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ELVIS SILVA LIMA

DIVERSIDADE E SAZONALIDADE DE PARASITOS METAZOÁRIOS EM *Eleotris pisonis* (ELEOTRIDAE), *Colomesus asellus* (TETRAODONTIDAE) E *Pimelodus ornatus* (PIMELODIDAE) DO RIO AMAZONAS, ESTADO DO AMAPÁ, BRASIL

Macapá - AP

2023

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Dedico este trabalho aos meus amados pais, Raimundo Silva, Socorro Silva; irmão Kelvis Silva e a minha esposa Joicyelle Silva por terem acreditado junto comigo na realização desse sonho.

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“Nunca deixe que lhe digam que não vale a pena acreditar no sonho que se tem”.

Renato Russo

RESUMO

O objetivo do presente estudo foi avaliar a influência das variações sazonais e anuais nas comunidades componentes e infracomunidades de parasitos metazoários em *Eleotris pisonis, Colomesus asellus* e *Pimelodus ornatus* do Rio Amazonas durante 2020 e 2021. Para *E. pisonis* foi registrado pela primeira vez a ocorrência de larvas de *Contracaecum* sp. e *Pseudoproleptus* sp., e indivíduos adultos de *Spirocamallanus inopinatus*, *Genarchella genarchella*, *Neoechinorhynchus* sp., Acari gen. sp. e *Ergasilus* sp. Larvas de *Contracecum* sp. foi o táxon dominante durante todo o estudo. A riqueza de espécies de parasitos e o índice de Brillouin foram maiores em 2021, não apresentando diferenças entre os períodos sazonais. Porém, *G. genarchella* apresentou diferenças entre anos e entre estações sazonais, enquanto *Ergasilus* sp. apresentou diferenças entre períodos sazonais. Para *C. asellus* foi feito o primeiro registro de *Contracaecum* sp., *G. genarchella*, *Clinostomum marginatum*, *Brasacanthus* sp. e *Argulus pestifer. Ergasilus colomesus* foi o táxon dominante ao longo do estudo. A riqueza de espécies e o índice de diversidade de Brillouin foram maiores em 2021 e na estação de estiagem. A infecção de *E. colomesus* e *Contracaecum* sp. apresentou diferenças entre estações sazonais, enquanto a prevalência de *E. colomesus* apresentou diferenças intra anual. Foi também descrito e caracterizado *Brasacanthus* n. sp. em *C. asellus* e essa descrição foi feita com base na morfologia e dados de biologia molecular usando parte da região do gene ribossomal 18S (pequena subunidade SSU). Apesar das semelhanças entre essa nova espécie de acantocéfalo e seu congênere *Brasacanthus sphoeroides*, o novo táxon difere pelo tamanho do corpo, comprimento da probóscide, receptáculo da proboscide, lemniscos, glândulas de cemento, testículos, bolsa de Saefftigen, bolsa copulatória e folículos ovarianos, os quais são menores em *Brasacanthus* n. sp. Análises morfológicas, moleculares e filogenéticas apoiam a proposição de *Brasacanthus* n. sp., que é a segunda espécie do gênero e a primeira espécie descrita para peixes de água doce. Para *P. ornatus* foram feitos novos relatos de parasitos como *S. inopinatus, Contracaecum* sp., *G. genachella* e *Telotha henselii.* Além disso, houve alternância na dominância de parasitos entre os anos, onde em 2020, *S. inopinatus* foi a espécie dominante, enquanto em 2021, foram larvas de *Contracaecum* sp. A diversidade de Brillouin, riqueza de espécies de parasitos e uniformidade foram maiores em 2021, para *P. ornatus*. A riqueza de espécies de parasitos e a diversidade de Brillouin foram maiores na estação chuvosa. Em *P. ornatus,* houve diferenças na infecção por *Contracaecum* sp. intra anual e na prevalência de *Demidospermus* sp. e *Cucullanus pinnai* entre períodos sazonais.

Palavras-chave: Amazônia; comunidade; diversidade; infecção; parasitos.

ABSTRACT

The aim of the present study was to evaluate the influence of seasonal and annual variations on the component communities and infracommunities of metazoan parasites in *Eleotris pisonis*, *Colomesus asellus* and *Pimelodus ornatus* from the Amazon River during 2020 and 2021. For *E. pisonis* the occurrence of larvae of *Contracaecum* sp. and *Pseudoproleptus* sp., and adult of *Spirocamallanus inopinatus*, *Genarchella genarchella*, *Neoechinorhynchus* sp., Acari gen. sp. and *Ergasilus* sp. were registered for the first time. Larvae of *Contracecum* sp. was the dominant taxon throughout the study. Parasite species richness and Brillouin index were higher in 2021, showing no differences between seasonal periods. However, *G. genarchella* showed differences between years and between seasonal periods, while *Ergasilus* sp. showed differences between seasonal periods. For *C. asellus*, *Contracaecum* sp., *G. genarchella*, *Clinostomum marginatum*, *Brasacanthus* sp. and *Argulus pestifer* were registered for first time. *Ergasilus colomesus* was the dominant taxon throughout the study. Parasite species richness and Brillouin diversity index were higher in 2021 and in the dry season. Infection by *E. colomesus* and *Contracaecum* sp. showed differences between seasonal periods, while the prevalence of *E. colomesus* showed differences intrannual. In *C. asellus*, it was also described and characterized *Brasacanthus* n. sp. and this description was based on morphology and molecular biology data using part of the 18S ribosomal gene region (SSU small subunit). Despite the similarities between this new species of acanthocephalan and its congener *Brasacanthus sphoeroides*, the new taxon differs in body size, proboscis length, proboscis receptacle, lemnisci, cement glands, testis, Saefftigen's pouch, copulatory poucha, and ovarian follicles, which were smaller in *Brasacanthus* n. sp. Morphological, molecular and phylogenetic analyzes support the proposition of *Brasacanthus* n. sp., which is the second specie of this genus and the first species described for freshwater fish. For *P. ornatus*, *S. inopinatus*, *Contracaecum* sp., *G. genachella* and *Telotha henselii* were new reports. In addition, there was a variation in the dominance of parasites between years, where in 2020, *S. inopinatus* was the dominant species, while in 2021, larvae of Contracaecum sp. was the dominant species. Brillouin diversity, parasite species richness and evenness were higher in 2021. Parasite species richness and Brillouin diversity were higher in the rainy season. There were differences in infection by *Contracaecum* sp. intrannual and in the prevalence of *Demidospermus* sp. and *Cucullanus pinnai* in seasonal periods for *P. ornatus*.

Key words: Amazon; community; diversity; infection; parasites.

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SUMÁRIO

1. INTRODUÇÃO GERAL

O sistema do Rio Amazonas tem como característica a variação sazonal nos níveis de água devido às chuvas sazonais da região andina, que influenciam a troca de matérias entre ecossistemas terrestres e aquáticos, afetando, portanto, a proporção de material suspenso e dissolvido na água e, consequentemente, as características físicas e químicas desse rio e seus tributários (AFFONSO et al., 2011).

A diversidade de espécies de peixes do sistema do Rio Amazonas é de grande importância econômica para as populações ribeirinhas que vivem da pesca e a utiliza para sua subsistência (SILVA et al., 2011; LYNCH et al., 2023). Essa diversidade de peixes se deve as características desse grande rio e seus tributários, os quais são ecossistemas ricos com teias tróficas complexas e cobertura vegetal que varia de acordo com o regime de inundação e clima regional (SOUSA et al., 2017). Estima-se que cerca de 200 espécies de peixes são usadas para consumo do homem, mas cerca de 6 a 12 espécies representam 80% dos desembarques nas grandes cidades ao longo do sistema do Rio Amazonas (BARTHEM; FABRÉ, 2004). Por exemplo, Sousa et al. (2017) estimando a produção pesqueira e sua relação com a sazonalidade hidrológica do desembarque no Porto de Panairzinho em Manacapuru (estado do Amazonas), mostraram maior ocorrência de cinco espécies de importância comercial como *Colossoma macropomum* (Cuvier 1816), *Cichla monoculus* (Spix, 1831), *Prochilodus nigricans* (Agassiz, 1829), *Astronotus ocellatus* (Cuvier, 1829) e *Osteoglossum bicirrhosum* (Cuvier, 1829. Porém, muitas outras espécies de peixes também compõe a grande diversidade da ictiofauna da Amazônia.

A região amazônica é conhecida mundialmente pela sua grande biodiversidade (LESSMANN et al., 2016; WINEMILLER et al., 2016; VIEIRA et al., 2018; CLERICI et al., 2019). Porém, muitas espécies que compõe essa biodiversidade amazônica e sua biologia são pouco conhecidas, como é o caso das relações entre peixes e parasitos dessa região em que os peixes são de fundamental importância para a cadeia produtiva local (CASTELLO; MACEDO, 2016; FREDERICO; OLDEN; ZUANON, 2016; HURD et al., 2016; LEES et al., 2016; BREJÃO et al., 2018; SIEBERT; DA SILVA, 2019).

Paralelamente, a diversidade de peixes da Amazônia tem sido ameaçada por diferentes impactos tais como mudanças no uso da terra que afetam processos biogeoquímicos relacionados a hidrologia e distribuição de sedimentos (WARD et al., 2013); construção de usinas hidrelétricas que modificam os sedimentos, fluxo de água, fauna aquática e a perda de vastas áreas de florestas de várzea causada pelo desmatamento (FEARNSIDE, 2016; CASTELLO et al., 2018; BREJÃO et al., 2018; SILVA el al., 2020), além do garimpo e intensificação da contaminação por mercúrio (JUNIOR et al., 2023; MESQUITA et al., 2023). Essas são ações antrópicas que levam a perda de ambientes importantes para as espécies de peixes nativos e, consequentemente, afetam as populações ribeirinhas locais. Além disso, há a dependência dos peixes de pequeno porte em relação aos insumos fornecidos pela floresta ciliar, os quais acabam tendo a sua dispersão limitada devido ao aumento do desmatamento nos sistemas da bacia do Rio Amazonas. Esses são impactos ambientais que podem afetar também as interações parasito-hospedeiro, por isso tais interações são bons indicadores desses impactos (BREJÃO et al., 2018). Porém, pouco se conhece sobre a fauna de parasitos em peixes do Rio Amazonas, considerando a grande diversidade de sua ictiofauna e sua importância para a cadeia produtiva da pesca local (CASTELLO; MACEDO, 2016; LEES et al., 2016; BREJÃO et al., 2018). Vários estudos têm mostrado a importância acerca da diversidade e os aspectos ecológicos dos parasitos em espécies de peixes da Amazônia (TAVARES-DIAS et al., 2014; CARVALHO; TAVARES-DIAS, 2017; TAVARES-DIAS; OLIVEIRA, 2017; OLIVEIRA et al., 2017a; NEVES; SILVA; TAVARES- DIAS, 2021; OLIVEIRA et al., 2022; MÜLLER et al., 2023), pois são informações importantes para auxiliar nas estratégias de manejo para a conservação das espécies. Além disso, os parasitos são reconhecidos, atualmente, como parte importante da biodiversidade do planeta (CARLSON et al., 2020).

Fatores bióticos (exemplos: hospedeiros, parasitos, entre outros) e abióticos (exemplos: pH, temperatura, sazonalidade, oxigênio, entre outros) são comumente responsáveis pela diversidade, prevalência e abundância de infecção dos parasitos em populações naturais de peixes (TAVARES-DIAS et al., 2014; BLASCO-COSTA; ROUCO; POULIN, 2015; OLIVEIRA et al., 2017b; GIARI; CASTALDELLI; TIMI, 2022). Além disso, sabe-se que entre os processos responsáveis pela distribuição espacial dos parasitos, podem estar os constantes cenários de mudanças no ambiente e as invasões biológicas, os quais podem levar a doenças emergentes (POULIN et al., 2011). Diante deste cenário, são necessários estudos sobre a diversidade das comunidades e infracomunidades de parasitos e seus padrões sazonal e temporal em peixes nativos da Amazônia como *Eleotris pisonis* (Gmelin, 1789); *Colomesus asellus* (Müller & Troschel, 1849) e *Pimelodus ornatus* (Kner, 1857), os quais têm sido negligenciados até o presente momento.

1.1. O sistema do Rio Amazonas

O Rio Amazonas tem sua origem datada de 9,0-9,4 milhões de anos, atingindo a forma e tamanho atual no final do plioceno (FIGUEIREDO et al., 2009; HOORN et al., 2017). É um dos maiores rios do mundo com 6,400 km de sua nascente na região Andina (no Peru), até a sua foz no estado do Amapá. Tem a maior vazão fluvial do mundo (até 6,6 × 10^3 km^3 (KOROSOV et al., 2015), transporta aproximadamente 1,2 x 10^9 de toneladas de sedimento e 50% da água doce que vai para o Oceano Atlântico (DAI; TRENBERTH, 2002; HOORN; WESSELINGH, 2010; CONTOS; TRIPCEVICH, 2014; KOROSOV; COUNILLON; JOHANNESSEN, 2015). Porém, alterações na qualidade da água podem estar relacionadas as mudanças no padrão de vazão do Rio Amazonas (SANTOS et al., 2018). É o maior responsável pela transferência de massa continental para o oceano, com 10% da carga dissolvida (GAILLARDET et al., 1997) e 3% da carga suspensa (MILLIMAN; SYVITSKI, 1992), números superados apenas pelos rios localizados no sul e sudeste asiático como rios Huanghe e Ganges-Brahmaputra, respectivamente (CALVÈS et al., 2019).

Na foz do Rio Amazonas há uma pluma que exerce influência no fluxo de calor, formação de camada de barreira e sequestro de carbono pela produção primária (FOLTZ; MCPHADEN, 2008; SUBRAMANIAM et al., 2008; BALAGURU et al., 2012; STUKEL et al., 2014; MEDEIROS et al., 2015; GOUVEIA et al., 2019; VALERIO et al., 2021) (Figura 1). A camada de barreira é formada por água de baixa salinidade (<35 ppm) que aumenta a estratificação do oceano, criando uma camada de barreira espessa que inibe a mistura de água termoclina fria com águas superficiais (LUKAS; LINDSTROM, 1991). Possui altas concentrações de material em suspensão e nutrientes que se espalham por milhares de quilômetros sobre a plataforma Continental Norte Brasileira e águas abertas adjacentes no Oceano Atlântico Equatorial carregadas pelas fortes correntes na área (NITTROUER; DEMASTER, 1996). O serviço de sequestro de carbono realizado na região não é regra, já que áreas do Rio Amazonas que sofrem influência de maré contribuem com 5% de toda emissão de CO_2 da bacia (SAWAKUCHI et al., 2017).

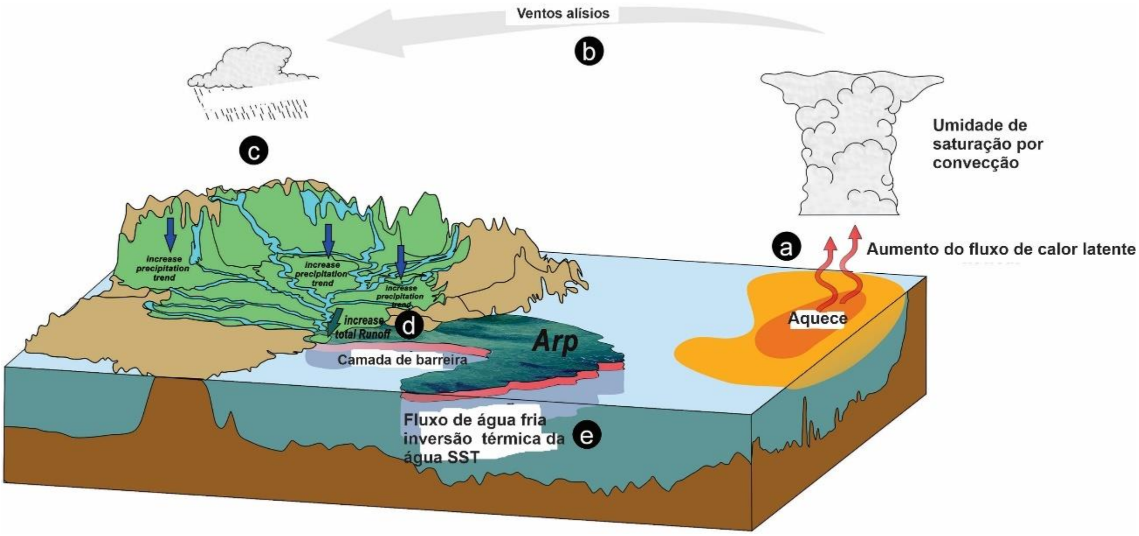

Figura 1. Representação da pluma do Rio Amazonas e sua influência no fluxo de calor, formação de camada de barreira. Fonte: Gouveia et al. (2019).

A variabilidade de água doce da Amazônia associada às mudanças de salinidade do oceano podem afetar interações como ar-mar do Atlântico tropical, variabilidade da zona de convergência intertropical do Atlântico e altura do nível do mar, exercendo assim influência sob as propriedades físicas dos oceanos costeiros vizinhos (LUKAS; LINDSTROM, 1991; SMITH JR; DEMASTER, 1996; MASSON; DELECLUSE, 2001; COLES et al., 2013; OLIVEIRA et al., 2018; MOUYEN et al., 2018; RUDZIN et al., 2019; DURAND et al., 2019; GIFFARD et al., 2019; GOUVEIA et al., 2019; JAHFER et al., 2020; LIANG et al., 2020; PIECUCH; WADEHRA, 2020). Tosetto et al. (2022) compararam a diversidade dos filos do Reino Animalia das ecorregiões (Figura 2) ao longo da plataforma continental do Atlântico Ocidental Tropical para testar a hipótese de que a pluma do Rio Amazonas estruturava espacialmente a diversidade de espécies de animais, e os resultados mostraram que a descarga do Rio Amazonas (Pluma do Rio Amazonas) atua como barreira biogeográfica para a dispersão animal das ecorregiões do Mar do Caribe e do Brasil Tropical (Figura 3).

A bacia Amazônica possui uma expressiva diversidade de peixes reconhecida mundialmente (TISSEUIL et al., 2013), com cerca de 2.700 espécies descritas, representando cerca de 15% de todas as espécies de água doce (JÉZÉQUEL et al., 2020; TENCATT et al., 2020) e, aproximadamente 1.000 ainda estão por ser descritas. Processos de diversificação de espécies ainda estão ocorrendo (ALBERT; VAL; HOORN, 2018; DUTRA et al., 2020; LEITÃO et al., 2021). Outros grupos aquáticos estão em processo de especiação semelhante, salientando a importância da Amazônia como foco de biodiversidade neotropical. Essa riqueza de espécies supera outras grandes bacias hidrográficas tais como a do Rio Mekong (1.200-1.700 espécies), Rio Zaire (cerca de 700 espécies), Rio Paraná/Paraguai/La Plata (591 espécies), Rio Mississippi (375 espécies), Rio Yangtze Kiang (322 espécies), Rio Nilo (129 espécies), Rio Danúbio (103 espécies) e Rio Volga (88 espécies) (WATER RESOURCES EATLA, 2006; OBERDORFF et al., 2019). A grande diversidade e padrões de distribuição podem estar relacionados a heterogeneidade ambiental, clima, contingência histórica da região, tamanho, localização equatorial e alta precipitação pluviométrica (JUNK et al., 2007; OBERDORFF et al., 2019).

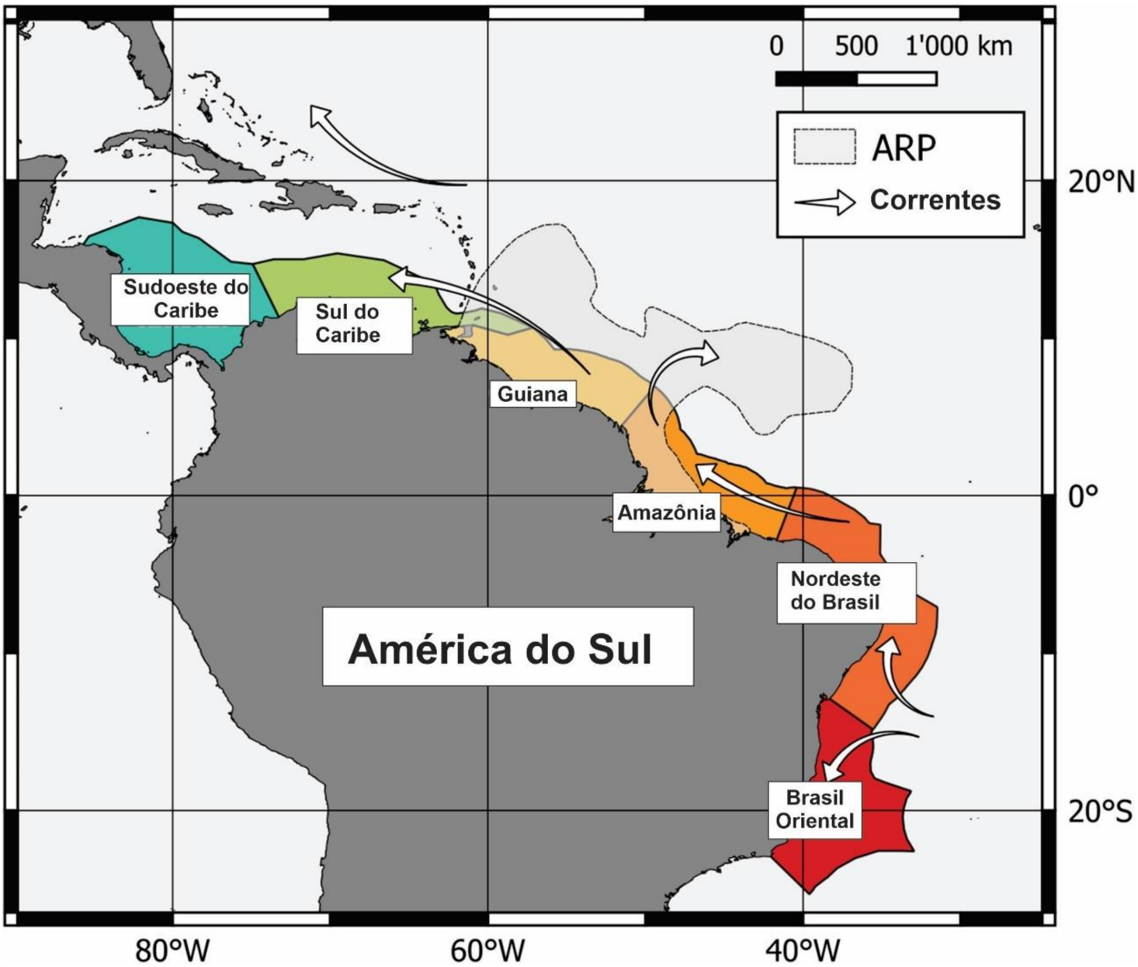

Figura 2. Ecorregiões do Oceano Atlântico Tropical Ocidental. Área sombreada representa aproximadamente as influências da área pela Pluma do Rio Amazonas (ARP) ao longo do ano. As setas indicam a direção geral das correntes de superfície. Fonte: Tosetto et. al. (2022).

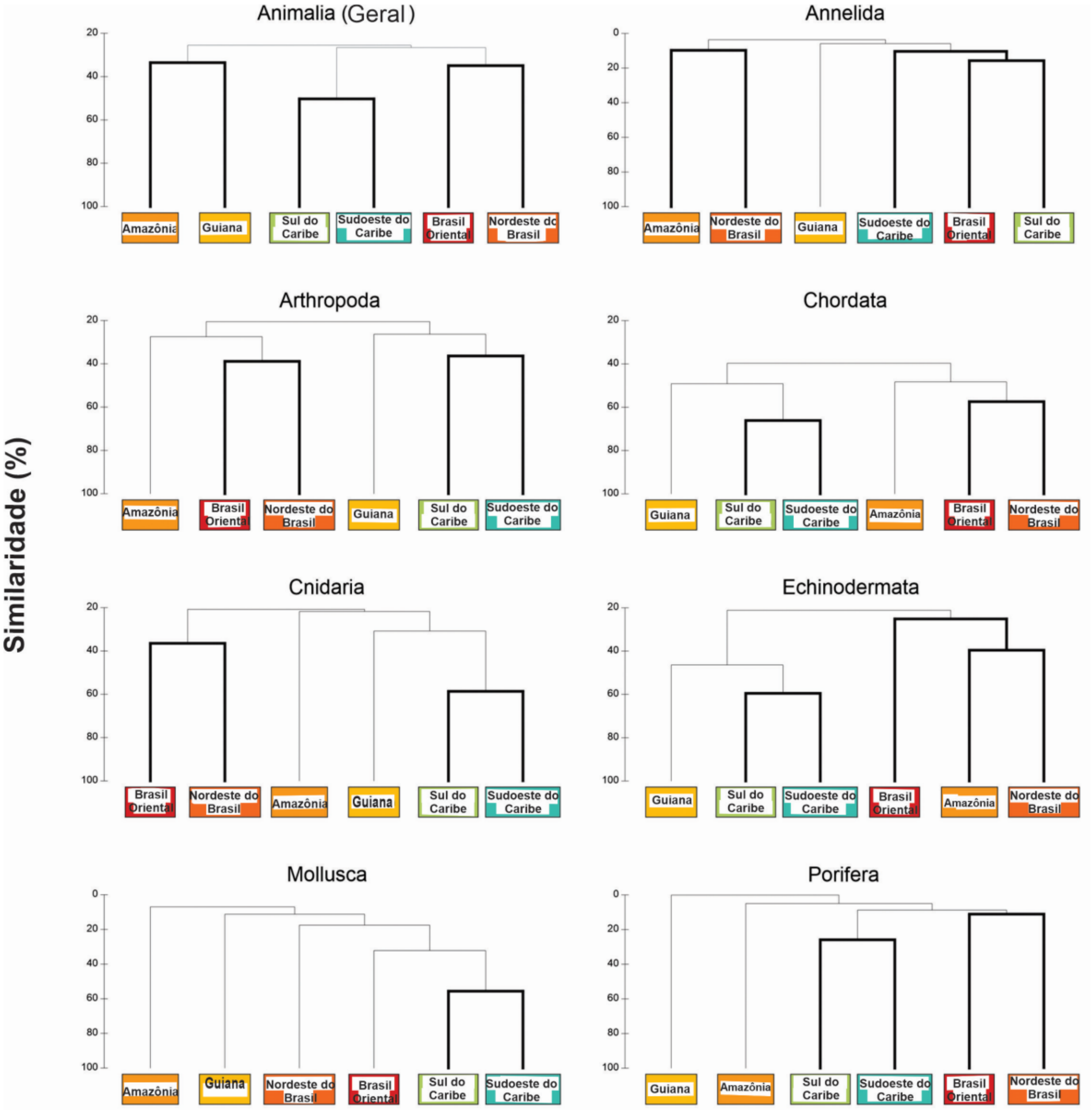

Figura 3. Dendrograma de análise de cluster indicando padrões na estrutura de composição de espécies para o todo reino animal e os filos mais ricos entre as seis ecorregiões do Oceano Atlântico Tropical Ocidental. Fonte: Tosetto et al. (2022).

Impactos ecológicos causados pelas mudanças climáticas previstas, mostram que a Amazônia é muito vulnerável (CASTELO; MACEDO, 2016; OLIVEIRA et al., 2021) e enfrentará menos chuvas, períodos mais quentes e secos e mais eventos extremos, incluindo secas e inundações (REBOITA et al., 2022), podendo causar o desaparecimento da floresta no leste, sul e centro da Amazônia. Situação agravada pelo desmatamento direto de 12% da floresta tropical até 2017, causada principalmente pela exploração madeireira e agricultura (STROPP et al., 2020) e incêndios florestais (OLIVEIRA et al., 2023).

Estudos indicam que macrófitas aquáticas e matéria orgânica particulada fina são importantes fontes basais que contribuem com a biomassa de peixes em planícies de inundação da Amazônia (ARANTES et al., 2018), além das variações nas paisagens, condições ambientais e fatores abióticos que podem alterar as características da variabilidade e complexidade das estruturas das teias alimentares existentes (WARREN, 1989; WINEMILLER, 1989, 1996; FINLAY; POWER; CABANA, 1999; AMUNDSON et al., 2003; ISHIKAWA; DOI; FINLAY, 2012).

A segurança alimentar de muitas populações tradicionais de países tropicais e subtropicais depende da pesca (LYNCH et al., 2016; MYERS et al., 2022). Porém, as principais ameaças não climáticas para esse recurso natural são represas, desvios, poluição, invasões biológicas, luz, ruído e desenvolvimento urbano (FREDERICO et al., 2016; REID et al., 2019). Além disso, a diminuição das áreas de várzeas do baixo Rio Amazonas pode também ter relação direta com a diminuição da produção pesqueira na região amazônica (CASTELLO; MACEDO, 2016; RÖPKE et al., 2022; PEREIRA et al., 2023). Quando os níveis da água estão altos, muitas espécies de peixes de várzeas dependem de matéria orgânica produzida *in situ* constituída de frutas e insetos que compõe uma parcela considerável de suas dietas (GOULDING, 1980; SOARES; ALMEIDA; JUNK, 1986; OLIVEIRA et al., 2006; FLEISCHMANN et al., 2023).

As mudanças climáticas podem causar impactos ecológicos na bacia do Rio Amazonas onde modelos apontam que mais de um terço das espécies de peixes de água poderão ser perdidas devido as mudanças climáticas, podendo também alterar a reprodução, migração e persistência das espécies (XENOPOULOS et al., 2005; LEIGH et al., 2015; PACIFICI et al., 2015; CASTELLO; MACEDO, 2016).

As temperaturas elevadas associadas a vazão reduzida do rio podem promover a redução dos níveis de oxigênio dissolvido, afetando assim a produtividade primária, tolerância fisiológica, desempenho e padrões de ocupação espacial de muitas espécies de peixes do Rio Amazonas (POFF et al., 2002; BARLETTA et al., 2010; LEIGH et al., 2015). Em 2005 e 2010 a bacia do Rio Amazonas passou por duas severas secas sazonais e uma enchente recorde em 2009 (MARENGO et al., 2012). Freitas et al. (2013) estimaram que os impactos ambientais associados as mudanças climáticas podem levar a perda de 7 a 12% das espécies de peixes do Rio Amazonas até 2070. Porém, esses números podem ser ainda maiores, uma vez que 90% das espécies de peixes dessa bacia são sensíveis às mudanças ocasionadas pelo clima nos períodos de estiagem (FREDERICO et al., 2016). O aumento da frequência desses períodos extremos pode ter implicações sob a biodiversidade de água doce, podendo extinguir táxons antes mesmo que se possa conhecê-los (JAEGER et al., 2014; SEGADELLI et al., 2020). Horn & Wesselingh (2010) destacaram que não há números exatos que expressam essa diversidade, uma vez que várias áreas e grupos ainda não foram estudados ou foram espaçadamente amostrados. Assim, é necessário estudar a variação sazonal de parasitos em *E. pisonis, C. asellus* e *P. ornatus* do Rio Amazonas.

2.2. Interações parasito-hospedeiro-ambiente

As interações parasito-hospedeiro são resultados de co-evoluções que levaram a adaptações recíprocas, permitindo que os parasitos pudessem explorar aspectos de seus hospedeiros, assim garantindo a sua sobrevivência, transmissão e manutenção de populações viáveis (TIMI; POULIN, 2020). Em populações de peixes, a composição da fauna de parasitos pode estar relacionada à distribuição geográfica, mudança de habitat, modo de vida, idade, posição na cadeia trófica, quantidade de alimentos ingeridos, longevidade e probabilidade de contato dos peixes com hospedeiros intermediários, além da densidade populacional dos hospedeiros intermediários e definitivos no ambiente (TAVARES-DIAS et al., 2014; BLASCO-COSTA; ROUCO; POULIN, 2015; OLIVEIRA et al., 2017b; TAVARES-DIAS; OLIVEIRA, 2017; CRACCO; LEHUN; TAKEMOTO, 2022), além de fatores ambientais como a sazonalidade.

Vários estudos vêm sendo realizados para investigar possíveis danos que os parasitos podem causar em seus hospedeiros. As respostas dos peixes e parasitos aos impactos antropogênicos tem mostrado que esses podem ser afetados pelos níveis de poluição nos hábitats aquáticos, os quais podem afetar a estrutura de ambos as comunidades, e os efeitos são refletidos em ectoparasitos adultos ou estágios larvais que estão em contato direto com o ambiente (Figura 4). Porém, endoparasitos também podem ser afetados. Wood et al. (2018) testaram a influência da pressão da pesca na diversidade de peixes e seus parasitos em recife de coral em três ilhas no Pacífico. Os resultados mostraram que a diversidade de parasitos aumentou na ausência da pesca e a comunidade de parasitos em pequenos peixes apresentou maior diversidade. Isso pode ter ocorrido porque peixes maiores são mais propensos a passar pela pressão da pesca e consequentemente diminuição da comunidade de parasitos, enquanto peixes menores são menos suscetíveis a sofrer impactos da pesca.

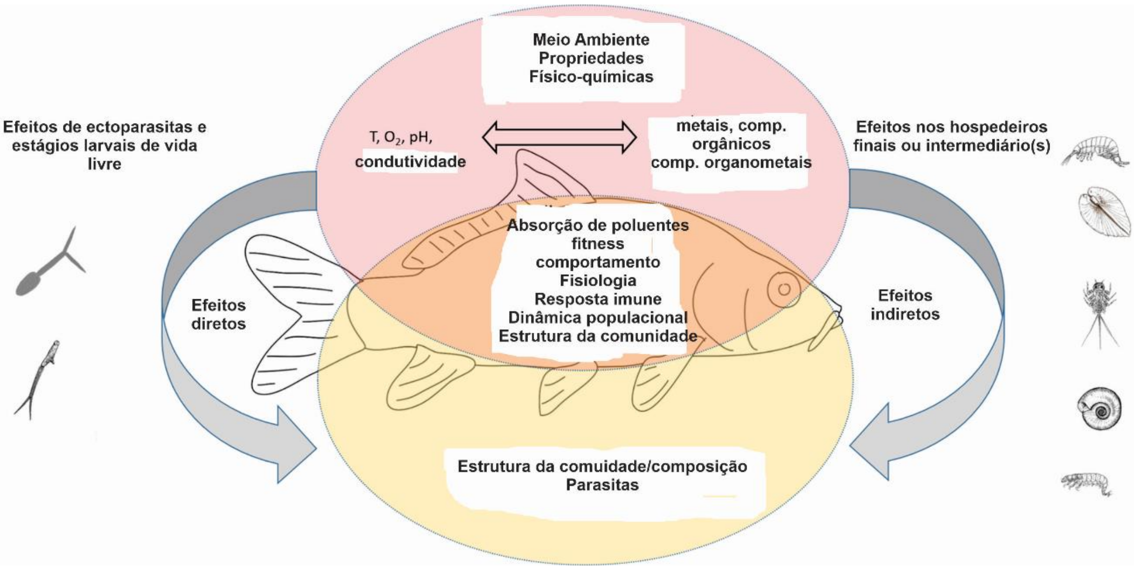

Figura 4. Poluição dos habitats aquáticos podem afetar direta ou indiretamente a estrutura e composição das comunidades de parasitos de peixes de ambientes naturais. O modo de ação direta inclui principalmente efeitos em ectoparasitos adultos ou estágios larvais que estão em contato direto com o meio ambiente. Fonte: Sures; Nachev (2022).

Gilbert; Avenant-Oldewage (2021) relataram que a contaminação do ambiente afeta os parasitos de forma variável, resultando em alterações de população e de organismo. Esses mostraram que os monogenéticos, em sua maioria, são influenciados pela exposição à eutrofização, efluentes de fábricas de celulose e metais. A comparação dos monogenéticos mostrou forte efeitos negativos para metais e forte efeito positivo para a eutrofização (Figura 5).

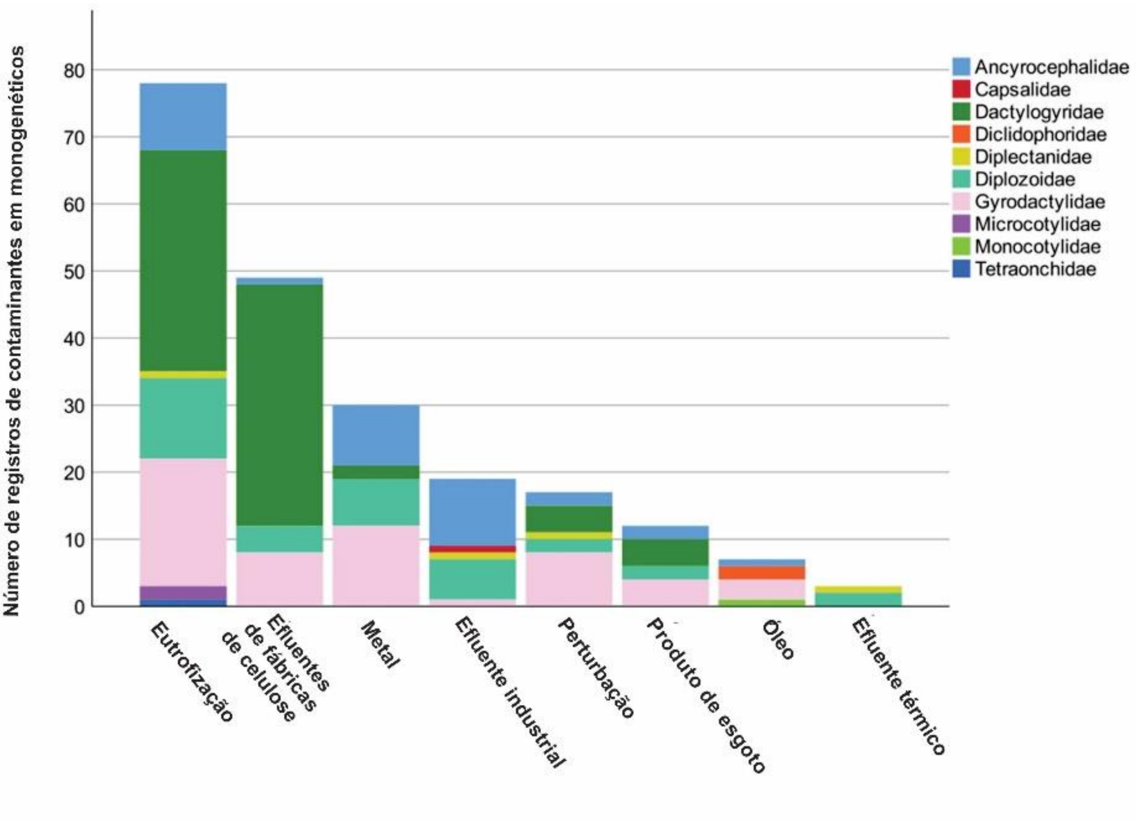

Figura 5. Registros de contaminantes e monogenéticos em relação às famílias desses parasitos. A legenda na imagem indica a cor da barra correspondente às famílias de monogenéticos. Fonte: Adapatado de Gilbert; Avenant-Oldewage (2021).

Timi; Poulin (2020) afirmaram que vieses e conclusões errôneas sobre padrões e processos em nível de indivíduo e comunidade podem ocorrer se estudos de ecologia de peixes não levarem em consideração os parasitos, fortalecendo a importância de incorporá-los nas análises de estudos ecológicos de peixes de ambientes naturais (Figura 6).

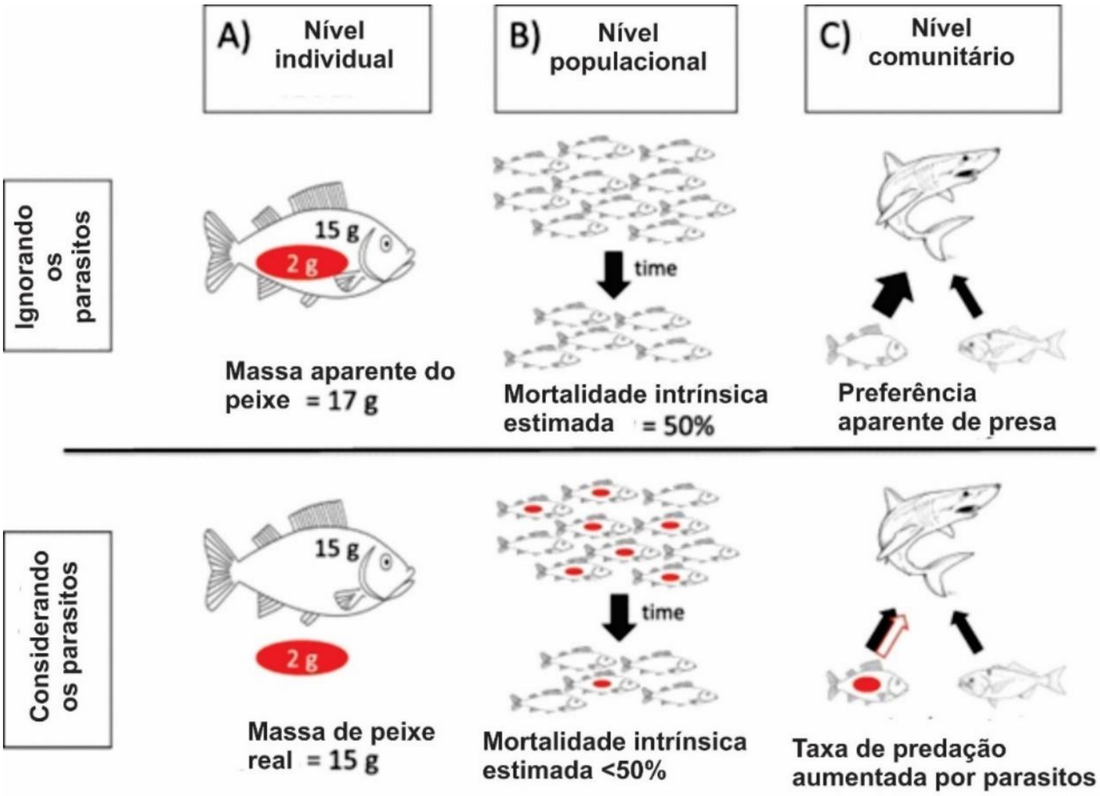

Figura 6. Alguns erros de interpretação em algumas análises de ecologia de peixes de ambiente natural. Fonte: Timi; Poulin (2020).

Os parasitos podem viver dentro (endoparasitos) ou sobre (ectoparasitos) seus hospedeiros, e a sua presença pode alterar o peso dos peixes hospedeiros devido aos seus efeitos na sua fisiologia e saúde, por isso o peso e comprimento corporal são rotineiramente utilizados para calcular índices de condição corporal (bem estar), com a finalidade de avaliar a saúde dos peixes parasitados (TIMI; POULIN, 2020). Esses índices corporais são indicadores indiretos de sucesso no forrageamento e habilidade para lidar com pressões ambientais (JAKOB; MARSHALL; UETZ, 1996) e infecções parasitárias.

Em estudos sobre parasitos de populações naturais de peixes é comum a análise de correlação da diversidade, abundância e riqueza de espécies de parasitos com o comprimento, peso e fator de condição dos hospedeiros (BLASCO-COSTA et al., 2015). Alguns estudos mostram que o tamanho do corpo de peixes amazônicos tem sido um fator determinante na diversidade e riqueza de espécies de parasitos (TAVARES-DIAS et al., 2014; TAVARES-DIAS; OLIVEIRA, 2017; NEVES et al., 2020). Por exemplo, em *Pseudoplatystoma fasciatum* Linnaeus, 1766 do Rio Jamari, em Rondônia, a abundância de *Peltidocotyle* sp., *Nominoscolex* sp. e *Eustrongylides* sp. apresentou correlação positiva com o comprimento dos hospedeiros (CARVALHO et al., 2019). Em *Calophysus* *macropterus* Lichtenstein, 1819 do Rio Acre, o comprimento teve correlação positiva somente com a abundância de metacercárias de *Diplostomulum* Brandes, 1892, enquanto em *C. macropterus* do Rio Iaco, correlação positiva ocorreu com a abundância de monogenea *Demidospermus luckyi* (Kritsky, Thatcher & Boeger, 1987), *Demidospermus macropteri* (Mendoza-Franco & Scholz 2009), *Demidospermus pinirampi* (Kritsky, Thatcher & Boeger, 1987) e *Ameloblastella unapi* (Mendoza-Franco & Scholz 2009) (NEGREIROS; PEREIRA; TAVARES-DIAS, 2019).

O tamanho do peixe pode estar correlacionado com essas variáveis porque geralmente a carga parasitária aumenta com a idade ou tamanho do hospedeiro (DOGIEL; PETRUSHEVSKII, 1958; POULIN, 2000), tendo como resultado padrões acumulativos de abundância de parasitos conforme o crescimento dos peixes, no qual peixes maiores são capazes de acomodar maior abundância de parasitos, podendo também ingerir maior quantidade de alimentos, que pode levá-los a uma maior exposição aos estágios infecciosos de parasitos no ambiente (POULIN, 2000; TIMI; LUQUE; POULIN, 2010; VALTONEN; MARCOGLIESE; JULKUNEN, 2010).

Apesar das correlações em algumas espécies, vários estudos não encontraram correlação do tamanho dos peixes com abundância, índice de diversidade, riqueza de espécie e fator de condição em algumas espécies de peixes hospedeiros (TAVARES-DIAS; NEVES, 2017; FERREIRA; PASSADOR; TAVARES-DIAS, 2019). O comprimento de *Corydoras melanistius* Regan 1912 e C*orydoras spilurus* Norman 1926 não mostrou correlação com a abundância de *Spirocamallanus inopinatus* (Travassos, Artigas & Pereira, 1928), *Camallanus* sp. Railliet & Henry, 1915) e metacercárias de Digenea gen. sp. (FERREIRA et al., 2019). Porém, tais estudos ainda não foram realizados em *E. pisonis*, *C. asellus* e *P. ornatus* do Rio Amazonas.

2.3. Variação sazonal e diversidade de parasitos metazoários em populações naturais de peixes da Amazônia

Apesar dos peixes serem considerados uma boa fonte de proteína, várias doenças causadas por parasitos podem acometer populações naturais, levando a perdas econômicas para a pesca (TAVARES-DIAS et al., 2014; BEHRINGER et al., 2020; ERKINHARJU et al., 2021). Para desenvolver programas de desenvolvimento pesqueiro é importante obter informações sobre a saúde das espécies de peixes, pois muitos parasitos podem influenciar o tamanho, estrutura populacional, alimentação, crescimento e reprodução dos peixes hospedeiros (MAGURRAN, 2004; OLIVEIRA et al., 2017b;

TAVARES-DIAS et al., 2014; TAVARES-DIAS & OLIVEIRA, 2017; TIMI; POULIN, 2020). Portanto, deve-se estudar os parasitos nas diferentes espécies de peixes de ambientes naturais, além da influência sazonal nas comunidades e infracomunidades de parasitos (AVENANT-OLDEWAGE; MILNE, 2014; ALHO et al., 2015; KONDO et al., 2016; KUHN et al., 2016; MARTINS et al., 2017; KALOGIANNI et al., 2017; RIJIN et al., 2018; HOSHINO; TAVARES-DIAS, 2019; NEGREIROS et al., 2019b; NEVES et al., 2021) e as interações do sistema parasito-hospedeiro-ambiente (ALCANTARA; TAVARES-DIAS, 2015).

Em geral, Monogenea, Nematoda, Trematoda, Crustacea, Acanthocephala e Cestoda são os principais táxons de parasitos metazoários encontrados em populações de peixes de ambiente natural na Amazônia (ALCANTARA; TAVARES-DIAS, 2015; BAIA et al., 2018; TAVARES-DIAS et al., 2014; TAVARES-DIAS; OLIVEIRA, 2017). São táxons de parasitos com ciclos de vida distintos e que dependendo do seu estágio de vida parasitam diferentes espécies de hospedeiros. O ciclo de vida desses táxons pode ser direto (ex. Monogenea e Crustacea) ou indireto, usando mais de uma espécie de hospedeiro, como é o caso das espécies de nematoides, trematoides, acantocéfalos e cestoides (BLASCO-COSTA; POULIN, 2017).

Segundo Luque et al. (2017), em diferentes países da América do Sul, incluindo o Brasil, Monogenea é o grupo mais diversificado com 835 espécies, seguido por Trematoda com 662 espécies relatadas. Assim, os peixes podem ser parasitados por diversas espécies de diferentes táxons como ocorreu em *Hoplerythrinus unitaeniatus*Spix & Agassiz, 1829 e *Calophysus macropterus* Lichtenstein 1819 de um afluente do Rio Amazonas, estado do Amapá e Rio Iaco, estado do Acre, respectivamente, que foram infectados por espécies de Protozoa, Monogenea, Acanthocephala, Cestoda, Crustacea, Digenea, Nematoda e Hirudinea (GONÇALVES et al., 2015; NEGREIROS; PEREIRA; TAVARES-DIAS, 2019)

Na bacia amazônica, a sazonalidade é caracterizada pela ocorrência de dois períodos distintos: chuvoso e estiagem. Em geral, o período chuvoso compreende aos meses de dezembro a maio, com precipitação variando de 125 a 400 mm/mês, enquanto a estiagem compreende aos meses de junho a novembro com precipitação variando de 50 a 150 mm/mês (SOUZA et al., 2017). A Tabela 1, mostra alguns táxons de parasitos da região amazônica que tiveram influencia sazonal.

Tabela 1: Influência da sazonalidade nas infracomunidades de parasitos em espécies de peixes da região amazônica.

Local	**Hospedeiro**	**Parasito**	**Período sazonal**	**Referência**
Igarapé Fortaleza	*Hoplerythrinus unitaeniatus*	Maior prevalência e abundância média de *Ichthyophthirius multifiliis*; *Whittingtonocotyle caetei*; *Whittingtonocotyle jeju*; *Urocleidoides* sp. e *Anacanthorus* sp.	Chuvoso	Gonçalves et al., (2015)
Igarapé Fortaleza	*Hoplias malabaricus*	Maior abundância de *Urocleidoides eremitus*;	Chuvoso	Gonçalves et al., (2015)
Igarapé Fortaleza	*Hemibrycon surinamensis*	Maior abundância *I. multifiliis*;	Chuvoso	Hoshino; Tavares-Dias (2019)
Igarapé Fortaleza	*Hemibrycon surinamensis*	Maior abundância de *Trichodina* sp.;	Chuvoso	Hoshino; Tavares-Dias (2019)
Igarapé Fortaleza	*Metynnis lippincottianus*	Maior prevalência e abundância média de *I. multifiliis* e *P. pillulare*	Chuvoso	Hoshino; Tavares-Dias (2020)
Igarapé Fortaleza	*Cichlasoma amazonarum*	Maior diversidade e riqueza de *Gussevia disparoides* e *Posthodiplostomum* sp.;	Chuvoso	Carvalho; Tavares-Dias (2017)
Rio Xapuri	*Pimelodus blochii*	Maior prevalência de *Procamallanus.* (*S.*) *pimelodus*	Chuvoso	Cavalcante et al., (2020)

Igarapé Fortaleza	*Aquidens tetramerus*	Maior prevalência e abundância média de *Tripartiella tetramerii, Trichodina nobilis, Gussevia alioides* e *Gussevia disparoides*	Estiagem	Tavares-Dias et al., (2014)
		Maior prevalência e abundância média de *Piscinoodinium pillulare*	Estiagem	Gonçalves et al., (2015)
Igarapé Fortaleza	*Hemibrycon surinamensis*	Maior prevalência e abundância média de *P. pillulare*	Estiagem	Hoshino; Tavares-Dias (2019)
Igarapé Fortaleza	*Hemibrycon surinamensis*	Maior abundância média de *Jainus hexops*	Estiagem	Hoshino; Tavares-Dias (2019)
Igarapé da Fortaleza	*Metynnis lippincottianus*	Maior abundância média de *Anacanthorus jegui, Anacanthorus strongylophalus, Urocleidoides* sp., *Dadayus pacupeva, Dadaytrema oxycephalum* e *Spinoxyuris oxydoras*	Estiagem	Hoshino; Tavares-Dias (2020)
Rio Iaco, estado do Acre	*Calophysus macropterus*	Prevalência e Abundância média de Anisakidae gen. sp.	Estiagem	Negreiros; Pereira; Tavares-Dias (2019)
Rio Acre	*Calophysus macropterus*	Maior abundância média de *Alinema amazonicum*	Estiagem	Negreiros; Pereira; Tavares-Dias (2019)

Igarapé Fortaleza	*Cichlasoma amazonarum*	Maior abundância média de *I. multifiliis* e *P. pillulare*	Estiagem	Carvalho; Tavares-Dias (2017)
Rio Acre	*Pimelodus blochii*	Maior abundância média de *Procamallanus* (*S.*) *pimelodus*	Estiagem	Negreiros et al. (2018).
Rio Acre	*Pimelodus blochii*	Maior prevalência de *Orientatractis moraveci*	Estiagem	
Rio Iaco	*Pimelodus blochii*	*Demidospermus peruvianus*	Estiagem	Negreiros et al. (2018).
Rio Xapuri	*Pimelodus blochii*	*Maior prevalência de Rondonia rondoni*	Estiagem	Cavalcante et al., (2020)

Portanto, estes estudos mostraram que algumas infracomunidades de parasitos podem sofrer influência da sazonalidade, enquanto outras não, devido às diferenças nas respostas das espécies de parasitos relacionadas aos fatores abióticos (ambiente) e bióticos (ecologia dos hospedeiros). Porém, não se conhece a influência da sazonalidade na comunidade e infracomunidades dos parasitos metazoários de *E. pisonis*, C. *asellus* e *P. ornatus* do Rio Amazonas.

2.4. As espécies de peixes do presente estudo e seus parasitos

2.4.1. *Eleotris pisonis*

Eleotris pisonis (Figura 7) é um Perciforme da família Eleotridae amplamente distribuído no contienente americano de noroeste a oeste do Atlântico Central (Bermudas, Bahamas, Carolina do Sul e norte do Golfo do México nos Estados Unidos) e ao sudeste do Brasil (FROESE; PAULY, 2023) e no continente asiático. É conhecido popularmente como amoré, amorê, amoré-preto, amoré-pixuna ou macuco. Esse peixe prefere tributários estuarinos de água doce com faixa de salinidade de 0 a 36,6 ppm, e os indivíduos adultos são encontrados em fundos rasos, lamacentos ou arenosos (DARCY, 1980; CERVIGÓN, 1994; RAY; ROBINS, 2016).

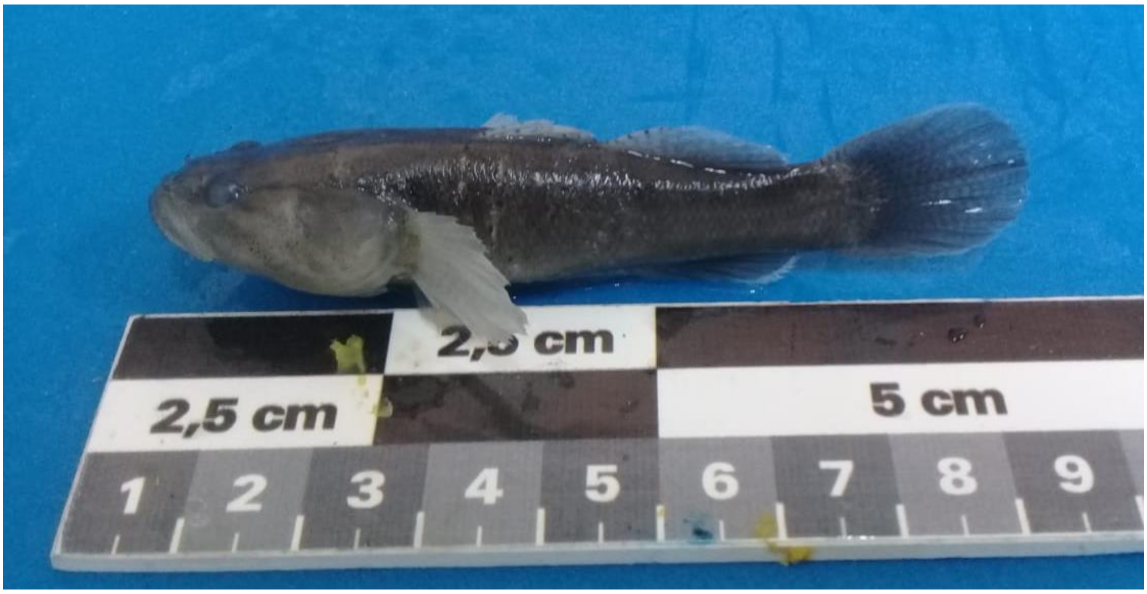

Figura 7. *Eleotris pisonis* coletado no Rio Amazonas, estado do Amapá, Brasil.

Peixe onívoro com tendência à carnivoria, alimenta-se de larvas e pupas de dípteros, pequenos crustáceos (caranguejos e camarões) e pequenos peixes. A sua dieta varia de acordo com a maturidade sexual e estação do ano. O tamanho médio da primeira maturação gonadal ocorre com cerca 5,7 cm para machos e 4,3 cm para fêmeas, com desova ocorrendo na estação de estiagem (NORDLIE, 1981; LE BAIL et al., 2000; SANTOS et al., 2004). Para o continente americano, *E. pisonis* tem registro de infecção por *Cucullanus caballeroi* Petter, 1976 em peixes coletados nas Ilhas Guadalupe (PETTER et al., 1977), enquanto na Ásia, acantocéfalos *Brentisentis uncinus* Leotta et al., 1982 e *Gorgorhynchus satoi* Morisita, 1937 (Leotta et al., 1982) foram coletados em hospedeiros da foz do Rio Keelung, Taiwan (Leotta et al., 1982).

2.4.2. *Colomesus asellus*

Colomesus asellus (Figura 8) é um Tetraodontidae da América do Sul conhecido como baiacu e encontrado na bacia do Rio Amazonas, do Peru à ilha do Marajó, Rio Tocantins, incluindo afluentes dos rios Araguaia, Guaporé, Orinoco e Essequibo (BARTOLETTE et al., 2018; FROESE; PAULY, 2023). Esse peixe é encontrado principalmente em riachos de água doce e costeiros, podendo tolerar água salobra (ANDERSON JR, 2001).

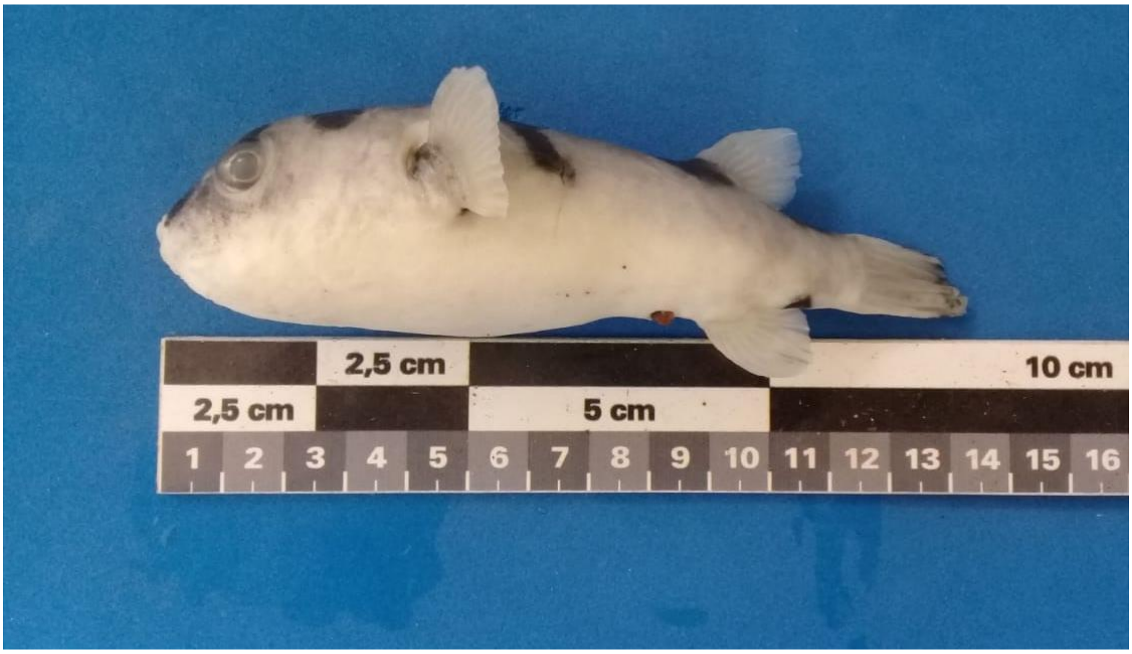

Figura 8. *Colomesus asellus* coletado no Rio Amazonas, estado do Amapá, Brasil.

Peixe onívoro, alimenta-se de moluscos, microcrustáceos copepodas e cladoceras e outros invertebrados aquáticos (Ceratopogonidae, Chironomidae, Trichoptera, Ephemeroptera, Hemiptera, Coleoptera, Odonata, Diptera e Hymenoptera), peixes e plantas (SANTOS et al., 2004; TORRENTE-VILARA et al., 2013; BARTOLETTE et al., 2018). A sua desova total ocorre no início do período chuvoso, porém há relatos de indivíduos em atividade reprodutiva no período de estiagem, no mês de julho (TORRENTE-VILARA et al., 2013). Esse peixe é usado na aquariofília.

Em *C. asellus* do Rio Amazonas, no estado Amazonas, e no Rio Môa, município de Cruzeiro do Sul, estado do Acre, foram encontrados *Ergasilus colomesus* Thatcher & Boeger, 1983 (THATCHER; BOEGER, 1983; VIRGÍLIO et al., 2021). Porém, nos estudos de Neves & Tavares-Dias (2019) e Neves et al. (2020) não foram encontrados parasitos nos espécimes de *C. asellus* coletados no Rio Matapi, um afluente do Rio Amazonas. Em *Colomesus psitacus* Bloch & Schneider, 1801 do arquipélago do Marajó e Rio Tocantins, município de Cametá, estado do Pará, foram relatos *Cucullanus marajoara* (Pinheiro et al., 2018), *Gnathostoma* sp. e *Rohdella amazonica* (Giese et al., 2015; Pinheiro et al., 2017; Pinheiro et al., 2018). Portanto, não há outros estudos sobre a diversidade de parasitos em *C. asellus*.

2.4.3. *Pimelodus ornatus*

Pimelodus ornatus (Figura 9) é um Pimelodidae conhecido popularmente como mandi-guaru, mandi-prata, mandi-pinini ou mandi-pintado. Esse Siluriformes pode ser encontrado nos rios Amazonas, Madeira, Parnaíba, Negro, Alto Paraná, Orinoco, grandes rios das Guianas e em rios de países como Paraguai, Bolívia, Peru e Venezuela (NOMURA, 1984; TORRENTE-VILARA et al., 2013). Pode chegar até a 38,5 cm de comprimento, tendo uma cabeça forte e óssea associado a um padrão colorido conspícuo que inclui a presença de uma mancha na nadadeira dorsal, permitindo assim que essa espécie seja diferenciada de outros pimelodídeos (TORRENTE-VILARA et al., 2013). Emitem sons altos e as fêmeas podem preservar os espermatozoides com as suas secreções maternas inserindo-os no epitélio do seu trato genital sendo indicativo de fertilização interna (BOUJARD, 1997; LE BAIL et al., 2000). Alimenta-se de invertebrados aquáticos (camarões e caranguejos) (Observação pessoal), mas tem tendência a ser piscívoro (SÁ-OLIVEIRA et al., 2015) (Figura 9).

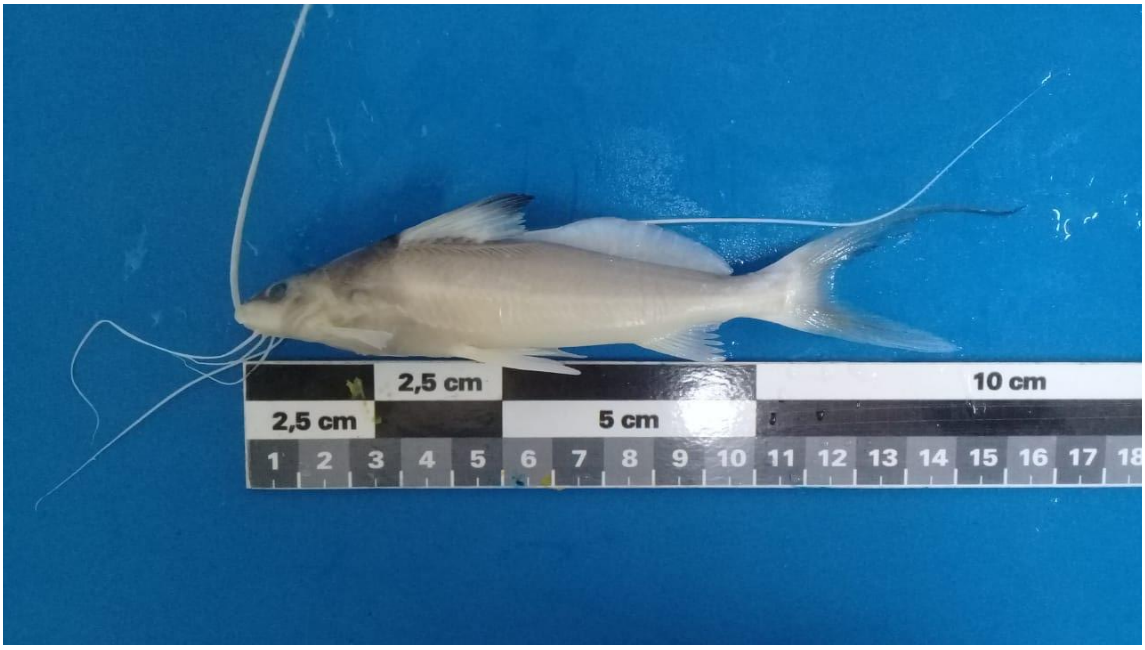

Figura 9. *Pimelodus ornatus* coletado no Rio Amazonas, estado do Amapá, Brasil.

Parasitos nematoides em fase larval e adulta são prevalentes em várias espécies de peixes de água doce (MORAVEC, 1998). Estudos mostraram que nematoides Cuculanídeos e Camallanídeos (*Cucullanus pinnai*, *Cucullanus* sp., *Procamallanus pimelodus, Procamallanus* sp. e *Procamallanus freitasi*) estão presentes em pimelodídeos como *Pimelodus maculatus, Pimelodus blochii* e *Pimelodus pohi* (KOHN; FERNANDES, 1987; SABAS; BRASIL-SATO, 2014; CAVALCANTE et al., 2020).

Porém, não conhece os efeitos da variação temporal e sazonal na diversidade parasitária de *P. ornatus*.

2.5. Análises moleculares em estudos de parasitos metazoários

A redução no custo do sequenciamento de DNA possibilitou obter e comparar sequências de genes como a citocromo oxidase subunidade I (COI), região espaçadora do transcrito interno (ITS 1 e 2), 18S rDNA e 28S rDNA ajudando assim a elucidar perguntas de espécies de parasitos em diferentes estágios de vida (BLASCO-COSTA; POULIN, 2017; LEVY et al., 2020; RANA; KAUR, 2021; GARCÍA-VARELA; ANDRADE-GÓMEZ, 2021; MÜLLER et al., 2022). Técnicas moleculares também são usadas para evitar armadilhas associadas às espécies crípticas de parasitos os quais são geneticamente distintas, mas são morfologicamente indistinguíveis (POULIN, 2010; NADLER; DE LEÓN, 2011; PEREIRA et al., 2018; MENDOZA-PALMERO et al., 2019).

A utilização de estudos ecológicos aliados a taxonomia integrativa, usando análises moleculares pode ser uma ferramenta promissora na descoberta de novas espécies. Portanto, há a necessidade de estudos que visem não só os aspectos ecológicos e influência da sazonalidade, mas também investigações dos ambientes onde os peixes e parasitos são encontrados, já que as características ambientais são de fundamental importância para a composição da estrutura das comunidades e infracomunidade de parasitos em populações naturais de peixes.

Nos estudos de Pérez-Del-Olmo et al. (2023) foi aplicado uma abordagem de taxonomia integrativa para a identificação de parasitos metazoários em uma grande amostra de espécies de peixes de profundidade do Mediterrâneo Ocidental. Fragmentos parciais do gene mitocondrial *nad* 1 foram amplificados para amostras representativas por morfotipo para auxiliar na delimitação das espécies. Assim como também foi avaliado a diversidade de *Lepidapedon* sp. (Digenea) e os padrões de variação genética e epidemiológico das populações de parasitos em relação ao hospedeiro, proveniência geográfica e de batimetria. O censo das infrapopulações de *Lepidapedon* sp. foram analisados a partir de cinco espécies de peixes de profundidade (*Coelorinchus mediterraneus*, *Lepidion lepidion*, *Phycis blennoides*, *Trachyrincus scabrus* e *Mora moro)* coletados por arrasto de fundo em profundidades de 400–200m. Concluindo que a combinação de dados moleculares com análises ecológicas fornecem informações sobre os fatores e possíveis mecanismos que determinam os padrões de conectividade entre as populações de parasitos no fundo do mar (Figura 10).

Alguns estudos vêm utilizando diferentes marcadores moleculares na descrição de novas espécies de parasitos de peixes, como ocorreu nos estudos de Oliveira et al. (2021) que descreveu uma nova espécie de *Therodamas* (Copepoda) em *Leporinus fasciatus* do Rio Jarí, utilizado fragmentos de 18S rDNA. Estudos de Yamada et al. (2018) utilizaram o 28S rDNA para descrever uma nova espécie de *Aphanoblastella* (Monogenea) das brânquias de *Pemelodella avanhandavai* Eigenmann, 1917 da bacia do Rio Paraná.

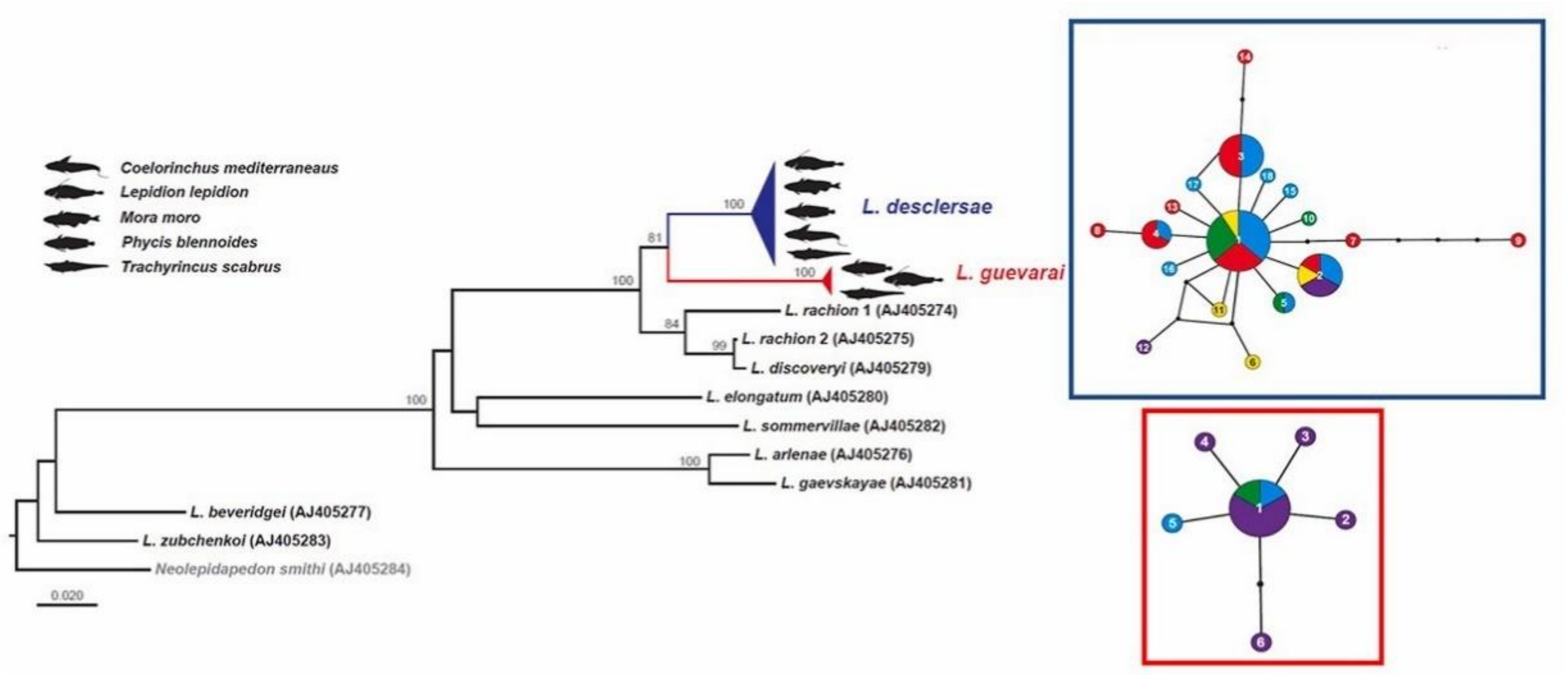

Figura 10. Filograma de junção de vizinhos com base no conjunto de dados *nad* 1 para *Lepidapedon* sp. incluindo as sequências recém-geradas para isolados de cinco hospedeiros de peixes de profundidade no Mediterrâneo Ocidental, na costa da Espanha. Fonte: Pérez-Del-Oomo et al. (2023)

3. PROBLEMAS

Como estão compostas as comunidades e infracomunidades de parasitos metazoários em *E. pisonis, C. asellus* e *P. ornatus* do Rio Amazonas? *Eleotris pisonis, C. asellus* e *P. ornatus* do Rio Amazonas são hospedeiros de parasitos ainda não descritos? A variação sazonal (ciclo chuvoso/estiagem) influencia a estrutura das comunidades e infracomunidades parasitária de *E. pisonis, C. asellus* e *P. ornatus* do Rio Amazonas?

4. HIPÓTESES

1) As comunidades e infracomunidades de parasitos metazoários em *E. pisonis, C. asellus* e *P. ornatus* do Rio Amazonas estão compostas por espécies de Monogenea, Crustacea, Nematoda, Acanthocephala e Trematoda, os táxons mais comuns em peixes da região.

2) *Eleotris pisonis*, *C. asellus* e *P. ornatus* do Rio Amazonas são infectados por espécies de parasitos já descritas para outros Eleotridae, Tetraodontidae e Pimelodidae e também por novas espécies, pois as espécies de parasitos desses peixes não têm sido estudadas.

3) A variação sazonal do ciclo chuvoso/estiagem (taxas pluviométricas), influencia na estrutura das comunidades e infracomunidades de parasitos metazoários em *E. pisonis*, *C. asellus* e *P. ornatus* do Rio Amazonas, devido a influência dos níveis de chuvas da região.

5. OBJETIVOS

5.1 GERAL

Investigar a diversidade, variação temporal e sazonal de parasitos metazoários em *E. pisonis*, *C. asellus* e *P. ornatus* do Rio Amazonas, no estado do Amapá, Brasil.

5.2 Objetivos específicos

- Identificar e realizar análises morfológicas das espécies de parasitos metazoários em *E. pisonis, C. asellus* e *P. ornatus* do Rio Amazonas;
- Descrever novas espécies de parasitos metazoários usando caracteres morfológicos, bem como auxílio da biologia molecular para grupos com taxonomia mais complexa;
- Avaliar a estrutura das comunidades e infracomunidades de parasitos metazoários em *E. pisonis, C. asellus* e *P. ornatus* do Rio Amazonas;
- Avaliar se o ciclo sazonal influencia na estrutura das comunidades e infracomunidades de parasitos metazoários em *E. pisonis, C. asellus* e *P. ornatus* do Rio Amazonas;
- Determinar o padrão de dispersão das espécies dos parasitos metazoários em *E. pisonis, C. asellus* e *P. ornatus* do Rio Amazonas;
- Avaliar se o peso e comprimento dos hospedeiros influenciam na abundância, diversidade e riqueza de espécies de parasitos metazoários em *E. pisonis, C. asellus* e *P. ornatus* do Rio Amazonas;
- Avaliar se o parasitismo influencia no fator de condição (Kn) de *E. pisonis, C. asellus* e *P. ornatus* do Rio Amazonas;

6. REFERÊNCIAS DA INTRODUÇÃO GERAL

AFFONSO, A.; BARBOSA, C.; NOVO, E. Water quality changes in floodplain lakes due to the Amazon River flood pulse: Lago Grande de Curuaí (Pará). **Brazilian Journal of Biology**, v. 71, p. 601–610, 2011.

AILÁN-CHOKE, L. G. et al. An integrative approach assesses the intraspecific variations of *Procamallanus* (*Spirocamallanus*) *inopinatus*, a common parasite in Neotropical freshwater fishes, and the phylogenetic patterns of Camallanidae. **Parasitology**, v. 147, n. 14, p. 1752–1764, 2020.

ALBERT, J. S.; VAL, P.; HOORN, C. The changing course of the Amazon River in the Neogene: center stage for Neotropical diversification. **Neotropical Ichthyology**, v. 16, 2018.

ALCANTARA, N. M.; TAVARES-DIAS, M. Structure of the parasites communities in two Erythrinidae fish from Amazon River system (Brazil). **Revista Brasileira de Parasitologia Veterinária**, v. 24, n. 2, p. 183–190, 2015.

ALHO, C. J.; REIS, R. E.; AQUINO, P. P. Amazonian freshwater habitats experiencing environmental and socioeconomic threats affecting subsistence fisheries. **Ambio**, v. 44, n. 5, p. 412–425, 2015.

AMUNDSON, R. et al. Global patterns of the isotopic composition of soil and plant nitrogen. **Global biogeochemical cycles**, v. 17, n. 1, 2003.

ANDERSON JR., W. D. National Audubon Society Field Guide To Tropical Marine Fishes of the Caribbean, the Gulf of Mexico, Florida, the Bahamas, and Bermuda. **Copeia**, v. 2001, n. 3, p. 884–886, 2001.

ARANTES, C. C. et al. Relationships between forest cover and fish diversity in the Amazon River floodplain. **Journal of applied ecology**, v. 55, n. 1, p. 386–395, 2018.

AVENANT-OLDEWAGE, A.; MILNE, S. Aspects of the morphology of the juvenile life stages of *Paradiplozoon ichthyoxanthon* Avenant-Oldewage, 2013 (Monogenea: Diplozoidae). **Acta Parasitologica**, v. 59, n. 2, p. 247–254, 2014.

BAIA, R. R. J. et al. Patterns of the parasite communities in a fish assemblage of a river in the Brazilian Amazon region. **Acta parasitologica**, v. 63, n. 2, p. 304–316, 2018.

BALAGURU, K. et al. The barrier layer of the Atlantic warmpool: Formation mechanism and influence on the mean climate. **Tellus A: Dynamic Meteorology and Oceanography**, v. 64, n. 1, p. 18162, 2012.

BARLETTA, M. et al. Fish and aquatic habitat conservation in South America: a continental overview with emphasis on neotropical systems. **Journal of fish biology**, v. 76, n. 9, p. 2118–2176, 2010.

BARTHEM, R. B.; FABRÉ, N. N. Biologia e diversidade dos recursos pesqueiros da Amazônia. **A pesca e os recursos pesqueiros na Amazônia brasileira**, v. 1, p. 17–62, 2004.

BARTOLETTE, R. et al. Seasonal and ontogenetic diet patterns of the freshwater pufferfish *Colomesus asellus* (Müller & Troschel, 1849) in the upper-middle Tocantins River. **Acta Scientiarum. Biological Sciences**, v. 40, p. 1–6, 2018.

BEHRINGER, D. C. et al. Disease in fisheries and aquaculture. Em: **Marine Disease Ecology**. [s.l.] Oxford University Press, 2020. p. 183–210.

BLASCO-COSTA, I.; POULIN, R. Parasite life-cycle studies: a plea to resurrect an old parasitological tradition. **Journal of Helminthology**, v. 91, n. 6, p. 647–656, 2017.

BLASCO-COSTA, I.; ROUCO, C.; POULIN, R. Biogeography of parasitism in freshwater fish: spatial patterns in hot spots of infection. **Ecography**, v. 38, n. 3, p. 301–310, 2015.

BOUJARD, T. **Poissons de Guyane: Guide écologique de l'Approuague et de la réserve des Nouragues**. Paris, Editions Quae, 1997.

BREJÃO, G. L. et al. Threshold responses of Amazonian stream fishes to timing and extent of deforestation. **Conservation biology**, v. 32, n. 4, p. 860–871, 2018.

CALVÈS, G. et al. Past Amazon Basin fluvial systems, insight into the Cenozoic sequences using seismic geomorphology (Marañón Basin, Peru). **Journal of South American Earth Sciences**, v. 90, p. 440–452, 2019.

CARLSON, C. J. et al. A global parasite conservation plan. **Biological Conservation**, v. 250, p. 108596, 2020.

CARVALHO, A. A.; TAVARES-DIAS, M. Diversity of parasites in *Cichlasoma amazonarum* Kullander, 1983 during rainy and dry seasons in eastern Amazon (Brazil). **Journal of applied ichthyology**, v. 33, n. 6, p. 1178–1183, 2017.

CARVALHO, A. L. **Diagnóstico e aspectos ecológicos dos parasitos de duas espécies de *Cichla* (Perciformes: Cichlidae) e *Pseudoplatystoma* (Siluriformes: Pimelodidae) na sub-bacia do rio Jamari-Ariquemes, estado de Rondônia**. 49 f. Tese (Doutorado em Ciências Veterinárias) - Instituto de Veterinária, Universidade Federal Rural do Rio de Janeiro, Seropédica, RJ. 2019.

CASTELLO, L. et al. Fishery yields vary with land cover on the Amazon River floodplain. **Fish and Fisheries**, v. 19, n. 3, p. 431–440, 2018.

CASTELLO, L.; MACEDO, M. N. Large-scale degradation of Amazonian freshwater ecosystems. **Global Change Biology**, v. 22, n. 3, p. 990–1007, 2016.

CRACCO, A.; LEHUN, A. L.; TAKEMOTO, R. M. Composition and structure of the parasitic fauna of *Hypostomus* spp. (Loricariidae: Hypostominae) from a Neotropical river in Brazil. **Parasitology Research**, v. 121, n. 8, p. 2253–2262, 2022.

CAVALCANTE, P. H. DE OLIVERA et al. Helminth diversity in *Pimelodus blochii* Valenciennes, 1840 (Osteichthyes: Pimelodidae) in two Amazon Rivers. **Parasitology Research**, v. 119, n. 12, p. 4005–4015, 2020.

CERVIGÓN, F. Los Peces Marinos de Venezuela. Tomo III. **Ex-Libris. Caracas, Venezuela**, 1994.

CLERICI, N. et al. Peace in Colombia is a critical moment for Neotropical connectivity and conservation: Save the northern Andes-Amazon biodiversity bridge. **Conservation Letters**, v. 12, n. 1, p. e12594, 2019.

COLES, V. J. et al. The pathways and properties of the Amazon River Plume in the tropical North Atlantic Ocean. **Journal of Geophysical Research: Oceans**, v. 118, n. 12, p. 6894–6913, 2013.

CONTOS, J.; TRIPCEVICH, N. Correct placement of the most distant source of the A mazon River in the Mantaro River drainage. **Area**, v. 46, n. 1, p. 27–39, 2014.

DAI, A.; TRENBERTH, K. E. Estimates of freshwater discharge from continents: Latitudinal and seasonal variations. **Journal of hydrometeorology**, v. 3, n. 6, p. 660–687, 2002.

DARCY, G. H. Comparison of ecological and life history information on gobiid fishes, with emphasis on the southeastern United States. 1980.

DE AGUIAR, J. C. C. et al. Morphology and molecular phylogeny of *Pauciconfibuloides amazonica* gen. n. sp. n. (Platyhelminthes, Monogenoidea) parasitizing the Amazonian croaker Plagioscion squamosissimus. **Parasitology International**, v. 87, p. 102489, 1 abr. 2022.

DOGIEL, V. A.; PETRUSHEVSKII, G. K. **& YI Polyanski 1958. Parasitology of Fishes**. Leningrad University Press, , 1958.

DURAND, F. et al. Impact of continental freshwater runoff on coastal sea level. **Surveys in Geophysics**, v. 40, n. 6, p. 1437–1466, 2019.

DUTRA, G. M. et al. Rapid assessment of the ichthyofauna of the southern Guiana Shield tributaries of the Amazonas River in Pará, Brazil. **Acta Amazonica**, v. 50, p. 24–36, 2020.

ERKINHARJU, T. et al. Cleaner fish in aquaculture: review on diseases and vaccination. **Reviews in Aquaculture**, v. 13, n. 1, p. 189–237, 2021.

FEARNSIDE, P. M. Environmental and social impacts of hydroelectric dams in Brazilian Amazonia: Implications for the aluminum industry. **World Development**, v. 77, p. 48–65, 2016.

FERREIRA, M. M.; PASSADOR, R. J.; TAVARES-DIAS, M. Community ecology of parasites in four species of *Corydoras* (Callichthyidae), ornamental fish endemic to the eastern Amazon (Brazil). **Anais da Academia Brasileira de Ciências**, v. 91, 2019.

FIGUEIREDO, J. et al. Late Miocene onset of the Amazon River and the Amazon deep-sea fan: Evidence from the Foz do Amazonas Basin. **Geology**, v. 37, n. 7, p. 619–622, 2009.

FINLAY, J. C.; POWER, M. E.; CABANA, G. Effects of water velocity on algal carbon isotope ratios: implications for river food web studies. **Limnology and Oceanography**, v. 44, n. 5, p. 1198–1203, 1999.

FLEISCHMANN, A. S. et al. Increased floodplain inundation in the Amazon since 1980. **Environmental Research Letters**, v. 18, n. 3, p. 034024, 2023.

FOLTZ, G. R.; MCPHADEN, M. J. Seasonal mixed layer salinity balance of the tropical North Atlantic Ocean. **Journal of Geophysical Research: Oceans**, v. 113, n. C2, 2008.

FREDERICO, R. G.; OLDEN, J. D.; ZUANON, J. Climate change sensitivity of threatened, and largely unprotected, Amazonian fishes. **Aquatic conservation: marine and freshwater ecosystems**, v. 26, p. 91–102, 2016.

FREITAS, C. E. et al. An initial assessment of drought sensitivity in Amazonian fish communities. **Hydrobiologia**, v. 705, n. 1, p. 159–171, 2013.

FROESE, R.; PAULY, D. FishBase. World Wide Web electronic publication. 2 jan. 2023.

GARCÍA-VARELA, M.; ANDRADE-GÓMEZ, L. First steps to understand the systematics of Echinorhynchidae Cobbold, 1876 (Acanthocephala), inferred through nuclear gene sequences. **Parasitology international**, v. 81, p. 102264, 2021.

GIARI, L.; CASTALDELLI, G.; TIMI, J. T. Ecology and effects of metazoan parasites of fish in transitional waters. **Parasitology**, v. 149, n. 14, p. 1829–1841, 2022.

GIESE, E. et al. *Rohdella amazonica* n. sp. (Aspidogastrea: Aspidogastridae) from the Amazoninan banded puffer fish *Colomesus psittacus* (Bloch & Schneider, 1801). **Journal of helminthology**, v. 89, n. 3, p. 288–293, 2015.

GIFFARD, P. et al. Contribution of the Amazon river discharge to regional sea level in the tropical Atlantic Ocean. **Water**, v. 11, n. 11, p. 2348, 2019.

GILBERT, B. M.; AVENANT-OLDEWAGE, A. Monogeneans as bioindicators: A meta-analysis of effect size of contaminant exposure toward Monogenea (Platyhelminthes). **Ecological Indicators**, v. 130, p. 108062, 1 nov. 2021.

GOULDING, M. **The fishes and the forest**. London, England, University of California press, 1980.

GOUVEIA, N. A.; GHERARDI, D. F. M.; ARAGÃO, L. E. O. C. The Role of the Amazon River Plume on the Intensification of the Hydrological Cycle. **Geophysical Research Letters**, v. 46, n. 21, p. 12221–12229, 2019.

GONÇALVES, R. A. et al. Seasonal pattern in parasite infracommunities of Hoplerythrinus unitaeniatus and Hoplias malabaricus (Actinopterygii: Erythrinidae) from the Brazilian Amazon. **Acta Parasitologica**, v. 61, n. 1, p. 119-129, 2016.

HOORN, C. et al. The Amazon at sea: Onset and stages of the Amazon River from a marine record, with special reference to Neogene plant turnover in the drainage basin. **Global and Planetary Change**, v. 153, p. 51–65, 2017.

HOORN, C.; WESSELINGH, F. P. Introduction: Amazonia, landscape and species evolution. **Amazonia: landscape and species evolution: a look into the past**, p. 1–6, 2010.

HOSHINO, É. DE M.; TAVARES-DIAS, M. Interannual and seasonal variation in protozoan and metazoan parasite communities of *Hemibrycon surinamensis,* a characid fish inhabiting the Brazilian Amazon. **Acta parasitologica**, v. 64, n. 3, p. 479–488, 2019.

HOSHINO, É. DE M.; TAVARES-DIAS, M. Temporal and seasonal variations in parasites of *Metynnis lippincottianus* (Characiformes: Characidae), a host from the eastern Amazon (Brazil). **Journal of Natural History**, v. 53, n. 43–44, p. 2723–2736, 2020.

HURD, L. E. et al. Amazon floodplain fish communities: habitat connectivity and conservation in a rapidly deteriorating environment. **Biological Conservation**, v. 195, p. 118–127, 2016.

ISHIKAWA, N. F.; DOI, H.; FINLAY, J. C. Global meta-analysis for controlling factors on carbon stable isotope ratios of lotic periphyton. **Oecologia**, v. 170, n. 2, p. 541–549, 2012.

JAEGER, K. L.; OLDEN, J. D.; PELLAND, N. A. Climate change poised to threaten hydrologic connectivity and endemic fishes in dryland streams. **Proceedings of the National Academy of Sciences**, v. 111, n. 38, p. 13894–13899, 2014.

JAHFER, S.; VINAYACHANDRAN, P.; NANJUNDIAH, R. S. The role of Amazon river runoff on the multidecadal variability of the Atlantic ITCZ. **Environmental Research Letters**, v. 15, n. 5, p. 054013, 2020.

JAKOB, E. M.; MARSHALL, S. D.; UETZ, G. W. Estimating fitness: a comparison of body condition indices. **Oikos**, p. 61–67, 1996.

JÉZÉQUEL, C. et al. Freshwater fish diversity hotspots for conservation priorities in the Amazon Basin. **Conservation biology**, v. 34, n. 4, p. 956–965, 2020.

JUNIOR, R. P. M. et al. Riscos Potenciais à Saúde Humana e Ambiental com o uso do Mercúrio na Atividade de Garimpo Artesanal de Ouro no Alto Rio Madeira, Amazônia Ocidental. **Revista Eletrônica Acervo Saúde**, v. 23, n. 6, p. e12840–e12840, 2023.

JUNK, W. J.; SOARES, M. G. M.; BAYLEY, P. B. Freshwater fishes of the Amazon River basin: their biodiversity, fisheries, and habitats. **Aquatic Ecosystem Health & Management**, v. 10, n. 2, p. 153–173, 2007.

KALOGIANNI, E. et al. Occurrence and effect of trematode metacercariae in two endangered killifishes from Greece. **Parasitology research**, v. 116, n. 11, p. 3007–3018, 2017.

KOHN, A.; FERNANDES, B. M. Estudo comparativo dos helmintos parasitos de peixes do Rio Mogi Guassu, coletados nas excursões realizadas entre 1927 e 1985. **Memórias do Instituto Oswaldo Cruz**, v. 82, n. 4, p. 483–500, 1987.

KOROSOV, A.; COUNILLON, F.; JOHANNESSEN, J. A. Monitoring the spreading of the A mazon freshwater plume by MODIS, SMOS, A quarius, and TOPAZ. **Journal of Geophysical Research: Oceans**, v. 120, n. 1, p. 268–283, 2015.

KUHN, J. A. et al. Temporal changes and between-host variation in the intestinal parasite community of Arctic charr in a subarctic lake. **Hydrobiologia**, v. 783, n. 1, p. 79–91, 2016.

LAPOLA, D. M. et al. The drivers and impacts of Amazon forest degradation. **Science**, v. 379, n. 6630, p. eabp8622, 2023.

LE BAIL, P.; KEITH, P.; PLANQUETTE, P. Atlas des poissons d'eau douce de Guyane. Tome 2, fascicule II: Siluriformes. **Patrimoines naturels (MNHN/SPN)**, v. 43, p. 307, 2000.

LEES, A. C. et al. Hydropower and the future of Amazonian biodiversity. **Biodiversity and conservation**, v. 25, n. 3, p. 451–466, 2016.

LEIGH, C. et al. Ecological effects of extreme climatic events on riverine ecosystems: insights from Australia. **Freshwater Biology**, v. 60, n. 12, p. 2620–2638, 2015.

LEITÃO, C. S. DE S. et al. River reorganization affects populations of dwarf cichlid species (*Apistogramma* genus) in the lower Negro River, Brazil. **Frontiers in Ecology and Evolution**, v. 9, p. 760287, 2021.

LEOTTA, S. H.; SCHMIDT, G. D.; KUNTZ, R. E. *Brentisentis uncinus* gen. et sp. n. and *Gorgorhynchus satoi* (Morisita 1937) Yamaguti 1963 from Taiwan. **The Journal of Parasitology**, v. 68, n. 1, p. 134–137, 1982.

LESSMANN, J. et al. Large expansion of oil industry in the Ecuadorian Amazon: biodiversity vulnerability and conservation alternatives. **Ecology and evolution**, v. 57, n. 14, p. 4997–5012, 2016.

LEVY, E. et al. *Profilicollis chasmagnathi* (Acanthocephala) parasitizing freshwater fishes: paratenicity and an exception to the phylogenetic conservatism of the genus? **Parasitology Research**, v. 119, p. 3957–3966, 2020.

LIANG, Y.-C. et al. Amplified seasonal cycle in hydroclimate over the Amazon river basin and its plume region. **Nature communications**, v. 11, n. 1, p. 1–11, 2020.

LUKAS, R.; LINDSTROM, E. The mixed layer of the western equatorial Pacific Ocean. **Journal of Geophysical Research: Oceans**, v. 96, n. S01, p. 3343–3357, 1991.

LUQUE, J. L. et al. Helminth parasites of South American fishes: current status and characterization as a model for studies of biodiversity. **Journal of Helminthology**, v. 91, n. 2, p. 150–164, 2017.

LYNCH, A. J. et al. The social, economic, and environmental importance of inland fish and fisheries. **Environmental Reviews**, v. 24, n. 2, p. 115–121, 2016.

LYNCH, A. J. et al. People need freshwater biodiversity. **WIREs Water**, v. 10, n. 3, p. e1633, 2023.

MAGURRAN A. **Measuring biological diversity**. Oxford: Blackwell Publishing, 2004.

MARENGO, J. A. et al. Extreme climatic events in the Amazon basin. **Theoretical and Applied Climatology**, v. 107, n. 1, p. 73–85, 2012.

MARTINS, W. M. DE O. et al. Seasonality of parasitic helminths of *Leporinus macrocephalus* and their parasitism rates in farming systems in the Amazon. **Revista Brasileira de Parasitologia Veterinária**, v. 26, n. 4, p. 419–426, 2017.

MASSON, S.; DELECLUSE, P. Influence of the Amazon river runoff on the tropical Atlantic. **Physics and Chemistry of the Earth, Part B: Hydrology, Oceans and Atmosphere**, v. 26, n. 2, p. 137–142, 2001.

MEDEIROS, P. M. et al. Fate of the Amazon River dissolved organic matter in the tropical Atlantic Ocean. **Global Biogeochemical Cycles**, v. 29, n. 5, p. 677–690, 2015.

MENDOZA-PALMERO, C. A. et al. *Walteriella* ng (Monogenoidea: Dactylogyridae) from the gills of pimelodid catfishes (Siluriformes: Pimelodidae) from the Peruvian Amazonia based on morphological and molecular data. **Systematic parasitology**, v. 96, n. 6, p. 441–452, 2019.

MENDOZA-PALMERO, C. A.; BLASCO-COSTA, I.; SCHOLZ, T. Molecular phylogeny of Neotropical monogeneans (Platyhelminthes: Monogenea) from catfishes (Siluriformes). **Parasites & Vectors**, v. 8, n. 1, p. 1–11, 2015.

MESQUITA, G. V. et al. Determinação de mercúrio total em peixes frescos em alguns dos principais rios de Roraima por geração de vapor frio e espectrometria de fluorescência atômica (cv AFS). **Ambiente: Gestão e Desenvolvimento**, v. 16, n. 1, p. 45–56, 2023.

MILLIMAN, J. D.; SYVITSKI, J. P. Geomorphic/tectonic control of sediment discharge to the ocean: the importance of small mountainous rivers. **The journal of Geology**, v. 100, n. 5, p. 525–544, 1992.

MORAVEC, F. **Nematodes of freshwater fishes of the Neotropical Region.** Academia, Publishing House of the Academy of Sciences of the Czech Republic, 1998.

MOUYEN, M. et al. Assessing modern river sediment discharge to the ocean using satellite gravimetry. **Nature communications**, v. 9, n. 1, p. 1–9, 2018.

MÜLLER, M. et al. Integrative taxonomy suggests that South American freshwater nematodes *Echinocephalus* and their host stingrays co-originated in late Oligocene to early Miocene. **Journal of Helminthology**, v. 96, p. e68, 2022.

MÜLLER, M. I. et al. *Henneguya correai* n. sp.(Cnidaria, Myxozoa) Parasitizing the Fins of the Amazonian Fish *Semaprochilodus insignis*. **Diversity**, v. 15, n. 6, p. 702, 2023.

MYERS, S. et al. Current guidance underestimates risk of global environmental change to food security. **bmj**, v. 378, 2022.

NADLER, S. A.; DE LEÓN, G. P.-P. Integrating molecular and morphological approaches for characterizing parasite cryptic species: implications for parasitology. **Parasitology**, v. 138, n. 13, p. 1688–1709, 2011.

NEGREIROS, L. P. et al. Long-term temporal variation in the parasite community structure of metazoans of *Pimelodus blochii* (Pimelodidae), a catfish from the Brazilian Amazon. **Parasitology research**, v. 118, n. 12, p. 3337–3347, 2019.

NEGREIROS, L. P. et al. Community structure of metazoan parasites from *Pimelodus blochii* in two rivers of the western Brazilian Amazon: same seasonal traits, but different anthropogenic impacts. **Parasitology research**, v. 117, n. 12, p. 3791–3798, 2018.

NEGREIROS, L. P.; PEREIRA, F. B.; TAVARES-DIAS, M. Metazoan parasites of *Calophysus macropterus* (Siluriformes: Pimelodidae) in the Acre and Iaco rivers in the western Amazon region of Brazil: diversity, similarity and seasonal variation. **Journal of Natural History**, v. 53, n. 23–24, p. 1465–1479, 2019.

NEVES, L. R. et al. Diversity of monogenean parasites on gills of fishes from the Matapi River, in the Brazilian Amazon. **Revista Brasileira de Parasitologia Veterinária**, v. 29, 2020.

NEVES, L. R.; SILVA, L. M. A.; TAVARES- DIAS, M. Diversity and Ecology of Endohelminth Parasites in a Fish Assemblage of an Amazon River Tributary in Brazil. **Acta Parasitologica**, p. 1–11, 2021.

NEVES, L. R.; TAVARES-DIAS, M. Low levels of crustacean parasite infestation in fish species from the Matapi River in the state of Amapá, Brazil. **Revista Brasileira de Parasitologia Veterinária**, v. 28, p. 493–498, 2019.

NITTROUER, C. A.; DEMASTER, D. J. The Amazon shelf setting: tropical, energetic, and influenced by a large river. **Continental shelf research**, v. 16, n. 5–6, p. 553–573, 1996.

NOMURA, H. **Dicionário dos peixes do Brasil.** Rio de Janeiro: Technical Books Livraria, 1984.

NORDLIE, F. G. Feeding and reproductive biology of eleotrid fishes in a tropical estuary. **Journal of Fish Biology**, v. 18, n. 1, p. 97–110, 1981.

OBERDORFF, T. et al. Unexpected fish diversity gradients in the Amazon basin. **Science advances**, v. 5, n. 9, p. eaav8681, 2019.

OLIVEIRA, A. C. B. et al. Carbon sources of fish in an Amazonian floodplain lake. **Aquatic Sciences**, v. 68, n. 2, p. 229–238, 2006.

OLIVEIRA, D. J. DE et al. Conservation status of the "Piracanjuba" *Brycon orbignyanus* (Valenciennes, 1850) (Characiformes, Bryconidae): basis for management programs. **Biodiversidade Brasileira**, v. 7, n. 1, p. 18–33, 19 jul. 2017a.

OLIVEIRA, J. C. et al. A climatology of the annual cycle of river discharges into the Brazilian continental shelves: from seasonal to interannual variability. **Environmental earth sciences**, v. 77, n. 5, p. 1–17, 2018.

OLIVEIRA, M. S. et al. Integrative taxonomy of a new species of *Therodamas* (Ergasilidae) infecting the Amazonian freshwater fish Leporinus fasciatus (Anostomidae). **Parasitology Research**, p. 1–11, 2021.

OLIVEIRA, B. F. A et al. Deforestation and climate change are projected to increase heat stress risk in the Brazilian Amazon. **Communications Earth & Environment**, v. 2, n. 1, p. 207, 2021.

OLIVEIRA, G. et al. Increasing wildfires threaten progress on halting deforestation in Brazilian Amazonia. **Nature Ecology & Evolution**, p. 1-2, 2023.

OLIVEIRA, M. S. et al. Morphological and functional structure of two Ergasilidae parasites determine their microhabitat affinity on the gills of an Anostomidae fish from the Amazon. **Parasitology Research**, v. 121, n. 8, p. 2295–2305, 2022.

OLIVEIRA, M. S. B. et al. Metazoan parasite communities of wild *Leporinus friderici* (Characiformes: Anostomidae) from Amazon River system in Brazil. **Studies on neotropical fauna and environment**, v. 52, n. 2, p. 146–156, 2017b.

PACIFICI, M. et al. Assessing species vulnerability to climate change. **Nature climate change**, v. 5, n. 3, p. 215–224, 2015.

PEREIRA, D. V. et al. Relationships between fishery catch rates and land cover along a longitudinal gradient in floodplains of the Amazon River. **Fisheries Research**, v. 258, p. 106521, 2023.

PEREIRA, E. S. et al. Fish parasite diversity in the Amambai river, State Mato Grosso do Sul, Brazil. **Acta Scientiarum. Biological Sciences**, v. 40, 2018.

PÉREZ-DEL-OLMO, A. et al. Linking integrative taxonomy and ecology: Diversity and population structure of two platyhelminth parasites (Digenea: Lepidapedidae) of sympatric deep-sea fishes in the Western Mediterranean. **Deep Sea Research Part I: Oceanographic Research Papers**, v. 192, p. 103948, 2023.

PETTER, A. J.; GOLVAN, Y. J.; TCHEPRAKOFF, R. Nématodes de poissons de rivière en Guadeloupe. **Bulletin du Muséum National d’Histoire Naturelle, Paris, 3e série, No. 428, Section A, Zoologie**, v. 298, p. 159–171, 1977.

PIECUCH, C. G.; WADEHRA, R. Dynamic sea level variability due to seasonal river discharge: a preliminary global ocean model study. **Geophysical Research Letters**, v. 47, n. 4, p. e2020GL086984, 2020.

PINHEIRO, R. H. DA S. et al. Gnathostomatidae nematode parasite of *Colomesus psittacus* (Osteichthyes, Tetraodontiformes) in the Ilha de Marajó, Brazilian Amazon. **Revista Brasileira de Parasitologia Veterinária**, v. 26, p. 340–347, 2017.

PINHEIRO, R. H. DA S. et al. *Cucullanus marajoara* n. sp.(Nematoda: Cucullanidae), a parasite of *Colomesus psittacus* (Osteichthyes: Tetraodontiformes) in the Marajó, Brazil. **Revista Brasileira de Parasitologia Veterinária**, v. 27, p. 521–530, 2018.

POFF, N.; BRINSON, M. M.; DAY, J. Aquatic ecosystems and global climate change. **Pew Center on Global Climate Change, Arlington, VA**, v. 44, p. 1–36, 2002.

POULIN, R. Variation in the intraspecific relationship between fish length and intensity of parasitic infection: biological and statistical causes. **Journal of Fish Biology**, v. 56, n. 1, p. 123–137, 2000.

POULIN, R., PATERSON, R.A., TOWNSEND, C.R., TOMPKINS, D.M. E KELLY, D.W. Invasões biológicas e dinâmica de doenças endêmicas em ecossistemas de água doce. **Freshwater Biology**, *56* (4), 676-688, 2011.

POULIN, R. Uneven distribution of cryptic diversity among higher taxa of parasitic worms. **Biology Letters**, v. 7, n. 2, p. 241–244, 2010.

RANA, K.; KAUR, H. Phylogenetic analysis of *Pallisentis nagpurensis* (Acanthocephala: Quadrigyridae) infecting snakehead murrel Channa striata in Himachal Pradesh, India. **Journal of Parasitic Diseases**, v. 45, n. 3, p. 797–805, 2021.

RAY, C.; ROBINS, C. R. **A field guide to Atlantic coast fishes: North America**. Houghton Mifflin Harcourt, 2016.

REID, A. J. et al. Emerging threats and persistent conservation challenges for freshwater biodiversity. **Biological Reviews**, v. 94, n. 3, p. 849–873, 2019.

REBOITA, M. S. et al. South America climate change revealed through climate indices projected by GCMs and Eta-RCM ensembles. **Climate Dynamics**, v. 58, n. 1-2, p. 459-485, 2022.

RIJIN, K. et al. Seasonal Variation in the Occurrence of Parasitic Isopods and Copepods (Crustacea) Infecting the Clupeidaen Fishes of Malabar Coast, India. **Turkish Journal of Fisheries and Aquatic Sciences**, v. 19, n. 3, p. 241–249, 2018.

RÖPKE, C. et al. Effects of climate-driven hydrological changes in the reproduction of Amazonian floodplain fishes. **Journal of Applied Ecology**, v. 59, n. 4, p. 1134–1145, 2022.

RUDZIN, J. E.; SHAY, L. K.; JAIMES DE LA CRUZ, B. The impact of the Amazon–Orinoco River plume on enthalpy flux and air–sea interaction within Caribbean Sea tropical cyclones. **Monthly Weather Review**, v. 147, n. 3, p. 931–950, 2019.

SABAS, C. S. S.; BRASIL-SATO, M. C. Helminth fauna parasitizing *Pimelodus pohli* (Actinopterygii: Pimelodidae) from the upper São Francisco River, Brazil. **Revista Brasileira de Parasitologia Veterinária**, v. 23, p. 375–382, 2014.

SÁ-OLIVEIRA, J. C. et al. Upstream and downstream responses of fish assemblages to an eastern Amazonian hydroelectric dam. **Freshwater biology**, v. 60, n. 10, p. 2037-2050, 2015.

SANTOS, G. M. et al. Peixes do baixo rio Tocantins. 20 anos depois da Usina Hidrelétrica Tucuruí. 2004.

SAWAKUCHI, H. O. et al. Carbon dioxide emissions along the lower Amazon River. **Frontiers in Marine Science**, v. 4, p. 76, 2017.

SEGADELLI, S. et al. Predicting extreme-precipitation effects on the geomorphology of small mountain catchments: Towards an improved understanding of the consequences for freshwater biodiversity and ecosystems. **Water**, v. 12, n. 1, p. 79, 2020.

SIEBERT, T. H. R.; DA SILVA, R. A. Levantamento dos principais peixes comercializados na feira do pescado–Santarém–PA. **Revista Brasileira de Engenharia de Pesca**, v. 12, n. 1, p. 62–74, 2019.

SILVA, A. et al. Parasite diversity in *Oxydoras niger* (Osteichthyes: Doradidae) from the basin of Solimões River, Amazonas state, Brazil, and the relationship between monogenoidean and condition factor. **Brazilian Journal of Biology**, v. 71, n. 3, p. 791–796, 2011.

SILVA, G. C. X. et al. Environmental impacts of dam reservoir filling in the East Amazon. **Frontiers in Water**, v. 2, p. 11, 2020.

SMITH JR, W. O.; DEMASTER, D. J. Phytoplankton biomass and productivity in the Amazon River plume: correlation with seasonal river discharge. **Continental Shelf Research**, v. 16, n. 3, p. 291–319, 1996.

SOARES, M. G. DA M.; ALMEIDA, R. G.; JUNK, W. J. The trophic status of the fish fauna in Lago Camaleão, a macrophyte dominated floodplain lake in the middle Amazon. **Amazoniana: Limnologia et Oecologia Regionalis Systematis Fluminis Amazonas**, v. 9, n. 4, p. 511–526, 1986.

SOUSA, R. G. C. et al. Seasonal Dynamic Of Amazonian Small-scale Fisheries Is Dictated By The Hydrologic Pulse. **Boletim do Instituto Pesca**, v. 43(2), p. 207–221, 2017.

SURES, B.; NACHEV, M. Effects of multiple stressors in fish: how parasites and contaminants interact. **Parasitology**, v. 149, n. 14, p. 1822–1828, 2022.

STROPP, J. et al. The ghosts of forests past and future: deforestation and botanical sampling in the Brazilian Amazon. **Ecography**, v. 43, n. 7, p. 979–989, 2020.

STUKEL, M. R. et al. Top-down, bottom-up and physical controls on diatom-diazotroph assemblage growth in the Amazon River plume. **Biogeosciences**, v. 11, n. 12, p. 3259–3278, 2014.

SUBRAMANIAM, A. et al. Amazon River enhances diazotrophy and carbon sequestration in the tropical North Atlantic Ocean. **Proceedings of the National Academy of Sciences**, v. 105, n. 30, p. 10460–10465, 2008.

SURES, B.; NACHEV, M. Effects of multiple stressors in fish: how parasites and contaminants interact. **Parasitology**, v. 149, n. 14, p. 1822–1828, 2022.

TAVARES-DIAS, M. et al. Ecology and seasonal variation of parasites in wild *Aequidens tetramerus*, a Cichlidae from the Amazon. **Acta Parasitologica**, v. 59, n. 1, p. 158–164, 2014.

TAVARES-DIAS, M.; NEVES, L. R. Diversity of parasites in wild *Astronotus ocellatus* (Perciformes, Cichlidae), an ornamental and food fish in Brazil. **Anais da Academia Brasileira de Ciências**, v. 89, p. 2305–2315, 2017.

TAVARES-DIAS, M.; OLIVEIRA, M. S. B. Structure of parasites community in *Chaetobranchopsis orbicularis* (Cichlidae), a host from the Amazon River system in northern Brazil. **Parasitology research**, v. 116, n. 8, p. 2313–2319, 2017.

TENCATT, L. F. et al. A journey through the Amazon Middle Earth reveals *Aspidoras azaghal* (Siluriformes: Callichthyidae), a new species of armoured catfish from the rio Xingu basin, Brazil. **Journal of Fish Biology**, v. 97, n. 4, p. 1072–1086, 2020.

THATCHER, V. E.; BOEGER, W. A. The parasitic crustaceans of fishes from the Brazilian Amazon. 4. *Ergasilus colomesus* n. sp. (Copepoda: Cyclopoida) from an ornamental fish, *Colomesus asellus* (Tetraodontidae) and aspects of its pathogenicity. **Transactions of the American Microscopical Society**, p. 371–379, 1983.

TIMI, J. T.; LUQUE, J. L.; POULIN, R. Host ontogeny and the temporal decay of similarity in parasite communities of marine fish. **International Journal for Parasitology**, v. 40, n. 8, p. 963–968, 2010.

TIMI, J. T.; POULIN, R. Why ignoring parasites in fish ecology is a mistake. **International Journal for Parasitology**, v. 50, n. 10–11, p. 755–761, 2020.

TISSEUIL, C. et al. Global diversity patterns and cross-taxa convergence in freshwater systems. **Journal of Animal Ecology**, v. 82, n. 2, p. 365–376, 2013.

TOSETTO, E. G. et al. The Amazon River plume, a barrier to animal dispersal in the Western Tropical Atlantic. **Scientific Reports**, v. 12, n. 1, p. 537, 2022.

TORRENTE-VILARA, G.; QUEIROZ, L. DE; OHARA, W. M. Um breve histórico sobre o conhecimento da fauna de peixes do Rio Madeira. **LJ Queiroz, G. Torrente-**

Vilara, WM Ohara, THS Pires, J. Zuanon & CRC Doria. Peixes do Rio Madeira, São Paulo: Santo Antônio Energia SA, p. 19–45, 2013.

VALTONEN, E.; MARCOGLIESE, D. J.; JULKUNEN, M. Vertebrate diets derived from trophically transmitted fish parasites in the Bothnian Bay. **Oecologia**, v. 162, n. 1, p. 139, 2010.

VALERIO, N. D. et al. CO2 partial pressure and fluxes in the Amazon River plume using in situ and remote sensing data. **Continental Shelf Research**, v. 215, p. 104348, 2021.

VIEIRA, R. et al. Identification of Tannins in Amazon Biodiversity Plants: Application Possibilities as a Natural Coagulant. **Journal of Applied Pharmaceutical Sciences–JAPHAC**, v. 5, n. 1, p. 17–23, 2018.

VIRGILIO, L. R. et al. *Ergasilus colomesus* (Copepoda: Ergasilidae) parasitizing gills of *Colomesus asellus* (Tetraodontiformes: Tetraodontidae) in the western Brazilian Amazon. **Annals of Parasitology**, v. 67, n. 1, p. 123–127, 2021.

WARREN, P. H. Spatial and temporal variation in the structure of a freshwater food web. **Oikos**, v. 55, n. 3, p. 299–311, 1989.

WARD, N. D. et al. Degradation of terrestrially derived macromolecules in the Amazon River. **Nature Geoscience**, v. 6, n. 7, p. 530-533, 2013.

WATER RESOURCES EATLA. **Watersheds of the World** 2006.

WINEMILLER, K. O. Ontogenetic diet shifts and resource partitioning among piscivorous fishes in the Venezuelan ilanos. **Environmental Biology of fishes**, v. 26, n. 3, p. 177–199, 1989.

WINEMILLER, K. O., MCINTYRE, P. B., CASTELLO, L., FLUET-CHOUINARD, E., GIARRIZZO, T., NAM, S., Saenz, L. Balancing hydropower and biodiversity in the Amazon, Congo, and Mekong. ***Science***, v.351, n. 6269, 128-129, 2016.

WOOD, C. L. et al. Human impacts decouple a fundamental ecological relationship - The positive association between host diversity and parasite diversity. **Global change biology**, v. 24, n. 8, p. 3666–3679, 2018.

XENOPOULOS, M. A. et al. Scenarios of freshwater fish extinctions from climate change and water withdrawal. **Global change biology**, v. 11, n. 10, p. 1557–1564, 2005.

YAMADA, F. H. et al. A new species of *Aphanoblastella* Kritsky, Mendoza-Franco and Scholz, 2000 (Monogenea, Dactylogyridae) parasitic on heptapterid catfish (Siluriformes) in the Neotropical region. **Acta parasitologica**, v. 63, n. 4, p. 772–780, 2018.

CAPÍTULO I

Component communities and annual and seasonal variations of metazoan parasites in *Eleotris pisonis* (Gmelin, 1789) (Gobiiformes: Eleotridae) in the Amazon River, Brazil

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Comunidades componentes, variação anual e sazonal de parasitos metazoários em *Eleotris pisonis* (Gmelin, 1789) (Gobiiformes: Eleotridae) no Rio Amazonas, Brasil

Running Head: Parasites of *Eleotris pisonis*

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Abstract

Our hypothesis for this study was that annual and seasonal variations do not influence the structure of the component communities and the diversity of metazoan parasites of spinycheek sleeper (*Eleotris pisonis*) in the Amazon River, state of Amapá, Brazil. A total of 164 fish were collected between 2020 and 2021, from which 888 parasites were found. In 2020, five species of parasites were found (one Nematoda, one Digenea, one Acanthocephala, one Arachnida and one Crustacea); and in 2021, five species were also found (three Nematoda, one Digenea and one Crustacea). Larvae of *Contracaecum* sp.

were the dominant taxon throughout the study. The parasite species richness and Brillouin diversity index were higher in 2021, without significant differences between seasonal periods. Some component communities of parasites showed differences between years and between seasonal periods. These facts do not support the hypothesis that such variables would not influence the component communities of the parasites. Lastly, this report provides the first records of *Spirocamallanus inopinatus*, *Genarchella genarchella*, Acari, *Ergasilus* sp., *Neoechinorhynchus* sp., larvae of *Pseudoproleptus* sp. and larvae of *Contracaecum* sp. in *E. pisonis*.

Keywords: Aggregation, freshwater fish, infection parasites, seasonality.

Resumo

Nossa hipótese é que variações anuais e sazonais não influenciam a estrutura das comunidades componentes e a diversidade dos parasitos metazoários de amoré *Eleotris pisonis* do Rio Amazonas, estado do Amapá (Brasil). Um total de 164 peixes foram coletados de 2020 e 2021; e 888 parasitos foram encontrados nos peixes estudados. Em 2020, foram encontradas cinco espécies de parasitos (um Nematoda, um Digenea, um Acanthocephala, um Arachnida e um Crustacea); e em 2021, também foram encontradas cinco espécies três Nematoda, um Digenea e um Crustacea). Larvas de *Contracaecum* sp. foram o táxon dominante ao longo do estudo. A riqueza de espécies de parasitos e o índice de diversidade de Brillouin foram maiores em 2021, enquanto entre as estações sazonais não houve diferenças significativas. Algumas comunidades componentes de parasitos apresentaram diferenças entre os anos e entre os períodos sazonais. Esses fatos não corroboram a hipótese de que tais variáveis não influenciariam as comunidades componentes dos parasitos. Por fim, este é o primeiro registro de *Spirocamallanus inopinatus*, *Genarchella genarchella*, Acari, *Ergasilus* sp., *Neoechinorhynchus* sp., larvas de *Pseudoproleptus* sp. e larvas de *Contracaecum* sp. para *E. pisonis*.

Palavras-chave: Agregação, peixe de água doce, infecção por parasitos, sazonalidade.

Introduction

The spinycheek sleeper *Eleotris pisonis* (Gmelin, 1789) (Gobiiformes: Eleotridae) is widely distributed along the western margin of the central Atlantic Ocean, from Bermuda, Bahamas, South Carolina and the northern Gulf of Mexico in the United States at the northern end of its range to Brazil at the southern end of its range (Froese & Pauly, 2023). Spinycheek sleepers prefer freshwater estuarine tributaries with a salinity range from 0 to 36.6 psu (Darcy, 1980; Ray & Robins, 2016), and adult individuals are found in shallow water with a muddy or sandy bottom (Cervigón, 1994). They are carnivorous fish that feed on dipteran larvae and pupae, small crustaceans such as crabs and shrimps, and small fish. In addition, cannibalism has also been reported, but with low frequency (Perrone & Vieira, 1991). Their diet varies according to their state of sexual maturity and with the seasons. They are small-sized fish that reach sexual maturity at lengths of 5.7 cm for males and 4.3 cm for females. Spawning occurs in the dry season (Nordlie, 1981; Planquette et al., 2000; Santos et al., 2004). However, studies on the communities and infracommunities of metazoan parasites in this fish species are scarce.

The parasite communities of freshwater fish are important components of biodiversity, as they provide information about their environments such as water quality. In addition, they influence the productivity and food web of ecosystems (Negreiros et al., 2019a; Lehun et al., 2022). Therefore, studies on wild fish parasite communities can generate information on how these parasite-host-environment relationships can respond to ecological actions such as host diet, environmental characteristics, existence of infective stages in the ecosystem and annual and seasonal variations (Negreiros et al., 2019a; Hoshino & Tavares-Dias, 2019; Hoshino & Tavares-Dias, 2020; Lima et al., 2021, 2022, 2023; Lehun et al., 2022), and also the strategies used by different taxa of parasites in relation to these variables.

In temperate climate regions, it is known that water temperature and the behavior of wild fish populations play important roles in the dynamics of infection caused by parasites (Schade et al., 2016; Yang et al., 2016). Many aquatic invertebrates in these regions are potential intermediate, paratenic or definitive hosts for fish. These characteristics contrast with those of tropical regions, where aquatic ecosystems do not have extreme temperatures, which can fluctuate during the year.

Fish can harbor ectoparasites and endoparasites of different species with different life cycles. The species of parasites harbored are often related to the behavior and diet of the host. In this way, species occupying different niches are exposed to different parasites, thus potentially resulting in different patterns of infection (Tavares-Dias et al., 2014; Tavares-Dias & Oliveira, 2017; Baia et al., 2018; Cavalcante et al., 2020; Lima et al., 2022). Studies have shown that the rainy and dry seasons can influence the behavior of host fish, as well as the diversity of parasites and invertebrates in ecosystems. Thus, identifying the factors that can influence the structure of parasite communities is important for better understanding of the parasite ecology of host fish.

Abiotic and biotic factors in the environment may be related to temporal and seasonal variations in the structure of parasite communities in wild fish populations. Studies have shown that wild fish in the Amazon basin may present variations in their parasite communities according to temporal and seasonal periods (Hoshino & Tavares-Dias, 2019; Negreiros et al., 2019a; Hoshino & Tavares-Dias, 2020; Lima et al., 2023).

The tropical climate of the eastern Amazon region is mainly influenced by the Amazon rainforest, where the rainy season occurs from December to May and the dry season from June to November (Souza & Cunha, 2010). Rainfall levels influence the physicochemical characteristics of aquatic ecosystems during seasonal periods, consequently influencing fish populations and parasite communities. However, information on the effects of seasonality on the dynamics of infections in wild fish populations in the Amazon is scarce (Negreiros et al., 2019a; Hoshino & Tavares-Dias, 2020; Lima et al., 2022, 2023). For example, there are no studies on annual and seasonal variations in the communities of metazoan parasites in *E. pisonis*.

Organisms can respond similarly to abiotic and biotic factors, resulting in parallel patterns in community structures across taxonomic groups, called community concordance (Jackson & Harvey, 1993). However, temperature is an example of a variable that can influence both the immune system of host fish populations and the reproduction rate of parasites: innate immunity is more active at low temperatures, while adaptive immunity is suppressed at low temperatures (Ondračková et al., 2015). Thus, in temperate climate regions, seasonal variations in temperature can alter not only fish metabolism but also parasite communities (Ondračková et al., 2015; Rohlenová et al., 2011). In tropical climate regions, communities are expected to have a stable structure throughout the year (Dias & Tavares-Dias, 2015). Thus, the hypothesis of the present study was that annual and seasonal variations do not influence the parasite communities of *E. pisonis*. The aim of this study was to characterize the community of metazoan parasites in *E. pisonis* in the Amazon River and to study the effects of annual and seasonal variations on the structure of their parasite communities.

Materials and methods

Study area and fish collection

In bimonthly collections from January 2020 to November 2021, a total of 164 specimens of *E. pisonis* [7.77 ± 1.77 cm (4.1-12.2 cm) and 7.98 ± 6.10 g (0.8-38.9 g)] were collected from the Amazon River, near to Santana Island, municipality of Santana, state of Amapá, Brazil (Figure 1). These fish were collected using gillnets of different sizes and meshes (15, 20, 25, 30 and 35 mm between knots), cast nets (20 mm mesh between knots) and hand lines. The fish were then sacrificed using the medullary transection method, preserved in 10% formalin and transported to the Aquaculture and Fisheries Laboratory of Embrapa Amapá, Macapá, state of Amapá, Brazil, in order to analyze their parasites.

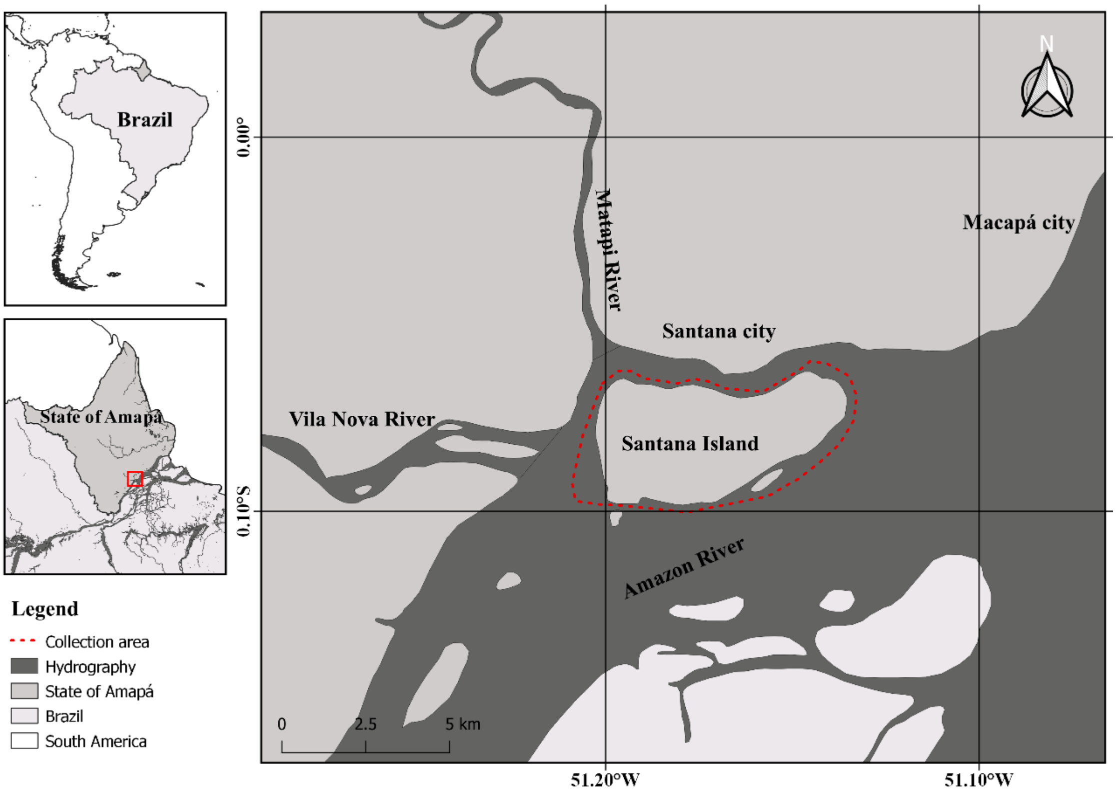

Figure 1. Collection site of *Eleotris pisonis* in the Amazon River, state of Amapá, northern Brazil.

The water quality parameters of electrical conductivity, pH and total dissolved solids were measured at the fish sampling sites using a multiparameter device (AKSO, model COMBO5-02-1016). Dissolved oxygen levels and water temperature were measured using an oximeter (Instrutherm, model MO-900). Rainfall data from the fish collection region were obtained from the Center for Hydrometeorology and Renewable Energy (NHMET) of the Institute of Scientific and Technological Research of the State of Amapá (IEPA).

Parasite analysis procedures

In the laboratory, the total length (cm) and weight (g) of the fish were measured, and necropsies were performed to collect metazoan parasites. The mouth, opercular cavity, gills and fins were examined with the aid of a stereomicroscope to collect ectoparasites, while internal organs such as the gastrointestinal tract and viscera were examined for endoparasites. The parasites found were preserved in 70% ethyl alcohol in order to subsequently make permanent slides, following previous recommendations (Eiras et al., 2006).

Data analysis

The parasite indices of prevalence, mean intensity and mean abundance of infracommunities were calculated (Bush et al., 1997). The dispersion index (DI) was determined and the DI significance was calculated using *d*-statistics (Ludwig et al., 1988). The Poulin discrepancy index (D) was calculated using the Quantitative Parasitology 3.0 software. To describe the parasite community, the species richness, Brillouin diversity index (*HB*), evenness (*E*) and Berger-Parker dominance index (*d*) were calculated using the Diversity software. The alternation of parametric and non-parametric tests was done in accordance with the normality tests for each set of data analyzed here. Spearman's correlation coefficient (*rs*) was used to evaluate possible correlations of host body length and weight with parasite abundance, species richness and Brillouin diversity index (Zar, 2010).

Fish weights and lengths were used to determine the relative condition factor (Kn) (Le Cren, 1951) for each year (2020 and 2021), for both the rainy and the dry season. To determine whether the total length (cm), weight (g), relative condition factor (Kn), physical-chemical parameters, prevalence, abundance, species richness, uniformity, Brillouin diversity index and Berger-Parker dominance index followed patterns of normal distribution and homoscedasticity, the Shapiro-Wilk and Bartlett tests were used, respectively. To ascertain whether there was any difference in the prevalence of parasites between 2020 and 2021, and between the seasonal periods (rainy and dry), the chi-square test (χ^2) with Yates correction was used. To determine any differences in the abundance of parasites, the Mann-Whitney test (*U*) was used (Zar, 2010). To estimate any differences in diversity indices (species richness, evenness, Brillouin diversity index and Berger-Parker dominance index) among the metazoan parasites between 2020 and 2021 and between seasonal periods, the Kruskal-Wallis test was used, followed by the Dunn test. These analyses were carried out in the R software (R Core Team, 2021).

Permutation Analysis of Variance (PERMANOVA) was used to detect any differences in the parasite communities between the years 2020 and 2021 and between the rainy and dry seasons. Annual and seasonal variables were adjusted by means of principal coordinate analysis (PCoA) ordering based on the Bray-Curtis distance, using the envfit function from the vegan package (Oksanen et al., 2020), and p-values were calculated using the permutation test (number of permutations = 999) in the R software, version 4.2.2, using the vegan package (Oksanen et al., 2020).

To analyze how differences in sampling effort might influence the results, we plotted species accumulation curves (observed and expected) for 2020 and 2021 and for the rainy and dry seasons. To compare the diversity between the years studied and between the rainy and dry seasons, diversity profiles were generated based on Hill numbers (Hill, 1973), which on a q scale incorporates species richness ($q = 0$), exponential value ($q = 1$) and the inverse of the Shannon-Weaver and Simpson indices ($q = 2$). An increase in the q scale represents an increase in the weight given to common species in the diversity indices (Magurran & McGill, 2011), thus making it possible to identify how diversity is ordered and whether changes occur in relation to dominant or rare species (Tóthmérész, 1995). The diversity profile was built using the 'renyi' function from the vegan package (Oksanen et al., 2020). These analyses were carried out in the R software (R Core Team, 2021).

Results

Physicochemical parameters in the years 2020 and 2021 and seasonal periods

The pH and total dissolved solids measured in the years 2020 and 2021 showed significant differences ($t = 2.4242$, $p < 0.05$; $U = 2.0$, $p < 0.05$), but the other parameters analyzed did not show any significant differences between the years (Table 1). Between the seasonal periods (rainy and dry), there were significant differences in precipitation and temperature, respectively ($t = 3.9908$, $p< 0.001$; $t = 6.8690$, $p < 0.001$), but the other parameters analyzed did not show any significant differences (Table 1).

Table 1. Physicochemical parameters of water from the Amazon River, eastern Amazon region, Brazil, during periods of collection of *Eleotris pisonis*.

			Tests	
Parameters	**2020**	**2021**	***t***	***U***
Rainfall (mm)	195.7 ± 119.5	231.5 ± 153.2	-0.8197	-
Temperature (°C)	29.0 ± 1.0	29.1 ± 1.1	0.0284	-
Dissolved oxygen (mg/L)	5.8 ± 0.2	4.2 ± 1.8	2.2699	-
pH	7.1 ± 0.2	6.8 ± 0.3	2.4242*	-
Total dissolved solids (mg/L)	56.8 ± 10.7	107 ± 60.7	-	2.0*
Electrical conductivity (µS/cm)	90.4 ± 12.2	148.1 ± 111.4	-	10.5
	Rainy season	**Dry season**		
Rainfall (mm)	311.5 ± 106.7	131.0 ± 116.3	3.9908**	-
Temperature (°C)	28.3 ± 0.3	30.0 ± 0.5	6.8690**	-
Dissolved oxygen (mg/L)	5.2 ± 0.8	4.6 ± 2.12	0.4952	-
pH	6.9 ± 0.2	6.9 ± 0.3	-0.2389	-
Total dissolved solids (mg/L)	72.6 ± 34.6	91.4 ± 62.5	-	16.00
Electrical conductivity (µS/cm)	98.8 ± 61.4	139.6 ± 98.7	-	15.50

t: t test; *U:* Mann-Whitney test; *p < 0.05; **p < 0.001

Component communities of metazoan parasites

Eleotris pisonis was found to be parasitized by larvae of *Contracaecum* Railliet & Henry, 1912; *Pseudoproleptus* Khera, 1953 and *Spirocamallanus inopinatus* Travassos, Artigas & Pereira, 1928 (Nematoda); *Genarchella genarchella* Travassos, Artigas & Pereira, 1928 (Digenea); *Neoechinorhynchus* Stiles & Hassall, 1905 (Acanthocephala); *Ergasilus* Nordman, 1832 (Ergasilidae); and Acari Krantz, 1978 (Arachnida) (Voucher: 171P-177P-IEPA). *Contracaecum* sp. was the dominant species, and no parasite was found in the mouth or fins of the hosts (Table 2).

Table 2. Spatial distribution of metazoan parasites of *Eleotris pisonis* in the Amazon River, eastern Amazon region, Brazil

Parasite species	**P (%)**	**MA ± SD**	**MI ± SD**	**TNP**	**FD (%)**	**SI**
Nematoda						
Contracaecum sp. (larvae)	7.9	0.1± 0.5	0.6 ± 1.1	22	2.4	Intestine
Contracaecum sp. (larvae)	48.7	2.1 ± 4.8	0.2 ± 6.3	354	39.8	Stomach
Contracaecum sp. (larvae)	28.0	0.9 ± 2.1	0.3 ± 2.9	147	16.5	Abdominal cavity
Contracaecum sp. (larvae)	0.6	0.01 ± 0.08	1 ± 0	1	0.1	Liver
Pseudoproleptus sp. (larvae)	0.6	0.01 ± 0.08	1 ± 0	1	0.1	Intestine
Spirocamallanus inopinatus	0.6	0.01 ± 0.08	1 ± 0	1	0.1	Abdominal cavity
Digenea						
Genarchella genarchella	21.9	0.9 ± 2.5	0.2 ± 3.8	155	17.4	Pharynx
Genarchella genarchella	10.9	0.2 ± 1.3	0.4 ± 3.3	45	5.0	Intestine
Genarchella genarchella	16.4	0.5 ± 1.5	0.3 ± 2.6	84	9.4	Stomach
Genarchella genarchella	1.8	0.06 ± 0.5	0.3 ± 2.5	10	1.1	Abdominal cavity
Genarchella genarchella	0.6	0.04 ± 0.4	0.1 ± 0	6	0.6	Pyloric cecum
Acanthocephala						
Neoechinorhynchus sp.	0.6	0.01 ± 0.08	1.0 ± 0	1	0.1	Intestine
Neoechinorhynchus sp.	1.2	0.02 ± 0.17	0.6 ± 0.7	3	0.3	Stomach
Crustacea						
Ergasilus sp.	17.0	0.3 ± 1.4	0.4 ± 3.06	57	6.4	Gills
Arachnida						
Acarina gen. sp.	0.6	0.01 ± 0.08	1.0 ± 0	1	0.1	Gills

P: prevalence; MA: mean abundance; MI: mean intensity; TNP: total number of parasites; FD: frequency of dominance; SI: sites of infection; SD: standard deviation.

The parasite community showed low species richness, low HB and predominance of endoparasites (Table 3). Only *Contracaecum* sp., *G. genarchella* and *Ergasilus* sp. presented prevalence above 10%, and these were analyzed separately. The hosts were predominantly infected by only one species of parasite. Larvae of *Contracaecum* sp. (DI= 4.83, *d* = 21.59 and D = 0.87), *G. genarchella* (DI = 5.03, *d* = 22.49 and D = 0.74) and *Ergasilus* sp. (DI = 2.07, *d* = 6.77 and D = 0.87) showed highly aggregated distribution patterns.

Table 3. Component community of metazoan parasites in *Eleotris pisonis* from the Amazon River, state of Amapá, in Brazil.

Paramenters	Values
Number of fish examined	164
Total number of parasites	888
Total prevalence (%) of parasites	82.3
Percentage of endoparasites (%)	93.4
Percentage of ectoparasites (%)	6.5
Percentage of larvae	59.1
Species richness of parasites	1.3 ± 0.9
Brillouin diversity index	0.2 ± 0.2
Evenness	0.8 ± 0.4

Species richness (*rs* = 0.28, p = 0.0004 and *rs* = 0.28, p = 0.0004), Brillouin diversity index (*rs* = 0.25, p = 0.0013 and *rs* = 0.24, p = 0.0013), abundance of *Contracaecum* sp. (*rs* = 0.46, $p \leq 0.0001$ and *rs* = 0.47, $p \leq 0.0001$), abundance of *Ergasilus* sp. (*rs* = 0.27, p = 0.0003 and *rs* = 0.29, p = 0.0001) showed weak but significant positive correlations with host length and weight, respectively.

Annual variation of metazoan parasites

Host fish collected in 2020 had a mean length of 8.0 ± 1.8 cm and those collected in 2021 had a mean length of 7.5 ± 1.6 cm, which was a significant difference (*t* = 2.02, p = 0.04). Fish collected in 2020 had a mean weight of 9.2 ± 7.1 g and those collected in 2021 had a mean weight of 6.8 ± 4.7 g, which was also a significant difference (*U* = 2745.5, p = 0.04). The relative condition factor (Kn) of hosts collected in 2020 (Kn = 1.02 ± 0.39) and 2021 (Kn = 0.85 ± 0.67) were significantly different (*U* = 2434.0, p = 0.0013).

Among all the hosts examined, a total of 368 parasites were recovered in 2020, while 520 were recovered in 2021. In 2020, hosts were predominantly infected by one parasite species, while in 2021 they were infected by one or two parasite species (Figure 2). In 2020, the total prevalence of parasites was 75.3% and in 2021 it was 88.5%. In both years, *Contracaecum* sp. was the dominant parasite, with higher prevalence in 2021. However, between these two years, there were significant differences in the prevalence and mean abundance of *G. genarchella* (Table 4).

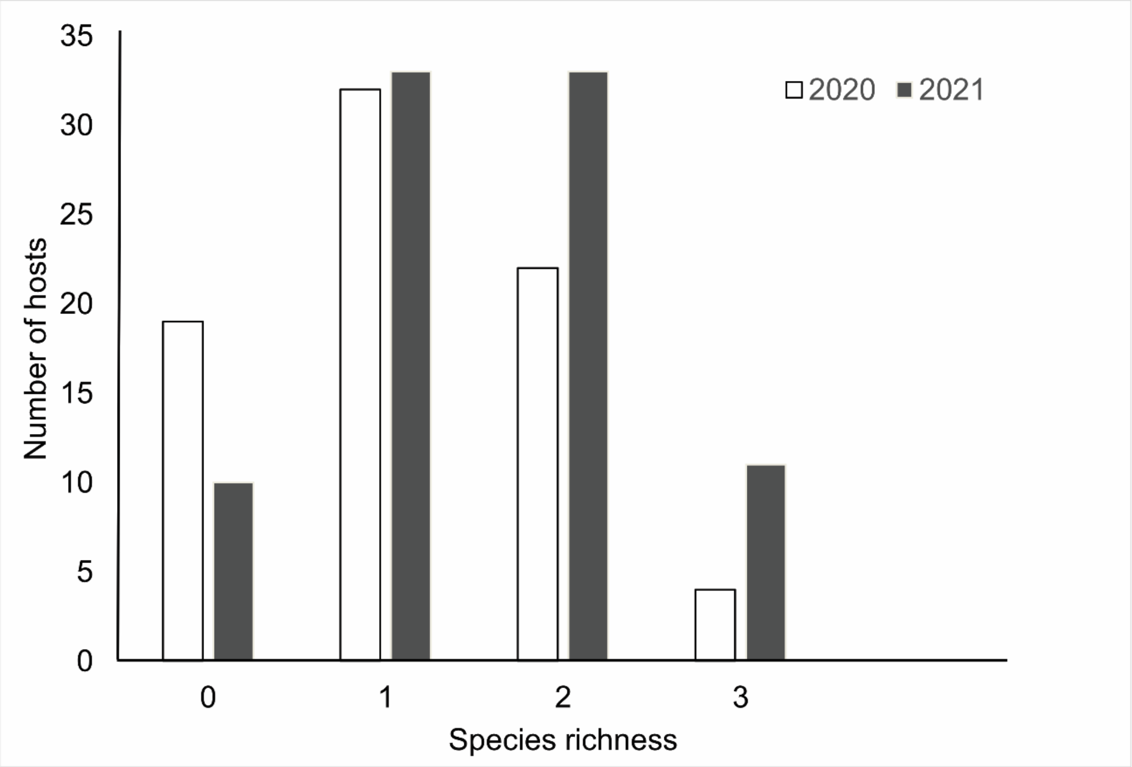

Figure 2. Species richness of metazoan parasites in *Eleotris pisonis* collected from the Amazon River, Brazil, over a two-year period.

Table 4. Metazoan parasites in *Eleotris pisonis* in the Amazon River, eastern Amazon region, Brazil, collected over a two-year period.

		2020 (n = 77)			**2021 (n = 87)**				
Parasite species	**Infection sites**	**P (%)**	**MA ± SD**	**TNP**	**P (%)**	**MA ± SD**	**TNP**	χ^2	***U***
Contracaecum sp. (larvae)	Intestine, stomach abdominal cavity and liver	63.6	3.6 ± 6.9	281	70.1	2.7 ± 3.6	243	0.7	3200.0
Pseudoproleptus sp. (larvae)	Intestine	0	0	0	1.1	0.01 ± 0.1	1	-	-
Spirocamallanus inopinatus	Abdominal cavity	0	0	0	1.1	0.01± 0.1	1	-	-
Genarchella genarchella	Pharynx, intestine, stomach, abdominal cavity and cecum pyloric	24.6	0.6 ± 1.7	47	65.5	2.9 ± 3.7	253	27.4**	1804.5**
Neoechinorhynchus sp.	Intestine and stomach	3.9	0.05 ± 0.2	4	0	0	0	-	-
Acarina gen. sp.	Gills	1.3	0.01 ± 0.11	1	0	0	0	-	-
Ergasilus sp.	Gills	24.6	0.4 ± 1.9	35	13.7	0.2 ± 0.7	22	0.3	3142.5

P: prevalence; MA: mean abundance; SD: standard deviation; TNP: total number of parasites; χ^2: chi-square test; U: Mann-Whitney test; *$p < 0.05$; **$p < 0.00$

The PCoA axes 1 and 2 were responsible for 65% of the total variation in abundance composition in 2020 and 2021. Although the PCoA showed overlapping and species sharing during these years, there were significant differences (PERMANOVA: F = 7.7445, $p \leq 0.001$) in the parasite infracommunities, influenced mainly by variations in the abundance of *Contracaecum* sp. ($R^2 = 0.4275$, $p \leq 0.001$), *G. genarchella* ($R^2 = 0.3443$, $p \leq 0.001$) and *Ergasilus* sp. ($R^2 = 0.0701$, $p = 0.002$) (Figure 3).

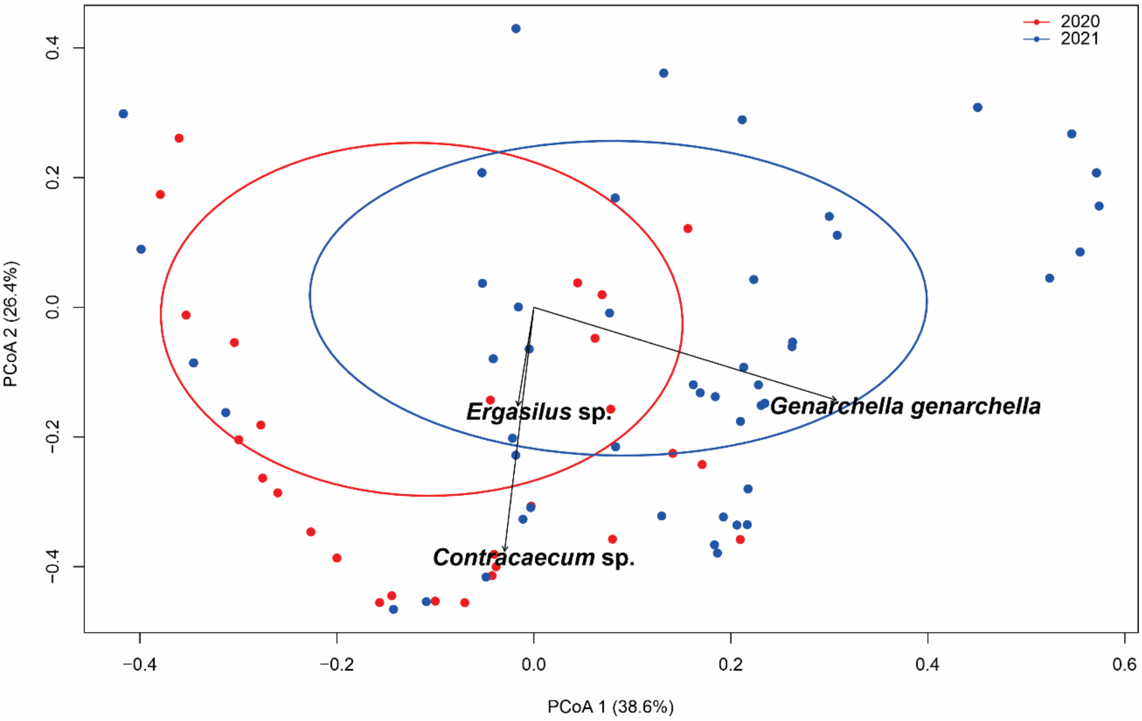

Figure 3. Principal coordinate analysis (PCoA) using a Bray-Curtis distance matrix for communities of metazoan parasites of *Eleotris pisonis* in the Amazon River, state of Amapá, Brazil, during 2020 and 2021. The percentage of the variation explained by the principal coordinates plotted is indicated on the axes.

Parasite species richness ($\chi^2 = 7.23$, $p = 0.0071$) and Brillouin diversity index ($\chi^2 = 4.6729$, $p = 0.0306$) were higher in 2021, but evenness ($\chi^2 = 0.0069$, $p = 0.9335$) and Berger-Parker dominance index ($\chi^2 = 0.5514$, $p = 0.4577$) showed no differences between the years studied (Figure 4). The species accumulation curve showed that the number of hosts found in 2020 was sufficient for the number of parasite species collected to reach representativeness, however, the 2021 species accumulation curve did not demonstrate a tendency towards stability, not being enough so that the number of parasite species collected would reach representativeness, after collecting the samples (Figure 5).

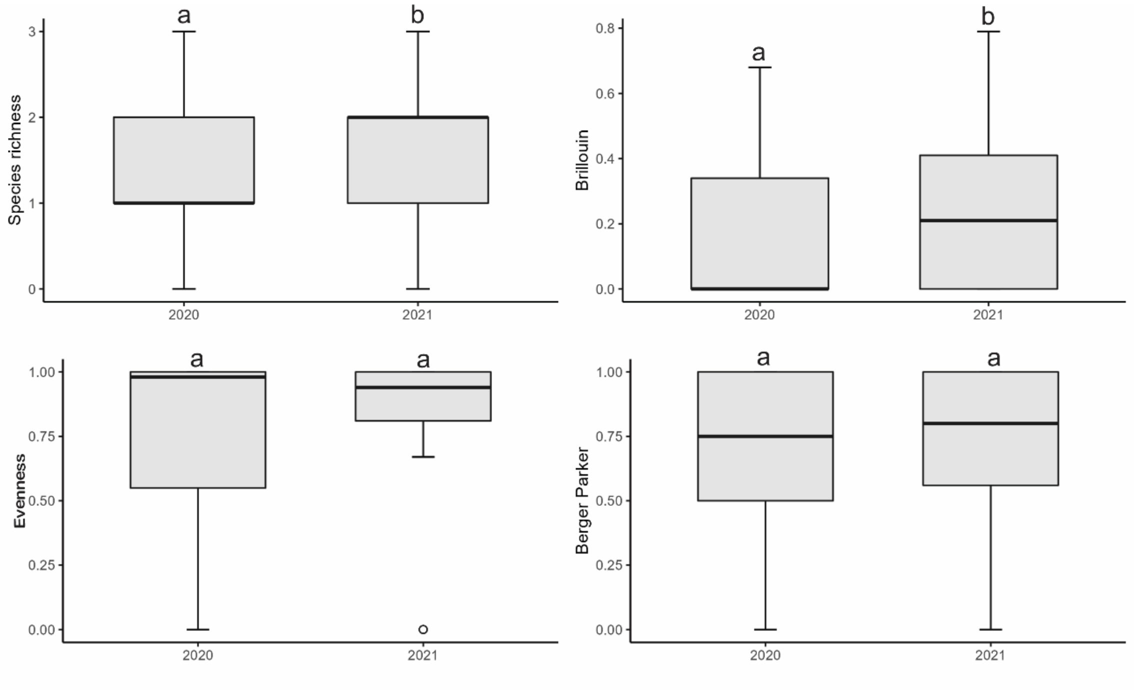

Figure 4. Diversity parameters for metazoan parasites in *Eleotris pisonis* in the Amazon River, eastern Amazon region, Brazil, collected in 2020 and 2021. (Box plots show medians, interquartile ranges, minimum-maximum ranges and outliers. Different letters indicate differences between the medians according to Dunn's test ($p < 0.001$).

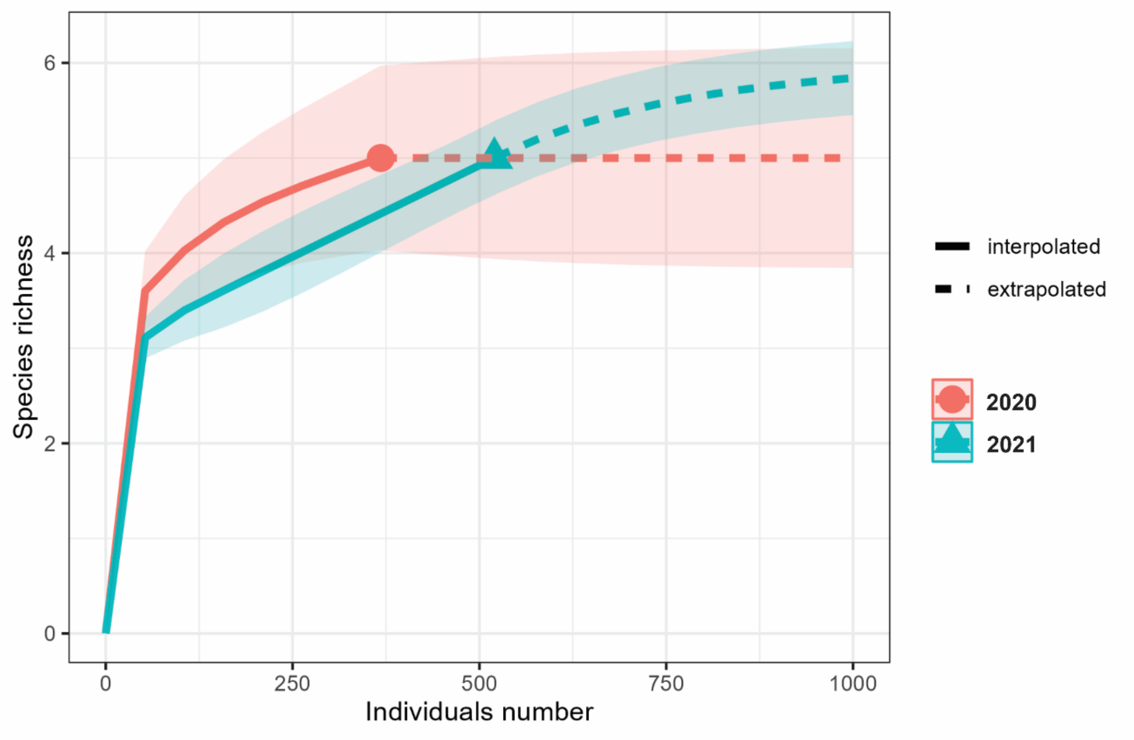

Figure 5. Species accumulation curve for metazoan parasites in *Eleotris pisonis* in the Amazon River, state of Amapá, Brazil, collected in 2020 and 2021.

Hill's diversity profile demonstrated that parasite species richness was similar between 2020 and 2021 (0 on the q scale). However, the Shannon-Weaver index (1 on the q scale) was higher in 2021, suggesting that the increased importance of common species influenced the differences detected by the diversity index between years. The Simpson index (2 on the q scale) showed greater dominance in 2021. These results suggest that species richness was similar between the years, but the other indices that use abundance values showed that there was greater diversity in 2021 than in 2020 (Figure 6).

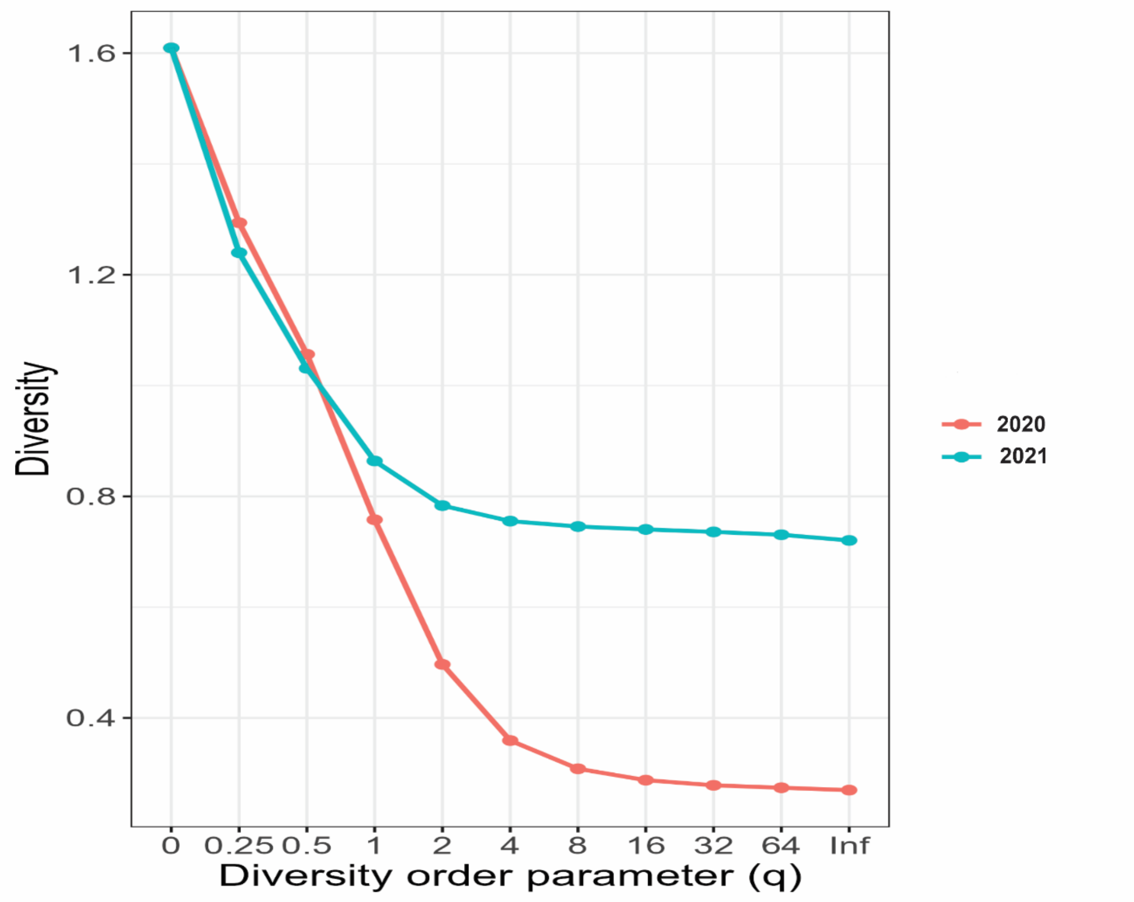

Figure 6. Hill diversity profile for diversity of parasites in *Eleotris pisonis* in 2020 and 2021. On the horizontal axis (left) rare species become more important, while towards the opposite side (right) there is more evenness of proportions. Some indices can be observed on the horizontal axis: 0 = species richness; 1 = Shannon index; 2 = Simpson's index; Inf = Berger-Parker index.

Seasonal variation of metazoan parasites

The fish collected in the rainy season had a mean length of 8.3 ± 1.7 cm, and those collected in the dry season had a mean length of 7.1 ± 1.5 cm, which was a significant difference ($t = 4.9091$, $p \leq 0.0001$) between these seasonal periods. The fish collected in the rainy season weighed 10.1 ± 6.7 g, while those collected in the dry season weighed 5.5 ± 4.0 g, which was also a significant difference ($U = 1751.50$; $p \leq 0.0001$) between these seasonal periods. The relative condition factor of the hosts collected in the rainy season ($Kn = 1.18 \pm 1.58$) and in the dry season ($Kn = 1.07 \pm 0.85$) were significantly different ($U = 2477.00$; $p = 0.002$).

Totals of 495 parasites were collected in the rainy season and 383 in the dry season. In both the rainy and the dry season, hosts were predominantly infected by one parasite species (Figure 7). In the rainy season, 80.6% of the fish were parasitized; while in the dry season, 85.5% of the fish were parasitized. *Contracaecum* sp. was the dominant species in both seasonal periods. There was higher prevalence and abundance of *G. genarchella* in the dry season, while *Ergasilus* sp. had higher prevalence in the rainy season. The other species did not show any significant differences between seasonal periods (Table 5).

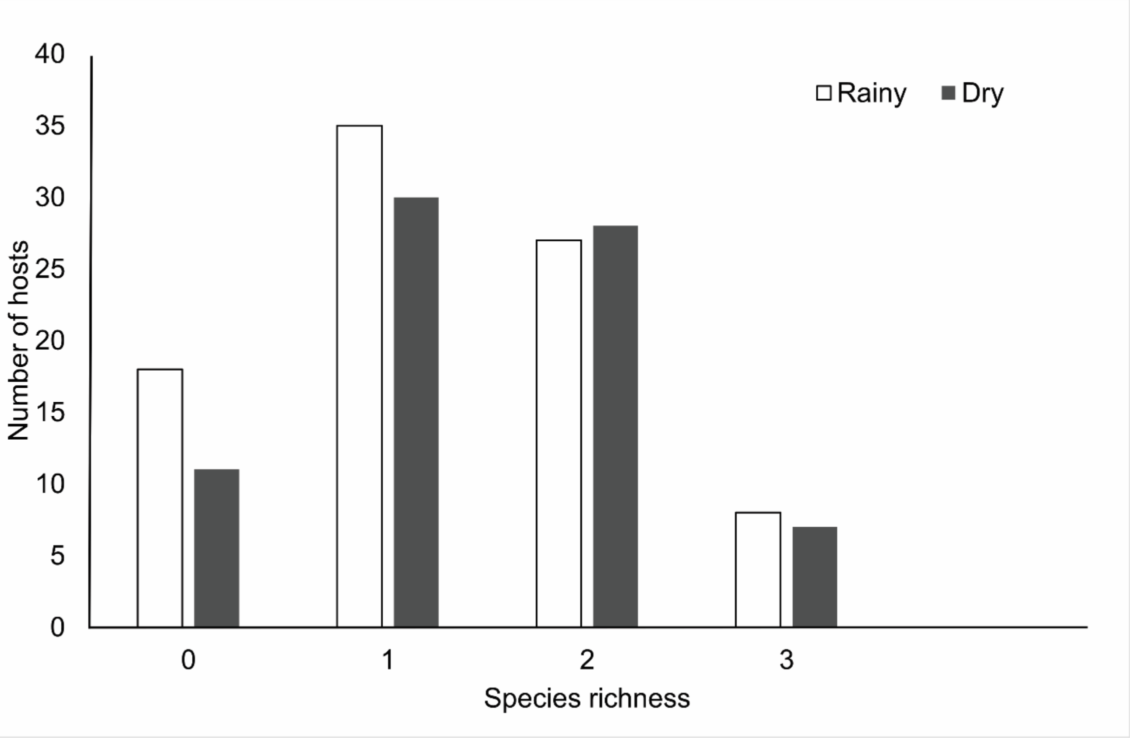

Figure 7. Species richness of metazoan parasites in *Eleotris pisonis* in the Amazon River during the rainy and dry seasons.

Table 5. Metazoan parasites in *Eleotris pisonis* in the Amazon River, eastern Amazon region, Brazil, collected during the rainy and dry seasons.

		Rainy (n = 88)			**Dry (n = 76)**				
Parasite species	**Infection sites**	**P (%)**	**MA ± SD**	**TNP**	**P (%)**	**MA ± SD**	**TNP**	χ^2	***U***
Contracaecum sp. (larvae)	Intestine, stomach abdominal cavity and liver	69.3	3.6 ± 6.6	323	64.4	2.6 ± 3.7	203	0.43	3181.5
Pseudoproleptus sp. (larvae)	Intestine	0	0	0	1.3	0.01 ± 0.1	1	-	-
Spirocamallanus inopinatus	Abdominal cavity	0	0	0	1.3	0.01 ± 0.1	1	-	-
Genarchella genarchella	Pharynx, intestine, stomach, abdominal cavity and cecum pyloric	31.8	1.3 ± 2.8	119	63.1	2.3 ± 3.4	181	17.6**	2354.5**
Neoechinorhynchus sp.	Intestine and stomach	3.4	0.05 ± 0.2	4	0	0	0	-	-
Acarina gen. sp.	Gills	1.1	0.5 ± 1.9	1	0	0	0	-	-
Ergasilus sp.	Gills	22.7	0.01 ± 0.1	48	10.5	0.1 ± 0.3	9	5.9*	2912.0

P: prevalence; MA: mean abundance; SD: standard deviation; TNP: total number of parasites; χ^2: chi-square test; *U*: Mann-Whitney test; *p < 0.05; **p < 0.00

Axes 1 and 2 of the PCoA results (Figure 8) were responsible for 94.3% of the total variation in the composition of parasite abundance in the rainy and dry seasons. Despite the overlap due to sharing of some species, the PCoA showed that there were significant differences (PERMANOVA: F = 6.6655; $p \leq 0.003$) in the parasite infracommunities between the seasonal periods, mainly influenced by variations in the abundance of *G. genarchella* ($R^2 = 0.7782$; $p \leq 0.001$), larvae of *Contracaecum* sp. ($R^2 = 0.6065$; $p \leq 0.001$) and *Ergasilus* sp. ($R^2 = 0.1533$; $p \leq 0.001$) (Figure 8).

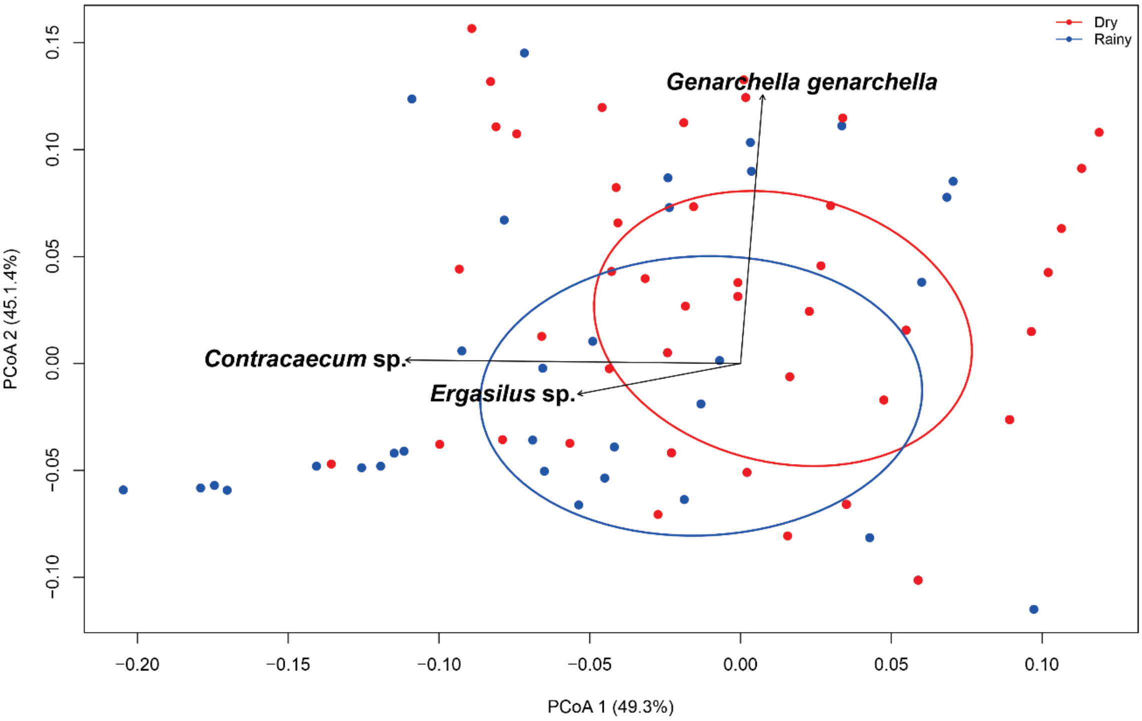

Figure 8. Principal coordinate analysis (PCoA) using a Bray-Curtis distance matrix for communities of metazoan parasites of *Eleotris pisonis* in the Amazon River, eastern Amazon region, Brazil, during the rainy and dry seasons. The percentage of the variation explained by the principal coordinates plotted is indicated on the axes.

Parasite species richness ($\chi^2 = 0.8869$, p = 0.346), Brillouin diversity index ($\chi^2 = -1.27$, p = 0.203), evenness ($\chi^2 = -1.76$, p = 0.0778) and Berger-Parker dominance ($\chi^2 = 0.424$, p = 0.672) did not show any significant differences between seasonal periods (Figure 9). The parasite species accumulation curve did not show any differences in species richness between the seasonal periods. This showed that the representativeness of the species collected, regarding parasite richness, was not affected by the sampling effort between the seasonal periods, given that the two curves tended towards stability (Figure 10).

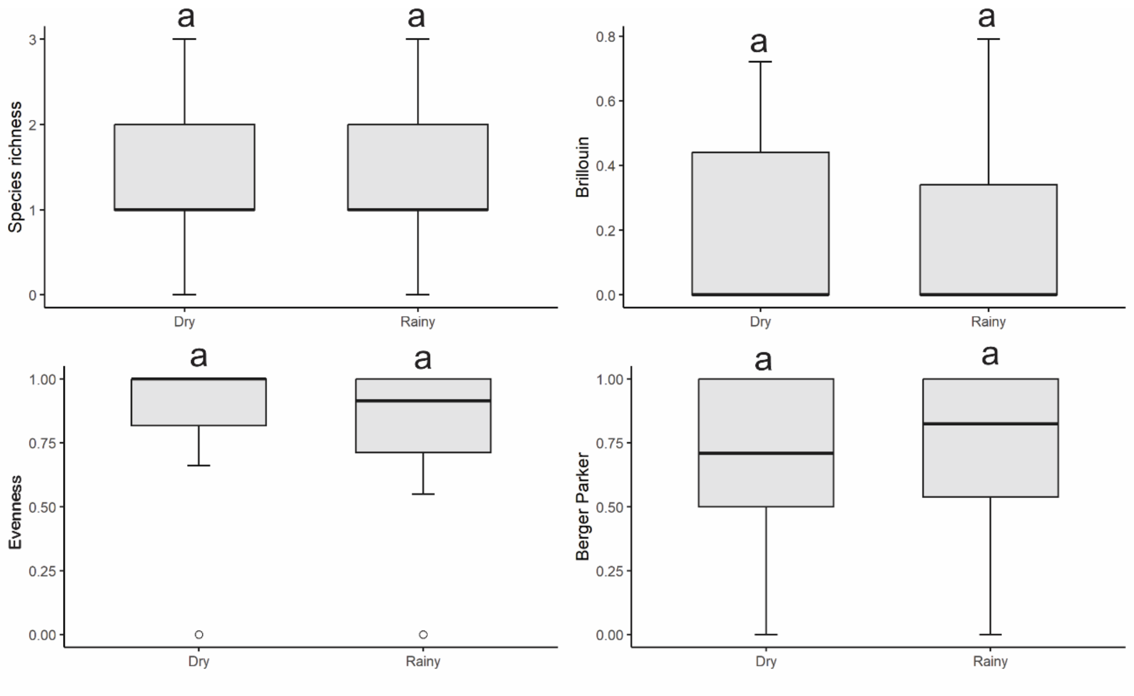

Figure 9. Diversity parameters of metazoan parasites in *Eleotris pisonis* in the Amazon River, eastern Amazon region, Brazil, during the rainy and dry seasons. (Box plots represent medians, interquartile ranges, minimum–maximum ranges and outliers). Different letters indicate differences between the medians according to Dunn's test ($p < 0.001$)

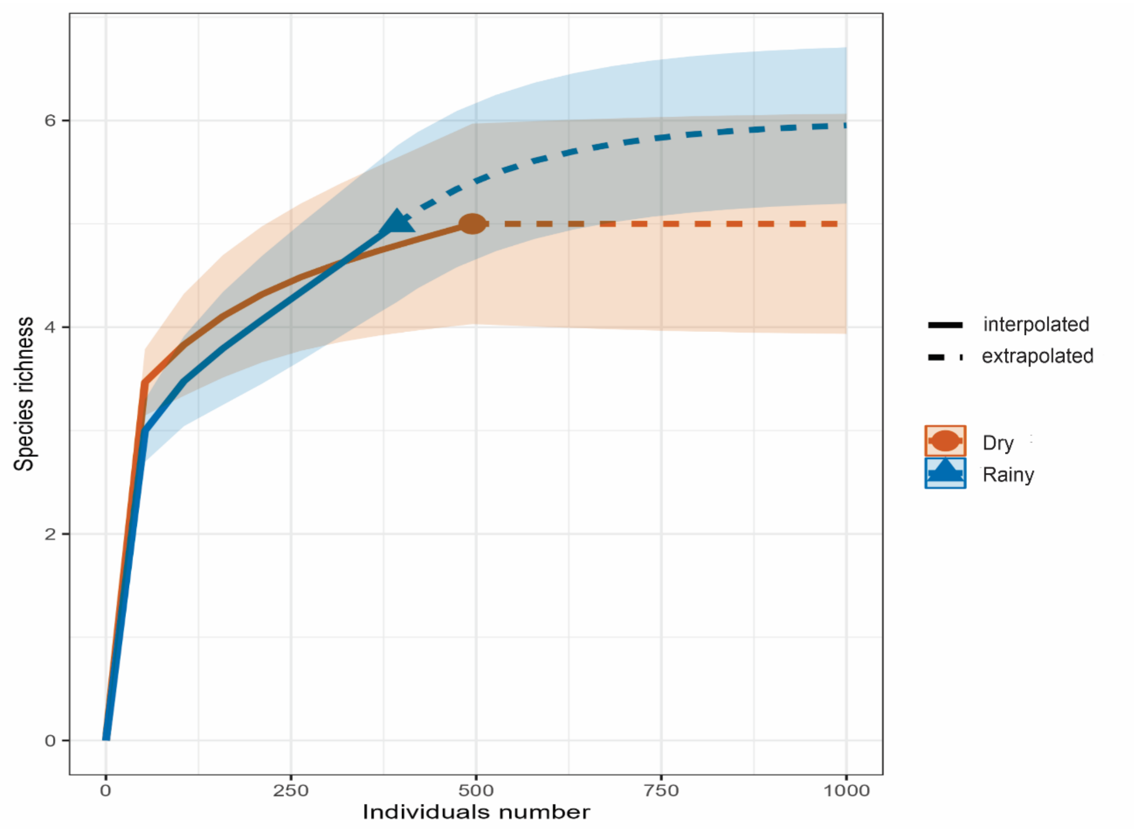

Figure 10. Species accumulation curve for metazoan parasites in *Eleotris pisonis* in the Amazon River, eastern Amazon region, Brazil, during the rainy and dry seasons.

Hill's diversity profile showed that parasite species richness did not differ between the seasons (rainy and dry) (0 on the q scale). However, the Shannon-Weaver index (1 on the q scale) for the dry season was slightly higher, suggesting that the increased importance of common species influenced the differences detected by the diversity index between seasonal periods. On the other hand, the Simpson index (2 on the q scale) for dominance was higher in the rainy season. These results suggest that species richness was also similar between seasonal periods. However, the other indices that use abundance values alternated between seasonal periods (Figure 11).

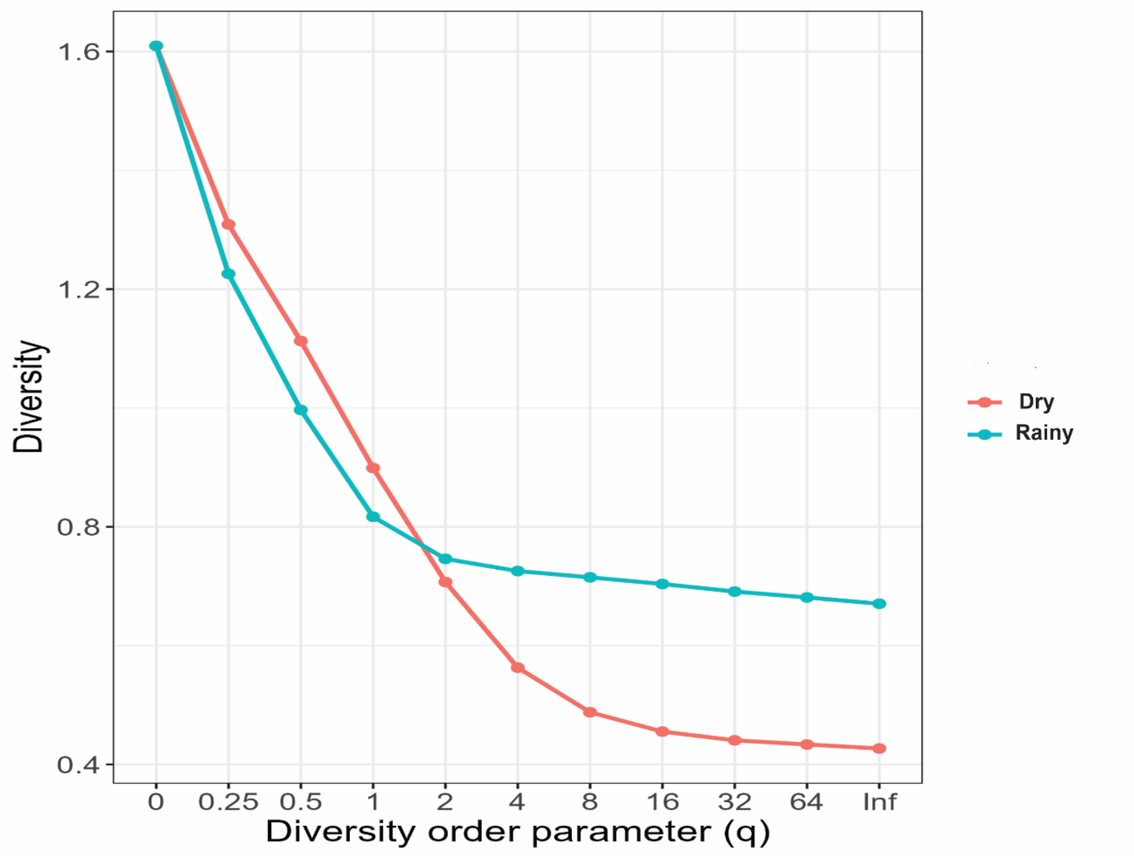

Figure 11. Hill diversity profile for parasite diversity in the rainy and dry seasons in *Eleotris pisonis*. On the horizontal axis (left), rare species become more important, while on the opposite side (right) there is more evenness of proportions. Some indices can be observed on the horizontal axis: 0 = species richness; 1 = Shannon index; 2 = Simpson's index; Inf = Berger-Parker index.

Discussion

Component communities of metazoan parasites

The component community of metazoan parasites in *E. pisonis* in the Amazon River comprised three species of Nematoda, one Digenea, one Acanthocephala, one Crustacea and one Arachnida. However, in *E. pisonis* collected from the Guadeloupe islands in the Caribbean, only *Cucullanus caballeroi* Petter, 1976, was found (Petter et al., 1977); in hosts from the mouth of Keelung River, Taiwan, the acanthocephalans *Brentisentis uncinus* Leotta et al., 1982 and *Gorgorhynchus satoi* Morisita, 1937, were found (Leotta et al., 1982); and in *E. pisonis* in the Matapi River, a tributary of the Amazon River, no crustacean parasite was found (Neves & Tavares-Dias, 2019). These differences in the component communities and richness of host species can be attributed to differences in the environment, diet, seasonal period and sampling effort. Regarding sampling effort, the number of specimens of *E. pisonis* sampled in the present study was higher than in the previous studies cited above. In addition, new records of metazoan parasites for *E. pisonis* are reported in the present study.

The presence of these endoparasites in *E. pisonis* may be related to its carnivorous feeding habit (Froese & Pauly, 2023), since infections by endoparasites have mainly been related to the diet of the host fish (Oliveira et al., 2017; Negreiros et al., 2019a,b; Lima et al., 2021, 2022, 2023). However, infection by the ectoparasite *Ergasilus* sp. may be related to the specificity of the parasite and the reproductive period, as observed in *Colomesus asellus* Thatcher & Boeger, 1983, from the Môa River, in the eastern Brazilian Amazon region (Virgilio et al., 2021) and from the Amazon River (Lima et al., 2023). Infections by mites may be associated with environmental conditions, host stress levels and accidental infections in the environment (Olmeda et al., 2011; Lizama et al., 2013).

Highly aggregated dispersion patterns were found for *Contracaecum* sp., *G. genarchella* and *Ergasilus* sp. in *E. pisonis*. These distribution patterns are common among freshwater fish species living in different natural environments (Tavares-Dias & Neves, 2017; Oliveira et al., 2017; Neves et al., 2021). Aggregated dispersion has been linked to the genetic variability of the host population, decreased interspecific competition between parasites, decreased damage to the host and environmental factors (Poulin, 2011; Tavares-Dias & Oliveira, 2017).

The presence of larvae of *Contracaecum* sp. and *Pseudoproleptus* sp. was an indication that *E. pisonis* is an intermediate host due to its carnivorous feeding habit (Bartolette et al., 2018; Froese & Pauly, 2023). Shrimps, gastropods and crabs were found in the stomach of *E. pisonis* (personal observation), thus indicating that this host occupies a superior position in the food web. Larvae of *Contracaecum* sp. were the dominant species, presenting a higher level of infection than that of other nematodes such as *Pseudoproleptus* sp. and *S. inopinatus*. Infection by *Contracaecum* sp. was also reported in *Pimelodus ornatus* Kner, 1958 (Lima et al., 2021, 2022) and *C. asellus* (Lima et al., 2023), collected in the same study area from which the specimens of *E. pisonis* were collected. However, there have been reports of occurrence of *Contracaecum* sp. in other species of Amazonian fish such as *Hemibrycon surinamensis* Géry, 1962 (Hoshino et al., 2014), *Metynnis lippincottianus* Cope, 1879 (Hoshino & Tavares-Dias, 2014), *Astronotus ocellatus* Agassiz, 1831 (Tavares-Dias & Neves, 2017) and *Astronotus crassipinnis* Heckel, 1840 (Santos et al., 2018).

Spirocamalanus inopinatus is a nematode with wide geographic distribution and with records in different fish species in Brazil (Neves et al., 2020), but the present study provided the first record of this nematode in *E. pisonis.* However, the infection levels in *E. pisonis* were low in comparison with those reported in *Pimelodus blochii* Valenciennes 1840 in the Iaco and Acre Rivers (Negreiros et al., 2018, 2019b), *Pimelodus maculatus* Lacepéde, 1803, in the Guandu River (Albuquerque et al., 2008) and *P. ornatus* in the Amazon River (Lima et al., 2021). These findings demonstrate that *S. inopinatus* is a common nematode in Amazonian fish, but with low levels of infection in *E. pisonis*.

Larvae of *Pseudoproleptus* sp. were found in *E. pisonis*, and this paratenic nematode has also been well documented in several species of Amazonian fish (Melo et al., 2011; Tavares-Dias et al., 2014; Oliveira et al., 2018; De Souza et al., 2020) and in the Amazonian shrimp (*Macrobrachium amazonicum* Heller, 1862) and some aquatic insects that are used as intermediate hosts (Moravec & Santos, 2009). However, some fish species are used as definitive hosts for this nematode, such as *Hoplias malabaricus* Bloch, 1794, which feeds on the cichlids *Satanoperca jurupari* Heckel, 1840, and *Aequidens tetramerus* Heckel, 1840. In turn, these are intermediate hosts for the larvae of *Pseudoproleptus* sp. (Melo et al., 2011). This pattern can also be found in *E. pisonis*, given that we found an individual of *E. pisonis* feeding on another specimen of this species. Occurrence of cannibalism among *E. pisonis* has previously been documented for this species, with low occurrence (0.6%) (Perrone & Vieira, 1991). Nonetheless, the possibility that *E. pisonis* also occupies higher levels in the food web due to its infrequent cannibalistic habit cannot be ruled out.

In *E. pisonis,* the presence of *G. genarchella* and *Neoechinorhynchu*s sp. indicated that this fish is a definitive host for these endoparasites (Cardoso et al., 2017; Ferrari-Hoeinghaus et al., 2007). Mites presented low levels of infection in the gills of *E. pisonis* in the Amazon River, but have been widely found on a variety of vertebrates, including fish and piscivorous birds (Lizama et al., 2013). In Amazonian fish, mite infection has been reported in *Colossoma macropomum* Cuvier, 1816 (Gonçalves et al., 2018), *Brachyplatystoma vaillantii* Valenciennes, 1840 (Brito-Junior & Tavares-Dias, 2021) and *Hemiodus unimaculatus* Bloch, 1794 (Almeida et al., 2021).

Ergasilus sp. are generally ectoparasites with a certain degree of host specificity. Their level of occurrence in *E. pisonis* was lower than that of *C. asellus* (Lima et al., 2023). Ergasilid species have wide distribution in the Amazon River system, with occurrence in several Amazonian fish species (Thatcher & Boeger, 1983; Vasconcelos &

Tavares-Dias, 2016; Borges et al., 2018; Sousa et al., 2019; Lima et al., 2023); however, it was recorded for the first time in *E. pisonis* in the present study.

Annual variation in communities and infracommunities of metazoan parasites

The specimens of *E. pisonis* collected in 2020 were larger and heavier, and had a better relative condition factor than those collected in 2021. Despite these differences in *E. pisonis* populations, five species of parasites were found in both years, with predominance of larvae of *Contracaecum* sp. This may have been related to the low specificity and high reproductive and infection rates of this generalist nematode (Neves et al., 2013). Although the body size of the hosts is one of the determining factors for the diversity, richness and abundance of parasites (Marcogliese et al., 2016; Baia et al., 2018), such differences in *E. pisonis* may also be related to the spawning peaks of this host fish species after the Amazonian floods. These factors would cause a difference in diet between juveniles and adults, and would show that sexual maturation is likely to be a factor influencing the trophic ontogeny of the species. This would cause a decrease in intraspecific competition (Perrone & Vieira, 1991), thus generating better feeding and reproduction conditions.

Species richness of parasites and Brillouin's diversity were higher in 2021. Similar findings were reported for *P. ornatus* and *C. asellus* collected from the Amazon River in 2020 and 2021(Lima et al., 2022, 2023). Such differences may have been influenced by variations in physicochemical characteristics of these environments.

For *E. pisonis*, the differences in the parasite infracommunities between the years 2020 and 2021 indicated in the PCoA were mainly due to the abundance of *Contracaecum* sp., *G. genarchella* and *Ergasilus* sp. Similar results were reported by Lima et al. (2023) in *C. asellus* collected from the Amazon River. This indicates that such differences may be related to the availability of intermediate hosts of the endoparasites in the environment, and to the reproductive period of the ergasilids (Villalba-Vasquez et al., 2018; Hoshino & Tavares-Dias, 2019; Lima et al., 2022, 2023). In addition, the levels of *G. genarchella* infection were higher in 2021, and this variation may have been more related to seasonal variation than to any influence of the availability of infective stages of these digeneans in the environment. In *P. ornatus* (Lima et al., 2022) and *C. asellus* (Lima et al., 2023) in the Amazon River, short-term annual variations in parasite communities and infracommunities have also been correlated with the seasonal cycle (rainy/dry), availability of infectious stages, changes to the parasite species recruitment process, urban eutrophication and host body size.

Genarchella genarchella and larvae of C*ontracaecum* sp. were present in both of the years studied, thus indicating that contact between *E. pisonis* and the infective forms of these endoparasites did not vary between these two years. Similar findings were reported by Hoshino et al. (2014) in *H. surinamensis* collected from a tributary of the Amazon River, in which the levels of infection by *G. genarchella* and larvae of *Contracaecum* sp. were stable among the years studied, as also were the levels in *C. asellus* in the Amazon River (Lima et al., 2023).

In *E. pisonis*, infestations by *Ergasilus* sp. were observed in both years of the present study. In *C. asellus*, infestation by *E. colomesus* also occurred in both years studied, due to segregation of the hosts by size for feeding (Lima et al., 2023). The absence of segregation by size for feeding and reproduction among *E. pisonis* may have facilitated encounters with these ergasilids during the years studied, and may have facilitated their attachment to the hosts' gills for reproduction (Williams & Bunkley-Williams, 2019).

Mites were observed on the gills of *E. pisonis* only in 2020. These parasites are usually found on the gills, integuments and digestive tracts of their host fish (Olmeda et al., 2011; Lizama et al., 2013). However, some authors have considered them to be unusual parasites in fish, and others have taken the view that mites are not fish parasites, since the habitats and behavior of fish do not contribute to the infestations found (Olmeda et al., 2011; Lizama et al., 2013; Brito-Junior & Tavares-Dias, 2021). However, mites can proliferate and infect weak or stressed fish, under certain environmental conditions, thereby causing serious damage to the host. In fish in Australia, Europe and North America, some genera of mites have been isolated and correlated with high host mortality (Olmeda et al., 2011; Lizama et al., 2013).

Seasonal variation of communities and infracommunities of metazoan parasites

Specimens of *E. pisonis* collected in the rainy season were larger and heavier and thus presented a better relative condition factor. This result indicated that the fish were feeding better during this seasonal period and/or were in the reproductive period, as indicated by some individuals in which mature gonads were found. Such observations were also reported by Perrone & Vieira (1990) in *E. pisonis* collected from the estuarine region of the Jucu River, in the State of Espírito Santo, Brazil, where females with mature ovaries occurred frequently from February to June and, specifically, soon after the river flood peak. This result emphasized in rivers, such that changes in water volume caused by seasonal changes directly affected the existing community, influencing changes mainly with regard to the feeding, reproduction and sizes of fish populations (Lowe-McConnell, 1967). In the Amazon basin, the rainy and dry seasons generally influence the communities of invertebrates and fish that serve as food for many carnivorous fish such as *E. pisonis*. In the rainy season, there is greater diversity of the zooplankton and other invertebrates that form part of the diet of these fish, thus improving their body condition (Gonçalves et al., 2016; Tavares-Dias et al., 2014).

Populations of *E. pisonis* do not show spatial segregation between adults and juveniles. However, there is a difference in feeding habits between juveniles and adults, caused by sexual maturation, which influences the trophic ontogeny of this species (Perrone & Vieira, 1990). Thus, adult individuals (larger and heavier) reproduce during the rainy season, while occupying the same space as young individuals, at a time when conditions for development are better. Spawning peaks occurred in the dry season, at a time when young individuals (smaller and less heavy) with less favorable feeding conditions are found. This corroborated the fact that smaller and less heavy individuals were found in the dry season.

In *E. pisonis*, parasite species richness (five species in each seasonal period), diversity, evenness and Berger-Parker dominance index did not differ between seasonal periods. In addition, *Contracaecum* sp. was the dominant species in both seasonal periods. Similar results were reported in relation to *P. blochii* in the Acre River, where these diversity parameters were not influenced by seasonality (Cavalcante et al., 2020). However, mites and *Neoechinorhynchus* sp. occurred only in the rainy season, while *Pseudoproleptus* sp. and *S. inopinatus* occurred only during the dry season. Among the possible influences on the seasonal pattern found in our data, the absence of a spatial seasonal segregation pattern (Perrone & Vieira, 1990) and the abundance of hosts in both seasons can be cited.

The PCoA showed seasonal differences in the infracommunities of *Contracaecum* sp., *G. genarchella* and *Ergasilus* sp. in *E. pisonis*. The period of intense rainfall (rainy season) and the less rainy period (dry season) in the Amazon region are well defined. These seasonal variations may be responsible for variations in parasite species recruitment, food availability for hosts and, consequently, infective stages in the environment (Neves et al., 2013; Gonçalves et al., 2016; Hoshino & Tavares-Dias, 2020). They may also influence the reproductive period of Amazonian fish (Cavalcante et al., 2020). These variations can alter the habitats of fish populations and water velocity, thus increasing the stress levels among host fish and their susceptibility to parasitic infections and, consequently, altering the structure of parasite communities and infracommunities in fish host populations (Gonçalves et al., 2016).

In *E. pisonis*, G. *genarchella* was found in both seasonal periods, but the highest levels of infection were observed in the dry season. The infective stages of this digenean are present during both seasonal periods (rainy and dry), but more frequently in the dry season. In *C. asellus* in the Amazon River, *G. genarchella* infection also occurred in both seasons (Lima et al., 2023). In contrast, for *P. ornatus* in the Amazon River, infection by this digenean only occurred in the rainy season (Lima et al., 2022), when the chances of host fish encountering the infective stages of this digenean are greater.

In *E. pisonis*, the presence of *Ergasilus* sp. was observed only in the rainy season, as also were *Telotha henselli* Von Martens, 1869, in *P. ornatus* (Lima et al., 2022) and *Argulus pestifer* Ringuelet, 1948, in *C. asellus* (Lima et al., 2023). On the other hand, in *C. asellus*, infestation by *E. colomesus* occurred in both seasons, but with higher levels occurring in the dry season. Females of the genus *Ergasilus* attach themselves to the gills and remain there until their eggs are mature, after which they detach from their hosts and release the eggs into the environment (Williams & Bunkley-Williams, 2019). Our results indicate, therefore, that the ergasilid species found in *E. pisonis* in the Amazon River presents higher reproduction levels during the rainy season.

Conclusions

About 59.1% of the parasites were larvae, thus indicating that *E. pisonis* is an intermediate or paratenic host. Our results, based on sampling over two years and in both seasons (rainy and dry), indicated that the parasites differed between the years and seasons regarding the diversity of some infracommunities. The little effects of annual and seasonal variations on the diversity and levels of infection were related to variations in rainfall levels and, consequently, to the availability of infective stages of parasites with direct and indirect life cycles and changes in the recruitment of parasite species in the environment caused by seasonality. Hence, the results do not corroborate the hypothesis that seasonal cycle (rainy/dry) would not influence the communities of parasites. Furthermore, this study was the first to investigate the effects of annual and seasonal variations of metazoan parasites on *E. pisonis*. Consequently, this study provides the first record of occurrences of larvae of *Contracaecum* sp. and *Pseudoproleptus* sp., and adult individuals of *S. inopinatus*, *G. genarchella*, *Neoechinorhynchu*s sp., mites and *Ergasilus* sp., in *E. pisonis*.

Ethics declaration

Ethics approval to obtain access to genetic heritage was authorized by the Brazilian Ministry of the Environment (SISBio n° 73550-1 and SisGen no AA4B6BA). This study was developed in accordance with the principles adopted by the Brazilian College of Animal Experimentation (COBEA) and was conducted under authorization from the Ethics Committee for Animal Use of Embrapa (protocol no 014/2018).

Conflict of interest

The authors declare no competing interests.

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References

Albuquerque MC, Santos MD, Monteiro CM, Martins AN, Ederli NB, Brasil-Sato MC. Helmintos endoparasitos de *Pimelodus maculatus* Lacépède, 1803 (Actinopterygii, Pimelodidae) de duas localidades (lagoa e calha do rio) do rio Guandu, Estado do Rio de Janeiro, Brasil. *Rev Bras Parasitol Vet* 2008; 17(Suppl 1): 113–119.

Almeida OM, Oliveira MSB, Tavares-Dias M. Community and infracommunities of metazoan parasites in *Hemiodus unimaculatus* (Hemiodontidae) from Jari River basin, a tributary of Amazon River (Brazil). *Rev Bras Parasitol Vet* 2021; 30(4): e016521. https://doi.org/10.1590/S1984-29612021090.

Baia RRJ, Florentino AC, Silva LMA, Tavares-Dias M. Patterns of the parasite communities in a fish assemblage of a river in the Brazilian Amazon region. *Acta Parasitol* 2018; 63(2): 304–316. https://doi.org/10.1515/ap-2018-0035.

Bartolette R, Rosa DCO, Beserra DA, Soares BE, Albrecht MP, Brito MFG. Seasonal and ontogenetic diet patterns of the freshwater pufferfish *Colomesus asellus* (Müller & Troschel, 1849) in the upper-middle Tocantins River. *Acta Sci Biol Sci* 2018; 40: e35282. https://doi.org/10.4025/actascibiolsci.v40i1.35282.

Borges WF, Oliveira MSB, Santos GG, Tavares-Dias M. Parasites in Loricariidae from Brazil: checklist and new records for fish from the Brazilian Amazon. *Acta Sci Biol Sci* 2018; 40: e40621. https://doi.org/10.4025/actascibiolsci.v40i1.40621.

Brito-Junior IA, Tavares-Dias M. Diversity of ectoparasites and endoparasites infecting *Brachyplatystoma vaillantii* (Siluriformes: Pimelodidae), a large migratory catfish from the Amazon. *Acta Amaz* 2021; 51(2): 122–128. https://doi.org/10.1590/1809-4392201901321.

Bush AO, Lafferty KD, Lotz JM, Shostak AW. Parasitology meets ecology on its own terms: Margolis et al. revisited. *J Parasitol* 1997; 83(4): 575–583. https://doi.org/10.2307/3284227.

Cardoso ACF, Oliveira MSB, Neves LR, Tavares-Dias M. Metazoan fauna parasitizing *Peckoltia braueri* and *Pterygoplichthys pardalis* (Loricariidae) catfishes from the northeastern Brazilian Amazon. *Acta Amaz* 2017; 47(2): 147–154. https://doi.org/10.1590/1809-4392201603232.

Cavalcante PHO, da Silva MT, Pereira ANS, Gentile R, Santos CP. Helminth diversity in *Pimelodus blochii* Valenciennes, 1840 (Osteichthyes: Pimelodidae) in two Amazon Rivers. *Parasitol Res* 2020; 119(12): 4005–4015. https://doi.org/10.1007/s00436-020-06906-x.

Cervigón F. *Los Peces Marinos de Venezuela*. Vol. 3. 2nd ed. Caracas, Venezuela: Fundación Científica Los Roques - Fundación Polar - Universidad de Oriente; 1994.

Darcy GH. *Comparison of ecological and life history information on gobiid fishes, with emphasis on the southeastern United States*. Florida; Southeast Fisheries Center (U.S.); 1980.

De Souza DC, Eiras JC, Adriano EA, Corrêa LL. Metazoan parasites of *Plagioscion squamosissimus* (Osteichthyes: Sciaenidae) of two rivers from the eastern Amazon (Brazil). *Ann Parasitol* 2020; 66(2): 217-225. doi: 10.17420/ap6602.257.

Dias MKR, Tavares-Dias M. Seasonality affects the parasitism levels in two fish species in the eastern Amazon region. *J Appl Ichthyol* 2015; 31(6): 1049–1055. https://doi.org/10.1111/jai.12865.

Eiras JC, Takemoto RM, Pavanelli GC. *Métodos de estudo e técnicas laboratoriais em parasitologia de peixes.* 2nd ed. Maringá: Eduem; 2006.

Ferrari-Hoeinghaus AP, Takemoto RM, Pavanelli GC. Digenetic trematode parasites of *Loricariichthys platymetopon* (Loricariidae, Siluriformes) of the upper Paraná river floodplain, Brazil. *Acta Sci Biol Sci* 2007; 29(3): 327–329.

Froese R, Pauly D. *FishBase. Version (2/2023).* USA: FishBase [online]. 2023 [cited 2020 Feb 5]. Available from: www.fishbase.org

Gonçalves BB, Oliveira MSB, Borges WF, Santos GG, Tavares-Dias M. Diversity of metazoan parasites in *Colossoma macropomum* (Serrasalmidae) from the lower Jari River, a tributary of the Amazonas River in Brazil. *Acta Amaz* 2018; 48(3): 211–216. https://doi.org/10.1590/1809-4392201704371.

Gonçalves RA, Oliveira MSB, Neves LR, Tavares-Dias M. Seasonal pattern in parasite infracommunities of *Hoplerythrinus unitaeniatus* and *Hoplias malabaricus* (Actinopterygii: Erythrinidae) from the Brazilian Amazon. *Acta Parasitol* 2016; 61(1): 119–129. https://doi.org/10.1515/ap-2016-0016.

Hill MO. Diversity and evenness: a unifying notation and its consequences. *Ecology* 1973; 54(2): 427–432. https://doi.org/10.2307/1934352.

Hoshino ÉM, Tavares-Dias M. Interannual and seasonal variation in protozoan and metazoan parasite communities of *Hemibrycon surinamensis,* a characid fish inhabiting the Brazilian Amazon. *Acta Parasitol* 2019; 64(3): 479–488. https://doi.org/10.2478/s11686-019-00057-5.

Hoshino ÉM, Tavares-Dias M. Temporal and seasonal variations in parasites of *Metynnis lippincottianus* (Characiformes: Characidae), a host from the eastern Amazon (Brazil). *J Nat Hist* 2020; 53(43–44): 2723–2736. https://doi.org/10.1080/00222933.2020.1733121.

Hoshino MDFG, Hoshino ÉM, Tavares-Dias M. First study on parasites of *Hemibrycon surinamensis* (Characidae), a host from the eastern Amazon region. *Rev Bras Parasitol Vet* 2014; 23(3): 343–347. https://doi.org/10.1590/S1984-29612014069.

Hoshino MDFG, Tavares-Dias M. Ecology of parasites of *Metynnis lippincottianus* (Characiformes: Serrasalmidae) from the eastern Amazon region, Macapá, State of Amapá, Brazil. *Acta Sci Biol Sci* 2014; 36(2): 249–255. https://doi.org/10.4025/actascibiolsci.v36i2.19876.

Jackson DA, Harvey HH. Fish and benthic invertebrates: community concordance and community–environment relationships. *Can J Fish Aquat Sci* 1993;5 0(12): 2641–2651. https://doi.org/10.1139/f93-287.

Le Cren ED. The Length-Weight Relationship and Seasonal Cycle in Gonad Weight and Condition in the Perch (*Perca fluviatilis*). *J Anim Ecol* 1951; 20(2): 201–219. https://doi.org/10.2307/1540.

Lehun AL, Cavalcanti LD, Lizama MAP, Silva JOS, Casali GP, Takemoto RM. Temporal effects and changes in the parasitic community of *Prochilodus lineatus* (Valenciennes, 1837) (Characiformes: Prochilodontidae) in a floodplain. *J Helminthol* 2022; 96: e4. https://doi.org/10.1017/S0022149X21000742.

Leotta SH, Schmidt GD, Kuntz RE. *Brentisentis uncinus* gen. et sp. n. and *Gorgorhynchus satoi* (Morisita 1937) Yamaguti 1963 from Taiwan. *J Parasitol* 1982; 68(1): 134–137. https://doi.org/10.2307/3281336.

Lima ES, Oliveira MSB, Tavares-Dias M. Diversity and community ecology of metazoan parasites in *Pimelodus ornatus* (Siluriformes: Pimelodidae) from the Amazonas River in Brazil. *Rev Bras Parasitol Vet* 2021; 30(3): e006021. https://doi.org/10.1590/S1984-29612021065.

Lima ES, Oliveira MSB, Tavares-Dias M. Temporal and seasonal variation of metazoan parasites in *Pimelodus ornatus* (Siluriformes: Pimelodidae) from the Amazon River, Brazil. *Parasitol Res* 2022; 121: 3171–3181. https://doi.org/10.1007/s00436-022-07637-x.

Lima ES, Santos CBR, Tavares-Dias M. Metazoan parasites in *Colomesus asellus* (Pisces: Tetraodontidae) from Amazon River, in Brazil: an ecological, annual and seasonal study. *J Nat Hist* 2023; 57(13–16): 885–903. https://doi.org/10.1080/00222933.2023.2220173.

Lizama M, Monkolski J, Carniel M, Costa A. Mollusca, Hirudinea, Pentastomida e Acari parasitos de peixes. In: Pavanelli, GC, Takemoto RM, Eiras JC (Org.) *Parasitologia de peixes de água doce do Brasil* Maringá: Eduem; 2013. p. 399–435.

Lowe-McConnell R. Some factors affecting fish populations in Amazonian waters. *Atas do Simp, sobre a Biota Amazónica* 1967; 7: 177–186.

Ludwig JA, Reynolds LJF. S*tatistical ecology: A primer in methods and computing* (Vol. 1). John Wiley & Sons, New York; 1988.

Magurran AE, McGill BJ. Challenges and opportunities in the measurement and assessment of biological diversity. Oxford University Press. In: Magurran AE & McGill, BJ; 2011. p. 1–7 p.

Marcogliese DJ, Locke SA, Gélinas M, Gendron AD. Variation in parasite communities in spottail shiners (*Notropis hudsonius*) linked with precipitation. *J Parasitol* 2016; 102(1): 27–36. https://doi.org/10.1645/12-31.

Melo MFC, Giese EG, Santos JN, Santos CP. First record of larval *Pseudoproleptus* sp. (Nematoda: Cystidicolidae) in fish host. *Acta Trop* 2011; 117(3): 212–215. https://doi.org/10.1016/j.actatropica.2010.12.011.

Moravec F, Santos CP. Larval *Pseudoproleptus* sp. (Nematoda: Cystidicolidae) found in the Amazon river prawn *Macrobrachium amazonicum* (Decapoda: Palaemonidae) in Brazil. *J Parasitol* 2009; 95(3): 634–638. https://doi.org/10.1645/GE-1887.1.

Negreiros LP, Florentino AC, Pereira FB, Tavares-Dias M. Long-term temporal variation in the parasite community structure of metazoans of *Pimelodus blochii* (Pimelodidae), a catfish from the Brazilian Amazon. *Parasitol Res* 2019a; 118(12): 3337–3347. https://doi.org/10.1007/s00436-019-06480-x.

Negreiros LP, Pereira FB, Tavares-Dias M, Tavares LEr. Community structure of metazoan parasites from *Pimelodus blochii* in two rivers of the western Brazilian Amazon: same seasonal traits, but different anthropogenic impacts. *Parasitol Res* 2018; 117(12): 3791–3798. https://doi.org/10.1007/s00436-018-6082-5.

Negreiros LP, Pereira FB, Tavares-Dias M. Metazoan parasites of *Calophysus macropterus* (Siluriformes: Pimelodidae) in the Acre and Iaco rivers in the western Amazon region of Brazil: diversity, similarity and seasonal variation. *J Nat Hist* 2019b; 53(23–24): 1465–1479. https://doi.org/10.1080/00222933.2019.1657195

Neves LR, Pereira FB, Tavares-Dias M, Luque JL. Seasonal influence on the parasite fauna of a wild population of *Astronotus ocellatus* (Perciformes: Cichlidae) from the Brazilian Amazon. *J Parasitol* 2013; 99(4): 718–721. https://doi.org/10.1645/12-84.1.

Neves LR, Silva LMA, Florentino AC, Tavares-Dias M. Distribution patterns of *Procamallanus* (*Spirocamallanus*) *inopinatus* (Nematoda: Camallanidae) and its interactions with freshwater fish in Brazil. *Rev Bras Parasitol Vet* 2020; 29(4): e012820. https://doi.org/10.1590/S1984-29612020092.

Neves LR, Silva LMA, Tavares-Dias M. Diversity and Ecology of Endohelminth Parasites in a Fish Assemblage of an Amazon River Tributary in Brazil. *Acta Parasitol* 2021; 66: 1431-1441. https://doi.org/10.1007/s11686-021-00413-4.

Neves LR, Tavares-Dias M. Low levels of crustacean parasite infestation in fish species from the Matapi River in the state of Amapá, Brazil. *Rev Bras Parasitol Vet* 2019; 28(3): 493–498. https://doi.org/10.1590/S1984-29612019006.

Nordlie FG. Feeding and reproductive biology of eleotrid fishes in a tropical estuary. *J Fish Biol* 1981; 18(1): 97–110. https://doi.org/10.1111/j.1095-8649.1981.tb03764.x.

Oksanen J, Blanchet FG, Kindt R, Legendre P, Minchin P, O'hara R, Simpson G, Solymos P, Stevens M, Wagner H. Vegan: community ecology package. R package. 2020; Available from: https://CRAN.R-project.org/package=vegan.

Oliveira MSB, Corrêa LL, Prestes L, Neves LR, Brasiliense ARP, Ferreira DO, et al. Comparison of the endoparasite fauna of *Hoplias malabaricus* and *Hoplerythrinus unitaeniatus* (Erythrinidae), sympatric hosts in the eastern Amazon region (Brazil). *Helminthologia* 2018; 55(2): 157-165. https://doi.org/10.2478/helm-2018-0003.

Oliveira MSB, Gonçalves RA, Ferreira DO, Pinheiro DA, Neves LR, Dias MKR, et al. Metazoan parasite communities of wild *Leporinus friderici* (Characiformes: Anostomidae) from Amazon River system in Brazil. *Stud Neotrop Fauna Environ* 2017; 52(2): 146–156. https://doi.org/10.1080/01650521.2017.1312776.

Olmeda AS, Blanco MM, Pérez-Sánchez JL, Luzón M, Villarroel M, Gibello A. Occurrence of the oribatid mite *Trhypochthoniellus longisetus longisetus* (Acari: Trhypochthoniidae) on tilapia *Oreochromis niloticus*. *Dis Aquat Org* 2011; 94(1): 77–81.

Ondračková M, Valová Z, Hudcová I, Michálková V, Šimková A, Borcherding J, et al. Temporal effects on host-parasite associations in four naturalized goby species living in sympatry. *Hydrobiologia* 2015; 746: 233–243. https://doi.org/10.1007/s10750-014-1967-5.

Perrone EC, Vieira F. Ocorrência e Período Reprodutivo de *Eleotris pisonis* (Teleostei: Eleotrididae) na região estuarina do Rio Jucu, Espírito Santo, Brasil. *Ciênc Cult* 1990; 42(9): 701–709.

Perrone EC, Vieira F. Hábito alimentar de *Eleotris pisonis* (Teleostei: Eleotridae) na região estuarina do rio Jucu, Espírito Santo, Brasil. *Rev Bras Biol* 1991; 51(4): 867–872.

Petter AJ, Golvan YJ, Tcheprakoff R. Nématodes de poissons de rivière en Guadeloupe. Bulletin du Muséum National d’Histoire Naturelle, Paris, 3e série, No 428, Section A, *Zoology* 1977; 298: 159–171.

Planquette P, Keith P,Le Bail PY. *Atlas des poissons d’eau douce de Guyane*. Paris: Muséum National d’Histoire Nattrelle; 2000.

Poulin R. *Evolutionary ecology of parasites.* 2nd ed. Princeton university press; London; 2011.

Team RC. R CoreTeam: a language and environment for statistical computing. Vienna, Austria; 2021.

Ray C, Robins CR. *A field guide to Atlantic coast fishes: North America*. 1nd 1ed.; Boston: Massachusetts;Houghton Mifflin Harcourt; 2016.

Rohlenová K, Morand S, Hyršl P, Tolarová S, Flajšhans M, Šimková A. Are fish immune systems really affected by parasites? An immunoecological study of common carp (*Cyprinus carpio*). *Parasit Vectors* 2011; 4(1): 120. https://doi.org/10.1186/1756-3305-4-120.

Santos GG, Oliveira MSB, Neves LR, Tavares-Dias M. Parasites community of *Astronotus crassipinnis* (Cichlidae), a fish from the Brazilian Amazon. *Ann Parasitol* 2018; 64(2): 121-128. https://doi.org/10.17420/ap6402.143.

Santos GM, Juras AA, Mérona B, Jégue M. *Peixes do baixo rio Tocantins. 20 anos depois da Usina Hidrelétrica Tucuruí*. Eletronorte , Brasília, Brasil; 2004.

Schade FM, Raupach MJ, Wegner KM. Seasonal variation in parasite infection patterns of marine fish species from the Northern Wadden Sea in relation to interannual temperature fluctuations. *J Sea Res* 2016; 113: 73–84. https://doi.org/10.1016/j.seares.2015.09.002.

Sousa BO, Oliveira MS, Tavares-Dias M. Community structure of metazoan parasites in *Hemibrycon surinamensis* (Characidae) from the Matapi River in the State of Amapá, Brazil. *An Acad Bras Ciênc* 2019; 91(2): e20180393. https://doi.org/10.1590/0001-3765201920180393.

Souza EB, Cunha AC. Climatologia de precipitação no Amapá e mecanismos climáticos de grande escala. In: Cunha AC, Souza EB, Cunha HFA. *Tempo, Clima e Recursos Hídricos*. Macapá: IEPA; 2010. p. 177–195.

Tavares-Dias M, Neves LR. Diversity of parasites in wild *Astronotus ocellatus* (Perciformes, Cichlidae), an ornamental and food fish in Brazil. *An Acad Bras*

Ciênc 2017; 89(3): 2305–2315. https://doi.org/10.1590/0001-3765201720160700.

Tavares-Dias M, Oliveira MSB, Gonçalves RA, Silva LMA. Ecology and seasonal variation of parasites in wild *Aequidens tetramerus*, a Cichlidae from the Amazon. *Acta Parasitol* 2014; 59(1): 158–164. https://doi.org/10.2478/s11686-014-0225-3.

Tavares-Dias M, Oliveira MSB. Structure of parasites community in *Chaetobranchopsis orbicularis* (Cichlidae), a host from the Amazon River system in northern Brazil. *Parasitol Res* 2017; 116(8): 2313–2319. https://doi.org/10.1007/s00436-017-5539-2.

Thatcher VE, Boeger WA. The parasitic crustaceans of fishes from the Brazilian Amazon. 4. *Ergasilus colomesus* n. sp. (Copepoda: Cyclopoida) from an ornamental fish, *Colomesus asellus* (Tetraodontidae) and aspects of its pathogenicity. *Trans Am Micro Soc* 1983; 102(4): 371–379. https://doi.org/10.2307/3225850.

Tóthmérész B. Comparison of different methods for diversity ordering. *J Veg Sci* 1995; 6(2): 283–920. https://doi.org/10.2307/3236223.

Vasconcelos HCG, Tavares-Dias M. Host-parasite interaction between crustaceans of six fish species from the Brazilian Amazon. *Acta Sci Biol Sci* 2016; 38(1): 113–123. https://doi.org/10.4025/actascibiolsci.v38i1.29601

Villalba-Vasquez PJ, Violante-González J, Monks S, Marino-Romero JU, Ibáñez SG, Rojas-Herrera AA, et al. Temporal and spatial variations in the metazoan parasite communities of the Panama spadefish, *Parapsettus panamensis* (Pisces: Ephippidae), from the Pacific coast of Mexico. *Invertebr Biol* 2018; 137(4): 339–354. https://doi.org/10.1111/ivb.12232.

Virgilio LR, Machado EO, Freire AN, Takemoto RM, Camargo LMA, Meneguetti D. *Ergasilus colomesus* (Copepoda: Ergasilidae) parasitizing gills of *Colomesus asellus* (Tetraodontiformes: Tetraodontidae) in the western Brazilian Amazon. *Ann Parasitol* 2021;67(1): 123–127. https://doi.org/10.17420/ap6701.320.

Williams EH, Bunkley-Williams L. Life cycle and life history strategies of parasitic Crustacea. In: *Parasitic Crustacea*. Berlim: Springer; 2019. p. 179–266.

Yang BJ, Zou H, Zhou S, Wu SG, Wang GT, Li WX. Seasonal dynamics and spatial distribution of the *Dactylogyrus* species on the gills of grass carp (*Ctenopharyngodon idellus*) from a fish pond in Wuhan, China. *J Parasitol* 2016; 102(5): 507–513. https://doi.org/10.1645/15-931.

Zar J. *Biostatistical analysis*. 5th ed. Upper Saddle River: Prentice-Hall; 2010.

CAPÍTULO II

Original Article

Metazoan parasites in *Colomesus asellus* (Pisces: Tetraodontidae) from Amazon River, in Brazil: an ecological, annual and seasonal study

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Metazoan parasites in *Colomesus asellus* (Pisces: Tetraodontidae) from Amazon River, in Brazil: an ecological, annual and seasonal study

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ABSTRACT

Our hypothesis is that annual and seasonal variations influence the structure of component communities and diversity of metazoan parasites from *Colomesus asellus* from the Amazon River. A total of 107 fish were collected during 2020 and 2021, and 932 metazoan parasites were recovered. In 2020, four species of parasites (one Nematoda, two Digenea and one Crustacea) were found; and in 2021, five species (one Nematoda, one Digenea, one Acanthocephala and two Crustacea) were recorded. *Ergasilus colomesus* was the most dominant throughout the study. Species richness and Brillouin diversity index were higher in 2021 and in the dry season. Some parasite component communities showed differences between years and between seasonal periods. The structure of parasite communities was mainly influenced by rainfall levels, seasonal availability of infective stages in the environment, and body size of the fish host. These facts corroborate the hypothesis that such variables could influence the component communities of parasites. Lastly, this is the first record of *Contracaecum* sp., *Genarchella genarchella, Clinostomum marginatum, Brasacanthus* sp. and *Argulus pestifer* in *C. asellus*.

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Aggregation; Amazon; freshwater fish; infection; parasites; seasonality; Amazon puffer

Introduction

Fish parasites are important components of biodiversity as they can provide information about environments (eg water quality) and influence the productivity and structure of the food web of an ecosystem. Parasites affect growth, reproduction and survival of host populations (Baia *et al.* 2018; Negreiros *et al.* 2019a; Lehun *et al.* 2022). Therefore, studies on fish parasite infracommunities (all parasites from all species in one host individual) and component community (all parasites from all species in all hosts) enrich our knowledge about parasite–host–environment relationships and the strategies used by different parasite species (Lehun *et al.* 2022). Parasite diversity and infection patterns in wild Amazonian

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Abstract

Our hypothesis is that annual and seasonal variations influence the structure of component communities and diversity of metazoan parasites from *Colomesus asellus* from the Amazon River. A total of 107 fish were collected during 2020 and 2021; and 932 metazoan parasites were recovered. In 2020, four species of parasites were found (one Nematoda, two Digenea and one Crustacea); and in 2021, five species (one Nematoda, one Digenea, one Acanthocephala and two Crustacea were recorded). *Ergasilus colomesus* was the most dominant throughout the study. Species richness and Brillouin diversity index were higher in 2021 and in the dry season. Some parasite component communities showed differences between years and between seasonal periods. The structure of parasite communities was mainly influenced by rainfall levels, seasonal availability of infective stages in the environment, and body size of the fish host. These facts corroborate the hypothesis that such variables could influence the component communities of parasites. Lastly, this is the first record of *Contracaecum* sp., *Genarchella genarchella*, *Clinostomum marginatum*, *Brasacanthus* sp. and *Argulus pestifer* in *C. asellus*.

Keywords: Aggregation, Amazon, freshwater fish, infection, parasites, seasonality, Amazon puffer.

Introduction

Fish parasites are important components of biodiversity as they can provide information about environments (e.g. water quality) and influence the productivity and structure of the food web of an ecosystem, Parasites affect growth, reproduction and survival of host populations (Baia et al., 2018; Negreiros et al., 2019; Lehun et al., 2022). Therefore, studies on fish parasite infracommunities (all parasites from all species in one host individual) and component community (all parasites from all species in all hosts) enrich the knowledge about parasite-host-environment relationships and the strategies used by different parasite species (Lehun et al., 2022). Parasite diversity and infection patterns in wild Amazonian fish populations have been associated with several ecological features such as host diet, environmental characteristics, the existence of infective stages in the ecosystem, and temporal and seasonal variations (Hoshino & Tavares-Dias, 2019; Hoshino & Tavares-Dias, 2020; Negreiros et al., 2019; Lehun et al., 2022). These fish populations may harbor ecto- and endoparasites from different taxa. With different life cycles that may be related to the behavior and diet of the host. Thus, species that occupy different ecological niches are exposed to different parasites, potentially resulting in different patterns of infection (Tavares-Dias et al., 2014; Tavares-Dias, 2017; Baia et al., 2018; Cavalcante et al., 2020). These studies show that rainy and dry seasons may influence host behavior, as well as the diversity of parasites and invertebrates in the ecosystem. Therefore, identifying the factors influencing the structure of parasite communities is fundamental to the thorough understanding of the parasite ecology of fish host.

Temporal variations in the structure of parasite communities may be related to the abiotic and biotic factors of the local environment. The few studies published regarding parasite communities of wild freshwater fish from the Amazon have indicated that these communities may these communitites may show stable structures over time (Hoshino & Tavares-Dias, 2019; Negreiros et al., 2019; Hoshino & Tavares-Dias, 2020).

The eastern Amazon region has tropical climate influenced by of the Amazon rainforest. In this region, the rainy season generally occurs from December to May and the dry one from June to November (Souza & Cunha, 2010). These variations in rainfall levels influence fish populations and, consequently, their communities and parasites, due to fluctuations in the hydrodynamics and physicochemical characteristics of the aquatic ecosystems. Several studies have shown fluctuations on parasite diversity, richness and infection patterns according to seasonal variations (rainy and dry cycles) in regard to parasite communities of wild Amazonian fish (Neves, et al., 2013; Tavares-Dias et al., 2014; Gonçalves et al., 2016; Carvalho & Tavares-Dias, 2017; Negreiros et al., 2018; Hoshino & Tavares-Dias, 2019; Hoshino & Tavares-Dias, 2020). However, information on the effects of seasonality in the dynamics of parasitic infection in tropical fish populations is scarce (Gonçalves et al., 2016; Hoshino & Tavares-Dias, 2020; Negreiros et al., 2019; Violante-González et al., 2009). It is well illustrated by the fact that temporal and seasonal variations in the community of metazoan parasites of the Amazon puffer *Colomesus asellus* Müller & Troschel, 1849, have never been investigated.

Colomesus asellus is a freshwater fish belonging to the family Tetraodontidae that is found in the basins of Amazon River, from Peru to the island of Marajó, and in Tocantins River, including tributaries of Araguaia, Guaporé, Orinoco and Essequibo (Bartolette et al., 2018; Froese & Pauly, 2023). It inhabits freshwater and coastal streams, but also tolerates brackish environments (Smith, 1997). This fish feeds on mollusks, copepods and cladocerans microcrustaceans, among other aquatic invertebrates, small fish and plants (Santos et al., 2004; Torrente-Vilara et al., 2013; Bartolette et al., 2018). Its total reproductive period occur at the beginning of the flood period, but there are reports of fish showing reproductive activity in the ebb tide period in July (Torrente-Vilara et al., 2013). The hypothesis of the present study is that temporal and seasonal variations can influence the parasite communities of *C. asellus* in the Brazilian Amazon.

Materials and methods

Study area

From January 2020 to November 2021, a total of 107 specimens of *C. asellus* [7.5 ± 3.1 cm (2.3-13.4 cm), and 21.7 ± 21.4 g (1.0-81.2 g)] were collected from the Amazon River, near the island of Santana, in the municipality of Santana, in the state of Amapá, northern Brazil (Fig. 1). In bimonthly sampling (at least 15 fish every two months, but we don't always get it), fish were caught using gill nets of different sizes and meshes (15, 20, 25, 30 and 35 mm), cast nets (20 mm mesh) and hand lines. Hosts were then euthanized through sectioning the medulla, preserved in formalin (10%), and transported to the Aquaculture and Fisheries Laboratory of Embrapa Amapá, Macapá, Brazil, for analysis of their parasites.

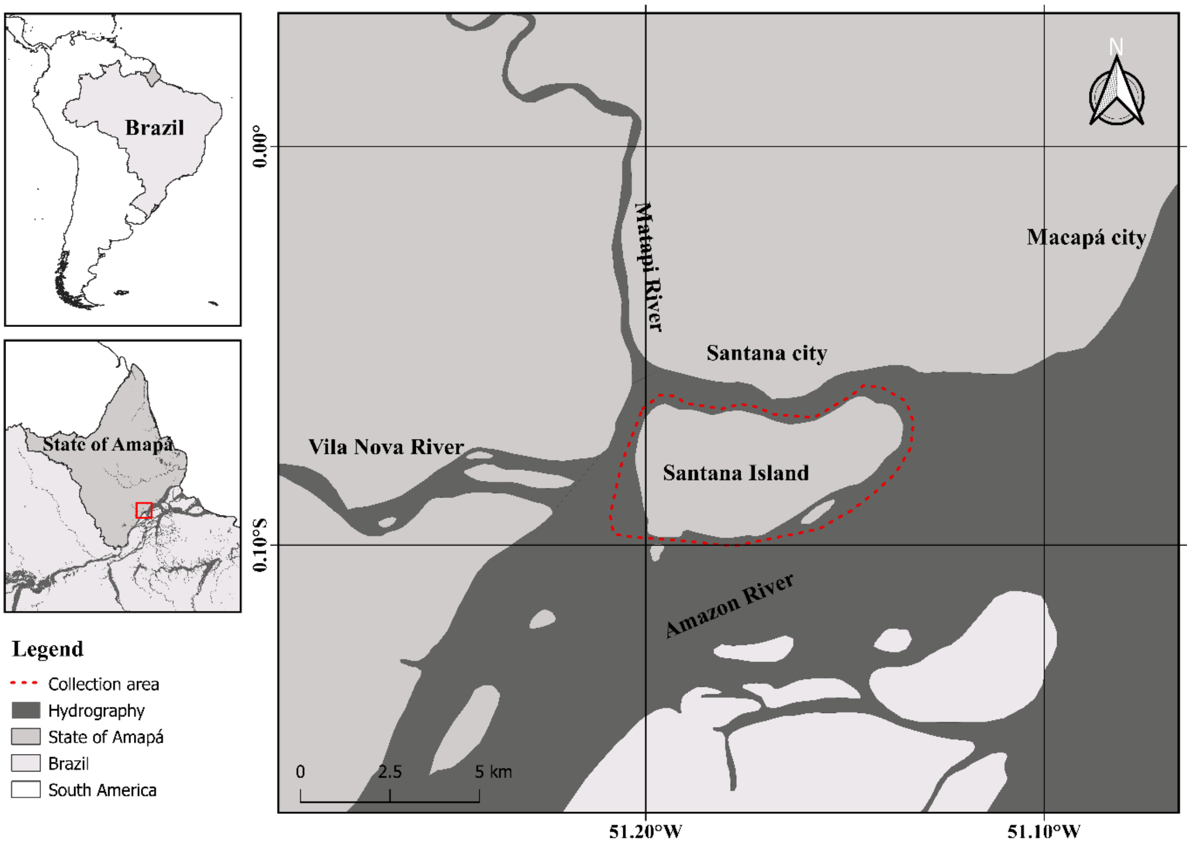

Fig.1 Collection area for *Colomesus asellus* in the Amazon River, in the state of Amapá, in the eastern Amazon region, Brazil.

At the fish sampling sites, the water quality parameters of electrical conductivity, pH and total dissolved solids were measured using a multiparameter meter (Akso, model Combo5-02-1016). Dissolved oxygen levels and water temperature were measured using an oximeter (Instrutherm, model MO-900). Rainfall data in the region were obtained from the Hydrometeorology and Renewable Energy Center (NHMET) of the Institute of Scientific and Technological Research of the state of Amapá (IEPA) (Table 1).

Parasite collection and processing

In the laboratory, the fish were weighed (g), their total lengths were measured (cm), and they were necropsied to collect metazoan parasites. Their mouths, opercular cavities, gills and fins were analyzed for ectoparasites. Internal organs such as the gastrointestinal tract and viscera were examined for endoparasites with the aid of a stereomicroscope. The parasites found were preserved in 70% ethanol for preparation of permanent slides (Eiras et al., 2006). Voucher specimens were deposited at IEPA, Macapá, AP, Brazil, in the Scientific Collection Curation Office for the Fauna of Amapá, under IEPA accession numbers 165P-170P.

Table 1. Physicochemical parameters of water from the Amazon River, in the eastern Amazon region, Brazil, during the periods of collection of *Colomesus asellus*.

			Test	
Parameters	**2020**	**2021**	***t***	***U***
Rainfall (mm)	195.7 ± 119.5	231.5 ± 153.2	-0.8197	-
Temperature (°C)	29.0 ± 1.0	29.1 ± 1.1	0.0284	-
Dissolved oxygen (mg/L)	5.8 ± 0.2	4.2 ± 1.8	2.2699	-
pH	7.1 ± 0.2	6.8 ± 0.3	2.4242*	-
Total dissolved solids (mg/L)	56.8 ± 10.7	107 ± 60.7	-	2.0*
Electric conductivity (µS/cm)	90.4 ± 12.2	148.1 ± 111.4	-	10.5
	Rainy season	**Dry season**		
Rainfall (mm)	311.5 ± 106.7	131.0 ± 116.3	3.9908**	-
Temperature (°C)	28.3 ± 0.3	30.0 ± 0.5	6.8690**	-
Dissolved oxygen (mg/L)	5.2 ± 0.8	4.6 ± 2.12	0.4952	-
pH	6.9 ± 0.2	6.9 ± 0.3	-0.2389	-
Total dissolved solids (mg/L)	72.6 ± 34.6	91.4 ± 62.5	-	16.00
Electric conductivity (µS/cm)	98.8 ± 61.4	139.6 ± 98.7	-	15.50

t: t test; *U:* Mann- Whitney test; *p < 0.05; **p < 0.001

Data analysis

Parasite indices such as prevalence and mean abundance of parasites at the infrapopulation level were calculated (Bush et al., 1997). Poulin discrepancy index (D) (Poulin, 1993) were calculated with *d*-statistics using Quantitative Parasitology 3.0 software (Reiczigel et al., 2019). These parameters were calculated for species with prevalence > 10% (Bush, 1990). To describe the parasite community, the species richness, Brillouin diversity index (*HB*), evenness (*E*) and Berger-Parker dominance index (*d*) were calculated at the infracommunity level using PAST software (Hammer et al., 2001). These analyzes were carried out with the objective of evaluating the dynamics and structure of the infracommunities parasites, examining their relationship with annual variations and seasonal and the diversity of the respective infracommunities parasites. The obtained parasite indices were used to evaluate the descriptors ecological. The descriptors used in the data analysis were divided here into groups according to their purpose: measures of diversity, dominance, similarity and dispersion (Magurran, 1988). The Spearman correlation coefficient (*rs*) was used to evaluate possible correlations between host body length and weight with parasite abundance, species richness and diversity (Zar 2010).

The total length (cm) and weight (g) of fish were used to determine the relative condition factor (Kn) (Le Cren, 1951) in the years 2020 and 2021 during both rainy and dry seasons. The Shapiro-Wilk and Bartlett tests were used to determine if data on length, weight, relative condition factor, prevalence, abundance, species richness, evenness (*E*), Brillouin diversity index (*HB*) and Berger-Parker dominance index (*d*) followed a normal distribution pattern and if there was homoscedasticity among groups. To verify possible differences in the prevalence of parasites between the years 2020 and 2021 and between the seasonal periods (rainy and dry), the chi-square test (χ^2) with Yates's correction was used. To investigate any differences in the abundance of parasites, length, weight and Kn, the Mann-Whitney test (*U*) was used (Zar, 2010). T-test was used to evaluate differences in length, weight and Kn of hosts according to year and season. To estimate any differences in the diversity indices (species richness, *E*, *HB* and *d*) of metazoan parasites between 2020 and 2021 and seasonal periods, the Kruskal-Wallis test was used, followed by the Dunn test. These analyses were performed in the R software (R CoreTeam, 2021).

Variance analysis of permutation (PERMANOVA) was used to detect any differences in the parasite community between the years 2020 and 2021 and the rainy and dry seasons. Abundance data, as well as yearly and seasonal data, used as separate factors to evaluate similarity between the parasitic communities**,** were adjusted to fit within the ordering of principal coordinate analysis (PCoA) based on Bray-Curtis distance, using the envfit function of the vegan package (Oksanen et al., 2020); and p-values were calculated using the permutation test (number of permutations = 999) in the R software. To analyze how differences in sampling effort can influence the results, we plotted a species accumulation curve (observed and expected) for the years 2020 and 2021 and for the rainy and dry seasons. These analyses were performed using the R software, version 4.2.2, using the vegan package (Oksanen et al., 2020).

Results

Parasite component community

Colomesus asellus was found to be parasitized by Nematoda, Digenea, Ergasilidae, Acanthocephala and Crustacea. The dominant species was the ergasilid *E. colomesus*. No parasites was found in the mouths or fins of the hosts (Table 2). The community of parasites showed low species richness, low *HB* and predominance of ectoparasites (Table 2). Only *Contraecum* sp. Railliet & Henry, 1912 and *E. Colomesus* Thatcher & Boeger, 1983 were prevalent above 10%, and they were analyzed separately. Hosts were predominantly infected by just one species of parasite. Larvae of *Contracaecum* sp. (*d*= 5.5 and D= 0.83) showed an aggregated distribution pattern, whereas of *E. colomesus* (*d*=-14.5 and D= 0.19) had a uniform pattern.

Species richness (*rs* = 0.23, p = 0.01 and *rs* = 0.23, p = 0.01), Brillouin diversity (*rs* = 0.19, p = 0.04 and *rs* = 0.19, p = 0.04), abundance of *Contracaecum* sp. (*rs* = 0.38, p = 0.0001 and *rs* = 0.37, p = 0.0001) and abundance of *E. colomesus* (*rs* = 0.40; p = 0.0001 and *rs* = 0.40, p = 0.0001) showed a weak and significant positive correlation with host length and weight, respectively.

Table 2. Metazoan parasites of *Colomesus asellus* from the Amazon River, in the eastern Amazon region, Brazil.

Parasite species	P (%)	MA ± SD	MI ± SD	TNP	FD (%)	SI
Nematoda						
Contracaecum sp. (larvae)	7.5	0.2 ± 1.1	2.5 ± 3.5	20	2.1	Intestine
Contracaecum sp. (larvae)	13.1	0.2 ± 0.9	1.9 ± 1.9	26	2.8	Stomach
Contracaecum sp. (larvae)	3.7	0.2 ± 1.2	4.3 ± 5.2	17	1.8	Abdominal cavity
Digenea						
Genarchella genarchella	0.9	0.01 ± 0.1	1 ± 0	1	0.1	Pharynx
Genarchella genarchella	3.7	0.4 ± 2.9	11 ± 11.7	44	4.7	Intestine
Genarchella genarchella	0.9	0.01 ± 0.1	1 ± 0	1	0.1	Stomach
Genarchella genarchella	0.9	0.01 ± 0.1	1 ± 0	1	0.1	Abdominal cavity
Clinostomum marginatum	0.9	0.01 ± 0.1	1 ± 0	1	0.1	Stomach
Acanthocephala						
Brasacanthus sp.	1.9	0.02 ± 0.1	1 ± 0	2	0.2	Pharynx
Brasacanthus sp.	2.8	0.1 ± 0.9	5.0 ± 2.6	15	1.6	Intestine
Brasacanthus sp.	4.7	0.7 ± 5.0	14.8 ± 20.1	74	7.9	Stomach
Brasacanthus sp.	0.9	0.01 ± 0.1	1.0 ± 0	0	0.1	Abdominal cavity
Crustacea						
Argulus pestifer	0.9	0.01 ± 0.1	1.0 ± 0	1	0.1	Gills
Ergasilus colomesus	80.4	6.8 ± 13.8	8.5 ± 15	728	78.1	Gills
Number of fish examined	107					
Total number of parasites	932					
Total prevalence (%) of parasites	82.2					
Percentage of endoparasites (%)	66.6					
Percentage of ectoparasites (%)	33.3					
Percentage of larvae	6.7					
Species richness of parasites	1.3 ± 0.9					
Brillouin diversity index	0.1 ± 0.2					
Evenness	0.8 ± 0.4					

P: prevalence; MA: mean abundance; MI: mean intensity; TNP: total number of parasites; FD: frequency of dominance; SI: site of infection; SD: standard deviation.

Annual variations in the parasite community

The fish host collected in 2020 measured an average of 7.9 ± 3.1 cm and those collected in 2021 measured an average of 6.8 ± 3.1 cm, showing no significant difference (U = 1046.5; p = 0.058). The fish collected in 2020 weighed an average of 24.4 ± 21.3 g and those collected in 2021 weighed an average of 17.1 ± 20.8 g, showing significant difference (U = 998.5; p = 0.02). The relative condition factors (Kn) of the hosts collected in 2020 (Kn = 0.96 ± 0.23) and 2021 (Kn = 0.86 ± 0.62) were not significantly different (U = 1133.0; p = 0.49).

Among the hosts examined, a total of 485 parasites were collected in 2020, while 447 were collected in 2021. In both 2020 and 2021, hosts were predominantly infected by only one species of parasite (Fig. 2). In 2020, the total prevalence of parasites was 74.6%, and in 2021 it was 95%. In both years, *E. colomesus* was the most dominant parasite, with higher prevalence in 2021. However, the prevalence of the other species was not different between the years (Table 3).

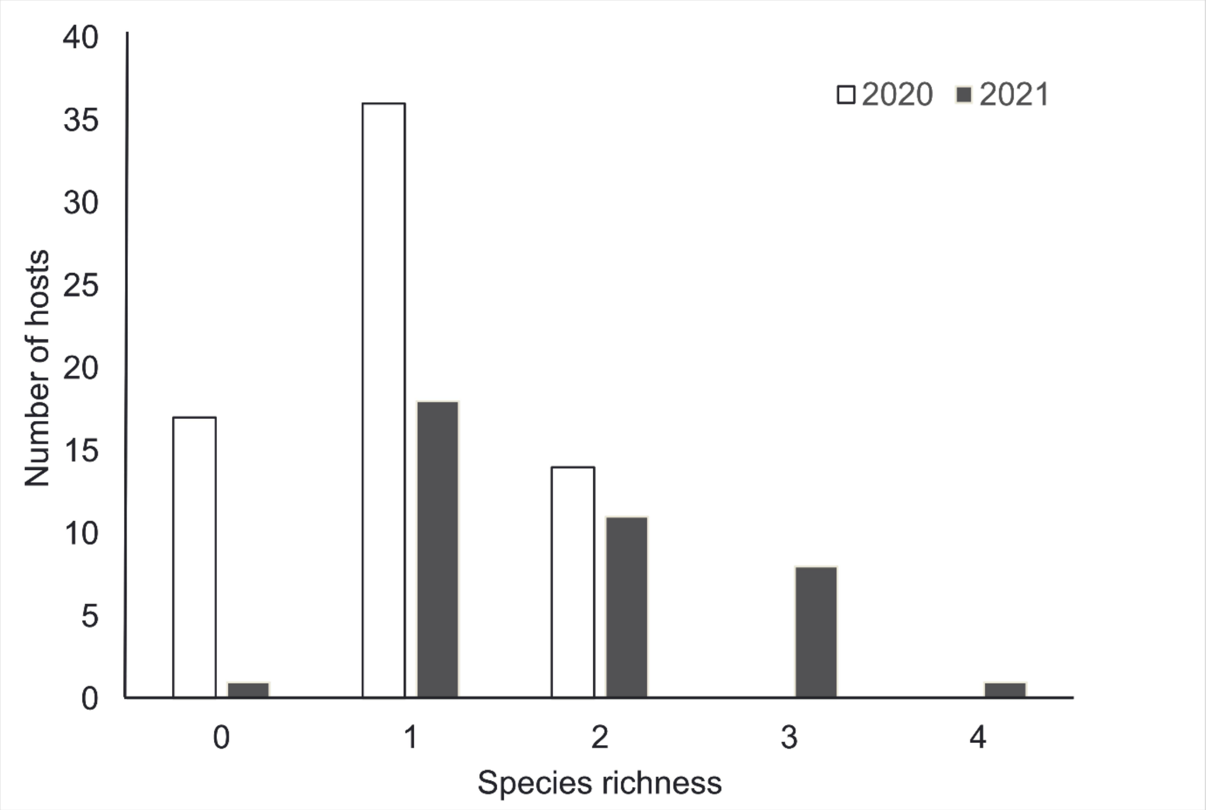

Fig. 2 Species richness of metazoan parasites in *Colomesus asellus* from the Amazon River, Brazil, during the two years of sample collection.

Table 3. Metazoan parasites in *Colomesus asellus* from the Amazon River, in the eastern Amazon region, Brazil, collected over a two-year period.

		2020 (n = 67)			**2021 (n = 40)**				
Parasite species	**Infection site**	**P (%)**	**MA ± SD**	**TNP**	**P (%)**	**MA ± SD**	**TNP**	**χ^2**	***U***
Contracaecum sp. (larvae)	Intestine, stomach and abdominal cavity	17.9	0.5 ± 1.8	36	27.5	0.7 ± 2.0	27	1.3	1216.0
Genarchella genarchella	Pharynx, intestine, stomach and abdominal cavity	6.0	0.7 ± 3.7	45	2.5	0.05 ± 0.3	2	0.6	1293.5
Clinostomum marginatum	Stomach	1.5	0.01 ± 0.1	1	0	0	0	-	-
Brasacanthus sp.	Pharynx, intestine, stomach and abdominal cavity	0	0	0	22.5	2.3 ± 8.3	92	-	-
Argulus pestifer	Gills	0	0	0	2.5	0.03 ± 0.2	1	-	-
Ergasilus colomesus	Gills	73.1	6.0 ± 12.0	403	92.5	8.1 ± 16.5	325	4.7*	1136.0

P: prevalence; MA: mean abundance; SD: standard deviation; TNP: total number of parasites; χ^2: chi-square test; U: Mann-Whitney test; *p < 0.05; **p < 0.001

Axes 1 and 2 of PCoA were shown to be responsible for 65.8% of the total variation of the data in the composition of abundance in 2020 and 2021. Although PCoA showed overlapping and sharing of species during these years, there was a significant difference (PERMANOVA: F = 3.014, p = 0.034) in the parasite infracommunities, influenced mainly by variation in the abundance of *E. colomesus* ($R^2 = 0.451$, $p = 0.001$), *Contracaecum* sp. ($R^2 = 0.163$, $p = 0.001$), *Genarchella genarchella* Travassos, Artigas & Pereira, 1928 ($R^2 = 0.087$, $p = 0.015$) and *Brasacanthus* sp. Thatcher, 2001 ($R^2 = 0.072$, $p = 0.026$) (Fig. 3).

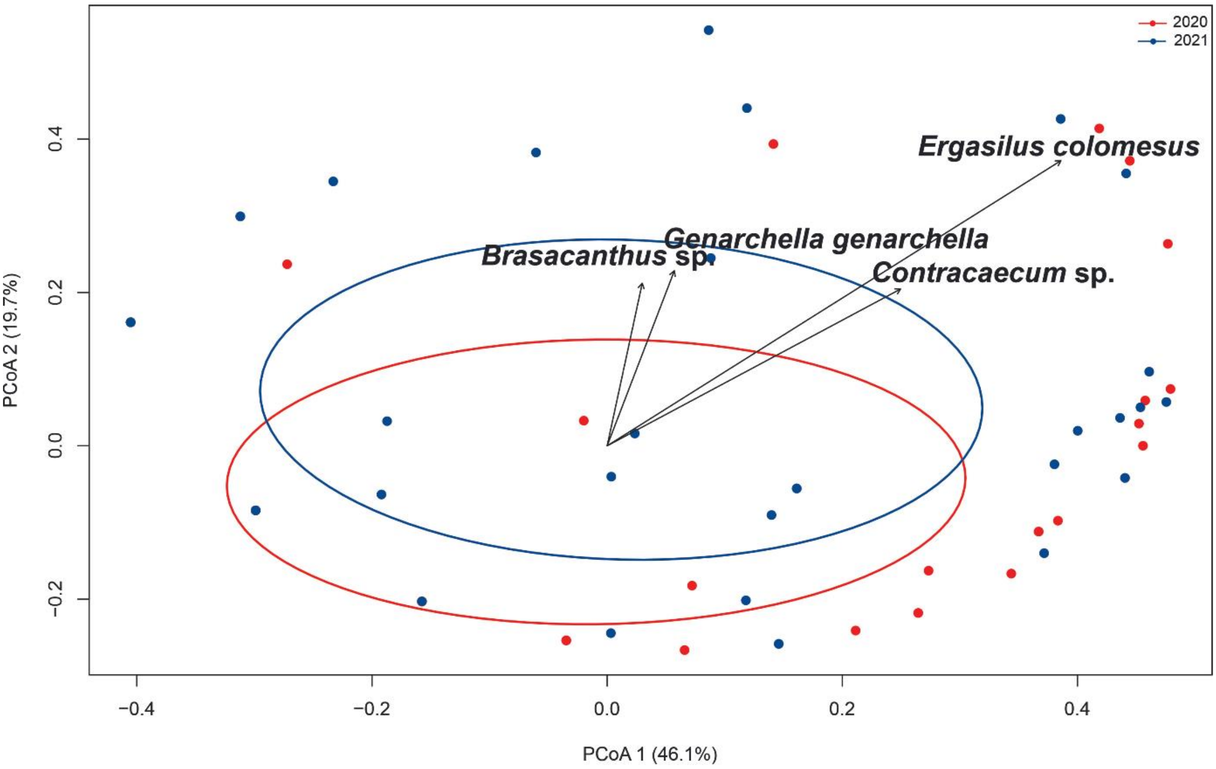

Fig. 3 Principal coordinate analysis (PCoA) using a Bray-Curtis distance matrix for communities of metazoan parasites of *Colomesus asellus* from the Amazon River, in the state of Amapá, Brazil, during 2020 and 2021. The percentage of the variation explained by the plotted principal coordinates is indicated on the axes.

The richness of parasite species ($\chi^2 = 19.186$, $p = 0.00001$) and Brillouin diversity index ($\chi^2 = 15.371$, $p = 0.00008$) were higher in 2021, but evenness ($\chi^2 = 0.288$, $p = 0.591$) and Berger-Parker dominance ($\chi^2 = 0.006$, $p = 0.939$) showed no difference between the years studied (Fig. 4). The species accumulation curve showed that the number of hosts collected was sufficient for the parasite species collected to reach representativeness, because the curves for both years showed a trend of stability (Fig. 5).

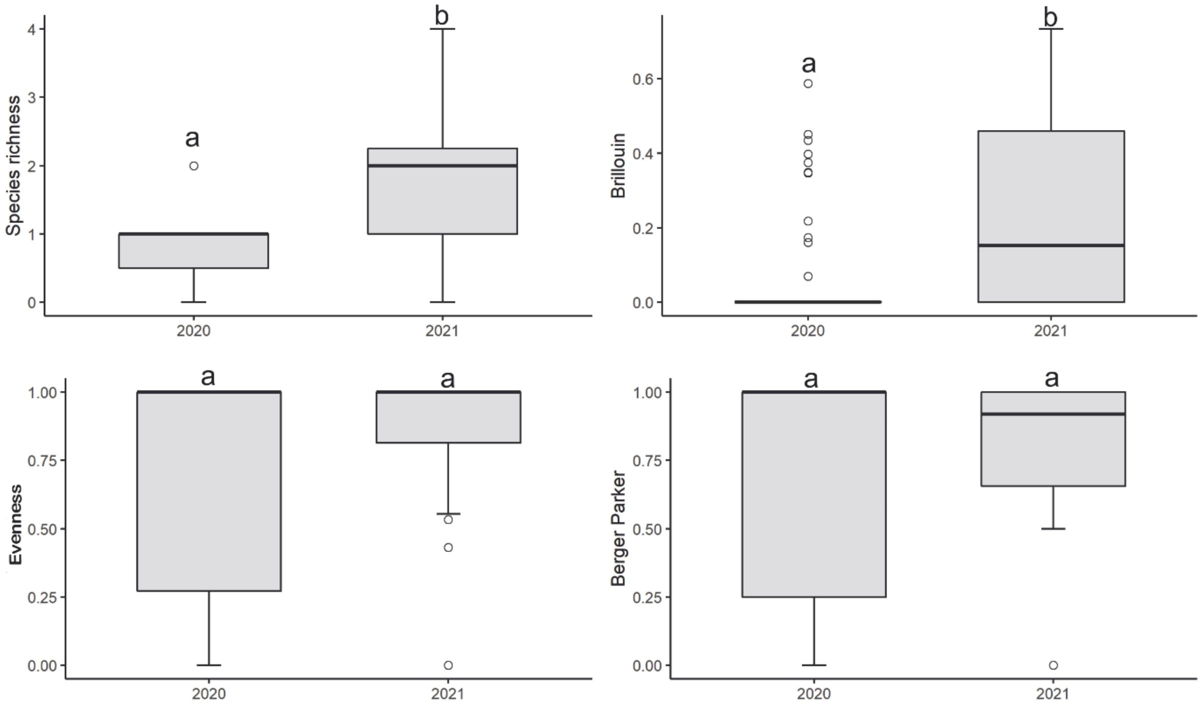

Fig. 4 Diversity parameters for metazoan parasites in Colomesus asellus from the Amazon River, in the eastern Amazon region, Brazil, collected in 2020 and 2021 (box plots show medians, interquartile ranges, minimum-maximum ranges and outliers). Different letters indicate differences between the medians according to Dunn's test ($p < 0.001$).

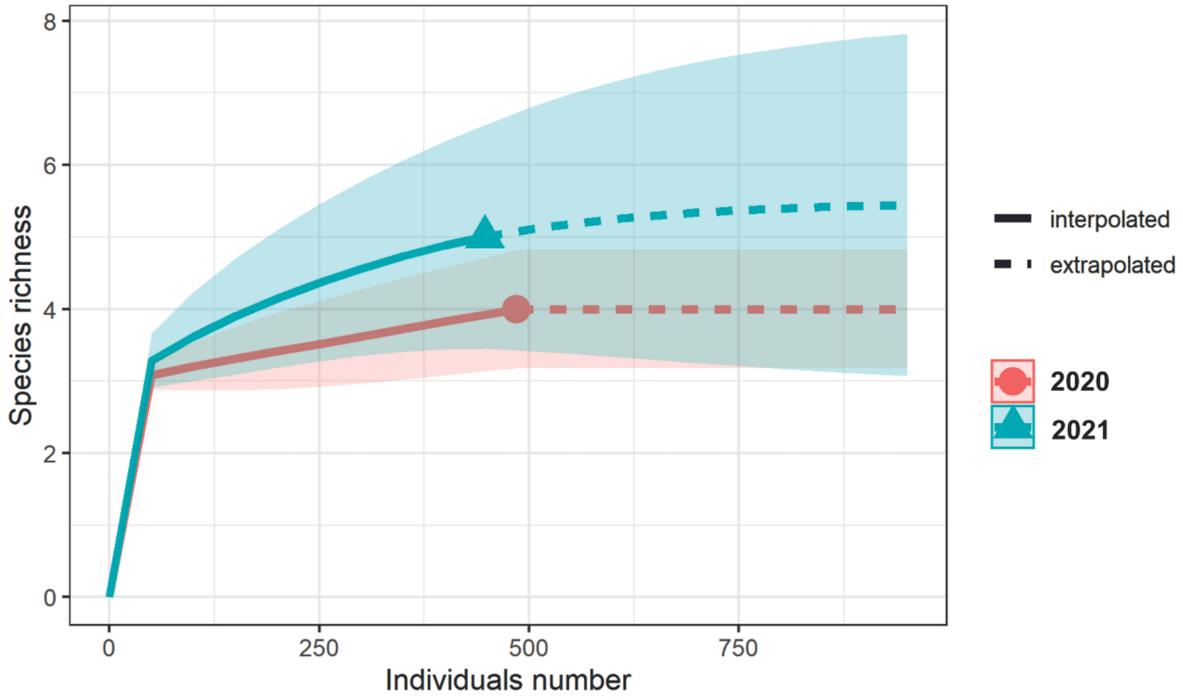

Fig. 5 Species accumulation curve for metazoan parasites in *Colomesus asellus* from the Amazon River, in the state of Amapá, Brazil, collected in 2020 and 2021.

Seasonal variations in the parasite community

Fish collected in the rainy season measured an average of 5.6 ± 2.6 cm, and those collected in the dry season measured an average of 9.9 ± 2.1 cm, showing significant difference (*U* = 344; p = 0.0001) between these seasonal periods. Fish collected in the rainy season weighed 10.0 ± 12.9 g, while those collected in the dry season weighed 37.7 ± 20.3 g, which also showed a significant difference (*U* = 297; p = 0.0001) between these seasonal periods. The relative condition factors of the hosts collected during the rainy season (Kn = 0.94 ± 0.27) and dry season (Kn = 1.00 ± 0.04) did not show any significant difference (*U* = 1178.0; p = 0.17).

A total of 246 parasites were collected in the rainy season and 686 in the dry season. In the rainy season, hosts were infected by one parasite species, while in the dry season, hosts were infected by two parasite species (Fig. 6). In the rainy season, 69.3% of the fish were parasitized; and in the dry season, 100% of the fish were parasitized. *E. colomesus* was predominant in both seasonal periods. There was higher prevalence and abundance of *E. colomesus* and *Contracaecum* sp. in the dry season, while the other species did not present significant differences between the seasons (Table 4).

Axes 1 and 2 shown in the PCoA results (Fig. 7) were responsible for 68.2% of the total variation in the composition of parasite abundance in the rainy and dry seasons. Despite the overlap due to species sharing, PCoA showed that there were significant differences (PERMANOVA: F = 9.915; p = 0.001) in the parasite infracommunities between the seasonal periods, influenced mainly by the variations in abundance of *E. colomesus* (R^2 = 0.5429; p = 0.001) and larvae of *Contracaecum* sp. (R^2 = 0.173; p = 0.002) (Fig. 7).

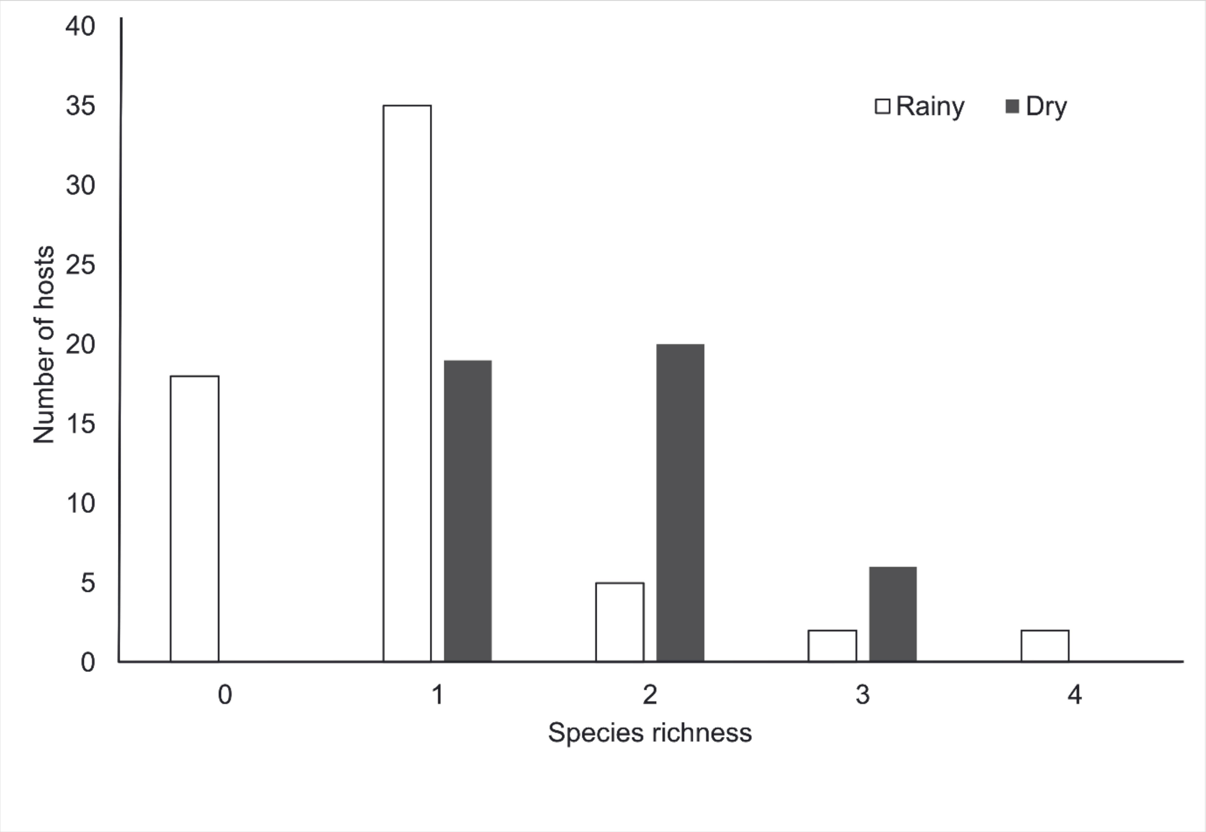

Fig. 6 Species richness of metazoan parasites in *Colomesus asellus* from the Amazon River during the rainy and dry seasons.

The richness of parasite species (χ^2 = 25.791; p = 0.0000004) and Brillouin diversity index (χ^2 = 18.301; p = 0.00002) were higher in the dry season, while evenness (χ^2 = 0.175; p = 0.676) and Berger-Parker dominance (χ^2 = 0.312; p = 0.577) did not show any significant differences between the seasonal periods (Fig. 8). The curve of accumulation of parasite species showed differences in species richness between the seasonal periods, while the representativeness of the richness of parasite species collected was not affected by the sampling effort between the seasonal periods, as the two curves showed a trend of stability (Fig. 9).

Table 4. Metazoan parasites in *Colomesus asellus* from the Amazon River, in the eastern Amazon region, Brazil, collected during the rainy and dry seasons.

		Rainy season (n = 62)			**Dry season (n = 45)**				
Parasite species	**Site of infection**	**P (%)**	**MA ± SD**	**TNP**	**P (%)**	**MA ± SD**	**TNP**	**χ^2**	***U***
Contracaecum sp. (larvae)	Intestine, stomach and abdominal cavity	4.8	0.08 ± 0.4	5	44.4	1.3 ± 2.7	58	21.9**	838.5**
Genarchella genarchella	Pharynx, intestine, stomach and abdominal cavity	1.6	0.02 ± 0.1	1	8.9	1.0 ± 4.5	46	3.0	1292.0
Clinostomum marginatum	Stomach	0	0	0	2.2	0.02 ± 0.1	1	-	-
Brasacanthus sp.	Pharynx, intestine, stomach and abdominal cavity	12.9	1.47 ± 6.8	91	2.2	0.02 ± 0.1	1	3.8	1242.5
Argulus pestifer	Gills	1.6	0.02 ± 0.1	1	0	0	0	-	-
Ergasilus colomesus	Gills	67.7	2.3 ± 3.5	148	97.8	12.8 ± 19.4	580	13.0**	545.5**

P: prevalence; MA: mean abundance; SD: standard deviation; TNP: total number of parasites; χ^2: chi-square test; U: Mann-Whitney test; *p < 0.05; **p < 0.001

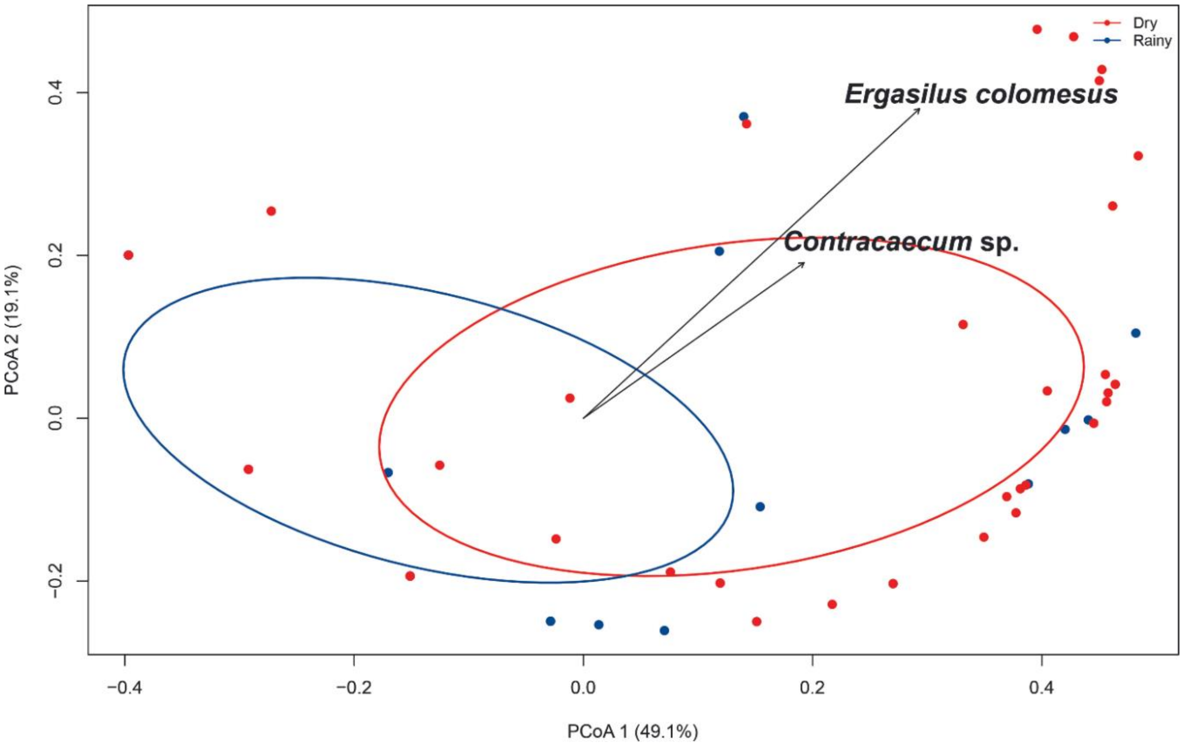

Fig. 7 Principal coordinate analysis (PCoA) using a Bray-Curtis distance matrix for communities of metazoan parasites of *Colomesus asellus* from the Amazon River, in the eastern Amazon region, Brazil, during the rainy and dry seasons. The percentage of the variation explained by the plotted principal coordinates is indicated on the axes.

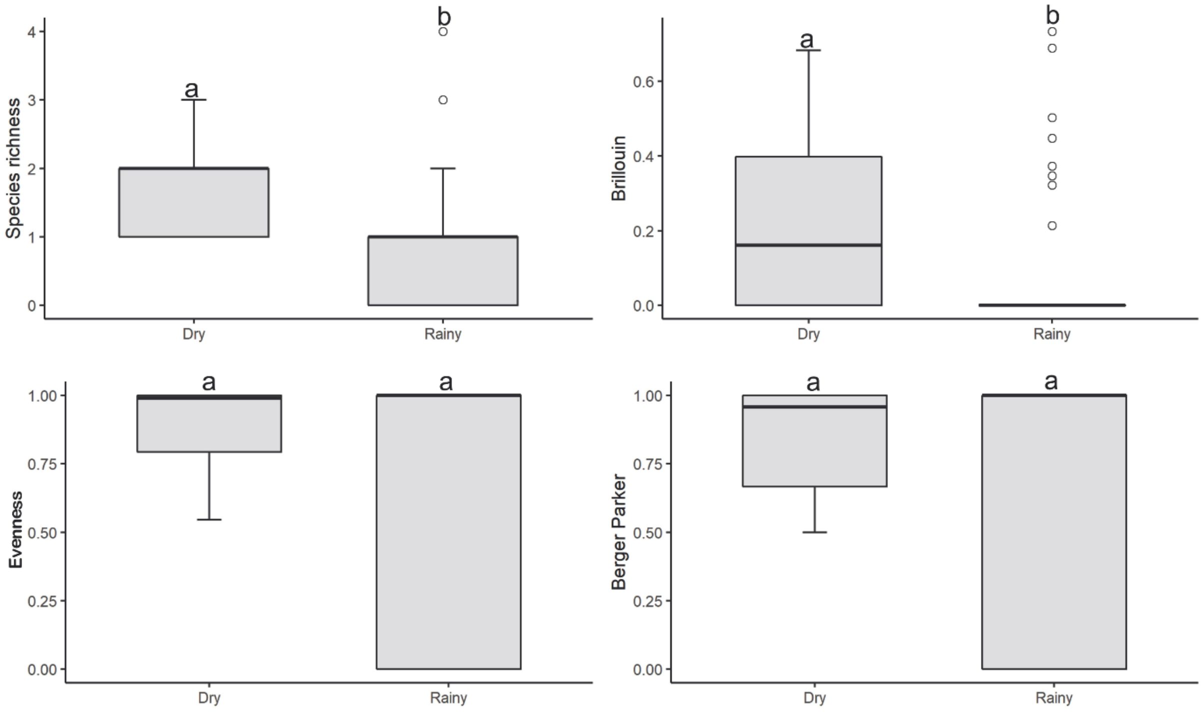

Fig. 8 Diversity parameters of metazoan parasites in *Colomesus asellus* from the Amazon River, in the eastern Amazon region, Brazil, during the rainy and dry seasons (box plots

represent medians, interquartile ranges, minimum–maximum ranges and outliers). Different letters indicate differences between the medians according to Dunn's test ($p < 0.001$).

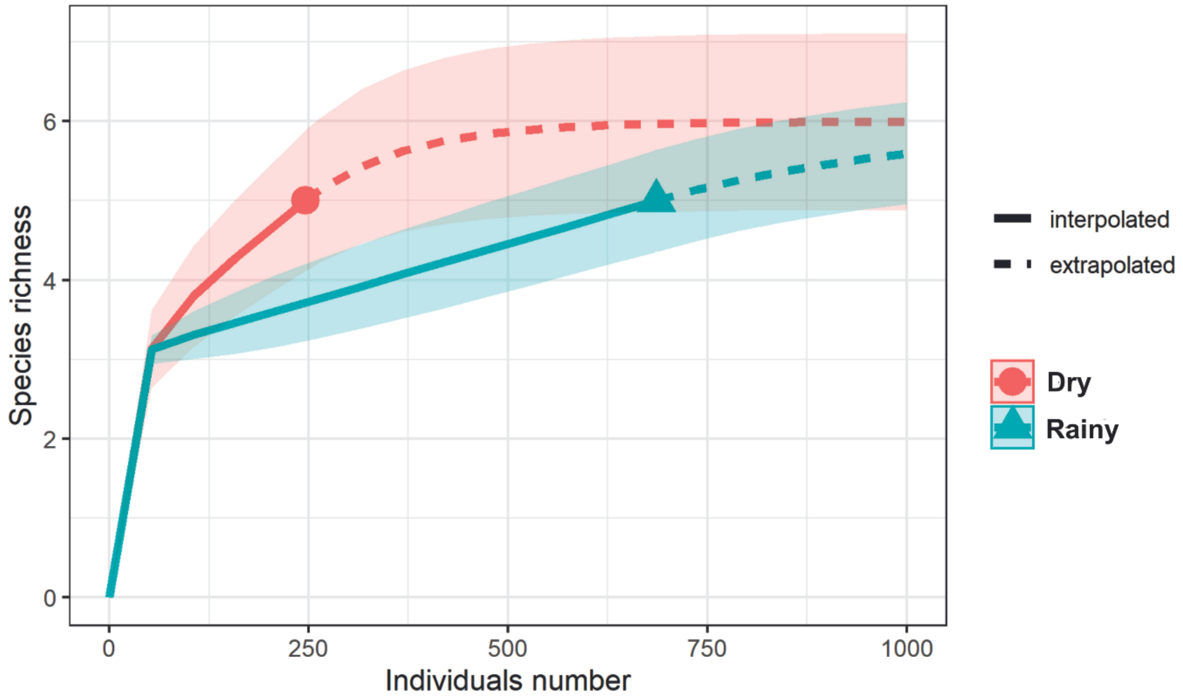

Fig. 9 Species accumulation curve for metazoan parasites in *Colomesus asellus* from the Amazon River, in the eastern Amazon region, Brazil, during the rainy and dry seasons.

Discussion

The community of metazoan parasites of *C. asellus* from the Amazon River was represented by one species of Nematoda, two species of Digenea, one species of Acanthocephala and two species of Crustacea. No monogenea was found in the gills of *C. asellus*, similar to the study by Neves et al., (2020), indicating that there is probably no Monogenea infestation or the occurrence is so low that it is not appearing in the samples. *Contracaecum* sp., *G. genarchella*, *Clinostomum marginatum* Braun, 1899, *Brasacanthus* sp. and *Argulus pestifer* Ringuelet, 1948, were new records for *C. asellus*. Comparatively, for *Colomesus psittacus* Bloch & Schneider, 1801 from the Marajó and Tocantins River archipelago, in the state of Pará, Brazil, two species of Nematoda (*Cucullanus marajoara* and *Gnathostoma* sp.) and one of Digenea (*Rohdella amazonica*) have been reported (Giese et al., 2015; Pinheiro et al., 2017; Pinheiro et al., 2018). These differences probably be attributed to differences in host species, an their diet composition, local environmental characteristics and sampling period, considering that in the present study, the community of parasites in *C. asellus* was investigated over a two-year period. The presence of these endoparasites (Nematoda, Digenea and Acanthocephala) may be related to the omnivorous feeding habits (mollusks, copepods, cladocerans and other aquatic invertebrates) of *C. psittacus* and *C. asellus* (Froese & Pauly, 2022), since endoparasite infection has been correlated with the diet of fish host, in which prey items usually are intermediate hosts (Sabas & Brasil-Sato, 2014; Blasco-Costa & Poulin, 2017; Oliveira et al., 2017; Tavares-Dias & Neves, 2017; Negreiros et al., 2019). In the case of the ectoparasite *E. colomesus*, the infestation may be related to host specificity and to the continuous reproductive period of this crustacean, as was reported in *C. asellus* from the Môa River, in the eastern Amazon region of Brazil (Virgílio et al., 2021).

Larvae of Contracaecum sp. showed aggregated distribution within the population of C. asellus, can be attributed to different factors such as host size, host density, factors that can be influenced by environmental variations (Vasconcelos & Tavares-Dias, 2017). This is a common pattern for parasites of several species of wild freshwater fish in different environments (Oliveira et al., 2017; Tavares-Dias & Oliveira, 2017a; Neves et al., 2021; Lima et al., 2021). Aggregated distribution of parasites may be, and associated to genetic variability of host population, decreased interspecific competition between parasites, decreased host damage, and variation in environmental factors (Poulin, 2013; Tavares-Dias & Oliveira, 2017; Salgado-Maldonado et al., 2019). The distribution of *E. colomesus* was uniform. Although uniform dispersion for parasites is less frequent in wild fish populations, this has also been reported for *Cosmetocleithrum striatuli* Abdallah, Azevedo & Luque, 2012, *Contracaecum* sp. and *Neoechinorhynchus pterodoridis* Thatcher, 1981 in osts from the Amazon (Goncalves et al., 2018; Carvalho et al., 2020).

The presence of *Contracaecum* sp. larvae in *C. asellus* indicates that this fish is an intermediate or paratenic in the local ecosystem, serving as prey for fish-eating birds and othes larger carnivorous fish (Bartolette et al., 2018; Froese & Pauly, 2022). In the stomach of *C. asellus*, we found larvae of Trichoptera, Chironomidae, mites, crabs, gastropods and shrimps, thus indicating that this host occupies an intermediary position in the food web.

Argulus pestifer presented low levels of infestation in the gills of *C. asellus* from the Amazon River, while *E. colomesus* presented high levels. *Ergasilus colomesus*, a crustacean with host specificity high, has wide distribution in the Amazon River system, since it has been found in *C. asellus* along this river and in some of its tributaries (Thatcher & Boeger, 1983; Virgilio et al., 2021). So far, there is no record of *E. colomesus* infesting other hosts. So far, there is no record of *E. colomesus* infesting other hosts.

The specimens of *C. asellus* collected in 2020 weighed more than those collected in 2021; however, host Kn was not different between these years. Such differences in host body weight may be related to different levels of rainfall between years and temporal segregation of young and adult fish, influenced by seasonality. In the rainy season, young *C. asellus* are predominantly found at the margins of the river, taking advantage of the best feeding conditions, while in the dry season adult *C. asellus* in the reproductive phase predominate (Bartolette et al., 2018). These adults consume a higher amount of food, but diet composition of juvenile and adult fish is similar (Bartolette et al., 2018). In 2020, the lower rainfall may have resulted in higher sampling of adult individuals during their reproductive period, since we observed specimens of *C. asellus* with mature gonads, possibly consuming higher amounts of food. These factors may have influenced the differences in the weight of these hosts.

The present diversity and species richness of parasites were higher in 2021, caused by the presence of *A. pestifer* and *Brasacanthus* sp., which were found only in the hosts collected during this period. Similar studies have also reported annual differences in the diversity and richness of parasites in *P. blochii* from the Iaco River (Negreiros et al. 2019) and in *H. surinamensis* from the Igarapé Fortaleza basin, Amazon region (Hoshino and Tavares-Dias 2019). Such differences may have been influenced by variation in the levels of precipitation between the years, altering the outflow of Amazonian rivers and, consequently, the physicochemical characteristics of these environments.

The differences in the parasite communities of *C. asellus* between 2020 and 2021 indicated through PCoA were mainly due to the abundance of *Contracaecum* sp., *G. genarchella*, *Brasacanthus* sp. and *E. colomesus*. These differences may have been related to the availability of intermediate hosts of these endoparasites in the environment, as also reported for other wild fish species (Hoshino & Tavares-Dias, 2019; Villalba-Vasquez et al., 2018; Lehun et al., 2022). In addition, the prevalence of *E. colomesus* was higher in 2021, and this temporal variation was more related to seasonal variation. In *Hemibrycon surinamensis* Gery, 1962 and *M. lippincottianus* (Hoshino & Tavares-Dias, 2019; Hoshino & Tavares-Dias, 2020) from the eastern Brazilian Amazon, short-term annual variations in parasite communities have been correlated with the seasonal cycle, availability of infectious stages, changes on the process of recruitment of parasite species, urban eutrophication in the environment and host body size.

In both years, infection by *G. genarchella* and *Contracaecum* sp. larvae was present, indicating that contact between *C. asellus* and infective stages of these endoparasites occurred annually without distinction. Similar results were previously reported for *H. surinamensis* from a tributary of the Amazon River, where the levels of infection by *G. genarchella* and *Contracaecum* sp. larvae was also common among the years (Hoshino & Tavares-Dias, 2019).

However, infection by *C. marginatum* was low and occurred only in 2020, when the hosts had contact with the infective stages in the environment.

Argulus pestifer was observed only in 2021, whereas *E. colomesus* occurred in both years, but with higher levels of infection occurring in 2021. In *H. surinamensis* infestations by *Argulus* sp. also occurred in one of the years of study. The higher population density and aggregating behavior of *C. asellus* for its feeding and reproduction (Bartolette et al., 2018) seem to have facilitated the encounters with *E. colomesus*, which became attached to these hosts' gills for reproduction (Williams & Bunkley-Williams, 2019). However, it is also possible that *E. colomesus* just might be more successful in infecting hosts in the dry season when the water level is lower, increasing the frequency of the encounters between the fish and parasites.

The specimens of *C. asellus* collected in the dry season were larger and heavier than those of the rainy season. This indicated that the fish fed more in this season and/or were in the reproductive period, as indicated by the presence of mature gonads in 12 of the fish examined. The Amazon basin has periods of flood and drought that influence the communities of invertebrates and fish that serve as food for *C. asellus*. Populations of *C. asellus* show temporal segregation between juvenile and adult, in which the rainy season young individuals (smaller and lighter) predominantly occupy the margins of rivers, where conditions for development are better (Bartolette et al., 2018). On the other hand, in the dry season, the margins are predominantly occupied by adult individuals (larger and heavier), for feeding and reproduction (Bartolette et al., 2018). Consequently, we collected larger and heavier individuals in the dry season. In addition, adult fish consume larger amounts of food in the dry season (Bartolette et al., 2018). I suggest including this sentence here: "These observations probably driven similar results obtained in the annual comparisons.

In *H. surinamensis*, the richness of parasite species showed seasonal changes, being higher in the dry season (Hoshino & Tavares-Dias, 2019). In *P. blochii* from Acre and Xapuri rivers, this parameter had no influence of seasonality (Cavalcante et al., 2020). Among the causes that may have influenced have influenced the differences in parasite richness among seasons are environmental variations (e.g. decreased dissolved oxygen in the dry season, accumulation of organic matter, and increased turbidity and suspended solids in water during the rainy season) as a consequence of cyclic seasonality in the Amazon (Cunha et al., 2003; Takiyama et al., 2004). This seems to have increased the chances of contact between the present hosts and some of their parasites, due to a reduction in habitat range that occurs during the dry season, affecting the abundance and richness of parasite species (Gonçalves et al., 2016;

Tavares-Dias et al., 2014; Lima et al., 2021). However, the variations in rainfall and temperature seem to have altered the recruitment of parasites of *C. asellus*.

In the Amazon region seasonality is well defined by two periods, one of intense rainfall (rainy season) and one of less intensity (dry season). These seasonal fluctuations may be responsible for variations in the recruitment of parasite species, availability of food to host fish and, presence of infectious stages in the environment (Neves et al., 2013; Gonçalves et al., 2016; Negreiros et al., 2019; Hoshino & Tavares-Dias, 2020), in addition to influencing the breeding period of Amazonian fish (Cavalcante et al., 2020). These effects can increase stress levels between fish host and, consequently, their susceptibility to parasitic infections, thus altering the structure of parasite communities (Gonçalves et al., 2016). In this sense, the PCoA showed seasonal differences in the abundance of *Contracaecum* sp. and *E. colomesus*, along with greater richness of parasite species and higher Brillouin diversity in the dry season. This may be related to the number of adult individuals collected in the dry season, consuming a greater variety of foods, thus being able to come into contact with different infectious stages of parasites in the environment.

In *C. asellus*, larvae of *Contracaecum* sp. were found in both seasonal periods, but the highest levels of infection were observed in the dry season. In *Hoplias malabaricus* Bloch, 1794 and *Hoplerythrinus unitaeniatus* Spix & Agassiz, 1829 from the eastern Brazilian Amazon region, larvae of *Contracaecum* sp. were found in the dry and rainy season (Gonçalves et al., 2016). Infection by *G. genarchella* occurred also in both seasons, while *C. marginatum* occurred only in the dry season, when the chances of host fish finding the infective stage of this digenean are higher.

Conclusions

This study contributes to knowledge of the metazoan parasite community of *C. asellus*, which was characterized by the presence of ectoparasites and endoparasites. It can be said that the parasite diversity of *C. asellus* is lower than that observed for other fish species in the Amazon, with low prevalence and low abundance, and with dominance of *E. colomesus*. Furthermore, only *Contracaecum* sp. was found larval stage, which indicates that *C. asellus* acts both as an intermediate/paratenic host and as a definitive host for the acanthocephalans and digenean found in adult stages. These results, based on a two-year time span, indicated that the infection patterns of some parasite communities differed between years and seasons, corroborating the hypothesis that annual and seasonal variation could influence the patterns of infection of parasite species. The seasonal variations in parasite diversity and infection levels were related to variations in rainfall levels, and host body size. Lastly, in this study, except by *E. colomesus*, all parasites species represented new records for *C. asellus.*

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Statements and Declarations

Declaration of authors' contributions

All authors contributed to the conception and design of the study. Material preparation, data collection and analysis were performed by Elvis Silva Lima. The first version of the manuscript was written by Elvis Silva Lima and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Declaration of data availability

DECLARATIONS FOR PUBLICATION

Conflict of interests

The authors declare that there was no conflict of interest.

Ethical guidelines

Ethics approval for obtaining access to genetic heritage was authorized by the Brazilian Ministry of the Environment (SISBio no. 73550-1 and SisGen no. AA4B6BA). This study was developed in accordance with the principles adopted by the Brazilian College of Animal Experimentation (COBEA) and was conducted under authorization from the Ethics Committee for Animal Use of Embrapa (protocol no. 014/2018).

Data availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

References

Baia, R. R. J., A. C. Florentino, L. M. A. Silva & M. Tavares-Dias, 2018. Patterns of the parasite communities in a fish assemblage of a river in the Brazilian Amazon region. Acta Parasitologica 63: 304–316. https://doi.org/10.1515/ap-2018-0035.

Bartolette, R., D. C. O. Rosa, D. A. Beserra, B. E. Soares, M. P. Albrecht & M. F. G. Brito, 2018. Seasonal and ontogenetic diet patterns of the freshwater pufferfish *Colomesus asellus* (Müller & Troschel, 1849) in the upper-middle Tocantins River. Acta Scientiarum. Biological Sciences 40: 1–6. https://doi.org/10.4025/actascibiolsci.v40i1.35282.

Blasco-Costa, I. & R. Poulin, 2017. Parasite life-cycle studies: A plea to resurrect an old parasitological tradition. Journal of Helminthology 91: 647–656. https://doi.org/10.1017/S0022149X16000924.

Bush, A. O., K. D. Lafferty, J. M. Lotz & A. W. Shostak, 1997. Parasitology meets ecology on its own terms: Margolis et al. Revisited. The Journal of parasitology 83: 575–583. https://doi.org/10.2307/3284227.

Cardoso, A. C. F., M. S. B. Oliveira, L. R. Neves & M. Tavares-Dias, 2017. Metazoan fauna parasitizing *Peckoltia braueri* and *Pterygoplichthys pardalis* (Loricariidae) catfishes from the northeastern Brazilian Amazon. Acta Amazonica 47: 147–154. https://doi.org/10.1590/1809-4392201603232.

Carvalho, A. A. & M. Tavares-Dias, 2017. Diversity of parasites in *Cichlasoma amazonarum* Kullander, 1983 during rainy and dry seasons in eastern Amazon (Brazil). Journal of applied ichthyology 33: 1178–1183. https://doi.org/10.1111/jai.13451.

Carvalho, A., R. L. Ferreira, P. Araujo, M. Tavares-Dias, E. Matos & M. N. Videira, 2020. Condition factor and ecology of endohelminths in *Metynnis lippincottianus* from the Curiaú river, in eastern Amazon (Brazil). Boletim do Instituto de Pesca 46: e559. https://doi.org/10.20950/1678-2305.2020.46.2.559

Cavalcante, P. H. D. O., M. T. Silva, A. N. S. Pereira, R. Gentile & C. P. Santos, 2020. Helminth diversity in *Pimelodus blochii* Valenciennes, 1840 (Osteichthyes: Pimelodidae) in two Amazon Rivers. Parasitology Research 119: 4005–4015. https://doi.org/10.1007/s00436-020-06906-x.

Cunha, A., J. Sousa, W. L. Gomes, J. S. F. Baía & H. F. A. Cunha, 2003. Estudo preliminar Sobre a Variação Espaço-Temporal de Parâmetros de Qualidade de Água no Igarapé da Fortaleza. Diagnóstico das Ressacas do Estado do Amapá*:* Bacias do Igarapé da Fortaleza e Rio Curiaú, Macapá-AP, CPAQ/IEPA e DGEO/SEMA, 105–136.

Eiras J.C., R. M. Takemoto & G. C. Pavanelli 2006. Métodos de estudo e técnicas laboratoriais em parasitologia de peixes, 2 nd edn. Editora da Universidade Estadual de Maringá, Maringá.

Ferrari-Hoeinghaus, A. P., R. M. Takemoto & G. C. Pavanelli, 2007. Digenetic trematode parasites of *Loricariichthys platymetopon* (Loricariidae, Siluriformes) of the upper Paraná River floodplain, Brazil. Acta Scientiarum. Biological Sciences 29: 327–329.

Froese R., & D. Pauly 2023. FishBase. World wide web electronic publication. www.fishbase.org, version (05/2022).

Giese, E.G, M. V. O. Silva, M. N. Videira, A.P. Furtado, E. R. Matos, E. C. Gonçalves, F,T.V. Melo & J. N. Santos, 2015. *Rohdella amazonica* n. sp. (Aspidogastrea: Aspidogastridae) from the Amazoninan banded puffer fish *Colomesus psittacus* (Bloch & Schneider, 1801). Journal of helminthology 89: 288–293. https://doi.org/10.1017/S0022149X14000054.

Goncalves, B. B., M. S. B. Oliveira, W. F. Borges, G. G. Santos & M. Tavares-Dias, 2018. Diversity of metazoan parasites in *Colossoma macropomum* (Serrasalmidae) from the lower Jari River, a tributary of the Amazonas River in Brazil. Acta Amazonica 48: 211–216. https://doi.org/10.1590/1809-4392201704371.

Gonçalves, R. A., M. S. B. Oliveira, L. R. Neves & M. Tavares-Dias, 2016. Seasonal pattern in parasite infracommunities of *Hoplerythrinus unitaeniatus* and *Hoplias malabaricus* (Actinopterygii: Erythrinidae) from the Brazilian Amazon. *Acta Parasitologica* 61: 119–129. https://doi.org/10.1515/ap-2016-0016.

Hammer O, D. A. T. Harper & P. D. Ryan, 2001. PAST: paleontological statistics software package for education and data analysis. Palaeont Elect 4:9

Hoshino, E. M. & M. Tavares-Dias, 2019. Interannual and seasonal variation in protozoan and metazoan parasite communities of *Hemibrycon surinamensis*, a characid fish inhabiting the Brazilian Amazon. Acta parasitologica 64: 479–488. https://doi.org/10.2478/s11686-019-00057-5.

Hoshino, E. M. & M. Tavares-Dias, 2020. Temporal and seasonal variations in parasites of *Metynnis lippincottianus* (Characiformes: Characidae), a host from the eastern Amazon (Brazil). Journal of Natural History 53: 2723–2736. https://doi.org/10.1080/00222933.2020.1733121.

Le Cren, E. D. 1951. The Length-Weight Relationship and Seasonal Cycle in Gonad Weight and Condition in the Perch (Perca fluviatilis). Journal of Animal Ecology 20: 201–219. https://doi.org/10.2307/1540.

Lehun, A. L., L. D. Cavalcanti, M. los A. P. Lizama, J. O. S. Silva, G. P. Casali, & R. M. Takemoto, 2022. Temporal effects and changes in the parasitic community of *Prochilodus lineatus* (Valenciennes, 1837)(Characiformes: Prochilodontidae) in a floodplain. Journal of Helminthology 96: e4. https://doi.org/10.1017/S0022149X21000742.

Lima, E. S., M. S. B. Oliveira & M. Tavares-Dias, 2021. Diversity and community ecology of metazoan parasites in *Pimelodus ornatus* (Siluriformes: Pimelodidae) from the Amazonas River in Brazil. Revista Brasileira de Parasitologia Veterinária 30: e006021. https://doi.org/10.1590/S1984-29612021065.

Ludwig, J. A., J. F. Reynolds, L. Quartet, & J. Reynolds, 1988. *Statistical ecology: A primer in methods and computing* (Vol. 1). John Wiley & Sons.

Magurran, A. E. 1988. Ecological diversity and its measurement. Croom Helm, London, 179p.

Morey, G. A. M., A. C. Moreira, A. M. Morais, F. M. P. B. Atroch, H. P. Santana, N. R. Brandão, J. C. Dumbo, J. F. Vital, J. O. Malta, 2016. Copepods (Crustacea: Ergasilidae) fish parasites of floodplain lakes of Central Amazon, Brazil. Neotropical Helminthology 10: 281–294.

Negreiros, L. P, F. B. Pereira, M. Tavares-Dias & L. E. Tavares, 2018. Community structure of metazoan parasites from *Pimelodus blochii* in two rivers of the western Brazilian Amazon: Same seasonal traits, but different anthropogenic impacts. Parasitology research 117: 3791–3798. https://doi.org/10.1007/s00436-018-6082-5.

Negreiros, L. P., A. C. Florentino, F. B. Pereira & M. Tavares-Dias, 2019. Long-term temporal variation in the parasite community structure of metazoans of *Pimelodus blochii* (Pimelodidae), a catfish from the Brazilian Amazon. Parasitology research 118: 3337–3347. https://doi.org/10.1007/s00436-019-06480-x.

Negreiros, L. P., F. B. Pereira & M. Tavares-Dias, 2019. Metazoan parasites of *Calophysus macropterus* (Siluriformes: Pimelodidae) in the Acre and Iaco rivers in the western Amazon region of Brazil: diversity, similarity and seasonal variation. Journal of Natural History 53: 1465–1479. https://doi.org/ 10.1080/00222933.2019.1657195.

Neves, L. R., F. B. Pereira, M. Tavares-Dias & J. L. Luque, 2013. Seasonal influence on the parasite fauna of a wild population of *Astronotus ocellatus* (Perciformes: Cichlidae) from the Brazilian Amazon. The Journal of parasitology 99: 718–721. https://doi.org/10.1645/12-84.1.

Neves, L. R., & M. Tavares-Dias, 2019. Low levels of crustacean parasite infestation in fish species from the Matapi River in the state of Amapá, Brazil. Revista Brasileira de Parasitologia Veterinária, *28*, 493-498. https://doi.org/10.1590/S1984-29612019006

Neves, L. R., L. M. A. Silva & M. Tavares- Dias, 2021. Diversity and Ecology of Endohelminth Parasites in a Fish Assemblage of an Amazon River Tributary in Brazil. Acta Parasitologica 66: 1431–1441. https://doi.org/10.1007/s11686-021-00413-4.

Oliveira, M. S. B., R. A. Gonçalves, D. O. Ferreira, D. A. Pinheiro, L. R. Neves, M. K. R. Dias & M. Tavares-Dias, 2017. Metazoan parasite communities of wild *Leporinus friderici* (Characiformes: Anostomidae) from Amazon River system in Brazil. Studies on neotropical fauna and environment 52: 146–156. https://doi.org/10.1080/01650521.2017.1312776.

Pinheiro, R. H. S., R. L. S. Santana, F. T. V. Melo, J. N. dos Santos & E. G. Giese, 2017. Gnathostomatidae nematode parasite of *Colomesus psittacus* (Osteichthyes, Tetraodontiformes) in the Ilha de Marajó, Brazilian Amazon. Revista Brasileira de Parasitologia Veterinária 26: 340–347. https://doi.org/10.1590/S1984-29612017047.

Pinheiro, R. H. S., R. L. S. Santana, S. Monks, J. N. Santos & E. G. Giese, 2018. *Cucullanus marajoara* n. sp. (Nematoda: Cucullanidae), a parasite of *Colomesus psittacus* (Osteichthyes: Tetraodontiformes) in the Marajó, Brazil. Revista Brasileira de Parasitologia Veterinária 27: 521–530. https://doi.org/10.1590/S1984-296120180072.

Poulin R., 2013. Explaining variability in parasite aggregation levels among host samples. Parasitology 140: 541–546. https://doi.org/10.1017/s0031182012002053.

Poulin, R., 1993. The disparity between observed and uniform distributions: a new look at parasite aggregation. International journal for parasitology,*23*(7): 937-944.

R CoreTeam 2021. R: a language and environment for statistical computing. Vienna, Austria. Available at https://www.R-project.org/

Reiczigel, J., Marozzi, M., Fábián, I., & Rózsa, L. 2019. Biostatistics for parasitologists–a primer to quantitative parasitology. Trends in parasitology, *35*(4): 277-281.

Sabas, C. S. S. & M. C. Brasil-Sato, 2014. Helminth fauna parasitizing *Pimelodus pohli* (Actinopterygii: Pimelodidae) from the upper São Francisco River, Brazil. Revista Brasileira de Parasitologia Veterinária 23: 375–382. https://doi.org/10.1590/S1984-29612014067.

Santos, G. M., A. A. Juras, B. Mérona & M. Jégue, 2004. Peixes do baixo rio Tocantins. 20 anos depois da Usina Hidrelétrica Tucuruí.

Salgado-Maldonado G., E. F. Mendoza-Franco, J. M. Caspeta-Mandujano & C Ramírez-Martínez 2019. Aggregation and negative interactions in low-diversity and unsaturated monogenean (Platyhelminthes) communities in *Astyanax aeneus* (Teleostei) populations in a Neotropical river of Mexico. International Journal for Parasitology: Parasites and Wildlif 8: 203-215. https://doi.org/10.1016/j.ijppaw.2019.02.005.

Smith, C. L. 1997. National Audubon Society field guide to tropical marine fishes of the Caribbean, the Gulf of Mexico, Florida, the Bahamas, and Bermuda. Alfred A. Knopf.

Souza E. B., A. C. Cunha 2010. Climatologia de precipitação no Amapá e mecanismos climáticos de grande escala. In: Ecodesign T (ed) Tempo, Clima e Recursos Hídricos. pp 177–195.

Oksanen, J., F. G. Blanchet, M. Friendly, R. Kindt, P. Legendre, D. McGlinn, P. R. Minchin, R. B. O'Hara, G.L. Simpson, P. Solymos, M. H. H. Stevens, E. Szoecs and H. Wagner, 2020. vegan: Community Ecology Package. R package version 2.5-7. https://CRAN.R-project.org/package=vegan

Takiyama, L. R., A. Silva, W. J. P. Costa & H. Nascimento, 2004. Qualidade das águas das ressacas das bacias do Igarapé da Fortaleza e do Rio Curiaú. *Diagnóstico das ressacas de Estado do Amapá: Bacias do Igarapé da Fortaleza e do Curiaú. Macapá: CPAQ/IEPA*, 81–104.

Tavares-Dias, M. 2017. Community of protozoans and metazoans parasitizing Auchenipterus nuchalis (Auchenipteridae), a catfish from the Brazilian Amazon. Acta Scientiarum. Biological Sciences 39: 123-128. https://doi: 10.4025/actascibiolsci.v39i1.32836.

Tavares-Dias, M. & L. R. Neves, 2017. Diversity of parasites in wild *Astronotus ocellatus* (Perciformes, Cichlidae), an ornamental and food fish in Brazil. Anais da Academia Brasileira de Ciências 89: 2305–2315. https://doi.org/10.1590/0001-3765201720160700.

Tavares-Dias, M., & M. S. B. Oliveira, (2017a). Structure of parasites community in *Chaetobranchopsis orbicularis* (Cichlidae), a host from the Amazon River system in northern Brazil. Parasitology research 116*:* 2313–2319. https://doi.org/10.1007/s00436-017-5539-2.

Tavares-Dias, M., M. S. B. Oliveira, R. A. Gonçalves & L. M. A. Silva, 2014. Ecology and seasonal variation of parasites in wild *Aequidens tetramerus*, a Cichlidae from the Amazon. Acta Parasitologica 59: 158–164. https://doi.org/10.2478/s11686-014-0225-3.

Thatcher, V. E. & W. A. Boeger, 1983. The parasitic crustaceans of fishes from the Brazilian Amazon. 4. *Ergasilus colomesus* n. sp.(Copepoda: Cyclopoida) from an ornamental fish, *Colomesus asellus* (Tetraodontidae) and aspects of its pathogenicity. Transactions of the American Microscopical Society 102: 371–379. https://doi.org/10.2307/3225850.

Torrente-Vilara, G., L. de Queiroz, & W. M. Ohara, 2013. Um breve histórico sobre o conhecimento da fau*na de peixes do Rio Madeira. LJ Queiroz, G. Torrente-Vilara, WM Ohara, THS Pires, J. Zuanon & CRC Doria. Peixes do* Rio Madeira, São Paulo: Santo Antônio Energia SA, 19–45.

Vasconcelos, H. C.G., & M. Tavares-Dias,.2016. Host-parasite interaction between crustaceans of six fish species from the Brazilian Amazon. Acta Scientiarum: Biological Sciences, 38(1). 10.4025/actascibiolsci.v38i1.29601

Villalba-Vasquez, P. J., J. Violante-González, S. Monks, J. U. Marino-Romero, S. G. Ibáñez, A. A. Rojas-Herrera, R. Flores-Garza & V. Rosas-Guerrero, 2018. Temporal and spatial variations in the metazoan parasite communities of the *Panama spadefish*, *Parapsettus panamensis* (Pisces: Ephippidae), from the Pacific coast of Mexico. Invertebrate Biology 137: 339–354. https://doi.org/10.1111/ivb.12232.

Violante-González, J., Ma. L. Aguirre-Macedo, A. Rojas-Herrera & S. G. Guerrero, 2009. Metazoan parasite community of blue sea catfish, *Sciades guatemalensis* (Ariidae), from Tres Palos Lagoon, Guerrero, Mexico. Parasitology Research 105: 997–1005. https://doi.org/10.1007/s00436-009-1488-8.

Virgilio, L. R., E. O. Machado, A. N. Freire, R. M. Takemoto, L. M. A. Camargo & D. Meneguetti, 2021. *Ergasilus colomesus* (Copepoda: Ergasilidae) parasitizing gills of *Colomesus asellus* (Tetraodontiformes: Tetraodontidae) in the western Brazilian Amazon. Annals of Parasitology 67: 123–127. https:// doi: 10.17420/ap6701.320.

Williams, E. H. & L. Bunkley-Williams, 2019. Life cycle and life history strategies of parasitic Crustacea. Em Parasitic Crustacea (p. 179–266). Springer.

Zar J. 2010. Biostatistical analysis 5th ed Upper Saddle River, NJ: Prentice-Hall 557–558.

Zemmer, S. A., J. Wyderko, J. S. Neto, I. Cedillos, L. Clay, E. Benfield & L. K. Belden, 2017. Seasonal and annual variation in trematode infection of stream snail *Elimia proxima* in the Southern Appalachian Mountains of Virginia. Journal of Parasitology 103: 213–220. https://doi.org/10.1645/16-82.

CAPÍTULO III

Caracterização morfológica e molecular de uma nova espécie de *Brasacanthus* Thatcher, 2001 (Acanthocephala) com evidências de relações filogenéticas e posição sistemática do gênero

Artigo será submetido no periódico "Parasitology Research"

Caracterização morfológica e molecular de uma nova espécie de *Brasacanthus* Thatcher, 2001 (Acanthocephala) com evidências de relações filogenéticas e posição sistemática do gênero

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Abstract

Este estudo descreve uma nova espécie de acantocéfalo do gênero *Brasacanthus* Thatcher, 2001 com base na morfologia e dados de biologia molecular. Este endoparasito foi encontrado infectando *Colomesus asellus* (Tetraodontidae: Tetradontifomes) do Rio Amazonas, na Amazônia oriental brasileira. *Brasacanthus* n. sp. é caracterizada por ter a forma do corpo esférica, testículos paralelos ou diagonais, lemniscos longos e dobrados na região anterior e sino uterino proeminente que o distingue de todas as espécies de acantocéfalos. Apesar das semelhanças entre a nova espécie e o seu congênere *Brasacanthus sphoeroides*, o novo táxon difere pelo tamanho do corpo, comprimento e receptáculo da probóscide, comprimento dos lemniscos, comprimento das glândulas de cemento, diâmetro dos testículos, comprimento da bolsa de Saefftigen, bolsa copulatória e folículos ovarianos, os quais são menores na nova espécie de *Brasacanthus* aqui descrita. Além disso, as análises moleculares e filogenéticas usando parte da região do gene ribossomal 18S (pequena subunidade SSU), dão suporte para a proposição da nova espécie. Observamos que o novo táxon formou um clado irmão com *Calakmulrhynchus amini* dentro da família Arhythmacanthidae. A nova espécie de *Brasacanthus* é a segunda espécie do gênero e a primeira espécie descrita para peixe de água doce. Baseado nas características morfológicas e evidências moleculares apresentadas, sugere-se que *Calakmulrhynchus* deve ser sinonimizado para o gênero *Brasacanthus*. Também chamamos a atenção para a pouca utilidade da classificação em subfamílias para membros da família Arhythmacanthidae, esta afirmação é baseada em nossos achados e de outros autores que consideram duvidosos os caracteres que são adotados para a distinção das subfamílias, onde espinhos podem não estar ausentes no tronco de alguns paracantocefaloidinas e, além disso esta classificação não reflete a filogenia do grupo. Assim, reforçamos que a subdivisão em subfamílias na família Arhythmacanthidae deve ser abandonada.

Palavras-chave: Acantocéfalo, Amazônia, Filogenia molecular, gene 18S.

Introdução

Acantocéfalos são endoparasitos que infectam principalmente o trato digestivo de diferentes vertebrados e são comumente encontrados parasitando peixes (Pinacho-Pinacho *et al.,* 2020, Mohd-Agos *et al.,* 2021). A interação parasitária entre os acantocéfalos e seu hospedeiro pode fornecer informações valiosas sobre os ecossistemas, o ciclo de vida dos parasitas, as interações parasita-hospedeiro e a posição do hospedeiro na cadeia alimentar (Mohd-Agos *et al.,* 2021). O interesse na diversidade de acantocéfalos está crescendo, pois a integridade de muitos habitats e ecossistemas está em sério risco. Entretanto, a diversidade de acantocéfalos de peixes amazônicos ainda é pouco estudada; consequentemente, é esperada a descoberta de novas espécies. Estudos recentes da biodiversidade de parasitos acantocéfalos tem utilizado uma abordagem mais integrativa, nos quais a descrição de novas espécies têm utilizado dados morfológicos e moleculares (Costa Fernandes *et al.* 2019; Reier *et al.* 2019; Keidel *et al.* 2019; García-Varela e Andrade-Gómez 2021; Lynggaard *et al.* 2021; Montes *et al.* 2021;). No entanto, uma abordagem integrativa para espécies do gênero *Brasacanthus* Thatcher, 2001 (Acanthocephala) é inexistente até o momento e, ainda não sabemos qual a real posição sistemática que esse gênero ocupa, assim como não existem dados sobre as interrelações filogenéticas com as demais famílias e gêneros.

O gênero *Brasacanthus* foi proposto para alocar *Brasacanthus sphoeroides* Thatcher, 2001 encontrado parasitando o intestino do baiacu marinho *Sphoeroides greeleyi* Gilbert, 1900 (Tetraodontiformes: Tetraodontidae) da Baia de Paranaguá, estado do Paraná, Brasil (Thatcher, 2001). Porém, após essa primeira descrição da espécie não há outros relatos de espécies de *Brasacanthus*, até o presente momento.

Em *Colomesus asellus* Müller & Troschel, 1849 (Tetraodontiformes: Tetraodontidae:) do Rio Amazonas, na região norte do Brasil, nós encontramos uma nova espécie de acantocéfalo do gênero *Brasacanthus*. Descrevemos esta nova espécie com base em microscopia de luz e eletrônica de varredura, diferenciação molecular e análises filogenéticas moleculares usando sequências da pequena subunidade (SSU) do rDNA nuclear. Este estudo representa os primeiros dados moleculares para o gênero *Brasacanthus*, permitindo algumas alterações a partir de reconstruções filogenéticas e classificação taxonômica a nível de família e gênero.

Material e métodos

Amostragem de hospedeiros, coleta e identificação dos parasitos

Sessenta e sete espécimes de *C. asellus* foram coletados de junho de 2022 a Julho de 2022, no Rio Amazonas, nas proximidades da ilha de Santana, município de Santana, estado do Amapá, norte do Brasil (Fig. 1). Os peixes foram coletados com redes de emalhar de diferentes tamanhos (15, 20, 25, 30, 35 mm entre nós), tarrafas (malha de 20 mm entre nós) e linhas de mão. Os espécimes obtidos foram transportados vivos para o Laboratório de Aquicultura e Pesca da Embrapa Amapá, Macapá, estado do Amapá (Brasil). Em seguida, os peixes foram eutanasiados pelo método da secção medular e preservados em gelo para busca dos acantocéfalos. A metodologia de eutanásia foi aprovada pelo comitê de Ética para uso de Animais da Embrapa (Protocol N°014/2018). Além disso, o acesso ao patrimônio genético foi autorizado pelo Ministério do Meio Ambiente do Brasil (SISBio nº 73550-1 e SisGen nº AA4B6BA).

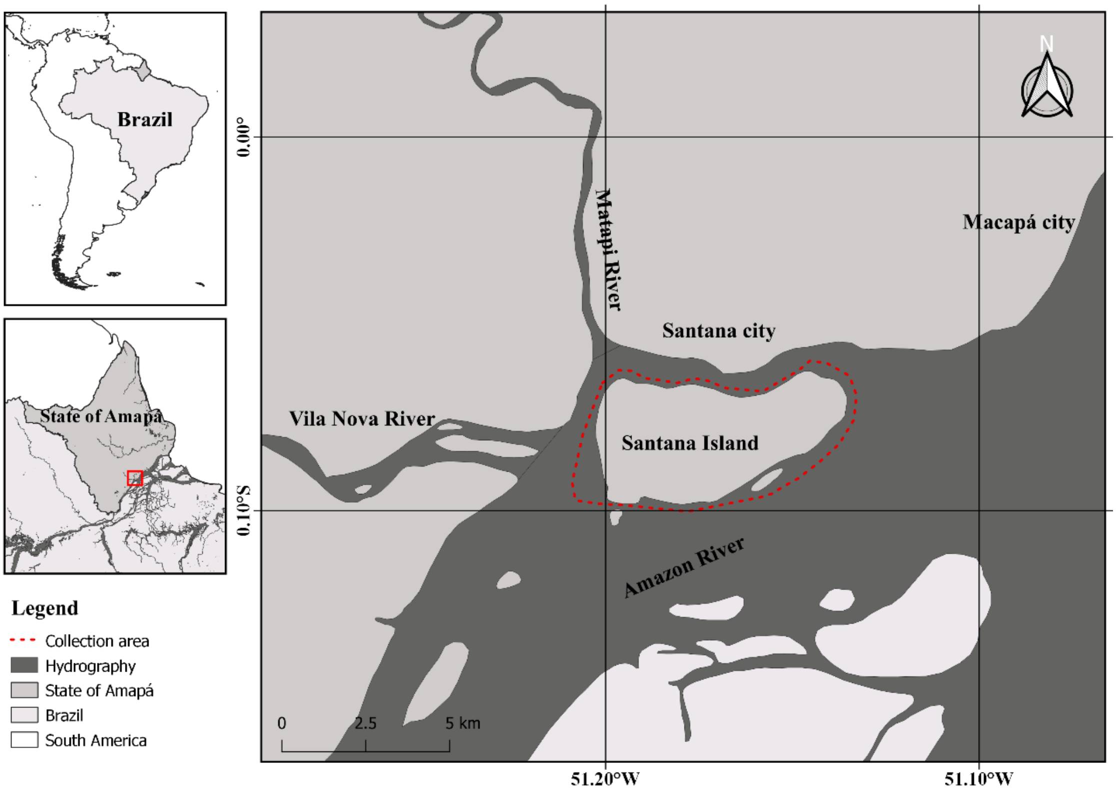

Fig. 1. Área de **c**oleta de *Colomesus asellus* no Rio Amazonas, estado do Amapá, na Amazônia oriental, Brasil.

Os acantocéfalos adultos coletados da faringe, intestino, estômago e cavidade abdominal de *C. asellus* foram colocados em água destilada gelada, fixados em álcool 70% para descrição da morfologia e em álcool 80% para biologia molecular. Os espécimes selecionados foram corados com Carmim ácido de Langeron, desidratado em uma série de etanol graduada (80, 90 e 100%), diafanizado em eugenol e montado em entelan e/ou goma de Damar. Os espécimes de acantocéfalos foram examinados em microscópio com câmara clara (Olympus BX41, Tokyo, Japan), para confecção dos desenhos taxonômicos.

Para análises de microscopia eletrônica de varredura, os espécimes de acantocéfalos foram lavados em água destilada por 20 minutos e, posteriormente, pós-fixados em tetróxido de ósmio a 1% (OsO_4), durante 2 horas. As amostras foram desidratadas em séries crescentes de etanol e levados a secagem na câmara do aparelho de ponto crítico de CO_2. Posteriormente, os acantocéfalos foram montados cuidadosamente em stubs e metalizados com uma fina camada de ouro-paládio. As fotomicrografias foram feitas em microscópio eletrônico de varredura (VEGA 3T-ESCAN, Brno, Czech Republic) do Laboratório de Biologia Celular e Helmintologia, Instituto de Ciências Biológicas (ICB), Universidade Federal do Pará, Belém, PA (Brasil).

Os espécimes-tipo serão depositados na Curadoria da *Coleção Helmintológica do Instituto Oswaldo Cruz (CHIOC*), sob número de acesso (XXXX), do Instituto Oswaldo Cruz, Fundação Oswaldo Cruz, Rio de Janeiro, RJ, Brasil.

Extração de DNA, amplificação por PCR, sequenciamento

O DNA genômico foi extraído de dois espécimes de acantocéfalos usando Dneasy® Blood & Tissue Kit, seguindo recomendações do fabricante (QIAGEN, CA, EUA). A técnica da reação em cadeia da polimerase (PCR) foi utilizada para isolar e amplificar parte da região gene 18S rDNA usando os seguintes iniciadores fw.18iB (AGATTAAGCCATGCATG) e ver.lg18iB (CAAAGGGGGACTTAATC) desenhado por Braicovich *et al*. (2014). A PCR foi realizada com volume final de 25 µL, consistindo em 18.5 µL de Platinum® PCR SuperMix 96 (Invitrogen, França) contendo Taq DNA polimerase anticorpo, Mg++, desoxirribonucleotídeo trifosfatos (dNTP) e recombinante Taq DNA Polimerase, além de 2 µL de cada primer e 2.5 µL de DNA do parasito.

Parte da região 18S foram amplificados usando o seguinte protocolo: desnaturação inicial de 3 minutos a 94ºC, seguida de 35 ciclos com 94ºC por 1 minuto (desnaturação), 57.7º C por 1 minuto (hibridização) e 72ºC por 1 minuto (extensão). Após os 35 ciclos foi realizada uma extensão final de 10 minutos a 72ºC. Com a conclusão das reações de PCR, as amostras foram visualizadas em gel de agarose (1%).

Os produtos de PCR foram purificados usando colunas de rotação GE Healthcare (GFX^{TM} PCR DNA and Gel Band Purification Kit), e posteriormente quantificados em espectrofotômetro NanoDrop 2000 (Thermo Fisher Scientific, MA, EUA) a 260 nm. Os amplicons foram sequenciados pelo método de dideoxitermal no ABI 3500 (Applied Biosystems) seguindo as instruções do fabricante para sequenciamento dos amplicons. Os amplicons foram sequenciados para ambas as fitas de DNA usando os primers da PCR.

Análise filogenética molecular

As sequências obtidas foram editadas e alinhadas usando software Bioedit 7.2 (Hall 1999) aplicando o algoritmo ClustalW (Thompson *et al.*, 1997) e depositados no GenBank sob o número de acesso (**a sequência ainda será depositada no GenBank**). Para avaliar as ocorrências de saturação de substituição, o índice ISS foi estimado com a utilização do Software DAMBE 5 (Xia 2013). O número de substituições de bases por sítio entre as sequências foi calculado e estimativas de erro padrão foram obtidas usando uma inicialização procedimento com 2.000 réplicas. No programa MEGA versão 6.06 (Tamura et al., 2013) foi calculado a divergência genética entre as sequências gênicas usadas no presente estudo.

A sequência de parte da região 18S foi incluída e alinhada em uma matriz de sequências de representantes das principais famílias de acantocéfalos da ordem Echinorhynchida Southwell and Macfine, 1925. Detalhes das sequências incluídas nas análises de filogenia podem ser encontrados na Tabela 1.

No programa MrBayes 3.ª.7a (Ronquist *et al.,* 2012) foi realizado a análise bayesiana (BI) usando a plataforma CIPRES Science Gateway (Miller *et al.,* 2010) aplicando o modelo de evolução GTR + I + G obtido pela análise jModelTest sob o Critério de informação bayesiano (BIC) (Posada 2008). *Floridosentis mugilis* Machado Filho (1951) foi escolhido como grupo externo. Foram estimadas a partir de 10 milhões de gerações as probabilidades posteriores através de algoritmos Markov Chain Monte Carlo (MCMC), no qual 25% das gerações foram definidos como burn-ins e descartados posteriormente.

Uma árvore de consenso foi estimada com base nas demais topologias criadas pelas análises (Miller *et al.,* 2010). Os nós dos clados formados com probabilidade posterior (PP), são apresentados com nós de (PP) apenas para os clados acima de 0.5. Para visualização da árvore filogenética foi utilizado o programa FigTree versão 1.4.3 (Rambaut et al. 2018) e editadas no programa CorelDRAW 2021.

Tabela 1. Informações do espécime e números de acesso do GenBank para as espécies estudadas. A sequência marcada em negrito foi obtida neste estudo.

Nome do Táxon	Família	Host	SSU	Referência
***Brasacanthus* n. sp.**	**Arhythmacanthidae**	***Colomesus asellus***	**Código gba**	**Este estudo**
Echinorhynchus truttae	Echinorhynchidae	*Thymallus thymallus*	AY830156	García-Varela and Nadler (2005)
Echinorhynchus cotti	Echinorhynchidae	*Cottus reinii*	MW172280	García-Varela and Andrade-Gomez (2021)
Echinorhynchus borealis	Echinorhynchidae	*Perca fluviatilis*	MW172281	García-Varela and Andrade-Gomez (2021)
Rhadinorhynchus mariserpentis	Rhadinorhynchidae	*Regalecus russelii*	MK014866	Steinauer *et al.*, (2019)
Rhadinorhynchus gerberi	Rhadinorhynchidae	*Trachinotus botla*	MN105739	Lisitsyna *et al.*, (2019)
Sclerocollum australe	Cavisomatidae	*Siganus argenteus*	MN705831	Huston *et al.*, (2020)
Sclerocollum robustum	Cavisomatidae	*Siganus lineatus*	MN705832	Huston *et al.*, (2020)
Corynosoma enhydri	Polymorphidae	*Enhydra lutris*	AF001837	García-Varela and Nadler (2005)
Profilicollis altmani	Polymorphidae	*Enhydra lutris*	AF001838	Near *et al*,. (1998); García-Varela and Nadler (2005)
Polymorphus brevis	Polymorphidae	*Nycticorax nycticorax*	AF064812	García-Varela *et al.*, (2000); García-Varela and Nadler (2005)
Plagiorhynchus cylindraceus	Plagiorhynchidae		AF001839	Near *et al.*, (1998); García-Varela and Nadler (2005)
Centrorhynchus sp.	Centrorhynchidae	*Falco peregrinus*	AY830155	García-Varela and Nadler (2005)
Acanthocephalus amini	Echinorhynchidae	*Mayaheros urophthalmus*	EU732662	García-Varela and Andrade-Gomez (2021)
Acanthocephaloides propinquus	Arhythmacanthidae	*Gobius bucchichi*	AY830149	García-Varela and Nadler (2005)
Heterosentis sp.	Arhythmacanthidae	*Acanthopagrus australia*	MN705825	Huston et al., (2020)
Heterosentis pseudobagri	Arhythmacanthidae	*Pelteobagrus fulvidraco*	OP286859	Gao *et al.*, (2023)

Filisoma rizalinum	Cavisomidae	*Scatophagus argus*	JX014229	Verweyen *et al.*, (2011)
Filisoma filiforme	Cavisomidae	*Kyphosus bigibbus*	MN705826	Huston *et al.*, (2020)
Acanthocephalus anguillae	Echinorhynchidae	*Anguilla anguilla*	MN394412	Amin *et al.*, (2019)
Acanthocephalus anguillae	Echinorhynchidae	*Proteus anguinus*	MN394414	Amin *et al.*, (2019)
Acanthocephalus dirus	Echinorhynchidae	*Semotilus atromaculatus*	MW172276	García-Varela and Andrade-Gomez (2021)
Acanthocephalus lucii	Echinorhynchidae	*Perca fluviatilis*	MW172277	García-Varela and Andrade-Gomez (2021)
Acanthocephalus clavula	Echinorhynchidae	*Anguilla anguilla*	MW172278	García-Varela and Andrade-Gomez (2021)
Acanthocephalus rhinensis	Echinorhynchidae	*Anguilla anguilla*	MW172279	García-Varela and Andrade-Gomez (2021)
Acanthocephalus nanus	Echinorhynchidae	*Cynops pyrrhogaster*	LC129889	Nakao (2016)
Outgroup				
Floridosentis mugilis	Neoechinorhynchidae	*Mugil cephalus*	AF064811	García-Varela *et al.*, (2000); García-Varela and Nadler (2005)

Resultados

Systematica

Classe: Palaeacanthocephala Meyer, 1931

Ordem: Echinorhynchida Southwell and Macfine, 1925

Família: Arhythmacanthidae Yamaguti, 1935

Gênero: *Brasacanthus* Thatcher, 2001

***Brasacanthus* n. sp. (Figs. 2-4)**

Sumário taxonômico

Hospedeiro: *Colomesus asellus* Müller & Troschel, 1849 (Tetraodontiformes: Tetraodontidae)

Localidade: Rio Amazonas, nas proximidades da ilha de Santana, município de Santana, estado do Amapá, Brasil (00º 04'00''S; 51°08'00" W - 00°06'00"S 51º12' 30''W)

Sítio de infecção: Faringe, intestino, estômago e cavidade abdominal.

Um total de 45 acantocéfalos foram encontrados em 67 hospedeiros: Prevalência = 19.4%, Intensidade média: 5 e Abundância média: 0.67.

Material tipo: Holótipo e alótipo (código de depósito) e 19 parátipos (código de depósito) serão depositados na Curadoria da *Coleção Helmintológica do Instituto Oswaldo Cruz (CHIOC*), sob o número: Holótipo: (ainda serão depositados) e parátipos: (ainda serão depositados), Números de acesso do GenBank: (ainda será depositado).

Registro no ZooBank: The Life Science Identifier (LSID) para *Brasacanthus* n. sp. é (XXXXXXXX).

Etimologia: Epíteto específico será usado em homenagem ao rio em que os parasitos foram coletados.

Descrição

Com base em 21 espécimes (10 machos e 11 fêmeas) obtidos de *C. asellus* naturalmente infectados são apresentadas as medidas como os valores do holótipo/alótipo seguidos pela amplitude e média dos parátipos entre parênteses (relatados em micrômetros, a menos que indicado de outra forma).

Corpo pequeno, tronco arredondado, dimorfismo sexual evidente com fêmeas maiores que os machos (Figs. 2B e 3A). Tegumento contendo numerosos pequenos núcleos hipodermais, armado com espinhos restritos à extremidade anterior (Figs. 4B, 4E e 4F). Sistema lacunar com anastomoses reticulares. Probóscide curta, subcilíndrica com 12-14 fileiras longitudinais de 10-12 ganchos cada; ganchos aumentam de tamanho medialmente e, em seguida, diminuem de tamanho em direção à base. Ganchos maiores enraizados e ganchos menores não enraizados (Figs. 2A, 3B e 4B). Pescoço curto, desarmado (Figs. 2A, 2B e 3A). Bainha da probóscide cilíndrica, de parede dupla, com gânglio na base (Figs. 2A, 2B e 3A). Lemniscos longos, planos, irregulares, frequentemente dobrado para trás anteriormente (Figs. 2A, 2B e 3A). Testículos esféricos, paralelos ou diagonais, posteriores ao meio do corpo; glândulas de cemento piriformes (Fig. 2B). Útero de formato cilíndrico alongado ou reservatório de ovo esférico (Figs. 3C); ovos alongados, fusiformes, com prolongamentos polares da membrana de fertilização (Fig. 3D).

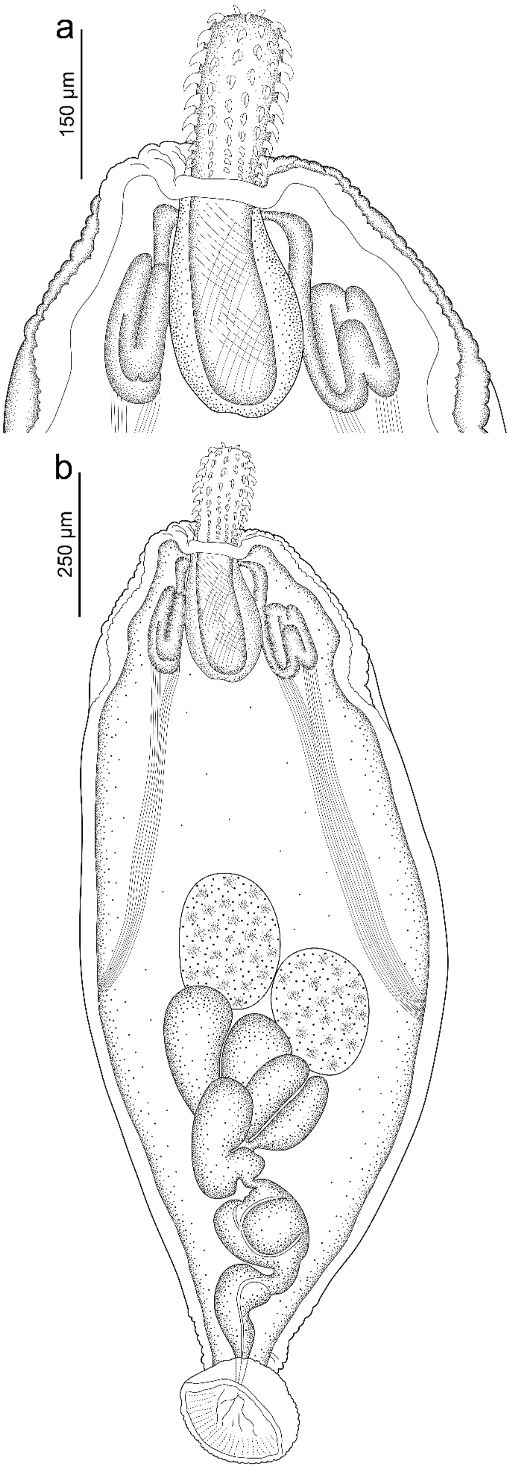

Fig. 2. *Brasacanthus* n. sp. em *Colomesus asellus*. (a) Detalhes da proboscide, receptáculo e lemnisco do macho. (b) Macho de corpo inteiro.

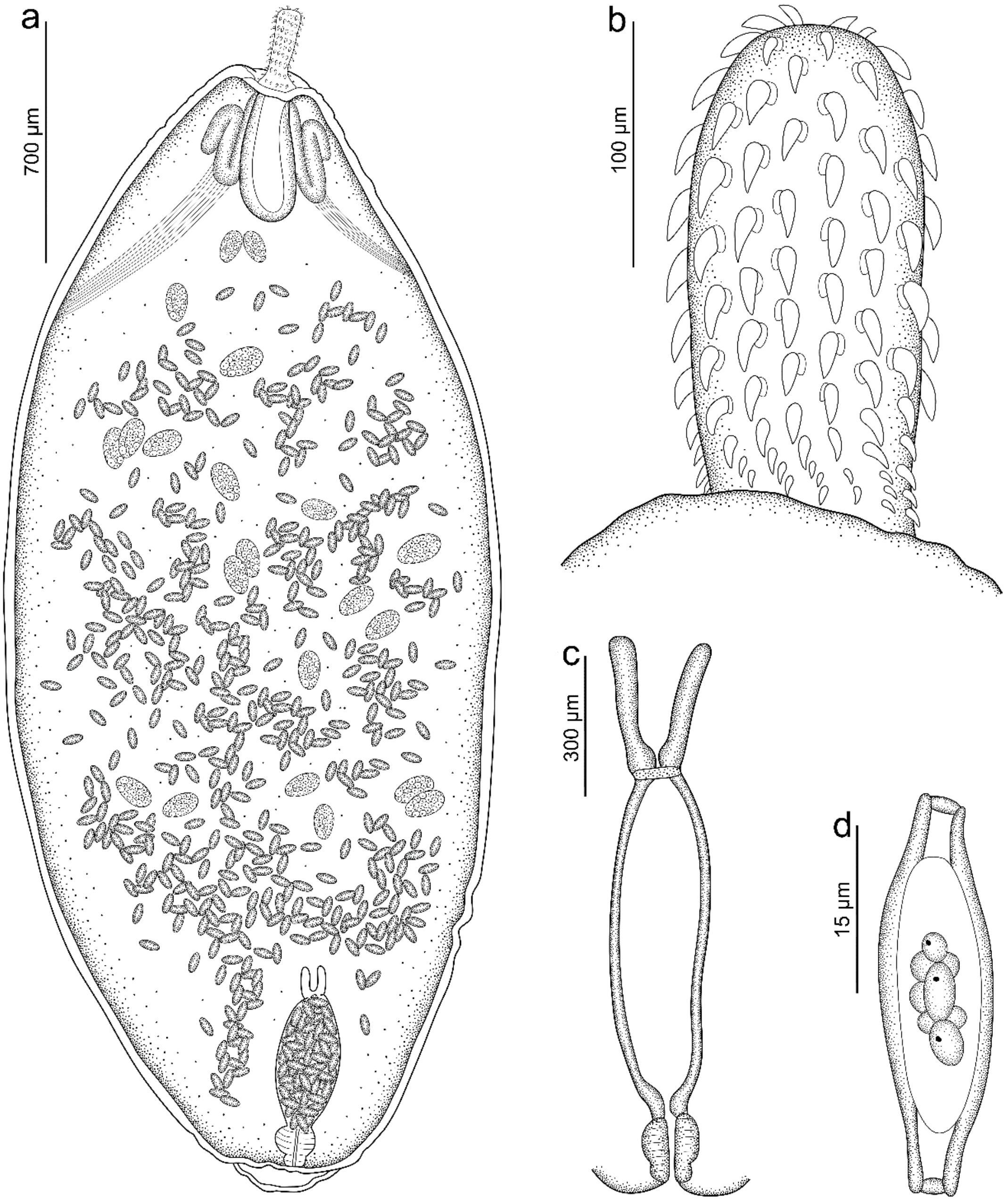

Fig. 3. *Brasacanthus* n. sp. em *Colomesus asellus*. (*a*) Fêmea de corpo inteiro. (b) Probóscide de fêmea. (c) Sistema reprodutivo da fêmea. (d) ovo maduro.

Machos (baseado em holótipo e nove parátipos, todos espécimes maduros). Tronco expandido medialmente, medindo 1.387 (0.94–2.25, 1.59) mm de comprimento e 0.64 (0.56–1.31, 0.86) mm de diâmetro máximo (Fig. 2B). Probóscide subcilíndrica, 210 (137–410, 230) de comprimento e 90 (80–200, 110) de diâmetro (Figs. 2A-B) Ganchos da probóscide perto do ápice, medindo cerca de 15 (15–25, 19) de comprimento; perto do meio 50 (22–50, 31) e perto da base 30 (13–35, 19) (Fig. 2A). Receptáculo da probóscide mede 230 (180–538, 270) de comprimento e 130 (100–310, 0.14) de largura (Figs. 2A-B). Lemnisco mede 530 (400–690, 515) de comprimento e 30 (20–84, 46) de largura (Figs. 2A e 2B). Testículos subesféricos; testículo direito mede 200 (136–321, 233) de diâmetro; testículo esquerdo mede 180 (133–320, 233) de diâmetro (Fig. 2B). Glândulas de cemento piriformes, em número de 6, medindo 80 (86–180, 138) de maior diâmetro (Fig. 2B). A bolsa de Saefftigen mede 236 (236–594, 380) de comprimento e 105 (105–202, 145) de maior diâmetro (Fig. 2B). A bolsa copuladora mede 190 (173–505, 290) de comprimento e 221 (221–300, 249) de largura (Fig. 2B). Complexo genital ocupa cerca de 55% do comprimento do corpo.

Fêmeas (baseado em alótipa e 10 parátipos, todas grávidas). Tronco expandido medialmente, medindo 2.8 (1.34–2.80, 2.05) mm de comprimento e 1.3 (0.82–1.52, 1.20) mm no máximo diâmetro (Fig. 3A). Probóscide subcilíndrica, 250 (114–320, 269) de comprimento e 70 (50–146, 77) de largura (Figs. 3A-B). Ganchos da probóscide perto do ápice medem cerca de 13 (13–23, 19) de comprimento; perto do meio 47 (16–47, 27) e perto da base 32 (9–32, 14) (Fig. 3B). Ganchos maiores enraizados, ganchos menores não enraizados (Fig. 3B). Receptáculo da probóscide mede 340 (125–418, 282) de comprimento e 160 (40–168, 135) de diâmetro máximo (Fig. 3A). Lemniscos medem 530 (306–530, 458) de comprimento e 50 (27–57, 34) largura (Fig. 3A). Os folículos ovarianos medem 95 (57–217, 73) de comprimento e 50 (24–70, 40) de largura (Fig. 3A). Os ovos medem 41 (19–57, 40) de comprimento e 10 (4–13, 9) de largura (Fig. 3D). Complexo genital ocupa cerca de 27,3% do comprimento do tronco.

Remarks

A nova espécie é alocada no gênero *Brasacanthus* com base nas seguintes características morfológicas: possui forma do corpo esférica, testículos paralelos ou diagonais, lemniscos em comprimento variável geralmente dobrado para trás e um reservatório de ovo uterino proeminente, conforme características propostas por Thatcher (2001). No entanto, observamos ainda que *Brasacanthus* n. sp. possui espinhos tegumentares na região anterior do corpo (Fig. 4E and 4F).

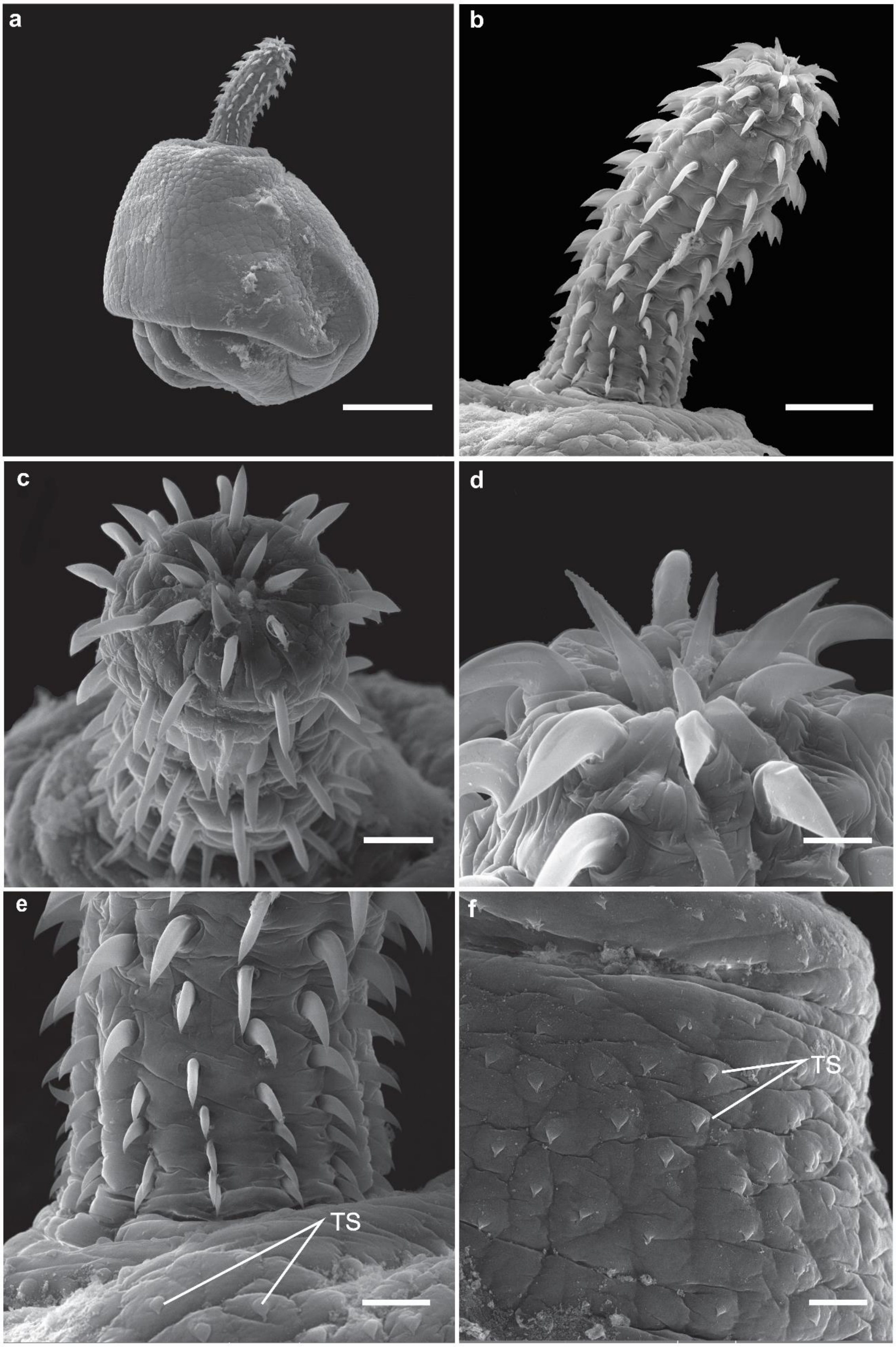

Fig. 4. Microscopia eletrônica de varredura de *Brasacanthus* n. sp. em *Colomesus asellus*. (a) Proboscide e corpo de um macho adulto; (b) Proboscide de um macho adulto; (c e d) Ganchos apicais da proboscide, vista apical; (e) Detalhes dos ganchos basais da proboscide; e dos

espinhos tegumentares da região anterior do corpo (TS); (F) Detalhes dos espinhos tegumentares (TS). Barras de escala: a = 200 µm; b = 50 µm; c = 50 µm; d = 10 µm; e = 20 µm; f = 20 µm.

Atualmente, o gênero *Brasacanthus* é monotípico e representado pela espécie *B. sphoeroides,* que foi descrita de *S. greeleyi* (Thatcher, 2001). *Brasacanthus* n. sp. é a segunda espécie do gênero e difere da espécie-tipo por apresentar menor tamanho corporal, comprimento da probóscide e receptáculo da probóscide. Além disso, o lemnisco de *Brasacanthus* n. sp. é menor em comprimento, e na maioria das vezes está dobrado múltiplas vezes ao lado do receptáculo da proboscide, enquanto que em *B. sphoeroides* os leminiscos são maiores e, apesar de dobrarem posteriormente, estão livres no corpo desse parasito.

Apesar de *Brasacanthus* n. sp. apresentar similaridade com *B. sphoeroides* quanto as características dos ganchos da probóscide (os ganchos apicais da probóscide aumentam de tamanho em direção da região medial da probóscide e, em seguida, diminuem de tamanho em direção a base), se diferem no tamanho das glândulas de cemento, testículos, bolsa de Saefftigen e bolsa copulatória, as quais são menores em *Brasacanthus* n. sp. Adicionalmente, os folículos ovarianos e ovos também são menores nessa nova espécie quando comparados a *B. sphoeroides*.

Divergência Genética

Observamos que a divergência genética entre as sequências gênicas utilizadas no presente estudo variou de 0.003 a 0.183 entre espécies do gênero *Echinorhynchus*; quando comparadas entre espécies do gênero *Sclerocollum* a variação foi de 0.003; gênero *Heterosentis* a variação foi de 0.011 e para o gênero *Acanthocephalus* variou de 0.002 a 0.011.

Quando comparamos sequências gênicas entre os gêneros da mesma família, observamos que em Echinorhychidae a média de divergência foi de 0.010; Rhadinorhynchidae 0.020; Cavisomatidae 0.003; Polymorphidae 0.048; e Paracanthocephalidae 0.006; enquanto que a variação entre as famílias foi de 0.024 a 0.170. Entre os gêneros da família Arhythmacanthidae a divergência variou de 0.011 a 0.034, com média de divergência de 0.031. A distância genética entre *Brasacanthus* n. sp. e *Calakmulrhynchus amini* Salgado-Maldonado & Novelo-Turcotte (2009) foi de 0.009.

Análises moleculares e estudo filogenético

O sequenciamento de parte da região ribossomal 18S (SSU-DNAr) de um espécime de *Brasacanthus* n. sp. resultou em uma sequência com 950 (pb). Realizamos uma pesquisa com a sequência obtida no BLAST® a qual indicou semelhança (95.24-98.20%) com quatro espécies: *Echinorhynchus* sp. (EU732662.1), *Acanthocephaloides propinquus* Dujardin 1845

(AY830149), *Heterosentis pseudobagri* Wang & Zhang (1987) (OP286859) e *Heterosentis* sp. Van Cleave, 1931. Todas as outras demais sequências de SSU de acantocéfalos do GenBank apresentaram grau de similaridade inferior a 95.13%.

A reconstrução filogenética gerou uma árvore de consenso baseada na matriz contendo sequencias referentes a 27 táxons e com 931 bp. Observamos a formação de nove clados (com valor de PP de 100%) representados por nove famílias de acantocéfalos parasitos de diferentes hospedeiros.

A árvore filogenética apresenta dois grandes clados, o primeiro é formado por membros da família Echinorhynchidae, Rhadinorhynchidae, Cavisomatidae, Polymorphidae, Plagiorhynchidae e Centrorhynchidae que apresentou baixos valores de suporte (PP de 60%). Entretanto, dentro desse grande clado as sequencias de espécies da família Rhadinorhynchidae e Cavisomatidae formaram um grupo monofilético com alto valor de suporte (PP de 100%), assim como, Polymorphidae, Plagiorhynchidae e Centrorhynchidae (PP de 100%). O segundo grande clado teve um valor de suporte de PP de 100% e engloba os subclados de membros da família Paracanthocephalidae, Cavisomidae e Arhythmacanthidae. As famílias Paracanthocephalidae e Cavisomidae apresentaram-se como clados-irmão com alto suporte (PP de 100%).

As sequências de representantes da família Arythmacanthidae (PP de 80%) apresentaram cinco táxons subdividido em dois clados. Um formado por *Heterosentis* sp. da subfamília Arhythmacanthinae Yamaguti, 1935 parasitando *Acanthopagrus australis* Günther, 1859 (Teleostei: Sparidae) da Austrália e *Heterosentis pseudobagri* parasitando *Pelteobagrus fulvidraco* Richardson, 1846 (Siluriformes: Bagridae) da China. O segundo clado é formado por *Acanthocephaloides propinquus* pertencente a subfamília Neoacanthocephalloidinae Golvan, 1960, obtido de *Gobius bucchichii* Steindachner, 1870 (Teleostei: Gobiidae) do Mar Mediterrâneo; *Calakmulrhynchus amini* (García-Varela & Andrade-Gómez, 2021) da subfamilia Paracanthocephaloidinae (Golvan, 1969) de sequência obtida de *Mayaheros urophthalmus* (Günther, 1862) (Teleostei: Cichlidae) no México e *Brasacanthus* n. sp. formaram um clado irmão dentro da família Arhythmacanthidae com alto suporte (PP = 100%) (Fig. 5).

Discussão

Diferenciação de espécies

Brasacanthus n. sp. é a segunda espécie do gênero e a primeira descrita parasitando peixes de água doce. Thatcher (2001) propôs um novo gênero para alocar *B. sphoeroides* de *S. greeleyi* do litoral Sul do Brasil. *Brasacanthus* n. sp. possui corpo expandido medialmente, quase em formato oval; ganchos apicais e mediais similares em tamanho, e os basais são menores e um sino uterino proeminente. Tais características morfológicas correspondem as relatadas por Thatcher (2001), para o gênero *Brasacanthus*.

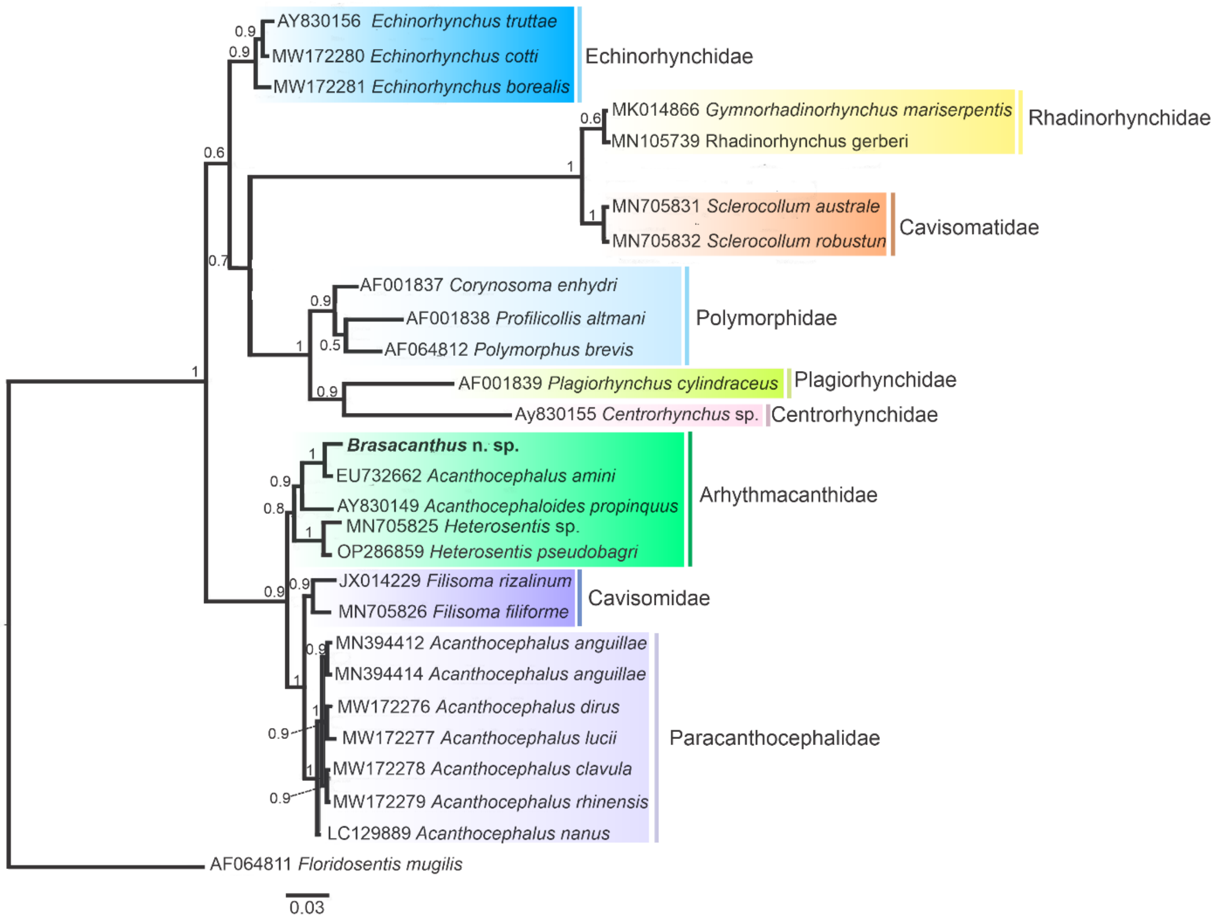

Fig. 5. Árvore bayesiana com dados de parte da região SSU 18S-DNAr de 9 famílias de acantocéfalos. Os números de acesso do GenBank são indicados ao lado dos nomes das espécies. As diferentes cores representam as famílias de acantocéfalos. Os valores de suporte nodais são indicados para BI com probabilidades posteriores para análises bayesianas. A barra de escala do comprimento do ramo indica o número de substituições por local.

Brasacanthus n. sp. apesar de apresentar formato corporal muito semelhante a espécie-tipo, possui diferenças morfológicas. O corpo em formato oval, é dilatado na região mediana; o que parece ser um caráter morfológico comum para o gênero *Brasacanthus*, podendo ser encontrado em espécies descritas no futuro. Além disso, o lemnisco de *Brasacanthus* n. sp. é menor em comprimento, e na maioria das vezes está dobrado múltiplas vezes ao lado do receptáculo da proboscide. Esse carácter morfológico é de difícil visualização e pode ser facilmente confundido com lemniscos curtos. Porém, Thatcher (2001) destacou que os lemniscos longos são características marcantes de *B. sphoeroides*, podendo este caráter ser compartilhado por outras espécies do gênero.

Como este é o primeiro estudo com dados de biologia molecular para uma espécie do gênero *Brasacanthus,* isto impossibilita a comparação genética e evolutiva com *B. sphoeroides*. Portanto, o presente estudo estabelece uma linha de base para estudos futuros sobre o gênero *Brasacanthus* e sua relação com a família Arhythmacanthidae.

Relocação do gênero Brasacanthus para Arhythmacanthidae e notas sobre o sistema baseado em subfamílias

Brasacanthus n. sp. agrupou em um clado com *C. amini* dentro da família Arhythmacanthidae, uma espécie de acantocéfalo de *M. urophthalmus,* ciclídeos de água doce da região Neotropical do México. Desta forma sugerimos que o gênero *Brasacanthus* seja realocado da família Echinorhynchidae para a família Arhythmacanthidae. Atualmente, a família Arhythmacanthidae é composta por 10 gêneros, que são parasitos de peixes teleósteos distribuídos mundialmente. As espécies de acantocéfalos alocadas nessa família são caracterizadas por possuírem seis glândulas de cemento e transição abrupta ou gradual de pequenos ganchos na região basal da probóscide (sem raízes) ou espinhos para ganchos grandes na região medial e apical da probóscide (com raízes) (Pichelin & Cribb,1999). Assim, além da evidência filogenética, verificamos que *Brasacanthus* spp. compartilham os mesmos caracteres morfológicos de representantes da família Arhythmacanthidae (ou seja, seis glândulas de cemento e morfologia dos ganchos e espinhos da proboscide), reforçando que o gênero *Brasacanthus* deve ser alocado nessa família.

Tradicionalmente, a família Arhythmacanthidae é classificada em três subfamílias de acordo com a disposição de espinhos tegumentares pelo corpo (Golvan, 1969). No entanto, observamos que *Brasacanthus* n. sp. possui espinhos na porção anterior do corpo, enquanto *B. sphoeroides* não possui espinhos pelo corpo. Adicionalmente, destacamos que a sequência de *A. propinquus* (Neoacanthocephalloidinae) agrupou formando um clado monofilético com o clado *Calakmulrhynchus* (Paracanthocephaloidinae) e *Brasacanthus* n. sp.

Pichelin & Crib (1999) relataram que os espinhos do corpo dos espécimes da subfamília Paracanthocephaloidinae podem ser pequenos e difíceis de visualizar, ou podem ser perdidos. Além disso, Golvan (1969) também observou essa dificuldade e relata espinhos tegumentares em espécie do gênero *Acanthocephaloide*s que se pensava anteriormente não ter espinhos no corpo, além das descobertas de pequenos espinhos na região posterior do corpo em *Heterosentis parasiluri* Yu & Wu, 1989 e *H. hirsutus* Pichelin & Crib, 1999. Observamos que os espinhos tegumentares de *Brasacanthus* n. sp. foram notados e descritos apenas utilizando microscopia eletrônica de varredura (MEV). Assim, a ausência de espinhos tegumentares nas espécies *B. sphoeroides* e *C. amini* podem não ter sido observadas visto que para essas espécies de parasitos não foi realizado MEV.

Baseado em nossos achados e naqueles relatados pelos autores acima citados consideramos que a utilização da classificação em subfamílias não reflete exatamente a filogenia do grupo, podendo ser um caractere que pouco reflete a evolução das espécies dentro da família Arhythmacanthidae. Adicionalmente, Pichelli & Cribb (1999) chamaram a atenção para a pouca utilidade da classificação em subfamílias para membros da família Arhythmacanthidae diante do pequeno número de gêneros, já que eles consideram duvidoso os caracteres que são adotados para distinção das subfamílias, onde espinhos podem não estar ausentes no tronco de alguns paracantocefaloidinas. Desta forma, isso sugere que a subdivisão em subfamílias deve ser abandonada.

Variação genética e notas sobre gêneros da família

Brasacanthus n. sp. agrupou com alto suporte com a sequência de *C. amini* (Fig. 5) e apresentou baixa divergência genética. A baixa divergência pode estar relacionada a característica do gene que parece representar melhor as topografias entre famílias do que as variações entre espécies, isso é evidenciado nos cálculos das distâncias genéticas de membros do mesmo gênero da nossa árvore filogenética, onde espécies do gênero *Echinorhynchus, Sclerocollum, Acanthocephalus* apresentaram divergências menores que a apresentada entre *C. amini* e *Brasacanthus* n. sp.

Calakmulrhynchus amini foi inicialmente alocada no gênero *Acanthocephalus* por Salgado-Maldonado & Novelo-Turcotte (2009). Posteriormente, Garcia-Varella & Andrade-Gomes (2021) propuseram o gênero *Calakmulrhynchus* para alocar *C. amini* na família Arhythmacanthidae, utilizando dados morfológicos e moleculares. Reforçamos que *C. amini* agrupou com a sequência de *Brasacanthus* n. sp. demonstrando que essas duas espécies são filogeneticamente muito próximas.

Além disso, *C. amini* compartilha diversas características morfológicas com *B. sphoeroides* e *Brasacanthus* n. sp. Ambas as espécies possuem um receptáculo da proboscide com uma dupla parede muscular e o gânglio na base, seis glândulas de cemento, além de ganchos apicais e mediais que são quase uniformes em tamanho e forma, enquanto os ganchos basais são menores. Adicionalmente, Salgado-Maldonado & Novelo-Turcotte (2009) chamam atenção para o grande, robusto e cilíndrico útero (reservatório de óvulos uterinos) presentes em *C. amini* e reforçamos que tal caractere morfológico também é compartilhado por *Brasacanthus* n. sp. Dessa forma, as características morfológicas e evidências moleculares apresentadas mostram que *Calakmulrhychus* deve ser sinonimizado para o gênero *Brasacanthus*.

Salgado-Maldonado & Novelo-Turcotte (2009) relataram que a forma do corpo, tamanho da proboscide e formato do útero de *Brasacanthus amini* n. comb. são muito semelhantes a *B. sphoeroides*. Porém, destacam que o tamanho do corpo de *B. sphoeroides* é maior que *Brasacanthus amini* n. comb. Já quando comparado o tamanho do corpo de *Brasacanthus amini* n. comb., este é maior que o de *Brasacanthus* n. sp. A proboscide de *Brasacanthus amini* n. comb. é subglobular, enquanto em *B. sphoeroides* e *Brasacanthus* n. sp. é subcilíndrica. Os lemniscos de *Brasacanthus amini* n. comb. são mais curtos se comparados aos de *Brasacanthus* n. sp. A ausência de espinhos no tegumento da região anterior do corpo de *Brasacanthus amini* n. comb. também difere de *Brasacanthus* n. sp.

Brasacanthus sphoeroides possui 14 fileiras longitudinais de ganchos com linhas alternadas de 10 e 11 ganchos por fileira. *Brasacanthus amini* n. comb. possui 12 fileiras longitudinais com 11 a 14 ganchos por fileiras, enquanto *Brasacanthus* n. sp. 12 fileiras longitudinais com 10 a 12 ganchos por fileiras. *Brasacanthus sphoeroides* possui ganchos próximo do ápice que medem cerca de 22-23 de comprimento; perto do meio 31-38 e os próximos da base 14-16. Os ganchos apicais de *B. amini* n. comb medem cerca 37-46, mediais medem cerca de 19-54 e os basais de 20-33, enquanto em *Brasacanthus* n. sp. os gachos apicais medem cerca de 13-25, mediais medem cerca 16-50, basais medem cerca de 9-32. Essas diferenças morfológicas reforçam a nova combinação e o status de *Brasacanthus amini* n. comb.

Em *Brasacanthus amini* n. comb., outra característica morfológica que deve ser destacada é o tamanho dos lemniscos. Apesar dos autores relatarem que nessa espécie os lemniscos são menores do que o receptáculo da proboscide, ressaltamos que tal carácter em *Brasacanthus* n. sp. foi de difícil observação. Conforme destacado anteriormente, os lemniscos estão frequentemente dobrados sobre si e podem ser facilmente confundidos com lemniscos curtos. Desta forma, os autores podem não ter notado essa característica em *Brasacanthus amini* n. comb.

Inferências biogeográficas

A filogenia obtida no presente trabalho apresenta evidências adicionais para entendermos um pouco mais sobre a evolução e distribuição das espécies da família Arhythmacanthidae e do gênero *Brasacanthus*. Desta forma, observamos que as espécies desse gênero podem ser divididas em uma linhagem que foi encontrada parasitando peixes Tetraodontidae (*Brasacanthus* n. sp e *B. sphoeroides*) de água doce e salgada, respectivamente, e, uma linhagem parasitando ciclídeo de água doce na América Central.

Observamos ainda que os parasitos *Brasacanthus* n. sp. e *Brasacanthus amini* n. comb. são espécies irmãs e que apresentam proximidade genética. Esses achados podem estar relacionados ainda com a história evolutiva dos peixes hospedeiros. Dessa forma, observamos que os Tetraodontidae apresentam distribuição circumglobal, sendo representados por 189 espécies agrupadas em 19 gêneros, ocorrendo em mares, estuários e rios nas regiões tropicais e temperadas (Tyler, 1964; Dekkers 1975; Ebert, 2001; Nelson, 2006). De acordo com Yamanoue et al. (2013) durante a dispersão dos baiacus tetraodontídeos, estes animais diversificaram primeiro em águas costeiras e foram migrando para mares abertos, mares profundos e, por último, para a água doce. Além disso, essas espécies invadiram ambientes de água doce em regiões costeiras em diferentes continentes, pelo menos três vezes e em momentos distintos.

Colomesus asellus divergiu há 10 milhões de anos atrás durante o Mioceno (cerca de 9-17 MA) (Yamanoue et al., 2011). Nesse período, houve o registro de duas incursões marinhas, nas quais, um sistema lacustre ou pantanoso que drenava para o norte até o Mar do Caribe se desenvolveu na Amazônia Ocidental (Hoorn, 1993;1994). Desta forma, os ancestrais (marinho/salobra) das espécies do gênero *Colomesus* invadiram a Amazônia Ocidental através da última incursão marinha no Mioceno (11-12 MA) divergindo posteriormente para linhagem com a adaptação a habitats de água de doce, no caso de *C. asellus* (Yamanoue et al., 2011). Além disso, a separação de *C. asellus* das espécies *Sphoeroides parvus, Sphoeroides annulatus, Sphoeroides testudineus*, *Sphoeroides pachigaster* ocorreu entre 30 a 20 milhões de anos (Yamanoue et al. 2011), apontando que *Sphoeroides* spp. diversificaram primeiro, sendo consideradas mais antigas em relação as espécies do gênero *Colomesus*. Desta forma podemos levantar a hipótese de que *Brasacanthus* n. sp. e *B. sphoeroides* foram espécies que co-especiaram com seus hospedeiros a partir de um ancestral comum.

Colomesus asellus está distribuído por toda a Amazônia, bacias do Tocantins-Araguaia e ambientes costeiros que vão da Foz do Rio Amazonas até a Venezuela. Este peixe junto com *Colomesus tocantinensis*, que ocorre na bacia do Rio Tocantins, são os únicos baiacus de água doce da América do Sul (Amaral et al., 2013). O ciclídeo *M. urophthalmus* é um peixe neotropical muito frequente e abundante em ambientes de água salobra e de água doce, mas também tolera à salinidade (Miller et al. 2009), o que facilita sua dispersão ao longo da costa. Esses peixes encontram-se distribuídos do sul de Veracruz, no México, até a Nicarágua, habitando rios, lagos, lagoas e dolinas cársticas (cenotes) na Península de Yucatan (México), incluindo lagoas costeiras nas ilhas de Contoy e Mujeres.

A colonização dos peixes ciclídeos na América Central a partir da América do Sul ocorreu durante o Oligoceno (29-24 Ma), no curso do evento da ponte terrestre das Grandes Antilhas–Aves Ridge (GAARlandia) (Říčan et al., 2013). Assim, a atual distribuição de *Brasacanthus* n. sp. e *Brasacanthus amini* n. comb. e sua proximidade filogenética pode estar associada com o compartilhamento de ambientes ao longo da história evolutiva dos hospedeiros *M. urophtalmus* e *C. asellus*, ou mesmo com espécies marinhas como *S. greeleyi* que tem ampla distribuição (Santa Catarina no Brasil a América do Norte). Vale ressaltar que *S. greeleyi* possui o mesmo ancestral comum de *C. psitacus* (marinho/salobra) e *C. asellus* (água doce), possibilitando levantar a hipótese de que o *B. amini* n. comb. também pode ter origem marinha, assim como *Brasacanthu*s n. sp.

A monofília apresentada por *Brasacanthus* n. sp. e *B. amini* n. comb. mostra um padrão de distribuição descontinua, separadas por uma área onde ainda não há registros de *Brasacanthus* spp. (América Central). No entanto, padrões similares de distribuição são observados em outros parasitos, por exemplo, em membros do gênero *Pomphorhynchu*s Monticelli, 1905, os quais apresentaram a mesma distribuição que o gênero *Brasacanthus*, com algumas espécies ocorrendo na América do Sul e outras ocorrendo na América do Norte, mas nenhuma espécie na América Central (García-Varela et al. 2017). No entanto, talvez a distribuição descontinua do gênero *Brasacanthus* deva estar relacionada a falta de estudo sobre esses acantocéfalos na América Central.

Conclusões

Este primeiro estudo de biologia molecular para uma espécie do gênero *Brasacanthus* na América do Sul, aumentou para três o número de espécies desse gênero sendo*: Brasacanthus sphoeroides*, *Brasacanthus amini* n. comb. e *Brasacanthus* n. sp., e esta última espécie é a segunda parasitando baiacus. Nossos estudos não corroboraram a alocação do gênero *Brasacanthus* na família Echinorinchidae, como proposto por Thatcher (2001), pois baseado em evidências morfológica e moleculares realocamos este gênero na família Arhythmacanthidae.

Com a inclusão do gênero *Brasacanthus* na família Arhythmacanthidae, aumenta para 10 o número de gêneros desta família, uma vez que propomos que o gênero *Calakmulrhynchus* seja sinonimizado *Brasacanthus.* Assim, a família Arhythmacanthidae fica composta pelos seguintes gêneros: *Brasacanthus* Thatcher, 2001; *Heterosentis* Van Cleave, 1931; *Hypoechinorhynchus* Yamaguti, 1939, *Spiracanthus* Munoz & George-Nascimento, 2002, *Bolborhynchoides* Achmerow & Dombrovskaja, 1959; *Acanthocephaloides* Meyer, 1932; *Breizacanthus* Golvan, 1969; *Euzetacanthus* Golvan & Houin, 1964; *Paracanthocephaloides* Golvan, 1969 e *Solearhynchus* Buron & Maillard, 1985. Além disso, a classificação em subfamílias de membros da família Arhythmacanthidae não deve ser considerada, uma vez que os caracteres que classificam as subfamílias são duvidosos e não refletem a filogenia dessa família.

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Conflitos de interesse

Os autores declaram não haver conflito de interesses.

Disponibilidade de dados e materiais

Não aplicável' para essa seção.

Disponibilidade de Código

Não aplicável' para essa seção.

Aprovação ética

A aprovação ética para obtenção de acesso ao patrimônio genético foi autorizada pelo Ministério do Meio Ambiente do Brasil (SISBio nº 73550-1 e SisGen nº AA4B6BA). Este estudo foi desenvolvido de acordo com os princípios adotados pelo Colégio Brasileiro de Experimentação Animal (COBEA) e foi conduzido sob autorização do Comitê de Ética no Uso de Animais da Embrapa (protocolo nº 014/2018).

Consentimento para participar

Não aplicável' para essa seção.

Consentimento para publicação

Os autores declaram ter conhecimento da publicação deste estudo.

Contribuições dos autores

Todos os autores contribuíram para a concepção e desenho do estudo. A preparação do material, coleta e análise dos dados foram realizadas por Elvis Silva Lima. A primeira versão do manuscrito foi escrita por Elvis Silva Lima e todos os autores comentaram as versões anteriores do manuscrito. Todos os autores leram e aprovaram o manuscrito final.

Referências

Amaral CR, Brito PM, Silva DA, Carvalho EF (2013) A new cryptic species of South American freshwater pufferfish of the genus *Colomesus* (Tetraodontidae), based on both morphology and DNA data. PLoS One 8(9): e74397. https://doi.org/10.1371/journal.pone.0074397

Amin OM, Heckmann RA, Fišer Ž, Zakšek,V, Herlyn H (2019) Description of *Acanthocephalus anguillae balkanicus* subsp. n. (Acanthocephala: Echinorhynchidae) from *Proteus anguinus* Laurenti (Amphibia: Proteidae) and the cave ecomorph of A*sellus aquaticus* (Crustacea: Asellidae) in Slovenia. Folia Parasitol 66: 1–13. doi: 10.14411/fp.2019.015

Braicovich PE, Lanfranchi AL, Farber MD, Marvaldi A, Luque JL, Timi JT (2014) Genetic and morphological evidence reveals the existence of a new family, genus and species of Echinorhynchida (Acanthocephala). Folia Parasitol: 1-8. doi: 10.14411/fp.2014.044

Cazier EC, Cooper MA, Eaton SG, Pulham AJ (1997) Basin development and tectonic history of the Llanos Basin, Eastern Cordillera, and Middle Magdalena Valley, Colombia: Reply. AAPG Bulletin 81: 1332–1335

Costa Fernandes VS, Amin O, Borges JN, Santos CP (2019) A new species of the Acanthocephalan genus *Filisoma* (Cavisomidae) from Perciform fishes in Rio de Janeiro, Brasil. Acta Parasitol 64: 176–186. https://doi.org/10.2478/s11686-018-00019-3

Cooper MA, Addison FT, Alvarez R, Coral M, Graham RH, et al. (1995) Basin development and tectonic history of the Ilanos Basin, eastern Cordillera, and Middle Magdalena Valley, Colombia. AAPG Bulletin 79: 1421–1443.

Dekkers WJ (1975) Review of the Asiatic freshwater puffers of the genus *Tetraodon* Linnaeus, 1758 (Pisces, Tetraodontiformes, Tetraodontidae). Bijdragen tot de Dierkunde, 45: 87–142.

Ebert K (2001) The puffers of fresh and brackish waters. Mo¨rfelden-Walldorf: A. C. S. Glaser. 96 p.

Gao JW, Yuan XP, Jakovlić I, Wu H, Xiang CY, Xie M, Song R, Xie ZG, Yuan-AN W, Ou, DS (2023) The mitochondrial genome of *Heterosentis pseudobagri* (Wang & Zhang, 1987) Pichelin & Cribb, 1999 reveals novel aspects of tRNA genes evolution in Acanthocephala. BMC genomics 24(1):1-15. https://doi.org/10.1186/s12864-023-09177-9

García-Varela M, and Andrade-Gómez L (2021) First steps to understand the systematics of Echinorhynchidae Cobbold, 1876 (Acanthocephala), inferred through nuclear gene sequences. Parasitol Int 81: 102264. https://doi.org/10.1016/j.parint.2020.102264

García-Varela M, de León GP-P, de la Torre P, Cummings,MP, Sarma SSS, Laclette JP (2000) Phylogenetic relationships of Acanthocephala based on analysis of 18S ribosomal RNA gene sequences. J Mol Evol 50(6): 532-540. https://doi.org/10.1007/s002390010056

García-Varela M, Mendoza-Garfias B, Choudhury A, de León GP-P (2017) Morphological and molecular data for a new species of *Pomphorhynchus* Monticelli, 1905

(Acanthocephala: Pomphorhynchidae) in the Mexican redhorse *Moxostoma austrinum* Bean (Cypriniformes: Catostomidae) in central Mexico. Syst Parasitol 94: 989–1006. https://doi.org/10.1007/s11230-017-9756-y

García-Varela M, Nadler SA (2005) Phylogenetic relationships of Palaeacanthocephala (Acanthocephala) inferred from SSU and LSU rDNA gene sequences. J Parasitol 91(6): 1401–1409. https://doi.org/10.1645/GE-523R.1

Golvan YJ (1969) Systématique des Acanthocéphales (Acanthocephala Rudolphi 1801).: L'ordre des Palæacanthocephala Meyer 1931. la super-famille des Echinorhynchoidea (Cobbold 1876)-Golvan et Houin 1963. Éditions du Muséum.

Hall, TA (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis program for 95/98/NT. Nucleic acids symp 41: 95–98.

Hoorn, C (1993) Marine incursions and the influence of Andean tectonics on the Miocene depositional history of northwestern Amazonia: results of a palynostratigraphic study. Paleogeogr. palaeoclimatol. palaeoecol 105: 267–309. https://doi.org/10.1016/0031-0182(93)90087-Y

Hoorn, C (1994) An environmental reconstruction of the palaeo-Amazon River system (Middle–Late Miocene, NW Amazonia). Paleogeogr. palaeoclimatol palaeoecol 112: 187–238. https://doi.org/10.1016/0031-0182(94)90074-4

Hoorn C (2006) Mangrove forests and marine incursions in neogene Amazonia (Lower Apaporis River, Colombia). Palaios 21: 197–209. https://doi.org/10.2110/palo.2005.p05-131

Huston DC, Cribb TH, Smales LR (2020) Molecular characterisation of acanthocephalans from Australian marine teleosts: proposal of a new family, synonymy of another and transfer of taxa between orders. Syst Parasitol 97(1):1–23. https://doi.org/10.1007/s11230-019-09896-2

Keidel L, García-Varela M, Brener B, de León GP-P, Santos, CP (2019) Integrative taxonomy reveals a new species of *Dollfusentis* (Acanthocephala: Illiosentidae), in *Orthopristis ruber* (Osteichthyes: Haemulidae) from Rio de Janeiro, Brazil. Parasitol Int 71: 132–142. https://doi.org/10.1016/j.parint.2019.04.003

Lisitsyna OI, Kudlai O, Cribb TH, Smit NJ (2019) Three new species of acanthocephalans (Palaeacanthocephala) from marine fishes collected off the East Coast of South Africa. Folia Parasitol 66:1–20. doi:10.14411/fp.2019.012.

Lynggaard C, García-Prieto L, Guzmán-Cornejo, C, García-Varela, M (2021) Description of a new species of *Moniliformis* (Acanthocephala: Moniliformidae) from *Peromyscus hylocetes* (Rodentia: Cricetidae) in Mexico. Parasitol Int 83: 102315. https://doi.org/10.1016/j.parint.2021.102315

Miller MA, Pfeiffer W, Schwartz, T (2010) Creating the CIPRES Science Gateway for inference of large phylogenetic trees. In 2010 gateway computing environments workshop (GCE*)*, Ieee, 1–8.

Miller RR, Minckley WL, Norris SM (2009) Peces dulceacuícolas de México. CONABIO, SIMAC, ECOSUR, DFC, Mexico City.

Mohd-Agos S, Mohd-Husin N, Zakariah et al. (2021) Three new species of Acanthocephala from *Acanthogyrus* (Acanthosentis)(Acanthocephala: Quadrigyridae) from tinfoil barb fish, *Barbonymus schwanenfeldii* in Lake Kenyir, Terengganu, Malaysia. Trop Biomed 38(3): 387–395.

Montes MM, Arredondo NJ, Marcotegui P, Ferrari W, Solari A, Martorelli SR (2021) New insights on *Pomphorhynchus sphaericus* Gil de Pertierra, Spatz et Doma, 1996 (Acanthocephala: Pomphorhynchidae). Parasitol Res 120(11): 3725–3737. https://doi.org/10.1007/s00436-021-07331-4

Nakao M (2016) *Pseudoacanthocephalus toshimai* sp. nov. (Palaeacanthocephala: Echinorhynchidae), a common acanthocephalan of anuran and urodelan amphibians in Hokkaido, Japan, with a finding of its intermediate host. Parasitol Int 65(4): 323–332. https://doi.org/10.1016/j.parint.2016.03.011

Near TJ, Garey JR, Nadler SA (1998) Phylogenetic relationships of the Acanthocephala inferred from 18S ribosomal DNA sequences. Mol Phylogenet Evol 10(3): 287–298. https://doi.org/10.1006/mpev.1998.0569

Nelson JS (2006) Fishes of the world. New York: Wiley. 601 p

Pinacho-Pinacho CD, Sereno-Uribe AL, García-Varela M, de León GP-P (2020) A closer look at the morphological and molecular diversity of *Neoechinorhynchus* (Acanthocephala) in Middle American cichlids (Osteichthyes: Cichlidae), with the description of a new species from Costa Rica. J Helminthol 94: e23. https://doi.org/10.1017/S0022149X18001141

Pichelin S, Cribb TH. A review of the Arhythmacanthidae (Acanthocephala) with a description of *Heterosentis hirsutus* n. sp. from *Cnidoglanis macrocephala* (Plotosidae) in Australia. Parasite 6 (4): 293–302.

Posada D (2008) jModelTest: phylogenetic model averaging. Mol Biol Evol 25(7): 1253–1256. https://doi.org/10.1093/molbev/msn083

Rambaut A, Drummond AJ, Xie D, Baele G, Suchard MA (2018) Posterior Summarization in Bayesian Phylogenetics Using Tracer 1.7. Syst Biol 67(5): 901–904. https://doi.org/10.1093/sysbio/syy032

Reier S, Sattmann H, Schwaha T, Harl,J, Konecny,R, Haring E (2019) An integrative taxonomic approach to reveal the status of the genus *Pomphorhynchus* Monticelli, 1905 (Acanthocephala: Pomphorhynchidae) in Austria. Int J Parasitol: Parasites and Wildlife 8**:** 145–155. https://doi.org/10.1016/j.ijppaw.2019.01.009

Říčan O, Piálek L, Zardoya R, Doadrio I, Zrzavý J (2013) Biogeography of the Mesoamerican Cichlidae (Teleostei: Heroini): colonization through the GAARlandia land bridge and early diversification. J Biogeogr 40(3): 579-593. https://doi.org/10.1111/jbi.12023

Ronquist F, Teslenko M, van der Mark P, et al. (2012) MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. Syst Biol 61(3): 539–542. https://doi.org/10.1093/sysbio/sys029

Salgado-Maldonado G, Novelo-Turcotte MT (2009) *Acanthocephalus amini* n. sp. (Acanthocephala: Echinorhynchidae) from the freshwater fish *Cichlasoma urophthalmus* (Günther)(Cichlidae) in Mexico. Syst Parasitol 73: 193–198. https://doi.org/10.1007/s11230-009-9189-3

Steinauer ML, Garcia-Vedrenne AE, Weinstein SB, Kuris AM (2019) Acanthocephalan parasites of the oarfish, *Regalecus russelii* (Regalecidae), with a description of a new species of Gymnorhadinorhynchus (Acanthocephala: Gymnorhadinorhynchidae). J Parasitol 105(1): 124–132. https://doi.org/10.1645/17-53

Tamura K, Stechier G, Peterson D, Filipski A, Kumar S (2013). MEGA6: Molecular Evolutionary Genetics Analysis Version 6.0. Mol Biol Evol 30: 2725–2729. https://doi.org/10.1093/molbev/mst197

Thatcher VE (2001) *Brasacanthus sphoeroides* gen. n., sp. n. (Acanthocephala, Echinorhynchidae) from a coastal marine fish of Parana State, Brazil. Revta bras Zool 18: 1319–1323. https://doi.org/10.1590/S0101-81752001000400024

Tyler JC (1964) A diagnosis of the two species of South American puffer fishes (Tetraodontidae, Plectognathi) of the genus *Colomesus*. Proc Acad Nat Sci 116:119–148. https://www.jstor.org/stable/4064623

Thompson JD, Gibson TJ, Plewniak F, Jeanmougi F, Higgins DG (1997) The Clustal X windows inteface: flexible stratagies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Res 24: 4876–4882. https://doi.org/10.1093/nar/25.24.4876

Verweyen L, Klimpel S, Palm HW (2011) Molecular phylogeny of the Acanthocephala (class Palaeacanthocephala) with a paraphyletic assemblage of the orders Polymorphida and Echinorhynchida. PLoS One 6(12): e28285. https://doi.org/10.1371/journal.pone.0028285

Wilkens H (1982) Regressive evolution and phylogenetic age: the history of colonization of freshwaters of Yucatan by fish and crustacea. Texas Meml Museum Bull 28: 237–243.

Xia X (2013) DAMBE5: a comprehensive software package for data analysis in molecular biology and evolution. Mol Biol Evol 30(7): 1720–1728. https://doi.org/10.1093/molbev/mst064

Yamanoue Y, Miya M, Doi H, Mabuchi K, Sakai H, Nishida M. (2011). Multiple invasions into freshwater by pufferfishes (Teleostei: Tetraodontidae): a mitogenomic perspective. PLoS One 6(2): e17410. https://doi.org/10.1371/journal.pone.0017410

CAPÍTULO IV

Original Article

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Lima, E. S., Oliveira, M. S. B., & Tavares-Dias, M. (2021). Diversity and community ecology of metazoan parasites in *Pimelodus ornatus* (Siluriformes: Pimelodidae) from the Amazonas River in Brazil. *Revista Brasileira de Parasitologia Veterinária*, *30*. DOI: https://doi.org/10.1590/S1984-29612021065

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Diversidade e ecologia da comunidade de parasitos metazoários em *Pimelodus ornatus* (Siluriformes: Pimelodidae) do Rio Amazonas no Brasil

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Abstract

The present study investigated the metazoan parasite community in *Pimelodus ornatus* from the Amazon River, in the state of Amapá (Brazil). Of 71 fish examined, 70.4% were parasitized by *Demidospermus* sp. (Monogenea), *Cucullanus pinnai*, *Procamallanus* (*Spirocamallanus*) *inopinatus* and *Contracaecum* sp. (Nematoda) and plerocercoids from Proteocephalidae gen. sp. (Cestoda). The dominance was of nematode species such as *Procamallanus* (*S.*) *inopinatus* and *Contracaecum* sp. The parasites showed a highly aggregated dispersion and a predominance of hosts infected by one species of parasite. The parasite community was characterized by a low Shannon diversity index, low evenness and low species richness. The richness of parasite species, Shannon's diversity index, abundance of *P.* (*S.*) *inopinatus* and Proteocephalidae gen. sp. showed a positive correlation with the length of the hosts. Therefore, the size of the hosts had an influence on the parasite community and infracommunities, as well as their intermediate position in the food web. This is the first record of *P.* (*S.*) *inopinatus* and *Contracaecum* sp. for *P. ornatus*.

Keywords: Aggregation, ectoparasites, endoparasites, freshwater fish, infection.

Resumo

Este estudo investigou a comunidade de parasitos metazoários em *Pimelodus ornatus* do Rio Amazonas, no estado do Amapá (Brasil). De 71 peixes examinados, 70,4% estavam parasitados. Um total de 147 parasitos foi coletado, entre *Demidospermus* sp. (Monogenea), *Cucullanus pinnai* (Nematoda), *Procamallanus* (*Spirocamallanus*) *inopinatus* e *Contracaecum* sp. (Nematoda) e plerocercoides de Proteocephalidae gen. sp. (Cestoda). A dominância foi de nematoides como *Procamallanus* (*S.*) *inopinatus* e *Contracaecum* sp. Os parasitos apresentaram dispersão altamente agregada e predomínio de hospedeiros infectados por uma espécie de parasito. A comunidade de parasitos foi caracterizada por baixo índice de diversidade de Shannon, baixa equitabilidade e baixa riqueza de espécies. A riqueza de espécies de parasitos, índice de diversidade de Shannon, abundância de *P.* (*S.*) *inopinatus* e Proteocephalidae gen. sp. apresentaram correlação positiva com o comprimento dos hospedeiros. Portanto, o tamanho dos hospedeiros teve influência sobre a comunidade e infracomunidades de parasitos, bem como sua posição intermediária na cadeia alimentar. Este é o primeiro registro de *P.* (*S.*) *inopinatus* e *Contracaecum* sp. para *P. ornatus*.

Palavras-chave: Agregação, ectoparasitos, endoparasitos, peixes de água doce, infecção.

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Original Article

Diversity and community ecology of metazoan parasites in *Pimelodus ornatus* (Siluriformes: Pimelodidae) from the Amazonas River in Brazil

Diversidade e ecologia da comunidade de parasitos metazoários em *Pimelodus ornatus* (Siluriformes: Pimelodidae) do Rio Amazonas no Brasil

Short title: Parasites in *Pimelodus ornatus*

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Abstract

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Este estudo investigou a comunidade de parasitos metazoários em *Pimelodus ornatus* do Rio Amazonas, no estado do Amapá (Brazil). De 71 peixes examinados, 70,4% estavam parasitados e um total de 147 parasitos foram coletados, entre *Demidospermus* sp. (Monogenea), *Cucullanus pinnai* (Nematoda), *Procamallanus* (*Spirocamallanus*) *inopinatus* e *Contracaecum* sp. (Nematoda) e plerocercoides de Proteocephalidae gen. sp. (Cestoda). A dominância foi de nematoides como *Procamallanus* (*S.*) *inopinatus* e *Contracaecum* sp. Os parasitos apresentaram dispersão altamente agregada e predomínio de hospedeiros infectados por uma espécie de parasito. A comunidade de parasitos foi caracteriza por baixo índice de diversidade de Shannon, baixa equitabilidade e baixa riqueza de espécies. A riqueza de espécies de parasitos, índice de diversidade de Shannon, abundância de *P.* (*S.*) *inopinatus* e Proteocephalidae gen. sp. apresentaram correlação positiva com o comprimento dos hospedeiros. Portanto, o tamanho dos hospedeiros teve influência sobre a comunidade e infracomunidades de parasitos, bem como sua posição intermediária na cadeia alimentar. Este é o primeiro registro de *P.* (*S.*) *inopinatus* e *Contracaecum* sp. para *P. ornatus*.

Palavras-chave: Agregação, ectoparasitos, endoparasitos, peixes de água doce, infecção.

Introduction

The Amazon River is the largest drainage basin in the world and accounts for 20% of the global freshwater, 1.2 x 10^9 tons of sediment (Nittrouer & DeMaster, 1986; Milliman, 2001;

Chong et al., 2016), 10% of the dissolved loads and 3% of the suspended loads that enter the ocean (Milliman & Syvitski, 1992; Gaillardet et al., 1997; Calvès et al., 2019). In addition, it has a high diversity of fish species.

The diversity of fish in the Amazon River is of economic importance for many riverine populations that live of fishing and use different species of fish for their subsistence (Salo et al., 2013). This diversity is due to the particular characteristics of this large river and its tributaries, which are rich ecosystems with complex trophic chains and with vegetation cover that vary according to the flood regime and regional seasonality (Costa Sousa et al., 2017; Arantes et al., 2019). However, many species of Amazonian fish and their biology are little known (Tavares-Dias & Oliveira, 2017; Negreiros et al., 2018), such as the *Pimelodus ornatus* (Kner, 1857).

Pimelodus ornatus is a Pimelodidae, popularly known as mandi-guaru, silver mandi, mandi-pinini or painted mandi. This Siluriformes can be found in the Amazon, Madeira, Parnaíba, Negro, Alto Paraná, Orinoco, which are large rivers in the Guianas, Paraguay, Bolivia, Peru and Venezuela (Nomura, 1984; Torrente et al., 2013; Froese & Pauly, 2021). Omnivorous fish, active at night, can occur in the main river channels, in rocky bottoms with dead tree trunks, upstream and downstream from rapids and backwaters (Froese & Pauly, 2021). Their first sexual maturation occurs from 15.4 cm in length (Vazzoler, 1996) and females can preserve sperm with their secretions by inserting them in the epithelium of their genital tract, being indicative of internal fertilization (Vazzoler, 1996; Boujard, 1997; Le Bail et al., 2000). This host fish has been parasitized by species of Monogenea, Nematoda, Trematoda, Cestoda and Pentastomida (Table 1). However, little is known about the ecological interactions of *P. ornatus* with its parasite community.

Table 1. List of metazoan parasites species reported for *Pimelodus ornatus* from South America.

Taxon/parasite species	Locality	References
Monogenea		
Demidospermus peruvianus	Peru	Mendoza-Palmero et al. (2019)
Demidospermus curvovaginatus	Peru	Mendoza-Palmero et al. (2019)
Nematoda		
Cucullanus pinnai	Brazil	Thatcher (2006), Kohn et al. (2011)
Pseudocladorchis cylindricus	Brazil	Travassos et al. (1928)
Trematoda		
Dadaytrema oxycephala	Brazil	Travassos et al. (1928)
Genarchella parva	Argentina	Kohn et al. (2007)
Genarchella genarchella	Brazil	Fernandes & Kohn (2001), Kohn et al. (2011)
Cestoda		
Mariauxiella pimelodi	Brazil	De Chambrier & Rego (1995)
Spasskyellina mandi	Brazil	Pavanelli & Takemoto (1996)
Nomimoscolex sp.	Peru	De Chambrier et al. (2015)
Pentastomida		
Porocephalus gracilis	Brazil	Travassos et al. (1928)
Leiperia gracile	Brazil	Luque et al. (2013)

Adequate knowledge of parasite biodiversity is crucial for environmental management and conservation initiatives (Poulin, 2004; Negreiros et al., 2019). It is known that among the processes responsible for the spatial distribution of the parasites, they can be related to the constant scenarios of changes in the environment and biological invasions, which can lead to diseases (McLeod & Wing, 2008; Harvell et al., 2009; Altizer et al., 2013). Therefore, biotic and abiotic factors are commonly responsible for the diversity, richness and infection rates by parasites in wild fish (Tavares-Dias et al., 2014; Blasco-Costa et al., 2015; Oliveira et al., 2017; Tavares- Dias & Oliveira, 2017; Negreiros et al., 2018), affecting the parasite-host relationship. Thus, the objective of the present study was to investigate the diversity and community ecology of metazoan parasites in *P. ornatus* in the Amazon River region of the state of Amapá, northern Brazil.

Materials and methods

Sampling area and fish collection

Seventy-one specimens of *P. ornatus* (15.8 ± 2.4 cm and 18.9 ± 8.1 g) were collected every two months, from January to September 2020, on the Amazon River near the municipality of Santana, Amapá state (Figure 1). The fish were collected with nets of different sizes and meshes (15, 20, 25, 30 and 35), throw nets (20 mm mesh) and hand lines. The collected specimens were conserved in 10% formaldehyde and transported to the Embrapa Aquaculture and Fisheries Laboratory, Macapá, Amapá state, Brazil.

During the fish collection, the water quality parameters of electrical conductivity, pH and total dissolved solids were measured using a multiparameter (COMBO5-02-1016), and the dissolved oxygen and temperature were measured using an oximeter (MO-900). The average water temperature was 29.4 ± 1.2 ° C, dissolved oxygen 5.9 ± 0.1 mg/L, pH 7.1 ± 0.2, total dissolved solids 52.2 ± 2.0 mg/L and electrical conductivity 85.8 ± 4.9 µS/cm.

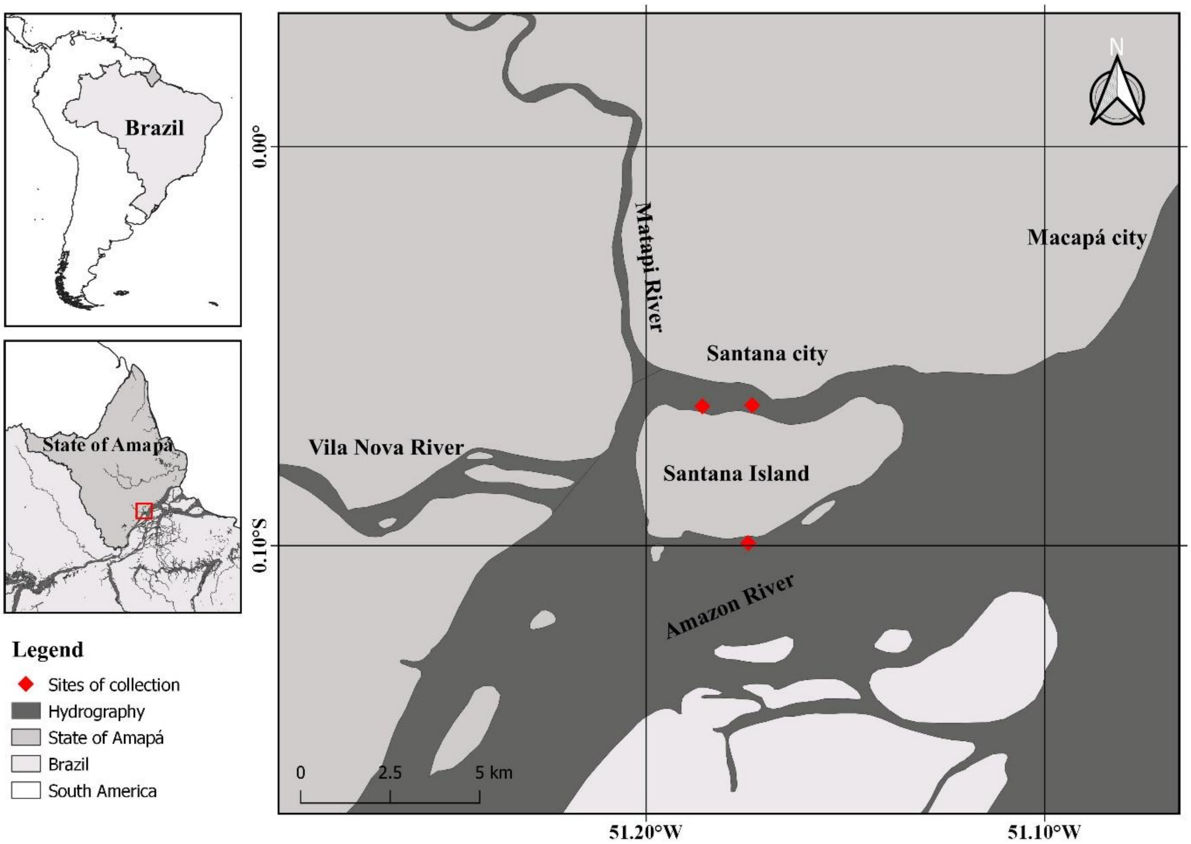

Figure 1. Collection site of *Pimelodus ornatus* in Amazonas River, State of Amapá, northern Brazil.

The present study was carried out according to the recommendations and guidelines of the Brazilian College of Animal Experimentation (COBEA) and with authorization from the Ethics Committee on the Use of Animals of Embrapa Amapá (Protocol N^{o} 014 - CEUA/CPAFAP).

Collection and analytical procedures of the parasites

All fish were weighed (g) and the total length (cm) was measured, and the gills, gastrointestinal tract and viscera were examined for the presence of metazoan parasites. The gills, gastrointestinal tract and viscera were examined with a stereomicroscope and the observed parasites were fixed with 70% ethyl alcohol. The parasites were prepared for identification using the methodology described in Eiras et al. (2006). Parasites were identified according to Moravec (1998) and Thatcher (2006), and specialized papers. Voucher specimens were deposited at Instituto de Pesquisas Cientificas e Tecnológicas do Estado do Amapá (IEPA), Macapá, AP, Brazil, in the Scientific Collection Curation Office for the Fauna of Amapá, under accession number IEPA:160-164P.

Data analysis

Prevalence, mean abundance, and mean intensity of parasite infracommunities (Bush et al., 1997) and frequency of dominance (Rohde et al., 1995) were calculated. The dispersion index (ID) and the significance of the ID were calculated using the *d*-statistic with the software Quantitative Parasitology 3.0, as well as the Poulin discrepancy index (D) (Ludwig et al., 1988). These parameters were calculated for species with a prevalence > 10%.

All data were previously evaluated on the assumptions of normality and homoscedasticity using the Shapiro-Wilk and Bartlett tests, respectively. The Shannon index (*H*), evenness (*E*) and species richness of parasites (Rohde et al., 1995, Magurran, 2004) were used to estimate parasite diversity. Spearman's correlation coefficient (*rs*) was used to investigate possible correlations between host body length and weight with the richness of parasite species, Shannon index and parasite abundance.

Results

Pimelodus ornatus specimens were parasitized by *Demidospermus* Suriano, 1983, *Cucullanus pinnai* (Travassos, Artigas & Pereira, 1928); *Procamallanus* (*Spirocamallanus*) *inopinatus* (Travassos, Artigas & Pereira, 1928), larvae of *Contracaecum* Railliet & Henry, 1912 and plerocercoids of Proteocephalidae La Rue, 1911 (Table 2). However, the dominance was of nematode species and no parasites were found in the mouth and operculum of the examined hosts. The parasites showed aggregate dispersion (Table 3).

Table 2. Parasitic helminths in *Pimelodus ornatus* from Amazonas River, State of Amapá, in Brazil.

Parasite species	**P (%)**	**AM ± SD**	**MI ± SD**	**TNP**	**FD (%)**	**SI**
Monogenea						
Demidospermus sp.	2.8	0.04 ± 0.3	1.5 ± 1.0	3	2.1	Gills
Nematoda						
Contracaecum sp. (larvae)	22.5	0.4 ± 1.0	1.9 ± 1.4	31	21.2	Intestine
Contracaecum sp. (larvae)	2.8	0.03 ± 0.2	1.0 ± 0	3	1.4	Stomach
Procamallanus (*S.*) *inopinatus*	52.1	1.2 ± 1.5	2.4 ± 1.3	88	60.3	Intestine
Procamallanus (*S.*) *inopinatus*	1.4	0.01 ± 0.1	1.0 ± 0	1	0.7	Stomach
Procamallanus (*S.*) *inopinatus*	1.4	0.01 ± 0.1	1.0 ± 0	1	0.7	Abdominal cavity
Cucullanus pinnai	5.6	0.1 ± 0.2	1.0 ± 0	4	2.7	Intestine
Cestoda						
Proteocephalidae gen. sp. (plerocercoids)	11.3	0.2 ± 0.6	1.6 ± 1.1	13	8.9	Intestine
Proteocephalidae gen. sp. (plerocercoids)	4.2	0.04 ± 0.2	1.0 ± 0	3	2.0	Stomach

P: Prevalence, MA: Mean abundance, MI: Mean intensity, TNP: Total number of parasites, FD: Frequency of dominance, SI: Site of infection, SD: Standard deviation.

Table 3. Index of dispersion (ID), *d*-statistical (*d*) and discrepancy index (D) of parasite infracommunities in *Pimelodus ornatus* from the Amazonas River, Amapá state, in Brazil.

Parasite species	**ID**	***d***	**D**	**Dispersion type**
Contracaecum sp.	2.74	5.5	0.83	Aggregated
Procamallanus (*S*.) *inopinatus*	1.77	4.0	0.60	Aggregated
Proteocephalidae gen. sp.	1.85	4.3	0.83	Aggregated

The parasite component community showed a low Shannon diversity index, low evenness and low species richness, and predominance of endoparasite species (Table 4). There was a predominance of hosts infected by one species of parasite (Figure 2).

The species richness of parasites (*rs* = 0.24; p = 0.04) and Shannon's diversity index (*rs* = 0.29; p = 0.01) showed a weak positive correlation with the length of the hosts. The richness of parasite species (*rs* = 0.18, p = 0.12) had no correlation with the weight of the hosts, but the Shannon's diversity index (*rs* = 0.27; p = 0.02) showed weak positive correlation with the weight of the hosts. The abundance of *P*. (*S*) *inopinatus* (*rs* = 0.21; p = 0.01) and Proteocephalidae gen. sp. (*rs* = 0.22; p = 0.05) showed a weak positive correlation with the length of the hosts. There was no correlation between the weight of the hosts and the abundance of *P*. (*S*) *inopinatus* (*rs* = 0.08; p = 0.50), Proteocephalidae gen. sp. (*rs* = 0.22; p = 0.06) and *Contracaecum* sp. (*rs* = -0.05; p = 0.66). The abundance *Contracaecum* sp. (*rs* = -0.01, p = 0.91) there was no correlation between host length.

Table 4. Component community of metazoan parasites in *Pimelodus ornatus* from the Amazon River, state of Amapá, in Brazil.

Parameters	Values
All species of parasites	
Number of hosts examined	71
Total prevalence (%) of parasites	70.4
Total number of parasites	147
Number species of parasites	5

Diversity of Shannon	0.2 ± 0.3
Evenness	0.1 ± 0.2
Species richness of parasites	1.0 ± 0.8
Species of endoparasites	
Number species of endoparasites	4
Percentage of endoparasites (%)	97.9
Species of endoparasites (larvae)	2
Species of endoparasites (adults)	2
Species of ectoparasites	1
Number species of ectoparasites	1
Percentage of ectoparasites (%)	2.1
Species of ectoparasites (larvae)	0

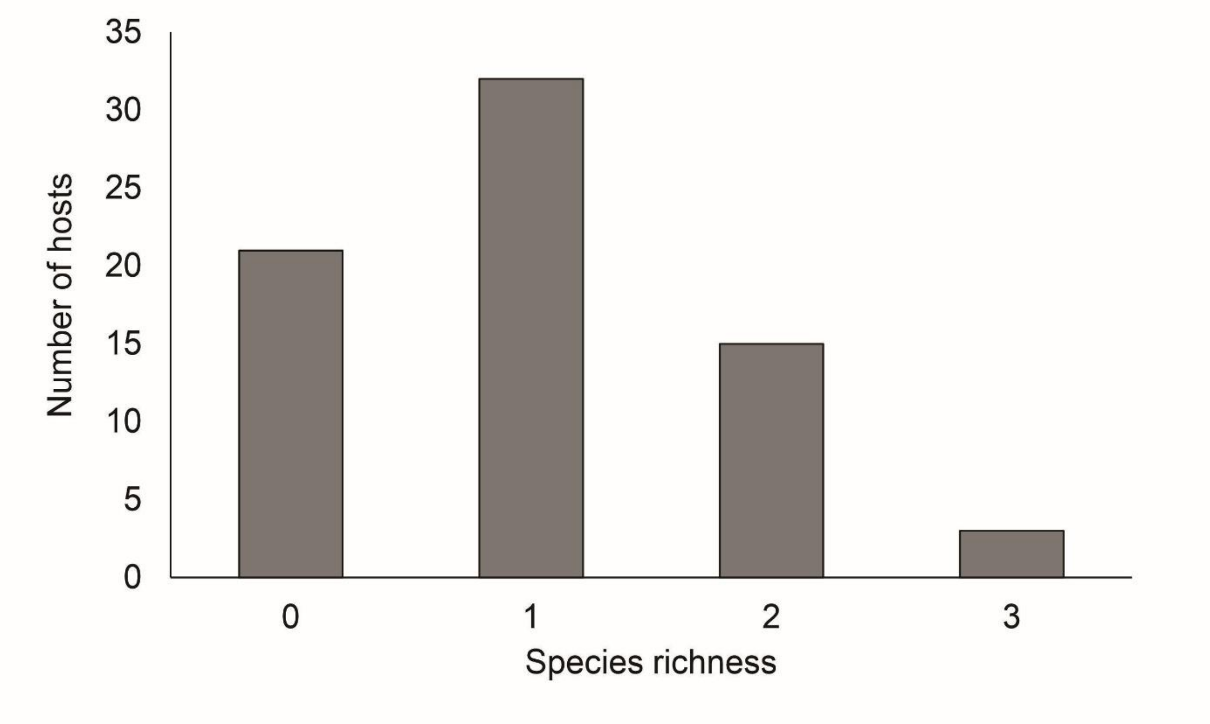

Figure 2. Species richness of metazoan parasites in *Pimelodus ornatus* from the Amazon River, Amapá of State, in Brazil.

Discussion

The component community of metazoan parasites in *P. ornatus* from the Amazon River consisted of one species of Monogenea, three species of Nematoda and one species of Cestoda. Few parasite species known for *P. ornatus* were found here (Table 1). For *P. ornatus* sampled from the reservoir of the Hydroelectric Power Station of Itaipu, State of Paraná (Brazil), Kohn et al. (2011) reported only one Nematoda species and one Digenea species. In contrast, the metazoan community in *Pimelodus blochii* (Valenciennes 1840) from the Acre and Iaco rivers, was made up of five species of Monogenea, 10 Nematoda, three Digenea, one Cestoda and three Crustacea (Negreiros et al., 2018); in *Pimelodus pohli* Ribeiro & Lucena, 2006 of the São Francisco river, for two species of Monogenea, three Nematoda, one Digenea and one Acanthocephala (Sabas & Brasil-Sato, 2014) and in *Pimelodus maculatus* Lacepède, 1803 of the Guandu River for one species of Nematoda and one species of Cestoda (Albuquerque et al., 2008). These results are probably related to omnivorous fed habit of these host fish species (Froese & Pauly, 2021), considering that acquiring of nematodes and other endoparasites are linked to the diet (Sabas & Brasil-Sato, 2014; Blasco- Costa et al., 2015; Oliveira et al., 2017; Tavares-Dias & Oliveira, 2017; Negreiros et al., 2018). However, such differences in the component community and parasite richness may be attributed to the difference in host species, diet and different environments.

In *P. ornatus*, the parasites showed an aggregate dispersion pattern, which is common in several species of freshwater fish from various natural environments (Sabas & Brasil-Sato, 2014; Tavares-Dias & Oliveira, 2017; Oliveira et al., 2019; Neves et al., 2020a). This aggregation may be associated with the genetic variability of the host population, decreased interspecific competition between parasites, decreased damage to the host and other environmental factors (Poulin, 2013; Tavares-Dias & Oliveira, 2017; Salgado-Maldonado et al., 2019).

Monogeneans are parasites that may serve as indicators of environmental quality, since they are generally present in greater abundance in aquatic environments with low conditions in water (Dogiel, 1961; Oliveira et al., 2017; Negreiros et al., 2018; Oliveira et al., 2019). In addition, most monogenean parasites are species-specific, parasitizing a host or phylogenetically related hosts. The depth of the water body and flow velocity limit the exploitation of monogenean species in the host fish (Negreiros et al., 2018). In *P. ornatus* of the Amazon River, an environment with a high average water flow (1.0 x $10^5 m^3/s$), which prevents the accumulation and permanence of pollutants and eutrophication (Abreu et al., 2020), and with a high level of oxygen, there was a low level of infection by monogeneans *Demidospermus* sp. Negreiros et al. (2018) reported that anthropogenic development of the Rio Acre influenced the levels of infection by *Demidospermus peruvianus* Mendoza-Palermo & Scholz, 2011; *Demidospermus striatus* Mendoza-Palermo & Scholz, 2011; *Demidospermus* sp. and *Ameloblastella* Kritsky, Mendoza-Franco & Schoz, 2000 in the gills of *P. blochii*.

In *P. ornatus*, larval stage of *Contracaecum* sp. and Proteocephalidae gen. sp. were present, indicating that this fish is an important intermediate host in the transmission of these endoparasites perhaps due to its omnivorous feeding habit (Sánchez-Botero & Araújo-Lima, 2001; Froese & Pauly, 2021). We observed that *P. ornatus* was feeding on small crustaceans such as crabs and shrimp. In addition, *P. ornatus* also feeds on small fish (Sá-Oliveira et al., 2014). These results indicate that *P. ornatus* occupies a lower position in the food chain, thus facilitating these infections by such endoparasites with different life cycles. However, this was the first record of *Contracaecum* sp. for *P. ornatus*.

Procamallanus (*S.*) *inopinatus,* a nematode with wide geographic distribution and is found in different species of fish in Brazil (Neves et al. 2020b), was recorded here for the first time in *P. ornatus*. This endoparasite was the dominant species in *P. ornatus* and had a higher level of infection when compared to *Contracaecum* sp. and *C. pinnai*, and occurred in the intestine, stomach and abdominal cavity. However, the levels of *C. pinnai* infection in *P.*

ornatus were low when compared to *P. blochii* from the Iaco and Acre rivers (Negreiros et al., 2018; Negreiros et al., 2019) and *P. maculatus* from the Rio Guandu (Albuquerque et al., 2008). *Cucullanus pinnai* has also been reported to parasitize *Pimelodus albicans* (Valenciennes, 1840) (Chemes & Takemoto, 2011) and *Pimelodus clarias* (Linnaeus, 1758) (Kohn & Fernandes, 1987), demonstrating that this is a common nematode infecting species of *Pimelodus*.

No crustacean parasite species was found on the gills of *P. ornatus* from the Amazonas River. Similar findings were reported by Neves & Tavares-Dias (2019) for *Ageneiosus ucayalensis* (Castelnau, 1855), *Pimelodella eigenmanni* (Boulenger, 1891), *Colomesus asellus* (Muller & Troschel, 1849), *Pimelodus blochii* (Valenciennes, 1840), *P. ornatus*, *Platynematichthys notatus* (Jardine, 1841) and *Peckoltia lineola* (Armbruster, 2008) from Matapi River, a tributary of the Amazonas River, in Amapá State (Brazil). This absence of parasitic crustacean species may be due to the water velocity and daily tides from the Amazonas River (Abreu et al., 2020), given that these ectoparasites need to swim to find adequate hosts, while others depend on the flow of water and swimming speed (Neves & Tavares-Dias, 2019).

In *P. ornatus*, regarding the host-parasite relationship, there was a positive correlation of the species richness of parasites and Shannon diversity index with the total length of the host, demonstrating that the size of the fish influenced these parameters. Similar findings have been reported for *Chaetobranchopsis orbicularis* (Steindachner, 1975) (Tavares-Dias & Oliveira, 2017), *Colossoma macropomum* Cuvier, 1816 (Gonçalves et al., 2018) and *Acestrorhynchus falcirostris* (Cuvier, 1819) (Hoshino et al., 2016). Moreover, the body length of *P. ornatus* also influenced in the abundance of *P.* (*S*) *inopinatus* and Proteocephalidae gen. sp., in which larger fish tended showed more parasites when compared to smaller ones, although these correlations explicit only 22% of occurrence of this parasite. Determining the factors that affect the parasites present in wild fish populations is important for parasite ecology studies. However, in fish populations, the influence of the body size on parasite load may vary, and the causes of variations are little understood.

Conclusions

The parasite community of *P. ornatus* in the Amazon River was composed of helminth species with low prevalence, low abundance, low diversity and low species richness, predominance of ectoparasites and with aggregate dispersion. The size of the host fish influenced the diversity of parasites, explaining less than 25% of the occurrence of parasites. In addition, new reports of parasites have been recorded for *P. ornatus*. The data obtained here emphasize the importance of the Amazon River as a source of biodiversity. It was possible to contribute to an increase in the knowledge of the freshwater biodiversity from the Amazon, expanding the ecological interactions of parasites and biological information on this Amazonian siluriform with gaps in the literature. This information may be used for future comparisons in studies on the impacts of anthropogenic actions on the parasitic diversity of *P. ornatus* in the Amazon River.

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References

Abreu CHM, Barros MLC, Brito DC, Teixeira MR, Cunha AC DA. Hydrodynamic modeling and simulation of water residence time in the estuary of the lower Amazon River. *Water* 2020; 12(3): 660. https://*doi*.org/*10.3390*/*w12030660.*

Altizer S, Ostfeld RS, Johnson PT, Kutz S, Harvell CD. Climate change and infectious diseases: from evidence to a predictive framework. *Science* 2013; 341(6145): 514–519. https://doi.org/10.1126/science.1239401

Albuquerque MC, Santos MD, Monteiro CM, Martins AN, Ederli NB, Brasil-Sato MAC. Helmintos endoparasitos de *Pimelodus maculatus* Lacépède, 1803, (Actinopterygii, Pimelodidae) de duas localidades (lagoa e calha do rio) do Rio Guandu, Estado do Rio de Janeiro, Brasil. *Rev Bras Parasitol Vet* 2008; 17(Suppl1): 113-119.

Arantes CC, Winemiller KO, Petrere M, Freitas CEC. Spatial variation in aquatic food webs in the Amazon River floodplain. Fresh Sci 2019; 38(1): 213-228. https://doi.org/10.1086/701841.

Blasco-Costa I, Rouco C, Poulin R. Biogeography of parasitism in freshwater fish: spatial patterns in hot spots of infection. *Ecography* 2015; 38(3): 301-310. https://doi.org/10.1111/ecog.01020.

Boujard T. P*oissons de Guyane: Guide écologique de l'Approuague et de la réserve des Nouragues*. Editions Quae, Paris,1997.

Bush AO, Lafferty KD, Lotz JM, Shostak AW. Parasitology meets ecology on its own term. *J Parasitol* 1997; 83(4): 575-583. https://doi.org/10.2307/3284227.

Calvès G, Calderon Y, Roso V, Bonnel C, Roddaz M, Brusset S, et al. Past Amazon Basin fluvial systems, insight into the Cenozoic sequences using seismic geomorphology (Marañón Basin, Peru). *J South Am Earth Sci* 2019; 90: 440–452. https://doi.org/10.1016/j.jsames.2018.12.019.

Chemes SB, Takemoto RM. Diversity of parasites from middle Paraná system freshwater fishes, Argentina. *Int J Biodivers Conserv* 2011; 3(7): 249–266. https://doi.org/10.5897/IJBC.9000044.

Chong LS, Berelson WM, Hammond DE, Fleisher MQ, Anderson RF, Rollins NE, et al. Biogenic sedimentation and geochemical properties of deep-sea sediments of the demerara slope/abyssal plain: influence of the Amazon River Plume. *Mar Geol* 2016; 379: 124–139. https://doi.org/10.1016/j.margeo.2016.05.015.

Costa Sousa RG, Souza LA, Frutuoso ME, Freitas CEC. Seasonal dynamic of Amazonian small-scale fisheries is dictated by the hydrologic pulse. *Bol Inst Pesca* 2017; 43(2): 207–221. https://doi.org/10.20950/1678-2305.2017v43n2p207

De Chambrier A, Rego AA. *Mariauxiella pimelodi* n. g., n. sp. (Cestoda: Monticelliidae): a parasite of pimelodid siluroid fishes from South America. *Syst Parasitol* 1995; 30(1): 57–65. https://doi.org/10.1007/BF00009245.

De Chambrier A, Waeschenbach A, Fisseha M, Scholz T, Mariaux J. A large 28S rDNA-based phylogeny confirms the limitations of established morphological characters for classification of proteocephalidean tapeworms (Platyhelminthes, Cestoda). *ZooKeys* 2015; 500: 25-59. 10.3897/zookeys.500.9360.

Dogiel VA. Ecology of the parasites of freshwater fishes. In: Dogel VA, Petrushevski GK, Polyanski YI, editors. *Parasitology of fishes.* Leningrad: University Press; 1961. p. 1-47.

Eiras JC, Takemoto RM, Pavanelli GC. *Métodos de estudos e técnicas laboratóriais em parasitologia de peixes.* Maringá: Eduem, 2006.

Fernandes BMM, Kohn A. On some trematodes parasites of fishes from Paraná River. *Braz J Biol* 2001; 61(3): 461-466. http://dx.doi.org/10.1590/S1519-69842001000300016.

Froese R, Pauly D. FishBase. Version (2/2021). USA: FishBase. [online]2021 [cited 2020 February 5]. Available from: www.fishbase.org

Gaillardet J, Dupre B, Allegre CJ, Négrel P. Chemical and physical denudation in the Amazon River Basin. *Chem Geol* 1997; 142(3): 141–173. https://doi.org/10.1016/S0009-2541(97)00074-0.

Gonçalves BB, Oliveira MSB, Borges WF, Santos GG, Tavares-Dias M. Diversity of metazoan parasites in *Colossoma macropomum* (Serrasalmidae) from the lower Jari River, a tributary of the Amazonas River in Brazil. *Acta Amaz* 2018; 48(3): 211–216. https://doi.org/10.1590/1809-4392201704371.

Harvell D, Altizer S, Cattadori IM, Harrington L, Weil E. Climate change and wildlife diseases: when does the host matter the most? *Ecology* 2009; 90(4): 912–920. https://doi.org/10.1890/08-0616.1.

Hoshino MDFG, Neves LR, Tavares-Dias M. Parasite communities of the predatory fish, *Acestrorhynchus falcatus* and *Acestrorhynchus falcirostris,* living in sympatry in Brazilian Amazon. *Rev Bras Parasitol Vet* 2016; 25(2): 207–216. https://doi.org/10.1590/S1984-29612016038.

Kohn A, Fernandes BM. Estudo comparativo dos helmintos parasitos de peixes do Rio Mogi Guassu, coletados nas excursões realizadas entre 1927 e 1985. *Mem Inst Oswaldo Cruz* 1987; 82(4): 483–500. http://dx.doi.org/10.1590/S0074-02761987000400006.

Kohn A, Fernandes BMM, Cohen SC. *South American trematodes parasites of fishes.* Imprinta Express Ltda: Rio de Janeiro; 2007.

Kohn A, Moravec F, Cohen SC, Canzi C, Takemoto RM, Fernandes BM. Helminths of freshwater fishes in the reservoir of the Hydroelectric Power Station of Itaipu, Paraná, Brazil. *Check List* 2011; 7(5): 681-690. https://doi.org/10.15560/7.5.681.

Le Bail PY, Keith P, Planquette P. Atlas des poissons d'eau douce de Guyane. Paris: Muséum National d' Histoire Nattrelle;2000.

Ludwig JA, Quartet L, Reynolds JF, Reynolds J. S*tatistical ecology: a primer in methods and computing.* New York: John Wiley & Sons; 1988.

Luque JL, Vieira F, Takemoto R, Pavanelli G, Eiras J. Checklist of Crustacea parasitizing fishes from Brazil. *Check List* 2013; 9(6): 1449-1470. https://doi.org/10.15560/9.6.1449

Magurran A. *Measuring biological diversity*. Oxford: Blackwell Publishing; 2004.

McLeod RJ, Wing SR. Influence of an altered salinity regime on the population structure of two infaunal bivalve species. *Estuar Coast Shelf Sci* 2008; 78(3): 529–540. https://doi.org/10.1016/j.ecss.2008.01.019.

Mendoza-Palmero CA, Mendoza-Franco EF, Acosta AA, Scholz T. *Walteriella* n. g. (Monogenoidea: Dactylogyridae) from the gills of pimelodid catfishes (Siluriformes: Pimelodidae) from the Peruvian Amazonia based on morphological and molecular data. Syst Parasitol 2019; 96(6): 441–452. https://doi.org/10.1007/ s11230-019-09866-8.

Milliman JD, Syvitski JPM. Geomorphic/tectonic control of sediment discharge to the ocean: the importance of small mountainous Rivers. *J Geol* 1992; 100(5): 525–544. https://doi.org/10.1086/629606.

Milliman JD. River inputs. In: Steele JH, Trorpe SA, Turekian KK, editors. *Encyclopedia of Ocean Sciences*. San Diego: Academic Press; 2001. p. p.2419-2427.

Moravec F. Nematodes of freshwater fishes of the Neotropical Region. Praha, Vydala Academia; 1998.

Negreiros LP, Florentino AC, Pereira FB, Tavares-Dias M. Long-term temporal variation in the parasite community structure of metazoans of *Pimelodus blochii* (Pimelodidae), a catfish from the Brazilian Amazon. *Parasitol Res* 2019; 118(12): 3337–3347. https://doi.org/10.1007/s00436-019-06480-x.

Negreiros LP, Pereira FB, Tavares-Dias M, Tavares LER. Community structure of metazoan parasites from *Pimelodus blochii* in two rivers of the western Brazilian Amazon: same seasonal traits, but different anthropogenic impacts. Parasitol Res 2018; 117(12): 3791–3798. https://doi.org/10.1007/s00436-018-6082-5.

Neves LR, Negreiros LP, Silva LMA, Tavares-Dias M. Diversity of monogenean parasites on gills of fishes from the Matapi River, in the Brazilian Amazon. *Rev Bras Parasitol Vet* 2020a; 29(4): e013520. https://doi.org/10.1590/S1984-29612020081.

Neves LR, Silva LMA, Florentino AC, Tavares-Dias M. Distribution patterns of *Procamallanus* (*Spirocamallanus*) *inopinatus* (Nematoda: Camallanidae) and its interactions with freshwater fish in Brazil. *Rev Bras Parasitol Vet* 2020b; 29(4): e012820. https://doi.org/10.1590/s1984-29612020092.

Neves LR, Tavares-Dias M. Low levels of crustacean parasite infestation in fish species from the Matapi River in the state of Amapá, Brazil. *Rev Bras Parasitol Vet* 2019; 28(3): 493-498. https://doi.org/10.1590/S1984-29612019006.

Nittrouer CA, DeMaster DJ. Sedimentary processes on the Amazon continental shelf: past, present and future research. *Cont Shelf Res* 1986; 6(1-2): 5-30. https://doi.org/10.1016/0278-4343(86)90051-8.

Nomura H. *Dicionário dos peixes do Brasil*. Technical Books Livraria: Rio de Janeiro; 1984.

Oliveira MSB, Adriano EA, Tavares-Dias M, Corrêa LL. Monogenoidea parasites of *Cichla monoculus* and *Cichla pinima* (Osteichthyes: Cichlidae), sympatric fish in lower Tapajós River, Northern Brazil. Ann Parasitol 2019; 65(4): 371–380. https://doi.org/10.17420/ap6504.223.

Oliveira MSB, Gonçalves RA, Ferreira DO, Pinheiro DA, Neves LR, Dias MKR, et al. Metazoan parasite communities of wild *Leporinus friderici* (Characiformes: Anostomidae) from Amazon River system in Brazil. *Stud Neotrop Fauna Environ* 2017; 52(2): 146–156. https://doi.org/10.1080/01650521.2017.1312776.

Pavanelli GC, Takemoto RM. *Spasskyellina mandi* n. sp. (Proteocephalidea: Monticelliidae), parasite of *Pimelodus ornatus* Kner, 1857 (Pisces: Pimelodidae) of the Paraná River, Paraná, Brazil. *Mem Inst Oswaldo Cruz* 1996; 91(6): 723-726. http://dx.doi.org/10.1590/S0074-02761996000600013.

Poulin R. Macroecological patterns of species richness in parasite assemblages. *Basic Appl Ecol* 2004: 5(5): 423–434. https://doi.org/10.1016/j.baae.2004.08.003.

Poulin R. Explaining variability in parasite aggregation levels among host samples. *Parasitology* 2013: 140(4): 541–546. https://doi.org/10.1017/s0031182012002053.

Rohde K, Hayward C, Heap M. Aspects of the ecology of metazoan ectoparasites of marine fishes. *Int J Parasitol* 1995; 25(8): 945-970. https://doi.org/10.1016/0020-7519(95)00015-T.

Salo M, Sirén A, Kalliola R. Diagnosing wild species harvest: resource use and conservation. New York: Academic Press; 2013.

Sabas CSS, Brasil-Sato MC. Helmintofauna de Pimelodus pohli (Actinopterygii: Pimelodidae) do alto Rio São Francisco, Brasil. *Rev Bras Parasitol Vet* 2014; 23(3): 375-382. https://doi.org/10.1590/S1984-29612014067.

Sánchez-Botero JI, Araújo-Lima CARM. As macrófitas aquáticas como berçário para a ictiofauna da várzea do rio Amazonas. *Acta Amaz* 2001; 31(3): 437-437. https://doi.org/10.1590/1809-43922001313447.

Sá-Oliveira JC, Angelini R, Isaac-Nahum VJ. Diet and niche breadth and overlap in fish communities within the area affected by an Amazonian reservoir (Amapá, Brazil). *An Acad Bras Ciênc* 2014; 86(1): 383-406. http://dx.doi.org/10.1590/0001-3765201420130053.

Salgado-Maldonado G, Mendoza-Franco EF, Caspeta-Mandujano JM, Ramírez-Martínez C. Aggregation and negative interactions in low-diversity and unsaturated monogenean (Platyhelminthes) communities in *Astyanax aeneus* (Teleostei) populations in a Neotropical river of Mexico. Int J Parasitol Parasites Wildl 2019; 8: 203-215. https://doi.org/10.1016/j.ijppaw.2019.02.005

Thatcher VE. *Amazon fish parasites*. Sophia: Pensoft Publishers; 2006.

Tavares-Dias M, Oliveira MSB. Structure of parasites community in *Chaetobranchopsis orbicularis* (Cichlidae), a host from the Amazon River system in northern Brazil. Parasitol Res 2017; 116(8): 2313–2319. https://doi.org/10.1007/s00436-017-5539-2.

Tavares-Dias M, Oliveira MSB, Gonçalves RA, Silva LMA. Ecology and seasonal variation of parasites in wild *Aequidens tetramerus*, a Cichlidae from the Amazon. *Acta Parasitol* 2014; 59(1): 158–164. https://doi.org/10.2478/s11686-014-0225-3.

Torrente LJ DE QG, Ohara VWM, Jansen TH DA SP, Costa Doria ZCR. Peixes do Rio madeira. São Paulo: INPA;2013.

Travassos L, Artigas P, Pereira C. Fauna helmintológica dos peixes de água doce do Brasil. *Arq Inst Biol* 1928; 1: 5-82.

Vazzoler A. *Biologia da reprodução de peixes teleósteos: teoria e prática*. Marigá: Eduem; 1996.

CAPÍTULO V

Original Article

Temporal and seasonal variation of metazoan parasites in *Pimelodus ornatus* (Siluriformes: Pimelodidae) from the Amazon River, Brazil

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RESEARCH

Temporal and seasonal variation of metazoan parasites in *Pimelodus ornatus* (Siluriformes: Pimelodidae) from the Amazon River, Brazil

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Abstract

This study investigated the influence of temporal and seasonal (i.e., rainy and dry seasons) in communities and infracommunities of metazoan parasites in *Pimelodus ornatus*, a Siluriformes from the Amazon River in the state of Amapá, in northern Brazil. A total of 144 fish were collected during 2020 and 2021. In 2020, five species of parasites were found (1 Monogenea, 3 Nematoda, and 1 Cestoda), and in 2021, seven species of parasites were found (1 Monogenea, 3 Nematoda, 1 Cestoda, 1 Digenea, and 1 Crustacea). In 2020, *Spirocamallanus inopinatus* was dominant, while in 2021, *Contracaecum* sp. was dominant. Brillouin diversity, species richness, and evenness were higher in 2021. There were differences for some parasite infracommunities between years and between seasonal periods. Brillouin diversity and species richness were higher in the rainy season, but there were no differences in evenness and Berger-Parker dominance between the seasonal periods. Temporal and seasonal variations were mainly influenced by rainfall levels, oxygen levels, and environmental temperature and, later, by the availability of infective stages of parasites in the environment and the size of the hosts. Lastly, the influence of the temporal and seasonal variations on the structure of communities and infracommunities of parasites was weaker than expected due to the low infection rates of the majority of parasites found.

Keywords Amazon · Freshwater fish · Infection · Parasites · Seasonality

Introduction

The Amazon River basin, which is about 8.7 to 11.8 million years old (Gorini et al. 2014; Hoorn et al. 2017; van Soelen et al. 2017), is one of the most complex ecosystems in the world. It has a great biodiversity of fish that feeds riverside populations that live from fishing (Salo et al. 2013). This fish biodiversity is due to the particular characteristics of this large hydrographic basin and its tributaries, which have complex food webs and large vegetation cover, both influenced by the seasonal cycles (rainy/dry) of the Amazon region (Sousa et al. 2017; Arantes et al. 2019). In the Amazon River system, parasites also represent a numerically and functionally important component of this biodiversity. Understanding which factors drive variations in the diversity of parasites in the region has been of interest in fish parasitology. Thus, efforts have been made to understand annual and seasonal effects on diversity, richness, and parasite communities in wild Amazonian fish populations (Neves et al. 2013; Tavares-Dias et al. 2014; Gonçalves et al. 2016; Negreiros et al. 2019a,b; Hoshino and Tavares-Dias 2019, 2020; Cavalcante et al. 2020).

Variation in the structure of parasite communities can be expected over time. Information about these dynamics in Amazonian fish has been little addressed (Negreiros et al. 2019a; Hoshino and Tavares-Dias 2019, 2020). Variations in the diversity and abundance of parasites with complex life cycles can be strongly altered by the population density of intermediate and definitive hosts, which can vary in space, time, and seasonal period, thus playing an important role in

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Temporal and seasonal variation of metazoan parasites in *Pimelodus ornatus* (Siluriformes: Pimelodidae) from the Amazon River, Brazil

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Abstract

This study investigated the influence of temporal and seasonal (i.e., rainy and dry seasons) in communities and infracommunities of metazoan parasites in *Pimelodus ornatus*, a Siluriformes from the Amazon River in the state of Amapá, in northern Brazil. A total of 144 fish were collected during 2020 and 2021. In 2020, five species of parasites were found (1 Monogenea, 3 Nematoda, and 1 Cestoda), and in 2021, seven species of parasites were found (1 Monogenea, 3 Nematoda, 1 Cestoda, 1 Digenea, and 1 Crustacea). In 2020, *Spirocamallanus inopinatus* was dominant, while in 2021, *Contracaecum* sp. was dominant. Brillouin diversity, species richness, and evenness were higher in 2021. There were differences for some parasite infracommunities between years and between seasonal periods. Brillouin diversity and species richness were higher in the rainy season, but there were no differences in evenness and Berger-Parker dominance between the seasonal periods. Temporal and seasonal variations were mainly influenced by rainfall levels, oxygen levels, and environmental temperature and, later, by the availability of infective stages of parasites in the environment and the size of the hosts. Lastly, the influence of the temporal and seasonal variations on the structure of communities and infracommunities of parasites was weaker than expected due to the low infection rates of the majority of parasites found.

Keywords: Amazon, Freshwater fish~~,~~ Infection, Parasites, Seasonality

Introduction

The Amazon River basin, which is about 8.7 to 11.8 million years old (Gorini et al. 2014; Hoorn et al. 2017; van Soelen et al. 2017), is one of the most complex ecosystems in the world. It has a great biodiversity of fish that feeds riverside populations that live from fishing (Salo et al. 2013). This fish biodiversity is due to the particular characteristics of this large hydrographic basin and its tributaries, which have complex food webs and large vegetation cover, both influenced by the seasonal cycles (rainy/dry) of the Amazon region (Sousa et al. 2017; Arantes et al. 2019). In the Amazon River system, parasites also represent a numerically and functionally important component of this biodiversity. Understanding which factors drive variations in the diversity of parasites in the region has been of interest in fish parasitology. Thus, efforts have been made to understand annual and seasonal effects on diversity, richness, and parasite communities in wild Amazonian fish populations (Neves et al. 2013; Tavares-Dias et al. 2014; Gonçalves et al. 2016; Negreiros et al. 2019a,b; Hoshino and Tavares-Dias 2019; Hoshino and Tavares-Dias 2020; Cavalcante et al. 2020).

Variation in the structure of parasite communities can be expected over time. Information about these dynamics in Amazonian fish has been little addressed (Negreiros et al. 2019a; Hoshino and Tavares-Dias, 2019; Hoshino and Tavares-Dias, 2020). Variations in the diversity and abundance of parasites with complex life cycles can be strongly altered by the population density of intermediate and definitive hosts, which can vary in space, time, and seasonal period, thus playing an important role in the structure of the parasite communities of wild fish populations (Blasco-Costa et al. 2015). These variations can occur mainly in the Amazon basin, where seasonality is characterized by the occurrence of two distinct periods: rainy and dry seasons. The rainy season includes the months from December to May, while the dry season includes the months from June to November (Souza and Cunha 2010). Thus, seasonal variation is an abiotic factor that can influence the life of many Amazonian fish species, including *Pimelodus ornatus* (Kner, 1857), a Siluriformes fish whose parasite diversity has recently been studied by Lima et al. (2021). *Pimelodus ornatus* is a catfish found in the Amazon, Madeira, Parnaiba, Negro, upper Parana, Orinoco rivers, as well as large rivers from Guyanas, Paraguay, Bolivia, Peru, Venezuela, and Ecuador (Nomura 1984; Torrente et al. 2013; Lima et al. 2021; Froese and Pauly 2022).

This omnivorous fish is active at night and can be captured in main river beds, on rocky bottoms with dead tree trunks, upstream and downstream of rapids and backwaters (Lima et al. 2021; Froese and Pauly 2022). As it is a small size fish, its first sexual maturation occurs from 15.4 cm (Vazzoler 1996).

In Amazonian systems, invertebrates that are intermediate hosts for parasite species with a complex life cycle can also respond to environmental variations in different ways, from the individual level to the component community level (Gonçalves et al. 2016; Negreiros et al. 2019b; Hoshino and Tavares-Dias, 2019; Hoshino and Tavares-Dias, 2020). Parasites play an intrinsic role, however, hidden in the Amazonian ecosystems. Thus, understanding many aspects of the ecology of parasites in wild fish from this important Neotropicalregion has been hampered by the scarcity of annual and seasonal datasets and studies on almost allaspects of environment-host-parasite interactions. As the effects of temporal and seasonal variation on thecommunities and infra communities of *P. ornatus* metazoan parasites are not known, we hypothesizedthat (1) annual and seasonal variations are factors that act directly on the diversity, structure of communities and infracommunities of metazoan parasites in *P. ornatus* of the Amazon River, and (2) the diversity, communities and infracommunities of metazoan parasites are greater in the rainy season, when there is an increase in the availability and variety of food, and consequently greater contact of fish in the infective stages of the parasites in the environment.

Material and methods

Study area and fish collection

In bimonthly collections, a total of 144 *P. ornatus* were collected (18.8 ± 2.5 cm and 19.5 ± 8.8g) from January 2020 to November 2021 in the Amazon River, near the Island of Santana, in the municipality of Santana, Amapá, Brazil (Fig. 1). Fish were collected with gill nets with different sizes and meshes (15 mm, 20 mm, 25 mm, 30 mm, and 35 mm between knots), cast nets (mesh of 20 mm between knots) and hand lines. Then, the fish were euthanized by the spinal cord transection method, preserved in 10% formaldehyde, and transported to the Aquaculture and Fisheries Laboratory of Embrapa Amapá, Macapá, state of Amapá (Brazil), for analysis of the parasites.

At the fish sampling sites, water quality parameters such as electrical conductivity, pH, and total dissolved solids were measured using a multiparameter (Akso, model Combo5-02-1016). Dissolvedoxygen levels and water temperature were measured using an oximeter (Instrutherm, Model MO-900). Rainfall data from the fish collection region were obtained from the Hydrometeorology and Renewable Energy Center (NHMET) of The Institute of Scientific and Technological Research of the State of Amapá(IEPA) (Table 1).

Parasite analysis procedures

In the laboratory, fish were weighed (g) and measured in total length (cm), and were necropsied to collect the metazoan parasites. The mouth, opercular cavity, gills, and fins were analyzed for ectoparasites, and internal organs such as gastrointestinal tract and viscera were examined forendoparasites with the aid of a stereomicroscope. The parasites found were preserved in 70% ethyl alcohol for the preparation of permanent slides following previous recommendations (Eiras et al. 2006).

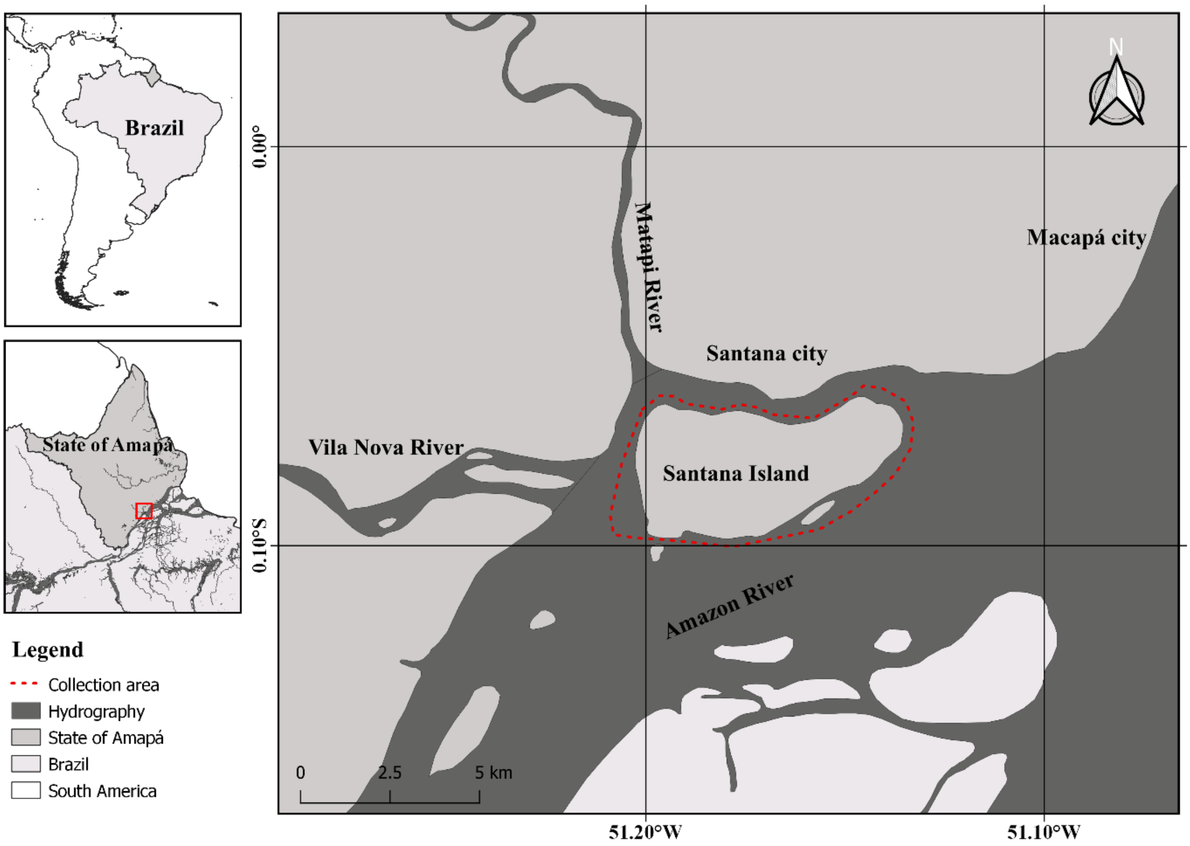

Fig. 1 Collection area of *Pimelodus ornatus* in the Amazon River, the state of Amapá, northern Brazil

Data analysis

Parasite indices such as prevalence and mean abundance of infracommunities were calculated (Bush et. al. 1997). To describe the parasite community, species richness, Brillouin diversity index (*HB*), evenness (*E*), and Berger-Parker dominance index (*d*) data were calculated using PAST software (Hammer et al. 2001). The Spearman correlation coefficient (*rs*) was used to evaluate possiblecorrelations of body length and weight of host with parasite abundance (Zar 2010).

Table 1. Physical-chemical parameters of water from the Amazon River, in the state of Amapá (Brazil), during periods of collection of *Pimelodus ornatus*.

			Test	
Parameters	**2020**	**2021**	***t***	***U***
Rainfall (mm)	195.7 ± 119.5	231.5 ± 153.2	-0.8197	-
Temperature (°C)	29.0 ± 1.0	29.1 ± 1.1	0.0284	-
Dissolved oxygen (mg/L)	5.8 ± 0.2	4.2 ± 1.8	2.2699	-
pH	7.1 ± 0.2	6.8 ± 0.3	2.4242*	-
Total dissolved solids (mg/L)	56.8 ± 10.7	107 ± 60.7	-	2.0*
Electric conductivity (µS/cm)	90.4 ± 12.2	148.1 ± 111.4	-	10.5
	Rainy season	**Dry season**		
Rainfall (mm)	311.5 ± 106.7	131.0 ± 116.3	3.9908**	-
Temperature (°C)	28.3 ± 0.3	30.0 ± 0.5	-6.8690**	-
Dissolved oxygen (mg/L)	5.2 ± 0.8	4.6 ± 2.12	0.4952	-
pH	6.9 ± 0.2	6.9 ± 0.3	-0.2389	-
Total dissolved solids (mg/L)	72.6 ± 34.6	91.4 ± 62.5	-	16.00
Electric conductivity (µS/cm)	98.8 ± 61.4	139.6 ± 98.7	-	15.50

t,: test t; *U*, Mann–Whitney test, $*p < 0.05$, , $**p < 0.001$

To determine whether total length (cm), weight (g), prevalence, abundance, species richness,evenness, Brillouin diversity index, and Berger-Parker dominance index data followed a normal distribution pattern, Shapiro-Wilk and Bartlett tests were used, respectively. To verify if there were differences in the prevalence of parasites between the years 2020 and 2021 and the seasonal periods(rainy and dry), the chi-square test (χ^2) with

Yates's correction was used, and to verify differences in the abundance of parasites, the Mann Whitney test (*U*) was used (Zar 2010). To estimate differences in diversity indices (species richness, evenness, Brillouin diversity index, and Berger-Parker dominance index) of metazoan parasites between 2020 and 2021 and seasonal periods, the Kruskal-Wallis followedby Dunn test was employed. These analyses were performed in R software (R CoreTeam 2021).

Variance analysis of permutation (PERMANOVA) was used to detect differences in the parasite community between the years 2020 and 2021 and the rainy and dry seasons. Temporal andseasonal variables were fitted in the ordering of principal coordinate analysis (PCoA) based on Bray-Curtis distance, using the envfit function of the vegan package (Oksanen et al. 2020), and *p*-values were calculated by the permutation test (number of permutations = 999) in R software. To analyze how differences in sampling effort can influence the results, we plotted a species accumulation curve (observed and expected) for the years 2020 and 2021 and for the rainy and dry seasons. These analyses were performed using R software (R CoreTeam 2021).

Results

Component community and parasite infracommunities in 2020 and 2021

Fish collected in 2020 measured 15.5 ± 2.4 cm, while those collected in 2021 measured 16.0 ± 2.5 cm, showing no significant differences ($t = 1.3$, $p = 0.18$) between years. Fish collected in 2020 weighed 18.9 ± 8.1 g, and those collected in 2021 weighed 20.0 ± 9.4 g, showing no significant differences ($t = -7.5$, $p = 0.4$) between the years.

Host length did not present correlation with the abundance of *S. inopinatus* ($rs = 0.16$, $p = 0.052$), *Contracaecum* sp. ($rs = 0.04$, $p = 0.57$), and Proteocephalidae gen. sp. ($rs = 0.01$, $p = 0.83$), nor did the weight with the abundance of *S. inopinatus* ($rs = 0.06$, $p = 0.43$), *Contracaecum* sp. ($rs = 0.01$, $p = 0.84$), and Proteocephalidae gen. sp. ($rs = 0.03$, $p = 0.70$).

Of the fish examined, 412 parasites were collected, those being 146 parasites in 2020 and 266 parasites in 2021. In 2020, 70.4% of fish were parasitized by *Demidospermus* Suriano, 1983; *Cucullanus pinnai* (Travassos, Artigas & Pereira, 1928); *Spirocamallanus inopinatus* (Travassos, Artigas & Pereira, 1928); larvae of *Contracaecum* Railliet & Henry, 1912; and plerocercoids of Proteocephalidae La Rue, 1911. In 2021, 86.3% of fish were parasitized by *Demidospermus* sp.; *C. pinnai*; *S. inopinatus*; larvae of *Contracaecum* sp.; Proteocephalidae gen. sp., *Genarchella genarchella* (Travassos, Artigas & Pereira, 1928); and *Thelotha henselli* (Von Martens, 1869).

There was a dominance of *S. inopinatus* in 2020 and *Contracaecum* sp. in 2021. Prevalence and abundance of *Contracaecum* sp. was higher in 2021, while the other parasite species did not differbetween the years (Table 2).

Axes 1 and 2 of PCoA of were responsible for 63% of the total data variation in the compositionof parasite abundance in 2020 and 2021. Despite the figure showing an overlap and sharing of species between the years, there was a significant difference (PERMANOVA: $F = 0.060$, $p = 0.001$) in parasite infracommunity, influenced mainly by the variation in the abundance of *S. inopinatus* ($R^2 = 0.356$, $p = 0.001$), *Contracaecum* sp. ($R^2 = 0.719$, $p = 0.001$), and Proteocephalidae gen. sp. ($R^2 = 0.339$, $p = 0.001$) (Fig. 2).

Table 2. Metazoan parasites in *Pimelodus ornatus* from Amazon River, in the state of Amapá (Brazil), collected in 2020 and 2021

		2020 (n = 71)			**2021 (n = 73)**			χ^2	***U***
Parasite species	**Sites of infection**	**P (%)**	**MA ± SD**	**TNP**	**P (%)**	**MA ± SD**	**TNP**		
Demidospermus sp.	Gills	2.8	0.04 ± 0.3	3	8.2	0.3 ± 1.2	20	1.1	2448.5
Cucullanus pinnai	Intestine, abdominal cavity	5.6	0.06 ± 0.2	4	11.0	0.3 ± 1.1	22	1.2	2410.0
Spirocamallanus inopinatus	Stomach abdominal cavity, intestine	53.5	1.3 ± 1.5	90	49.3	1.2 ± 1.8	85	0.1	2397.0
Contracaecum sp. (larvae)	Abdominal cavity, stomach, liver, intestine	22.5	0.4 ± 1.1	33	58.9	1.5 ± 2.1	108	18.2**	1621.0**
Genarchella genarchella	Pharynx	0	0	0	5.5	0.1 ± 0.2	4	-	-
Proteocephalidae gen sp. (plerocercoids)	Stomach, intestine	15.5	0.2 ± 0.6	16	16.4	0.4 ± 1.1	26	0.8	2559.5
Telotha henselli	Gills	0	0	0	2.7	0.03 ± 0.2	2	-	-

P,: prevalence;, *MA*,: mean abundance;, *SD*,: standard deviation;, *TNP*,: total number of parasites;, χ^2, chi-square test;, *U*, : Mann–Whitney test

, ***$p < 0.05$

, ****$p < 0.01$. -

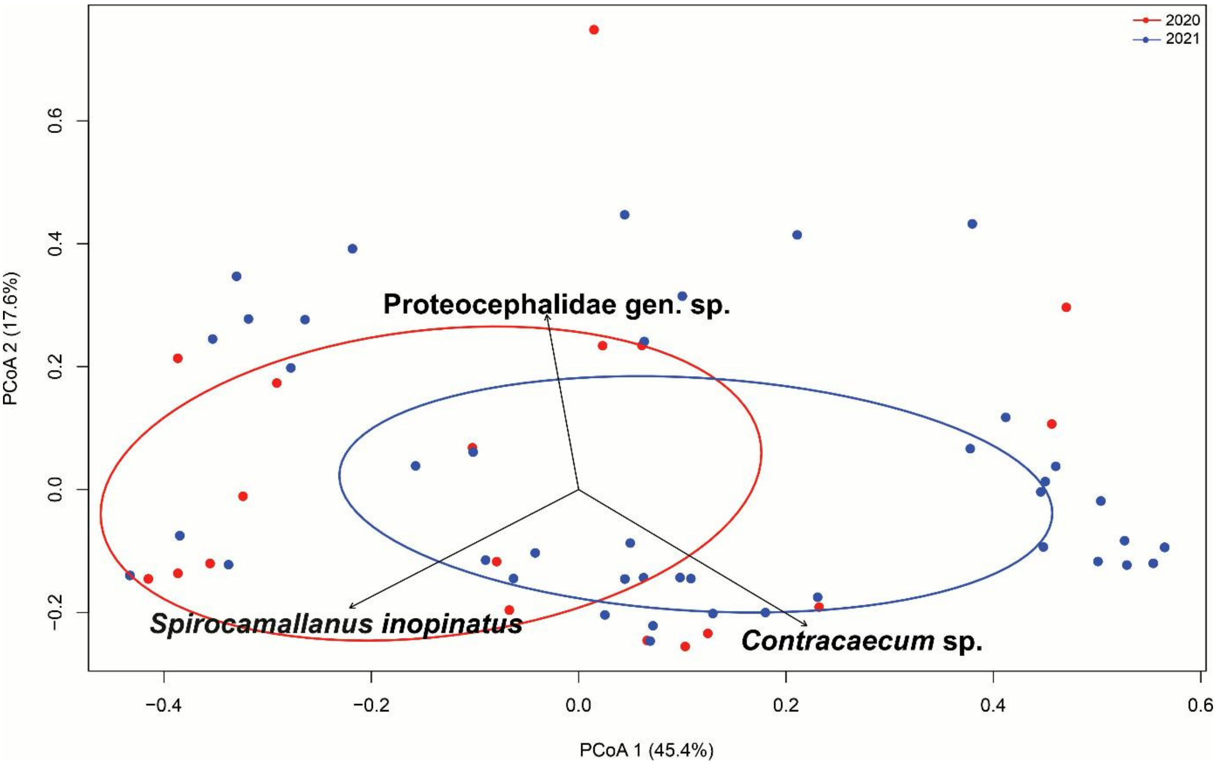

Fig. 2. Principal coordinate analysis (PCoA) using a Bray-Curtis distance matrix for communities of metazoan parasites of *Pimelodus ornatus* from Amazon River, in the state of Amapá (Brazil), collected in 2020 and 2021. The percentage of variation explained by the plotted principal coordinates is indicated on the axes.

The Brillouin diversity index, species richness of parasites, and evenness were higher in 2021, while Berger-Parker dominance showed no differences between years (Fig. 3). The parasite species accumulation curve showed that the species richness was different between years. It also showed that the parasite richness was not affected by the sampling effort between years (Fig. 4).

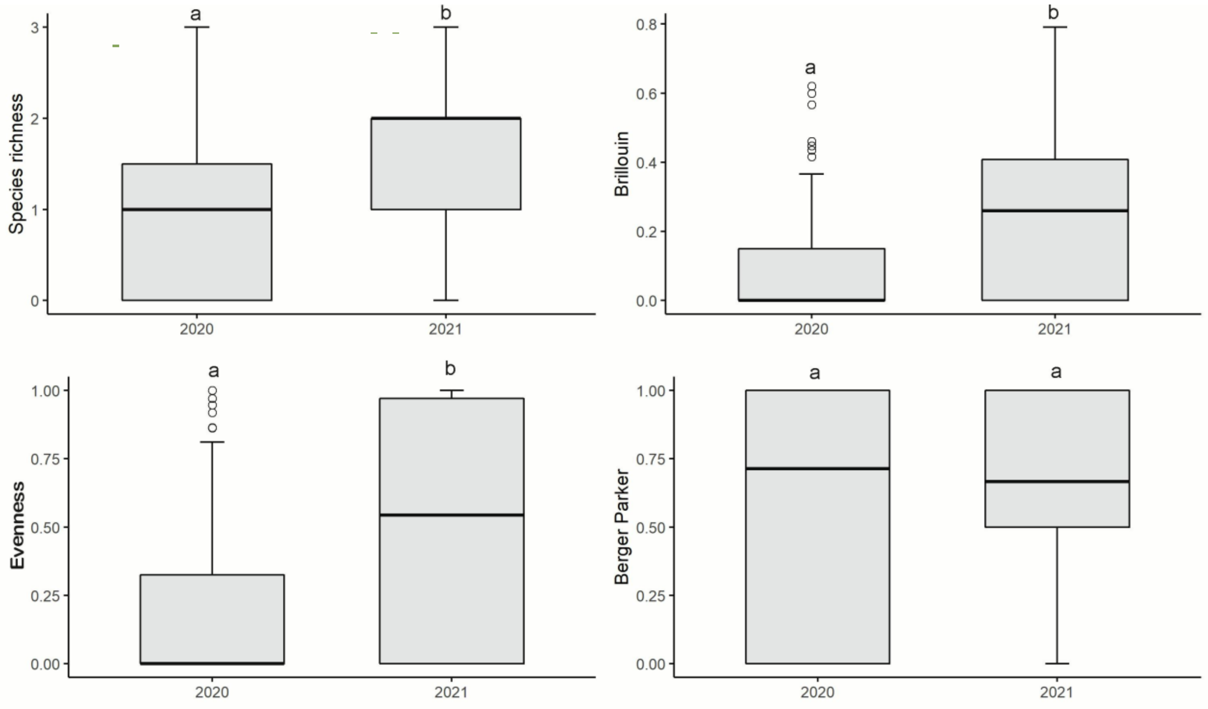

Fig. 3. Diversity parameters of metazoan parasites in *Pimelodus ornatus* from Amazon River, in the state of Amapá (Brazil), collected in 2020 and 2021 (Box plots represent medians, interquartile ranges, minimum–maximum ranges, and outliers). Different letters indicate differences between the medians according to Dunn’'s test ($p < 0.001$)

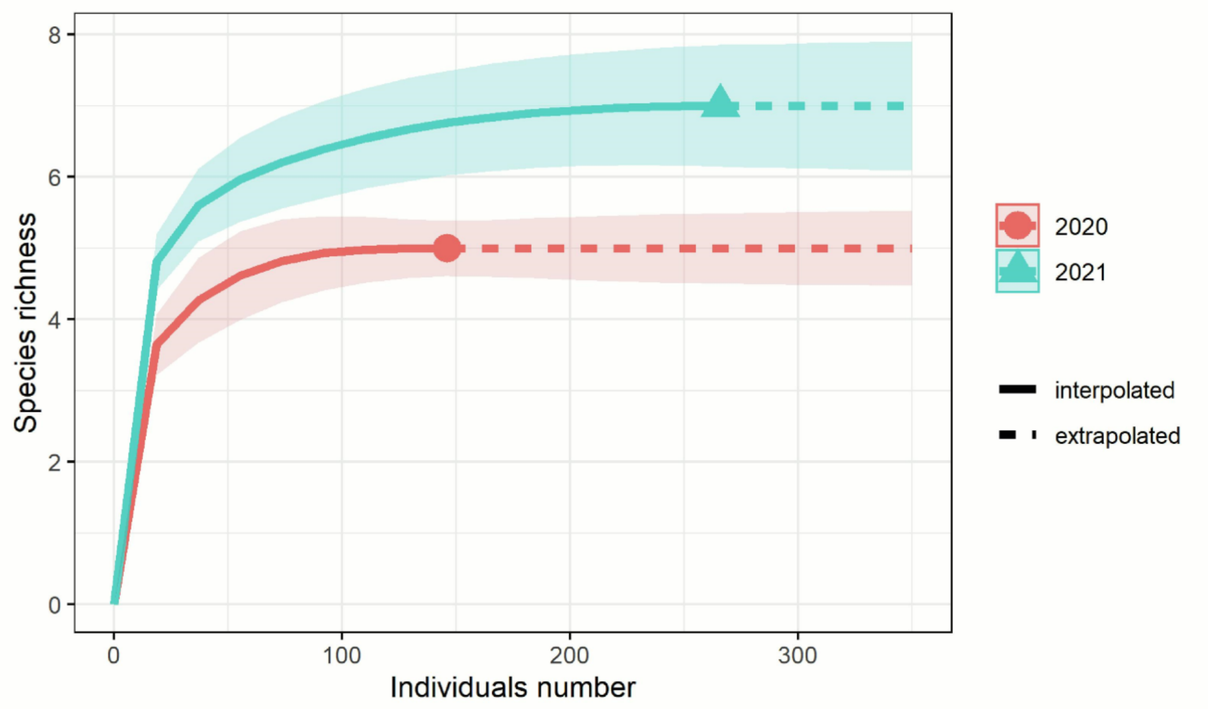

Fig. 4. Species accumulation curve of metazoan parasites in *Pimelodus ornatus* from Amazon River, in the state of Amapá (Brazil), collected in 2020 and 2021

Component community and infracommunity of parasites in the rainy and dry seasons

The fish collected in the rainy season measured 15.0 ± 2.6 cm, while those collected in the dryperiod measured 16.9 ± 1.8 cm, showing significant differences (*t* = 4.3, *p* = 0.0001) between the seasonal periods. The fish collected in the rainy season weighed 16.8 ± 8.5 g, and those collected in the dry season weighed 16.9 ± 1.8 g, showing significant differences (*t* = 4.6, *p* = 0.0001) between the seasonal periods.

Of the hosts examined in the rainy season, 74.1% were parasitized by *Demidospermus* sp., *C.pinnai*, *S. inopinatus*, *Contracaecum* sp., Proteocephalidae gen. sp., *G. genarchella*, and *T. henselli*. Of the fish examined in the dry period, 84.7% were parasitized by *Demidospermus* sp., *C. pinnai*, *S. inopinatus*, *Contracaecum* sp., and Proteocephalidae gen. sp. *Genarchella genarchella* and *T. henselli* were found only in the rainy season. In the rainy and dry seasons, *S. inopinatus* was the parasite with the highest prevalence and abundance. The prevalence of *Demidospermus* sp. and *C. pinnai* was higher in the dry season, while the other species did not differ between the seasonal periods (Table 3).

Axes 1 and 2 of PCoA were responsible for 63% of the total variation in the composition ofparasite abundance in the rainy and dry seasons (Fig. 5). This figure shows overlap and sharing of species, showing that there were no significant differences (PERMANOVA: $F = 0.009$, $p = 0.34$) in the parasite infracommunities between the seasonal periods. However, there was a significant variation in the abundance of *S. inopinatus* ($R^2 = 0.356$, $p = 0.001$), *Contracaecum* sp. ($R^2 = 0.405$, $p = 0.001$), and Proteocephalidae gen. sp. ($R^2 = 0.339$, $p = 0.001$).

Table 3. Metazoan parasites in *Pimelodus ornatus* from Amazon River, in the state of Amapá (Brazil), collected during rainy and dry seasons

		Rainy season (n = 85)			**Dry season (n = 59)**			χ^2	***U***
Parasite species	**Sites of infection**	**P (%)**	**MA ± SD**	**TNP**	**P (%)**	**MA ± SD**	**TNP**		
Demidospermus sp.	Gills	1.2	0.01 ± 0.1	1	11.9	0.4 ± 1.3	21	4.3*	2237.0
Cucullanus pinnai	Intestine, abdominal cavity	3.5	0.1 ± 0.7	10	16.9	0.3 ± 0.9	16	6.0*	3322.5
Spirocamallanus inopinatus	Stomach abdominal cavity, intestine	50.6	1.2 ± 1.5	98	52.5	1.3 ± 1.9	77	0.05	3237.5
Contracaecum sp. (larvae)	Abdominal cavity, stomach, liver, intestine	35.3	0.8 ± 1.4	65	49.2	1.3 ± 2.2	76	2.2	3510.0
Genarchella genarchella	Pharynx	4.7	0.05 ± 0.2	4	0	0	0	-	-
Proteocephalidae gen sp. (plerocercoids)	Stomach, intestine	16.5	0.4 ± 1.1	32	18	0.2 ± 0.4	10	0.03	3511.5
Telotha henselli	Gills	2.4	0.02 ± 0.2	2	0	0	0	-	-

P,: prevalence;, *MA*,: mean abundance;, *SD*,: standard deviation;, *TNP*,: total number of parasites;, χ^2, chi-square test;, *U*, : Mann–Whitney test., $*p < 0.05$, , $**p < 0.01$.

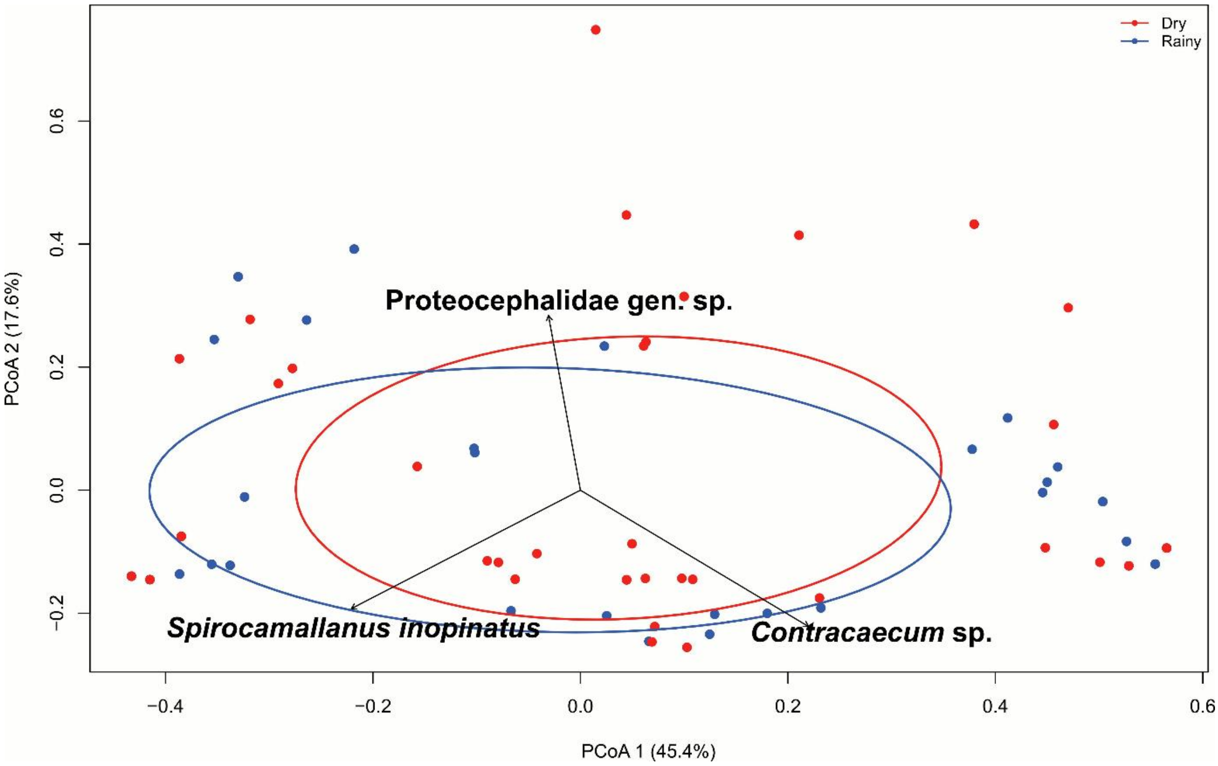

Fig. 5. Principal coordinate analysis (PCoA) using a Bray-Curtis distance matrix for communities of metazoan parasites of *Pimelodus ornatus* from the Amazon River, in the state of Amapá (Brazil), during rainy and dry seasons. The percentage of variation explained by the plotted principal coordinates is indicated on the axes.

The Brillouin diversity index and parasite species richness were higher in the dry season, while evenness and Berger-Parker dominance did not show significant differences between the seasons (Fig. 6). The species accumulation curve showed that the species richness of parasites diverged between the rainy and dry seasons, and that the richness of parasites was not affected by the sampling effort. The accumulation curve of parasite species in the rainy and dry seasons reached an asymptote (Fig. 7)

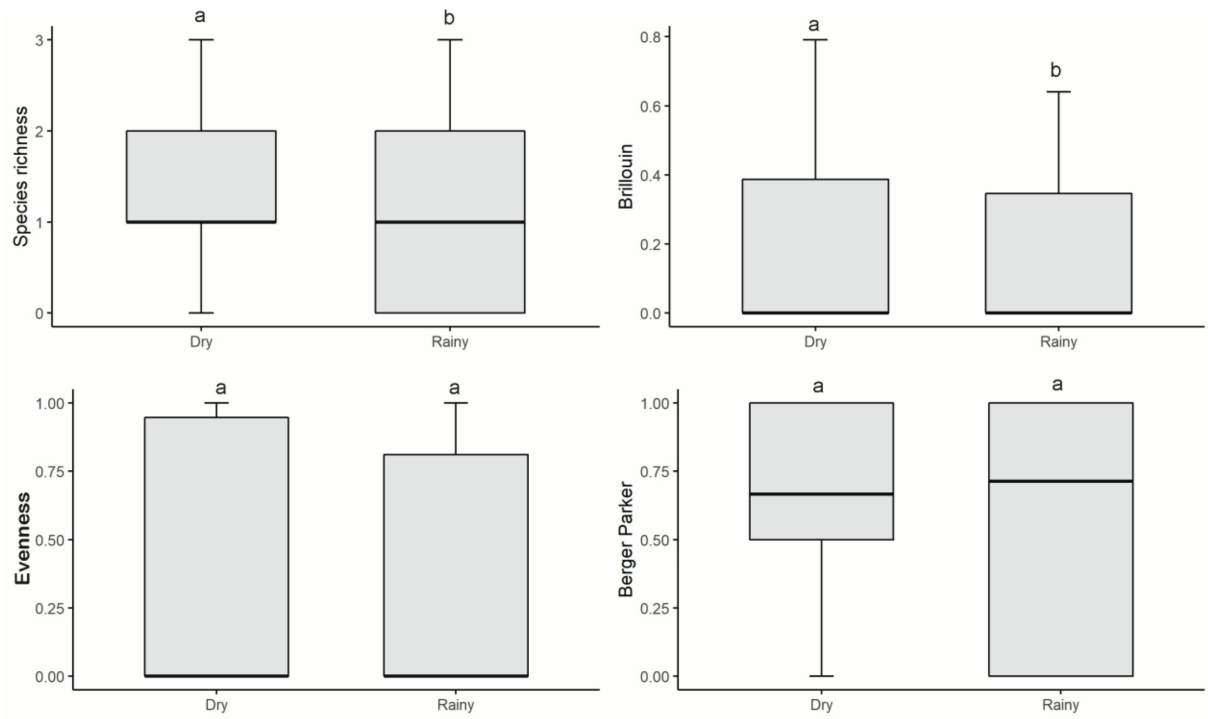

Fig. 6 Diversity parameters of metazoan parasites in *Pimelodus ornatus* from Amazon River, in the state of Amapá (Brazil), during rainy and dry seasons (Box plots represent medians, interquartile ranges, minimum–maximum ranges, and outliers). Different letters indicate differences between the medians according to Dunn’'s test ($p < 0.001$)

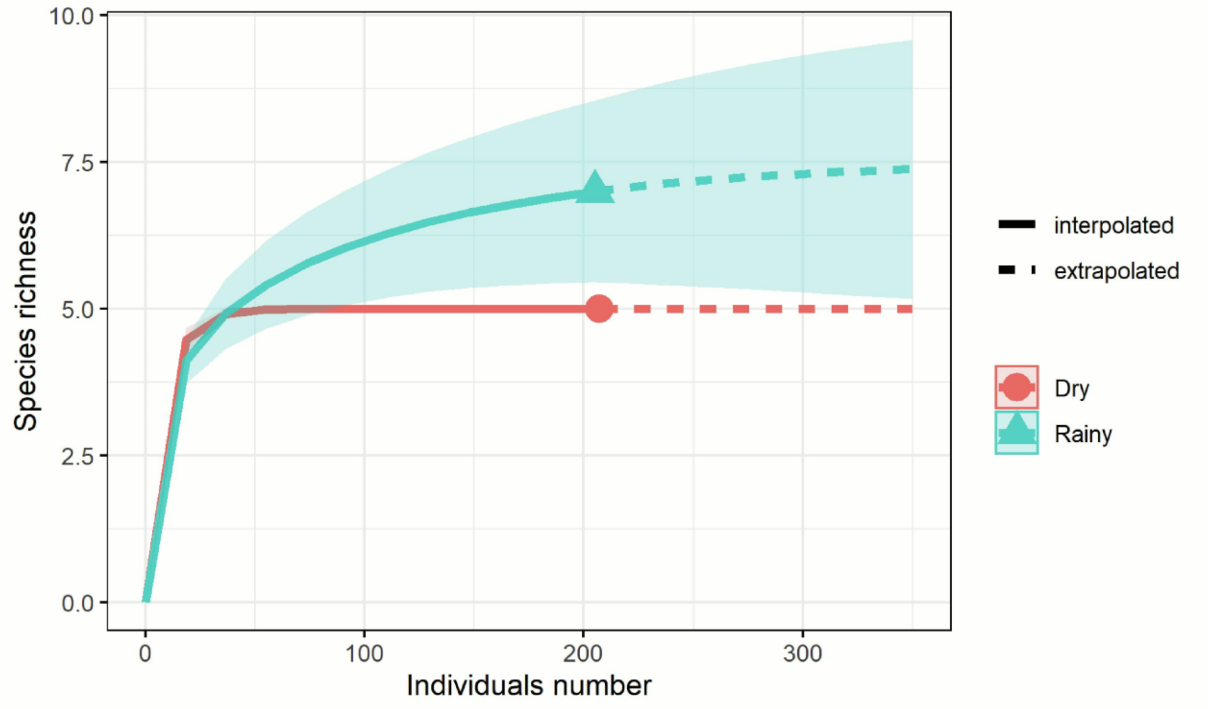

Fig. 7 Species accumulation curve of metazoan parasites in *Pimelodus ornatus* from Amazon River, in the state of Amapá (Brazil), during rainy and dry seasons

Discussion

Temporal variation of metazoan parasites

Surprisingly, no relationship between *P. ornatus* size and abundance of parasites between the years was observed in the present study; however, we also examined within the pooled parasite species. This indicated that, despite host size being a significant determining factor in the diversity, species richness, and abundance of parasites (Poulin and Leung 2011; Marcogliese et al. 2016; Baia et al. al. 2018; Lehun et al. 2022), it did not influence the temporal differences observed here, because other factors are related. Furthermore, many parasite communities experience temporal variations due to seasonal changes in biotic and abiotic environmental factors such as parasite life cycle, environmental dynamics, host-specific immune responses, and reproduction period (Cavalcante et al., 2020; Lehun et al. 2022).

As the Amazon River basin is one complex ecosystem, the temporal variations in the fish communities that inhabit this region remain poorly understood. The community and infracommunities of metazoan parasites in *P. ornatus* from the Amazon River did not show a similar pattern between the years studied due to seasonal variations. In *Pimelodus blochi* (Valenciennes, 1840) from the Iaco River, in western Amazon (Brazil), the long-term temporal variations in the community and infracommunity have been attributed to factors linked to the body size of the host and variations in the availability of infective stages in the environment. In *Hemibrycon surinamensis* (Gery, 1962) (Hoshino and Tavares- Dias 2019) and *Metynnis lippincottianus* (Hoshino and Tavares-Dias 2020) both host fishes from the eastern Amazon region (Brazil), short-period, temporal variation was associated with seasonal cycle (rainy/dry); availability of infective stages; changes in the recruitment process of parasite species; urban eutrophication in the environment; and host body size.

The results showed that there was an alternation in the dominance of parasite species in *P. ornatus*, with the dominance of *S. inopinatus* in 2020 and larvae of *Contracaecum* sp. in 2021. These nematodes in adult and larval stages, respectively, are related to: possible changes in the diet of *P. ornatus* or volume of food consumed or changes in availability and efficiency of infection by intermediate hosts in the environment (Sabas and Brasil-Sato 2014; Blasco-Costa et al. 2015; Tavares-Dias et al. 2017), since this host fish has an omnivorous feeding habit (Lima et al. 2021; Froese and Pauly 2022), and acts as an intermediate (*Contracaecum* sp.) and definitive host (*S. inopinatus*). In *P. blochii*, from the Iaco River in the western Amazon (Brazil), a long-term study has shown a dominance of larvae and adults of 11 species of nematodes (Negreiros et al. 2019a). However, in *H. surinamensis*, from the eastern Amazon, the dominance of metazoan parasites was of the monogenean species during the two years of study (Hoshino and Tavares-Dias 2019b). In *M. lippincottianus*, from eastern Amazon (Brazil), the dominance of metazoan parasites was of monogenean species in a year and digenean *Dadayus pacupeva* (Lacerda, Takemoto & Pavanelli, 2003) in the other year (Hoshino and Tavares-Dias 2020). Therefore, these results show a different pattern among these species of Siluriformes and Characiformes.

The PCoA indicated differences between 2020 and 2021, mainly due to differences in the abundance of *S. inopinatus*, *Contracaecum* sp., and Proteoceplalidae gen. sp. These differences may be related to variations in rainfall levels between the years studied, thus influencing the availability of intermediate hosts (e.g., crustaceans) and variations in environmental characteristics, favoring the recruitment of species with a complex life cycle, in addition to the position of *P. ornatus* in the food web, as has also been reported for other wild fish species (Villalba-Vasquez et al. 2018; Hoshino and Tavares-Dias, 2019; Hoshino and Tavares-Dias, 2020; Lehun et al. 2022).

Wild fish populations act as hosts for various taxa of parasites with varied life cycle strategies. Hence, the diversity and richness of parasites depends on the host species and other factors related to the host and environment such as parasite life cycle, feeding habits, and the reproductive period of host fish, (Tavares-Dias et al. 2014; Tavares-Dias et al. 2017; Baia et al. 2018; Cavalcante et al. 2020). For *P. ornatus*, Brillouin diversity, parasite species richness, and evenness were higher in 2021, caused by the presence of *G. genarchella* and *T. henselii*, which occurred only in 2021. Similar studies have also reported annual differences in the diversity and species richness of parasites in *P. blochii*, from the Iaco River (Negreiros et al. 2019a) and in *H. surinamensis*, from the Igarapé Fortaleza basin (Hoshino and Tavares-Dias 2019). These differences have been influenced by the precipitation levels between the years, thus altering the flow patterns of the rivers and the water quality of these Amazonian hydrographic basins.

Seasonal variation of metazoan parasites

The specimens of *P. ornatus* collected in the dry season were larger and heavier than in the rainy season, possibly indicating a greater feeding in this seasonal period. The Amazon River systems present periods of flooding and droughts, dynamics that also influence the communities of invertebrates and fish that serve as food for many omnivorous fish such as *P. ornatus*. As the diversity of zooplankton and other invertebrates is higher during the rainy season, this indicates ~~that~~ Amazonian fish have better feeding conditions and food diversity in this seasonal period (Tavares-Dias et al. 2014; Gonçalves et al. 2016), preparing for reproduction. As in wild fish populations, host size is an important factor influencing parasite communities and infracommunities, and may be associated with their trophic level, diet, and vulnerability (Poulin and Leung 2011). This factor usually accounts, in part, for the variation in infracommunities and diversity of parasites (Neves et al. 2013; Tavares-Dias et al. 2014; Baia et al. 2018).

The Amazonian region has a well-defined seasonality, with periods of intense rainfall (rainy season) and less rainy periods (dry season). Hence, in the Amazon River system, variations in communities and infracommunities of parasites in wild fish populations have been related to changes generated by the rainy/dry cycle, affecting the recruitment of parasite species, increased availability of food for the host fishes, and availability of infective stages in the environment (Neves et al. 2013; Gonçalves et al. 2018; Negreiros et al. 2019a; Hoshino and Tavares-Dias, 2019; Hoshino and Tavares-Dias, 2020), as well as by the season of reproduction of fishes (Cavalcante et al. 2020). Therefore, levels of rainfall can cause effects on aquatic ecosystems such as changes in fish habitat, water quality, and velocity, and consequently, increasing the stress on host fishes and susceptibility to parasite infections, affecting thus the structure of parasite communities and infracommunities and of intermediate hosts (Gonçalves et al. 2016).

The PCoA showed no seasonal difference in the infracommunity of *S. inopinatus*, *Contraceacum* sp., and Proteocephalidae gen. sp. However, parasite species richness and Brillouin diversity were higher in the dry season, while evenness and Berger-Parker dominance had no seasonal influence, because the dominant species did not vary between seasonal periods. In contrast, in *P. blochii* from the rivers Acre and Xapuri, these diversity parameters did not have a seasonal influence (Cavalcante et al. 2020). Environmental factors such as the decrease in dissolved oxygen levels and increase in water temperature during the dry period increased the species richness and parasite diversity in *P. ornatus* and, therefore, the recruitment processes of parasite species found here.

Nematode *S. inopinatus* was dominant in both seasonal periods studied. The prevalence of *Demidospermus* sp. and *C. pinnai* were higher in the dry season, when the water quality levels were lower, favoring the reproduction of monogeneans and nematodes, in contrast to what was detected in the rainy season, when there was a high oxygen level and an average flow of water of 1.0 × 105m^3/s, factors that can reduce the accumulation of pollutants and eutrophication of the studied area (Abreu et al. 2020). Similar results have been reported for *P. blochii* from Acre River, in western Amazon, infected by monogeneans *Demidospermus* spp. and *Ameloblastella* sp. in the dry season (Negreiros et al. 2019a). *Metynnis lippincottianus* from the Igarapé Fortaleza basin, in eastern Amazon, also presented a higher prevalence of *Contracaecum* sp. larvae in the dry season (Hoshino and Tavares-Dias 2020). In contrast, *H. surinamensis* from the Igarapé Fortaleza basin showed no seasonal variation in the levels of infection by *Contracaecum* sp. (Hoshino and Tavares-Dias 2019), therefore indicating a differentiated patternbetween host fish species.

Digenea is part of the parasite community of wild Amazonian fish populations (Neves et al. 2013; Negreiros et al. 2019a,b; Hoshino and Tavares-Dias, 2019; Hoshino and Tavares-Dias, 2020). The transmission of these endoparasites occurs when metacercariae are ingested by host fish such as *P. ornatus*, which has an omnivorous diet, which facilitates the infection by digeneans. Infection by *G. genarchella* and *T. henselii* occurred only during the rainy season when the increase in the water level of the Amazon Riverfacilitated the chances of contact of these parasites with *P. ornatus*. However, in *H. surinamensis* (Hoshino and Tavares-Dias 2019) and *M. lippincottianus* (Hoshino and Tavares-Dias 2020), both hosts of tributaries of the Amazon River system, infection by *G. genarchella* was not influenced by theseasonality, and *Ergasilus xinguensis* (Taborba, Paschoal & Luque, 2016) also occurred only in the rainy season.

In conclusion, about 43% of parasites were larvae, indicating that *P. ornatus* is the host intermediate. These results, based on a two-year sampling timescale, indicated that behaviors differedbetween years and between seasons, regarding diversity and some infracommunities of parasites. The effects of temporal and seasonal variations in the diversity and levels of infection were related to variations in rainfall levels and, consequently, the availability of infective stages of parasites with a direct and indirect life cycle and changes in the recruitment of parasite species in the environment caused by seasonality. Furthermore, this first study on the temporal and seasonal variations recorded the firstoccurrence of *G. genachella* and *T. henselii* for *P. ornatus*. Lastly, the studied region from the Amazon River has average water flow which, to a certain degree, prevents urban eutrophication; however, it has environmental factors that influence the infection rates of some metazoan parasites communities andinfracommunities, as well as diversity and species richness over the years and seasonal periods, thus, corroborating the hypotheses suggested.

Author contributions

All authors contributed to the conception and design of the study. Material preparation, data collection, and analysis were performed by Elvis Silva Lima. The first version of the manuscript was written by Elvis Silva Lima, and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Availability of data and material

Not applicable for that section.

Code availability

Not applicable for that section.

Declarations

Ethics approval

Ethics approval to obtain access to genetic heritage was authorized by the Brazilian Ministry of the Environment (SISBio n° 73550-1 and SisGen no AA4B6BA). This study was developed in accordance with the principles adopted by the Brazilian College of Animal Experimentation (COBEA) and was conducted under authorization from the Ethics Committee for Animal Use of Embrapa (protocol no 014/2018).

Consent to participate

Not applicable for that section.

Consent for publication

The authors declare that they are aware of the publication of this study.

Conflict of interest

The authors declare no competing interests.

References

Arantes CC, Winemiller KO, Petrere M, Freitas CE (2019) Spatial variation in aquatic food webs in the Amazon River floodplain. Freshw Sci 38:213–228. https://doi.org/10.1086/701841

Baia RRJ, Florentino AC, Silva LMA, Tavares-Dias M (2018) Patterns of the parasite communities in afish assemblage of a river in the Brazilian Amazon region. *Acta* Parasitol 63:304–316. https://doi.org/10.1515/ap-2018-0035

Blasco-Costa I, Rouco C, Poulin R (2015) Biogeography of parasitism in freshwater fish: spatial patterns in hot spots of infection. Ecography 38:301–310. https://doi.org/10.1111/ecog.01020

Bush AO, Lafferty KD, Lotz JM, Shostak AW (1997) Parasitology meets ecology on its own terms:Margolis et al. revisited. J Parasitol 575–583. https://doi.org/3284227

Cavalcante PH O, Silva MT, Pereira ANS, et al (2020) Helminth diversity in *Pimelodus blochii* Valenciennes, 1840 (Osteichthyes: Pimelodidae) in two Amazon Rivers. Parasitol Res 119:4005– 4015. https://doi.org/10.1007/s00436-020-06906-x

Abreu CHM, Barros M de LC, Brito DC, et al (2020) Hydrodynamic modeling and simulation of water residence time in the estuary of the Lower Amazon River. Water 12:660.https://doi.org/10.3390/w12030660

Eiras JC, Takemoto RM, Pavanelli GC (2006) Métodos de estudo e técnicas laboratoriais em parasitologia de peixes, 2 nd edn. Editora da Universidade Estadual de Maringá, Maringá

Froese R, Pauly D (2022) FishBase. World wide web electronic publication. www.fishbase.org, version (02/2022)

Gonçalves BB, Oliveira MSB, Borges WF, et al (2018) Diversity of metazoan parasites in *Colossoma macropomum* (Serrasalmidae) from the lower Jari River, a tributary of the Amazonas River in Brazil. Acta Amaz. 48:211–216. https://doi.org/10.1590/1809-4392201704371

Gonçalves RA, Oliveira MSB, Neves LR, Tavares-Dias M (2016) Seasonal pattern in parasite infracommunities of *Hoplerythrinus unitaeniatus* and *Hoplias malabaricus* (Actinopterygii: Erythrinidae) from the Brazilian Amazon. *Acta* Parasitol 61:119–129. https://doi.org/10.1515/ap-2016-0016

Gorini C, Haq BU, Reis AT, et al (2014) Late Neogene sequence stratigraphic evolution of the Foz do Amazonas basin, Brazil. Terra Nov 26:179–185. https://doi.org/10.1111/ter.12083

Hammer O, Harper DAT, Ryan PD (2001) PAST: paleontological statistics software package for education and data analysis. Palaeont Elect 4:9

Hoorn C, Bogotá-A GR, Romero-Baez M, et al (2017) The Amazon at sea: onset and stages of the Amazon River from a marine record, with special reference to Neogene plant turnover in the drainage basin. Global and Planetary Change 153:51–65. https://doi.org/10.1016/j.gloplacha.2017.02.005

Hoshino EM, Tavares-Dias M (2020) Temporal and seasonal variations in parasites of *Metynnis lippincottianus* (Characiformes: Characidae), a host from the eastern Amazon (Brazil). J. Nat. Hist 53:2723–2736. https://doi.org/10.1080/00222933.2020.1733121

Hoshino EM, Tavares-Dias M (2019) Interannual and seasonal variation in protozoan and metazoan parasite communities of *Hemibrycon surinamensis*, a Characid fish inhabiting the Brazilian Amazon. Acta Parasitol 64:479–488. https://doi.org/10.2478/s11686-019-00057-5

Lehun AL, Cavalcanti LD, Lizama M de los AP, et al (2022) Temporal effects and changes in theparasitic community of *Prochilodus lineatus* (Valenciennes, 1837) (Characiformes: Prochilodontidae) in a floodplain. J Helminthol 96: https://doi.org/10.1017/S0022149X21000742

Lima ES, Oliveira MSB, Tavares-Dias M (2021) Diversity and community ecology of metazoan parasitesin *Pimelodus ornatus* (Siluriformes: Pimelodidae) from the Amazonas River in Brazil. Rev Bras Parasitol Vet 30(3): e006021. https://doi.org/10.1590/S1984-29612021065

Marcogliese DJ, Locke SA, Gélinas M, Gendron AD (2016) Variation in parasite communities in spottail shiners (*Notropis hudsonius*) linked with precipitation. Int J Parasitol 102:27–36. https://doi.org/10.1645/12-31

Negreiros LP, Florentino AC, Pereira FB, Tavares-Dias M (2019a) Long-term temporal variation in theparasite community structure of metazoans of *Pimelodus blochii* (Pimelodidae), a catfish from the Brazilian Amazon. Parasitol Res 118:3337–3347. https://doi.org/10.1007/s00436-019-06480-x

Negreiros LP, Pereira FB, Tavares-Dias M (2019b) Metazoan parasites of *Calophysus macropterus* (Siluriformes: Pimelodidae) in the Acre and Iaco rivers in the western Amazon region of Brazil:diversity, similarity and seasonal variation. J. Nat. Hist 53:1465–1479. https://doi.org/ 10.1080/00222933.2019.1657195

Neves LR, Pereira FB, Tavares-Dias M, Luque JL (2013) Seasonal influence on the parasite fauna of a wild population of *Astronotus ocellatus* (Perciformes: Cichlidae) from the Brazilian Amazon. Int J Parasitol 99:718–721. https://doi.org/10.1645/12-84.1

Nomura H. (1984) Dicionário dos peixes do Brasil, Technical Books Livraria: Rio de Janeiro

Oksanen J, Blanchet FG, Kindt R, et al (2020) Vegan: community ecology package. R package.https://CRAN.R-project.org/package=vegan

Poulin R, Leung T (2011) Body size, trophic level, and the use of fish as transmission routes by parasites. Oecologia 166:731–738. https://doi.org/10.1007/s00442-011-1906-3

R CoreTeam (2021) R: a language and environment for statistical computing. Vienna, Austria. Availableat https://www.R-project.org/

Sabas CSS, Brasil-Sato MC (2014) Helminth fauna parasitizing *Pimelodus pohli* (Actinopterygii: Pimelodidae) from the upper São Francisco River, Brazil. Rev Bras Parasitol Vet 23:375–382. https://doi.org/10.1590/S1984-29612014067

Salo M, Sirén A, Kalliola R (2013) Diagnosing wild species harvest: resource use and conservation. Academic Press.

Sousa R, Souza LA, Frutuoso ME, Freitas CEC (2017) Seasonal dynamic of amazonian small-scale fisheries is dictated by the hydrologic pulse. Bol Inst *Pesca* 3:207–221. doi: https:// doi.org/10.20950/1678-2305.2017v43n2p207

Souza EB, Cunha AC (2010) Climatologia de precipitação no Amapá e mecanismos climáticos de grande escala. In: Ecodesign T (ed) Tempo, Clima e Recursos Hídricos. pp 177–195.

Tavares-Dias M, Oliveira MSB, Gonçalves RA, Neves LR (2017) Parasitic diversity of a wild *Satanoperca jurupari* population, an ornamental cichlid in the Brazilian Amazon. Acta Amaz47:155–162. https://doi.org/10.1590/1809-4392201602514

Tavares-Dias M, Oliveira MSB, Gonçalves RA, Silva LMA (2014) Ecology and seasonal variation of parasites in wild *Aequidens tetramerus*, a Cichlidae from the Amazon. Acta Parasitol 59:158–164. https://doi.org/10.2478/s11686-014-0225-3

Torrente LJ de QG, Ohara VWM, Jansen TH da SP, da Costa Doria ZCR (2013) Peixes do Rio madeira. São Paulo INPA

van Soelen EE, Kim J-H, Santos RV, et al (2017) A 30 Ma history of the Amazon River inferred from terrigenous sediments and organic matter on the Ceará Rise. Earth and Planetary Science Letters 474:40–48. https://doi.org/10.1016/j.epsl.2017.06.025

Vazzoler A (1996) Biologia da reprodução de peixes teleósteos: teoria e prática. Maringá: Eduem 169: VillalbaVasquez PJ, Violante-González J, Monks S, et al (2018)

Temporal and spatial variations in the metazoan parasite communities of the Panama spadefish, *Parapsettus panamensis* (Pisces: Ephippidae), from the Pacific coast of Mexico. Invertebr Biol 137:339–354. https://doi.org/10.1111/ivb.12232

Zar J (2010) Biostatistical analysis 5th ed Upper Saddle River, NJ: Prentice-Hall 557–558

7. CONCLUSÕES FINAIS

Os resultados da diversidade, sazonalidade de parasitos metazoários de *E. pisonis*, *C. asellus* e *P. ornatus* coletados no Rio Amazonas, nos permitiu concluir que:

-Cerca de 59,1% dos parasitos encontrados em *E. pisonis* eram larvas, assim este peixe é um hospedeiro intermediário ou paratênico;

- Em *E. pisonis, G. genarchella* e *Ergasilus* sp. diferiram entre estações, enquanto *G. genarchella* diferiu entre os anos estudados;

-*Contracaecum* sp. e *Pseudoproleptus* sp., e indivíduos adultos de *S. inopinatus*, *G. genarchella*, *Neoechinorhynchus* sp., ácaros e *Ergasilus* sp. foram registrados pela primeira vez em *E. pisonis*;

-A diversidade parasitária de *C. asellus* foi caracterizada por baixa prevalência e baixa abundância, com dominância de *E. colomesus;*

-Presença de larvas de *Contracaecum* sp. é um indicativo que *C. asellus* pode atuar como hospedeiro intermediário ou paratênico;

-Para *C. asellus*, *E. colomesus* e *Contracaecum* sp. diferiram entre estação sazonal, enquanto a prevalência de *E. colomesus* diferiu entre os anos estudados.

-As variações sazonais na diversidade dos parasitos em *C. asellus* e nos níveis de infecção foram relacionadas às variações nos níveis de chuva e ao tamanho do corpo dos hospedeiros.

- A presença de *Contracaecum* sp., *Genarchella genarchella*, *Clinostomum marginatum*, *Brasacanthus* sp. e *Argulus pestifer* são novos registros para *C. asellus*;

- Para *C. asellus*, uma nova espécie de acantocéfalo do gênero *Brasacanthus* foi descrita, e está é a segunda espécie para o gênero e a primeira encontrada em peixes de água doce;

- Baseado em dados de biologia molecular o gênero *Brasacanthus* deve ser realocado dentro da família Arhythmacanthidae;

- *Calakmulrhynchus amini* formou um clado irmão com a nova espécie de *Brasacanthus* aqui descrita;

-A comunidade parasitária de *P. ornatus* no Rio Amazonas foi composta por espécies de helmintos com baixa prevalência, baixa abundância, baixa diversidade e baixa riqueza de espécies, com predominância de ectoparasitos e com dispersão agregada;

- Em *P. ornatus*, o tamanho dos peixes hospedeiros influenciou na diversidade de parasitos;
- *Spirocamallanus inopinatus*, *Contracaecum* sp. *Genarchella genachella* e *T. henselii* são novos relatos de parasitos para *P. ornatus*;
- Em *P. ornatus*, cerca de 43% dos parasitos encontrados eram larvas, indicando que este é hospedeiro intermediário para algumas infracomunidade parasitárias;
- Em *P. ornatus*, a infecção por *Contracaecum* sp. diferiu entre os anos estudados, enquanto a prevalência de *Demidospermus* sp. e *Cucullanus pinnai* diferiu entre estação sazonal.