

First record of *Contracaecum* sp. (Nematoda: Anisakidae) larvae in *Eunectes notaeus* with a hypothesis on the role of yellow anaconda in the parasite's transmission

Primer registro de larvas de *Contracaecum* sp. (Nematoda: Anisakidae) en *Eunectes notaeus* de Argentina y una hipótesis sobre el rol de las anacondas amarillas en el ciclo de transmisión

Mariané B. Mañez^{1,2*} , Lucas E. Garbin^{1,3} , Juan F. Draque⁴, Mariano Barros⁴, Patricio Micucci⁴, Tomas Waller⁵, Julia I. Diaz^{1,2} 

ABSTRACT: Species of *Contracaecum* (Anisakidae) are widely distributed nematodes whose life cycle involves aquatic invertebrates and fishes as intermediate and/or paratenic hosts and piscivorous birds and mammals as definitive hosts. Although third-stage larvae have occasionally been reported from reptiles, no records of *Contracaecum* larvae have been documented in snakes of the genus *Eunectes*. This study documents for the first time the occurrence of *Contracaecum* third-stage larvae in the yellow anaconda, *Eunectes notaeus*, from Bañado La Estrella, Formosa Province, Argentina. One hundred and fifty adult *E. notaeus* (> 160 cm) collected during the Yellow Anaconda Management Program between 2019 and 2023 were examined for helminths. Larvae recovered from the gastric serosa were studied using light microscopy and scanning electron microscopy and identified based on morphological characters. A total of 196 third-stage larvae of *Contracaecum* were recovered, with a prevalence of 33.33%, a mean abundance of 1.30, and a mean intensity of 3.92. There were no statistically significant differences in prevalence between years. This study represents the first record of *Contracaecum* larvae infecting *E. notaeus* and the first for the genus *Eunectes*. The larval localisation and the position of these snakes in the trophic webs lead to hypothesising that small specimens of this semi-aquatic species can act as paratenic hosts, potentially contributing to the maintenance of *Contracaecum* within Neotropical wetland food webs. These findings expand the known host range of the genus and provide new insights into the ecological role of reptiles in anisakid transmission cycles.

Keywords: Nematodes, reptiles, snakes, wetlands

RESUMEN: Las especies del género *Contracaecum* (Anisakidae) son nematodos de amplia distribución cuyo ciclo de vida involucra invertebrados acuáticos y peces como hospedadores intermediarios y/o paraténicos, y aves y mamíferos piscívoros como hospedadores definitivos. Aunque ocasionalmente se han reportado larvas de tercer estadio en reptiles, el papel ecológico de las serpientes en la dinámica de transmisión de *Contracaecum* aún no se ha analizado en el género *Eunectes*. Este estudio documenta por primera vez larvas de tercer estadio de *Contracaecum* en la anaconda amarilla, *Eunectes notaeus*, en el Bañado La Estrella, provincia de Formosa, Argentina. Se examinaron en búsqueda de helmintos 150 ejemplares adultos de *E. notaeus* (> 160 cm) coleccionados durante un programa para la conservación y aprovechamiento sustentable de la boa curiyú en la Argentina (Programa Curiyú) entre 2019 y 2023. Las larvas halladas enquistadas en la serosa del estómago se estudiaron mediante microscopía óptica y microscopía electrónica de barrido, y se identificaron con base en caracteres morfológicos. Se recuperaron un total de 196 larvas del tercer estadio de *Contracaecum*, con una prevalencia de 33,33%, una abundancia media de 1,30 y una intensidad media de 3,92. No se observaron diferencias estadísticamente significativas en la prevalencia entre los años. Este estudio representa el primer registro de larvas de *Contracaecum* parasitando a *E. notaeus* y el primero para el género *Eunectes*. La localización larvaria y la posición de las serpientes en las redes tróficas permiten hipotetizar que individuos pequeños de esta serpiente semiacuática puedan actuar como hospedadores paraténicos, contribuyendo potencialmente al mantenimiento de *Contracaecum* dentro de las redes tróficas de los humedales

¹ Centro de Estudios Parasitológicos y de Vectores (CEPAVE) - Consejo Nacional de Investigaciones Científicas y Técnicas / Universidad Nacional de La Plata, Boulevard 120 y 60 s/n, La Plata, 1900, Argentina.

² Facultad de Ciencias Naturales y Museo (FCNyM), Universidad Nacional de La Plata, La Plata, Argentina.

³ Universidad Nacional Arturo Jauretche (UNAJ), Florencio Varela, Argentina.

⁴ Fundación Biodiversidad, Ciudad Autónoma de Buenos Aires, Argentina.

⁵ Independent Researcher, Ciudad Autónoma de Buenos Aires, Argentina.

*Corresponding author: marianemanez@cepave.edu.ar

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neotropicales. Estos hallazgos amplían el rango de hospedadores conocido del género y proporcionan nuevas hipótesis sobre el papel ecológico de los reptiles en los ciclos de transmisión de anisákidos.

Palabras clave: Nematodes, reptiles, serpientes, humedales

Citación: Mañez, M. B., Garbin, L. E., Draque, J. F., Barros, M., Micucci, P., Waller, T. y Díaz, J. I. (2026). First record of *Contracaecum* sp. (Nematoda: Anisakidae) larvae in *Eunectes notaeus* with a hypothesis on the role of yellow anaconda in the parasite's transmission. *Revista Argentina de Parasitología*, 15(2), 13–20.

INTRODUCTION

Anisakidae nematodes, particularly *Contracaecum* Railliet & Henry, 1912, are widely distributed parasites of aquatic ecosystems, infecting a broad range of vertebrate and invertebrate hosts worldwide (Anderson, 2000; Garbin et al., 2023). Although fishes constitute the main paratenic host, third-stage larvae of *Contracaecum* have also been reported encysted in amphibians and reptiles, indicating that these vertebrates may contribute to parasite transmission in aquatic food webs (Silva Trejo et al., 2025).

The yellow anaconda, *Eunectes notaeus* Cope, 1862, is a large semiaquatic snake widely associated with wetlands in South America (McCartney-Melstad et al., 2012). In Argentina, it is found in the provinces of Formosa, Chaco, Corrientes, northern Santa Fe, northern Entre Ríos, and southern Misiones (Micucci et al., 2006). Its broad generalist diet (Barros et al., 2011; Miranda et al., 2016; Waller et al., 2007) and dual role as both predator and prey integrate *E. notaeus* into multiple trophic pathways within wetland food webs, making it a potentially important paratenic host for parasites with indirect life cycles. Despite these ecological characteristics, parasitological and health records for *E. notaeus* remain remarkably scarce (Fernandes & Kohn, 2014; Mañez et al., 2026).

The present study documents for the first time the occurrence of *Contracaecum* third-stage larvae in adults of the yellow anaconda, *E. notaeus*, from Bañado La Estrella, Formosa Province, Argentina. Based on these findings we hypothesise about the role of semi-aquatic snakes as paratenic hosts in the transmission dynamics of anisakid nematodes within Neotropical wetlands.

MATERIALS AND METHODS

A total of 150 adult anacondas provided by the “Yellow Anaconda Management Program” (Programa Curiyú) were examined for parasites; therefore, no unnecessary deaths occurred during this research. The sampling was conducted in conjunction with the biological research campaigns of the Fundación Biodiversidad during June 2019, May 2021, June 2022, and May 2023. Each snake was categorised as an adult, defined as individuals with a snout-vent length > 160cm. Specimens were placed in a supine position and dissected by the hunters themselves

along the midline, beginning at the ventral region of the jaw and continuing to the cloaca, following the skinning protocol established by the Management Program for each harvest season (Micucci et al., 2006). The coelomic cavity, serous membranes, mesenteries, and musculature were inspected for nematode larvae, which were preserved individually in 4% formalin or 70% alcohol.

The nematodes were counted, cleared in lactophenol, mounted on temporary slides, and observed with an Olympus BX51® optical microscope (Tokyo, Japan), photographed with a Q Imaging Go3 camera using the Q Capture Pro 6.0 software, and measured using a drawing tube. To obtain greater detail on certain external structures, three specimens were dehydrated in a gradual series of alcohols, dried using the critical-point technique, metallised, and mounted for proper observation of their external characteristics under a Jeol/SET 100® scanning electron microscope (SEM) (Davidovich, 2022; Santana et al., 2023). Measurements are expressed in millimetres (unless otherwise indicated), with the mean followed by the range in parentheses. Larvae identification followed the morphological criteria of Anderson (2000).

The parasitological descriptors prevalence (P), mean abundance (MA) and mean intensity (MI) were interpreted and calculated overall and discriminated for each year according to Bush et al. (1997). To further characterise the distribution of parasite intensity among infected hosts, the median and mode were also calculated. The 95% confidence interval (CI) for prevalence was estimated using the exact binomial method, while 95% CIs for mean abundance and mean intensity were obtained using bias-corrected and accelerated (BCa) bootstrap procedures with 2000 replicates. The median and mode were calculated only among parasitized hosts, as descriptors of infection intensity. The 95% CI for the median was estimated using a non-parametric method based on order statistics, with RStudio (version 2026.08) and the ParasiteR package (Furlan et al., 2026; Reiczigel et al., 2019).

To evaluate whether parasite prevalence varied significantly across sampling years (2019, 2021, 2022, and 2023), we performed a Chi-squared test of independence using a 4×2 contingency table, with year as the grouping variable and parasitized/non-

parasitized individuals as the response categories. Statistical analyses were performed using RStudio, and statistical significance was set at $p < 0.05$.

Statement of Animal Ethics

The collection and use of biological samples were authorized by Resolution No. 40/15 of the Ministry of Production and Environment of Formosa, along with permits issued through provisions 0151/19, 050/21, 052/22, and 049/23. These permits established the requirements for accessing biological resources and their derivatives, under both *ex situ* and *in situ* conditions, within the jurisdiction of Formosa Province. All animals were treated following the Reference ethical framework for biomedical research: ethical principles for research on laboratory, farm, and wild animals (Consejo Nacional de Investigaciones Científicas y Técnicas [CONICET], 2005).

RESULTS

A total of 196 third-stage larvae of nematodes were found and identified as belonging to the genus *Contracaecum*.

Nematoda, Anisakidae Skrjabin & Karokhin, 1945

Contracaecum Railliet & Henry, 1912

Contracaecum sp. L3 (Figs.1-3)

Description. Based on 12 specimens. Larvae are creamy white, robust, and widest at the midbody. Total body length 18.56 (10.20–28.40); body width 0.6 (0.4–0.7); three rudimentary lips with a well-developed boring tooth; nerve ring from anterior end 0.27 (0.2–0.35), and body width at the level of the nerve ring 0.27 (0.24–0.45); excretory pore located at the base of the lips; oesophagus length 2.01 (1.30–2.60); intestinal caecum length 1.68 (0.90–2.00); ventricular appendix length 0.46 (0.38–0.56), tail length 0.13 (0.08–0.20) (excluding the mucron) (1% of body length). Oesophagus / body length ratio 10.83%, intestinal caecum / body length ratio 9.05%, ventricular appendix / body length ratio 2.48%, tail length / body length ratio 0.70%.

Host. *Eunectes notaeus* Cope, 1862.

Locality. Bañado La Estrella, Formosa, Argentina.

Site of infection. Larvae encysted in the serosa of the stomach.

Population parameters. Overall values and those for each year are presented in Tables 1 and 2. Parasite prevalence did not differ significantly among sampling years ($\chi^2 = 7.25$, $df = 3$, $p = 0.064$).

Remarks. The larvae recovered from *Eunectes notaeus* agree closely with previous *Contracaecum* L3 descriptions in their general morphology, including the presence of a robust body, a well-developed intestinal caecum and ventricular appendix, a short conical tail bearing a terminal mucron, and the relative positions

of the nerve ring and excretory pore. In the Neotropical region, third-stage larvae of *Contracaecum* have been reported encysted in several snake species, including the northern Mexican garter snake *Thamnophis eques* (Reuss) and the blackbelly garter snake *Thamnophis melanogaster* (Peters) from Mexico (Jiménez-Ruiz et al., 2002; Silva-Trejo et al., 2025); and the arrow-headed coral snake *Micrurus alleni* Schmidt and the double-banded false coral snake *Erythrolamprus bizona* Jan from Costa Rica (Goldberg & Bursey, 2004). Compared with the most comprehensive morphometric data currently available for snake-derived *Contracaecum* larvae, the specimens recovered from *E. notaeus* show broad overlap in body length, body width, intestinal caecum length, and tail length, but generally possess a longer oesophagus than larvae reported from *T. melanogaster* (1.30–2.60 vs. 0.39–0.57) (Silva-Trejo et al., 2025). The largest specimens recovered from *E. notaeus* also exceeded the maximum body length reported for third-stage larvae recovered from *T. melanogaster* (10.20–28.40 vs. 7.95–25.45).

DISCUSSION

Snakes are known to act as hosts for *Contracaecum* L3; however, reports of these larvae in snakes remain scarce, and most studies only document their occurrence without providing a detailed morphological description (Goldberg & Bursey, 2004; Jiménez-Ruiz et al., 2002; Silva-Trejo et al., 2025). To our knowledge, there are no previous records of *Contracaecum* larvae infecting species of *Eunectes*. Thus, the present finding represents the first record for this host genus and expands the known diversity of reptilian hosts for anisakid nematode larvae in the Neotropical region.

Various aquatic invertebrates, particularly crustaceans, together with fishes, act as intermediate hosts for *Contracaecum* species (Anderson, 2000). Parasitological studies on freshwater fish

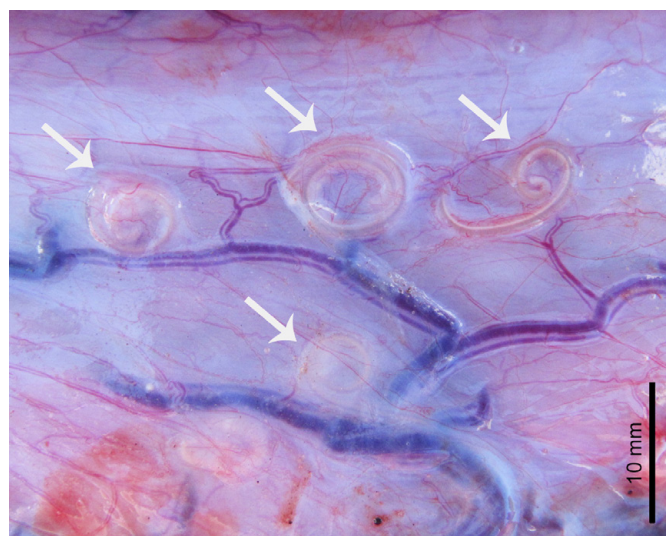


Figure 1. *Contracaecum* sp. L3 (arrows) encysted in the serosa of the stomach of *Eunectes notaeus*.

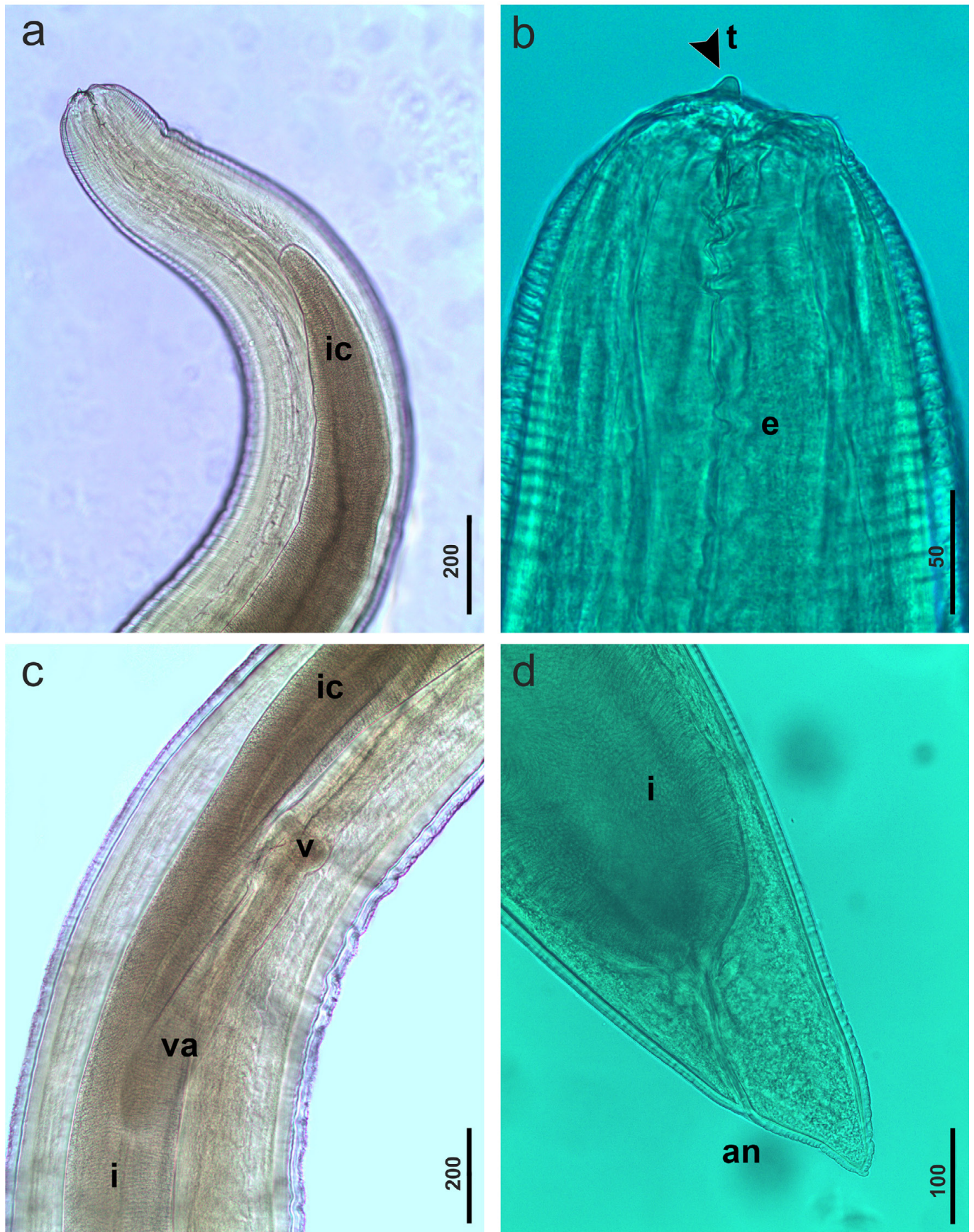


Figure 2. *Contracaecum* sp. L3 under optical microscope. a. Anterior end showing intestinal caecum. b. Anterior end showing three rudimentary lips with a well-developed boring tooth. c. Detail of ventriculus and ventricular appendix. d. Posterior end showing anus. Abbreviations: an, anus; e, oesophagus; i, intestine; ic, intestinal caecum; va, ventricular appendix; t, tooth.

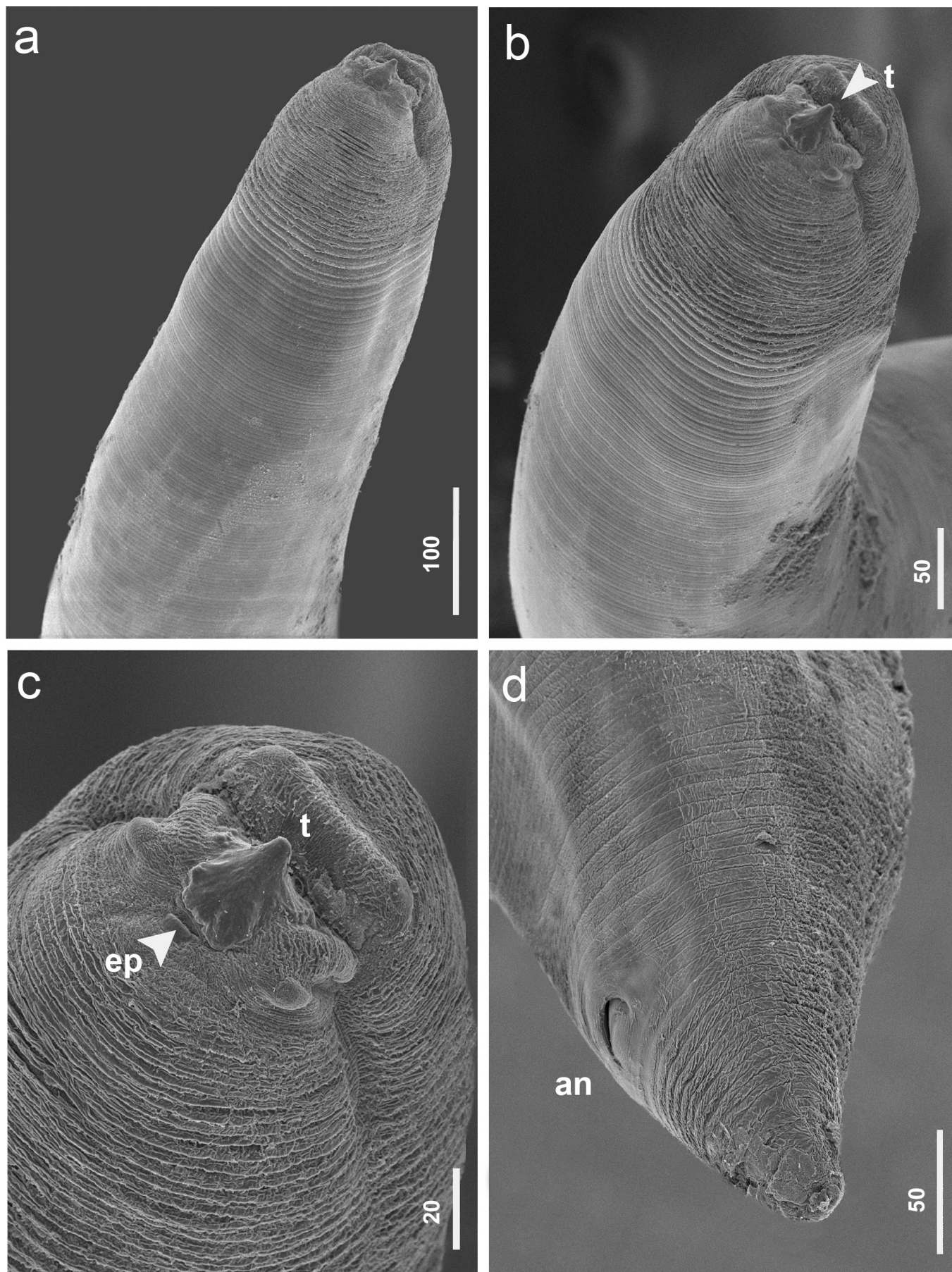


Figure 3. *Contracaecum* sp. L3 under scanning electron microscope. a-c. Anterior end showing rudimentary lips with a well-developed boring tooth and excretory pore. d. Posterior end showing anus. Abbreviations: an, anus; ep, excretory pore; t, tooth.

Table 1. Overall parasitological descriptors of *Contracaecum* L3 from *E. notaeus* (N=150) from Bañado La Estrella, Formosa, Argentina. P=prevalence; MA=mean abundance; MI=mean intensity; Me=median; Mo=mode; CI=95% confidence interval.

P [CI95%]	33.33	[25.86–41.48]
MA [CI95%]	1.31	[0.92–1.88]
MI [CI95%]	3.92	[3.08–5.20]
Me MI [CI95%]	3	[1–5]
Mo MI	1	—

from Formosa Province remain scarce. Larvae of *Contracaecum* sp. were recorded in the spotted sorubim *Pseudoplatystoma corruscans* (Spix & Agassiz), the red-bellied piranha *Pygocentrus nattereri* Kner, and the white pim catfish *Pimelodus albicans* Valenciennes in the Pilcomayo River, Salta Province (Ramallo et al., 2020). Similarly, *Contracaecum* sp. larvae have been reported in the trahira *Hoplias malabaricus* Bloch, the dorado *Salminus brasiliensis* Cuvier, and *P. albicans* in other provinces of Argentina (Mancini et al., 2014; Ramallo & Ailán-Choke, 2022). Although there are no published records of these parasites in fish from Formosa, the host species mentioned are part of the ichthyofauna of Bañado La Estrella (Dománico et al., 2014), suggesting that suitable intermediate or paratenic fish hosts are likely present in the wetland and may contribute to the local transmission cycle of *Contracaecum*.

Although fish represented a relatively small proportion of the diet of *E. notaeus* in samples collected during the high-water season at Bañado La Estrella (Waller et al., 2007), their consumption likely represents one of the routes by which this snake acquires *Contracaecum* larvae. Additionally, field reports indicate that yellow anacondas, like other snakes, occasionally consume dead fish (DeVault & Krochmal, 2002; Sazima & Strüßmann, 1990; Strüßmann, 1997). In this sense, between October and December, the Bañado La Estrella undergoes

progressive drying, which may result in localised fish mortality events (Miranda et al., 2016). Because *Contracaecum* larvae may remain viable in the tissues of recently deceased fish, scavenging during these events may represent an additional route of larval acquisition. In this context, the similar prevalence of larvae across annual samplings suggests that fish consumption is a sustained behaviour over the years.

The localisation of larvae encysted within the gastric serosa of snakes is compatible with the behaviour of anisakid larvae in paratenic hosts, in which larvae migrate through host tissues and remain encapsulated until transmission to a suitable definitive host (Anderson, 2000). Although there are no published records of predation on *E. notaeus* by birds in Argentina, several groups of birds – including herons, egrets, owls, and other raptors – have been documented preying upon snakes and other reptiles (Kahl, 1971; Martínez et al., 2019; Santana et al., 2025). Among these the Jabiru *Jabiru mycteria* (Lichtenstein), which occurs in sympatry with *E. notaeus* in Bañado La Estrella, is known to include snakes in its diet, and has been reported as a host of *Contracaecum* spp. (Pinto et al., 2004). These ecological associations provide a plausible link between *E. notaeus* and avian definitive hosts. In this extensive floodplain, seasonal flooding promotes trophic interactions among aquatic invertebrates, fishes, reptiles, and piscivorous birds, thereby facilitating parasite transmission (Dománico et al., 2014). However, the large body size of adult *E. notaeus* may act as an ecological filter for trophic transmission, as larger snakes are likely to be less frequently consumed by potential predators than smaller (juvenile) individuals, potentially representing a dead-end host for the parasite.

Beyond documenting a new host record, we hypothesise that juvenile semi-aquatic yellow anacondas may play a role as paratenic hosts in the transmission dynamics of *Contracaecum* in Neotropical wetlands. Further studies integrating morphological and molecular data on *Contracaecum* larvae, along with dietary analyses and parasitological surveys of sympatric fishes, reptiles, and birds, will

Table 2. Parasitological descriptors of *Contracaecum* L3 from *E. notaeus* from Bañado La Estrella, Formosa, Argentina, by year.

N=Host number; PH=parasitized hosts; nL= number of larvae; P=prevalence; MA=mean abundance; MI=mean intensity; Me=median; Mo=mode; CI=95% confidence interval.

Year	N	PH	nL	P [CI]	MA [CI]	MI [CI]	Me [CI]	Mo
2019	30	7	16	23.33 [9.93–42.28]	0.53 [0.17–1.20]	2.29 [1.14–3.86]	1 [1–5]	1
2021	30	16	55	53.33 [34.33–71.66]	1.83 [1.07–3.60]	3.44 [2.31–6.31]	2.5 [1–4]	1
2022	45	14	47	31.11 [18.17–46.65]	1.04 [0.56–2.07]	3.36 [2.07–5.86]	2 [1–5]	1
2023	45	13	78	28.89 [16.37–44.31]	1.73 [0.93–2.93]	6 [4.00–8.23]	5 [1–11]	1

clarify the role of semi-aquatic snakes in maintaining anisakid nematode transmission networks in Neotropical wetlands. Because prey availability and trophic interactions vary markedly throughout the annual flood pulse, year-round studies combining dietary and parasite data will also be important to clarify how seasonal hydrological dynamics influence parasite transmission in these systems.

ACKNOWLEDGEMENTS

The authors would like to thank Nicolas Oswald, and Victoria Lichtschein from the Yellow Anaconda Management Program; Guillermo Panisse, Noralí Pagnutti, Agustín Abba, Lautaro Fuentes, and Virginia Monges from CEPAVE, for their assistance. We thank Patricia Sarmiento of Scanning Electron Microscope Service at the Museo de La Plata (FCNyM, UNLP). This study was authorised by Resolutions No. 0151/19, 050/21, 052/22, and 049/23 of the *Dirección de Recursos Naturales y Manejo* of the *Ministerio de la Producción y Ambiente* of Formosa, Argentina (Ministry of Production and Environment's Department of Natural Resources and Management).

FUNDING

This research was partially funded by Universidad Nacional de La Plata (N996 to JID), and Fundación Biodiversidad (to the Yellow Anaconda Management Program).

COMPETING INTERESTS

The authors declare no conflicts of interest.

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Recibido: 26 de julio de 2026

Aceptado: 16 de septiembre de 2026
