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Morphological identification of endoparasites in *Brachyplatystoma vaillantii* intended for consumption, Iquitos-Peru

Identificación morfológica de endoparásitos en *Brachyplatystoma vaillantii* destinado al consumo, Iquitos-Perú

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ABSTRACT

The juvenile stage of *Brachyplatystoma vaillantii* (Valenciennes), commonly called Manitoa in Peru, has commercial value for direct human consumption and as an ornamental aquarium fish. Unidentified and little-studied pathogens limit the productivity of Amazonian fish species and, therefore, this study aimed to identify and describe morphologically the genera of endoparasites present in the visceral cavity, muscle, spleen, liver, and intestine of *B. vaillantii*. Sixty samples were collected from the three main local markets in Iquitos-Peru (20 fish per market), and examined by direct observation. Parasites were collected, fixed and cleared for identification using classical taxonomy methods. *Anisakis* sp., *Contracaecum* sp., *Procamallanus (Spirocamallanus) juana, Sebekia* sp. and *Genarchella* sp. were collected. *Anisakis* sp. larvae had the highest prevalence (P %= 93.33) and mean intensity (Im=55.97) and *Procamallanus* (*Spirocamallanus*) *juana* the lowest prevalence (P %=3.33) and mean intensity (Im=0.1). The parasites identified in this work represent a health risk to the host. It is recommended that more biological and pathological studies of these parasites be carried out since they can affect one of the main economic activities of the Peruvian Amazon.

Keywords: Amazonia, nematode infections, parasites, pentastomida, siluriformes

RESUMEN

Brachyplatystoma vaillantii (Valenciennes), comúnmente denominado en Perú como "Manitoa", es un recurso de valor comercial para el consumo humano directo y para la actividad ornamental en etapa juvenil. Los patógenos no identificados y poco estudiados limitan la productividad de las especies amazónicas; por lo tanto, este estudio tuvo como objetivo identificar y describir morfológicamente los géneros de endoparásitos presentes en la cavidad visceral, músculos, bazo, hígado e intestinos de *B. vaillantii*. Se adquirieron 60 muestras de los tres principales mercados locales de la ciudad de Iquitos-Perú (20 peces por mercado). Los peces fueron examinados mediante observación directa y los parásitos fueron recolectados, fijados y aclarados para su identificación mediante claves taxonómicas. *Anisakis* sp., *Contracaecum* sp., *Procamallanus (Spirocamallanus) juana*, *Sebekia* sp. y *Genarchella* sp. fueron colectados. Se calcularon índices parasitológicos, siendo las larvas de *Anisakis* sp. el parásito que tuvo la mayor prevalencia (P %= 93.33) e intensidad media (Im=55.97) y *P.* (*S.*) *juana* la menor prevalencia (P %=3.33) e intensidad media (Im=0.1). Los parásitos identificados en este trabajo representan un riesgo para la salud del hospedero. Se recomienda realizar más estudios biológicos y patológicos de estos parásitos ya que pueden afectar una de las principales actividades económicas en la Amazonía peruana.

Palabras clave: Amazonía, infecciones por nemátodos, parásitos, pentastomida, siluriformes

INTRODUCTION

The Amazon represents one of the areas with the greatest biodiversity on the planet, its geographical location and climate make it ideal for pisciculture (fish farming) raising fish for consumption and ornamental aquarium purposes (Álvarez, 2007). Expectations are high for the growth and development of the aquaculture sector to contribute to the economy at both the national and rural community levels (Vásquez Oliveira, 2020) and in this way contribute to food security as well as the preservation of species in their natural environments (Ramírez- Gastón *et al.,* 2018, Berger, 2020).

Commercial fishing catches in Iquitos, Peru, include mostly members of the Orders Characiformes and Siluriformes, fish that are characteristically scaly and leathery respectively. The Siluriformes, including *Brachyplatystoma vaillantii* (Pimelodidae) (Valenciennes 1840), commonly called Manitoa in Peru, taken for ornamental and consumption purposes, comprise 39 % of Neotropical fish species (Villamil-Rodríguez *et al.,* 2018).

As the aquaculture sector in Peru grows, both in demand and production, risks and health challenges will become increasingly greater. Infectious diseases of bacterial, viral, fungal and parasitic origin, in particular, have presented these greater risks in recent years (Murrieta- Morey et al. 2020). Government agencies have been acting to meet the goals set in the National Aquaculture Development Plan 2010-2021, which seeks to promote research and development of health control services for aquaculture production and marketing Plan nacional de desarrollo acuícola (PNDA, 2010).

All fish species are vulnerable to pathogenic agents, whether in their natural environment or controlled conditions, with disease leading to high mortality and morbidity rates and consequent economic losses (Murrieta-Morey, 2019). Host-parasite associations represent adaptations that have allowed parasites to develop biological qualities that ensure transmission from one host to the next (Buchmann, 2022). The requirements of each life cycle stage of the parasite may affect the host's morphology, physiology, reproduction, and/or behavior (Timi and Poulin, 2020). The effects of such infections can become much more severe in cases of fish raised under controlled conditions (Buchmann, 2022).

This baseline study of the parasitic fauna of *B. vaillantii* in the Peruvian Amazon was an opportunity to provide data on parasite-host interactions and to inform the aquaculture industry and the general public about potential zoonotic risks. Therefore, the present investigation aimed to identify morphologically the endoparasites present in the muscle, visceral cavity, liver, spleen, and intestine of *B. vaillantii* acquired from the fish markets of Iquitos, Peru. These data could then be used to inform the development of any relevant plan to support the aquaculture industry.

MATERIALS AND METHODS

Sample collection

Sixty specimens of *Brachyplatystoma vaillantii* were obtained between October 2020 and February 2021 from three fish markets (20 fish per market); namely "Modelo", "Itinerante", and "Belen" in Iquitos, Peru. Fresh fish with an average total length of 34 cm and averaging 450 g in weight were selected in the study. Parasitological analyses were carried out in the Laboratory of Parasitology and Aquaculture Health of the "Fernando Alcántara Bocanegra" Research Center (CIFAB) of the Research Institute of the Peruvian Amazon (IIAP), Iquitos, Peru.

Parasitological examination

Fish were cut longitudinally from the anus opening to the gill region. Internal organs were removed and separated individually in Petri dishes for observation under a stereoscope Leica

EZ4. Samples that could not be analyzed on the same day were placed in appropriately labeled plastic bottles of 70 % ethanol. Fresh muscle tissue was cut along the length of the fish and examined under a negatoscope and a dissecting microscope for parasites. Nematodes including *Anisakis* type 1 larvae were removed from tissues using tweezers and brushes carefully, washed in distilled water, fixed in 70 % ethanol,

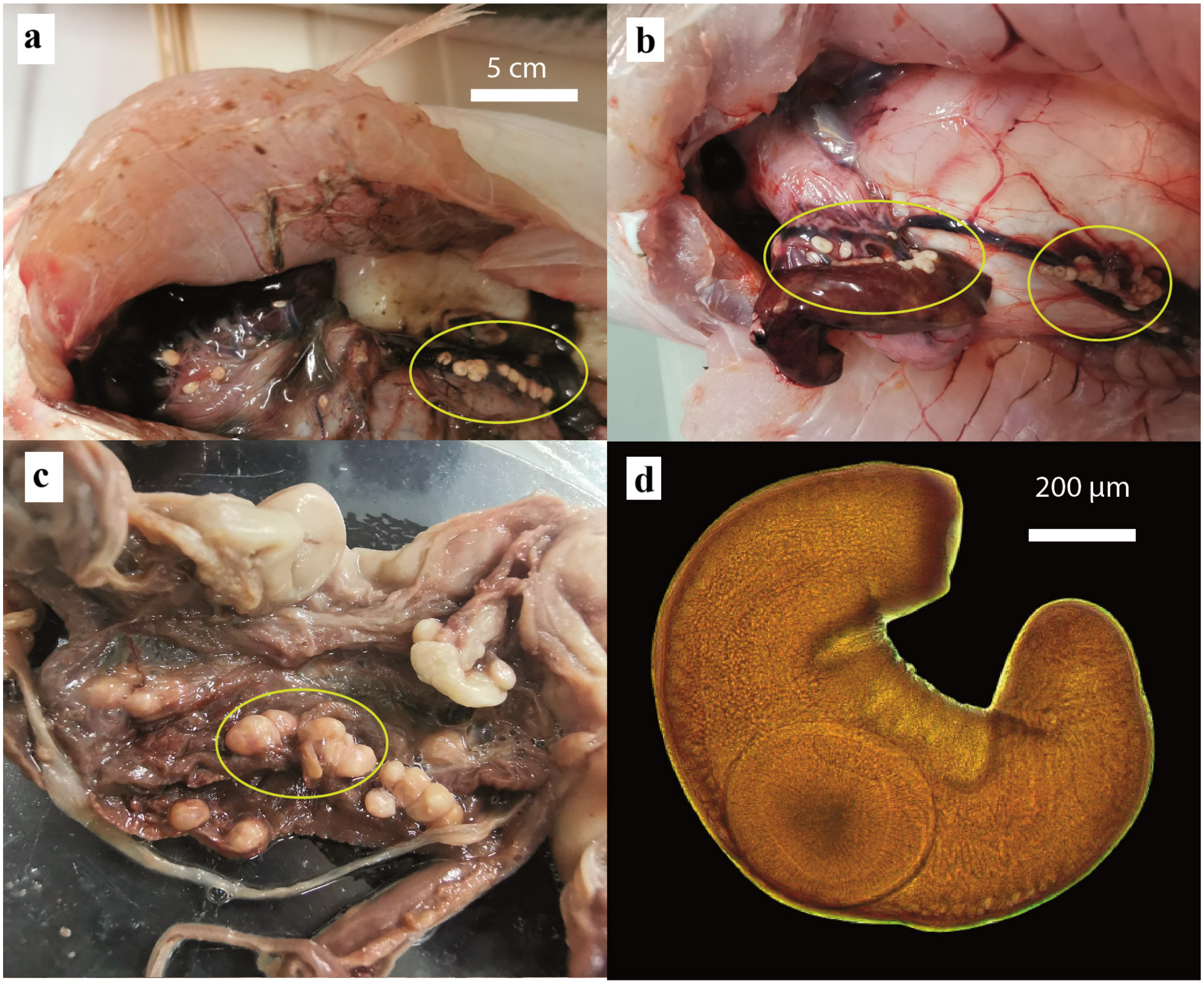

Figure 1. Metacercaria of *Genarchella* sp. in *Brachyplatystoma vaillantii*. a. Parasites in the visceral cavity (yellow circle). b. Parasites in the liver (yellow circle). c. Parasites in the stomach (yellow circle). d. Ventral view of specimen collected from the liver.

and cleared in lactic acid for five minutes before examination using an optical microscope (Leica DM70 microscope) mounted with a camera (Leica ICC50W) for digital image capture. Digenetic trematodes were compressed between two microscope glass slides and fixed in 70 % ethanol for 24 hours, transferred to hot AFA (70 % ethanol, 37 % formalin, and glacial acetic acid) then stained with acid carmine before microscopical examination. Pentastomids, decapsulated using tweezers and brushes, were washed in distilled water and cleared in Hoyer´s medium following Murrieta-Morey (2019). Measurements were taken in Leica ICC50W with a Leica Application Suite V4. 1. 0. program.

Identification of parasites

Selected specimens were photographed using a computerized image analysis system (Leica Application Suite V4.1.0 program and Leica DM70 microscope). All the parasites were identified, using morphological characters, concerning Moravec and Sey (1988), Moravec (1998), Riley et al. (1990), Gibson et al. (2002), and Murata et al. (2011).

Parasitological indices and statistical analysis

Indices of prevalence (P%), abundance (A), mean intensity of infection (MI), and mean abundance (MA) were calculated according to Bush et al. (1997). The community status of the parasites was calculated according to Bush and Holmes (1986). The software BioStat version 5.0 for Windows was used for descriptive analysis and the determination of significance between fish total length and mean abundance, using Spearman's correlation test.

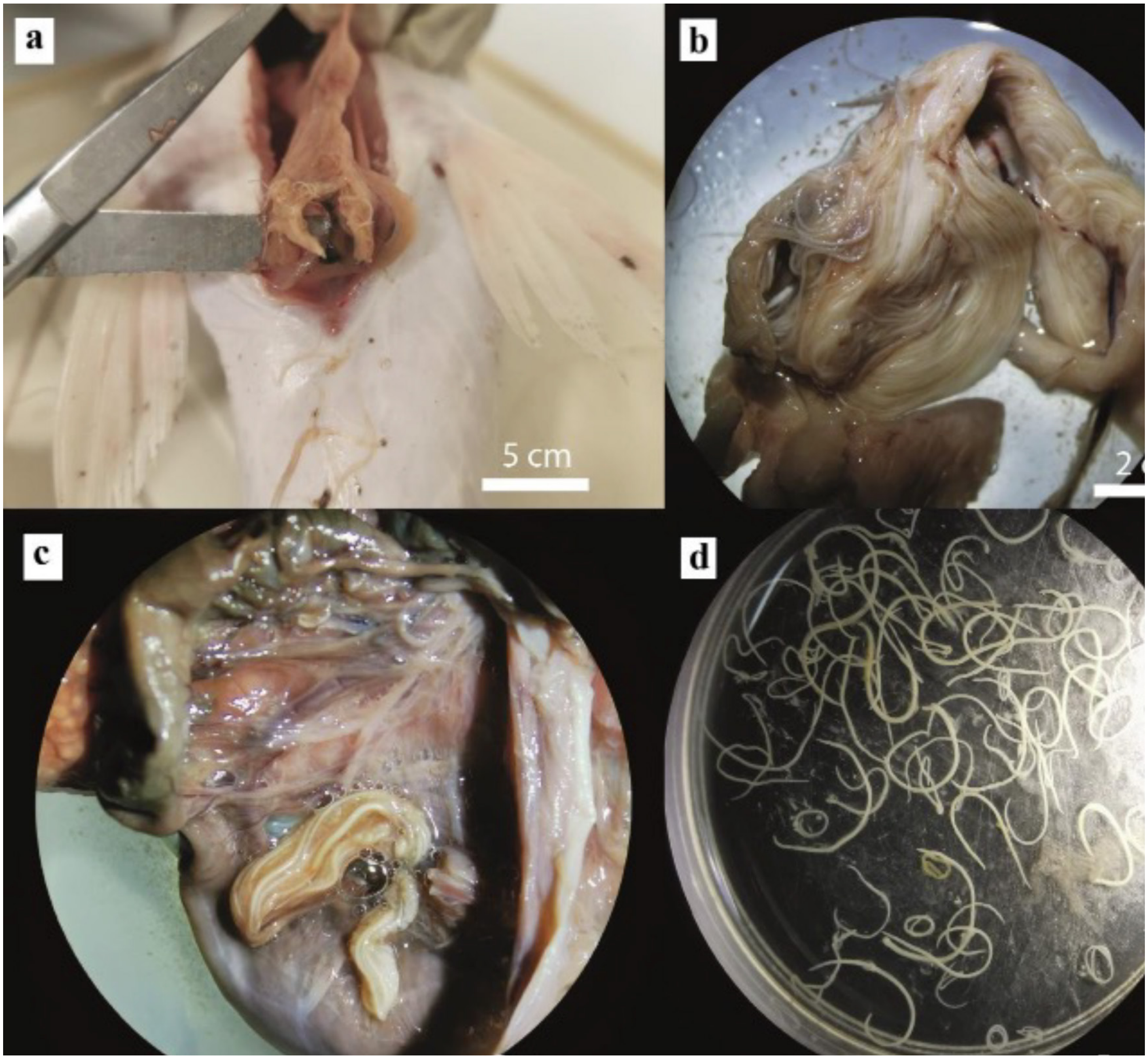

Figure 2. *Anisakis* sp. from *Brachyplatystoma vaillantii*. a, b. c. Parasite found in the intestine. d. Parasites in Petri dish.

RESULTS

Morphological identification

Of the 60 fishes analyzed, 56 (93.3 %) were infected by at least one species of parasite (Table 1). Digenean trematode metacercariae with an oral sucker, elongated body and a large central acetabulum (Fig. 1d) were found in the visceral cavity (Fig. 1a), liver (Fig. 1b), and stomach (Fig. 1c).

The trematode Genarchella sp.

These digeneans were encysted in the visceral cavity and the parenchyma of the liver and spleen of infected fish with a prevalence of 30 %

The Nematoda *Anisakis* type 1 larvae

Larvae L3 were found in the intestine (Fig. 2a, 2b, 2c). These nematodes had a whitish body (Fig. 2d); a larval tooth at the anterior end of the body (Fig. 3a), a mucron at the posterior end (Fig. 3b), and a prominent ventricle before the beginning of the intestine (Fig. 3c).

Contracaecum sp. larvae

Larvae L3 were found in the visceral cavity. These nematodes had an intestinal cecum leading to the anterior region of the body (Fig. 4a), a small larval tooth (Fig. 4b), and a tail without a mucron (Fig. 4c).

Procamallanus (Spirocamallanus) juana (Ramallo and Ailán Choke, 2017) These nematodes had a yellowish-brown buccal capsule with four to six thick rings (Fig. 4d). The muscular esophagus was slightly shorter than the glandular

Figure 3. Photomicrography of *Anisakis* sp. a. Anterior part of the body showing the larval tooth (lt). b. Posterior part of the body showing the mucrom (m). Ventral view of the body showing the ventricle (v).

esophagus. Male specimens had six pairs of papillae at the posterior end, three pre-anal and three post-anal, the right spicule larger than the left, and a hook-shaped terminal joining near the anal pore, tail with a conical end (Fig. 4e).

The Pentastomida *Sebekia* sp.

Immature pentastomids were found in the musculature of the fish (Fig. 5a). They were identified by the elongated body segmented by rings (Fig. 5b), the anterior part of the body having one oral cadre anteriorly and four hooks (Fig. 5c) and a rounded body posteriorly (Fig. 5d).

Parasite prevalence and localization

The highest prevalence was found in the *Anisakis* type 1 larvae (93.3 % (56/60)). These larvae, with a total number of parasites of 3,358, were found only in the intestine. Other parasites were *Genarchella* sp. ((30 %) 18/60) found in visceral cavity (98 parasites), liver (96 parasites) and stomach (70 parasites); *Contracaecum* sp. larvae (10 % (06/60)) found in visceral cavity (12 parasites), *Sebekia* sp. (6.67 % (04/60)) found in musculature (ten parasites) and visceral cavity (four parasites) and *Procamallanus* (*Spirocamallanus*) *juana* (3.33% (02/60)) found only in intestine (six parasites).

Parasitic indexes

Anisakis type 1 larva with the highest indices was a central species in the parasite community (Prevalence: 93.33, Abundance: 33.58, Mean intensity: 59.96, Mean abundance: 55.97), other parasites were *Genarchella* sp. (P%: 30, A: 264, MI: 14.67, MA: 4.4), *Contracaecum* sp. larvae (P%: 10, A: 12, MI: 2, MA: 0.2), *Sebekia* sp. (P%: 6.67, A: 14, MI: 3.5, MA: 0.23) and they were satellite species, while *P. (S.) juana,* with the lowest parasitic indices (P%: 3.33, A: 6, MI: 3, MA: 0.1), was a satellite species.

Parasitic co-infections

Most fish, 58.53 % (33/56), were infected with one parasite species (*Anisakis* type 1 larvae – 33 individuals), 32.14 % (18/56) were infected with two (*Anisakis type 1 larvae* + *Genarchella sp.* (12/18), *Anisakis* type 1 larvae + *Contracaecum* sp. larvae (4/18), *Anisakis* type 1 larvae + *Procamallanus* (*Spirocamallanus*) *juana* (1/18), *Anisakis* type 1 larvae + *Sebekia* sp. (1/18), 7.14 % (4/56)) with three (*Anisakis* type 1 larvae + *Procamallanus* (*Spirocamallanus*) sp + *Genarchella* sp. (1/4), *Anisakis* type 1 larvae + *Sebekia* sp. + *Genarchella* sp. (2/4), *Anisakis* type 1 larvae + *Contracaecum* sp. larvae + *Genarchella* sp. (1/4)), and 1.79 % (1/56) with four (*Anisakis* type 1 larvae + *Contracaecum* sp. larvae + *Procamallanus* (*Spirocamallanus*) *juana* + *Genarchella* sp. (1/1)).

Mean abundance and measures

Spearman's correlation showed no statistical difference in the mean abundance between size (p= -0.08, test value = -0.6) and weight (p= 0.098, test value = 0.75).

DISCUSSION

Genarchella sp. metacercariae were also reported by Brito-Junior and Tavares-Dias (2021) in juveniles of *B. vaillantii* from the Brazilian Amazon, at a lower prevalence (16.1 %) than found in our study from the Peruvian Amazon. In the Brazilian study, the sampling was carried out between November and December when the river flow increased. Fish's feeding usually decreases during this period, resulting in reduced parasite infestation probability. *Hemibrycon surinamensis* collected from channels of the Amazon River were found to have metacercaria of *Genarchella genarchella* in the gills with a prevalence of 66.7 %, and adults in the intestine with a prevalence of 5.4 % (Hoshino *et al.,* 2014), suggesting either the migration of these parasites or a greater affinity of *G. genarchella* for the gills of the fish.

Morphological characters are sufficient to identify the larval stages of the Anisakidae. By using known behavior within the biological cycle of marine anisakids, we were able to determine that both *Contracaecum* sp. larvae and *Anisakis* type 1 larvae had penetrated the intestinal wall, migrated to the visceral cavity and become encapsulated due to the

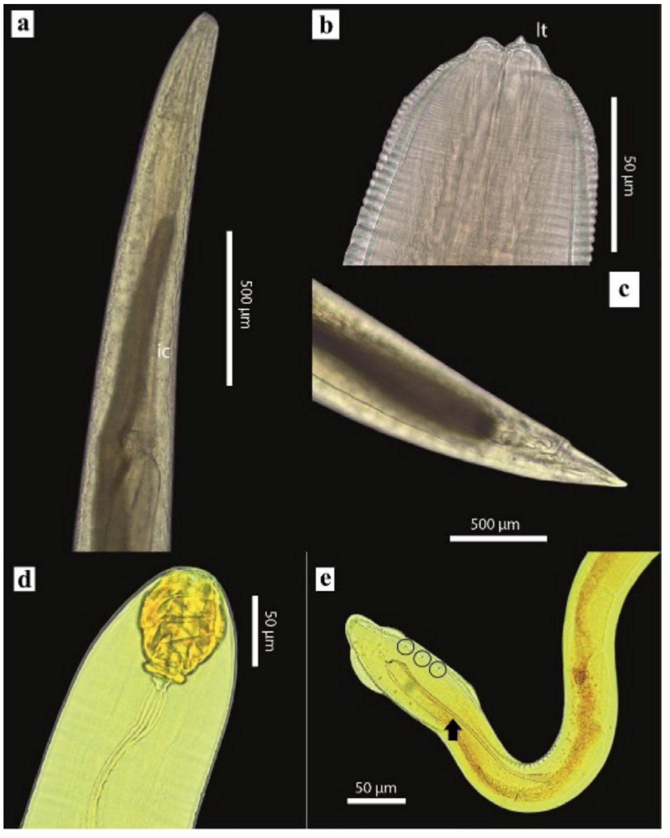

Figure 4. *Contracaecum* sp. from *Brachyplatystoma vaillantii*. a. ventral view of the body showing the intestinal cecum (ic). b. Anterior part of the body showing the larval tooth (lt). c. Posterior part of the body. d. *Procamallanus* (*Spirocamallanus*) *juana* from *Brachyplatystoma vaillantii*. Anterior part of the body showing the buccal capsule. e. Posterior part of the body showing the spicules (arrow) and the genital papillae (black circles).

host's response (Buchmann, 2012; Castellanos-Garzón *et al.,* 2020; Palomba *et al.,* 2020). Although the larvae (L3) of *Anisakis* sp. are of low pathogenicity and virulence (Levsen and Lunestad), their presence in culture conditions could negatively affect fish health. For example, Côrrea et al. (2013) noted changes in the hematological variables and Dezfuli et al. (2021) found lesions as cellular degeneration, cellular swelling, cytoplasmic vacuolization and granulomas in the livers of infected fish. In some instances, *Anisakis* type 1 larvae have also been found encapsulated close to the muscle, using UV press methodology (Cipriani *et al.,* 2021; Sánchez-Alonso *et al.,* 2021). The finding of *Anisakis* sp. larvae in the liver, intestine, and visceral cavity of *B. vallantii* is congruent with reports of larvae found in *Dormitator latifrons* (Richardson) and *Lesbiasina bimaculata* (Steindachner) from the Peruvian Coast (Airahuacho *et al.,* 2019).

Contracaecum sp. larvae (L3) with a prevalence of 10 % were isolated from the visceral cavity of *B. vaillantii*. *Contracaecum* sp. has also been reported from the surface of the abdominal muscles of the following species: *P. nattereri* (prevalence of 23.3 %), in *Pseudoplatystoma tigrinum* (13,3 %), *Pseudoplatystoma punctifer* (13.3 %), *Cichla monoculus* (46.6 %) and *Hypothalamus edentatus* (3.3 %) from Iquitos-Perú (García, 2021; Selis, 2022). Selis (2022) concluded that the prevalence of *Contracaecum* sp. infection is related to the feeding ecology. The infected fish were, however, sampled from the same locality in the same season. *Contracaecum* sp. L3 stages have also been reported from *Astronotus ocellatus* (Agassiz) in the Peruvian and the Brazilian Amazon (Neves *et al.,* 2013).

Contracaecum larvae are known to be zoonotic, a risk to public and aquaculture health (Pardo *et al.,* 2007), although clinical presentations in humans are rare (Shamsi, 2019). *Contracaecum multipapillatum* from mullet fish (*Mugil cephalus* Linnaeus) has been reported from the Colorado River, Baja California, Mexico, and rivers in Guatemala and Costa Rica (Valles-Ríos *et al.,* 2000; Choc *et al.,* 2020) and in piscivorous birds from Colombia (Mattiucci *et al.,* 2010).

The histological comparison of fish from Argentina with and without *C. multipapillatum* infection showed that melanomacrophage centers had developed in the liver, kidney, and spleen of infected fish (Motamedi *et al.,* 2018). Unfortunately, there are no morphometric or molecular data available to compare the *Contracaecum* sp. found in the Amazon in this study with known species of *Contracaecum* from the Americas. However, birds such as *Nannopterum brasilianus* (Gmelin, 1789), *Spheniscus magellanicus* (Forster, 1781), *Phalacrocorax atriceps* (King, 1828) *Pelecanus occidentalis* (Linneaus, 1766) and *Phalacrocorax brasilianus* (Gmelin, 1789) are known to be infected with *Contracaecum jorgei* (see Sardella, Mancini, Salinas, Simões and Luque, 2020), *Contracaecum pelagicum* (see Johnston and Mawson, 1942), *Contracaecum multipapillatum* (see Stossich, 1890), *Contracaecum bioccai* (Mattiucci *et al.,* 2008) or *Contracaecum australe* (see Garbin, Mattiucci, Paoletti, González-Acuña and Nascetti, 2011. One or more of these species of *Contracaecum* could also be present suitable bird hosts in the Peruvian Amazon with *B. vaillantii* acting as an intermediate host (Mattiucci *et al.,* 2008; Garbin *et al.,* 2011; Garbin *et al.,* 2013; Sardella *et al.,* 2020).

Procamallanus (Spirocamallanus) juana was previously reported in *Pimelodus albicans* (Valenciennes, 1840) (prevalence 75 %) and *Pimelodella gracilis* (Valenciennes), (prevalence 75 %) in Argentina (Ramallo and Ailán Choke, 2017). This nematode is here reported for the first time in *B. vaillanti* in Peru.

The pentastomid found in this study was identified as an immature stage of *Sebekia* sp., based on the shape and size of the hooks and the number of spines around the rings that segment the body (Hernández-Gómez, 2018). Although morphological comparisons allowed identification of genus, molecular studies are required to define the species. Pentastomids feed on mucus, fluids, and blood thereby decreasing the productivity rate of the hosts (Bunkley-Williams and Williams, 1995).

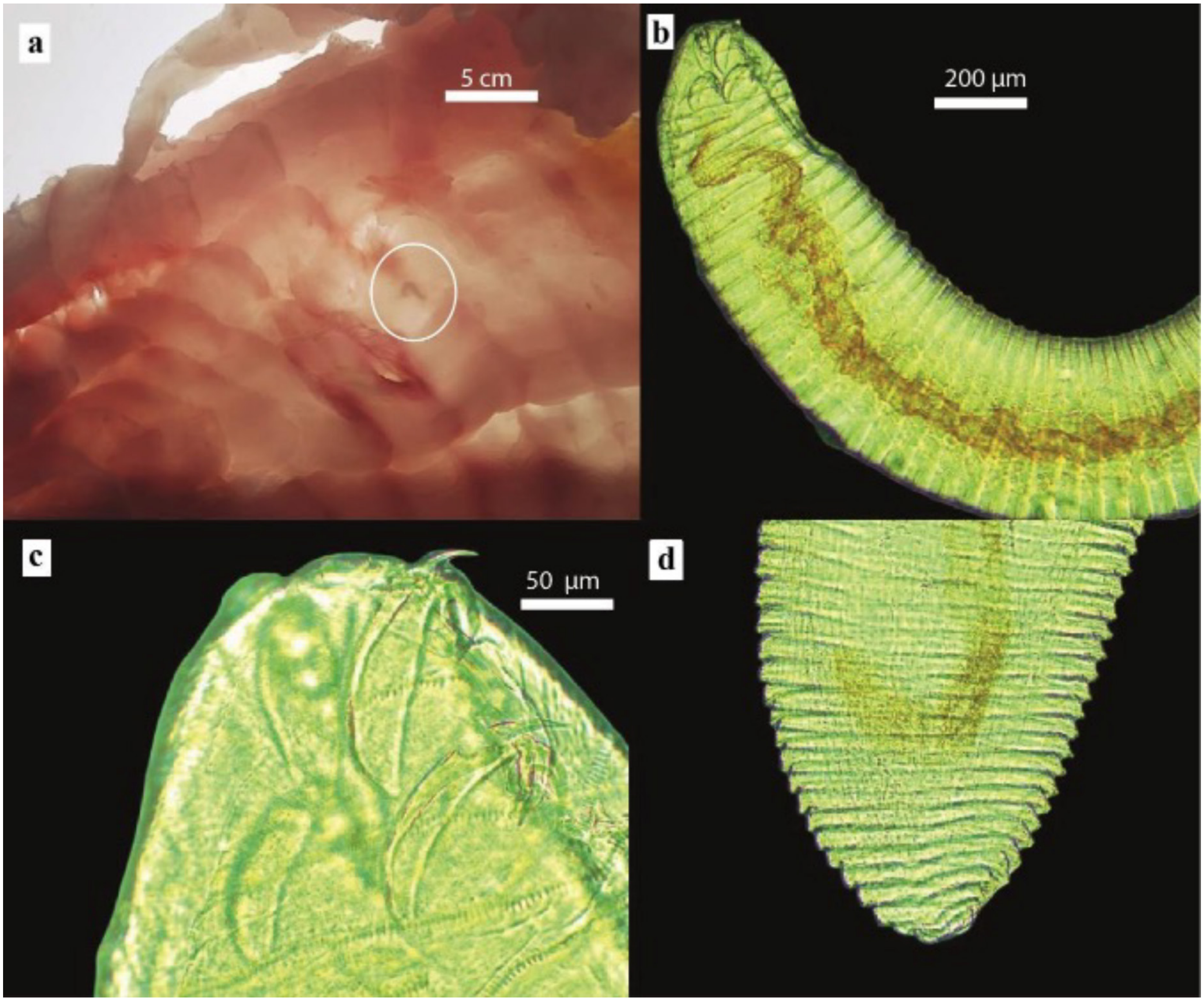

Figure 5. *Sebekia* sp. from *Brachyplatystoma vaillantii*. a. Parasite encysted in the musculature (white circle). b. Photomicrography of the body in ventral view. c. Anterior part of the body showing the hooks. d. Posterior part of the body.

Monoparasitic associations (55%) usually with *Anisakis* type 1 larvae were the most frequent, followed by biparasitic (30 %), tri-parasitic (6.67 %), and tetraparasitic associations (1.67 %). *Anisakis* type 1 larva were present in all coinfections, however, with the most frequent being biparasitism between *Anisakis* type 1 larva and *Genarchella* sp. (20 %) with 18 positive samples. *Genarchella genarchella* and *Pseudoterranova* sp., reported in *B. vaillantii* by Brito-Junior and Tavares-Dias (2021) were not found in our study. These authors did not indicate which of the parasites they listed were in association.

Brachyplatystoma vaillantii may be an intermediate host for a range of the larval stages of aquatic parasites. When *B. vaillantii* is then consumed by potential definitive hosts, piscivorous fish, or mammals, these larval stages can complete their development (Hoshino *et al.,* 2014).

CONCLUSIONS

The endoparasites present *in B. vaillantii* are represented by one species of the Trematoda, three of the Nematoda, and one pentastomid. Infection with any of these species has the potential to decrease the productivity of an aquaculture facility. Among the five parasite species recorded, *Anisakis* sp., *Contracaecum* sp., and *Sebekia* sp. are potentially zoonotic. In addition, *Sebekia* sp. was the only parasite recorded in the musculature of *B. vaillantii*. Although no other parasite species were found in the musculature, the finding of numerous larvae of the Anisakidae in the visceral cavity and intestine is of concern. The possible migration of anisakid larvae to the musculature of the fish and/or the secretion of toxins by larvae in the intestine could cause some imbalance in the health of people who accidentally ingest contaminated fish meat.

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AUTHOR'S PARTICIPATION

Contributions A.M.E.M y GAMM.: Investigation, Data curation; Formal analysis; Writing - original draft - review &editing, Prepared all figures V.P.N.G.: Data curation; Formal analysis; Investigation; Writing - original draft - review &editing. J.L.C.G.: Writing - review & editing. L.R.S : Data curation; review & editing.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

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