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ORIGINAL PAPER

Larval Digenean Parasitizing Amphibian Hosts from the Argentinian Chaco Region

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Abstract

Purpose The diversity of larval digenetic trematodes can provide information on one or more intermediate hosts prior to maturation into the definitive host; on host activity and distribution; and in some cases, on environmental perturbations. In this context, the goal of the present study was to analyse the trematode larvae found in adult amphibians collected from the Argentinian Chaco Region. Few studies have been reported on the systematics of larval digeneans in amphibians.

Methods A total of 167 specimens of frogs (*Leptodactylus macrosternum*, *Leptodactylus latinasus*, *Lepidobatrachus laevis*, *Lepidobatrachus llanensis* and *Lysapsus limellum*) and toads (*Rhinella bergi*) were examined for larval digeneans. Frogs and toads were collected in two Argentinian ecoregions (Dry Chaco and Humid Chaco); between June 2002 and December 2019. Morphology of each taxon was studied in detail using light microscopy and scanning electron microscopy (SEM).

Results Fourteen different taxa of the families Diplostomidae (Alariinae gen sp., *Didelphodiplostomum* sp., *Pharingostomoides* sp. and *Tylodelphys* sp.), Proterodiplostomidae (*Heterodiplostomum lanceolatum*), and Strigeidae (*Strigea* spp.) were found infecting the body cavity, mesentery, muscle, fluid in the spinal cord canal, and cranial cavity of amphibians. All species are described and illustrated, and their life cycles are briefly discussed.

Conclusion These infections provide new information on the morphology, morphometry, and composition of the diversity of mesocercariae and metacercariae in amphibians, thus contributing to the knowledge of potential intermediate hosts. In addition, our results serve as an indicator of parasite and host biodiversity in different ecoregions of the Gran Chaco.

Keywords Trematoda · Biodiversity · Transmission strategies · Amphibian · Argentina

Introduction

From a parasitological perspective, understanding parasite transmission routes is a major issue in the study of larval digeneans (metacercariae and mesocercariae) in amphibians. These amphibian hosts exploit several types of microhabitats, act as effective links between aquatic and terrestrial environments through multiple trophic levels, and are in contact with different larval parasite species whose potential final hosts include reptiles, birds, and mammals [1]. Thereby, amphibians from the Humid Chaco Ecoregion could be potential intermediate hosts in the life cycle of different families of digeneans such as Diplostomidae Poirier, 1886, Proterodiplostomidae Dubois, 1936, Echinostomatidae Poche, 1926, Ophistogonimidae Freitas, 1956, Plagiorchiidae (Lühe, 1901), and Strigeidae Railliet, 1919 [2–7] whose infections involve planorbid snails as first intermediate hosts to complete their life cycle.

The cercariae that emerge from the snails invade different development stages (tadpole or adult) of amphibians as second intermediate hosts and develop as encysted or free metacercariae before maturating in the definitive host, a terrestrial vertebrate such as amphibians, birds, mammals, among others. Likewise, some parasite species of the alariid and strigeid groups that penetrate various amphibian hosts develop into mesocercariae, a motile larval stage between cercariae and metacercariae [8–10].

The approach used in the present study predicts that the probabilities of infection by larval digenetic trematodes are dictated by the surrounding diversity of invertebrate and vertebrate animals and the variability of the

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microhabitat conditions, i.e. wet periods favour the ecological opportunity for interactions between aquatic and semiaquatic species and the establishment of new host-parasite associations. Thus, the diversity of larval digenetic trematodes can provide information on one or more intermediate hosts prior to maturation into the definitive host; on host activity and distribution; and in some cases, on environmental perturbations such as eutrophication, contamination, and habitat alteration, among others [1, 7, 11]. Few studies have been reported on the systematics of larval digeneans in tadpoles [12] and adult amphibians [6] from different environmental conditions in the Argentinian Chaco Region.

In this context, our aim was to provide new morphological and metric information on mesocercariae and metacercariae, and to present quantitative descriptors of larval infections in naturally infected adult amphibians from Dry and Humid Chaco Ecoregions.

Materials and Methods

Amphibians were collected in two ecoregions from Argentina [13], between June 2002 and December 2019: Ingeniero Juárez, Formosa Province in the Dry Chaco Ecoregion (DC), situated at 24° 12′ S, 61° 58′ W (sampling area: non-agricultural); and Corrientes, Corrientes Province in the Humid Chaco Ecoregion (HC), situated at 27° 30′ S, 58° 45′ W (sampling area: non-agricultural), and at 27° 48′ S, 58° 43′ W (sampling area: agricultural) (Fig. 1). A total of 167 adult specimens of 6 species were examined: *Leptodactylus macrosternum* Miranda-Ribeiro,

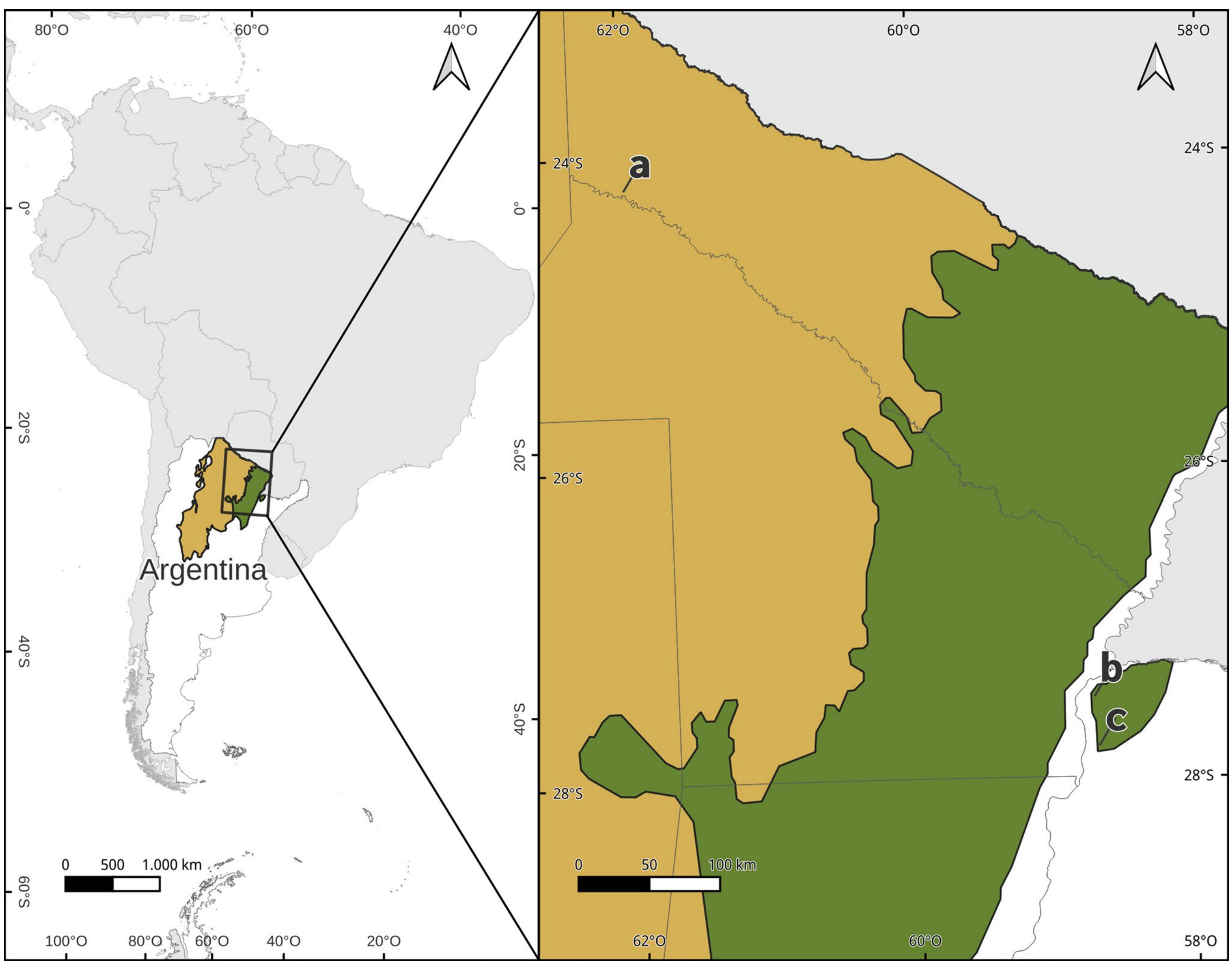


Fig. 1 Map of study localities in Dry Chaco (DC light brown) and Humid Chaco (HC green) from Argentina, using the program Qgis 3.26.3 (QGIS.org. 2022. QGIS Geographic Information System. QGIS Association. http://www.qgis.org). **a** Ingeniero Juárez, Formosa Province, DC; **b** Corrientes, Corrientes Province (a non-agricultural area), HC; **c** Corrientes, Corrientes Province (an agricultural area), HC

1926, *Leptodactylus latinasus* Jiménez de la Espada, 1875 (Leptodactylidae), *Rhinella bergi* (Céspedez, 2000) (Bufonidae) and *Lysapsus limellum* Cope, 1862 (Hylidae) from HC; *Lepidobatrachus laevis* Budgett, 1899 and *Lepidobatrachus llanensis* Reig and Cei, 1963 (Ceratophryidae) from DC. The classification for amphibians is according to Frost [14]. All specimens were anaesthetized using topical 2% lidocaine cream, and then dissected following standard protocols [15]. The present study complies with all the regulations and ethical and legal considerations for the capture and use of animals established by the National Council of Scientific and Technical Research (CONICET) of Argentina; we also followed the recommendation of the ASIH/HL/SSAR Guidelines for the Use of Live Amphibians and Reptiles as well as the regulations detailed in Argentinean National Law #14,346. The mesentery, external layer of stomach, lungs, liver, kidneys, body cavity, musculature, integument, and brain were examined for parasites. Larval trematodes were counted and isolated from host tissues, and then removed from cysts by using preparation needles. They were studied either *in vivo* or mounted in 0.6% saline solution or killed in hot distilled water, fixed in 70% ethyl alcohol, stained with hydrochloric carmine, and mounted in Canada balsam. Measurements are given as mean and standard deviation, and minimum and maximum, in micrometres (µm). Parasites were identified following Yamaguti [16, 17] and Gibson et al. [18]. Photographs were taken with a Leica DFC 295 camera mounted on a Leica DM 2500 microscope (Leica Microsystems, Germany). For examination using scanning electron microscopy (SEM), some specimens were dehydrated through an ethanol series and acetone. The specimens were gold-coated and examined using a Jeol 5800LV (Jeol, Tokyo, Japan). Representative specimens were deposited at the Helminthological Collection of the Centro de Ecología Aplicada del Litoral (CECOAL), Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Corrientes, Argentina.

Larval trematodes are presented according to family in alphabetical order, each taxon contains information about host and locality, site of infection, data of collection and quantitative descriptors. Prevalence and intensity of infection were calculated according to Bush et al. [19].

Results

Fourteen different digenean taxa were found in six species of anuran. Morphometric data of larval trematodes (mesocercariae and metacercariae) are shown in Table 1. Regarding the description of each taxon, it is based on the most remarkable morphological character.

Diplostomidae

Alariinae gen. sp. (Fig. 2a).

Mesocercaria elongated with spines on tegument, being less dense at posterior end. Oral and ventral suckers present. Pharynx muscular. Oesophagus immediately bifurcates to form the ceca, which extend laterally to almost posterior end of body. Four large penetration glands located anterior to the ventral sucker. Genital primordia present in hindbody.

Comment: alariid mesocercaria is the first record in frogs for South America.

Site of infection: body cavity.

Date of collection and quantitative descriptors: February 2011; prevalence of infection, 100%; maximum intensity, 6 mesocercariae (*L. laevis*). December 2019; 19%; 20 mesocercariae (*L. llanensis*).

Voucher material: CECOAL 19120209.

Host and locality: *L. laevis* ($n=1$) and *L. llanensis* ($n=21$) collected from Ingeniero Juárez, Formosa Province, Argentina.

Didelphodiplostomum sp. (Fig. 2b, c).

Larva diplostomulum surrounded by an elastic layer apparently formed by the intermediate host as a reaction against the parasite encapsulated, without cyst of parasite origin. Body bipartite, division into fore and hindbody. Forebody concave ventral. Hindbody short, ovoid to conical. Pseudosuckers present, muscular well developed. Oral and ventral suckers well developed. Pharynx ellipsoidal and strongly muscular. Holdfast organ round or transversely oval, overlying ventral sucker.

Comment: metacercaria of genus *Didelphodiplostomum* is the first record for South America.

Site of infection: body cavity.

Date of collection and quantitative descriptors: February 2018; prevalence of infection, 5%; maximum intensity, 24 diplostomula encapsulated.

Voucher material: CECOAL 18021915.

Host and locality: *L. llanensis* ($n=21$) collected from Ingeniero Juárez, Formosa Province, Argentina.

Pharyngostomoides sp. (Fig. 2 d, e, f, g).

Cyst oval to elliptical, 920—1130 (966 ± 71) long by 550—980 (581 ± 25) wide. Fibrous capsule with larva diplostomulum encapsulated, surrounded by a thin elastic layer apparently formed by the intermediate host as a reaction against the parasite. Larvae easily released. Body indistinctly bipartite, oval, forebody spatulate. Pseudosuckers present. Oral and ventral suckers of similar size. Pharynx muscular. Holdfast organ large, oval, anteriorly bilobed. Excretory pore at posterior tip of body; the protonephridial system with numerous corpuscles in forebody.

Comment: metacercariae of genus *Pharingostomoides* is the first recorded in South America.

Table 1 Morphometric data of larval trematodes found in amphibians from Argentinian Chaco Region (mean values ± SD, and minimum–maximum provided in parentheses)

	Total body		Forebody	Hindbody	Oral sucker		Ventral sucker		Pharynx	
	L	W	L	L	L	W	L	W	L	W
Mesocercariae										
Alariinae gen. sp. (*n* = 10)	606 ± 222 (300–850)	244 ± 58 (165–330)	– –	– –	74 ± 20 (55–100)	80 ± 28 (50–120)	71 ± 18 (50–100)	76 ± 13 (60–100)	26 ± 12 (15–50)	27 ± 12 (15–50)
Metacercariae										
Didelphodiplostomum sp. (*n* = 10)	513 ± 80 (405–580)	338 ± 83 (230–450)	394 ± 60 (320–460)	94 ± 11 (80–110)	48 ± 6 (42–55)	48 ± 6 (40–55)	45 ± 6 (40–53)	56 ± 8 (50–70)	48 ± 12 (40–60)	31 ± 8 (23–40)
Pharyngostomoides sp. (*n* = 5)	868 ± 207 (500–1000)	539 ± 176 (240–700)	810 ± 113 (700–950)	208 ± 46 (140–250)	76 ± 18 (50–100)	74 ± 14 (52–90)	75 ± 21 (45–100)	81 ± 19 (50–100)	31 ± 2 (30–35)	35 ± 8 (30–49)
Tylodelphys sp. (*n* = 10)	679 ± 238 (465–1150)	162 ± 122 (80–450)	– –	– –	35 ± 10 (25–55)	37 ± 6 (30–48)	40 ± 11 (30–60)	42 ± 15 (32–75)	27 ± 11 (20–50)	27 ± 10 (20–48)
Heterodiplostomum lanceolatum (*n* = 3)	6067 ± 569 (5600–6700)	757 ± 81 (700–850)	3567 ± 450 (3100–4000)	2500 ± 200 (2300–2700)	45 ± 7 (40–50)	48 ± 6 (44–52)	163 ± 32 (110–200)	162 ± 47 (75–150)	70 ± 7 (65–75)	68 ± 11 (50–75)
Strigea sp. 4 (*n* = 8)	433 ± 52 (390–495)	314 ± 14 (300–330)	374 ± 44 (335–435)	60 ± 17 (40–80)	65 ± 6 (55–70)	66 ± 4 (59–70)	90 ± 9 (70–100)	99 ± 7 (87–106)	31 ± 2 (30–33)	29 ± 2 (27–30)
Strigea sp. 5 (*n* = 5)	397 ± 6 (390–400)	275 ± 9 (270–285)	305 ± 9 (300–315)	92 ± 8 (85–100)	62 ± 5 (58–80)	65 ± 9 (62–70)	70 ± 3 (65–73)	82 ± 4 (75–85)	36 ± 5 (30–40)	32 ± 4 (28–35)
Strigea sp. 7 (*n* = 5)	480 ± 78 (360–552)	364 ± 55 (325–460)	358 ± 111 (240–460)	68 ± 18 (55–80)	69 ± 13 (50–88)	81 ± 13 (60–98)	75 ± 15 (54–100)	94 ± 17 (80–120)	24 ± 1 (24–25)	25 ± 4 (20–25)
Strigea sp. 8 (*n* = 5)	462 ± 79 (405–585)	336 ± 33 (300–385)	400 ± 74 (340–525)	70 ± 12 (60–90)	75 ± 6 (70–83)	71 ± 7 (65–80)	99 ± 10 (90–115)	103 ± 10 (90–115)	38 ± 3 (35–40)	39 ± 1 (38–40)
Strigea sp. 9 (*n* = 10)	406 ± 70 (330–520)	263 ± 32 (220–300)	301 ± 59 (250–430)	96 ± 40 (70–160)	62 ± 10 (40–73)	62 ± 9 (50–80)	60 ± 9 (50–75)	78 ± 7 (70–90)	32 ± 3 (28–35)	30 ± 3 (24–34)
Strigea sp. 11 (*n* = 2)	468 ± 25 (450–485)	383 ± 11 (375–390)	413 ± 18 (400–425)	55 ± 7 (50–60)	79 ± 13 (70–88)	73 ± 11 (65–80)	90 ± 1 (89–90)	99 ± 1 (98–100)	30 ± 1 (29–30)	25 ± 1 (24–25)
Strigea sp. 12 (*n* = 10)	371 ± 57 (335–470)	297 ± 24 (270–325)	273 ± 19 (255–300)	74 ± 8 (70–85)	69 ± 11 (60–89)	65 ± 7 (58–72)	85 ± 12 (71–100)	99 ± 10 (88–120)	28 ± 2 (25–30)	27 ± 4 (22–30)
Strigea sp. 13 (*n* = 1)	370	260	290	80	53	55	60	62	20	17
Strigea sp. 14 (*n* = 10)	449 ± 44 (400–550)	327 ± 31 (26–360)	361 ± 17 (332–430)	96 ± 18 (75–120)	67 ± 4 (60–70)	60 ± 5 (50–65)	70 ± 10 (58–85)	99 ± 9 (80–114)	22 ± 3 (18–25)	23 ± 2 (19–24)

(L) Length; (W) Width

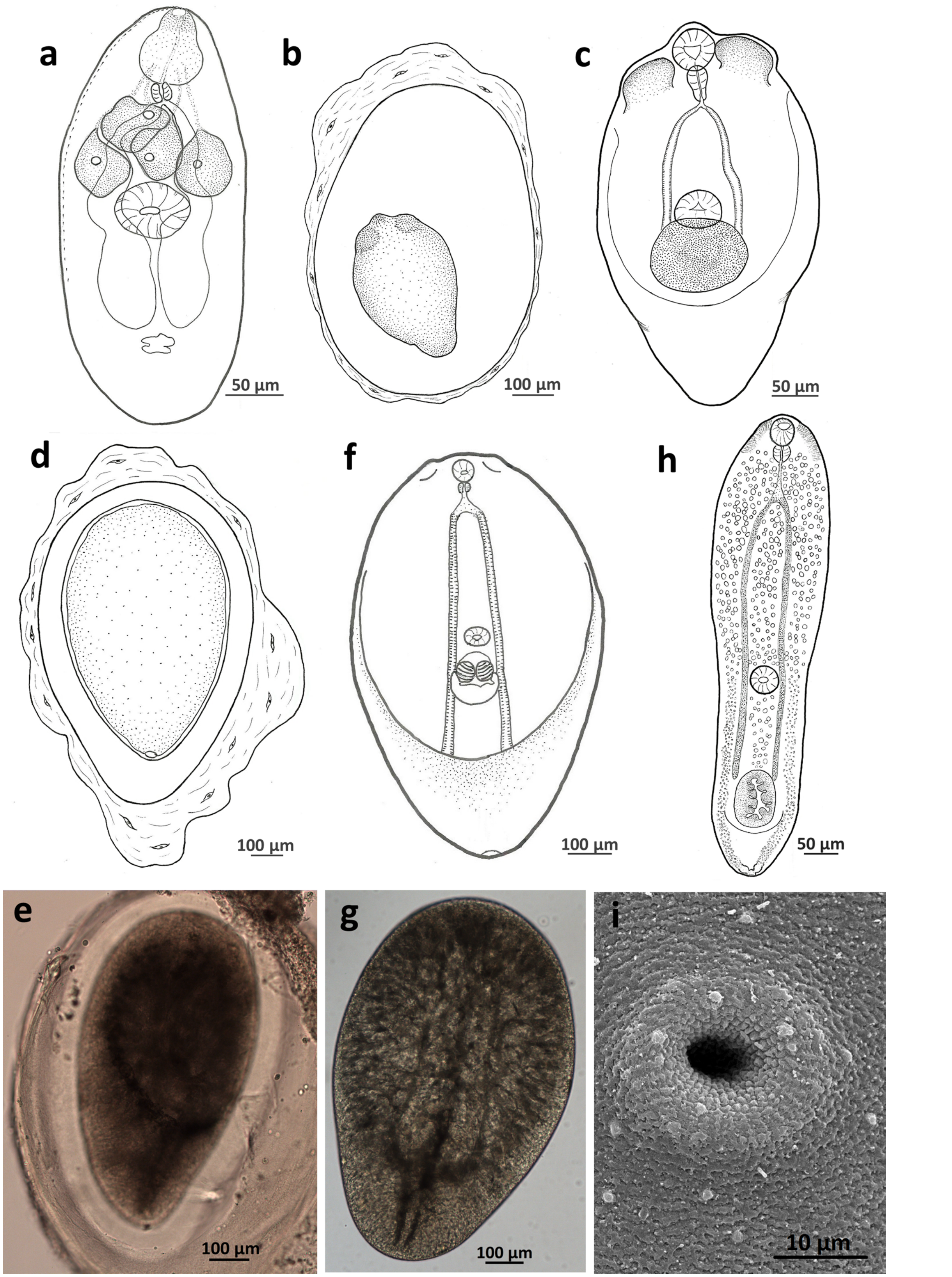


Fig. 2 Alariinae gen. sp. **a** whole-mount of mesocercariae, ventral view. *Didelphodiplostomum* sp. **b** encapsulated metacercaria; **c** whole-mount of excysted larva, ventral view. *Pharyngostomoides* sp. **d**, **e** fibrous capsule with metacercaria; **f**, **g** whole- mount of excysted larva, ventral view. *Tylodelphys* sp. **h** whole-mount of metacercaria, ventral view; (**i**) tegument with papillae

Site of infection: body cavity and muscle.

Date of collection and quantitative descriptors: February 2018; prevalence of infection, 33%; maximum intensity, 17 cysts.

Voucher material: CECOAL 18021935.

Host and locality: *L. llanensis* ($n=21$) collected from Ingeniero Juárez, Formosa Province, Argentina.

Tylodelphys sp. (Fig. 2h, i).

Larva tylodelphylus type free in cranial cavity and fluid of spinal cord canal. Body linguiform, indistinctly bipartite, hindbody conical; tegument with papillae. Pharynx, oral sucker and ventral sucker small. Holdfast organ oval with median slit. Excretory bladder V-shaped with pore at posterior tip of body.

Comment: the specimens in the present study closely resemble *T. excavata* (Rudolphi, 1803) in size (total body length = 319–1061; body width = 162–335; oral sucker = 33–55 × 40–48; ventral sucker = 37–44 × 20–30; pharynx = 21–46 × 19–22), body structure (position of ventral sucker, and short and conical hindbody) and intermediate hosts (frogs). Additionally, it shows some resemblance to *T. jenynsiae* Szidat, 1969 and *T. cardiophilus* Szidat, 1969; however, the intermediate hosts (fishes) and sites of infection (body cavity and pericardial cavity) are very different [20].

Site of infection: fluid of spinal cord canal and cranial cavity.

Date of collection and quantitative descriptors: March 2017; prevalence of infection, 3%; maximum intensity, 32 larvae.

Voucher material: CECOAL 17030102.

Host and locality: *L. limellum* ($n=39$) collected in the agricultural area of Corrientes, Corrientes Province, Argentina.

Proterodiplostomidae

Heterodiplostomum lanceolatum Dubois, 1936 (Fig. 3a, b).

Larva proterodiplostomid without cyst of parasite origin, free or encapsulated. Body distinctly bipartite, division into fore and hindbody; forebody flattened, lanceolate, hindbody cylindrical elongated. Oral sucker and pharynx very small. Ventral sucker larger. Holdfast organ longitudinally elongated, about 40% of forebody length. Oesophagus very short, caeca reaching almost to posterior end. Ovary pretesticular. Testes tandem. Uterus and ejaculatory ducts open by separate pores into small, ovoid copulatory bursa, which opens through a dorso-subterminal genital pore.

Comment: the present study does not show differences with the morphometric data obtained in proterodiplostomid larvae found in the same family of frogs (Leptodactylidae) from Brazil and Uruguay [21].

Site of infection: body cavity.

Date of collection and quantitative descriptors: April 2003; prevalence of infection, 5%; maximum intensity, 1 larva encapsulated (*L. macrosternum*). October 2017; 50%; 1 (*L. latinasus*).

Voucher material: CECOAL 03042807, 17101726.

Host and locality: *L. macrosternum* ($n=40$) collected in the non-agricultural area and *L. latinasus* ($n=2$) collected in the agricultural area from Corrientes, Corrientes Province, Argentina.

Strigeidae

Strigea Abildgaard, 1790.

Tetracotyliform larva not easily released. Body bipartite, division into forebody cup-shaped, with oblique aperture. Hindbody cylindrical curved dorsally. Oral and ventral suckers larger. Pseudosuckers present (e.g., *Strigea* sp. 12, Fig. 3c). Pharynx strongly muscular. Holdfast organ composed by dorsal and ventral lobes (e.g., *Strigea* sp. 4, Fig. 3d), with different morphology among species, and proteolytic gland posterior to holdfast organs. Genital primordia present in hindbody. Reserve bladder arranged as a net in the forebody, with the excretory bodies free in the canals of the reserve bladder.

Comment: of the features listed above, the pharynx, pseudosuckers, and non-rudimentary ventral sucker, as well as tetracotyle-type cysts in amphibians were considered sufficient to exclude the following genera: *Apharyngostrigea* (pharynx absent), *Schwartzitrema* (pseudosuckers in form of longitudinal muscular rolls), *Duboisella* (rudimentary ventral sucker), *Ophiosoma* and *Parastrigea* (pseudosuckers absent), *Pseudoapatemon* (no prominent pseudosuckers), *Ichtyocotylurus* and *Apatemon* (infect fish), *Australapatemon* (infect leeches), and *Cotylurus* (infect mainly snails).

Nine taxa (cysts and excysted metacercariae) were morphological and metrical described, and compared with three metacercariae (*Strigea* sp. 1, *Strigea* sp. 2 and *Strigea* sp. 3) previously recorded in frogs from the Chaco Region [6]. Of these 12 taxa of strigeids, the morphometric data of encysted metacercariae in relation to the different shape and size of the cysts and the sizes of the layers of the cyst wall are present in Table 2. The main difference among these larvae is in relation to the specific morphometric structure of the cyst (Table 2). The morphological layers of the cyst wall are the innermost hyaline layer originated by the parasite (indicated in black) and the outer radially laminated layer (indicated by the lamellae), which is surrounded by an elastic protective layer, apparently formed by the host. Also, the morphological characters of the excysted metacercaria, such as the morphology of the holdfast organ, the shape and location of the pseudo-suckers, and the relationships between suckers are present in Table 3.

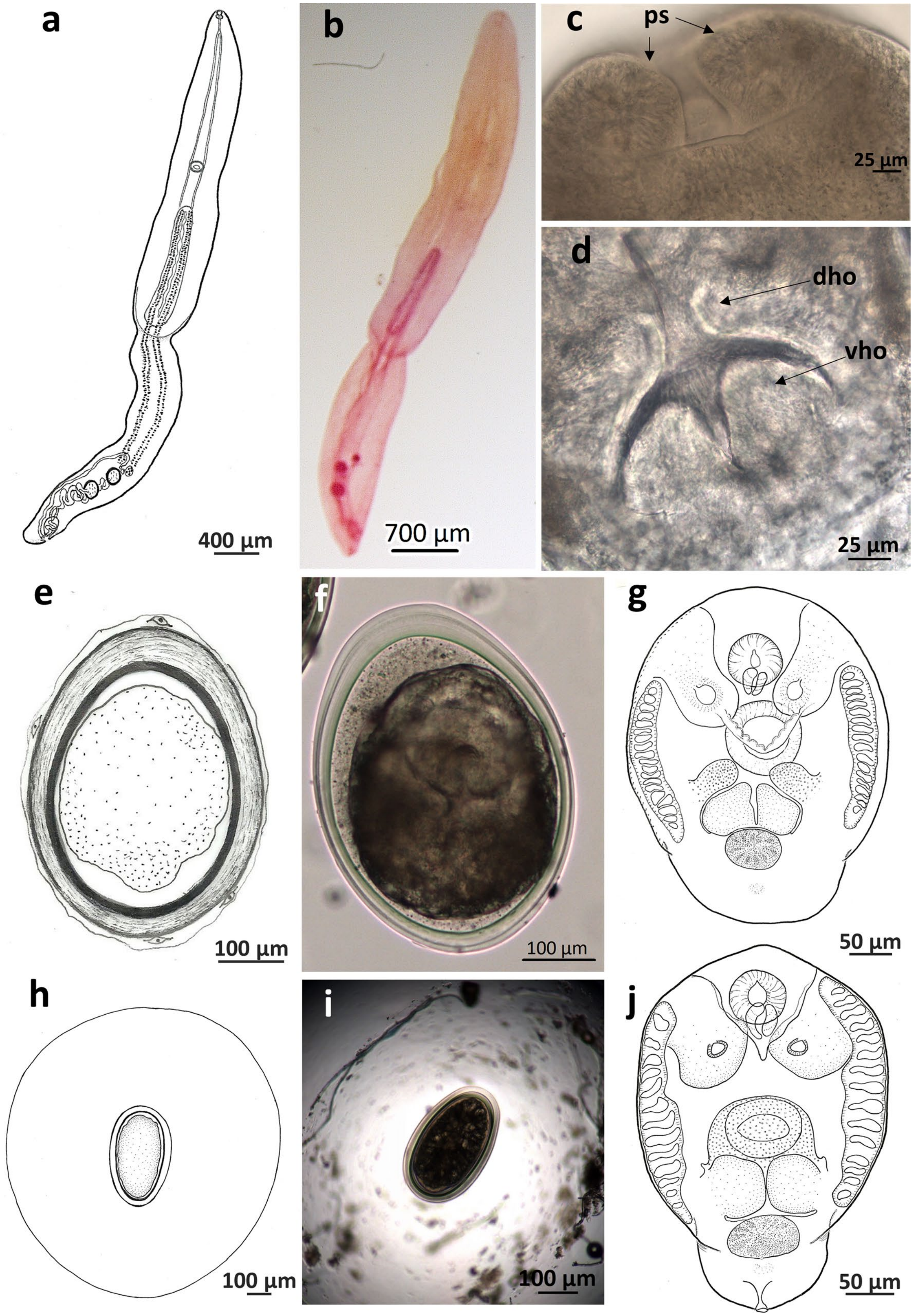


Fig. 3 *Heterodiplostomum lanceolatum* **a**, **b** whole- mount of excysted metacercaria, ventral view. Metacercaria **c** pseudosuckers, ps and **d** ventral holdfast organ, vho; dorsal holdfast organ, dho; *Strigea* sp. 4 **e**, **f** cyst; **g** whole-mount of excysted larva, ventral view. *Strigea* sp. 5 **h**, **i** cyst; **j** whole-mount of excysted larva, ventral view

Table 2 Comparison of morphometric data of encysted strigeids among shape and size of cysts, and sizes of cyst wall layers (measurement as mean ± standard deviation)

Metacercaria	Cyst-shaped long/wide ($n=10$)	Cyst wall		Protective elastic layer (? formed by host) ($n=5$)
		Inner hyaline layer ($n=5$)	Outer radiate layer ($n=5$)	
Strigea sp. 4	Cyst egg 513 ± 36/374 ± 34	12 ± 2	39 ± 8 [d]	~ 15 [e]
Strigea sp. 5	Cyst oval 579 ± 159/359 ± 82	9 ± 1	23 ± 3	Spherical shaped ~ 300
Strigea sp. 7	Cyst egg 1044 ± 174/672 ± 107	36 ± 8	114 ± 21	~ 15 [e]
Strigea sp. 8	Cyst almost spherical 706 ± 48/583 ± 54	9 ± 1	92 ± 13	~ 20 [e]
Strigea sp. 9	Cyst elongated-egg 1074 ± 169/653 ± 123	122 ± 37 [d]	155 ± 21 [d]	~ 40 [e]
Strigea sp. 11	Cyst egg [b] 408 ± 15/288 ± 10	11 ± 1	16 ± 5	~ 35 [e]
Strigea sp. 12	Cyst elliptical 444 ± 53/328 ± 39	8 ± 1	12 ± 3	~ 25 [e]
Strigea sp. 13	Cyst elongated-oval [c] 850/450	4	20	~ 230 [e]
Strigea sp. 14	Cyst oval 607 ± 50/442 ± 25	5 ± 2	68 ± 12 [d]	Spherical shaped ~ 130
Strigea sp. 1 [a]	Cyst egg 950 ± 116/594 ± 38	3 ± 1	63 ± 15	~ 110 [e]
Strigea sp. 2 [a]	Cyst oval 455 ± 33/325 ± 39	4 ± 1	43 ± 10 [d]	Spherical shaped ~ 60
Strigea sp. 3 [a]	Cyst elongated-oval 378 ± 34/227 ± 17	20 ± 5	83 ± 19 [d]	~ 20 [e]

[a] Species cited in Hamann et al. [6];
[b] $n=4$
[c] $n=1$
[d] Maximum thickness of layer
[e] Same shape as the cyst

Strigea sp. 4 (Fig. 3e, f and g).

Cyst size (minimum–maximum): 470—565 long by 315—410 wide.

Site of infection: mesentery, body cavity and muscle.

Date of collection and quantitative descriptors: February 2019; prevalence of infection, 33%; maximum intensity, 55 cysts.

Voucher material: CECOAL 18021935.

Host and locality: *L. llanensis* ($n=21$) collected from Ingeniero Juárez, Formosa Province, Argentina.

Strigea sp. 5 (Fig. 3h, i and j).

Cyst size (minimum–maximum): 320—725 long by 200—425 wide.

Site of infection: mesentery, body cavity and muscle.

Date of collection and quantitative descriptors: February 2019; prevalence of infection, 14%; maximum intensity, 16 cysts.

Voucher material: CECOAL 18021915.

Host and locality: *L. llanensis* ($n=21$) collected from Ingeniero Juárez, Formosa Province, Argentina.

Strigea sp. 7 (Fig. 4a, b and c).

Cyst size (minimum–maximum): 920—1350 long by 600—860 wide.

Site of infection: mesentery, body cavity and muscle.

Date of collection and quantitative descriptors: February 2017; prevalence of infection, 50%; maximum intensity, 9 cysts. February 2018; 14%; 34 cysts. December 2019; 100%; 100 cysts approximately (*L. llanensis*). February 2018; 4%; 1 cyst (*L. macrosternum*).

Voucher material: CECOAL 18021911, 18020530.

Host and locality: *L. llanensis* ($n=21$) collected from Ingeniero Juárez, Formosa Province, Argentina: *L. macrosternum* ($n=27$) collected in the agricultural area of Corrientes, Corrientes Province, Argentina.

Strigea sp. 8 (Fig. 4d, e, f).

Cyst size (minimum–maximum) 650—800 long by 550—725 wide.

Site of infection: mesentery, body cavity and muscle.

Date of collection and quantitative descriptors: February 2018; prevalence of infection, 33%; maximum intensity, 130

Table 3 Morphology differences between metacercariae of *Strigea* species recorded in amphibians from Argentinian Chaco Region

	Holfast organ	Pseudosuckers	Relation suckers length	width
Strigea sp. 4	Both lobes not overlapping ventral sucker; not projected from opening	Anterolateral edges; cochleariform	1: 1.4	1: 1.5
Strigea sp. 5	Dorsal lobe overlapping ventral sucker; not reaching to anterior margin of forebody	Anterolateral edges; cochleariform	1: 1.1	1: 1.3
Strigea sp. 7	Dorsal lobe overlapping ventral sucker; not projected from opening	Posterolateral to oral sucker; cochleariform	1: 1.1	1: 1.2
Strigea sp. 8	Both lobes not overlapping ventral sucker; not reaching to anterior margin of forebody	Anterolateral edges; cochleariform	1: 1.3	1: 1.5
Strigea sp. 9	Both lobes not overlapping ventral sucker; not reaching to anterior margin of forebody	Anterolateral edges; cochleariform	1: 1.0	1: 1.3
Strigea sp. 11	Dorsal lobe overlapping ventral sucker; not projected from opening	Posterolateral to oral sucker; cochleariform	1: 1.1	1: 1.4
Strigea sp. 12	Both lobes overlapping ventral sucker; projected from opening	Anterolateral edges; glandulo-muscular	1: 1.2	1: 1.5
Strigea sp. 13	Both lobes overlapping ventral sucker; projected from opening	Posterolateral to oral sucker; glandulo-muscular	1: 1.1	1: 1.1
Strigea sp. 14	Dorsal lobe overlapping ventral sucker; not reaching to anterior margin of forebody	Posterolateral to oral sucker; glandulo-muscular	1: 1.0	1: 1.7
Strigea sp. 1*	Ventral lobe overlapping ventral sucker; reaching to anterior margin of forebody	Posterolateral to oral sucker; cochleariform	1: 1.1	1: 1.3
Strigea sp. 2*	Both lobes overlapping ventral sucker; not reaching to anterior margin of forebody	Anterolateral edges; glandulo-muscular	1: 1.2	1: 1.4
Strigea sp. 3*	Both lobes not overlapping ventral sucker; not reaching to anterior margin of forebody	Anterolateral edges; cochleariform	1: 1.0	1: 1.1

*Cited in Hamann et al.[6]

cysts. December 2019; 25%; 100 cysts (*L. llanensis*). February 2018; 4%; 1 cyst (*L. macrosternum*).

Voucher material: CECOAL 18021939¸ 18020530.

Host and locality: *L. llanensis* ($n=21$) collected from Ingeniero Juárez, Formosa Province, Argentina; *L. macrosternum* ($n=27$) collected in the agricultural area of Corrientes, Corrientes Province, Argentina.

Strigea sp. 9 (Fig. 4 g, h, i).

Cyst size (minimum–maximum): 850—1400 long by 525—960 wide.

Site of infection: mesentery, body cavity and muscle.

Date of collection and quantitative descriptors: February 2018; prevalence of infection, 35%; maximum intensity, 216 cysts.

Voucher material: CECOAL 18021936.

Host and locality: *L. llanensis* ($n=21$) collected from Ingeniero Juárez, Formosa Province, Argentina.

Strigea sp. 11 (Fig. 4j, k and l).

Cyst size (minimum–maximum): 390—420 long by 275—300 wide.

Site of infection: body cavity.

Date of collection and quantitative descriptors: April 2017; prevalence of infection, 4%; maximum intensity, 4 cysts.

Voucher material: CECOAL 1704061.

Host and locality: *L. macrosternum* ($n=27$) collected in the agricultural area of Corrientes, Corrientes Province, Argentina.

Strigea sp. 12 (Fig. 5a, b, c).

Cyst size (minimum–maximum): 350—550 long by 265—400 wide.

Site of infection: body cavity.

Date of collection and quantitative descriptors: November 2017; prevalence of infection, 51%; maximum intensity, 18 cysts.

Voucher material: CECOAL 17112208.

Host and locality: R. bergi ($n=37$) collected in the agricultural area of Corrientes, Corrientes Province, Argentina.

Strigea sp. 13 (Fig. 5d, e, f).

Cyst size (minimum–maximum): 850 long by 450 wide.

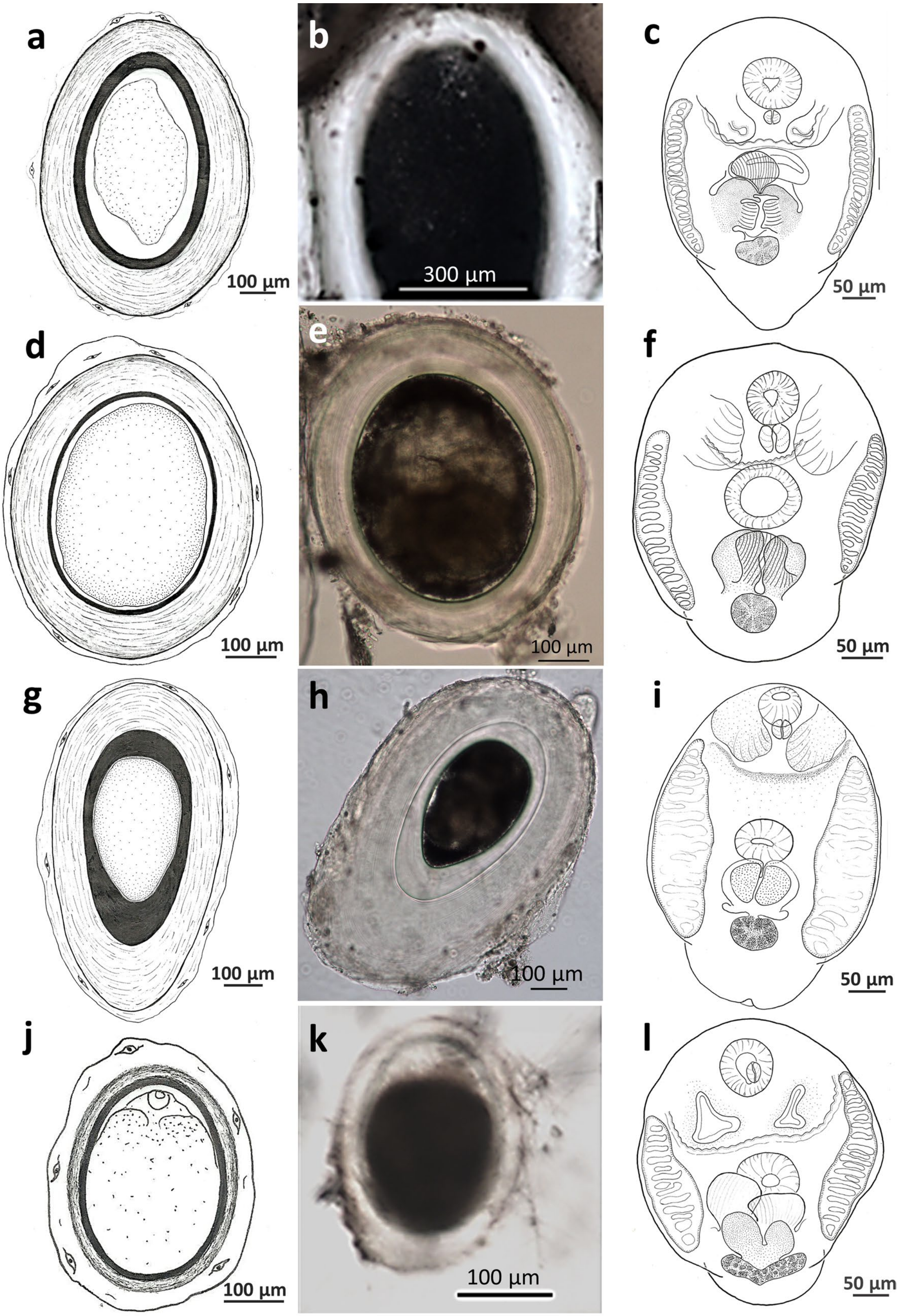
Site of infection: body cavity.

Date of collection and quantitative descriptors: October 2017; prevalence of infection, 50%; maximum intensity, 1 cyst.

Voucher material: CECOAL 17101726.

Host and locality: *L. latinasus* ($n=2$) collected in the agricultural area of Corrientes, Corrientes Province, Argentina.

Strigea sp. 14 (Fig. 5g, h, i).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◂**Fig. 4** *Strigea* sp. 7 **a**, **b** cyst; **c** whole-mount of excysted larva, ventral view. *Strigea* sp. 8 **d**, **e** cyst; **f** whole-mount of excysted larva, ventral view. *Strigea* sp. 9 **g**, **h** cyst; **i** whole-mount of excysted larva, ventral view. *Strigea* sp. 11 **j**, **k** cyst; **l** whole-mount of excysted larva, ventral view

Cyst size (minimum–maximum): 535—685 long by 400—490 wide.

Site of infection: mesentery, body cavity and muscle.

Date of collection and quantitative descriptors: December 2019; prevalence of infection, 4%; maximum intensity, 120 cysts.

Voucher material: CECOAL 191202209.

Host and locality: *L. llanensis* ($n = 21$) collected from Ingeniero Juárez, Formosa Province, Argentina.

Discussion

Some genera of mammalian Alariinae (e.g. *Alaria*, *Paralaria*, and *Procyotrema*) and Strigeidae (*Strigea*) have free swimming cercariae that penetrate the second intermediate hosts (adult frogs and tadpoles) through the skin and develop into mesocercariae [22]. Infected second intermediate hosts can be ingested by different paratenic hosts, such as amphibians, reptiles, birds, and mammals. Cannibalism has been reported for frogs infected with *Alaria alata* (Goeze, 1782) metacercariae [23]. In addition, humans have been proposed as potential paratenic hosts in the developmental cycle of *A. alata* [24]. In this regard, a fatal case from eating undercooked frog meat infected with *Alaria* metacercariae has been reported [25]. In the paratenic host, alariid larvae penetrate through the intestinal wall into the muscles of the anterior body region and internal organs, including lungs, liver, and kidney [26, 27]. In the present study, alariid mesocercariae were found in the body cavity of the frog species *L. llanensis* and *L. laevis*, making it the first record of this mesocercaria in amphibians from South America. To data, only one adult alariid species is known in non-domestic canids and felids from Argentina [28].

With regard to diplostomulum-type metacercariae, the genus *Didelphodiplostomum* Dubois, 1944 infects amphibians (e.g. *Ambystoma opacum*) as a second intermediate host, and mammals as definitive hosts [17]. In Argentina, Dubois [29] described the species *D. nunezae* Dubois, 1976 from *Didelphis azarae* Temminck, 1825 and, to date, it is the only adult species reported [28]. There are no reports of metacercarial infections. The genus *Pharyngostomoides* Harkema, 1942 includes species that also use mammals as definitive hosts (e.g., common raccoon) and annelids as second intermediate hosts, and would also have mesocercariae as part of their life cycle [22]. The present study shows anurans as potential paratenic intermediate hosts of this genus, and infection could occur through ingestion of intermediate hosts infected with larvae.

Diverse tylodelphylus-type metacercariae of the genus *Tylodelphys* Diesing, 1850 include species that infect the brain, cerebrospinal fluid, vitreous humour of the eyes, and body cavity of their second intermediate hosts, which are usually fish but sometimes amphibians [30]. The fluid in the spinal cord canal of many aquatic frogs (e.g., *Pelophylax lessonae*, *R. temporaria*, *R. arvalis* and *Bufo bufo*) from Europe is sometimes invaded by free swimming metacercariae of *T. excavata* which undergo their adult stage in the intestines of storks and buzzards [17, 31]. The heteroxenous life cycle of these parasites includes planorbids as first intermediate hosts and birds that feed on fish or amphibians as definitive hosts. To date, in Argentina, three adult species of this genus are known to parasitize birds [32] and seven species of metacercariae have been found at different infection site (pericardial cavity, body cavity, and brain) in fish [33–35]. This is the first report of tylodelphylus-type metacercariae found in the fluid of the spinal cord canal and cranial cavity of frogs from South America.

Another diplostomulum-type metacercaria was recorded for the genus *Heterodiplostomum* Dubois, 1936. It parasitizes amphibians as second intermediate hosts at different sites of infection (abdominal and hindlimb muscles, and abdominal cavity) and snakes as definitive hosts [22]. One species of adult trematode was found in snakes in Argentina [36, 37]. Metacercariae were first recorded in *L. macrosternum* frogs from Argentina [38] and the first DNA sequence and morphological data were reported for *H. lanceolatum* in *Leptodactylus podicipinus* Cope, 1862 frogs in Brazil [21]. Additionally, this proterodiplostomid larva was found in *L. latinasus* from Argentina in an agricultural environment [7]. Finally, the metacercaria described in *L. llanensis* represents a new host record for Dry Chaco Ecoregion.

About Strigeidae, three different metacercariae of the genus *Strigea* were previously recorded at various infection sites (liver, mesentery, body cavity, and muscle) in frogs [6]. Our results add nine strigeid larvae with tetracotyle-type cysts that infect amphibians as second intermediate hosts. The life cycle of these metacercariae depends on the host food web, i.e. the completion of the cycle depends on the predation behaviour of birds mainly on tadpoles, frogs, or toads [9]. Final hosts capture their prey near the aquatic environment, for example in flooded grass, which favours the return of the larvae (e.g., strigeids) to this ecosystem with the excretion of eggs in the faeces. Therefore, birds play a major role in the dissemination of digenean eggs. Some parasites will die due to environmental stresses such as desiccation, whereas others will find suitable intermediate hosts and develop. When the infected intermediate host is an amphibian, as in this study, the chances for the larvae to complete their life cycle are higher because amphibians

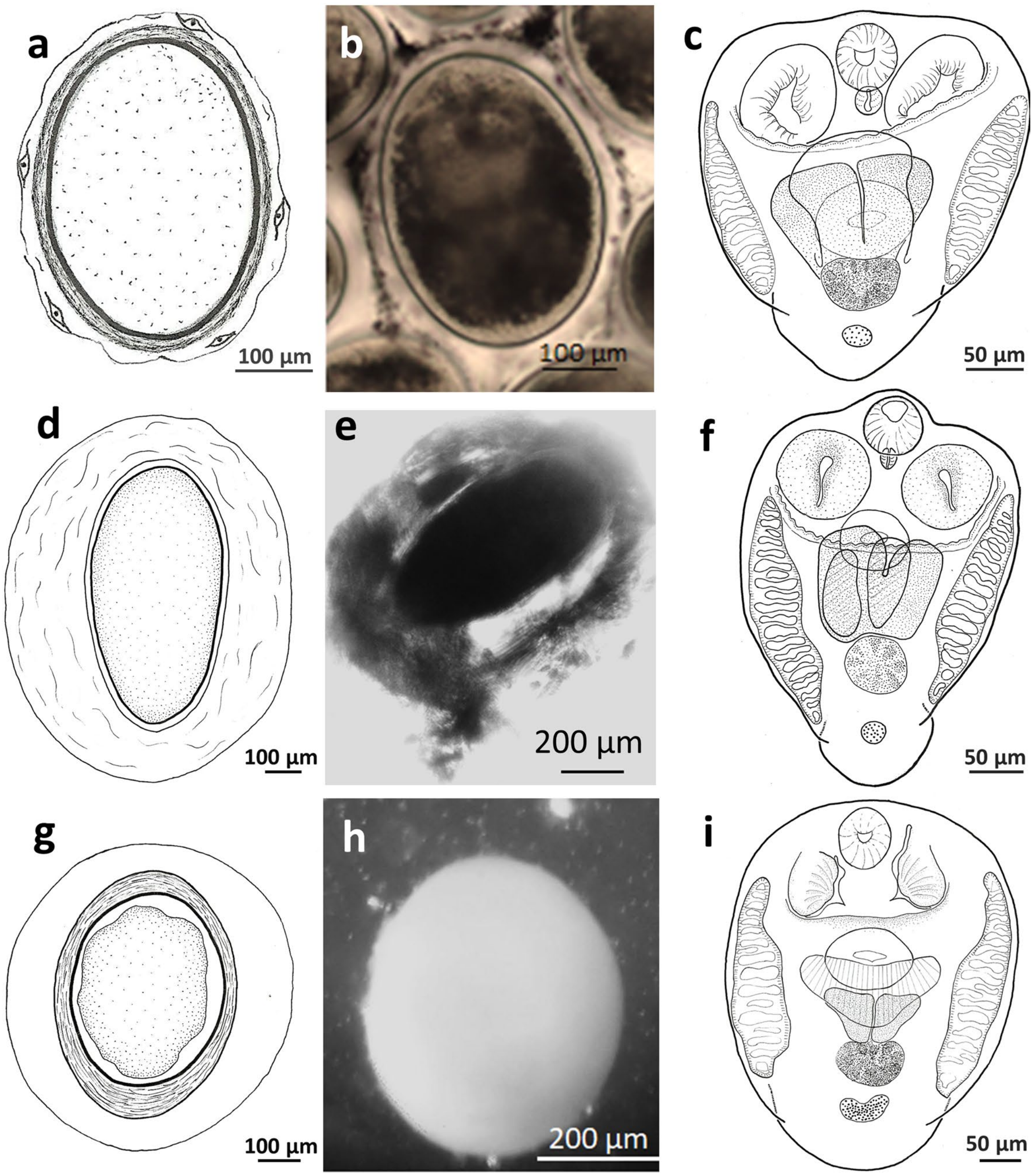

Fig. 5 *Strigea* sp. 12 **a**, **b** cyst; **c** whole-mount of excysted larva, ventral view. *Strigea* sp. 13 **d**, **e** cyst; **f** whole-mount of excysted larva, ventral view. *Strigea* sp. 14 **g**, **h** cyst; **i** whole-mount of excysted larva, ventral view

occupy an intermediate position in the food web and are easy prey of birds. The latter has been reported by several author [39–41]. Particularly, Panasci [39] found a high percentage of frogs as part of the diet of roadside hawks and some of these birds were described as definitive hosts of adult *Strigea* species [42, 43]. Based on their feeding preferences, we also concluded that some bird species of the families Accipitridae Vieillot, 1816 and Cariamidae Bonaparte, 1853 could act as potential definitive hosts in the indirect life cycles of the strigeids found as metacercariae in frogs. Only 10 adult species of *Strigea* genus are known to parasitize birds [32].

In conclusion, we found 14 taxa of larval trematodes in the Dry and Humid Chaco Ecoregions. All taxa recorded presumably have cercariae that use planorbid snails as a first intermediate host and infect amphibians at all stages of frog life [12] through natural orifices (such as the mouth) or by skin penetration [17]. These larval digeneans contribute to the list of those previously described parasitizing different amphibians in Argentina [6, 7, 12] and, in some cases, are new records. We are aware that all descriptions of larvae have many gaps in them and should be supplemented by future molecular and life cycle analyses. Specifically, we provide new information on morphological and metric data, which are currently only known for the adult stage in most of the species recorded from wild birds [32] and wild mammals [28] in Argentina.

Finally, although larval stages of metacercariae and mesocercariae are usually considered the least useful stages of development for identification purposes, this study provides information on possible intermediate hosts in the life cycles of digenetic trematodes. We present first records on different metacercariae and mesocercariae and several anuran amphibians as potential second intermediate hosts in the life cycle. Therefore, our research adds value by providing new records on the geographical distribution of larval trematodes and hosts and expands the knowledge of biodiversity in each particular environment. In addition, amphibians appear to be promising for further molecular screening in metacercariae of diplostomulum, tylodelphylus, proterodiplostomum, and tetracotyle types.

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Author Contributions All authors collected the data and provided critical feedback and helped shape the research, analysis and manuscript.

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Data Availability The data used in this study are included in the manuscript.

Declarations

Conflict of Interest The authors declare that they have no conflict of interest.

Ethical Approval We certify that anuran species reported in the study is not threatened, and all procedures were approved by the ethics committee of the institution where the study was conducted.

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