver) não devem exceder 25 palavras. Não deverá aparecer nenhuma abreviatura, e os nomes de espécies ou palavras em latim deverão vir em itálico. Evitar (por exemplo) títulos que iniciem com: Estudos preliminares; Observações sobre. Não usar o nome do autor e data de citação em nomes científicos.

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Devem conter no máximo 200 palavras, em um só parágrafo sem deslocamento. Não devem conter citações bibliográficas. Siglas e abreviações de instituições, ao aparecerem pela primeira vez no trabalho, serão colocadas entre parênteses e precedidas do nome por extenso, por exemplo, Indirect Fluorescence Assay (IFA). Devem ser informativos, apresentando o objetivo do trabalho, metodologia sucinta, os resultados mais relevantes e a conclusão. O abstract redigido em língua inglesa e o resumo em língua portuguesa, ambos seguidos por keywords e palavras-chave, respectivamente.

Keywords/ Palavras-chave

As palavras-chave devem expressar com precisão o conteúdo do trabalho. São limitadas em no máximo 6 (seis), e separadas por vírgula.

Introdução

Explanação clara e objetiva do estudo, da qual devem constar a relevância e objetivos do trabalho, restringindo as citações ao necessário.

Material e Métodos

Descrição concisa, sem omitir o essencial para a compreensão e reprodução do trabalho. Métodos e técnicas já estabelecidos devem ser apenas citados e referenciados. Métodos estatísticos devem ser explicados ao final dessa seção.

Resultados

O conteúdo deve ser informativo e não interpretativo: sempre que necessário devem ser acompanhados de tabelas, figuras ou outras ilustrações autoexplicativas.

Discussão

Deve ser limitada aos resultados obtidos no trabalho e o conteúdo deve ser interpretativo. Poderá ser apresentada como um elemento do texto ou juntamente aos resultados e conclusão. Enfatizar a importância de novos achados e novas hipóteses identificadas claramente com os resultados.

Conclusões

As conclusões podem estar inseridas na discussão ou em resultados e discussão, conforme a escolha dos autores. Nesse caso, esse item não será necessário.

Agradecimentos

Colaboradores que não atendam aos critérios de autoria, poderão ser listados nesta seção. Poderá haver agradecimento a pessoa ou instituição que forneceu auxílio técnico, sugestões, correção ou sugestão na escrita ou que de alguma forma colaborou para a elaboração do trabalho. Todo o suporte financeiro recebido deverá ser apresentado nesta seção.

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Os autores devem apresentar o número de protocolo de submissão e aprovação dos trabalhos em uma seção própria no final do artigo, antes das referências.

Declaração de Conflito de Interesse

O autor correspondente deve enviar uma declaração divulgando quaisquer potenciais conflitos de interesse, podendo ser eles de natureza pessoal, comercial, política ou acadêmica, envolvendo ou não compensação financeira, ou mesmo quando não houver qualquer conflito, para todos os autores.

Referências

Citações

As citações devem seguir o sistema autor-data:

Um autor: nome do autor e ano de publicação
Levine (1985) ou (Levine, 1985)

Dois autores: os nomes dos autores e ano da publicação
Paim & Souza (2011) ou (Paim & Souza, 2011)

Três ou mais autores: nome do primeiro autor seguido de "et al." e o ano de publicação
Araújo et al. (2002) ou (Araújo et al., 2002)

Só serão admitidas referências de fácil acesso aos leitores. Referências de difícil acesso poderão ser solicitadas aos autores, e em caso de não disponibilidade, deverão ser retiradas do texto. Não serão aceitas citações de trabalhos publicados em anais de congressos, e as teses devem estar disponíveis para consulta em sites oficiais, por exemplo, Banco de Teses da Capes: http://www.capes.gov.br/servicos/banco-de-teses. Todas as citações no texto devem ser cuidadosamente checadas em relação aos nomes dos autores e datas, exatamente como aparecem nas referências. Apresentar a lista de referências em ordem alfabética e, se necessário, em ordem cronológica. Mais de uma referência do(s) mesmo(s) autor(es) no mesmo ano deve ser identificada pelas letras "a", "b", "c", etc., inseridas após o ano de publicação. Títulos de periódicos devem ser abreviados conforme Index Medicus - http://www2.bg.am.poznan.pl/czasopisma/medicus.php?lang=eng. Para referências com 6 ou mais autores, apresentar os seis primeiros nomes seguidos da expressão et al.:

Livros

Levine JD. Veterinary protozoology. Ames: ISU Press; 1985.

Capítulo de livro

Menzies PI. Abortion in sheep: diagnosis and control. In: Youngquist RS, Threlfall WR. Current therapy in large animal theriogenology. 2nd ed. Philadelphia: Saunders; 2007. p. 667-680.

Artigo de periódico

Munhoz AD, Simões IGPC, Calazans APF, Macedo LS, Cruz RDS, Lacerda LC, et al. Hemotropic mycoplasmas in naturally infected cats in Northeastern Brazil. Rev Bras Parasitol Vet 2018; 27(4): 446-454. http://dx.doi.org/10.1590/s1984-296120180074

Tese e Dissertação

Araujo MM. Aspectos ecológicos dos helmintos gastrintestinais de caprinos do município de patos, Paraíba - Brasil [Dissertação]. Rio de Janeiro: Universidade Federal Rural do Rio de Janeiro; 2002.

Documento eletrônico

Centers for Disease Control and Prevention. Epi Info [online]. 2002 [cited 2003 Jan 10]. Available from: http://www.cdc.gov/epiinfo/ei2002.htm.

Tabelas

Elaboradas apenas com linhas horizontais de separação no cabeçalho e no final. A legenda (título) é precedida da palavra Tabela, seguida pelo número de ordem em algarismos arábicos, devendo ser descritivas, concisas e inseridas acima das mesmas. As tabelas devem estar limitadas a um número mínimo necessário.

Figuras

Figuras e elementos gráficos, utilizados ou elaborados pelos autores, devem seguir todas as orientações das seções abaixo.

Citações no texto

Gráficos, fotografias, esquemas, ilustrações etc. devem ser citados como figuras (Figura 1, Figura 2, Figuras 1, 2, 5-7, etc.) em ordem crescente à suas citações.

Resumo dos requisitos

A tabela abaixo resume os principais requisitos técnicos para arquivos de figuras.

Formato	TIFF ou JGP
Dimensões	Largura máxima: 2250 pixels (em 300 dpi). Altura máxima: 2625 pixels (em 300 dpi).
Resolução	300 – 600 dpi
Tamanho	<10 MB
Fontes	Arial, Helvetica, Times New Roman, Cambria Math ou Symbol em tamanho de 7,5 a 10 pt.
Nome	Fig1.tif, Fig2.tif, etc. Deve seguir a identificação das legendas.
Legenda	Deve estar inserida no texto do manuscrito e não dentro das figuras.

Uso de Cores

Embora a utilização de cores seja permitida, é importante que os autores se esforcem para garantir que o uso de cor não irá prejudicar o entendimento para leitores com algum distúrbio de visão. Recomendamos que os seguintes recursos sejam consultados antes de preparar figuras ou tabelas utilizando cores:

How to make scientific figures accessible to readers with color-blindness (2019, Science News,The American Society for Cell Biology)
Wong, B. Points of view: Color blindness. Nat Methods 8, 441 (2011). https://doi.org/10.1038/nmeth.1618

Edição e manipulação

Ao preparar suas imagens tome cuidado ao utilizar filtros ou outro tipo de edição para incluir destaques etc. As imagens não devem ser manipuladas ou ajustadas em excesso, de forma que possam causar a má interpretação das informações. Recomendamos que os autores observem as dicas e exemplos a seguir:

"What's in a picture? The temptation of image manipulation" (Mike Rossner, Kenneth M. Yamada. J Cell Biol 5 July 2004; 166 (1): 11–15. doi: https://doi.org/10.1083/jcb.200406019).
Digital Images and Misconduct. (Council of Science Editors, White Paper on Publication Ethics)
Preparing a Manuscript for Submission to a Medical Journal > Illustrations (Figures). (International Committee of Medical Journal Editors)

Dimensões, resolução e formatos

Gráficos, fotografias, esquemas, ilustrações etc. devem ser enviados nos formatos TIFF ou JPG, com alta resolução (300 ou 600 dpi) de acordo com o tipo e tamanho em que ela será utilizada na página, conforme requisitos abaixo:

Largura máxima: 17,5 cm ⇒ 2100 pixels (em 300 dpi), 4500 pixels (em 600 dpi) ou 9000 pixels (em 1200 dpi).
Altura máxima: 24,0 cm ⇒ 2850 pixels (em 300 dpi), 5650 pixels (em 600 dpi) ou 11300 pixels (em 1200 dpi).
Resolução de 1200 dpi (1 bit/canal): adequada para desenhos, gráficos ou diagramas de linha monocromáticos (apenas em preto e branco).
Resolução de 300 dpi (RGB 8 bits/canal ou escala de cinza): utilize para imagens coloridas ou em escala de cinza em que predominam meios tons ou gradientes, como em fotos, micrografias etc. e que não incluem muito texto.
Resolução de 600 dpi (RGB 8 bits/canal ou escala de cinza): mesma indicação anterior, mas para os casos de imagens que incluem mais texto ou para painéis ou combinações de imagens de meios tons combinados com desenhos, gráficos ou diagramas de linha.
Compressão: Para imagens JPG use a menor compressão possível para preservar a qualidade e para imagens em formato TIFF, se o seu programa de edição de imagens permitir, utilize a compactação de arquivos LZW para reduzir o tamanho do arquivo.

Texto dentro das figuras

Ao elaborar suas figuras, procure utilizar tipos e tamanhos de modo consistente entre todas as imagens, além de seguir as recomendações abaixo:

Fonte: as fontes relacionadas abaixo são indicadas pois apresentam melhor legibilidade em diferentes meios e se enquadram ao estilo adotado pela revista:
Arial ou Helvetica: para textos e eixos, exceto fórmulas matemáticas.
Times New Roman: textos, eixos e fórmulas matemáticas.
Cambria Math e Symbol: símbolos.
Tamanho: ao incluir textos nas figuras mantenha consistência entre todas e use tamanhos entre 7,5 pt e 10 pt e tenha certeza de que mesmo o menor texto permite a perfeita leitura de todos os textos e símbolos utilizados
Separadores decimais e de milhar: em artigos em inglês, se houver uso de marcador de casa decimal, este deve ser indicado por ponto e, havendo marcador de casa de milhar, este deve ser indicado por vírgula.
Destaques como setas, símbolos, abreviações não convencionais etc.: certifique-se de identificar seu uso claramente na legenda.
Não inclua legendas, citações ou indicação de fonte: essas informações devem ser incluídas da legenda da figura, enviadas com o manuscrito.

Legendas

As legendas devem ser explicativas, iniciando pela identificação em negrito, seguida por um ponto e texto descritivo. Caso necessário, incluir após a descrição uma explicação para eventuais destaques como setas, símbolos (*, †, ‡, §, etc.), letras, números etc. que tenham sido utilizados, incluindo a indicação da fonte e citações, quando pertinente.

Quando necessário, os autores são responsáveis por obter a correta autorização para uso das imagens, fotos, ilustrações etc. de outras fontes, diretamente com o proprietário do copyright, e incluir a citação correspondente.

Exemplo:

Figura 1.

Comparação entre uma figura original de outra fonte e a versão elaborada pelos autores após obter a correta autorização. (A) Figura original tal como publicada no artigo original de Silva et al. 2015. (B) Figura adaptada pelos autores após obtenção de autorização do detentor do copyright. Fonte: Silva et al. 2015.

Espaço em branco

Elimine espaços em branco em excesso no entorno do conteúdo de suas imagens, mas mantenha uma pequena margem de segurança de ~5 pixels.

Figuras múltiplas ou painéis

Painéis, pranchas ou imagens com múltiplas partes (a, b, c, etc.) devem sempre ser combinadas em um único arquivo.

Usando softwares especializados

Para imagens geradas ou preparadas com softwares ou ferramentas especializadas como Matlab, Prism, Stata, ChemDraw, PyMol, SPSS, GeneSpring ou Minitab, aconselhamos os autores a seguir a orientação da PLOS ONE disponível em https://journals.plos.org/plosone/s/figures#loc-creating-source-images-with-specialized-software.

Políticas

Representação de humanos ou animais

Imagens contendo fotos de pessoas devem garantir que elas não possam ser identificadas, exceto nos casos em que a sua utilização tenha sido autorizada especificamente para publicação no artigo.

Adicionalmente, os autores devem se certificar de que as imagens estão de acordo com nossas políticas sobre proteção dos direitos humanos e dos animais.

Licenças e copyright

Sempre que os autores utilizarem imagens de terceiros, seja na íntegra, redesenhada em nova versão, ou apenas como inspiração é necessário que seja obtida a devida autorização por escrito do detentor do copyright e incluída a respectiva citação e indicação da fonte. Veja mais em nossas políticas de Licenças e copyright.

ARTIGOS PUBLICADOS DURANTE O PERÍODO DO MESTRADO

- **NEVES, Jorge Kevin Silva**; CARDOSO, EVELYN LEBREGO ; REBÊLO, GABRIEL LIMA ; FÉLIX, ADRIANO JOSÉ SILVA ; MACHADO, SORAYA ALMEIDA ; COSTA-CAMPOS, CARLOS EDUARDO ; SANTOS, JEANNIE NASCIMENTO ; MELO, FRANCISCO TIAGO VASCONCELOS. Filling the gaps on parasites of *Osteocephalus*: Helminth community structure of *Osteocephalus cabrerai* (Anura: Hylidae) from the Brazilian Amazon. INTERNATIONAL JOURNAL FOR PARASITOLOGY: PARASITES AND WILDLIFE, v. 25, p. 100996, 2024. https://doi.org/10.1016/j.ijppaw.2024.100996

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