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SHORT COMMUNICATION

First report of the nematode *Eustrongylides* sp. (Nematoda: Dioctophymatidae) in the sardine *astyanax panamensis* in Darién, Panama

Primer reporte del nematodo *Eustrongylides* sp. (Nematoda: Dioctophymatidae) en la sardina *astyanax panamensis* en Darién, Panamá

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ABSTRACT

Astyanax panamensis is a species of sardine with an omnivorous diet and is often used as a subsistence food in various regions of the country. In this study, we report for the first time the presence of larvae of the genus *Eustrongylides* parasitizing this species in the Darién region. *Eustrongylides* sp. has previously been described from Lake Gatun in two host species; however, there is no recent evidence of its distribution in other provinces of Panama, which poses a risk due to its zoonotic potential. In this study, larvae of *Eustrongylides* sp. were morphologically identified, with an average length of 4.3 mm, and were found in the hosts' musculature. The

average number of larvae per individual was one; however, some individuals were found to be parasitized by up to three larvae simultaneously. The presence of Ciconiiformes birds in Darién suggests the possibility that the life cycle of the parasite may be sustained in this region. Further studies are needed to assess the prevalence of this and other parasites with zoonotic potential, both in Darién and in other provinces of Panama.

KEYWORDS

Darién, *Eustrongylides*, Nematoda, Panamá, Sardine.

RESUMEN

Astyanax panamensis es una especie de sardina con dieta omnívora y muchas veces utilizada como alimento de subsistencia en varias regiones del país. En este estudio, se da el primer reporte de larvas del género *Eustrongylides* parasitando a esta especie en el Darién. *Eustrongylides* sp. ha sido descrita en el lago Gatún en dos hospederos, sin embargo, no hay evidencia reciente de la distribución de este parásito en otras provincias de Panamá, lo que representa un riesgo debido a su potencial zoonótico. En este estudio se identificaron morfológicamente larvas de *Eustrongylides* sp., con un promedio de 4.3 mm de largo encontradas en la musculatura de los hospederos, el promedio de larvas por individuo fue de 1, sin embargo, se encontraron individuos parasitados con hasta 3 larvas a la vez. La presencia de aves Ciconiiformes en Darién indica la posibilidad de que el ciclo de vida se perpetúe en esta región. Se necesitan más estudios para evaluar la prevalencia de este y otros parásitos con potencial zoonótico, tanto en Darién como en las demás provincias de Panamá.

PALABRAS CLAVE

Darién, *Eustrongylides*, Nematoda, Panamá, Sardina.

INTRODUCTION

Among Characiformes, the genus *Astyanax* Baird & Girard, 1854 is characterized as one of the most diverse and widely distributed in the Americas (Lucena & Soares, 2016; Angulo et al., 2018). In Panama, four valid species of these sardines have been described: *A. anai*, *A. bimaculatus*, *A. orstedii*, and *A. panamensis* (Angulo et al., 2018; Gonzalez, 2021). The last one is a species that can reach up to 15 cm in length and inhabits various freshwater environments such as swamps, streams, rivers, and even reservoirs (Gonzalez, 2021). In many rural areas, it is used as a subsistence food resource.

One group of parasites that have been reported infecting *Astyanax* species are nematodes of the genus *Eustrongylides* Jägerskiöld, 1909 (Vieira-Menezes et al., 2017; Santacruz et al., 2023), which currently includes three valid species: *E. excisus*, *E. ignotus*, and *E. tubifex*. These species are distributed across all continents except in Arctic and sub-Arctic regions (Honcharov et al., 2022a). This genus of parasites has a wide range of intermediate and paratenic hosts, utilizing groups such as oligochaetes, fish, amphibians, reptiles, and

mammals to reach piscivorous birds, which serve as the definitive hosts (Honcharov et al., 2022b). These parasites, when accidentally ingested by humans, can cause a zoonotic disease known as eustrongylidiasis. This disease arises from the consumption of infected fish prepared raw, smoked, salted, or undercooked (Orihel & Ash, 1995; Eiras, 2024). It has been reported that there are a range of symptoms, including peritonitis due to larval perforation of the small intestine (Eberhard et al., 1989; Wittner et al., 1989), and cutaneous larval migration (Eberhard & Ruiz-Tiben, 2014).

Eustrongylidiasis is considered an underdiagnosed disease that primarily occurs in tropical and neotropical regions. This is largely due to limited awareness among healthcare professionals who may confuse its symptoms with other gastrointestinal diseases and the lack of studies on the parasite's presence across different countries and regions (Honcharov et al., 2022a; Morey et al., 2022).

In Panama, the genus *Eustrongylides* has been previously reported in the peacock bass *Cichla ocellaris* (Vasquez & Rogers, 1992) and in specimens of *Vieja maculicauda* (Roche et al., 2010), both from Lake Gatún. To date, it has not been reported in other areas of Panama, highlighting the need to expand knowledge on the distribution of this parasite.

This study reports the presence of this parasite in several individuals of the sardine species *A. panamensis*, collected from the Chucunaque River in the community of Yaviza, Darién Province.

MATERIALS AND METHODS

Study area

This study was conducted in the middle basin of the Chucunaque River, adjacent to the community of Yaviza (Fig. 1A), in the province of Darién, near coordinates 8° 9′ 30″ N; 77° 41′ 34″ W. The corregimiento of Yaviza is located in the district of Pinogana, approximately 220 km southeast of Panama City, and marks the end of the Pan-American Highway's overland route (Gabriel & Sherwood, 2022). This area lies near the Matusaragatí wetland, where the influence of tributary rivers such as the Chucunaque on the wetland’s dynamics has been studied (Carol et al., 2022).

COLLECTION OF INDIVIDUALS

Between March 25 and 29, 2025, wildlife rescue and relocation activities were carried out as part of the “Yaviza-Pinogana Road Construction Project” in the province of Darién. Five sampling points were established in association with the community of Yaviza (Fig. 1A), on both riverbanks (Fig. 1B, C), with the objective of rescuing and relocating as many fish as possible prior to construction activities.

A total of 55 individuals of the species *A. panamensis,* were captured using six-foot cast nets with a mesh size of 1 cm in areas of medium depth, with throws made from a boat. Subsequently, individuals were measured using a tape measure and photographed. Upon observing the presence of cysts in the musculature of some fish (Fig. 2A), with a small incision we were able to see the parasite (Fig. 2B). Once the presence of these parasites was confirmed, only visibly affected individuals were collected. Specimens were fixed in 70% alcohol and sent to the Parasitology Laboratory of the Faculty of Veterinary Medicine for processing

Figure 1.

Study area. A) Map with sampling points along the Chucunaque River, adjacent to the community of Yaviza. B) Left riverbank: market area, product loading and unloading zone. C) Right riverbank: residential area.

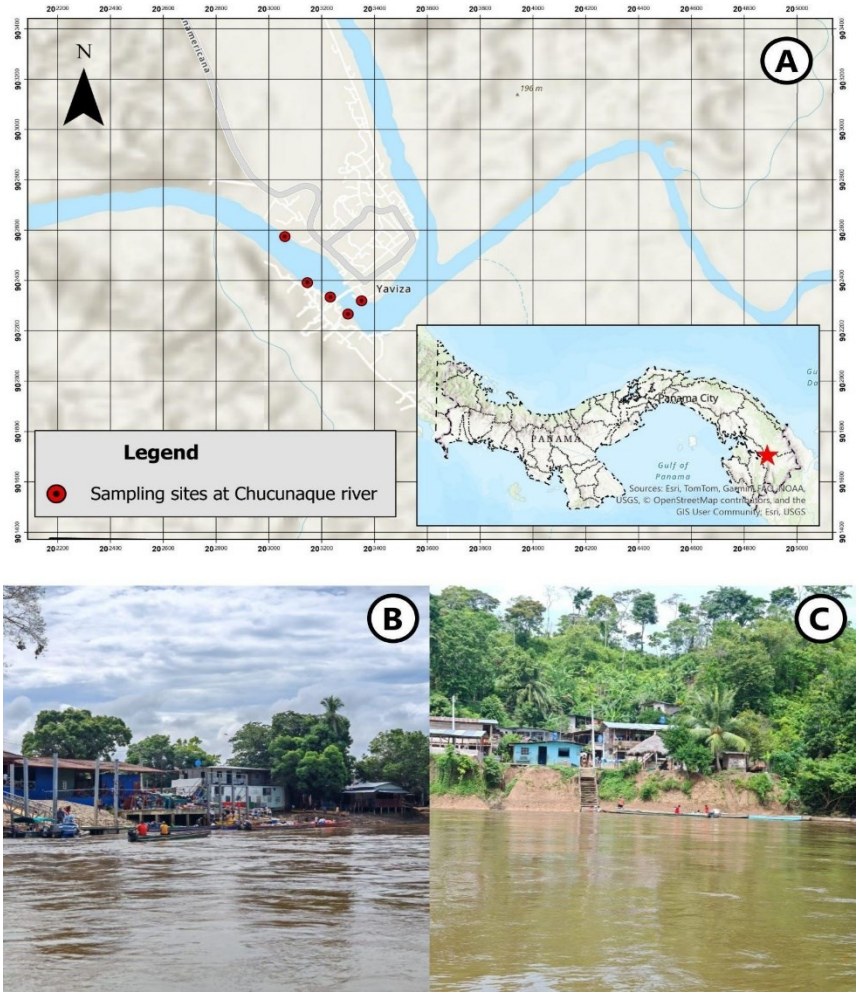
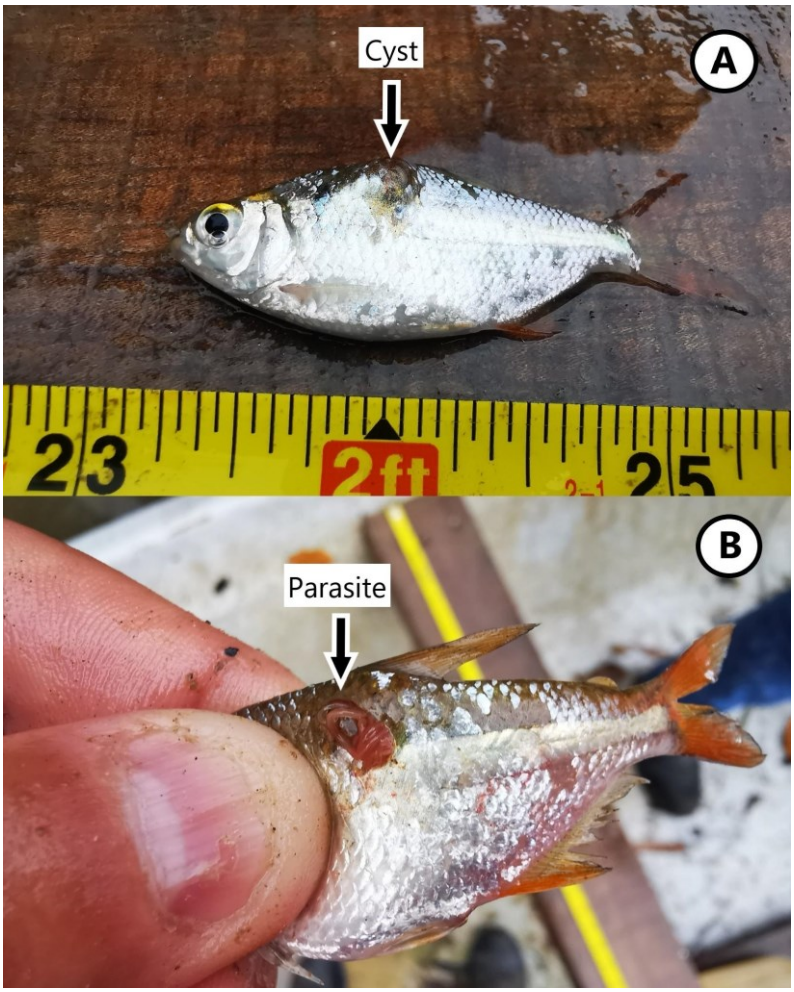


Figure 2.
Parasitized fish. A) Visible cyst formation in the musculature of the fish. B) Extraction of the parasite.



PROCESSING AND IDENTIFICATION OF PARASITES

Dissection of the specimens was conducted following standard protocols. The parasites found were measured in their entirety (mm) and placed in 2 mL Eppendorf tubes containing 95% ethanol for later identification. The posterior and cephalic sections were then separated for morphological identification. Both sections were cleared following the protocol of Seinhorst (1962), using a mixture of 99.5% glycerin and 95% ethanol, and allowed to evaporate over

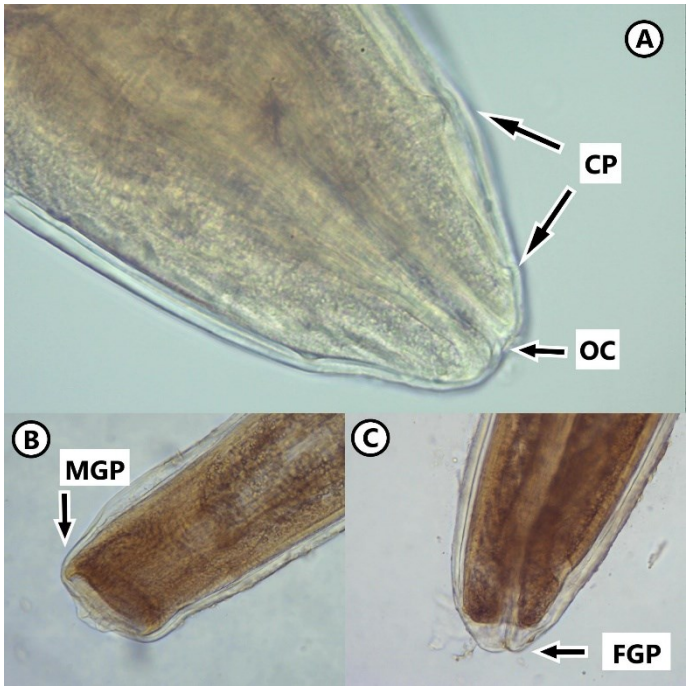
24 hours. Subsequently, internal, and external structures were observed under a light microscope (Amscope, model MD827S30L with integrated 3-megapixel digital camera), using the dichotomous keys provided by Mazzone et al. (2019) and Rahmati-Holasoo et al. (2024) as references.

RESULTS AND DISCUSSION

Seven individuals of *A. panamensis* were examined, and a total of 10 larvae of *Eustrongylides* sp. were found in the musculature. These larvae exhibited an average length of 4.3 mm and a reddish coloration (Fig. 2B). Among the hosts analyzed, only two individuals were parasitized by more than one larva, with a maximum of three larvae observed.

Upon clearing, the cephalic end revealed a small oral cavity and cephalic papillae (Fig. 3A), structures characteristic of parasites belonging to the genus *Eustrongylides*. Additionally, structures corresponding to both male (Fig. 3B) and female (Fig. 3C) genital primordia were observed.

Figure 3.
Micrographs of Eustrongylides sp. larva. A. Cephalic end showing the small oral cavity (O.C.) and cephalic papillae (C.P.). B. Posterior end showing the male genital primordium (M.G.P.). C. Posterior end showing the female genital primordium (F.G.P.).



Fish of the genus *Astyanax* are considered omnivorous, and their diet has been observed to include oligochaetes (Vilella et al., 2002; Silva et al., 2014), which have been described as the primary intermediate hosts in the life cycle of *Eustrongylides* (Honcharov et al., 2022a), as well as other parasitic helminths (Arepbaev et al., 2022). This could explain the parasitism observed in this species. Additionally, the presence of piscivorous birds plays a significant role in this distribution, since many species of these birds serve as definitive hosts. It is known that birds from the orders Ciconiiformes, Anseriformes, Gaviiformes, and Pelecaniformes have been reported as hosts of these nematodes (Honcharov et al., 2022b). In Darién, birds belonging to these orders have been reported (Walschburger et al., 2008; Méndez-Carvajal et al., 2021), which could indicate the perpetuation of the parasite's life cycle within the region. This genus of nematodes has also been reported infecting other *Astyanax* species, such as *A. fasciatus* in Brazil (Vieira-Menezes et al., 2017), and *A. mexicanus*, infecting the mesenteric region of these fish in Mexico (Santacruz et al., 2023).

CONCLUSIONS

This study constitutes the first report of *Eustrongylides* sp. larvae infecting sardines of the species *A. panamensis* from the Chucunaque River, Darién Province. It expands and updates previous information about the hosts and distribution of this genus of parasites in the country. It is generally known that this genus of parasites infects a wide variety of animal groups, including fish, and that accidental consumption can lead to zoonosis. However, further studies on parasitic biodiversity in the region are needed to clarify their zoonotic potential and thereby generate information that can be used for the awareness of society and healthcare professionals.

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