



New species and new record of the genus *Cheloniodiplostomum* (Trematoda, Proterodiplostomidae, Polycotylinae), parasites of freshwater turtles from Argentina

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Abstract

The aim of the present paper is to describe a new species of the genus *Cheloniodiplostomum* (Digenea, Proterodiplostomidae) in the Hilaire's toadhead turtle *Phrynops hilarii* from Argentina and to expand the geographic and host distribution of *Cheloniodiplostomum testudinis*. Additionally, we present a diagnostic key for *Cheloniodiplostomum*.

Keywords Digenea · Pleurodira · Freshwater · South America

Introduction

The trematode family Proterodiplostomidae Dubois, 1936 includes five subfamilies that are parasites of reptiles: Ophiodiplostominae Dubois, 1936 and Proalarioidinae Sudarikov, 1960 are parasites of snakes; Massoprostatinae Yamaguti, 1958 and Proterodiplostominae Dubois, 1936 are parasites of crocodiles; and members of Polycotylinae Monticelli, 1888 are parasites of crocodiles and turtles (Gibson et al. 2002). Apart from their different host distribution, several morphological features are used for their identification, such as the position of the vitellaria, the size and shape of the holdfast, and the presence of a pseudo sucker (Gibson et al. 2002). Within Polycotylinae, eight genera were described. Among them, members of *Cheloniodiplostomum* (Sudarikov, 1960) are parasites of Neotropical turtles containing only three recognized species: *Cheloniodiplostomum brevis* (MacCallum, 1921), *Cheloniodiplostomum delillei* (Zerecero, 1947), and *Cheloniodiplostomum testudinis*

(Dubois, 1936), being this last one the type species of the genus (Dubois 1979).

Cheloniodiplostomum brevis was described parasitizing two turtle species: *Phrynops geoffroanus* (Schweigger, 1812) in Colombia and *Kinosternon scorpioides* (L. 1766) from the Zoological Garden of New York, USA (MacCallum 1921; Dubois 1979). *Cheloniodiplostomum delillei* was recorded in the freshwater turtles *Chelydra serpentina* (L. 1758) and *Claudius angustatus* Cope, 1865 as well as in the diamondback water snake *Nerodia rhombifer* (Hallowell, 1852) in Mexico (Thatcher 1963, 1964). *Cheloniodiplostomum testudinis* was recorded in *Phrynops hilarii* (Dumeril & Bibron, 1835) in Corrientes, Argentina, in *Testudo* sp. in Rio Grande do Sul, Brazil, and in *P. geoffroanus* in São Paulo, Brazil (Dubois 1936; Lombardero and Moriena 1977; Fernandes and Kohn 2014; Silva 2014; Mascarenhas et al. 2016).

Knowledge of parasites in South American turtles is particularly scarce and fragmented (Lunaschi and Drago 2007; Fernandes and Kohn 2014; Silva 2014; Mascarenhas et al. 2016; Palumbo et al. 2016), especially regarding to the Argentinean species, which are the ones with the southernmost distribution of the region.

In this paper, we describe a new species of the genus *Cheloniodiplostomum* in freshwater turtles from Argentina. Additionally, we expand the geographic and host distribution of *C. testudinis*. We also present a diagnostic key for *Cheloniodiplostomum* species.

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Materials and methods

Specimens come from two turtle specimens analyzed, Argentine snake-necked turtle *Hydromedusa tectifera* Cope, 1869, and Hilaire's toadhead turtle *P. hilairei*, both found dead and collected in March and July 2016, respectively, near the stream Buñirigo (35° 01' 36" S, 57° 17' 24" W, datum: WGS84), Buenos Aires province, Argentina. The turtles were fixed in 10% formalin for their posterior examination. Viscera were fixed in 5% formalin for examination under a stereomicroscope. The helminth parasites found were conserved in 70% ethanol. Trematodes were stained with hydrochloric carmine or Tricromico Gomori, dehydrated in a graded ethanol series, cleared in eugenol, and mounted in natural Canada balsam for their study using a polarizing microscope (Olympus BX51®). The drawings were made with a camera lucida. Photographs were obtained taken with a Q-Imaging Go-3® digital camera. Some specimens were preserved for their study under a scanning electronic microscope (SEM) (Jeol/SET 100®), and photographed. In addition, serial histological sections of four specimens were made for a complete morphological study of the parasites. Specimens were identified according to Dubois (1936), Yamaguti (1961), Gibson et al. (2002), and Fernandes and Kohn (2014), and other specific bibliography was consulted. Measurements are given in micrometers unless otherwise indicated.

Voucher specimens were deposited in the Helminthological Collection of the Museo de La Plata. The analyzed hosts were deposited in the Herpetological Collection of the Museo de La Plata, numbers R-6376 and R-6402.

In addition, specimens of *Cheloniodiplostomum testudinis* and *Cheloniodiplostomum* sp. deposited in the Helminthological Collection of the Museo de La Plata under numbers MLP-He-6838 and MLP-He-6840 were analyzed.

Results

A total of 217 trematode specimens were recovered. *Hydromedusa tectifera* harbored 19 specimens in the first part of the intestine identified as *C. testudinis*, whereas *P. hilairei* harbored 198 specimens of two different species of *Cheloniodiplostomum* parasitizing the gut, 7 identified as *C. testudinis* and 191 belonging to a new species that is described below.

Proterodiplostomidae Dubois, 1936

Polycotylineae Monticelli, 1888

Cheloniodiplostomum Sudarikov, 1960

Cheloniodiplostomum testudinis (Dubois, 1936)

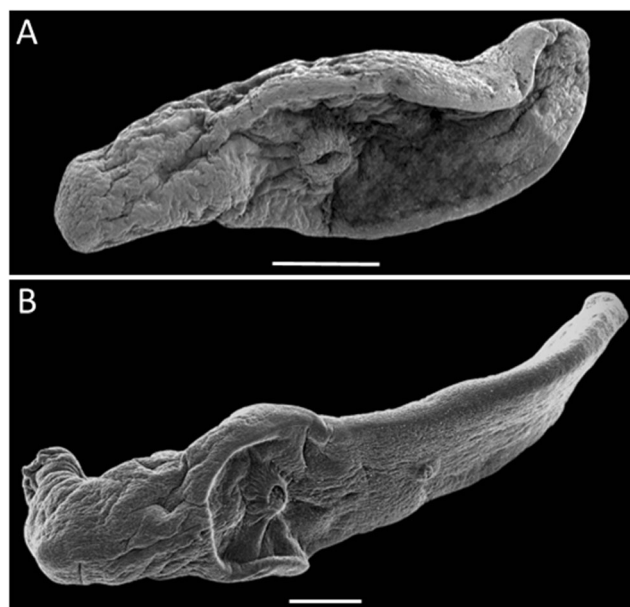


Fig. 1 Scanning electron micrographs of adults of *Cheloniodiplostomum* species found. **a** *Cheloniodiplostomum testudinis*. **b** *Cheloniodiplostomum argentinensis* n. sp. Scale bars 200 μ m



Fig. 2 Stained whole-mount micrographs of *Cheloniodiplostomum* spp. **a** *Cheloniodiplostomum testudinis*, showing holdfast (black arrow) and proteolytic glands (white arrow). **b** *Cheloniodiplostomum argentinensis* n. sp. showing holdfast (black arrow) and proteolytic glands (white arrow). **c** Detail of caudal end of *C. argentinensis* n. sp. **d** Egg (arrow) in uterus of *C. argentinensis* n. sp. Scale bars 100 μ m

Table 1 Measurements of *Chelonioidiplostomum* species, providing hosts, geographical distribution, and references. *N*, number of specimens measured; *L*, length; *W*, width

	<i>C. brevis</i> (MacCallum, 1921)	<i>C. delillei</i> (Zerecero, 1947)	<i>Chelonioidiplostomum</i> sp.	<i>C. testudinis</i> (Dubois, 1936)	<i>C. testudinis</i> (Dubois, 1936)	<i>C. testudinis</i> (Dubois, 1936)	<i>C. argentinensis</i> n. sp.
<i>N</i>	11	—	8	5	16	4	61
Length (mm)	2.70–5.34	2.31–3.39	1.32 (1.2–1.45)	1.84–2.07	1.71 (1.47–1.80)	1.45 (1.21–1.72)	2.10 (2.00–2.35) 3.44 (2.32–4.90)
Forebody L (mm)	0.75–2.85	1.68–2.28	0.85 (0.77–0.98)	1.05–1.29	1.25 (1.02–1.32)	0.97 (0.78–1.14)	1.50 (1.32–1.70) 2.45 (1.63–3.91)
Forebody W (mm)	1.07	0.92–1.13	0.49 (0.40–0.55)	0.52–0.66	0.33 (0.28–0.37)	0.47 (0.28–0.64)	0.42 (0.40–0.46) 0.27 (0.10–0.40)
Hindbody L (mm)	0.54–2.49	0.93–1.08	0.47 (0.38–0.56)	0.66–0.79	0.45 (0.41–0.52)	0.47 (0.25–0.70)	0.66 (0.65–0.68) 0.84 (0.55–1.25)
Hindbody W	730	475–616	330 (260–350)	410–500	270 (250–300)	292 (250–350)	400 (350–450) 288 (166–416)
Oral sucker L	65–81	81–84	50 (30–50)	53–62	50 (40–60)	67 (60–80)	55 (50–60) 65 (56–84)
Oral sucker W	60–80	71–93	50 (30–70)	48–75	50 (30–70)	53 (50–60)	52 (50–55) 53 (45–74)
Prepharynx	10–12	—	—	—	—	—	15 (13–16)
Pharynx L	60–77	71	40 (40–50)	50–65	55 (50–60)	49 (40–60)	65 (60–70) 67 (59–80)
Pharynx W	48–79	87	40 (20–50)	47–60	40 (30–50)	44 (40–50)	45 (40–50) 43 (35–53)
Esophagus L	72–200	140–178	50 (20–70)	65–120	80 (60–130)	55 (40–70)	115 (110–120) 140 (78–278)
Acetabulum L	62–122	98–111	50 (50–60)	66–71	50 (30–60)	65 (50–80)	75 (70–80) 80 (73–91)
Acetabulum W	72–132	115–131	70 (60–70)	72–87	50 (40–50)	55 (50–70)	75 (70–80) 66 (56–83)
Holdfast L	180–230	322–378	100 (70–120)	170–225	170 (150–190)	215 (160–260)	181 (180–185) 207 (169–320)
Holdfast W	180–270	466–502	110 (80–150)	170–225	130 (110–150)	186 (110–230)	160 (160–165) 134 (100–190)
Ovary L	135–180	84–115	60 (40–80)	90–100	80 (70–100)	95 (70–130)	70 (60–75) 103 (48–125)
Ovary W	180–240	129–147	100 (60–140)	117–140	80 (70–90)	77 (50–120)	80 (80–85) 85 (64–125)
Anterior testis L	330–450	158–211	100 (70–120)	140–207	180 (160–210)	194 (120–250)	140 (110–160) 198 (115–300)
Anterior testis W	460–580	211–255	170 (140–200)	216–305	220 (200–230)	151 (60–200)	200 (180–220) 154 (76–250)
Posterior testis L	280–420	132–237	100 (80–120)	135–200	140 (110–170)	198 (110–280)	120 (110–140) 215 (108–354)
Posterior testis W	400–630	255–352	200 (170–230)	200–265	230 (230–240)	146 (70–210)	210 (180–280) 168 (86–297)
Eggs	103–117 × 65–80	—	100 × 60	—	90 × 50	—	110 × 60
Host	<i>Kinosternon scorpionoides</i>	<i>Chelydra serpentina</i>	<i>Acanthocheilus spixii</i>	<i>Testudo</i> sp.	<i>Phrynops hilarii</i>	<i>Hydromedusa tectifera</i>	<i>Phrynops hilarii</i>
Locality	New York (USA)	Veracruz (Mexico)	Rio Grande do Sul (Brazil)	Brazil	Rio Grande do Sul (Brazil)	Buenos Aires (Argentina)	Buenos Aires (Argentina)
References	MacCallum (1921) Dubois (1938)	Zerecero (1947)	Mascarenhas et al. (2016)	Dubois (1938)	Mascarenhas et al. (2016)	Present study	Present study

Description (based on 16 whole-mounted specimens) (Figs. 1a and 2a and Table 1)

Short body divided in two parts by a constriction located at level of ovary and anterior testis, flattened forebody and cylindrical hindbody; oral sucker rounded, small, subterminal, prepharynx absent; pharynx rounded and short; esophagus short; caeca bifurcating above acetabulum and reaching posterior edge of posterior testis; acetabulum rounded, similar in size to oral sucker; holdfast rounded, located in forebody with papillae in posterior edge; testes in tandem; without cirrus and cirrus sac; ovary rounded pretesticular; uterus completely in hindbody; ootype intertesticular; vitellaria extending from near caeca bifurcation to ovary; eggs not observed.

Taxonomic summary

New host: *Hydromedusa tectifera*

Other host in this study: *Phrynops hilarii*

New locality: Arroyo Buñirigo (35°01'36" S, 57°17'24" W, datum: WGS84), Buenos Aires, Argentina

Location: anterior portion of intestine

Voucher specimens: MLP-He-7303

Cheloniodiplostomum argentinensis n. sp.

Description (based on 70 whole-mounted specimens) (Figs. 1b, 2b–d, and 3 and Table 1)

Very elongated body divided into tape-shaped forebody and cylindrical hindbody, without pronounced differentiation; lateral borders of forebody joining in its ventral and posterior margin generating a concavity; oral sucker rounded, small, subterminal; short prepharynx; pharynx rounded and short; esophagus short; caeca bifurcating above acetabulum and reaching end of body posterior to testes; acetabulum rounded, similar in shape and size to oral sucker; holdfast elliptical, located in the forebody, posterior edge of holdfast with proteolytic glands (Fig. 2c); testes in tandem; ovary rounded pretesticular; uterus completely in hindbody; ootype intertesticular; vitellaria extending widthwise in body from median point between caeca bifurcation and acetabulum to posterior edge of ovary, vitellaria ducts joined in a common vitelline duct behind anterior testis becoming yolk reservoir at intertesticular level; without cirrus and cirrus sac. Posterior end of body with bifurcated shape: blunt posterior end and dorsal funnel-like projection in which copulatory bursa opens (Fig. 3). Structure better observed in a lateral view. Genital cone rounded in which paraprostata opens independently, whereas uterus and seminal vesicle end in common hermaphroditic duct (Fig. 4). Few elliptical eggs observed (Fig. 2d).

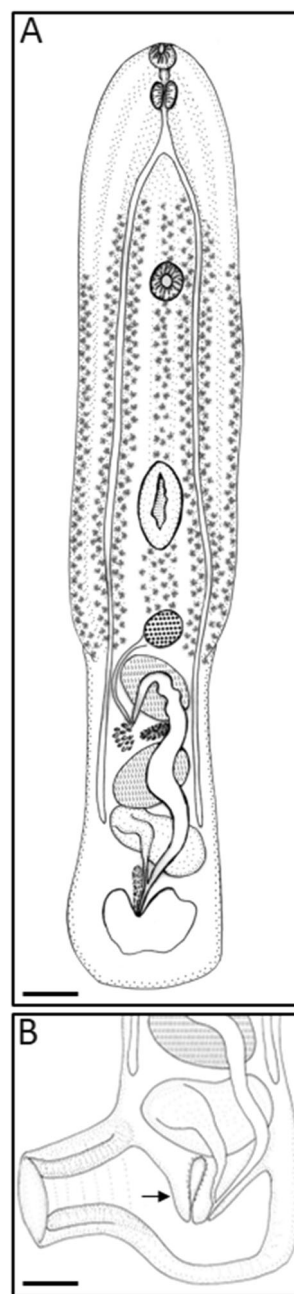


Fig. 3 Schematic illustration of *Cheloniodiplostomum argentinensis* n. sp. **a** Whole specimen in ventral view. **b** Detail of posterior end showing detail of the genital cone (arrow) in lateral view. Scale bars 100 μ m

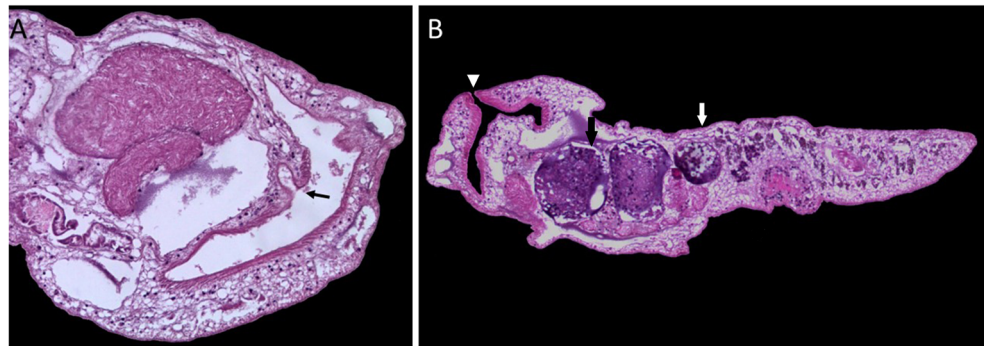
Taxonomic summary

Type host: *Phrynops hilarii*

Locality type: Arroyo Buñirigo (35°01'36" S, 57°17'24" W, datum: WGS84), Buenos Aires province, Argentina

Location: anterior portion of intestine

Fig. 4 Histological sections at optical microscope of *Cheloniodyplostomum argentinensis* n. sp. **a** Caudal end with detail of paraprostata (arrow). **b** Sagittal view showing ovary (white arrow), testis (black arrow), and bursa (arrow head). Scale bars 100 μ m



Holotype: MLP-He-7418

Paratypes: MLP-He-7419

Etymology: The specific epithet “*argentinensis*” refers to Argentina, the region where the specimens were collected.

Remarks

Cheloniodyplostomum argentinensis n. sp. can be distinguished from the other three species in the genus by the shape of the distal end and by having the forebody and hindbody of similar width. In the other species described, the forebody is wider than the hindbody. Additionally, in the new species, the vitellaria begins at the median point between the caeca bifurcation and the acetabulum, whereas in *C. brevis* and *C. testudinis*, it begins earlier, at caecal bifurcation level; in contrast, in *C. delillei*, the vitellaria begins posteriorly, at acetabulum level. Moreover, in *Cheloniodyplostomum argentinensis* n. sp., the holdfast is longitudinally elliptical, whereas in the rest of the species, it is described as circular in shape. Despite similar body size, *C. argentinensis* n. sp. has smaller testis than *C. brevis* and smaller holdfast than *C. delillei* (see Table 1). Additionally, in *Cheloniodyplostomum argentinensis* n. sp., testis is similar in size than holdfast, whereas in *C. brevis*, testis is larger than holdfast, and in *C. delillei*, testis is smaller than holdfast (see Table 1). Furthermore, the new species differ from *C. delillei* and *C. testudinis* mainly by the presence of prepharynx. The species here described also differ from *C. testudinis* in their general morphology, in the mean size of the body (3.44 vs. 1.84 mm), and in the size and shape of the genital atrium.

Following, a key for identification and differentiation of *Cheloniodyplostomum* species is presented.

1	Prepharynx absent	2	
1'	Prepharynx present	4	
2	Vitellaria only in forebody		<i>C. delillei</i> (Zerecero, 1947) Forebody wider than hindbody; rounded and big holdfast, bigger than testes
2'	Vitellaria in forebody and hindbody		<i>C. testudinis</i> (Dubois, 1936) Forebody wider than hindbody; vitellaria extending from caecal bifurcation to anterior testes; rounded holdfast, similar to testes in size
4	Holdfast much smaller than testes		<i>C. brevis</i> (MacCallum, 1921) Forebody wider than hindbody; forebody includes ovary and part of testicle; big testicles, almost three times the ovary; small and rounded holdfast
4	Holdfast similar in size than testes		<i>C. argentinensis</i> n. sp. Forebody and hindbody similar in width; vitellaria reaching posterior edge of ovary; elliptical holdfast similar in size than testes

Discussion

Knowledge of trematodes from freshwater turtles in Argentina contributes to the global understanding of wildlife parasites. In Argentina and with regard to *Cheloniodyplostomum*, only *C. testudinis* in *P. hilarii* from Corrientes has been recorded prior to this work (Lombardero and Moriena 1977). The

present paper enlarges the host range of *C. testudinis* finding it in *H. tectifera* and expands its geographical distribution.

The different reports about the species of the genus *Cheloniodyplostomum* in freshwater turtles have a Neotropical distribution, ranging from Mexico to Argentina. Although turtles are very well represented in North America and there are taxa within the subfamily Polycotylineae with a Nearctic distribution (e.g., *Crocodilicola* Poche, 1925; *Prolecithodyplostomum* Dubois, 1936; *Herpetodyplostomum* Dubois, 1936), *Cheloniodyplostomum* is restricted to Neotropic (Dubois 1982).

Cheloniodyplostomum testudinis has a disjunctive distribution in the south of Brazil and Corrientes and Buenos Aires in Argentina (Lombardero and Moriena 1977; Mascarenhas et al. 2016). Taking into account the distribution of *P. hilarii* (overlap with the area of Rio de la Plata Basin), the absence of records in intermediate areas (e.g., Uruguay and north-east of Argentina) can be related to the absence of parasitological studies in such places (Fig. 5).

In addition, Mascarenhas et al. (2016) report *Cheloniodyplostomum* sp. in *Acanthochelys spixii* (Duméril & Bibron, 1835) from Brazil. However, it differs from the species reported in the present paper by the general morphology of body, the position of the vitellaria, and other morphometric features shown in Table 1.

The life cycle of the species of this genus includes two intermediate hosts, a mollusk acting as first intermediate host in which rediae and cercariae develop, and fish acting as second intermediate hosts harboring metacercariae, mainly encysted in the eyeball. After reaching the final host, metacercariae develops into adult in the first portion of the intestine (Dubois 1968). There are no records of *Cheloniodyplostomum* metacercariae in fish of any of the tributary streams of the Rio de la Plata in which *P. hilarii* and *H. tectifera* inhabit. Future research on fish that share the environment with the studied turtles will help to elucidate the life cycle of *Cheloniodyplostomum* species described in the present paper.

Fig. 5 Map of South America showing the Rio de la Plata Basin (shaded area) and the historical records of *Cheloniodyplostomum testudinis* (black square) and new record of *C. testudinis* and type locality of *Cheloniodyplostomum argentinensis* n. sp. (white square)



Diagnostic features used to distinguish species are related to body shape, ratio between forebody and hindbody, vitellaria distribution, holdfast morphology, ovary and testis size, and copulatory bursa shape. By taking this work into account, new diagnostic features can be included, such as the presence of the prepharynx and the shape of the bursa and caudal end.

This study enlarges to four the number of known species of *Cheloniodiplostomum*, all of them reported in turtles of the Neotropical region, and expands the diversity, distribution, and host range of the genus.

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