

# Closing the knowledge gap: Helminth parasites of freshwater turtles from the Chaco-Pampa Plain, Southern South America

## Research Paper

**Cite this article:** Palumbo EO, Alcalde L, Bonino M, Lescano J, Montes M, Solari A and Inés Díaz J (2024). Closing the knowledge gap: Helminth parasites of freshwater turtles from the Chaco-Pampa Plain, Southern South America. *Journal of Helminthology*, **98**, e30, 1–15  
<https://doi.org/10.1017/S0022149X24000178>.

Received: 27 December 2023

Revised: 26 February 2024


Accepted: 28 February 2024

### Keywords:

Nematoda; Digenea; Cestoda; Pleurodira; Cryptodira

### Corresponding author:

Ezequiel Palumbo;  
Email: [epalumbo@cepave.edu.ar](mailto:epalumbo@cepave.edu.ar)

Ezequiel Oscar Palumbo<sup>1</sup> , Leandro Alcalde<sup>2</sup>, Marcelo Bonino<sup>3</sup>, Julián Lescano<sup>4</sup>, Martín Montes<sup>1</sup>, Agustín Solari<sup>5</sup> and Julia Inés Díaz<sup>1</sup>

<sup>1</sup>Centro de Estudios Parasitológicos y de Vectores (CEPAVE), FCNyM, UNLP, CONICET, Boulevard 120 s/n e/61 y 62 (1900), La Plata, Buenos Aires, Argentina; <sup>2</sup>Instituto de Limnología Dr. R. A. Ringuelet (ILPLA), FCNyM, UNLP, CONICET, Boulevard 120 s/n e/60 y 64 (1900), La Plata, Buenos Aires, Argentina; <sup>3</sup>Laboratorio de Ecología, Biología Evolutiva y Comportamiento de Herpetozoos (LEBECH) INIBIOMA (CONICET-UNCo), Centro Regional Universitario Bariloche Quintral 1250 (8400), Bariloche, Río Negro, Argentina; <sup>4</sup>Instituto de diversidad y ecología animal (IDEA), CENTRO CIENTIFICO TECNOLOGICO CONICET – CORDOBA (CCT, CORDOBA) (CONICET), Córdoba, Argentina and <sup>5</sup>Instituto de Biología Subtropical (IBS) (CONICET/UNAM) Av. 3 Fronteras 183, Puerto Iguazú, Misiones, Argentina

### Abstract

Six species of freshwater turtles dominate the Chaco-Pampa Plain in southern South America and their parasites have been relatively understudied, with most records concentrated in Brazil. Particularly in Argentina, there are only scattered records of parasites for most of the turtles that inhabit the region, leaving a large knowledge gap. The purpose of the present contribution is to increase the knowledge of the internal parasites of six species of freshwater turtles from Argentina, after 15 years of fieldwork, by providing new hosts and additional geographic records for many host-parasite relationships. Some molecular sequences of the studied parasites were provided as a tool for better species identification. We processed 433 stomach and fecal samples from live individuals and visceral and soft tissue samples from 54 dissected turtles collected from a wide range and different ecoregions. We found 6230 helminths belonging to 18 taxa (one cestode, 11 digeneans and six nematodes). Fourteen new parasite-host associations are reported here, and for the first time parasites are recorded for *Phrynops williamsi*. This work contributes significantly to the knowledge of the parasitofauna in freshwater turtles in Argentina, providing a detailed list of parasites present in each turtle species and reporting molecular characters for future studies.

### Introduction

Parasites have long been perceived as having a minor role in ecosystem functioning because of their relatively low biomass compared with other trophic groups (Hudson *et al.*, 2006). However, recent evidence has revealed they play a crucial role and have substantial impacts on the ecosystems. Parasites can greatly influence the dynamics of host populations, disrupt interspecific competition, and shape energy flow, among other critical aspects. Thus, parasites emerge as significant drivers of biological diversity by exerting profound effects on ecosystem functions and the structure of food webs (Hudson, 2002; Wood & Johnson, 2015).

The Chaco-Pampa Plain is a low land at the east of the south part of the Andes formed by sedimentary loessic soils. The area includes Central, North, and Northeast Argentina; South-east Bolivia; West, Northwest, and Southeast Paraguay; all of Uruguay; and South Brazil. The Pampa, Espinal, Dry Chaco, and Humid Chaco ecoregions are the dominant biomes (Morello *et al.*, 2012; Bencke *et al.*, 2016; Mereles *et al.*, 2020). Six species of freshwater turtles dominate the basins and related flood-plains in the area: the Chaco Side-necked turtle *Acanthochelys pallidipectoris* (Freiberg, 1945); the Black Spine-neck Swamp turtle *Acanthochelys spixii* (Duméril & Bibron, 1835); the Snake-necked turtle *Hydromedusa tectifera* Cope, 1870; the Hilaire's Side-necked turtle *Phrynops hilarii* (Duméril & Bibron, 1835) (Chelidae); the Scorpion Mud turtle *Kinosternon scorpioides* (L.) (Kinosternidae); and the Painted turtle *Trachemys dorbigni* (Duméril & Bibron, 1835) (Emydidae). These species usually arranged in assemblages formed by a single species (usually *H. tectifera* as in the Cordoba province or south Buenos Aires province: Cabrera *et al.*, 1986; Di Pietro *et al.*, 2012) or more commonly by two or three species (e.g., *A. pallidipectoris*-*K. scorpioides* in the Argentina Dry Chaco ecoregion: Cassano & Alcalde, 2022, *H. tectifera*-*P. hilarii*-*T. dorbigni*, or *H. tectifera*-*A. spixii* in Argentina, Uruguay and Brazil Pampa ecoregion: Bujes, 2008; Alcalde *et al.*, 2012). Some of these species are also found in neighbouring ecoregions limiting with the Chaco-Pampa Plain (e.g., Yungas ecoregion in Argentina and Bolivia: *K. scorpioides* in the Atlantic Rainforest ecoregion in Argentina and Brazil and Cerrado ecoregion in Brazil: *H. tectifera*: Alcalde *et al.*,

2021). The Chaco-Pampa plain may also house two turtle species that enter marginally from adjacent Atlantic Rainforest ecoregion in Argentina, Brazil and Uruguay: *Phrynops williamsi* Rhodin & Mittermeier, 1983, and the southernmost lineage (see de Carvalho *et al.*, 2022) of *Phrynops geoffroanus* (Schweigger, 1812).

Parasites of the South American freshwater turtles have been understudied, with most records (more than 60) coming from Brazil (see Mascarenhas & Müller, 2022). All turtle species from the Chaco-Pampa plain of Argentina have been studied for internal parasites, with deeper analysis conducted on *H. tectifera* and *P. hilarii*: seven species of digeneans and seven nematodes were recorded (Lombardero & Moriena, 1977; Palumbo *et al.*, 2016, 2019, 2020, 2021; Palumbo & Diaz, 2018; Palumbo *et al.*, 2024). Additionally, few ecological studies on some of these turtle species contemplate the analysis of host diet and their parasites (Pereira *et al.*, 2018; Mascarenhas *et al.*, 2021; Palumbo *et al.*, 2021).

The purpose of the present contribution is to increase the knowledge of the internal parasitic assemblages of six species of freshwater turtles from the Argentine Chaco-Pampa plain by providing new parasite-host associations, and additional geographic records for many parasite-host relationships. Some molecular sequences of the studied parasites were supplied as a tool for a better species identification.

## Materials and methods

### Sampling

Samplings were carried out in different ecoregions of the Argentine Chaco-Pampa plain between 2008 and 2023 (Table 1). Parasite samples proceeded from two sources: (1) necropsied turtles (n=54), most of which were road-kill and a few were from herpetological collections or were field collected individuals that have presumably died from ulcerative shell disease, and (2) field-caught turtles that were stomach flushed (n=433) (Table 1, Fig. 1).

Turtles found dead in the field were refrigerated and transferred to the laboratory for necropsy: the lateral processes, which join the plastron to the carapace, were cut with a grinder and a scalpel was used for the surrounding integument; after the plastron was removed, the musculature was stripped and the cavities were examined. All organs were removed and examined for internal parasites under a stereoscopic microscope (Olympus SZ61, Tokyo, Japan). All collected parasites were preserved in 70% ethanol for their identification (see the following section).

Trapped turtles for live study (using hookless trot line, netsm or by hand following Semeñiuk *et al.*, 2017), were sometimes processed in the field (if feces were not sampled), or carried to the laboratory to collect stomach flushing (according to Legler, 1977 with subtle modifications relative to water pumping; see Bonino *et al.*, 2009 and Alcalde *et al.*, 2010). Stomach samples were observed under a stereoscopic microscope and all parasites recovered were kept in individual vials with 70% ethanol for further identification (see the following section).

All studied turtles were straight-line carapace length measured (accuracy, 1 mm) and sexed according dimorphic characters and, if necessary, by penis eversion in alive individuals (following Rodrigues *et al.*, 2014) or trough dissection in necropsied turtles. Alive turtles were also weighted (accuracy, 1 g) and marked (following Cagle, 1939) before release at the site of capture.

Procedures used in this study comply with the current laws for working in Argentina. Permission to work in the study area and for

**Table 1.** Data from the sampled turtles, separated into eviscerated individuals and samples of the stomach contents

Host	Province	Year	N (Gender)
<b>Eviscerated specimens</b>			
<i>Acanthochelys pallidipectoris</i>	Santa Fe	2019	1 (M)
<i>Hydromedusa tectifera</i>	Buenos Aires	2016–2023	19 (9F:8M:2J)
	Córdoba	2022	1 (F)
<i>Kinosternon scorpioides</i>	Formosa	1982	1 (F)
<i>Phrynops hilarii</i>	Buenos Aires	2011–2022	6 (3F:2M:1J)
	Ciudad Autónoma Buenos Aires	2021	1 (?)
	Corrientes	2016	1 (F)
	Entre Ríos <sup>a</sup>	2010–2019	7 (6F:1M)
	Santa Fe <sup>a</sup>	2010–2021	8 (5F:2M:1J)
	Tucumán <sup>b</sup>	2016	2 (1F:1M)
<i>Phrynops williamsi</i>	Misiones	2021	2 (?)
<i>Trachemys dorbigni</i>	Buenos Aires	2019	1 (M)
	Entre Ríos	2018–2021	4 (4F)
<b>Stomach samples</b>			
<i>Acanthochelys pallidipectoris</i>	Santa Fe	2016–2019	18 (12F:6M)
	Salta	2018–2019	46 (23:19M:4J)
<i>Hydromedusa tectifera</i>	Buenos Aires	2008–2021	121 (53F:67M:1J)
	Córdoba	2008	154 (43F:59M:52J)
	San Luis	2014	3 (2F:1M)
<i>Kinosternon scorpioides</i>	Chaco	2020	8 (5F:3M)
	Formosa	2019	4 (1F:2M:1J)
	Salta	2018	5 (3F:2M)
<i>Phrynops hilarii</i>	Chaco	2020	2 (2F)
	Buenos Aires	2008–2018	71 (21F:32M:18J)
<i>Trachemys dorbigni</i>	Buenos Aires	2022	1 (?)

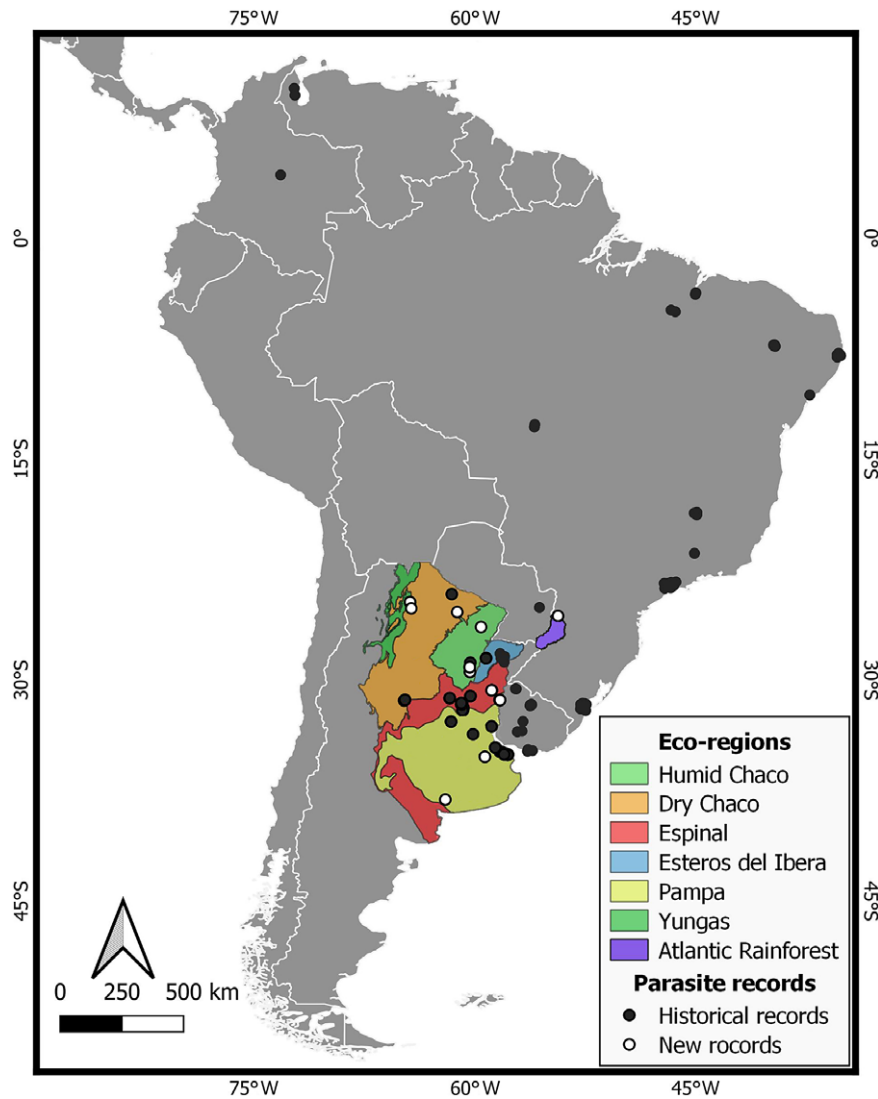
Hosts provided by <sup>a</sup>Centro de Investigaciones Científicas y Transferencia de Tecnología a la Producción (Yanina Prieto); <sup>b</sup>Alfredo Holley from anthropized environment

turtle handling was granted by the Dirección de Recursos Naturales of Buenos Aires (No. 102/2014-025 and 69/2016), Corrientes (No. 845), Chaco (No. 375), Entre Ríos (No. 001/17), Salta (No. 662), and Santa Fe provinces (No. 198), Argentina.

### Morphological identification of parasites

Nematodes were cleared in Amman's Lactophenol and temporarily mounted, flatworms were stained with hydrochloric carmine, dehydrated in a graded ethanol series, cleared in eugenol, and mounted in natural Canada balsam. Morphological studies were made under a compound microscope (Olympus BX51, Tokyo, Japan).

Adult parasites were determined using keys and specific bibliography (Hedrick, 1935; Cordero, 1946; Lombardero & Moriena, 1977; Baker, 1986; Font & Lotz, 2009; Mascarenhas & Müller, 2017; De Sousa *et al.*, 2018; Palumbo & Diaz, 2018; Palumbo *et al.*, 2020,



**Figure 1.** Records of helminth parasites studied in South American turtles.

2024). The prevalence, mean intensity, and abundance of parasites were calculated following Bush *et al.* (1997).

#### **Molecular and polymerase chain reaction methods**

A total of nine parasite specimens were isolated in Eppendorf tubes and frozen at  $-20^{\circ}\text{C}$  for subsequent DNA extraction and genotyping (Table 2). DNA was extracted from the isolates using 200  $\mu\text{L}$  of 5% Chelex solution (Bio-Rad Laboratories, CA, USA) containing 0.2 mg/mL Proteinase K (Roche), incubated at  $56^{\circ}\text{C}$  overnight, followed by 10 min at  $95^{\circ}\text{C}$ . Nuclear 18S, 28S, and ITS2 rDNA amplification were done using the appropriate primers. Each 50  $\mu\text{L}$  polymerase chain reaction (PCR) contained 25  $\mu\text{L}$  of GoTaq Green Master Mix (Promega, Madison, WI, USA), 2.5  $\mu\text{L}$  of each primer, 17  $\mu\text{L}$  of water, and 3  $\mu\text{L}$  of extracted DNA.

PCR amplification for 18S was performed using an Eppendorf Mastercycler ep gradient S, consisting of  $94^{\circ}\text{C}$  for 15 min, followed by 35 cycles of  $94^{\circ}\text{C}$  for 30 s,  $60^{\circ}\text{C}$  for 30 s, and  $72^{\circ}\text{C}$  for 70 s, with a final extension of  $72^{\circ}\text{C}$  for 240 s, for 28S amplification the program consisting of  $94^{\circ}\text{C}$  4 min, followed by 30 cycles of  $95^{\circ}\text{C}$  for 60 s,  $60^{\circ}\text{C}$

for 60 s, and  $72^{\circ}\text{C}$  for 120 s, with a final extension of  $72^{\circ}\text{C}$  for 4 min, and for ITS2 amplification was one cycle of  $95^{\circ}\text{C}$  3 min,  $42^{\circ}\text{C}$  2 min, and  $72^{\circ}\text{C}$  1 min, followed by six cycles of  $95^{\circ}\text{C}$  45 s,  $47^{\circ}\text{C}$  45 s, and  $72^{\circ}\text{C}$  1 min, followed with 35 cycles of  $95^{\circ}\text{C}$  20 s,  $50^{\circ}\text{C}$  20 s, and  $72^{\circ}\text{C}$  1 min, and a final extension of  $72^{\circ}\text{C}$  for 5 min.

PCR products were further purified and sequenced (Macrogen, South Korea). Sequences were edited and aligned using Chromas v2.6.6 and Gap v4.11.2 (Bonfield *et al.*, 1995) and then compared with the NCBI database using BLAST version 2.2.26 (Altschul *et al.*, 1997) to identify sequences with high similarity to DNA sequences obtained.

#### **Phylogenetic analyses**

The newly obtained 18S, 28S, and ITS2 sequences were aligned with GenBank available sequences using the CLUSTAL W program (Larkin *et al.*, 2007). The phylogenetic tree was reconstructed using the Maximum Likelihood method implemented in the Mega 7.0.26 program (Tables 2 and 3). The genetic differences in datasets were also calculated by using uncorrected *p* distances.

**Table 2.** Details of the primers used and method of analysis for each parasite species

Parasite species	Host	Locality	Gen	Primer	Model
<i>Caimanicola brauni</i>	<i>P. hylarii</i>	Santa Fe	28S	LSU5 (Littlewood 1994) 300F (Littlewood et al. 2000) 1500R (Snyder & Tkach 2001)	None
<i>Cheloniodiplostomum argentinense</i>		Entre Ríos			Kimura's (1980) two-parameter (K2-P)
<i>Prionosomoides phrynopsis</i>		Santa Fe			None
<i>Telorchis diaphanus</i>	<i>T. dorbigni</i>	Entre Ríos			Kimura's (1980) two-parameter (K2-P) + I + G
<i>Telorchis dubius</i>					
<i>Telorchis birabeni</i>	<i>P. williamsi</i>	Misiones			
	<i>P. hylarii</i>	Santa Fe			
<i>Caimanicola brauni</i>	<i>P. hylarii</i>	Santa Fe	ITS2	ITS2 (Cribb et al. 1998)	None
<i>Cheloniodiplostomum argentinense</i>				3S (Morgan and Blair 1995)	
<i>Thelandros</i> sp.	<i>P. hylarii</i>	Chaco	18S	Nem_18S_F (Floyd et al. 2005) Nem_18S_R (Floyd et al. 2005)	Tamura–Ney

**Table 3.** List of sequences extracted from GenBank used in the phylogenetic analysis

Parasite species	Host	Access number
<b>Proterodiplostomid taxa</b>		
<i>Cheloniodiplostomum argentinense</i>	<i>P. hylarii</i>	OR135714
<i>Cheloniodiplostomum</i> sp.	<i>H. tectifera</i>	OR135719
<i>Herpetodiplostomum vogti</i>	<i>Phrynops geoffroanus</i>	MW544286
<i>Proteroduboisia globulare</i>	<i>Caiman yacare</i>	MT622346
<i>Cystodiplostomum</i> sp.	<i>Caiman yacare</i>	MT622330
<i>Cystodiplostomum hollyi</i>	<i>Caiman yacare</i>	MT622327
<i>Proterodiplostomum longum</i>	<i>Caiman crocodilus</i>	MT622341
<i>Proterodiplostomum</i> sp.	<i>Caiman yacare</i>	MT622345
<i>Paraproterodiplostomum currani</i>	<i>Alligator mississippiensis</i>	MT622369
<i>Archaeodiplostomum overstreei</i>	<i>Alligator mississippiensis</i>	MT622323
<i>Pseudocrocodylicola americanense</i>	<i>Alligator mississippiensis</i>	MT622354
<i>Neocrocodylicola georgiana</i>	<i>Alligator mississippiensis</i>	MT622333
<i>Polycotyle ornata</i>	<i>Alligator mississippiensis</i>	MT622339
<i>Pseudoneodiplostomoides crocodilarum</i>	<i>Crocodylus johnsoni</i>	MT622357
<i>Pseudoneodiplostomum</i> sp.	<i>Crocodylus johnsoni</i>	MT622365
<i>Pseudoneodiplostomum thomasi</i>	<i>Crocodylus niloticus</i>	MT622366
<i>Mesodiplostomum gladiolum</i>	<i>Caiman yacare</i>	MT622332
<i>Crocodylicola pseudostoma</i>	<i>Rhambia guatemalensis</i>	MF398328
<i>Paradiplostomum abbreviatum</i>	<i>Caiman yacare</i>	MT622337
<i>Heterodiplostomum lanceolatum</i>	<i>Leptodactylus chaquensis</i>	MT622331
<i>Alaria mustelae</i>	<i>Lithobates sylvaticus</i>	JF820607

(Continued)

**Table 3.** (Continued)

Parasite species	Host	Access number
<b>Telorchidae taxa</b>		
<i>Telorchis birabeni</i>	<i>P. williamsi</i>	OR135715
	<i>P. hylarii</i>	OR135718
<i>Telorchis diaphanus</i>	<i>Trachemys dorbigni</i>	OR135720
<i>Telorchis dubius</i>		OR135716
<i>Telorchis bonnerensis</i>	<i>Lithobates sylvaticus</i>	JF820593
	<i>Chelydra serpentina</i>	JF820592
	<i>Ambystoma tigrinum</i>	JF820591
	<i>Chelydra serpentina</i>	JF820590
<b>Thelandros taxa</b>		
<i>Thelandros</i> sp.	<i>P. hylarii</i>	OR135723
<i>Thelandros</i> sp.	<i>Chioninia stangeri</i>	KY541834
<i>Thelandros tinerfensis</i>	<i>Tarentola gomerensis</i>	KJ778073
<i>Thelandros scleratus</i>	<i>Anolis cristatellus</i>	MH748148
<i>Parapharyngodon binae</i>	<i>Tropidurus torquatus</i>	MF102081
<i>Parapharyngodon echinatus</i>	<i>Tarentola parvicarinata</i>	AM943009
<i>Parapharyngodon cubensis</i>	<i>Anolis</i> sp.	KF029062
<i>Spauligodon saxicolae</i> (Outgroup)	<i>Darevskia unisexualis</i>	JF829227

## Results

### Parasite diversity

A total of 6230 helminths belonging to 18 taxa (one cestode, 11 digeneans, and six nematodes) were recovered (Table 4). Taxonomical position, host, site of infection, and localities are presented in the following section; additionally, a deep morphological diagnosis and analysis were included in those cases that the finding presents systematic contradictions or drawbacks, if not, only a brief description was provided.

**Table 4.** List of parasites recorded in this study with details of hosts, localities, number of host (N), prevalence (P), mean intensity (MI), and abundance and range (A)

Species	Host	Locality	N	P	MI	A
<b>Cestoda</b>						
<i>Ophiotaenia cohospes</i>	<i>H. tectifera</i>	Buenos Aires	19	31.5	12.5	75 (1–28)
			121 <sup>a</sup>	7.4	1.3	12 (1–8)
		Córdoba	1	100	5	5 (0–5)
<b>Digenea</b>						
<i>Caimanicola brauni</i>	<i>P. hilarii</i>	Buenos Aires	6	14.2	15	15 (0–15)
		Santa Fe	8	12.5	74	74 (0–74)
<i>Cheloniodiplostomum argentinense</i>	<i>P. hilarii</i>	Buenos Aires	6	14.2	10	10 (0–10)
		Entre Ríos	7	71.4	30.2	151 (4–64)
		Santa Fe	8	12.5	80	80 (0–80)
<i>Cheloniodiplostomum testudinis</i>	<i>P. hilarii</i>	Santa Fe	8	37.5	10	30 (3–24)
<i>Cheloniodiplostomum</i> sp.	<i>H. tectifera</i>	Buenos Aires	19	5	4	4 (0–4)
	<i>P. hilarii</i>	Buenos Aires	6	16.6	500	500 (0–500)
<i>Herpetodiplostomum duboisi</i>	<i>P. williamsi</i>	Misiones	2	50	96	96 (0–96)
<i>Nematophila grandis</i>	<i>P. hilarii</i>	CABA	1	100	1	1 (0–1)
<i>Prionosomoides phrynopsis</i>	<i>P. hilarii</i>	Buenos Aires	6	16.6	4	4 (0–4)
		Entre Ríos	7	85.7	24,6	148 (1–48)
		Santa Fe	8	25	6	23 (6–17)
		Tucumán	2	50	273	273 (0–273)
<i>Telorchis birabeni</i>	<i>P. hilarii</i>	Buenos Aires	6	16.6	1	1 (0–1)
		Entre Ríos	7	14.2	3	3 (0–3)
		Santa Fe	8	50	6.25	25 (4–10)
	<i>P. williamsi</i>	Misiones	2	50	43	43 (0–43)
<i>Telorchis devincenzii</i>	<i>H. tectifera</i>	Buenos Aires	19	5	21	21 (0–21)
<i>Telorchis diaphanus</i>	<i>P. hilarii</i>	Corrientes	1	100	15	15 (0–15)
	<i>T. dorbigni</i>	Entre Ríos	4	25	5	5 (0–5)
<i>Telorchis dubius</i>	<i>T. dorbigni</i>	Entre Ríos	4	50	7.5	15 (7–8)
<b>Nematoda</b>						
<i>Camallanus emydidi</i>	<i>T. dorbigni</i>	Entre Ríos	4	75	25.66	77 (4–60)
<i>Falcaustra affinis</i>	<i>T. dorbigni</i>	Buenos Aires	1	100	10	10 (0–10)
		Entre Ríos	4	100	209.25	837 (24–688)
<i>Hedruris dratini</i>	<i>H. tectifera</i>	Buenos Aires	121 <sup>a</sup>	20	137.3	3297 (15–541)
	<i>T. dorbigni</i>	Buenos Aires	1 <sup>a</sup>	100	25	25 (0–25)
<i>Spiroxys contortus</i>	<i>H. tectifera</i>	Córdoba	1	100	31	31 (0–31)
			154 <sup>a</sup>	13.6	8.47	178 (3–47)
	<i>P. hilarii</i>	Corrientes	1	100	20	20 (0–20)
		Entre Ríos	7	57.1	1.50	6 (1–2)
		Santa Fe	8	87,5	3.71	26 (1–11)
<i>T. dorbigni</i>	Entre Ríos	4	75	28	84 (2–55)	
Pharyngodonidae gen. sp.	<i>A. pallidipectoris</i>	Salta	46 <sup>a</sup>	2.17	1	1 (0–1)
	<i>K. scorpioides</i>	Chaco	8 <sup>a</sup>	12.5	1	1 (0–1)
		Salta	8 <sup>a</sup>	12.5	1	1 (0–1)
<i>Thelandros</i> sp.	<i>A. pallidipectoris</i>	Salta	46 <sup>a</sup>	2.1	2	2 (0–2)
		Santa Fe	18 <sup>a</sup>	5.5	2	2 (0–2)
	<i>P. hilarii</i>	Chaco	2 <sup>a</sup>	50	3	3 (0–3)

<sup>a</sup>Parameters calculated from regurgitated.

**Phylum** Platyhelminthes**Class** Cestoda**Family** Proteocephalidae La Rue, 1911*Ophiotaenia* La Rue, 1911*Ophiotaenia cohospes* Cordero, 1946

**Diagnosis:** Scolex with four equal, smooth, and elliptical suckers.

Acrospedot proglottids, the former is wider than longer, but they become longer changing their ratio when they are mature and gravid. Follicular testes distributed in two lateral fields, ovary bilobed, posterior in the proglottid. Lateral follicular vitellogenic glands. Medullary uterus. Lateral genital pore in median position.

**Site of infection:** Intestine.

**Host and localities:** *Hydromedusa tectifera* - Magdalena (35°01'36"S, 57°17'24"W) in Buenos Aires province (Pampa ecoregion) and Tanti (31°22'05"S, 64°34'34"W) and Flor Serrana (31°23'36"S, 64°35'42"W), in Córdoba province (Dry Chaco ecoregion).

**Voucher specimens:** MLP-He 8083

**Remarks:** This species was described parasitizing *H. tectifera* from Uruguay (Cordero, 1946) and subsequently found in anurans from Brazil, Ecuador, and Paraguay (McAllister *et al.*, 2010; Campião *et al.*, 2015). Until now, *O. cohospes* has not been recorded again in turtles since its description 70 years ago. It is also the first record for this parasite for Argentina.

**Class** Trematoda**Subclass** Digenea**Family** Cryptogonimidae Ward, 1917*Caimanicola* Freitas & Lent, 1938*Caimanicola brauni* (Mañé-Garzón & Gil, 1961)

**Diagnosis:** The presence of a crown of 24 spines surrounding the oral sucker, the globose pharynx, the distribution of the reproductive organs (ovary and testis in tandem at the posterior end), and the caecum that end up opening to the exterior at the posterior part of the body, are the main characteristics that identify the specimens as *C. brauni* (Table 4).

**Site of infection:** Intestine.

**Host and localities:** *Phrynosops hilarii* - Magdalena (35°01'36"S, 57°17'24"W) in Buenos Aires province (Pampa ecoregion), and Rafaela (31°15'04"S, 61°30'20"W) in Santa Fe province (Espinal ecoregion).

**GenBank acc. number:** OR135713 (ITS2), OR137955 (28S)

**Voucher specimens:** MLP-He 8084

**Remarks:** This species was described for *P. hilarii* from Uruguay (Mañé-Garzón & Gil, 1961a) and subsequently recorded for *A. spixii* and *P. hilarii* from Brazil (Chaviel *et al.*, 2020), and experimentally in *P. hilarii* from Buenos Aires, Argentina (Ostrowski de Núñez, 1987). The present record represents the first report for a wild turtle from Argentina. Unfortunately, no sequences are available on GenBank for this genus, so comparisons were precluded. This is the first molecular contribution for *Caimanicola*.

**Family** Proterodiplostomidae Dubois, 1936*Cheloniodiplostomum* Sudarikov, 1960*Cheloniodiplostomum argentinense* Palumbo & Diaz, 2018

**Diagnosis:** Elongated body, with a clear difference between forebody and hindbody, elliptical holdfast with proteolytic glands, the vitellaria begins anterior to the bifurcation of the cecum and ends at the posterior border of the ovary. The posterior end of the body is bifurcated: one portion blunt and the other with a funnel-shaped dorsal projection into which the copulatory pouch opens (Table 4).

**Site of infection:** Intestine.

**Host and localities:** *Phrynosops hilarii* - La Plata (34°53'26"S, 57°59'40"W) in Buenos Aires province (Pampa ecoregion), Aldea

Brasilera (31°53'17"S, 60°34'44"W) and Diamante (32°02'52"S, 60°38'10"W) in Entre Ríos province (Espinal ecoregion), and Helvecia (31°06'30"S, 60°05'48"W) in Santa Fe province (Espinal ecoregion).

**Historical records in Argentina:** *Phrynosops hilarii* - Magdalena (35°01'36"S, 57°17'24"W) in Buenos Aires province (Pampa ecoregion) (Palumbo & Diaz, 2018).

**GenBank acc. number:** OR135714 (28S)

*Cheloniodiplostomum testudinis* (Dubois, 1936)

**Diagnosis:** Short body divided into two parts by a constriction located at the level of the ovary and anterior testis, flattened forebody, and cylindrical hindbody; holdfast rounded; vitellaria extending from near caeca bifurcation to the ovary (Table 4).

**Site of infection:** Intestine.

**Host and localities:** *Phrynosops hilarii* - Cañada de Gomez (32°49'45"S, 61°22'57"W) (Pampa eco-region) and Santa Fe City (31°38'21"S, 60°40'54"W) in Santa Fe province (Espinal eco-region).

**Historical records in Argentina:** *Phrynosops hilarii* and *Hydromedusa tectifera* - Magdalena (35°01'36"S, 57°17'24"W) Buenos Aires province (Pampa eco-region) (Palumbo & Diaz, 2018). *Phrynosops hilarii* - unknown locality in Corrientes province (Lombardero & Moriena, 1977).

*Cheloniodiplostomum* sp.

**Diagnosis:** Elongated body divided into two parts by a constriction located at the level of the ovary, flattened forebody and cylindrical hindbody; oral sucker rounded, small, subterminal, pre pharynx absent, pharynx rounded and short, esophagus short, caeca bifurcating above acetabulum and reaching the posterior edge of the posterior testis; acetabulum rounded, similar in size to oral sucker; large and rounded holdfast located in forebody, with proteolytic glands in its posterior edge; testes in tandem, without cirrus and cirrus sac; ovary rounded pretesticular, ootype intertesticular; vitellaria extending from near pharynx to anterior testis; uterus completely in hindbody, few elliptical eggs observed; posterior end of the body with muscle pouch shape (Table 4, Fig. 2).

**Site of infection:** Intestine.

**Hosts and localities:** *Hydromedusa tectifera* and *Phrynosops hilarii* - La Plata (34°53'26"S, 57°59'40"W) in Buenos Aires province (Pampa eco-region).

**GenBank acc. number:** OR135719 (28S), OR135721 (ITS2).

**Voucher specimens:** MLP-He 8085

**Remarks:** Recently Achatz *et al.*, (2021) suggested that the genus *Cheloniodiplostomum* Sudarikov, 1960 should be considered as a junior synonym of *Herpetodiplostomum* Dubois, 1936, basing their proposal on the fact that the host specificity (turtles vs. alligators), the limits of the vitellarium (anterior to the acetabulum vs. posterior to the acetabulum), and the presence/absence of papillae on the holdfast would not be sufficient characteristics to differentiate both genera. Although these authors demonstrate that there are species of both genera that present papillae on the holdfast, they failed in finding any *Cheloniodiplostomum* species parasitizing alligators. Molecularly, they have sequenced a single species precluding any comparison between *Cheloniodiplostomum* and *Herpetodiplostomum*. We follow the classification first proposed by Sudarikov (1960) then followed by subsequent authors (Dubois, 1968, 1982; Niewiadomska, 2002; Mascarenhas & Müller, 2021).

Four species of *Cheloniodiplostomum* are recognized parasitizing neotropical turtles: *Ch. brevis* (Dubois, 1979) parasitizing *P. geoffroanus* (Colombia) and *K. scorpioides* (United States) (MacCallum, 1921; Dubois, 1979); *Ch. delillei* (Zeracer, 1947) recorded for *Chelydra serpentina* (L.) and *Claudius angustatus* Cope, 1875, both from Mexico (Thatcher, 1963, 1964);



**Figure 2.** Stained whole-mount micrographs of *Cheloniodiplostomum* sp. Scale bar 400  $\mu$ m.

*Ch. testudininis* described for a turtle (genus and species unknown), *P. geoffroanus* (Brazil), and *P. hilarii* and *H. tectifera* (Argentina) (Dubois, 1936; Lombardero & Moriena, 1977; Fernandes & Kohn, 2014; Silva, 2014; Mascarenhas *et al.*, 2016; Palumbo & Diaz, 2018); and *Ch. argentinense* parasitizing *P. hilarii* from Argentina (Palumbo & Diaz, 2018).

Phylogenetic analysis of partial 28S rRNA gene sequences shows that *Cheloniodiplostomum* and *Herpetodiplostomum* species form a strongly supported clade (Fig. 3A). This group composed only by species that parasitize turtles, while the other genera form a clade which species parasitize crocodiles, lizards and amphibians but not turtles.

*Herpetodiplostomum* Dubois, 1936

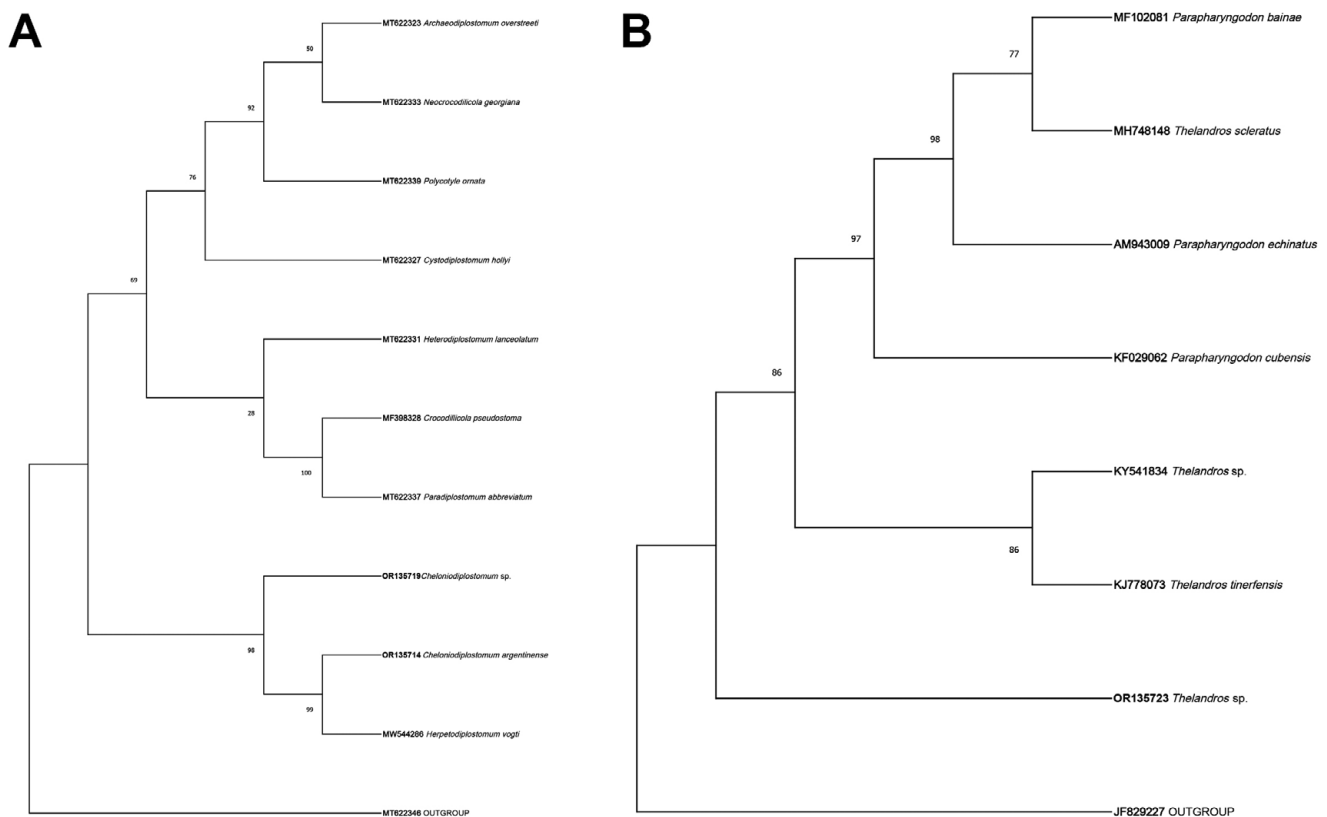
*Herpetodiplostomum duboisi* Mañé-Garzón & Holcman-Spector, 1969

**Diagnosis:** Elongated body, with a clear difference between fore-body and hindbody; rounded holdfast with proteolytic glands; the vitellaria start posteriorly to the bifurcation of the caecum and ends at posterior border of the anterior testis. The posterior end of the body is bifurcated: one portion blunt and the other with a funnel-shaped dorsal projection into which the copulatory pouch opens (Table 4).

**Site of infection:** Intestine.

**Host and localities:** *Phrynosops williamsi* - Comandante Andresito (25°40'22''S, 54°02'22''W) in Misiones province (Atlantic Rainforest eco-region).

**Voucher specimens:** MLP-He 8086



**Figure 3.** Phylogenetic tree based on *Cheloniodiplostomum* rDNA sequences newly obtained in this study compare with Diplostomidae sequences available in GenBank using the Maximum Likelihood method with a distance matrix calculation with K2P. The numbers at the nodes represent the percentages of 1000 bootstrap replicates (A). Phylogenetic tree based on *Thelandros* rDNA sequence newly obtained in this study compare with Pharyngodonidae sequences available in GenBank using the Maximum Likelihood method with a distance matrix calculation with T92. The numbers at the nodes represent the percentages of 1000 bootstrap replicates (B). Sequences are identified by GenBank accession numbers, taxa names, and hosts.

**Remarks:** There are six species recorded within the genus *Herpetodiplostomum*: *H. caimanicola* (Dollfus, 1935), *H. duboisi*, *H. gavialis* (Narain, 1930), *H. ovalis* Mañé-Garzón & Holcman-Spector, 1969, *H. vogti* Achatz et al., 2021, and *H. wolffhugeli* Mañé-Garzón & Holcman-Spector, 1969. Two of them are parasites of crocodiles, *H. caimanicola* (Brazil) and *H. gavialis* (India) and share the following characteristics: acetabulum twice as big as the oral sucker, vitellaria extend close to acetabulum than to the ovary; with proteolytic glands on the holdfast, but without proteolytic glands below it. On the other hand, the rest of the species have the acetabulum similar in size than the oral sucker; the vitellarium extends from anterior to the acetabulum to anterior testis; with proteolytic glands below the holdfast and, all have turtles as definitive host (Narain, 1930; Dubois, 1936; Mañé-Garzón & Holcman-Spector, 1969; Achatz et al., 2021), so they should probably be transferred to *Cheloniodiplostomum* depending on future phylogenetic analyses with sequences confirming what is presumed from morphology and hosts specificity.

The species *H. duboisi* has not been recorded since its original description parasitizing *P. hilarii* in Artigas, Uruguay (Mañé-Garzón & Holcman-Spector, 1969). Moreover, this is the first record of internal parasites for *P. williamsi*.

**Family** Cladorchiidae Fiscoe, 1901

*Nematophila* Travassos, 1934

*Nematophila grandis* (Diesing, 1839)

**Diagnosis:** Large, leaf-shaped body with a cup-shaped terminal oral sucker and a large muscular acetabulum at the posterior end of the body. Testis intracecal, anterior to the ovary. Vitellaria extra-cecal (Table 4).

**Site of infection:** Intestine.

**Host and localities:** *Phrynosoma hilarii*: Ciudad Autónoma de Buenos Aires (34°36'28"S, 58°21'28"W), Buenos Aires province (Pampa ecoregion).

**Historical records in Argentina:** *Phrynosoma hilarii* - unknow locality in Corrientes province (Lombardero & Moriena, 1977).

**Remarks:** This widely distributed species was previously recorded for several turtle species from Argentina, Brazil, Ecuador, French Guyana, Paraguay, Peru, and Venezuela (Mascarenhas & Müller, 2021). This finding represents the second record of the species in Argentina and the first for Buenos Aires.

**Family** Echinostomatidae Looss, 1899

*Prionosomoides* Freitas & Dobbin, 1967

*Prionosomoides phrynosopsis* (Mañé-Garzón & Gil, 1961)

**Diagnosis:** Peristomal disc with 47 teeth arranged in a single row, oral sucker smaller than the ventral sucker, ovary pretesticular, testis in tandem near the middle of the body (Table 4).

**Site of infection:** Intestine.

**Host and localities:** *Phrynosoma hilarii* - Magdalena (35°01'36"S, 57°17'24"W), Buenos Aires province (Pampa ecoregion), Rafaela (31°15'04"S, 61°30'20"W) and Santa Fe Capital (31°38'21"S, 60°40'54"W) in Santa Fe province (both at Espinal ecoregion), Gualaguaychú (Pampa ecoregion, 32°57'30"S, 58°34'59"W) and Diamante (Espinal ecoregion, 32°02'52"S, 60°38'10"W) both in Entre Ríos province; San Miguel de Tucumán (26°49'11"S, 65°12'52"W), in Tucumán province (ecoregion not assigned because the studied specimen came from unknown origin and proceed from an anthropized artificial pond).

**GenBank acc. number:** OR135717 (28S)

**Voucher specimens:** MLP-He 8087

**Remarks:** *Prionosomoides* includes three species that parasitize freshwater turtles: *Pr. scalaris* Freitas & Dobbin, 1967 parasitizes *P. geoffroanus* from Brazil (Freitas & Dobbin Jr., 1967) and *P. hilarii* from Argentina (Lombardero & Moriena, 1977); *Pr. taiwanensis* Fischthal & Kuntz, 1975 parasitizes *Mauremys reevesii* (Gray, 1831) from Taiwan (Fischthal & Kuntz, 1975); and *Pr. phrynosopsis* was reported for *P. hilarii* from Uruguay (Mañé-Garzón & Gil, 1961a). This finding is a new country record for *Pr. phrynosopsis* from Argentina.

**Family** Telorchiidae Loos, 1899

*Telorchis* Luhe, 1899

*Telorchis dubius* Mañé-Garzón & Holcman-Spector, 1968

**Diagnosis:** Lanceolate body shape, small oral sucker similar in size to the acetabulum, pre-pharynx absent, small pharynx, juxtaovarian large cirrus pouch, oval-shaped ovary, small testis arranged in tandem and vitellaria extending beyond the ovary (Table 4, Fig. 4A).

**Site of infection:** Intestine.

**Host and localities:** *Trachemys dorbigni* - Concordia (31°21'22"S, 58°05'42"W) and Federal (30°40'54"S, 58°47'20"W) in Entre Ríos province (Espinal eco-region).

**GenBank acc. number:** OR135716 (ITS2)

**Voucher specimens:** MLP-He 8088

**Remarks:** This species was recorded only for *T. dorbigni* from Uruguay (Mañé-Garzón & Holcman-Spector, 1968). This is the second register of this host for this parasite and the first record for Argentina. In addition, we present for the first time the sequence of the ITS2 gene of *T. dubius*. The p-distances analysis shows it is close to *T. diaphanus*.

*Telorchis devincenzii* Mañé-Garzón & Gil, 1961

**Diagnosis:** Oral sucker larger than acetabulum, presence of prepharynx, globose pharynx, juxtaovarian cirrus pouch and vitellogenetic glands of cecal and extracecal position (Table 4, Fig. 4B).

**Site of infection:** Intestine.

**Host and localities:** *Hydromedusa tectifera* - La Plata (34°51'38"S, 58°03'54"W) in Buenos Aires province (Pampa eco-region).

**Voucher specimens:** MLP-He 8089

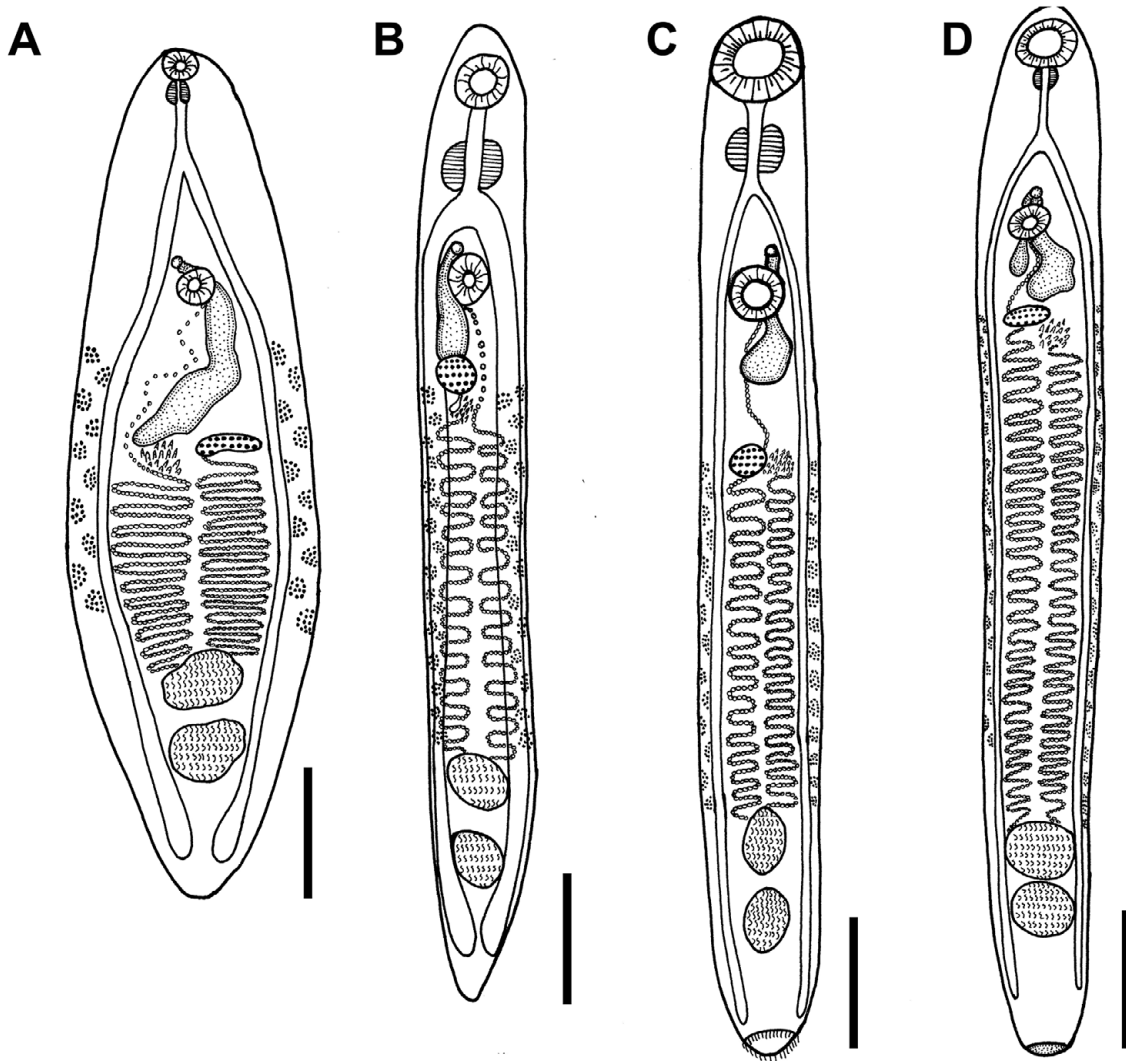
**Remarks:** After its finding and description parasitizing *H. tectifera* from Canelones, Uruguay (Mañé-Garzón & Gil, 1961b) the species was not recorded again. Catto & Amato, (1993) transferred some *Telorchis* species (but not *T. devincenzii*) to *Pseudotelorchis* Yamaguti, 1971 but late authors (Fernandes & Kohn, 2014; Mascarenhas & Müller, 2021) have erroneously interpreted that Catto & Amato, (1993) transferred *T. devincenzii* to *Pseudotelorchis*, and consequently they have mistakenly included their findings of *T. devincenzii* as *P. devincenzii*. It should be clearly stated that the morphological characteristics of *T. devincenzii* are proper to *Telorchis* and not fill those of *Pseudotelorchis*.

*Telorchis birabeni* Mañé-Garzón & Gil, 1961

**Diagnosis:** Oral sucker bigger than acetabulum, presence of a pre-pharynx and a globose pharynx, ovary and acetabulum widely separated, small testis in tandem, and vitellaria extending from the ovary to the anterior testis (Table 4, Fig. 4C).

**Site of infection:** Intestine.

**Host and localities:** *Phrynosoma hilarii* - La Plata (34°53'26"S, 57°59'40"W) in Buenos Aires province (Pampa eco-region); Gualaguaychú (32°57'30"S, 58°34'59"W) in Entre Ríos province (Pampa eco-region); Cañada de Gomez (32°49'45"S, 61°22'57"W), Helvecia (31°06'30"S, 60°05'48"W), Rafaela (31°15'04"S, 61°30'20"W) and Santa Fe Capital (31°38'21"S, 60°40'54"W) in Santa Fe province (Espinal eco-region). *Phrynosoma williamsi* - Comandante



**Figure 4.** Line drawings of *Telorchis* species. *T. dubius* (A), *T. devincenzii* (B), *T. birabeni* (C), and *T. diaphanus* (D). Scale bars 200  $\mu$ m.

**Table 5.** Number of per site base differences based on pairwise comparisons of the sequenced fragment at 5' end of ITS2 rDNA gene among the sequences newly obtained in this study and one sequence of *Telorchis* species available in GenBank. Positions containing gaps and missing data were eliminated

	OR135715 <i>T. birabeni</i>	OR135718 <i>T. birabeni</i>	OR135720 <i>T. diaphanus</i>	OR135716 <i>T. dubius</i>
OR135715 <i>T. birabeni</i>				
OR135718 <i>T. birabeni</i>	0.0087			
OR135720 <i>T. diaphanus</i>	0.1102	0.1127		
OR135716 <i>T. dubius</i>	0.1102	0.1127	0.0128	
JF820593 <i>T. bonnerensis</i>	0.1014	0.1038	0.0147	0.0147

Andresito (25°40'22''S, 54°02'22''W) in Misiones province (Atlantic Rainforest eco-region).

*Historical records in Argentina: Phrynos hilarii* - unknow locality in Corrientes province (Lombardero & Moriena, 1977).

*GenBank acc. number:* OR135715 (ITS2, PW), OR135718 (ITS2, PH)

*Voucher specimens:* MLP-He 8090

*Remarks:* The species was previously recorded for *P. hilarii* from Argentina, Brazil and Uruguay, and for *P. geoffroanus* from Brazil (Mañé-Garzón & Gil, 1961; Lombardero & Moriena, 1977; Silva, 2014; Mascarenhas *et al.*, 2016). This is the first record for *P. hilarii* for Buenos Aires, Entre Ríos and Santa Fe Argentine provinces, and the first record for *P. williamsi*. In addition, the two new ITS2 gene sequences provided a good complement to morphology to better differentiate species of the genus (Table 5).

*Telorchis diaphanus* Freitas & Dobbin Jr., 1959

*Diagnosis:* Oral sucker slightly larger than the acetabulum, prepharynx absent, small pharynx, cirrus sac reaches the upper edge of the ovary, oval-shaped ovary, large testis in tandem, extra cecal vitellaria with asymmetrical distribution (Table 4, Fig. 4D).

*Site of infection:* Intestine.

*Hosts and localities: Phrynos hilarii* - Bella Vista (28°30'46''S, 59°01'49''W) in Corrientes province (Esteros del Ibera ecoregion).

*Trachemys dorbigni* - Federal (30°40'54''S, 58°47'20''W) in Entre Ríos province (Espinal ecoregion).

*GenBank acc. number:* OR135722 (28S, TD), OR135720 (ITS2, TD)

*Voucher specimens:* MLP-He 8091

**Remarks:** This species was described for *K. scorpioides* from Pernambuco, Brazil (Freitas & Dobbin Jr., 1959). This is the first record of *T. diaphanus* for *P. hylarii* and *T. dorbigni* from Argentina. Table 5 shows that *T. diaphanus* is more closely related to *T. dubius* than to the rest of the species of the genus. The 28S gene sequences for the individuals found in both turtle species studied here constitute the first of this gene for *T. diaphanus*.

**Phylum** Nematoda

**Class** Chromadorea

**Family** Kathlaniidae Lane, 1914

*Falcaustra* Lane, 1915

*Falcaustra affinis* (Leidy, 1856)

**Diagnosis:** Three long vesicular lips; cephalic papillae present; small deirids; esophagus divided into pharynx, body, isthmus, and bulb. Males with a muscular ventral pseudoventosa; the ventral region between the cloaca and pseudoventosa contains 35 to 43 pairs of muscles. Ten pairs of caudal papillae present, three precloacal and seven postcloacal. Sclerotized spicules nearly equal in size. Well-developed gubernaculum. Females with vagina anteriorly directed, conical tail, with a pair of phasmidia near the tip. Eggs elliptical, thin-walled (Table 4).

**Site of infection:** Intestine.

**Host and localities:** *Trachemys dorbigni* - La Plata (35°01'18"S, 57°51'25"W) in Buenos Aires province (Pampa ecoregion), Concordia (31°21'22"S, 58°05'42"W) and Federal (30°40'54"S, 58°47'20"W) in Entre Ríos province (Espinal ecoregion).

**Voucher specimens:** MLP-He 8092

**Remarks:** This species was originally recorded from North American turtles and amphibians (Baker, 1986). Recently, Mascarenhas & Müller (2015) found this species parasitizing *T. dorbigni* from Rio Grande do Sul, Brazil. Our finding represents the southernmost record for the species and the first geographic record for Argentina.

**Family** Pharyngodonidae Travassos, 1919

*Thelandros* Wedl, 1862

*Thelandros* sp.

**General description** (based on four gravid females): Robust body 3.44 (2.7-4.25) mm long and 412 (298-554) µm wide, with cuticle annulations. Mouth opening triangular, surrounded by three bilobed lips, with a pedunculate amphid in each ventrolateral lobe. Total length of esophagus 382 (350-402) µm, corpus 250 (245-255) µm, isthmus 20 (16-22) µm, and bulb 94 (91-98) µm. Nerve ring and excretory pore at 120 (112-135) µm and 473 (418-544) µm from anterior end, respectively. Vulva post equatorial, 1.42 (1.10-1.84) mm from the posterior end. The anus opens at the end of the body, which ends in a caudal filament 366 (327-388) µm long. Eggs oval, 84 (81-88) µm x 41 (36-49) µm, with terminal operculum (Table 4, Fig. 5).

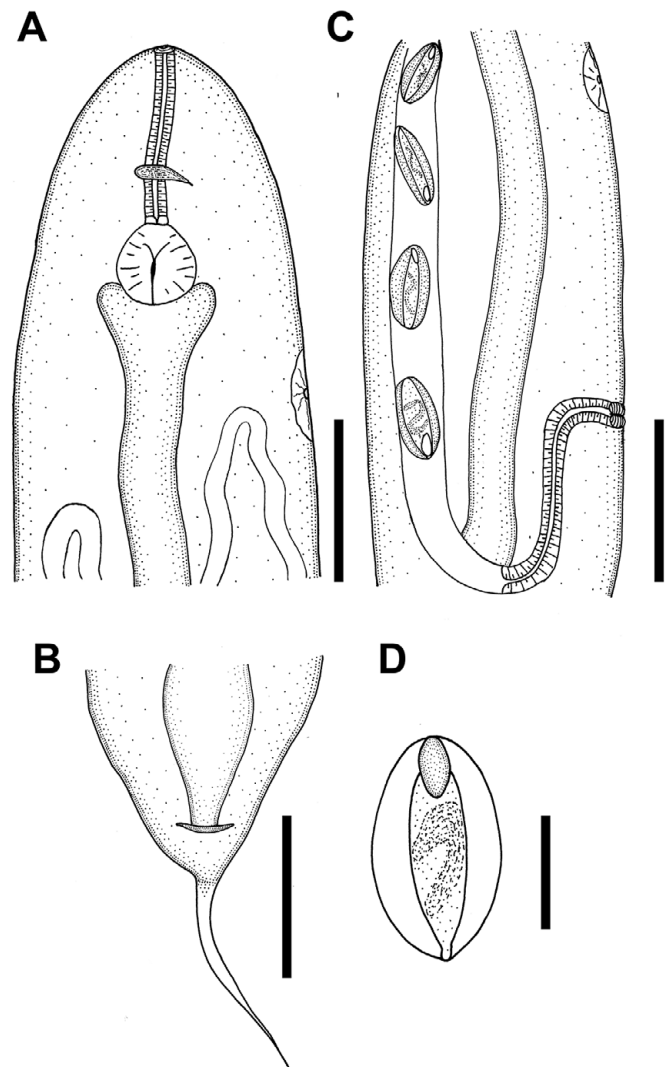
**Site of infection:** Stomach.

**Hosts and localities:** *Acanthochelys pallidipectoris* - Vera (29°26'14"S, 60°08'03"W) in Santa Fe province (Humid Chaco ecoregion); Las Lajitas (24°43'30"S, 64°09'32"W) in Salta province (Dry Chaco ecoregion). *Phrynops hylarii* - General José de San Martín (26°24'49"S, 59°22'02"W) in Chaco province (Dry Chaco ecoregion).

**GenBank acc. number:** OR135723 (18S)

**Voucher specimens:** MLP-He 8093

**Remarks:** The cephalic structures, the morphology of the esophagus, the position of the excretory pore and vulva, the pedunculate tail, and the shape of the eggs indicate that these specimens belong to *Thelandros* (De Sousa *et al.*, 2018). Unfortunately, only



**Figure 5.** Line drawings of *Thelandros* sp. detailing anterior end, lateral view (A), posterior end, ventral view (B), vulva (C), and egg (50 µm scale) (D). Scale bars 100 µm.

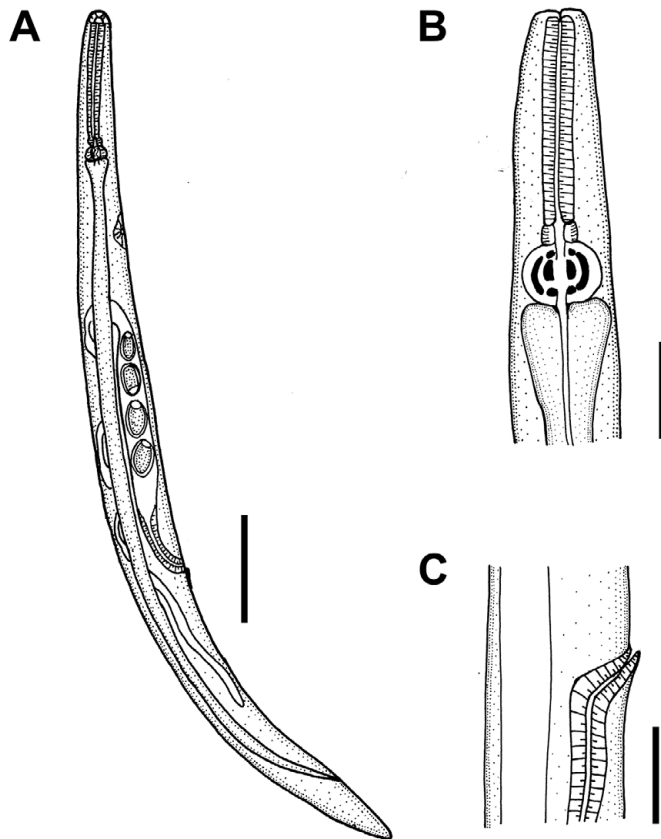
females were found, so it was not possible to reach their specific identity. This is the first record of the genus for freshwater turtles. The phylogenetic analysis made with 18S gene sequences of the family Pharyngodonidae shows a stronger association between the species of *Thelandros* recorded in lizards (KY541834 and KJ778073) and the species described here than with the rest of the species of the family (Fig. 3B).

Pharyngodonidae gen. *et* sp. indet.

**General description** (based on three gravid females): Small and cylindrical body, truncated anteriorly and narrow at the posterior end, 2.35-2.75 mm long and 113-125 mm wide. Mouth surrounded by three triangular lips. Without buccal capsule, esophagus 289-310 µm long, ending in a bulb 100-115 µm x 120-127 µm. Excretory pore opens at 355-389 µm from anterior end. Vulva opens 730-764 µm from caudal end, at mid-posterior part of body. One to five elliptical eggs 59-61 µm x 45-49 µm (Fig. 6).

**Site of infection:** Stomach.

**Hosts and localities:** *Acanthochelys pallidipectoris* - Joaquín V. González (29°26'14"S, 60°08'03"W) in Salta province (Dry Chaco ecoregion). *Kinosternon scorpioides* - Miraflores (25°



**Figure 6.** Line drawings of Pharyngodonidae gen. sp. indet. Complete individual (A) (scale 200  $\mu\text{m}$ ), detail anterior end (B), and vulva (C) in lateral view. Scale bars 100  $\mu\text{m}$ .

38°16'S, 60°56'03"W) in Chaco province and Las Lajitas (24°43'30"S, 64°09'32"W) in Salta province (Dry Chaco ecoregion).

*Voucher specimens:* MLP-He 8094

*Remarks:* One female was found in feces of *A. pallidipectoris* and two females were recovered from regurgitates of *K. scorpioides*. Because of the condition of the specimens and the fact that males were not found, it was not possible to reach genus and species level taxonomic identifications. This finding represents the first record of the family for *K. scorpioides*.

**Family** Camallanidae Railliet & Henry, 1915

*Camallanus* Railliet & Henry, 1915

*Camallanus emydidius* Mascarenhas & Müller, 2017

*Diagnosis:* The arrangement of ridges on the cephalic capsule, the number and distribution of caudal papillae (seven precloacal, two adcloacal, and four poscloacal), the morphology of the spicules in males, and the morphology of the vulva and number of mucrons in females, place these individuals within *C. emydidius* (Table 4).

*Site of infection:* Stomach and intestine.

*Host and localities:* *Trachemys dorbigni* - Federal (30°40'54"S, 58°47'20"W) in Entre Ríos province (Espinal ecoregion).

*Historical records in Argentina:* *Trachemys dorbigni* - Concordia (31°21'22"S, 58°05'42"W) in Entre Ríos province (Espinal ecoregion) (Palumbo *et al.*, 2024).

*Voucher specimens:* MLP He-8060

*Remarks:* *Camallanus emydidius* was originally described parasitizing *T. dorbigni* from Rio Grande do Sul, Brazil (Mascarenhas & Müller, 2017) and was later recorded on *H.*

*tectifera* from the same locality (Chaviel *et al.*, 2020). This record represents a new locality for *C. emydidius*. Additionally, there are records of other species of Camallanidae in Argentina: *C. pallidipectoris* Palumbo, Servián, Cassano & Diaz, 2024 in *A. pallidipectoris* and *P. hilarii* in Santa Fe and Entre Ríos, *C. spiculatus* Palumbo, Servián, Cassano & Diaz, 2024 in *A. spixii* and *P. hilarii* in Corrientes and *Serpinema* sp. in *K. scorpioides* in Formosa (Palumbo *et al.*, 2024).

**Family** Gnathostomatidae Railliet, 1895

*Spiroxys* Schneider, 1866

*Spiroxys contortus* (Rudolphi, 1819)

*Diagnosis:* Oral opening surrounded by two trilobed lips, the middle lobe has a truncated "tooth" at its apex; deirids located posterior to the excretory pore. Males with four pairs of precloacal papillae and seven pairs postcloacal. Spicules of equal size, supported by a cuticular gubernaculum. Vulva cuticular, located posterior to midbody. Vagina directed anteriorly, oviparous.

*Site of infection:* Stomach and intestine.

*Host and localities:* *Hydromedusa tectifera* - Tanti (31°22'05"S, 64°34'34"W) and Flor Serrana (31°23'36"S, 64°35'42"W) streams in Córdoba province (Dry Chaco ecoregion). *Phrynops hilarii* - Diamante (32°02'52"S, 60°38'10"W) in Entre Ríos province (Espinal ecoregion), Bella Vista (28°30'46"S, 59°01'49"W) in Corrientes province (Esteros del Ibera ecoregion) and Vera (29°26'14"S, 60°08'03"W) in Santa Fe province (Humid Chaco ecoregion). *Trachemys dorbigni* - La Plata (34°53'26"S, 57°59'40"W) in Buenos Aires province (Pampa ecoregion) and Concordia (31°21'22"S, 58°05'42"W) in Entre Ríos province (Espinal ecoregion).

*Historical records in Argentina:* *Hydromedusa tectifera* and *Phrynops hilarii* - Magdalena (35°01'36"S, 57°17'24"W) in Buenos Aires province (Pampa ecoregion) (Palumbo *et al.*, 2016).

*Remarks:* This species was previously recorded from *A. spixii*, *H. tectifera*, *P. hilarii*, and *T. dorbigni* from Brazil (Mascarenhas & Müller 2021), and from *H. tectifera* and *P. hilarii* from Argentina (Palumbo *et al.*, 2016). The 18S ribosomal gene of *S. contortus* found parasitizing a *H. tectifera* from Buenos Aires could be partially sequenced. Comparison with sequences obtained from specimens found parasitizing *Emys orbicularis* (L.) from Poland confirm the wide geographic range of *S. contortus* and the ability to adapt to different environments and hosts (Demkowska-Kutrzepa *et al.*, 2021). This is the first record of *S. contortus* for *T. dorbigni* from Argentina.

**Family** Hedruridae Railliet, 1916

*Hedruris* Nitzsch, 1821

*Hedruris dratini* Palumbo *et al.*, 2020

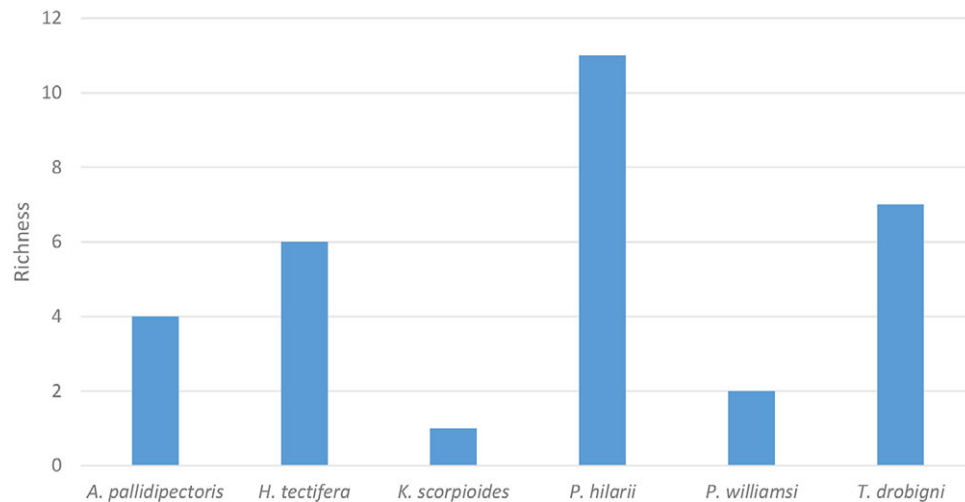
*Diagnosis:* The same position of the excretory pore and deirids with respect to the anterior end, the number and arrangement of the caudal papillae in males, and the shape of eggs, place these specimens within the species *H. dratini*.

*Site of infection:* Stomach and intestine.

*Host and localities:* *Hydromedusa tectifera* - La Plata (34°51'38"S, 58°03'54"W) in Buenos Aires province (Pampa ecoregion). *Trachemys dorbigni* - La Plata (34°53'26"S, 57°59'40"W) in Buenos Aires province (Pampa ecoregion).

*Historical records in Argentina:* *Hydromedusa tectifera* and *Phrynops hilarii* - La Plata (34°53'02"S, 58°02'30"W) in Buenos Aires province (Pampa ecoregion) (Palumbo *et al.*, 2020)

*Remarks:* *Hedruris dratini* was previously recorded for *H. tectifera* and *P. hilarii* from Argentina (Palumbo *et al.*, 2020). This is the first record of the species for *T. dorbigni*.



**Figure 7.** Number of species of endoparasites recorded per turtle species.

### Comments on parasite ecology

A total of 487 turtles were analysed, of which 433 were examined only from stomach contents, whereas the rest (54) were analysed entirely from viscera. Of the total number of turtles, 180 were parasitized ( $P = 37.03$ ) by at least one of the 18 taxa found and 6230 helminths specimens were counted. Most of the eviscerated turtles were parasitized ( $P = 92.59$ ,  $MI = 88.16$ ,  $A = 2733$ ). In contrast, parasites were found in 129 of the hosts analysed from stomach contents ( $P = 26.59$ ,  $MI = 27.32$ ,  $A = 3497$ ).

The Pampa ecoregion had the highest species richness ( $S = 14$ ), followed by Espinal ecoregion ( $S = 10$ ), the Humid ( $S = 5$ ) and Dry ( $S = 4$ ) Chaco, and finally Rainforest and Esteros del Ibera. According to the host the highest specific richness (Fig. 7) in this study was found in *P. hilarii* ( $S = 11$ ), followed by *T. dorbigni* ( $S = 8$ ), while the lowest richness was found in *K. scorpioides* ( $S = 1$ ). *Spiroxys contortus* was the species with the largest distribution (found in five ecoregions and three host species).

### Discussion

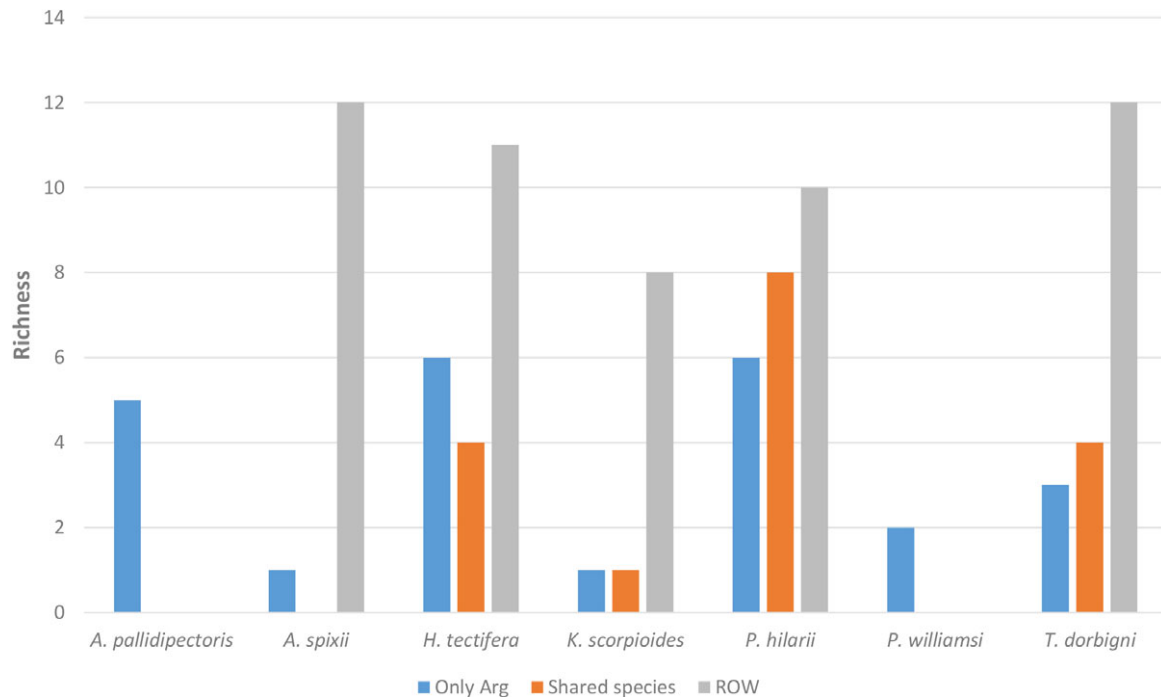
A total of 21 new geographic records were documented, increasing to 36 the total parasite records for freshwater turtles in different Argentine Chaco-Pampa plain ecoregions (Pampa, Espinal, Dry Chaco, Humid Chaco ecoregions) and neighbouring ones (Atlantic Rainforest and Esteros del Ibera ecoregions) (Mascarenhas & Müller, 2021). Fourteen new parasite-host associations were reported, with *A. pallidipectoris* having the highest number of records (4), followed by *T. dorbigni* (3), *P. hilarii*, *P. williamsi* and *H. tectifera* (2), and *K. scorpioides* (1). Notably, the findings in *A. pallidipectoris* and *P. williamsi* are significant as only two parasite species were reported previously for the former turtle species (Palumbo *et al.*, 2024), whereas no parasites were known for the second species throughout their distribution. Figure 8 illustrates the contribution of this study to each host species, highlighting the records for Argentina and the species documented in other countries but not yet in Argentina.

The turtle *P. hilarii* shares habitat and parasites with other turtle species along different Argentine provinces and ecoregions. Thus, *P. hilarii* shares four parasitic species with *H. tectifera*

(i.e., *C. testudinis*, *H. orestiae*, *H. dratini*, and *S. contortus*), two with *T. dorbigni* (i.e., *T. diaphanus* and *S. contortus*), and two with *A. pallidipectoris* (i.e., *Thelandros* sp. and *Camallanus pallidipectoris*). It is also observed that *A. pallidipectoris* and *K. scorpioides* were unique in this study harbouring the nematode Pharyngodonidae gen. et sp. indet. A recent study compared the parasitic fauna of three species of freshwater turtles from southern Brazil and mentioned 11 helminth species for *A. spixii* and eight species for both *H. tectifera* and *P. hilarii* (Mascarenhas *et al.* 2022). Authors stated that the parasite community of *A. spixii* is more similar to that of *H. tectifera* than that of *P. hilarii*. In Argentina, *A. spixii* does not share environments with *H. tectifera* but does with *P. hilarii*, enlarging the chances to acquiring the same nematode species (Palumbo *et al.*, 2024). The greater number of shared helminth species was recorded between *H. tectifera* and *P. hilarii* (4).

The most commonly used technique for searching endohelminth parasites is the capture and evisceration of hosts followed by processing of their internal organs. However, when working with larger vertebrates and considering that some species of turtles are endangered (e.g., *A. pallidipectoris*), euthanasia is not possible. The complete hosts analyzed in this study consisted of specimens found dead in the wild or housed in herpetological collections. Thus, to increase the collection of internal parasite samples, noninvasive technique such as stomach flushing was employed. Most of the parasitic species found in stomach contents were also found in eviscerated hosts of the same species and locations. Moreover, this procedure allowed us the detection of Pharyngodonidae gen. sp. in *A. pallidipectoris* and *K. scorpioides*, *Thelandros* sp. in *A. pallidipectoris* and *P. hilarii*, and *H. dratini* in *P. hilarii*, which could not be recorded through evisceration. These results indicate that flushing is a valuable technique for describing parasite fauna in this type of host.

Finally, it is important to emphasize how the integrative taxonomy (i.e., different morphological and molecular tools) helped us to solve and elucidate taxonomical hypothesis and systematic problems. For this reason, the contribution of sequences of different genes, even if not used in the work itself (such as the sequences of *Caimanicola brauni* or *Prionosomoides phrynopsis*) can be very useful to other researchers in future studies. Therefore, the most



**Figure 8.** Number of endoparasite species recorded only in Argentina (Only Arg), species recorded both in Argentina and elsewhere (Shared species) and species recorded outside Argentina (ROW), per turtle species.

commonly used sequences (28S, 18S, ITS, etc.) should be an additional character provided in each species description.

**Acknowledgments.** We thank Dr. Marina Ibáñez Shimabukuro for her collaboration in DNA extraction and replication and Dr. Adriana Manzano and Dr. Alfredo Holley for providing the hosts. This work is the ILPLA's scientific contribution N°1260.

**Funding.** This study was partially funding by UNLP N828 (Dir. JID).

**Competing interest.** Authors declare they have no financial interests.

**Ethical approval.** Not applicable.

## References

- Achatz, TJ, Brito, ES, Fecchio, A, and Tkach VV. (2021) Description and phylogenetic position of a new species of *Herpetodiplostomum* from *Phrynops geoffroanus* in Brazil and a reevaluation of *Cheloniodiplostomum*. *Journal of Parasitology* **107**, 455–462. <https://doi.org/10.1645/21-18>.
- Alcalde, L, Derocco, NN, Rosset, SD. (2010) Feeding in syntopy: diet of *Hydromedusa tectifera* and *Phrynops hilarii* (Chelidae). *Chelonian Conservation and Biology* **9**, 33–44. <https://doi.org/10.2744/CCB-0794.1>.
- Alcalde, L, Derocco, NN, Rosset, SD, and Williams, JD. (2012) Southernmost localities of *Trachemys dorbigni* and first record of *Trachemys scripta* for Argentina (Cryptodira: Emydidae). *Chelonian Conservation and Biology* **11**, 128–133. <https://doi.org/10.2744/02k.1>.
- Alcalde, L, Sánchez, RM, and Pritchard, P. (2021) *Hydromedusa tectifera* Cope 1870 – South American Snake-necked Turtle, Argentine Snake-necked Turtle, Tortuga Cuello de Vibora, Cágado Pescoço de Cobra. *Chelonian Research Monographs* **5**, 1–17.
- Altschul, SF, Madden, TL, Schäffer, AA, Zhang, J, Zhang, Z, Miller, W, and Lipman, DJ. (1997) Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Research* **25**, 3389–3402. <https://doi.org/10.1093/nar/25.17.3389>.
- Baker, MR. (1986) *Falcaustra* species (Nematoda: Kathlaniidae) parasitic in turtles and frogs in Ontario. *Canadian Journal of Zoology* **64**, 228–237.
- Bencke, GA, Chomenko, L, and Sant'Anna, DM. (2016) O que é o Pampa? In Chomenko, L, and Bencke, GA. (eds), *Nosso Pampa Desconhecido*. Rio Grande do Sul: Fundação Zoobotânica do Rio Grande do Sul, pp. 243–354.
- Bonfield, JK, Smith, KF, and Staden, R. (1995) A new DNA sequence assembly program. *Nucleic Acids Research* **23**, 4992–4999. <https://doi.org/10.1093/nar/23.24.4992>.
- Bonino, M, Lescano, J, Haro, J, and Leynaud, G. (2009) Diet of *Hydromedusa tectifera* (Testudines-Chelidae) in a mountain stream of Córdoba province, Argentina. *Amphibia-Reptilia* **30**, 545–554.
- Bujes, CS. (2008) *Biología e conservação de quelônios no delta do Rio Jacuí – RS: Aspectos da história natural de espécies em ambientes alterados pelo homem*. Universidad Federal do Rio Grande do Sul: Porto Alegre.
- Bush, AO, Lafferty, KD, Font, JM, and Shostak, AW. (1997) Parasitology meets ecology: definitions, clarifications, examples and Margolis *et al.* revisited. *Journal of Parasitology* **83**, 575–583.
- Cabrera, MR, Haro, JG, and Monguillot, JC. (1986) Presencia de *Hydromedusa tectifera* y *Phrynops hilarii* (Testudines: Chelidae) en la provincia de Córdoba, Argentina. *Academia Nacional de Ciencias* **73**, 1–10.
- Cagle, FR. (1939) A system of marking turtles for future identification. *Copeia* **1939**, 170–173.
- Campião, KM, Ribas, A, and Tavares, LER. (2015) Diversity and patterns of interaction of an anuran–parasite network in a neotropical wetland. *Parasitology* **142**, 1751–1757. <https://doi.org/10.1017/S0031182015001262>.
- Carvalho, VT, Vogt, RC, Rojas, RR, Silva Nunes, M, Fraga, R, Ávila, RW, Rhodin, AG, Mittermeier, RA, Hrbek, T, and Farias, IP. (2022) Four in one: cryptic diversity in Geoffroy's Side-Necked Turtle *Phrynops geoffroanus* (Schweigger, 1812) (Testudines: Pleurodira: Chelidae) in Brazil. *Diversity* **14**, 360.
- Cassano, MJ, and Alcalde, L. (2022) Diet and habitat of the Scorpion Mud Turtle (*Kinosternon scorpioides scorpioides*) in the southern limit of the species' distribution (Argentina). *Chelonian Conservation and Biology* **21**, 232–245.
- Catto, JB, and Amato, JFR. (1993) Two new species of *Pseudotolorchis* (Digenea, Telorchidae) parasites of the caiman, *Caiman crocodilus yacare*

- (Reptilia, Crocodylia) from the Pantanal Mato-Grossense, Brazil. *Memorias do Instituto Oswaldo Cruz* **88**, 561–566.
- Chaviel, BM, Mascarenhas, CS, Bernardon, FF, Coimbra, MAA, and Müller, G.** (2020) New records of helminths in Chelidae freshwater turtles (Testudines) in South America. *Revista Brasileira de Zootecia* **21**, 1–11. <https://doi.org/10.34019/2596-3325.2020.v21.28232>.
- Cope, ED.** (1875) Checklist of North American Batrachia and Reptilia. *Bulletin of the U.S. National Museum* **1**, 1–104.
- Cordero, EH.** (1946) *Ophiotaenia cohospes* n. sp. de la tortuga fluvial *Hydro-medusa tectifera* Cope, una larva plerocercóide en el parénquima de *Temnocephala brevicornis* Mont. y su probable metamorfosis. *Comunicaciones Zoológicas del Museo de Historia Natural de Montevideo* **2**, 1–15.
- Cribb, TH, Adlard, RD, and Bray, RA.** (1998) A DNA-based demonstration of a three-host life-cycle for the Bivesiculidae (Platyhelminthes: Digenea). *International Journal for Parasitology* **28**, 1791–1795. [https://doi.org/10.1016/S0020-7519\(98\)00127-1](https://doi.org/10.1016/S0020-7519(98)00127-1).
- De Sousa, A, Jorge, F, Carretero, MA, Harris, DJ, Roca, V, and Perera, A.** (2018) The importance of integrative approaches in nematode taxonomy: the validity of *Parapharyngodon* and *Thelandros* as distinct genera. *Journal of Helminthology* **93**, 1–13. <https://doi.org/10.1017/S0022149X1800069X>.
- Demkowska-Kutrzepa, M, Szczepaniak, K, Rocze-Karczmarz, M, Palumbo, EO, Studzinska, M, Różanski, P, and Tomczuk, K.** (2021) The first case of *Spiroxyx contortus* in European pond turtle (*Emys orbicularis*) in the wild in Poland. *International Journal for Parasitology Parasites Wildlife* **16**, 26–29. <https://doi.org/10.1016/j.ijppaw.2021.07.004>.
- Di Pietro, DO, Alcalde, L, Williams, JW, and Cabrera, MO.** (2012) *Hydro-medusa tectifera* (South American snake-necked turtle). *Herpetological Review* **43**, 303.
- Dollfus, RP.** (1935) Sur crocodilicola et autres hemistomes de crocodiliens. *Archives du Museum d'Historie Naturelle, Paris* **12**, 637–646.
- Dubois, G.** (1936) Les diplostomes de reptiles (Trematoda: Proterodiplostomidae nov. fam.) du Musée de Vienne. *Bulletin de la Société Neuchâteloise des Sciences Naturelles* **61**, 5–80.
- Dubois, G.** (1968) *Synopsis des strigeidae et des diplostomatidae (Trematoda)*. *Mémoires de la Société Neuchâteloise des Sciences Naturelles: Vol X*. Switzerland: Université de Neuchâtel.
- Dubois, G.** (1982) Répertoire des synonymes récents de genres et d'espèces de la superfamille des Strigeoidea Railliet, 1919 (Trematoda). *Bulletin de la Société Neuchâteloise des Sciences Naturelles* **105**, 163–183.
- Dubois, G.** (1979) Révision et nouvelle clé de détermination des Diplostomes de Reptiles (Trematoda: Proterodiplostomidae Dubois, 1936). *Bulletin de la Société Neuchâteloise des Sciences Naturelles* **102**, 39–48.
- Fernandes, BMM, and Kohn, A.** (2014) *South American trematodes parasites of amphibians and reptiles. Vol. 1*. Rio de Janeiro: Oficina de Livros.
- Fischthal, JH, and Kuntz, RE.** (1975) Some trematodes of amphibians and reptiles from Taiwan. *Proceedings of the Helminthological Society of Washington* **42**, 1–13.
- Floyd, RM, Rogers, AD, Lamshead, JD, and Smith, CR.** (2005) Nematode-specific PCR primers for the 18S small subunit rRNA gene. *Molecular Ecology Notes* **5**, 611–612.
- Font, WF, and Lotz, JM.** (2009) Family Telorchidae Looss, 1899. In Bray, RA, Gibson, DI, and Jones, A. (eds), *Keys to the Trematoda. Volume 3*. London: CAB International.
- Freiberg, MA.** (1945) Una nueva especie de Tortuga del genero *Platemys* Wagler. *Physis* **20**, 19–23.
- Freitas, JF, and Lent, H.** (1938) Pesquisas helminthológicas realizadas no Estado do Pará: II. dois novos trematódeos de *Caiman sclerops* Gray. *Memórias do Instituto Oswaldo Cruz* **33**, 53–56.
- Freitas, JFT, and Dobbin Jr., JE.** (1959) *Telorchis diaphanus* sp. n. trematódeo parasito de quelônio. *Anais da Sociedade de Biologia de Pernambuco* **16**, 191–199.
- Freitas, JFT, and Dobbin Jr., JE.** (1967) Sobre um novo trematódeo Echinostomatidae parasito de quelônio. *Memorias do Instituto Oswaldo Cruz* **65**, 37–39.
- Gray, JE.** (1831) A synopsis of the species of the class Reptilia. In Griffith, E, and Pidgeon, E. (eds), *The Animal Kingdom Arranged in Conformity with its Organization, by the Baron Cuvier*. Vol. **9**. London: The Class Reptilia Arranged by the Baron Cuvier, with Specific Descriptions, Whittaker, Treacher & Co.
- Hedrick, LR.** (1935) The life history and morphology of *Spiroxyx contortus* (Rudolphi); Nematoda: Spiruridae. *Transactions of the American Microscopical Society* **54**, 307–335.
- Hudson, PJ.** (2002) Parasites, diversity and the ecosystem. In Thomas, F, Renaud, F, and Guégan, JF. (eds), *Parasitism & Ecosystems Vol. 1*. New York: Oxford University Press.
- Hudson, PJ, Dobson, AP, and Lafferty, KD.** (2006) Is a healthy ecosystem one that is rich in parasites? *Trends in Ecology and Evolution* **21**, 381–385.
- Kimura, MA.** (1980) Simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *Journal of Molecular Evolution* **16**, 111–120. <https://doi.org/10.1007/BF01731581>.
- Larkin, MA, Blackshields, G, Brown, NP, et al.** (2007) Clustal W and Clustal X version 2.0. *Bioinformatics* **23**, 2947–2948. <https://doi.org/10.1093/bioinformatics/btm404>.
- Legler, JM.** (1977) Stomach flushing: a technique for Chelonian dietary studies. *Herpetologica* **33**, 281–284.
- Littlewood, DTJ.** (1994) Molecular phylogenetics of cupped oysters based on partial 28S rRNA gene sequences. *Molecular Phylogenetics and Evolution* **3**, 221–229. <https://doi.org/10.1006/mpev.1994.1024>.
- Littlewood, DTJ, Curini-Galletti, M, and Herniou, EA.** (2000) The interrelationships of Proseriata (Platyhelminthes: Seriata) tested with molecules and morphology. *Molecular Phylogenetics and Evolution* **16**, 449–466. <https://doi.org/10.1006/mpev.2000.0802>.
- Lombardero, OJ, and Moriena, A.** (1977) Nuevos trematodos para la Argentina en *Phrynos hilarii* (Duméril y Bibron). *Revista de Medicina Veterinaria* **58**, 64–68.
- MacCallum, M.** (1921) *Studies in helminthology*. New York: Zoopathologica.
- Mañé-Garzón, F, and Gil, O.** (1961a) Trematodos de las tortugas del Uruguay II. *Comunicaciones Zoológicas del Museo de Historia Natural de Montevideo* **97**, 1–13.
- Mañé-Garzón, F, and Gil, O.** (1961b) Trematodos de las tortugas del Uruguay IV: Tres nuevas especies del género *Telorchis* Luhe, 1900. *Comunicaciones Zoológicas del Museo de Historia Natural de Montevideo* **90**, 1–14.
- Mañé-Garzón, F, and Holcman-Spector, B.** (1968) Trematodos de las tortugas del Uruguay VII: Una nueva especie del género *Telorchis* (Lühe, 1900) del intestino de *Pseudemys dorsalis* (Dum. & Bib.). *Comunicaciones zoológicas del museo de historia natural de Montevideo* **9**, 1–4.
- Mañé-Garzón, F, and Holcman-Spector, B.** (1969) Trematodos de las tortugas del Uruguay IX: Tres nuevas especies del género *Herpetodiplostomum* Dubois, 1936. *Comunicaciones zoológicas del museo de historia natural de Montevideo* **126**, 1–4.
- Mascarenhas, CS, Bernardon, FF, and Müller, G.** (2016) Intestinal digenans of freshwater turtles *Phrynos hilarii* and *Acanthochelys spixii* (Testudines: Chelidae) from southern Brazil. *Revista Mexicana de Biodiversidad* **87**, 35–41. <https://doi.org/10.1016/j.rmb.2016.01.010>.
- Mascarenhas, CS, and Müller, G.** (2015) *Spiroxyx contortus* (Gnathostomatidae) and *Falcaustra affinis* (Kathlaniidae) from *Trachemys dorsalis* (Emydidae) in Southern Brazil. *Comparative Parasitology* **82**, 129–136. <https://doi.org/10.1654/4726.1>.
- Mascarenhas, CS, and Müller, G.** (2017) *Camallanus emydidius* n. sp. (Nematoda: Camallanidae) in *Trachemys dorsalis* (Duméril & Bibron, 1835) (Testudines: Emydidae) from Southern Brazil. *International Journal for Parasitology: Parasites Wildlife* **6**, 108–114. <https://doi.org/10.1016/j.ijppaw.2017.04.004>.
- Mascarenhas, CS, and Müller, G.** (2021) Checklist of helminths associated with continental Testudines from South America. *Neotropical Helminthology* **15**, 97–126. <https://doi.org/10.24039/rnh20211511047>.
- Mascarenhas, CS, Chaviel, BM, Bernardon, FF, Wolter, JH, Coimbra, MAA, and Müller, G.** (2022) Gastrointestinal helminths associated with three species of freshwater turtles in the Pampa biome, Southern Brazil. *Parasitology Research* **11**, 1–9. <https://doi.org/10.1007/s00436-021-07361-y>.
- McAllister, CT, Bursey, CR, and Freed, PS.** (2010) Helminth parasites (Cestoidea: Nematoda) of select Herpetofauna from Paraguay. *Journal of Parasitology* **96**, 222–224. <https://doi.org/10.1645/GE-2191.1>.
- Mereles, F, Céspedes, G, Egea-Elsam, J, and Spichger, R.** (2020) Estudios fitosociológicos en el gran chaco: estructura, composición florística y variabilidad del bosque de *Schinopsis balansae* en el chaco húmedo boreal, Paraguay. *Bonplandia* **29**, 39–55.

- Morello, JH, Matteucci, SD, Rodríguez, AF, and Silva, ME.** (2012) *Ecorregiones y Complejos Ecosistémicos Argentinos. Vol 1.* Orientación Gráfica Editora: Buenos Aires.
- Morgan, JAT, and Blair, D.** (1995) Nuclear rDNA ITS sequence variation in the trematode genus *Echinostoma*: an aid to establishing relationships within the 37-collar-spine group. *Parasitology* **111**, 609–615. <https://doi.org/10.1017/S003118200007709X>.
- Narain, D.** (1930) *Neodiplostomum gavialis* n. sp. from the crocodile. *Journal of Parasitology* **16**, 154–157.
- Niewiadomska, K.** (2002) Family Diplostomidae Poirier, 1886. In Gibson, DI, Jones, A, and Bray, RA. (eds), *Keys to the Trematoda Vol. I*. London: CAB International and Natural History Museum.
- Ostrowski de Núñez, VM.** (1987) The life history of *Acanthostomum brauni* Mañé-Garzón and Gil, 1961 (Trematoda, Acanthostomatidae). *Zoologischer Anzeiger* **218**, 273–286.
- Palumbo, EO, and Diaz, JI.** (2018) New species and new record of the genus *Cheloniodiplostomum* (Trematoda, Proterodiplostomidae, Polycotylineae), parasites of freshwater turtles from Argentina. *Parasitology Research* **117**, 767–773. <https://doi.org/10.1007/s00436-018-5750-9>.
- Palumbo, EO, Werneck, MR, and Diaz, JI.** (2019) Is *Amphiorchis* (Digenea: Spirorchidae) an exclusive parasite of sea turtles? *Helminthologia* **6**, 75–80. <https://doi.org/10.2478/helm-2018-0045>.
- Palumbo, EO, Capasso, SC, Cassano, MJ, Alcalde, L, and Diaz, JI.** (2016) *Spiroxyx contortus* (Rudolphi, 1819) and *Hedruris orestiae* (Moniez, 1889) in Argentine turtles. *Checklist* **12**, 1993. <https://doi.org/10.15560/12.6.1993>.
- Palumbo, EO, Servián, A, Sánchez, R, and Diaz, JI.** (2020) A new species of *Hedruris* (Nematoda: Hedruridae) from freshwater turtles, its life cycle and biogeographic distribution of the genus. *Journal of Helminthology* **94**, e93. <https://doi.org/10.1017/S0022149X19000877>.
- Palumbo, EO, Cassano, MJ, Alcalde, L, and Diaz, JI.** (2021) Seasonal variation of *Hedruris dratini* (Nematoda) parasitizing *Hydromedusa tectifera* (Chelidae), with focus on host's torpor state. *BMC Zoology* **6**, 10. <https://doi.org/10.1186/s40850-021-00078-6>.
- Palumbo, EO, Servián, A, Cassano, MJ, and Diaz, JI.** (2024) Camallaninae nematodes parasitizing endangered freshwater turtles from South America with the description of two new species. *South American Journal of Herpetology* **30**, 28–39. <https://doi.org/10.2994/SAJH-D-21-00051.1>.
- Rodrigues, JFM, Soares, DO, and Silva, JRF.** (2014) Sexing freshwater turtles: penile eversion in *Phrynops tuberosus* (Testudines: Chelidae). *Acta Herpetologica* **9**, 259–263. [https://doi.org/10.13128/Acta\\_Herpetol-14736](https://doi.org/10.13128/Acta_Herpetol-14736).
- Semeñiuk, MB, Alcalde, L, Sánchez, RM, and Cassano, MJ.** (2017) An easy, cheap, and versatile method to trap turtles, with calibrated sampling effort. *South American Journal of Herpetology* **12**, 107–116. <https://doi.org/10.2994/SAJH-D-16-00048.1>.
- Silva, LAF.** (2014) *Helminthofauna asociada a répteis provenientes da Reserva Particular do Patrimônio Natural Foz do Rio Aguapeí, Estado de São Paulo. Vol 1.* São Paulo: Universidade Estadual Paulista.
- Snyder, SD, and Tkach, VV.** (2001) Phylogenetic and biogeographical relationships among some holarctic frog lung flukes (Digenea: Haematoloecidae). *Journal of Parasitology* **87**, 1433–1440. [https://doi.org/10.1645/0022-3395\(2001\)087\[1433:PABRAS\]2.0.CO;2](https://doi.org/10.1645/0022-3395(2001)087[1433:PABRAS]2.0.CO;2)
- Sudarikov, VE.** (1960) Order Strigeata La Rue, 1926. Part 3. Superfamily Diplostomatoidea Nicoll, 1937. Families Alariidae Tubangui, 1922 and Bolbocephaloidea Strand, 1935. Superfamily Proterodiplostomatoidea Sudarikov, 1960. Trematodes of animals and man. *Osnovy Trematodologii* **18**, 451–694.
- Thatcher, VE.** (1963) Trematodes of turtles from Tabasco, Mexico, with a description of a new species of *Dadaytrema* (Trematoda: Paramphistomidae). *The American Midland Naturalist* **70**, 347–355.
- Thatcher, VE.** (1964) Estudio sobre los trematodos de reptiles de Tabasco, Mexico: lista de huéspedes y sus parasitos. *Anales de la Escuela Nacional de Ciencias Biológicas* **13**, 91–96.
- Wood, CL, and Johnson, PT.** (2015) A world without parasites: exploring the hidden ecology of infection. *Frontiers in Ecology Environment* **13**, 425–434.
- Zerecero, MC.** (1947) Posición sistemática de *Diplostomum brevis* y *D. cinosterni* MacCallum, 1921, y descripción de un nuevo trematodo parásito de *Chelydra serpentina* L. *Anales del Instituto de Biología, Universidad Nacional Autónoma de México* **18**, 507–516.