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Taxonomy and systematics

First report of *Aplectana hylambatis* (Nematoda: Cosmocercidae) in amphibians from the San Luis province, Argentina

Primer reporte de Aplectana hylambatis *(Nematoda: Cosmocercidae) en anfibios de la provincia de San Luis, Argentina*

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Abstract

The studies of helminth parasites in Argentine amphibians have focused on the Dry and Humid Chaco ecoregions; in the Dry Chaco the reports are from the Chaco and Formosa provinces. The aim of this work was to report the first record of *Aplectana hylambatis* (Baylis, 1927) Travassos, 1931 (Nematoda: Cosmocercidae) from San Luis province and the southernmost record for the Dry Chaco ecoregion. Specimens of *Rhinella arenarum* (Hensel, 1867) (n = 6), *Leptodactylus mystacinus* (Burmeister, 1861) (n = 13), and *Odontophrynus* cf. *asper* (n = 6) were analyzed. Specimens of *A. hylambatis* were collected from the large intestine of these hosts and were studied using light and scanning electron microscopy (SEM). In addition, relevant features are described, including the number and arrangement of caudal papillae, and of mamelon-like cuticular protuberances associated to the vulva. The morphological characteristics are compared and discussed in relation to those of specimens collected from other hosts in other localities of the country. The presence of *A. hylambatis* in localities of the Dry Chaco represents an expansion of the geographic range of this cosmocercid and the first record in *L. mystacinus* and *O.* cf. *asper* from Argentina.

Keywords: *Aplectana hylambatis*; Bufonidae; Cosmocercidae; Dry Chaco; Leptodactylidae; Odontophrynidae; Parasitic helminths

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Resumen

Los estudios de helmintos parásitos de anfibios argentinos se han centrado en las ecorregiones del Chaco Seco y Húmedo; en el Chaco Seco los reportes se realizaron en las provincias de Chaco y Formosa. El objetivo de este trabajo fue reportar el primer registro de *Aplectana hylambatis* (Baylis, 1927) Travassos, 1931 (Nematoda: Cosmocercidae) para la provincia de San Luis y el registro más austral para la ecorregión del Chaco Seco. Se analizaron ejemplares de *Rhinella arenarum* (Hensel, 1867) (n = 6), *Leptodactylus mystacinus* (Burmeister, 1861) (n = 13) y *Odontophrynus* cf. *asper* (n = 6). Los ejemplares de *A. hylambatis* fueron colectados del intestino grueso de estos hospedadores y se estudiaron mediante microscopía óptica y electrónica de barrido (MEB). Se describen características relevantes, como el número y disposición de las papilas caudales, y el número y disposición de las protuberancias cuticulares tipo mamelón asociadas a la vulva. Los caracteres morfológicos encontrados se comparan y discuten con los especímenes colectados en otros hospedadores de diferentes localidades del país. La presencia de *A. hylambatis* en localidades del Chaco Seco representa una ampliación del rango geográfico de este cosmocércido y el primer registro para *L. mystacinus* y *O.* cf. *asper* de Argentina.

Palabras clave: *Aplectana hylambatis*; Bufonidae; Cosmocercidae; Chaco Seco; Leptodactylidae; Odontophrynidae; Helmintos parásitos

Introduction

In 1927, Baylis described *Oxysomatium hylambatis* parasitizing the large intestine of *Leptopelis aubryi* (Duméril, 1856) from Macenta, Guinea, Africa (Baylis, 1927). In that work, the author established a synonymy between the genera *Oxysomatium* and *Aplectana*; however, he did not question the validity of the genus *Aplectana*, stating that it included species without a gubernaculum (called at that time "accessory piece").

Later, Travassos (1931) published a review of the family Cosmocercidae (Railliet, 1916) Travassos, 1925, where he established that the division between the 2 genera occurs mainly by the female reproductive system, which is prodelphic in the genus *Aplectana* and amphidelphic in *Oxysomatium*. In this way, it reproduces the description of the specimens made by Baylis (1927), but places them under the genus *Aplectana* due to the anterior location of the reproductive system of females.

Over the years, both genera have been studied in numerous works around the world. In some of them, these taxa were considered synonymous (Fotedar, 1960; Gutiérrez, 1945; Harwood, 1930; Hsü & Hoeppli, 1933; Skrjabin, 1910, 1951; Walton, 1940, 1941), whereas in others, they were separated on the basis of the size and shape of gubernaculum and spicules, the presence or absence of lateral alae, the number of cephalic papillae, the position of the vulva, and the stage of the eggs in utero (Bravo-Hollis, 1943), or on the basis of the presence or absence of gubernaculum in males, and prodelphic or amphidelphic condition in females (Ballesteros-Márquez, 1945).

Baker (1980) redescribes *A. hylambatis* (Baylis, 1927) Travassos, 1931 based on specimens collected from *Sclerophrys mauritanica* (Schlegel, 1841) (= *Bufo mauritanicus*), *Leptopelis aubryi* (Duméril, 1856), and *Rhinella achalensis* (Cei, 1972) (= *Bufo achalensis*). Subsequently, Baker and Vaucher (1986) synonymized *Oxysomatium bonariensis* (Gutiérrez, 1945) collected from *Rhinella arenarum* (Hensel, 1867) (= *Bufo arenarum*) in Argentina, and *A. pudenda* (Masi Pallarés and Maciel, 1974) collected from *Rhinella diptycha* (Cope, 1862) (= *Bufo paracnemis*), *Rana pacybrachion* (Steffen, 1815) (= *Leptodactylus latrans*), *Boana raniceps* (Cope, 1862), and *Boana punctata* (Schneider, 1799) (= *Hyla punctata*) in Paraguay, with *A. hylambatis*.

In South America, this cosmocercid has a wide geographic and host range; it was found in Argentina, Ecuador, Guyana, Paraguay, Peru, Brazil, and Uruguay (Campião et al., 2014; González, Hamann et al., 2021). In Argentina it is the nematode species of amphibians with the widest geographic distribution. There are records in 7 provinces, namely Buenos Aires, Córdoba, Corrientes, Chaco, Formosa, Salta, and San Juan, where it has been found parasitizing anurans of the families Bufonidae, Ceratophryidae, Hylidae, Leptodactylidae, Microhylidae, and Odontophrynidae (Draghi et al., 2015; González et al., 2019; González, Hamann et al., 2021).

Rhinella arenarum (Anura: Bufonidae) is distributed in Argentina, Bolivia, Brazil, and Uruguay (Frost, 2023). It is characterized by having terrestrial habits, reproducing in temporary or semi-permanent lentic and lotic environments where it deposits strings of pigmented eggs, and having a generalist diet (Babini et al., 2015; Bionda et al., 2011; de Oliveira et al., 2017; Quiroga et al., 2009). *Leptodactylus mystacinus* (Burmeister, 1861) (Anura: Leptodactylidae) is distributed in Argentina, Bolivia, Brazil, Paraguay, and Uruguay (Frost, 2023). It is a terrestrial anuran,

with fossorial characteristics; it breeds on foam nests built in temporary or semi-permanent lentic and lotic environments, and has a generalist diet (Babini et al., 2015; De-Carvalho et al., 2008; Heyer et al., 2010; López et al., 2005). These amphibian species are categorized as "Not Threatened" according to Vaira et al. (2012) and as "Least Concern" according to IUCN (2023). Finally, the genus *Odontophrynus* Reinhardt and Lütken, 1862 (Anura: Odontophrynidae), which includes 11 species, is distributed in southern and eastern South America (Frost, 2023). Recently, Argentinean species of this genus were revised by Rosset et al. (2022). These authors synonymized *Odontophrynus americanus* (Duméril and Bibron, 1841) with *O. occidentalis*, and established its distribution area, restricted to the center, west and south of the country, in the ecoregions of Monte, Dry Chaco, Patagonian steppe, and in the coastal dunes of the Humid Pampa ecoregion. On the other hand, these authors also proposed to name the cryptic diploid and tetraploid species currently grouped as the *Odontophrynus americanus* species group, as *Odontophrynus asper* (Philippi, 1902), with distribution in extensive areas of central and northern Argentina, Uruguay, southern Paraguay, southeastern Brazil, and probably also Bolivia (Rosset et al., 2022).

Studies on parasitic helminths in these 3 hosts, both in the larval and adult stages, refer to descriptions of new species, intraspecific variations, or extensions of host or geographic range (Campião et al., 2014; da Graça, et al., 2017; da Silva et al., 2018; González et al., 2019; González, Duré et al., 2021; González, Hamann et al., 2021; Queiroz et al., 2020; Ramallo et al., 2020). Throughout its geographic range, the helminth fauna of *R. arenarum* has been studied in Argentina, Brazil, and Uruguay (Campião et al., 2014). Specifically, in Argentina, *A. hylambatis* has been reported from Buenos Aires and San Juan provinces; *Rhabdias elegans* Gutiérrez, 1945 from Buenos Aires and Tucumán provinces; *Borrellostrongylus platensis* Gutiérrez, 1945 (= *Parabatrachostrongylus platensis* Tantalean and Naupay, 1974) from Buenos Aires province; *Oswaldocruzia proencai* Ben Slimane and Durette-Desset, 1995, *Ophidascaris* sp., *Aplectana tarija* Ramallo, Bursey and Goldberg, 2007, and *Cosmocercoides lilloi* Ramallo, Bursey and Goldberg, 2007 from Salta province; and *Bufonerakis andersoni* Baker, 1980 has been found but its locality was not reported (González and Hamann, 2015; González et al., 2019; Ramallo et al., 2020). For *L. mystacinus*, previous reports about its helminths have been made in Brazil and Paraguay (Campião et al., 2014); so far, no helminths have been found in this amphibian in Argentina. The previous records of helminths that have been made in the country for amphibians of the genus *Odontophrynus* include the nematodes *R. elegans*, *Cosmocerca podicipinus* Baker and Vaucher, 1984, and *C. parva* Travassos, 1925, and the digeneans Echinostomatidae gen. sp., and *Travtrema* aff. *stenocotyle* for *O. asper* (= *O. americanus*) from Corrientes province; *A. hylambatis* and *Falcaustra sanjuanensis* González, Sanabria and Quiroga, 2013 for *O. americanus* (= *O. occidentalis*) from San Juan province, and *A. hylambatis* for *O. lavillai* Cei, 1985 from Formosa province (Campião et al., 2014; González, Hamann et al., 2021; Piñeiro-Gómez et al., 2023).

The aim of this work was to report the first record of the cosmocercid *A. hylambatis* from the province of San Luis, Argentina, in the anurans *R. arenarum*, *L. mystacinus*, and *O.* cf. *asper*.

Materials and methods

San Luis, Argentina is part of the Dry Chaco ecoregion, and has xerophytic deciduous shrub vegetation with patches of *Larrea* (Morello et al., 2012). The sampled sites were located on the Central Sierras of San Luis. In the extreme west, these mountain ranges constitute an orographic barrier to humid winds from the east, which causes greater rainfall on the eastern slopes and drier climates to the west (Torrella & Adámoli, 2006). From November 2019 to February 2023, through active sampling techniques of direct visual recording and auditory detection (Vaira et al., 2021), 25 anuran amphibians of 3 species, belonging to 3 families, were captured: *R. arenarum* (Bufonidae) (n = 6), *L. mystacinus* (Leptodactylidae) (n = 13), and *O.* cf. *asper* (Odontophrynidae) (n = 6) (Table 1). Secretaría de Medio Ambiente, San Luis Province, authorized the capture of the specimens for this study (Resolution N°295-PRN-2022).

All organ systems were examined in the captured specimens, using helminthological techniques according to the criteria of Goater and Goater (2001) and González and Draghi (2021). Nematodes found in the large intestine, were counted, fixed with a hot 10% formalin solution, preserved in 70% alcohol, cleared with Amann's lactophenol and then, were morphometrically analyzed. Illustrations were made using a Leica DM2500 optical microscope with the aid of a drawing tube. For examination with scanning electron microscope (SEM), nematodes were dehydrated through a series of ethanol and acetone, and critical point-dried using CO_2. The specimens were coated with gold-palladium and examined with a JEOL JSM-5800 scanning electron microscope. Measurements along the text are given in micrometers unless otherwise indicated and are presented as the mean ± 1 standard deviation, with the minimum and maximum value in parentheses. Nematodes were deposited in the Helminthological Collection of

Museo de la Plata, La Plata, Argentina (MLP-He). The anuran specimens are stored in the Universidad Nacional de San Luis Herpetology Collection (CH-UNSL), San Luis, Argentina.

Results

Of the total amphibians analyzed, 11 were parasitized (total prevalence: 44%). In these, 338 specimens belonging to the species *Aplectana hylambatis* (Baylis, 1927) Travassos, 1931 (Nematoda, Cosmocercidae) were found (accession numbers: MLP-He 8044, 5 males and 5 females from *O.* cf. *asper*; MLP-He 8045, 5 males and 5 females from *L. mystacinus*; MLP-He 8046, 5 males and 5 females from *R. arenarum*).

Description
Aplectana hylambatis (Baylis, 1927) Travassos, 1931 [= *Oxysomatium bonariensis* Gutiérrez, 1945; = *Aplectana pudenda* Masi Pallarés and Maciel, 1974]
(Figs. 1-3)

General morphology (based on 82 adult specimens). Small nematodes, cuticle with transverse striations evenly distributed along the body. Lateral alae present in both sexes beginning at the level of the nerve ring and ending at the level of the anus in females and at the level of the first pairs of precloacal papillae in males. Triangular mouth surrounded by 3 lips, 1 dorsal with 2 cephalic papillae, and 2 ventral lips each with a papilla and an amphid (Fig. 1A). Well-differentiated esophageal bulb with chitinous valves. Very visible excretory pore, pre-bulbar and fringed (Fig. 2A). Prodelphic females with a highly developed ovijector (Fig. 2D, E); thin-membrane, morulated eggs and larvated eggs at the level of the vulva. Males with gubernaculum and equal spicules with characteristic articulation. The morphometric characters of males and females collected are provided in Table 2.

Male (based on 40 adult specimens). Specimens with 3 typical groups of caudal papillae present in this species, precloacal, adcloacal, and postcloacal, with a large medial unpaired papilla anterior to the cloaca. In the analyzed specimens, thirteen pairs of caudal papillae were counted with the following arrangement: 4 pairs

Table 1

Sample size (n), sex (F: females; M: males), collection date and accession numbers for 3 amphibian species from San Luis Province, Argentina.

Species	n	Sex	Collection data			Accession numbers
			Collection date	Locality-geographical coordinates	Elevation (m asl)	
Rhinella arenarum	2	1 F; 1 M	November/ 2019	33°06'38" S; 66°03'41" W El Trapiche	1,049	CH-UNSL 0644 CH-UNSL 0645
	1	1 M	October/ 2021	33°15'50" S; 66°12'32" W Cruz de Piedra	887	CH-UNSL 0646
	2	2 M	October/ 2021	33°16'35" S; 66°13'44" W Juana Koslay	850	CH-UNSL 0647
	1	1 F	December/ 2022	32°35'52" S; 66°06'37" W San Francisco del Monte de Oro	777	CH-UNSL 0636
Leptodactylus mystacinus	2	2 M	November/ 2019 October/ 2021	33°16'35" S; 66°13'44" W Juana Koslay	850	CH-UNSL 0638 CH-UNSL 0639
	11	2 F; 9 M	December/ 2021 January/ 2022 October/ 2022 November/ 2022 February/ 2023	33°6'22.6" S; 66°0'14.1" W La Florida	1,025	CH-UNSL 0634 CH-UNSL 0637 CH-UNSL 0631 CH-UNSL 0627 CH-UNSL 0630
Odontophrynus cf. *asper*	2	2 F	November/ 2019	33°11'53" S; 66°07'29" W Estancia Grande	1,071	CH-UNSL 0640
	2	1 F; 1 M	October/ 2021 November/ 2022	33°14'24.7" S; 66°10'46.4" W El Volcán	954	CH-UNSL 0635 CH-UNSL 0642
	2	2 M	January/ 2022 February/ 2022	33°6'22.6" S; 66°0'14.1" W La Florida	1,025	CH-UNSL 0632 CH-UNSL 0643

of ventral precloacal papillae; 5 pairs adcloacal (2 pairs ventrolateral, 3 pairs in anterior lip of the cloaca) and 1 unpaired papilla on anterior lip of the cloaca; 4 pairs of postcloacal papillae (2 pairs ventrolateral at the middle tail; 2 pairs close to its posterior end) (Figs. 1B-D; 2B, C).

Spicules and gubernaculum did not show morphological variation between specimens collected in different amphibian hosts and localities. Spicules showed the characteristic structure described for the species, long and chitinised, with a capitulum in the proximal end, and a pointed distal end that, when the spicules are outside the body, have a sickle or hockey stick appearance (Fig. 2H). Gubernaculum has an oval shape, chinitised, concave ventrally, with thickened edges, tapering towards the distal end (Fig. 2G).

Female (based on 42 adult specimens). Regarding the mamelon-like cuticular protuberances, most of the specimens analyzed showed between 1 large mamelon or 2 smaller mamelon-like protuberances located on the anterior lip of the vulva. However, in an analyzed

Table 2

Morphometric characters (mean ± SD and Min.-Max.) of males and females of *Aplectana hylambatis* collected in the 3 anuran species from San Luis Province, Argentina.

	San Francisco del Monte de Oro		La Florida		El Volcán	
	Rhinella arenarum		*Leptodactylus mystacinus*		*Odontophrynus* cf. *asper*	
	Males (n = 14)	Females (n = 11)	Males (n = 9)	Females (n = 10)	Males (n = 17)	Females (n = 21)
Total length	3.65±0.35mm (2.72-4.07)	4.37±0.36mm (3.55-4.85)	3.03±0.23mm (2.7-3.4)	3.53±0.20mm (3.22-3.87)	4.16±0.21mm (3.73-4.52)	5.0±0.30mm (4.5-5.4)
Greatest width	176.7±13.9 (145-205)	217.2±24.6 (185-265)	153.3±11.7 (135-170)	1047.5±133.5 (850-1250)	190.5±19.3 (150-220)	285.2±32.2 (235-360)
Oesophagus length	478.5±24.5 (415-505)	596.3±42.6 (490-645)	452.2±22.3 (405-480)	520.5±33.5 (455-550)	502.6±27.5 (450-550)	528.5±36.7 (430-575)
Oesophagus width	39.7±5.3 (31,2-48)	50.6±7.9 (33.6-60)	39.2±8.4 (28.8-57.6)	37.9±5.15 (31.2-45.6)	44.3±4.2 (38.7-53.2)	53.3±4.3 (45.2-61.1)
Bulb length	90.3±7.2 (74.4-98.4)	114.9±13.4 (84-129.6)	92±6.23 (84-100.8)	99.8±9.0 (84-117.6)	98.9±7.6 (91.77-118.37)	118.3±8.1 (106.4-113)
Bulb width	96.5±11.5 (72-110.4)	126.1±12.8 (98.4-148.8)	88.2±6.5 (76.8-98.4)	100.8±7.2 (88.8-115.2)	108±5.7 (99.7-119.7)	135.4±6.8 (122.3-148.9)
Nerve ring*	258.9±25.5 (205-295)	284.0±19.3 (245-305)	236.1±27.3 (200-275)	267.5±19.0 (245-300)	257.1±23.9 (200-295)	260.7±17.3 (240-300)
Excretory pore*	481±36.1 (400-515)	569.5±51.4 (450-630)	469.4±19.2 (435-495)	515.5±32.6 (470-560)	530.3±43.6 (475-636)	571.6±32.8 (505-640)
Tail length **	209.8±17.6 (172.8-235.2)	258.1±23.0 (210-285)	156.5±11.4 (144-172.8)	197±24.5 (150-225)	227.1±14.3 (205-260)	247.6±18.7 (210-280)
Gubernaculum length	76.1±8.33 (55.2-88.8)	-	68.5±6.4 (62.4-84)	-	85.9±7.1 (73-97)	-
Spicules length	242.4±21.7 (196.8-266.4)	-	208±18.8 (189.6–244.8)	-	284.8±23.3 (235-319)	-
Vulva*	-	2.9±0.2mm (2.4-3.25)	-	2.45±0.15mm (2.25-2.72)	-	3.32±0.22mm (2.86-3.71)
Egg length	-	90.5±5.4 (81.6-98.4)	-	84.2±5.1 (74.4-91.2)	-	105.83±11.9 (61.18-118.37)
Egg width	-	53.2±4.1 (48-60)	-	50.1±2.6 (48-55.2)	-	59.4±3.6 (53.2-65.1)

* From anterior end. ** In females, distance from anus to posterior end; in males, distance from cloaca to posterior end.

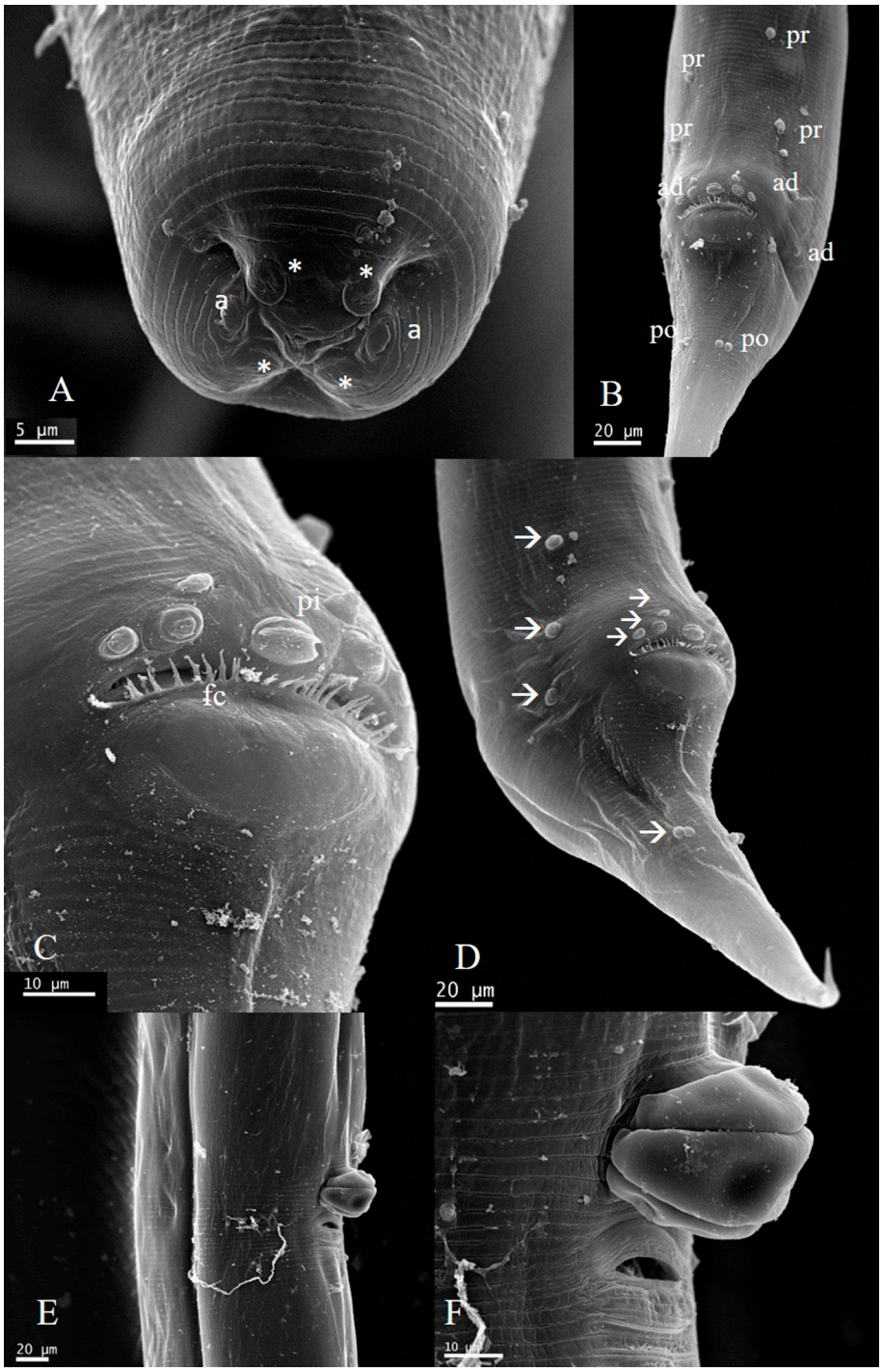

Figure 1. *Aplectana hylambatis* parasite of anurans from San Luis province, Argentina. A, Anterior end, apical view; B, male, posterior end, ventral view; C, detail of cloacal region, latero-ventral view; D, detail of paired papillae, latero-ventral view; E-F, female, mamelon-like cuticular, latero-ventral view. Abbreviations: a: amphid; ad: adcloacal papillae; fc: cuticular fringe; up: unpaired papilla; po: postcloacal papillae; pr: precloacal papillae; * cephalic papillae; closed arrows indicate paired papillae. A-D from *O.* cf. *asper*; E, F from *L. mystacinus*.

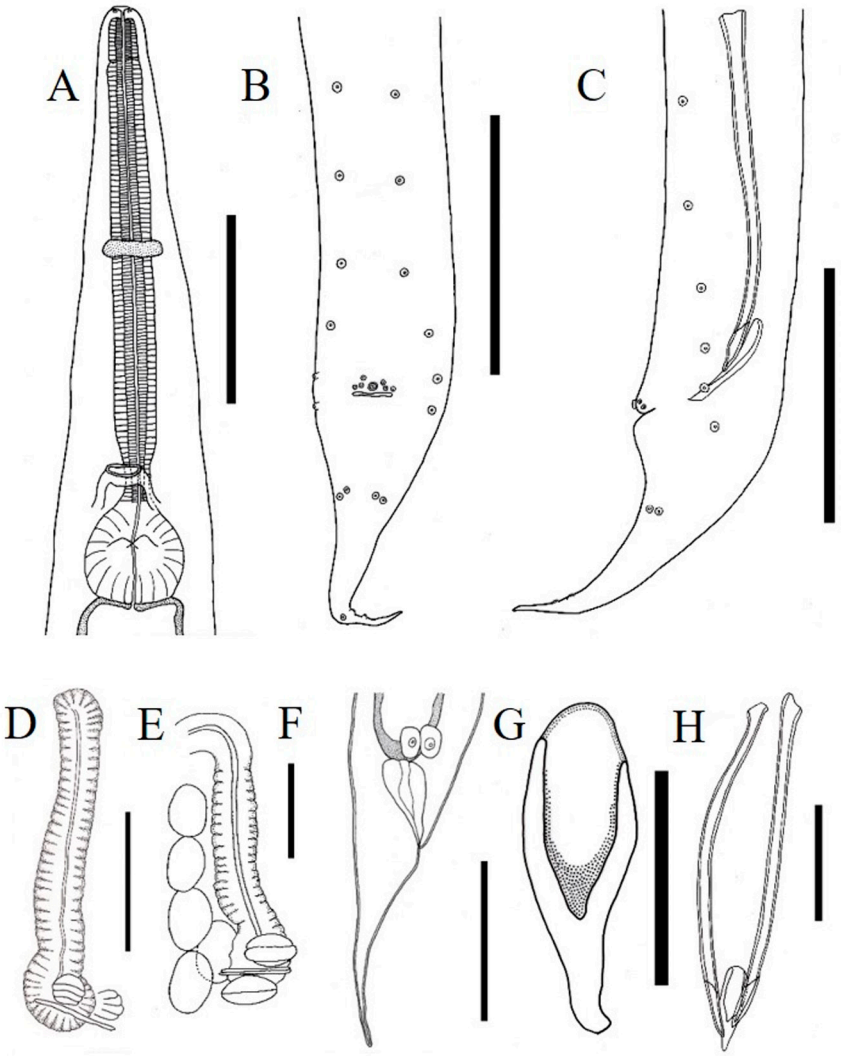

Figure 2. *Aplectana hylambatis* parasite of anurans from San Luis province, Argentina. A, Male, anterior end, ventral view; B, male, posterior end, ventral view; C, male, posterior end, lateral view; D, ovijector and 2 mamelon-like cuticular protuberances anterior to the vulva, ventral view; E, ovijector and 2 mamelon-like cuticular protuberances, 1 anterior and 1 posterior to the vulva, ventral view; F, female, posterior end, lateral view; G, gubernaculum, ventral view; H, spicules and gubernaculum, ventral view. Scale: A-D: 200 µm; E: 100 µm; F: 200 µm; G: 50 µm; H: 100 µm). A-C from *R. arenarum*; D-F from *L. mystacinus*; G, H from *O.* cf. *asper.*

specimen collected in *L. mystacinus*, 2 mamelon-like cuticular protuberances were observed, but with a different disposition than the one previously detailed; in this case, one protuberance was located anterior to the vulva and the other, posterior to it (Figs. 1E, F; 2D-F).

Some specimens collected from *R. arenarum* (2 couples) and from *L. mystacinus* (2 couples) were found in copulation (Fig. 3A). In these cases, a detailed examination of these specimens showed that only the right spicule enters the female reproductive system. In it, the distal end presented the typical articulation described in previous works. This articulation is in the elbow of the ovijector. The spicule that remains inside the male reproductive system was fully stretched. Although the specimens were clarified, it was not possible to identify the gubernaculum in the specimens in copulation (Fig. 3B).

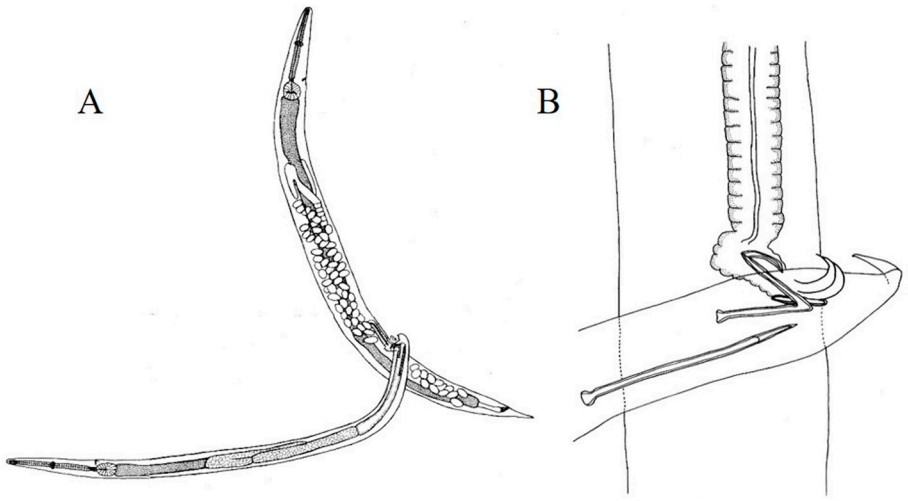

Figure 3. *Aplectana hylambatis* parasite of anurans from San Luis province, Argentina. A, Couples in copulation; B, detail of the arrangement of the spicules at the copulation time. Scale: A: 400 µm; B: 100 µm. A, B from *L. mystacinus*.

Discussion

The genus *Aplectana* is characterized by having lateral alae and somatic papillae in both sexes. Males lack plectanes or rosettes at the posterior end. Females have numerous thin-shelled eggs in the uterus and have prodelphic reproductive system (Gibbons, 2010). These characters were easily observable in the specimens studied here. The species of the genus *Aplectana* are grouped mainly according to the presence or absence of gubernaculum. Other features that distinguish these helminths are the number and arrangement of caudal papillae, and the size and morphology of the gubernaculum and spicules (Baker, 1980; Piñeiro-Gómez et al., 2017).

Morphological characters such as number and arrangement of caudal papillae in males, and mamelon-like cuticular protuberances on the anterior lip of the vulva in females, are presented in Table 3, and compared to those previously found in other hosts and other localities in Argentina. Regarding the number and arrangement of the caudal papillae, the males collected from the 3 host species presented a total of 13 pairs with the formula: 4:5:4+1. Compared with previous studies, only the specimens found in *T. typhonius* had a lower number of pairs of caudal papillae (12) (Draghi et al., 2015). Thirteen pairs of caudal papillae were also found in *P. santafecinus* and *R. major* from Taco Pozo (Table 3); however, their arrangement presented differences (*P. santafecinus*: 3:5:5; *R. major*: 4:4:5 *vs*. 4:5:4 in the present study). Finally, in males collected from other amphibians, a greater number of pairs of cloacal papillae were observed (up to 16 pairs in *D. muelleri*) (González et al., 2019) (Table 3).

The mamelon-like cuticular protuberances are structures that were not included in the original description of the species by Baylis (1927); however, subsequent studies have detailed their number and arrangement in specimens collected from different hosts and locations (Baker, 1980; González et al., 2019; Gutiérrez, 1945). In this study, these structures of the females varied between 1 and 2, and were located on the anterior lip of the vulva. This character of the females was like that found in the specimens studied in *R. arenarum* from La Plata, *R. major* from Concepción del Bermejo and Las Lomitas, and *L. bufonius* from Las Lomitas (Table 3) (González et al., 2019). However, one female presented a protuberance posterior to the vulva; and to our knowledge this had not been reported until now. Finally, of all the morphological characters studied, mamelon-like cuticular protuberances varied the most among hosts and localities (González et al., 2019; Gutiérrez, 1945) (Table 3).

In reference to the specimens in copulation, they were found with the typical arrangement of the male coiled by its posterior end to the female at the level of the vulva and forming an angle of approximately 90 degrees between them. In nematode mating, the spicules play an active role in copulation, while the gubernaculum, which is typically not everted during this process, acts as a guide for the spicules and prevents them from piercing the wall of the spicular pouch and cloaca when pressure is exerted on them (Chitwood & Chitwood, 1974).

In the 4 couples studied, it was observed that the right spicule is inserted into the vagina of the female and the articulation of the distal part is curved at the level of the ovijector's elbow, while the left spicule remains fully

Table 3

Some morphological characters of males and females of *Aplectana hylambatis* of the present study compared with specimens collected in different hosts in Argentina. n.e. = not specified.

	Total number of pair papillae	Number and arrangement of caudal papillae *	Number of mamelon-like cuticular protuberances	Locality	Reference
Bufonidae					
Rhinella achalensis		n.e.	n.e.	Córdoba	a
Rhinella arenarum	15	4:5:6+1	1-2	La Plata, Buenos Aires	b
Rhinella arenarum	15	4:5:6+1	1	Presidente Sarmiento, San Juan	c
Rhinella arenarum	15	4:5:6+1	1-2	La Plata, Buenos Aires	d
Rhinella arenarum	13	4:5:4+1	1-2	San Francisco del Monte de Oro, San Luis	Present study
Rhinella major	14	4:5:5+1	2	Ingeniero Juárez, Formosa	d
Rhinella major	13	4:4:5+1	2	Taco Pozo, Chaco	d
Rhinella major	14	4:5:5+1	1-2	Concepción del Bermejo, Chaco	d
Rhinella major	14	5:4:5+1	1-2	Las Lomitas, Formosa	d
Hylidae					
Trachycephalus typhonius (Linnaeus, 1758)	10-11	3-4:4:3+1	n.e	Pirané, Formosa	e
Leptodactylidae					
Leptodactylus bufonius (Boulenger, 1894)	14	5:4:5+1	1-2	Las Lomitas, Formosa	d
Leptodactylus bufonius (Boulenger, 1894)	15	4:5:6+1	1	Taco Pozo, Chaco	d
Leptodactylus bufonius (Boulenger, 1894)	14	5:4:6+1	2-3	Corrientes, Corrientes	d
Leptodactylus mystacinus	13	4:5:4+1	1-2	La Florida, San Luis	Present study
Physalaemus santafecinus (Barrio, 1965)	13	3:5:5+1	1	Corrientes, Corrientes	f
Microhylidae					
Dermatonotus muelleri (Boettger, 1885)	16	3:5:8+1	1	Las Lomitas, Formosa	d
Odontophrynidae					
Odontophrynus cf. *asper*	13	4:5:4+1	1-2	El Volcán, San Luis	Present study
O. americanus (= *O. occidentalis*)	14	4:5:5+1	2	Quebrada de las Flores, San Juan	g

* Precloacal papillae: adcloacal papillae, postcloacal papillae + unpaired papilla. References: a: Baker (1980); b: Gutiérrez (1945); c: González et al. (2013); d: González et al. (2019); e: Draghi et al. (2015); f: González and Hamann (2010); g: Piñeiro-Gómez et al. (2023).

elongated in the male without showing the articulation. Regarding this, the spicules serve to keep the vulva and vagina open. In trichostrongylids, the branching of the spicular lamina and the formation of twisted spicules seem to be adapted to this activity; in taxa with unequal spicules, the short spicule opens the vulva and the proximal region of the vagina, while the long one ensures sperm progression (Chitwood & Chitwood, 1974). In reference

to the gubernaculum, unfortunately, we could not observe its location in the specimens studied. Thus, it is necessary to find new mating specimens to elucidate the position it occupies during copulation.

Intraspecific variations in parasitic nematodes of amphibians have been recorded for different families such as Molineidae (Skrjabin & Schulz, 1937) Durette-Desset and Chabaud, 1977, Onchocercidae (Leiper, 1911), Pharyngodonidae (Travassos, 1919), and Cosmocercidae (González et al., 2019; Kirillova et al., 2021; Kuzmin et al., 2023; Rhoden & Bolek, 2011; Vhora & Bolek, 2013; Willkiens et al., 2023). Specifically, in *A. hylambatis*, previous studies carried out in different hosts from different locations of the Neotropical realm showed variations in 2 of the characters studied here, namely number and arrangement of mamelon-like cuticular protuberances and number and arrangement of cloacal papillae, in addition to variations in the total length of individuals of both sexes (Baker, 1980; González et al., 2019; Gutiérrez, 1945; Masi-Pallarés & Maciel, 1974). In this regard, intraspecific variation may be attributed to a number of factors related to the host, such as size, age, physical condition, metabolic rate, and food supply, and factors related to the parasite, such as the time of penetration (first, second or subsequent infection) and the crowding of the specimens, or related to different study techniques, in addition to ecological and geographic factors (Baker, 1980; Chitwood, 1957; Perkins et al., 2011; Vhora & Bolek, 2013).

The studies of helminth parasites of Argentine amphibians have focused on the Dry and Humid Chaco ecoregions; specifically, in the Dry Chaco the reports have been made in the northern provinces of the country, Chaco and Formosa. Until now, the southernmost records in this ecoregion correspond to Concepción del Bermejo in Chaco province (González, Duré et al., 2021; González, Hamann et al., 2021). In this way, the present study expands the knowledge on parasitic helminths from the South American Chaco by providing the southernmost record for the Dry Chaco ecoregion. Furthermore, it represents the first record of *A. hylambatis* in *L. mystacinus* and *O.* cf. *asper* from Argentina.

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