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SHORT COMMUNICATION

Occurrence of *Eustrongylides* sp. (Nematoda: Dioctophymatidae) in Fish Species Collected in the Peruvian Amazonia and Its Implications for Public Health

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Abstract

Purpose The present study aims to report the occurrence of *Eustrongylides* sp. in some neotropical fish species commercialized in the Peruvian Amazonia.

Methods Samples of young farmed *Arapaima gigas* were collected from a fish farmer; young *Brachyplatystoma tigrinum* were acquired from a fish exporter and samples of adult specimens of *Acestrorhynchus falcirostris, Pseudoplatystoma punctifer, Cichla monoculus, Hoplias malabaricus, Hydrolycus scomberoides, Raphiodon vulpinus,* and *Serrasalmus rhombeus* were acquired from a fish market of Iquitos, Loreto-Peru. Samples were transported to the "Laboratorio de Parasiología y Sanidad Acuícola" from "Instituto de Investigaciones de la Amazonía Peruana" located in Iquitos, Peru.

Results Larvae L4 of *Eustrongylides* sp. were found in the host body, in the muscle, and in the visceral cavity of studied fish species. Notorious damages were reported in young *A. gigas* (lumps in the muscle, inflammation and severe redness of the skin) and in young *B. tigrinum* (perforation of the abdominal cavity), while in adult specimens of the remaining species, no external damage was reported.

Conclusion The presence of *Eustrongylides* sp. in various fish species collected in the city of Iquitos-Peru contributes to the distribution of this parasite in the Peruvian Amazon, reporting it, for the first time in this country. Its presence in the muscle of various fish species commercialized in fish markets, warm a possible public health concern and implications for their marketability in the city of Iquitos, Peru.

Keywords Endoparasite · *Eustrongylides* sp. · Nematoda · Peruvian Amazonia · Zoonosis

Introduction

Nematodes of the genus *Eustrongylides* Jägersiköld, 1909 have been reported from different countries [1–4]. They use piscivorous birds as definitive hosts and aquatic oligochaetes as first intermediate hosts where the parasite develops into the second and third larval stages, which are infective to the second intermediate hosts, planktivorous and benthivorous fish species in which the third-stage larvae molt into the fourth stage and remain until ingestion by the definite hosts [5]. Carnivorous or piscivorous fish species that consume infected fish act as paratenic hosts [6].

Eustrongylides spp. present potential for transmission and pathogenicity [4] responsible for zoonotic infections [7]. *Eustrongylides* larvae are present in the viscera and in the muscle of various fishes [8], important for public health.

The presence of these parasites in fish hosts may cause serious damages than can deteriorate their health and makes fish products unfit for human consumption, by having a negative impact on their marketability [3]. The present study aims to report the occurrence of *Eustrongylides* sp. in some neotropical fish species used in the ornamental trade and commercialized in a fish markets from the Peruvian Amazon and also to discuss the potential public health issues and implications for local consumers.

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Materials and Methods

A private fish farmer noticed the presence of wounds, internal hemorrhage, protuberances, and ulcers on the body of *Arapaima gigas* (Schinz, 1822) (Fig. 1A, B). Ten specimens were analyzed. Oral communication of fish farmers mentioned that it is usual to notice those problems on young *A. gigas*. Fish samples were taken alive, placed in plastic bags with water, and transported to the laboratory of "Parasitología y Sanidad Acuícola" of "Instituto de Investigaciones de la Amazonía Peruana" in Iquitos, Peru

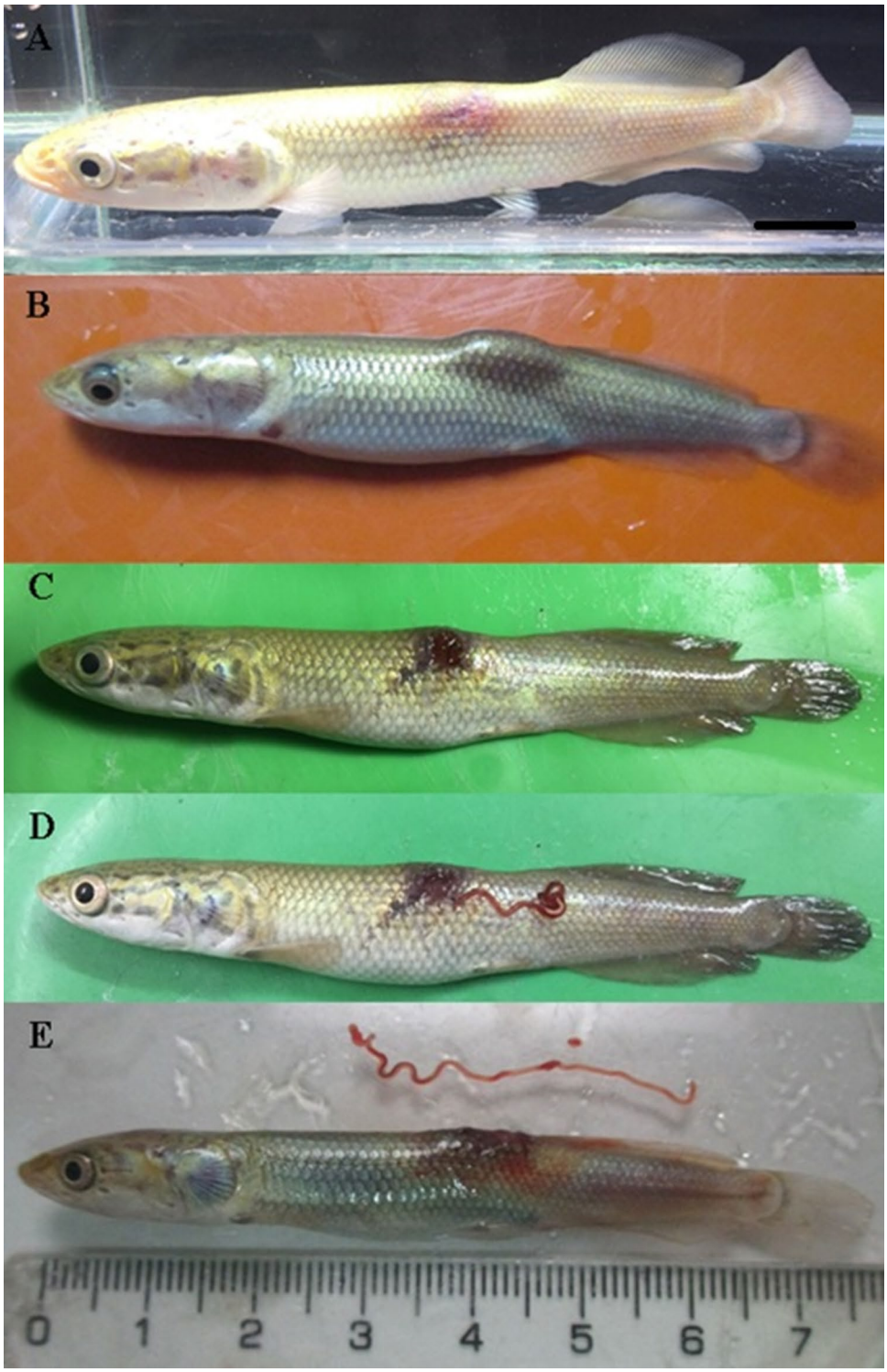

Fig. 1 External lesions in *Arapaima gigas* infected by *Eustrongylides* sp. **A** Internal bleeding; **B** Swelling of muscle; **C** Wound caused by parasite released; **D** Parasite emerging from the fish's musculature; **E** Parasite removed from *A. gigas.* Bar scale: 1 cm

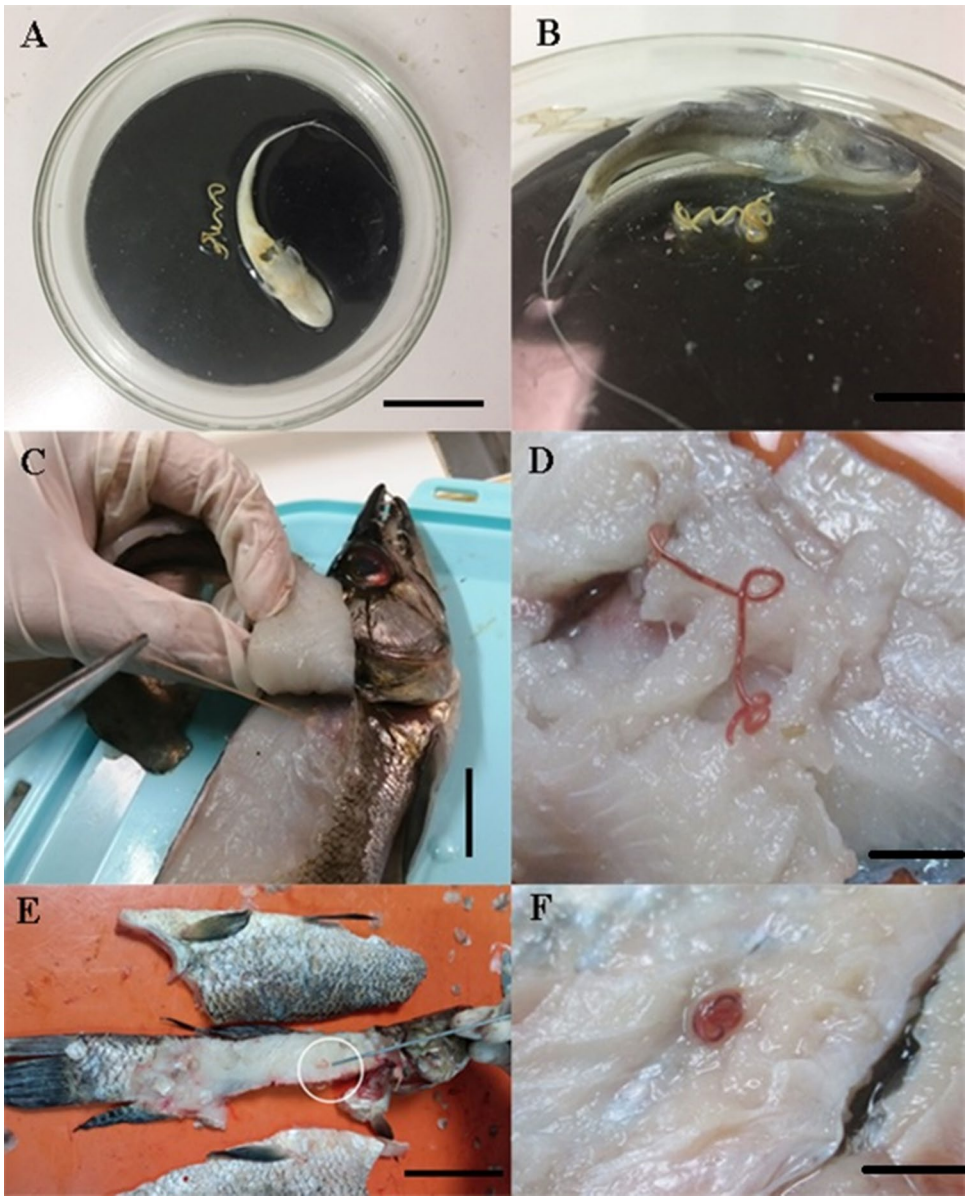

Fig. 2 **A** *Brachyplatystoma tigrinum* showing a perforation in the abdominal cavity caused by *Eustrongylides* sp. **B** Specimen of *B. tigrinum* with *Eustrongylides* sp. larvae removed from the abdominal cavity. **C** *Eustrongylides* sp. being collected from the muscle of *Acestrorrhynchus falcirostris*. **D** *Eustrongylides* sp. in the muscle of *A. falcirostris*. **E** and **F** *Eustrongylides* sp. encysted in the musculature of *Hoplias malabaricus*. Bar scale: **A**, **B**, **D**, **F** = 1 cm. **C** and **E** = 5 cm

for analyses. In the laboratory, the wounds and protuberances were observed with a stereoscope Leica EZ4 and the aid of dissection needles and tweezers, and a nematode was removed. From individuals of *Brachyplatystoma tigrinum* (Britsky, 1981), ten samples were taken alive for an ornamental fish exporter who also noticed the presence of wounds in the abdomen of infected fish. According to the fish exporter, this problem is frequently observed on young specimens that are fed with small fish and mainly with sludge worms (*Tubifex* spp.). Fish were sacrificed with a perforation in the skull for removal of the brain. The process was conducted using a hypodermic needle.

After the sacrifice, skin, muscle, and internal organs were analyzed. Nematodes from *A. gigas* were removed from the skin. Ten samples of *Acestrorhynchus falcirostris* (Cuvier, 1819), 10 of *Pseudoplatystoma punctifer* (Castelnau, 1855), 10 of *Cichla monoculus* Agassiz, 10 of *Hoplias malabaricus* (Bloch, 1794), 10 of *Hydrolycus scomberoides* (Cuvier, 1819), 10 of *Raphiodon vulpinus* Spix & Agassiz, 1829, and 10 of *Serrasalmus rhombeus* (Linnaeus, 1766) were collected dead from the Belen Market in Iquitos, Peru. Fish species were identified using the guide [9]. Fish were transported to the laboratory in styrofoam boxes with ice. The time of transportation from the market to the laboratory was 30 min. In the laboratory, internal organs were removed and preserved in ethanol 70% for analyses. The lateral musculature of the fish was analyzed by making fine cuts, which were carefully observed. Internal organs were analyzed using a stereoscope Leica EZ4. Organs were placed in Petri dishes with distilled water, and with the aid of tweezers

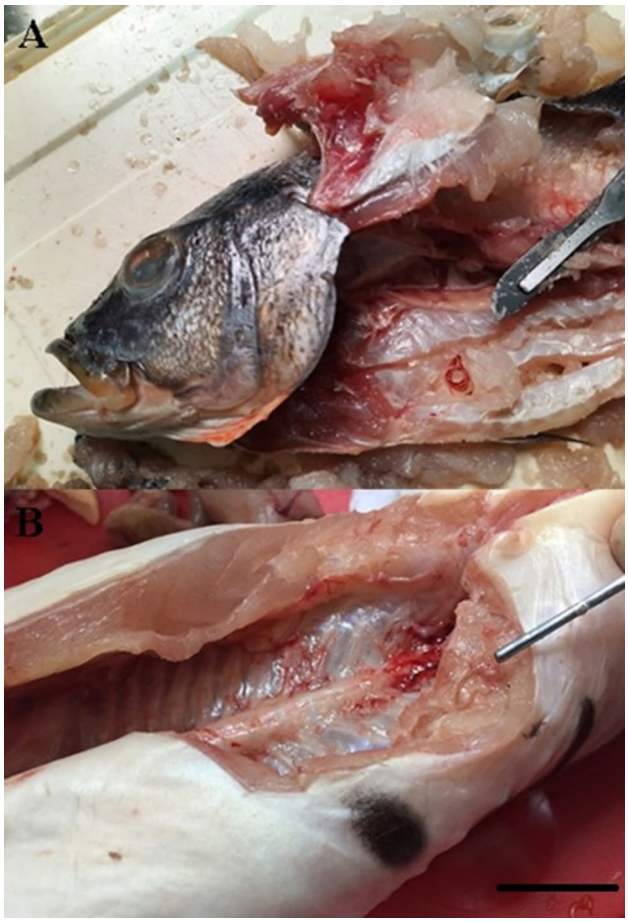

Fig. 3 **A** *Eustrongylides* sp. in the visceral cavity of *Cichla monoculus*; **B** *Eustrongylides* sp. in the musculature of *Pseudoplatystoma punctifer*. Bar scale: 5 cm

and needles, the tissue was examined by making fine cuts that allowed to observe inside them. In the presence of any parasites, these were removed with tweezers and preserved in ethanol 70% for posterior taxonomic identification. For taxonomic identification, parasites were clarified in Amman lactophenol [10]. For this, parasites were placed on a slide with a drop of Amman lactophenol, and then, they were covered with a cover slip and then observed under an optical microscope Leica DM750. Based on the morphological characters of the parasites, the identification was made using the information of [11, 12]. Parasitological indices followed those by [13] (Figs. 2 and 3).

Results

Biometric features as weight and length for each sampled fish species are presented in Table 1.

Taxonomic identification of the nematodes revealed the presence of larvae of *Eustrongylides* sp. in all infected fish. Fifty-two (52) larvae L4 were counted parasitizing 90 individuals of nine species. Of the total number of parasites recorded, 37 were found in the muscle and 15 in the visceral cavity. Non *Eustrongylides* larvae were found in internal organs. The main characteristics used for the identification were: reddish coloration, cephalic extremity presenting a small oral cavity surrounded by 12 cephalic papillae of similar size arranged in two concentric rings, and genital primordia well differentiated that allowed the identification of L4 larvae as male and female (Fig. 4). The presence of *Eustrongylides* sp. in fish hosts and their parasitological indices are showed in Table 2.

Discussion

In South America, *Eustrongylides* larvae were reported for the first time in Brazil by [12] who found this parasite in eight fish species. Later, it was reported from six additional species, highlighting the presence of the parasite in the tegument, musculature, and mesentery of *Serrasalmus natterei, Pseudoplatystoma fasciatum, Raphiodon vulpinus,* and *Hoplias malabaricus* [13]; in internal organs of characoid fishes [14]; in the musculature and mesentery of *Cichla ocellaris, Plagioscion squamosissimus,* and *H. malabaricus* [15]; in the intestine of *Mylossoma duriventre* [16]; in the musculature and mesentery of *H. malabaricus* and *Hoplerythrinus unitaeniatus*, [17, 18]; in the mesentery of *Pygocentrus nattereri* [19]; in the liver of *Gymnotus* sp. [20]; and in the abdominal cavity of *Astyanax fasciatus* [21]. In the present study, *Eustrongylides* sp. is reported for the first time in fish species from the Peruvian Amazon, expanding the geographical distribution of *Eustrongylides* spp. in South America.

Among species of the genus *Eustrongylides,* some morphological differences are used for their identification: form of the labial papillae, form of the termination of the caudal extremity, that can also be used to separate males and females, but these features are limited to adult specimens, making it difficult to identify larvae [3]. Larval stages of *Eustrongylides* spp. have been commonly reported from freshwater fish species worldwide based on the morphological features [22–24]. In the present study, it was possible to identify differences in the posterior extremity of specimens of *Eustrongylides* sp. separating males from females. The morphology of the posterior extremity is similar to *E. excisus* reported in Italy [2], but as there are no reports of adult forms of *Eustrongylides* in the Peruvian Amazon, it is difficult to guarantee the identification of a species based on morphological characteristics of the collected larvae.

In the life cycle of *Eustrongylides* spp., the first-stage larva develops in the egg passed with the infected bird faeces and is ingested by aquatic oligochaetes *Lumbriculus variegatus*, *Tubifex,* or *Limnodrilus* sp., which are the first

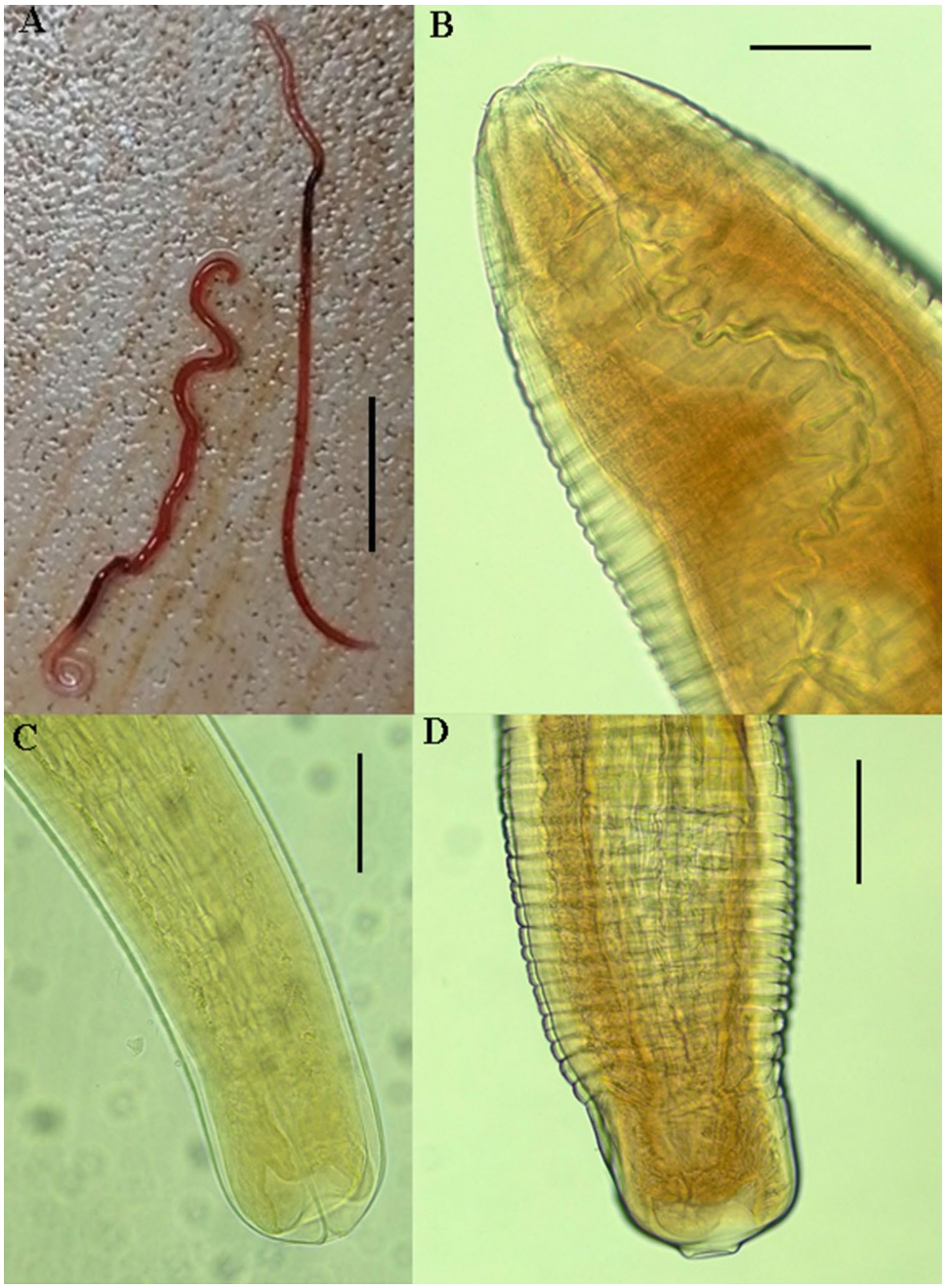

Fig. 4 Alive specimens of *Eustrongylides* sp. collected from *Brachyplatystoma tigrinum*; **B** Anterior part of the body of *Eustrongylides* sp; **C** Posterior part of the body of male specimen of *Eustrongylides* sp; **D** Posterior part of the body of female specimen of *Eustrongylides* sp. Scale bar **A**: 2 mm; **B**–**D**: 200 µm

intermediate hosts. In the coelom or the organs of the oligochaete, the second and third larval stages develop. The second intermediate host is a plankton- and benthos-eating fish species which on ingestion of infected oligochaetes serve as second intermediate hosts [25]; [26] originating larvae L4 that remain encysted until its consumption by the definitive host bird. In the Peruvian Amazon, *T. tubifex* is used as food for ornamental fish, justifying the presence of *Eustrongylides* sp. in young *A. gigas* and young *B. tigrinum* from commercial aquariums. In the same way, as *T. tubifex* inhabits the same environment of plankton- and benthos-eating fish species, it is probable that the transmission of *Eustrongylides* sp. was through food web transfer between *T. tubifex,* plankton- and benthos-eating fish species and top predator fish species. Considering that fish species act as paratenic host and birds as definitive, further investigations on the parasite occurrence in other fish species and some piscivorous birds inhabiting the same environment are needed to identify the species of *Eustrongylides* that are parasitizing fish hosts from the Peruvian Amazonia.

Parasitic infections in fish can cause changes in behaviour, vulnerability to predators, and a reduced capacity to resist stress [27]. The presence of a parasite generates a host strategy of inactivation or elimination through cellular

Table 1 Biometric features (standard length and weight) for each sampled fish species

Fish host species	Standard length (cm)	Total weight (g)
Arapaima gigas	15 ± 5	50 ± 4.5
Brachyplatystoma tigrinum	8 ± 2	24 ± 3.2
Acestrorhynchus falcirostris	28 ± 2.8	250 ± 3.5
Pseudoplatystoma punctifer	40 ± 10	980 ± 47.4
Cichla monoculus	35 ± 10	625 ± 6.5
Hoplias malabaricus	29 ± 6	320 ± 14.4
Hydrolycus scomberoides	32 ± 8.1	480 ± 24.7
Raphiodon vulpinus	34 ± 12	370 ± 14
Serrasalmus rhombeus	25 ± 5	660 ± 28.1

and humoral responses, which range from acute or chronic inflammation to severe necrosis [28]. In fish, *Eustrongylides* larvae develop in muscles and are found either free in the body cavity, or encapsulated in the liver or other visceral organs. Infected fishes usually fall prey to piscivorous fish species. On ingestion of the prey, the predator itself becomes infected. From its digestive tube, the larvae penetrate into its muscles and other organs, which they may seriously damage [29]. According to [30], the larvae are especially pathogenic to predator fish species, where they are capable of doing severe damage to various organs. In young fish, large scars are readily visible at the prior location of the parasites (muscles, gastric, and intestinal wall), sometimes even a complete destruction of the kidneys, or inflammatory lesions. In the present study, notorious damages were reported in young *A. gigas* (lumps in the muscle, inflammation, and severe redness of the skin) and in young *B. tigrinum* (perforation of the abdominal cavity), while in adult specimens of the remaining species, no external damage was reported, regardless of being parasitized by *Eustrongylides* sp. larvae, finding more larvae in the muscle than in the viscera. This suggests that young specimens are more sensitive to parasitism by this nematode and are more likely to show some sign of damage.

Eustrongylides spp. is worldwide distributed and have been found parasitizing the viscera, visceral cavity, and flesh of a wide variety of fish species [8]. In the present study, *Eustrongylides* sp. has been reported in the visceral cavity and flesh of nine fish species that are consumed as food in the Peruvian Amazonia. Their presence can have negative implications on the marketability of fish, decreasing their value in fish markets. Humans acquire the infection by consuming undercooked fish [31]. The nematodes cannot reach maturity in man, but remain in the fourth stage of larval development. The symptoms indicating the infection include gastritis and the perforation of intestines. The only possible means of cure is surgical removal of larvae [32].

The type of cooking in the preparation of fish can influence the risk of transmission of endoparasites to humans. In Peru, the tradition of consume raw or undercooked fish meat in the preparation of "ceviche" and the trend towards the adaptation of the oriental trend to consume "sushi" of oriental origin increase the probabilities of acquiring an accidental zoonotic infection [33].

According to [9], *A. gigas*, *B. tigrinum, A. falcirostris, P. punctifer, C. monoculus, H. malabaricus, H. scomberoides, R. vulpinus,* and *S. rhombeus* are traded in city markets and consumed by the local population. This fish species can be used in the preparation of "regional ceviche" or "regional sushi". In the preparation of "ceviche", the flesh is marinated with lemon. The marinating process does not erradicate nematode larvae and present a danger to public health [8]. The European Union Regulation No 853/2004 stipulates that all fish destined for raw consumption, cold-smoking processing with internal temperature below 60 ℃, marinated or salted process should be frozen at a temperature of no higher than – 20 ℃ for at least 24 h to prevent an accidental infection to consumers.

Although, in Peru, there are no records of parasitism in humans by *Eustrongylides* sp., this hypothesis cannot be ruled out, especially in the Amazon region, where it is common for children to ingest small raw fish as they

Table 2 Parasitological indices for *Eustrongylides* sp. collected from nine fish species from the Peruvian Amazon

Species	AF	PF	P%	I	mI	mA	Localization of the larvae
Arapaima gigas	10	5	50	1, 1, 1, 1, 1	1	0.5	Muscle
Brachyplatystoma tigrinum	10	6	60	1, 1, 1, 1, 1, 1	1	0.6	Visceral cavity
Acestrorhynchus falcirostris	10	5	50	1, 1, 1, 1, 1, 2	1.4	0.7	Muscle
Pseudoplatystoma punctifer	10	3	30	1, 1, 1	1	0.3	Muscle
Cichla monoculus	10	4	40	1, 1, 1, 2	1.2	0.5	Muscle (2) and visceral cavity (3)
Hoplias malabaricus	10	7	70	1, 1, 1, 1, 2, 2, 2	1.4	1	Muscle (4) and visceral cavity (6)
Hydrolycus scomberoides	10	6	60	1, 1, 1, 1, 1,1	1	0.6	Muscle
Raphiodon vulpinus	10	6	60	1, 1, 1, 1, 1, 1	1	0.6	Muscle
Serrasalmus rhombeus	10	4	40	1, 1, 1, 1	1	0.4	Viscera

AF analyzed fish, *PF* parasitized fish, *P%* prevalence, *I* intensity of infection, *mI* mean intensity of infection, and *mA* mean abundance of infection

believe that consumption will teach them to swim. A particular case occurred in New Jersey, United States, where live parasites were removed from the peritoneal cavity of a 17 year-old man complaining of intense abdominal pain. Examination revealed intestinal perforation and fourth-stage larvae were removed. The young reported that he had the habit of swallowing small fish alive while fishing [34].

Similar cases may be occurring in the Peruvian Amazon, where stomach problems caused by the accidental ingestion of parasites may be misdiagnosed, due to the lack of studies related to zoonotic parasites in fish, lack of specialists, and lack of interest on the part of the competent authorities to supervise the fish meat that is commercialized in the city markets. This study makes an alert call to local authorities and the population to consider the necessary measures to avoid the accidental ingestion of *Eustrongylides* spp. and other zoonotic parasites.

Conclusions

The presence of *Eustrongylides* sp. in various fish species collected in the city of Iquitos-Peru contributes to the distribution of this parasite in the Peruvian Amazon, reporting it, for the first time in this country. It occurs mainly in the muscle of fish, suggesting a possible public health concern and implications for their marketability in Iquitos. Considering that all analyzed fish species are used as food in the Peruvian Amazon, and the growing tendency of consumption of raw or undercooked fish (ceviche and sushi), it is necessary to expand the study in the other markets of Iquitos and analyzing other fish species of commercial interest, to generate information for relevant authorities to apply appropriate sanitary measures in fishing ports and city markets.

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Author Contributions GMM: data curation, formal analysis, and writing—original draft. CT: formal analysis and data curation. GR: formal analysis. CC: resources and supervision.

Data Availability The data that support the findings of this study are available from the corresponding author upon reasonable request.

Declarations

Conflict of interest The authors declare that they have no conflicts of interest.

References

1. Guagliardo S, Viozzi G, Brugni N (2019) Pathology associated with larval *Eustrongylides* sp. (Nematoda: Dioctophymatoidea) infection in *Galaxias maculatus* (Actinopterygii: Galaxiidae) from Patagonia, Argentina. Int J Parasitol Parasites Wildl 10:113–116. https://doi.org/10.1016/j.ijppaw.2019.08.004
2. Guardone L, Ricci E, Susini F, Polsinelli E, Guglielmone G, Armani A (2021) First detection of *Eustrongylides excisus* (Nematoda: Dioctophymatidae) in big-scale sand smelt (*Atherina boyeri*) from the lake Massaciuccoli (Northwest Tuscany, Italy): implications for public health and seafood quality. Food Control 120:107517. https://doi.org/10.1016/j.foodcont.2020.107517
3. Mazzone A, Caffara M, Gustinelli A, Agnetti F, Sgariglia E, Lo Vaglio G, Quaglio F, Fioravanti M (2019) Morphological and molecular characterization of larval and adult stages of *Eustrongylides excisus* (Nematoda: Dioctophymatoidea) with histopathological observations. Int J Parasitol 105(6):882–889. https://doi.org/10.1645/19-44
4. Xiong F, Wang G, Wu G, Nie P (2009) Development of *Eustrongylides ignotus* (Nematoda: Dioctophmida) in domestic ducks (*Anas platyrhynchos domestica* (L.). J Parasitol 95(5):1035–1039. https://doi.org/10.1645/GE-2057.1
5. Spalding M, Forrester GJ (2008) Parasitic diseases of wild birds. WileyBlackwell Publishing, Ames, pp 289–316
6. Goncharov SL, Soroka NM, Pashkevich IY, Dubovyi AI, Bondar AO (2018) Infection of predatory fish with larvae of *Eustrongylides excisus* (Nematoda, Dioctophymatidae) in the delta of the Dnipro River and the Dnipro-Buh Estuary in Southern Ukraine. Vestn Zool. https://doi.org/10.2478/vzoo-2018-0015
7. Eiras JC, Pavanelli GC, Takemoto RM, Nawa Y (2018) Fish-borne nematodiases in South America: neglected emerging diseases. J Helminthol 92(6):649–654. https://doi.org/10.1017/S0022149X17001006
8. Food and Drug Administration (2012) *Eustrongylides* species. In: Bad bug book, foodborne pathogenic microorganisms and natural toxins, 2nd edn, pp. 158–162. https://www.fda.gov/files/food/published/Bad-Bug-Book-2nd-Edition%28PDF%29.pdf. Accessed 21 Oct 2021
9. García C. *Peces de consumo de la Amazonía peruana*. Instituto de Investigaciones de la Amazonía Peruana, 2018. Accedido: 06 Jun 2021. [En línea]. Disponible en: http://repositorio.iiap.gob.pe/handle/20.500.12921/369
10. Amato J, Boeger (1991) Protocolos para laboratorio - coleta e processamento de parasitas do pescado. Imprensa Universitaria, Universidade Federal do Rio de Janeiro. 81pp
11. Kuraiem BP, Knoff M, Telleria E, Fonseca M, Machado L, Cunha N, Clemente S (2019). *Eustrongylides* sp. (Nematoda, Dioctophymatoidea) parasitizing *Hoplias malabaricus* (Actinopterygii: Erythrinidae) collected from the state of Rio de Janeiro, Brazil. Revista Brasileira de Parasitologia Veterinária, pp 29
12. Travassos L, Artigas PT, Pereira C (1928) *Fauna helmintológica dos peixes de água doce do Brasil*. São Paulo: Arch. Inst. Biol.
13. Rego A, Vicente J (1988) Excursão científica à zona do Pantanal, Estado de Mato Grosso, para coletas de helmintos. *Ciênc Cult São Paulo*, pp. 65–8.
14. Saraiva A, Rosim D, Silva-Souza A (2006) Nematode parasites of characoid fishes from Brazil. Bull Eur Assoc Fish Pathol 26:271–274
15. Martins ML, Santos R, Marengoni NG, Takahashi HK, Onaka EM (2009) Seasonality of *Eustrongylides* sp. (Nematoda: Dioctophymatidae) larvae in fishes from Paraná River, South-western Brazil. Bol Inst Pesca 35(1):29–37

16. Da Silva EF, Tavares M (2012) Infection by Helminthes in *Mylossoma duriventre* Cuvier, 1817, a characid from the Central Amazon, Brazil. Neotropical Helminthology,Lima,v.6,n.1,p.6773.http://www.alice.cnptia.embrapa.br/handle/doc/928941. Accessed 25 Oct 2021
17. Benigno RN, Clemente SC, Matos ER, Pinto RM, Gomes DC, Knoff M (2012) Nematodes in *Hoplerytrinus unitaeniatus*, *Hoplias malabaricus* and *Pygocentrus nattereri* (pisces characiformes) in Marajó Island, Brazil. Rev Bras Parasitol Vet Braz J Vet Parasitol Orgao Col Bras Parasitol Vet 21(2):165–170. https://doi.org/10.1590/s1984-29612012000200018
18. Meneguetti DU, Laray MP, Camargo LM (2013) Primeiro relato de larvas de *Eustrongylides* sp (Nematoda: Dioctophymatidae) em *Hoplias malabaricus* (Characiformes: Erythrinidae) no Estado de Rondônia, Amazônia Ocidental, Brasil. Rev Pan-Amaz Saúde 4(3):55–58. https://doi.org/10.5123/S2176-62232013000300008
19. Vicentin W, Vieira KR, Tavares LE, Costa FE, Takemoto RM, Paiva F (2013) Metazoan endoparasites of *Pygocentrus nattereri* (Characiformes: Serrasalminae) in the Negro River, Pantanal, Brazil. Rev Bras Parasitol Veterinária 22:331–338. https://doi.org/10.1590/S1984-29612013000300003
20. Ventura AS, Ishikawa MM, Gabriel AM, Silbiger HL, Cavichiolo F, Takemoto RM, (2016) Histopathology from liver of tuvira (Gymnotus spp.) parasitized by larvae of nematodes. *Ciênc. Rural*, vol. 46, pp. 1233–1239.: https://doi.org/10.1590/0103-8478cr20150881.
21. Vieira FG, Costa DP, Sato M (2017) Nematodes of *Astyanax fasciatus* (Actinopterygii: Characidae) and their parasitic indices in the São Francisco River, Brazil. Rev Bras Parasitol Vet 26:10–16. https://doi.org/10.1590/S1984-29612016074
22. Bjelić-Čabrilo O, Novakov N, Cirkovic M, Kostic D, Popovic E, Aleksic N, Lujic J (2013) The first determination of *Eustrongylides excisus* Jägerskiöld, 1909—larvae (Nematoda: Dioctophymatidae) in the pike-perch Sander lucioperca in Vojvodina (Serbia). Helminthologia 50(4):291–294. https://doi.org/10.2478/s11687-013-0143-1
23. Branciari R, Ranucci D, Miraglia D, Valiani A, Veronessi F, Urbani E, Lo Vaglio G, Pascucci L, Francheschini R (2016) Occurrence of Parasites of the Genus *Eustrongylides* spp. (Nematoda: Dioctophymatidae) in Fish Caught in Trasimeno Lake, Italy. Ital J Food Saf 5(4):6130. https://doi.org/10.4081/ijfs.2016.6130
24. Metin S, Didinen B, Boyaci Y, Kubilay A, Emre N, Didinen H, Emre Y (2014) Occurrence of *Eustrongylides excisus* Jägerskiöld, 1909 Larvae (Nematoda: Dioctophymatidae) in Pikeperch (*Sander lucioperca*, L.) in Lake Eğirdir. Acta Aqua Tr 10:20–24. https://doi.org/10.2239/egirdir.246371
25. Coyner DF, Schaack SR, Spalding MG, Forrester DJ (2001) Altered predation susceptibility of mosquitofish infected with *Eustrongylides ignotus*. J Wildl Dis 37(3):556–560. https://doi.org/10.7589/0090-3558-37.3.556
26. Pazooki J, Masoumian M, Yahyazadeh M, Abbasi J (2007) Metazoan parasites from freshwater fishes of Northwest Iran. J Agric Sci Technol 9(1):25
27. Hammond-Tooke CA, Nakagawa S, Poulin R (2012) Parasitism and behavioural syndromes in the fish *Gobiomorphus cotidianus*. Behaviour 149(6):601–622
28. Mitchell AJ (2009) *Eustrongylides ignotus* infecting commercial bass, Morone chrysops female X Morone saxatilis male, and other fish in the southeastern USA. J Fish Dis 32:795–799. https://doi.org/10.1111/j.1365-2761.2009.01051
29. Yanong RP (1969) Nematode (roundworm) infections in fish. EDIS. https://doi.org/10.32473/edis-fa091-2002
30. Moravec F (1994) Nematodes parasitic in fishes as larvae. In: Parasitic nematodes of freshwater fishes of Europe. Kluwer Acad Publ, pp. 374–384.
31. Mohammad M, Iraj M, Mahzad AM, Behyar J, Bagher AF, Saeed SS (2011) Occurrence and intensity rate of internal metazoan parasites in *Rutilus frisii* kutum and the first report Dioctophyma renale of (Nematoda: Dioctophymidae) in Iran. World J Zool 6(1):91–97
32. Cole R (2009) Eustrongylidosis en field manual of wildlife diseases—general field procedures and diseases of birds. www.nwhc.usgs.gov/publications/field_manual/chapter_29.pdf. Accessed 02 Nov 2021
33. Murrieta G (2019), *Parasitología en peces de la Amazonía: fundamentos y técnicas parasitológicas, profilaxis, diagnóstico y tratamiento*. http://repositorio.iiap.gob.pe/handle/20.500.12921/394. Accessed 02 Nov 2021
34. Eberhard ML, Hurwitz H, Sun A, Coletta D (1989) Intestinal perforation caused by Larval *Eustrongylides* (Nematoda: Dioctophymatoidae) in New Jerse. Am J Trop Med Hyg 40(6):648–650. https://doi.org/10.4269/ajtmh.1989.40.648