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

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Research Paper

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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; Southeast 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 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 Rios (No. 001/17), Salta (No. 662), and Santa Fe provinces (No. 198), Argentina.

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

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

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

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,

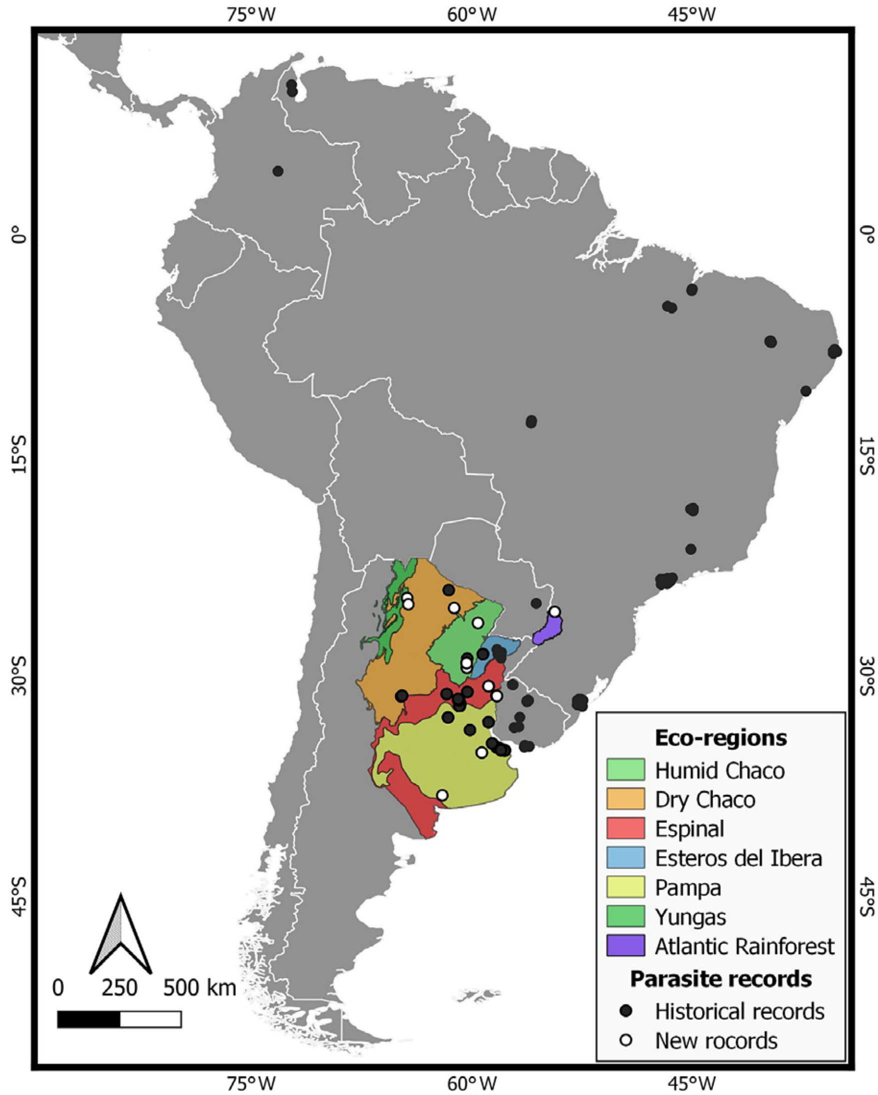

Figure 1. Records of helminths parasites studied in South American turtles.

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 °C for subsequent DNA extraction and genotyping (Table 2). DNA was extracted from the isolates using 200 μL of 5% Chelex solution (Bio-Rad Laboratories, CA, USA) containing 0.2 mg/mL Proteinase K (Roche), incubated at 56 °C overnight, followed by 10 min at 95 °C. Nuclear 18S, 28S, and ITS2 rDNA amplification were done using the appropriate primers. Each 50 μL polymerase chain reaction (PCR) contained 25 μL of GoTaq Green Master Mix (Promega, Madison, Wi, USA), 2.5 μL of each primer, 17 μL of water, and 3 μL of extracted DNA.

PCR amplification for 18S was performed using an Eppendorf Mastercycler ep gradient S, consisting of 94 °C for 15 min, followed by 35 cycles of 94 °C for 30 s, 60 °C for 30 s, and 72 °C for 70 s, with a final extension of 72 °C for 240 s, for 28S amplification the program consisting of 94 °C 4 min, followed by 30 cycles of 95 °C for 60 s, 60 ° C for 60 s, and 72 °C for 120 s, with a final extension of 72 °C for 4 min, and for ITS2 amplification was one cycle of 95 °C 3 min, 42 ° C 2 min, and 72 °C 1 min, followed by six cycles of 95 °C 45 s, 47 °C 45 s, and 72°C 1 min, followed with 35 cycles of 95 °C 20 s, 50 °C 20 s, and 72 °C 1 min, and a final extension of 72 °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.

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

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

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

Parasite species	Host	Access number
Proterodiplostomid taxa		
Cheloniodiplostomum argentinense	*P. hilarii*	OR135714
Cheloniodiplostomum sp.	*H. tectifera*	OR135719
Herpetodiplostomum vogti	*Phrynops geoffroanus*	MW544286
Proteroduboisia globulare	*Caiman yacare*	MT622346
Cystodiplostomum sp.	*Caiman yacare*	MT622330
Cystodiplostomum hollyi	*Caiman yacare*	MT622327
Proterodiplostomum longum	*Caiman crocodilus*	MT622341
Proterodiplostomum sp.	*Caiman yacare*	MT622345
Paraproterodiplostomum currani	*Alligator mississippiensis*	MT622369
Archaeodiplostomum overstreeti	*Alligator mississippiensis*	MT622323
Pseudocrocodilicola americaniense	*Alligator mississippiensis*	MT622354
Neocrocodilicola georgiana	*Alligator mississippiensis*	MT622333
Polycotyle ornata	*Alligator mississippiensis*	MT622339
Pseudoneodiplostomoides crocodilarum	*Crocodylus johnsoni*	MT622357
Pseudoneodiplostomum sp.	*Crocodylus johnsoni*	MT622365
Pseudoneodiplostomum thomasi	*Crocodylus niloticus*	MT622366
Mesodiplostomum gladiolum	*Caiman yacare*	MT622332
Crocodillicola pseudostoma	*Rhamdia guatemalensis*	MF398328
Paradiplostomum abbreviatum	*Caiman yacare*	MT622337
Heterodiplostomum lanceolatum	*Leptodactylus chaquensis*	MT622331
Alaria mustelae	*Lithobates sylvaticus*	JF820607

(*Continued*)

Table 3. (*Continued*)

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

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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
Cestoda						
Ophiotaenia cohospes	*H. tectifera*	Buenos Aires	19	31.5	12.5	75 (1–28)
			121[a]	7.4	1.3	12 (1–8)
		Córdoba	1	100	5	5 (0–5)
Digenea						
Caimanicola brauni	*P. hilarii*	Buenos Aires	6	14.2	15	15 (0–15)
		Santa Fe	8	12.5	74	74 (0–74)
Cheloniodiplostomum argentinense	*P. hilarii*	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)
Cheloniodiplostomum testudinis	*P. hilarii*	Santa Fe	8	37.5	10	30 (3–24)
Cheloniodiplostomum sp.	*H. tectifera*	Buenos Aires	19	5	4	4 (0–4)
	P. hilarii	Buenos Aires	6	16.6	500	500 (0–500)
Herpetodiplostomum duboisi	*P. williamsi*	Misiones	2	50	96	96 (0–96)
Nematophila grandis	*P. hilarii*	CABA	1	100	1	1 (0–1)
Prionosomoides phrynopsis	*P. hilarii*	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)
Telorchis birabeni	*P. hilarii*	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)
	P. williamsi	Misiones	2	50	43	43 (0–43)
Telorchis devincenzii	*H. tectifera*	Buenos Aires	19	5	21	21 (0–21)
Telorchis diaphanus	*P. hilarii*	Corrientes	1	100	15	15 (0–15)
	T. dorbigni	Entre Ríos	4	25	5	5 (0–5)
Telorchis dubius	*T. dorbigni*	Entre Ríos	4	50	7.5	15 (7–8)
Nematoda						
Camallanus emydidius	*T. dorbigni*	Entre Ríos	4	75	25.66	77 (4–60)
Falcaustra affinis	*T. dorbigni*	Buenos Aires	1	100	10	10 (0–10)
		Entre Ríos	4	100	209.25	837 (24–688)
Hedruris dratini	*H. tectifera*	Buenos Aires	121[a]	20	137.3	3297 (15–541)
	T. dorbigni	Buenos Aires	1[a]	100	25	25 (0–25)
Spiroxys contortus	*H. tectifera*	Córdoba	1	100	31	31 (0–31)
			154[a]	13.6	8.47	178 (3–47)
	P. hilarii	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)
	T. dorbigni	Entre Ríos	4	75	28	84 (2–55)
Pharyngodonidae gen. sp.	*A. pallidipectoris*	Salta	46[a]	2.17	1	1 (0–1)
	K. scorpioides	Chaco	8[a]	12.5	1	1 (0–1)
		Salta	8[a]	12.5	1	1 (0–1)
Thelandros sp.	*A. pallidipectoris*	Salta	46[a]	2.1	2	2 (0–2)
		Santa Fe	18[a]	5.5	2	2 (0–2)
	P. hilarii	Chaco	2[a]	50	3	3 (0–3)

[a]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. Acraspedot 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: Phrynops 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: *Phrynops 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: *Phrynops 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: *Phrynops 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: *Phrynops hilarii* and *Hydromedusa tectifera* - Magdalena (35°01′36″S, 57°17′24″W) Buenos Aires province (Pampa eco-region) (Palumbo & Diaz, 2018). *Phrynops hilarii* - unknow 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 *Phrynops 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 found 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* (Zerecero, 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 μm.

Ch. testudinis 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 forebody 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: *Phrynops williamsi* - Comandante Andresito (25°40′22″S, 54°02′22″W) in Misiones province (Atlantic Rainforest eco-region).

Voucher specimens: MLP-He 8086

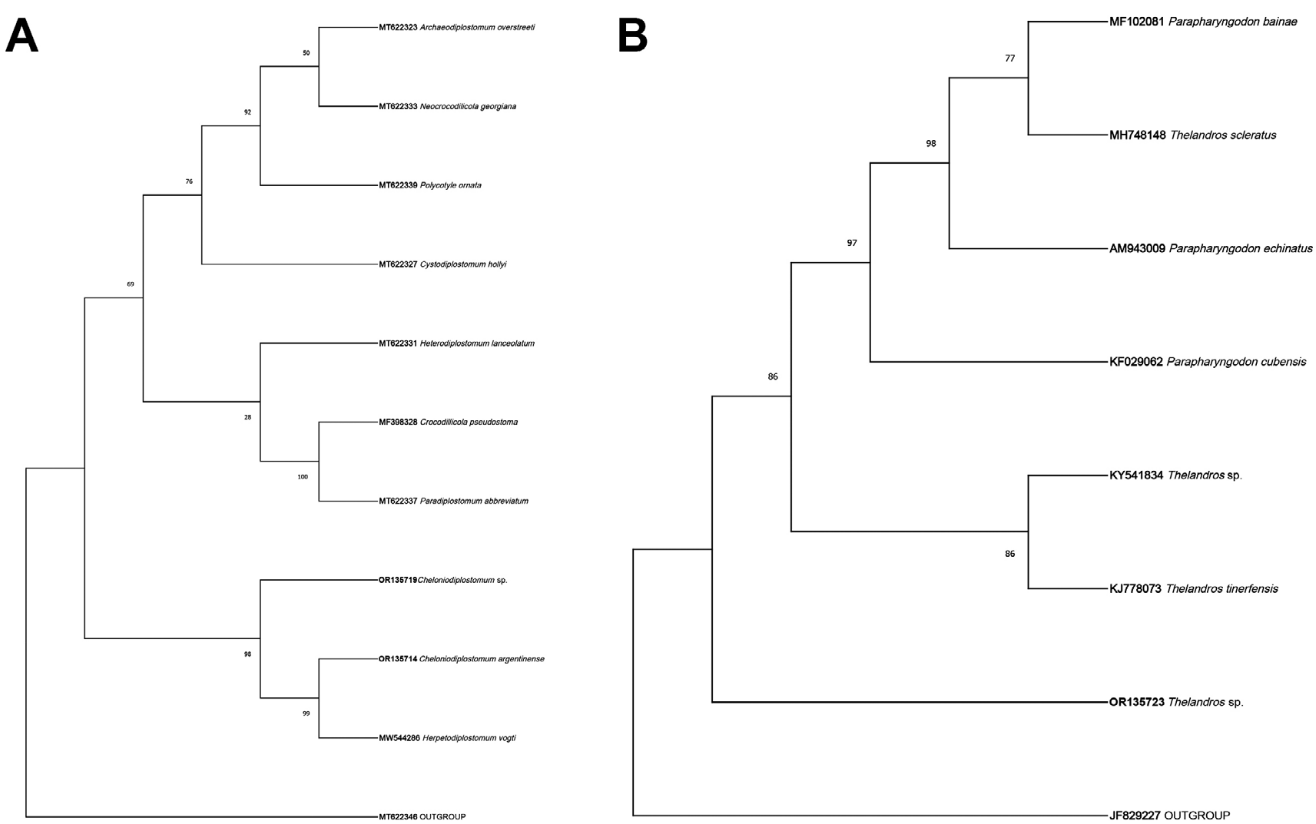

Figure 3. Phylogenetic tree based on *Cheloniodiplostomum* rDNA sequences newly obtained in this study compare with Diplostomidae sequences available in GenBank using the Maximum Likelihood method with a distance matrix calculation with K2P. The numbers at the nodes represent the percentages of 1000 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 Fischoeder, 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 extracecal (Table 4).

Site of infection: Intestine.

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

Historical records in Argentina: *Phrynops 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 phrynopsis (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: *Phrynops 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), Gualeguaychú (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* Fischathal & Kuntz, 1975 parasitizes *Mauremys reevesii* (Gray, 1831) from Taiwan (Fischthal & Kuntz, 1975); and *Pr. phrynopsis* was reported for *P. hilarii* from Uruguay (Mañé-Garzón & Gil, 1961a). This finding is a new country record for *Pr. phrynopsis* 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 vitellogenic 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: *Phrynops hilarii* - La Plata (34°53′26″S, 57° 59′40″W) in Buenos Aires province (Pampa eco-region); Gualeguaychú (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). *Phrynops williamsi* - Comandante

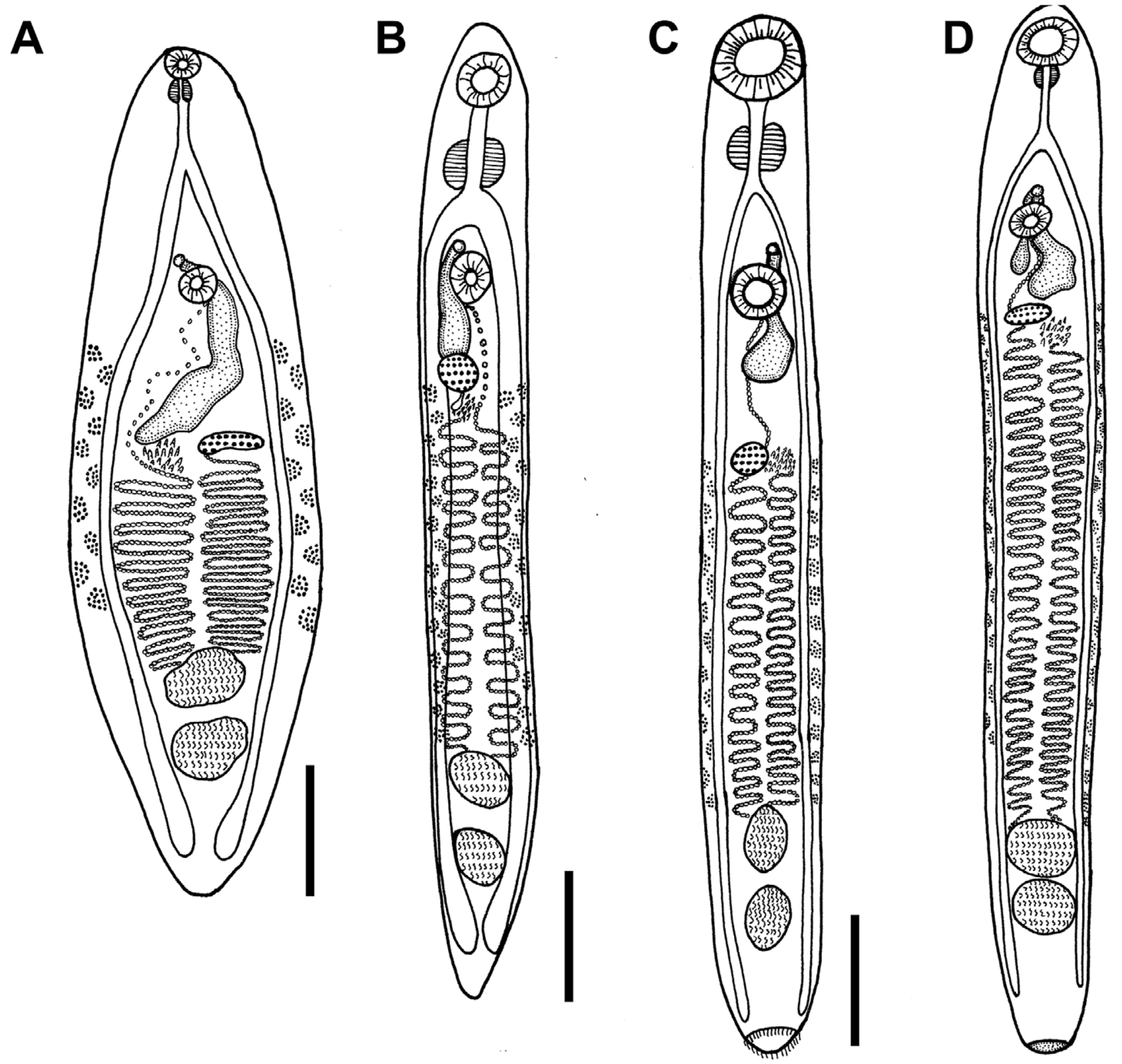

Figure 4. Line drawings of *Telorchis* species. *T. dubius* (A), *T. devincenzii* (B), *T. birabeni* (C), and *T. diaphanus* (D). Scale bars 200 μm.

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 *T. birabeni*	OR135718 *T. birabeni*	OR135720 *T. diaphanus*	OR135716 *T. dubius*
OR135715 *T. birabeni*				
OR135718 *T. birabeni*	0.0087			
OR135720 *T. diaphanus*	0.1102	0.1127		
OR135716 *T. dubius*	0.1102	0.1127	0.0128	
JF820593 *T. bonnerensis*	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: *Phrynops 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: *Phrynops 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

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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. hilarii* 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 poscloacal. 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 hilarii* - General José de San Martin (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 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).

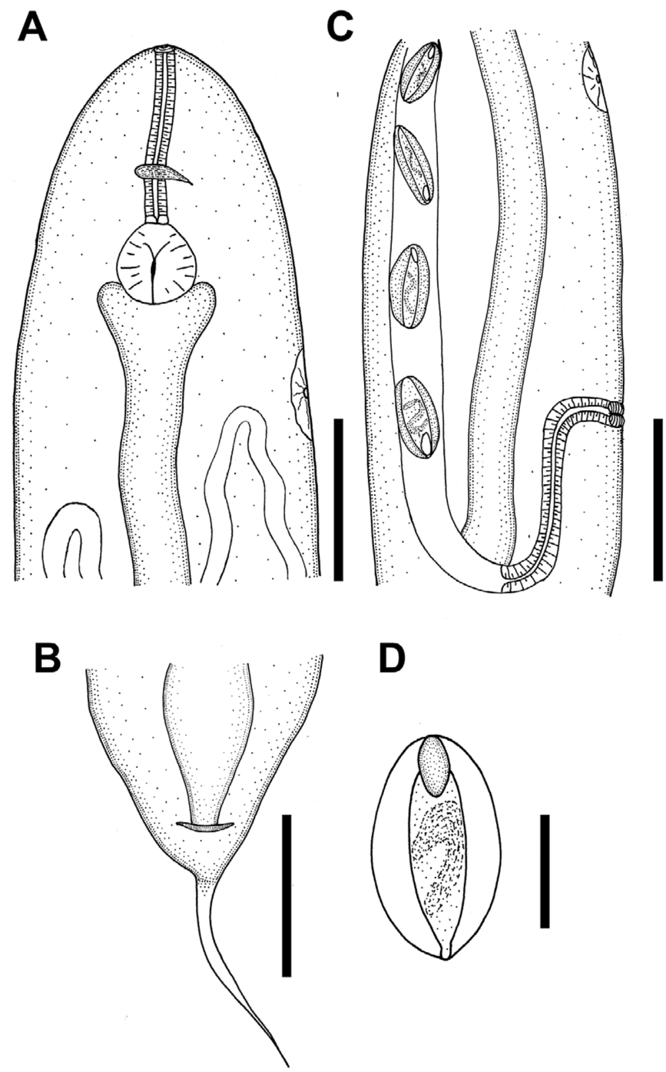

Figure 5. Line drawings of *Thelandros* sp. detailing anterior end, lateral view (A), posterior end, ventral view (B), vulva (C), and egg (50 µm scale) (D). Scale bars 100 µm.

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 × 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 × 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°

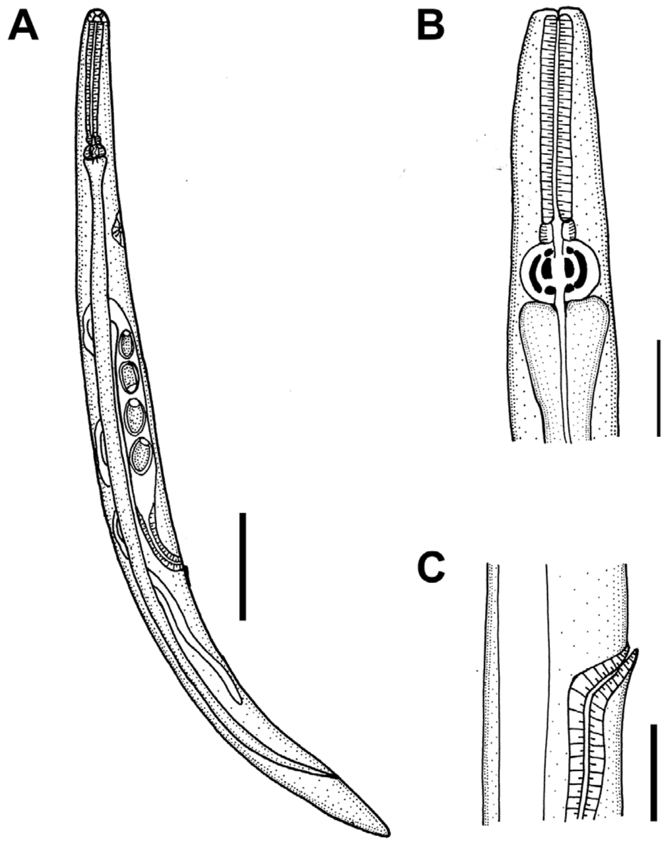

Figure 6. Line drawings of Pharyngodonidae gen. sp. indet. Complete individual (A) (scale 200 μm), detail anterior end (B), and vulva (C) in lateral view. Scale bars 100 μm.

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 Nitzch, 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.*

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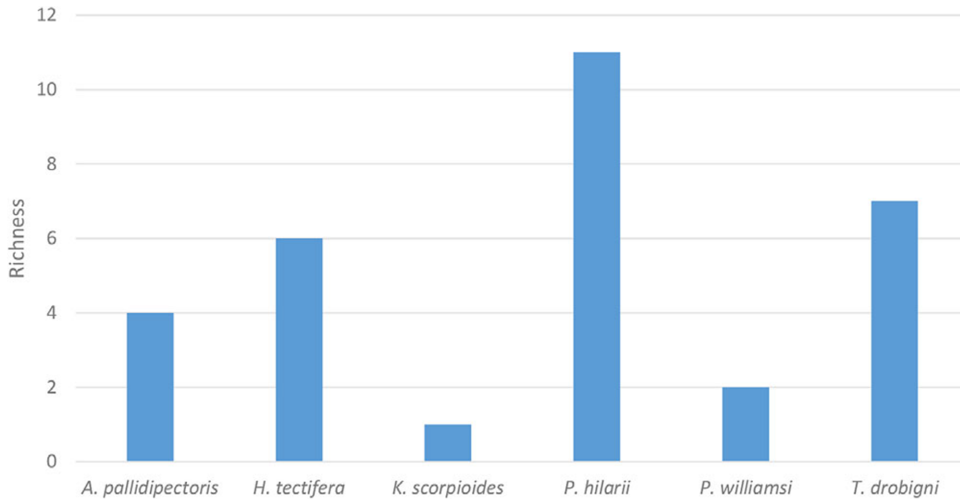

Figure 7. Number of species of endoparasites recorded per turtle species.

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

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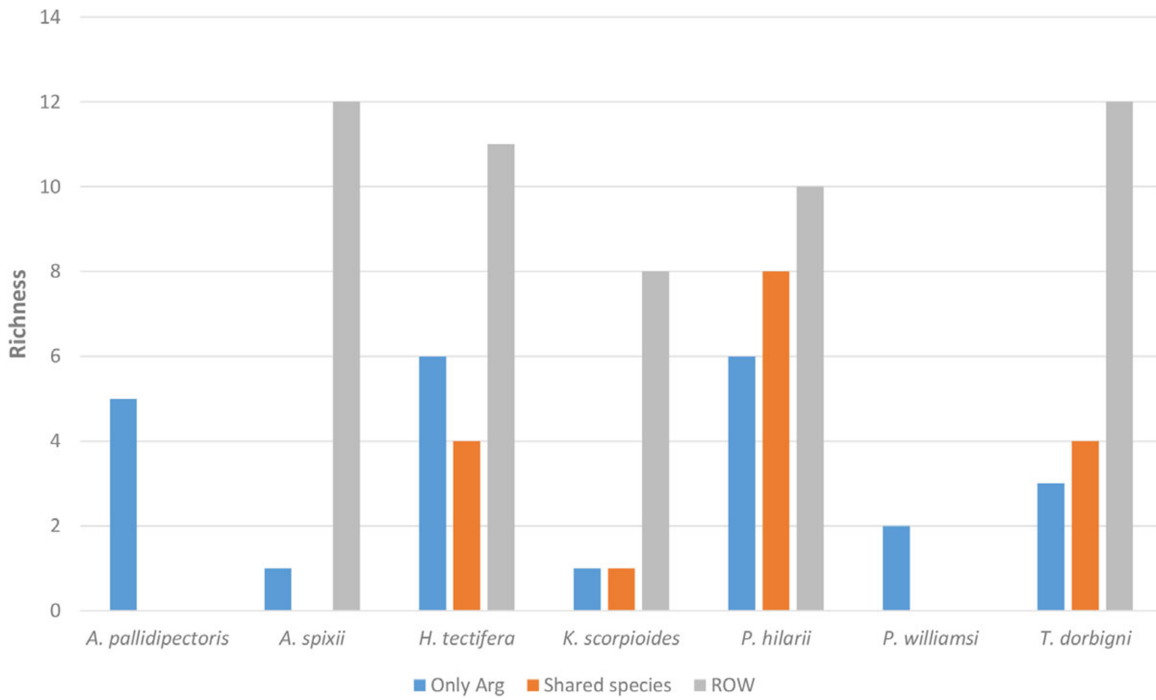

Figure 8. Number of endoparasite species recorded only in Argentina (Only Arg), species recorded both in Argentina and elsewhere (Shared species) and species recorded outside Argentina (ROW), per turtle species.

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

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