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Spiroxys contortus (Rudolphi, 1819) and *Hedruris orestiae* (Moniez, 1889) in Argentine turtles

Ezequiel Palumbo[1*], **Sofia Capasso**[1], **María Julia Cassano**[2], **Leandro Alcalde**[2] and **Julia Inés Diaz**[1]

1 Centro de Estudios Parasitológicos y de Vectores (CEPAVE), FCNyM, UNLP, CONICET, Boulevard 120 s/n e/61 y 62, 1900 La Plata, Argentina
2 Sección Herpetología, Instituto de Limnología Dr. Ringuelet (ILPLA), FCNyM, UNLP, CONICET, Boulevard 120 s/n e/61 y 62, 1900 La Plata, Argentina
* Corresponding author. E-mail: epalumbo@cepave.edu.ar

Abstract: Knowledge of parasites in turtles is scarce, particularly with regard to freshwater turtles of South America. Here, we describe the association of *Spiroxys contortus* (Rudolphi, 1819) in *Phrynops hilarii* (Duméril & Bibron, 1835) and *S. contortus* and *Hedruris orestiae* (Moniez, 1889) in *Hydromedusa tectifera* (Cope, 1870). The presence of *S. contortus* in *P. hilarii* represents a new host record and also the southernmost geographic record for this species. More interestingly, the presence of *H. orestiae* in *H. tectifera* represents the first record of this helminth species from a reptilian host.

Key words: helminths; *Spiroxys*; *Hedruris*; freshwater turtles; Neotropics

Knowledge of the parasite fauna in South American turtles is particularly scarce and fragmentary (Fernandes and Kohn 2014; Mascarenhas et al. 2016), especially with regard to the Argentine species, the southernmost species in the region (Brusa and Damborenea 2000; Alcalde et al. 2010).

Phrynops hilarii (Duméril & Bibron, 1835) inhabits a wide variety of aquatic environments in Uruguay, southern Brazil and Paraguay, and northeastern and central Argentina (Cabrera 1998). No natural populations of *P. hilarii* are known from the western Argentine provinces of Mendoza, San Juan and Tucumán (Prado et al. 2012). Excluding isolated populations, the westernmost occurrence of this species is in northwestern Cordoba province (Cabrera et al. 1986) and the southernmost records are from streams entering the Rio de La Plata shoreline, near Bahía Samborombón, Buenos Aires province, Argentina (Derocco et al. 2005; Williams and Kacoliris 2009). Although ecological studies of habitat usage and preferences are virtually lacking for this species, miscellaneous reports and observations indicate that *P. hilarii* is a swimming species that prefers deep, moderately vegetated waters; it is usually in association with large rivers such as the Paraná, Uruguay, Paraguay, and Río de la Plata rivers (Cabrera 1998).

Hydromedusa tectifera (Cope, 1870) is usually found in sympatry with *P. hilarii*, particularly in the southernmost part of its range. They occur in the same aquatic environments in Uruguay, southern Brazil, southeastern Paraguay, and northeastern and central Argentina. The southernmost occurrence of *H. tectifera* is in Río Sauce Grande (Sierra de la Ventana, Buenos Aires province), and the westernmost occurrence is in several mountain streams in Cordoba province, Argentina (Cabrera 1998; Di Pietro et al. 2012; Prado et al. 2012).

Reports on the diets of *P. hilarii* and *H. tectifera* indicate that these species ingest very similar food items, mainly adult and larvae of arthropods (Bonino et al. 2009; Alcalde et al. 2010), many of which have been reported as intermediate hosts for several parasite species (Moravec 1998).

Few species of nematodes have been reported parasitizing southern Brazilian populations of *P. hilarii* and *H. tectifera*. *Spiroxys* sp. (Gnathostomatidae) and *Camallanus* sp. (Camallanidae) were documented in *P. hilarii* (Bernardon et al. 2013). *Spiroxys contortus* (Rudolphi, 1819), *Camallanus* sp. (Mascarenhas et al. 2013) and *Spirocamallanus* sp. (Camallanidae) were reported in *H. tectifera* (Novelli et al. 2014). Knowledge of nematodes for Argentine populations of *H. tectifera* corresponds to the observations of Alcalde et al. (2010) who studied the diet of this turtle species and found nematodes in many of the stomach contents, but these helminthes remained unidentified until now.

Therefore, we present new geographical records of nematodes and new host–parasite associations for turtle populations from Argentina. This work highlights the fragmentary knowledge of the parasite fauna of the turtles in the region.

We analyzed for parasites the stomach contents washed from 47 *P. hilarii* and 25 *H. tectifera*. Samples were

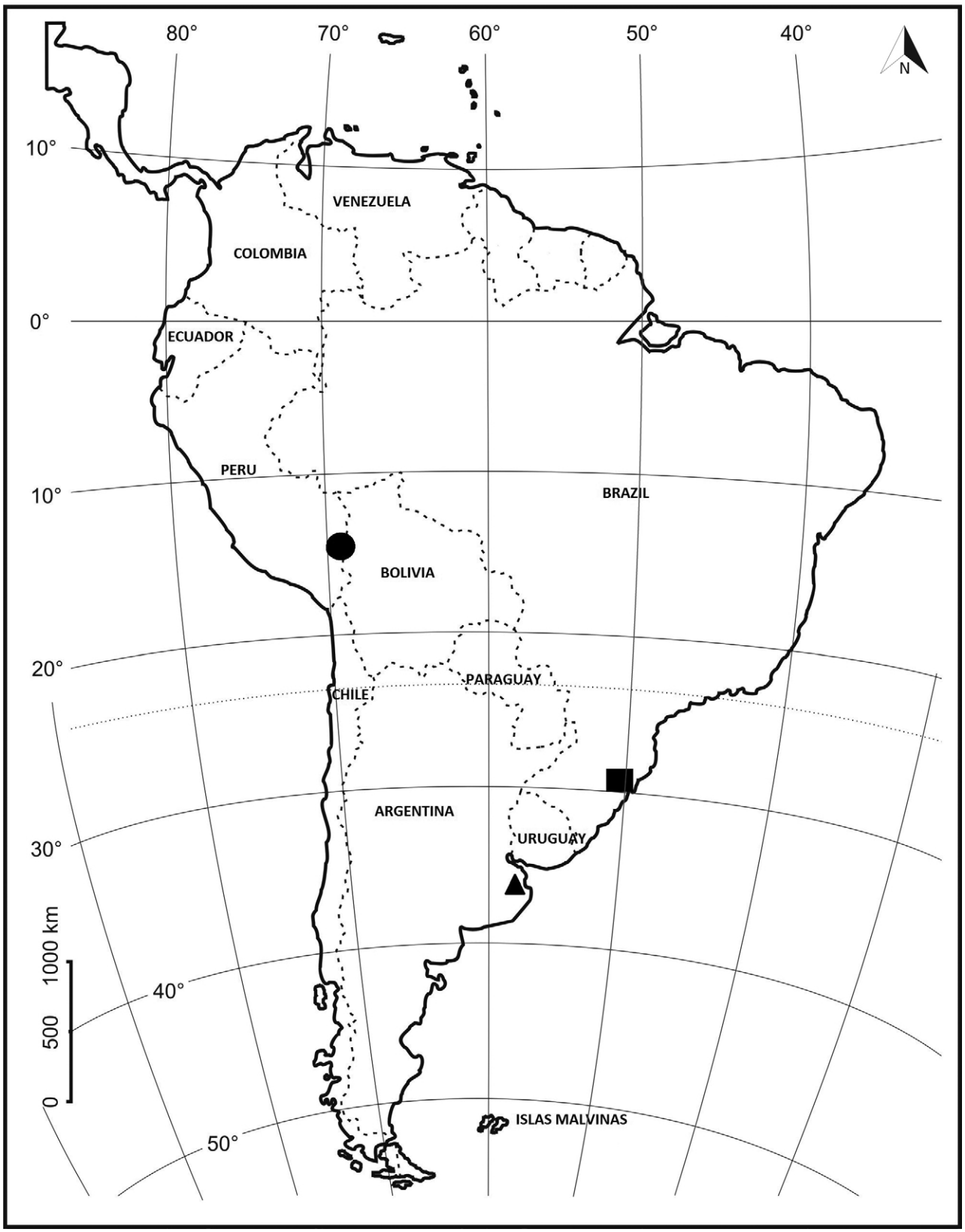
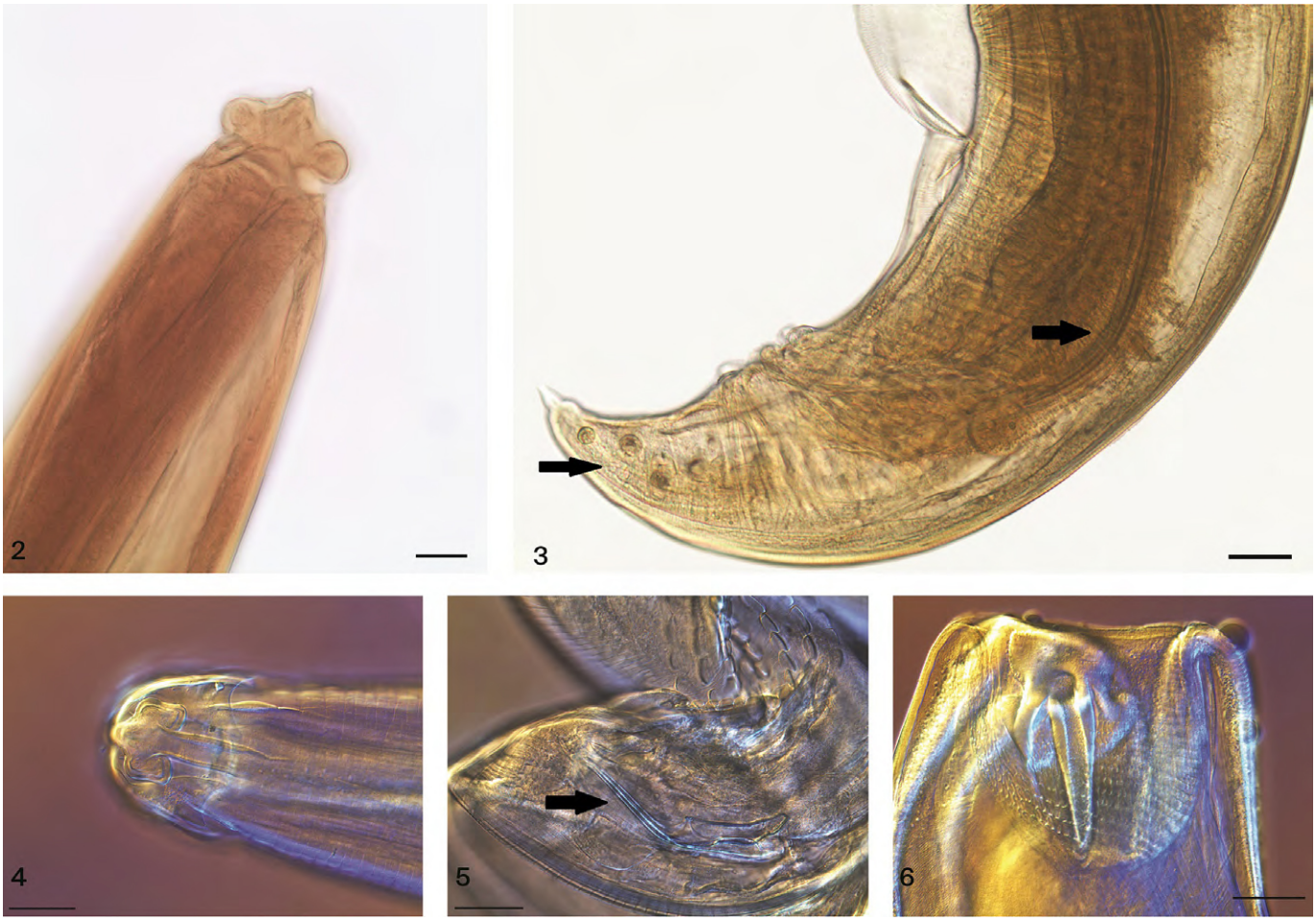


Figure 1. Geographic distribution of *Spiroxys contortus* (square = historical record, triangle = new record) and *Hedruris orestiae* (circle = historical record, triangle = new record) in South America.

taken between 2006 and 2008 from Arroyo Buñirigo, a stream in Buenos Aires province, Argentina (35°01′36″ S, 57°17′24″ W, datum: WGS84; Figure 1), a tributary of the Rio de la Plata. The stomach contents were collected following the procedures of Legler (1977) and preserved in 70% ethanol.

Helminthes were cleared with Lactophenol and mounted for study under a polarizing microscope (Olympus



Figures 2–6. Diagnostic features of the species. **2.** Anterior end of *Spiroxys contortus*. **3.** Caudal end of *S. contortus* (male), showing spicules and caudal papillae (arrows). **4.** *Hedruris orestiae* anterior end. **5.** Posterior end of *H. orestiae* (male), showing spicules (arrow). **6.** Posterior end of *H. orestiae* (female), detail of the hook. Scale bars = 50 µm.

BX51®). Photographs were obtained using a Q-Imaging Go-3® digital camera. Identification of the helminths was according to Hedrick (1935), Yamaguti (1961) Ibañez and Cordova (1976), and Moravec and Vargas-Vázquez (1998). Measurements are given in micrometers, unless otherwise indicated. The prevalence (P) and mean intensity (IM) of helminthes recovered from samples are calculated according to Bush et al. (1997) using the Quantitative Parasitology Program (QP 3.0, Reiczigel and Rózsa 2005). Voucher specimens are deposited in the Helminthological Collection of the Museo de La Plata (MLP He 7141–7143), La Plata, Buenos Aires, Argentina.

Forty-four nematodes were obtained from 20 stomach contents. A single helminth species, *Spiroxys contortus* (P = 70%; IM = 3) was obtained from *Phrynops hilarii*, and two nematode species, *S. contortus* (P = 70%; MI = 1.7) and *Hedruris orestiae* (Moniez, 1889) (P = 30%; MI = 3.33) were obtained from *Hydromedusa tectifera*. However, only two specimens of *H. tectifera* were found to have double infections (i.e., have both nematode species present).

Gnathostomatidae
Spiroxynae

Spiroxys contortus Rudolphi, 1819
(Figures 2 and 3; Table 1)

Description based on 17 adult specimens. Slender, colorless, semitransparent worms; intestine distinctly brown or approaching black in some individuals. Oral opening surrounded by two trilobed lips with a rather thick cuticular lining that projects anteriorly in each median lobe to form a blunt tooth (Figure 2). Each lip bears one lateral and two submedian papillae. Small cervical papillae located posterior from the excretory pore. Tail rather short in both sexes, ending in an abrupt, sharp, conical tip.

Male: Four pre-anal and seven post-anal pairs of papillae. Seventh and eighth pairs from the posterior end are sessile and located immediately posterior and anterior, respectively, to the anus. Long, slender, cylindrical, and equal spicule, without keel, distinctly transverse striated, ending in a sharp point. Gubernaculum composed of dense cuticular material.

Table 1. Comparative features on males and females of *Spiroxys contortus* found in freshwater turtles.

Host	*Trachemys scripta*	*Trachemys dorbigni*	*Phrynops hilarii*	*Hydromedusa tectifera*
Male	*n* = 2	*n* = 7	*n* = 4	*n* = 2
Length (mm)	11.85 (11.82–11.97)	19.55–28.15	14.65 (12.00–17.00)	20.40 (16.90–23.90)
Width	400 (340–476)	300–350	222 (200–240)	490 (600–750)
Excretory pore	650 (598–694)	480–810	573 (540–610)	820 (750–850)
Cervical papillae	830 (829–832)	—	850 (80–880)	1100 (1050–1200)
Esophagus (mm)	1.70 (1.47–1.93)	2.60–3.45	2.58 (2.30–2.80)	3.22 (3.15–3.30)
Spicules (mm)	1.90 (1.62–2.20)	2.77–3.88	1.34 (1.25–1.45)	2.30 (2.25–2.40)
Female	*n* = 2	*n* = 6	*n* = 4	*n* = 7
Length (mm)	14.40 (14.39–14.53)	29.1–42.87	17.00 (12.80–23.10)	23.41 (12.20–37.80)
Width	350 (340–354)	450–650	245 (180–300)	538 (290–750)
Excretory pore	590 (585–598)	590–1000	545 (480–600)	899 (650–1190)
Cervical papillae	860 (843–884)	—	775 (700–875)	1464 (1150–2000)
Esophagus (mm)	2.35 (2.31–2.38)	2.90–4.30	2.87 (2.20–4.01)	3.84 (2.40–5.20)
Vulva* (mm)	9.25 (8.84–9.66)	15.20–24.60	8.91 (8.20–10.00)	10.27 (5.80–15.00)
Eggs	—	60–67.5 × 40–47.5	—	80 × 50
Distribution	Yucatan, Mexico	Rio Grande do Sul, Brazil	Buenos Aires, Argentina	Buenos Aires, Argentina
Reference	Moravec and Vargas–Vázquez (1998)	Mascarenhas and Muller (2015)	Present study	Present study

*From anterior end.

Female: Two cuticular prominences guard the opening of the vulva, which is just posterior to the middle of the body. Vagina anteriorly directed, thick-walled, muscular, and annulated. No ovijectors.

Comments: *Spiroxys* species can be distinguished from one another by the number and distribution of male caudal papillae, the position of excretory pore and cervical papillae, presence or absence of gubernaculum and size of several features.

Hedruridae
Hedrurinae

Hedruris orestiae Moniez, 1889
(Figures 4–6; Table 2)

Description based on eight adults specimens: Body striated; anterior end slender; posterior end of females widened by a pouch; oral opening surrounded by four lips. Lateral lips with chitinized edges, with apical edge thicker (Figure 4).

Male: Nine pairs posterior subventral papillae; none precloacal; eight pairs postcloacal: one pair on anterior cloacal lip. Precloacal subventral surface with rows of rectangular, scale-like bosses extending from near cloaca anteriorly approximately. Spicules strongly chitinized (Figure 5).

Female: Prodelphic, didelphic, vulva at back of the body. When gravid, egg-filled uteri occupy entire body cavity. Tail curved dorsally and formed into a sucker-like apparatus armed with a large sclerotized, barbed hook (Figure 6). Eggs nonmammillated and cylindrical.

Comments: *Hedruris* species can be distinguished from each other, including those reported in *H. tectifera* (i.e., *Hedruris moniezi* Ibanez & Córdova, 1976 and *Hedruris basilichtensis* [Mateo, 1971]) by their general body shape, body length, number and distribution of male caudal papillae, spicule length, size and shape of the eggs, and hosts (Mateo 1971; Ibanez and Córbova 1976) (Table 2).

Spiroxys contortus is widely distributed in Eurasia, North Africa, and America (Baker 1987). In South America, the species was previously recorded from *H. tectifera* in Brazil (Mascarenhas et al. 2013) and *Trachemys dorbigni* (Duméril & Bibron, 1835) (Emydidae) (Mascarenhas and Müller, 2015) (Figure 1). Our find of *S. contortus* in a population of *P. hilarii* from Argentina represents a new host and the southernmost geographic distribution record of this nematode.

Dragonfly naiads are an important prey of turtles (Alcalde et al. 2010), and interestingly, dragonflies have been recorded as intermediate hosts of *S. contortus* in other regions of the world (Hedrick 1935).

Most species of *Hedruris* (Habronematoidea, Hedruridae) were reported parasitizing fishes, amphibians, and reptiles (Anderson 2000). In turtles, an unidentified species of *Hedruris* was reported from *Rhinoclemmys nasuta* (Boulenger, 1902) in Mexico (Dyer and Carr 1990). Moravec (1998) suggested that some species of nematodes, whose definitive hosts are usually amphibians and reptiles, could be found in the stomachs of fishes that accidentally acquired parasites by feeding on intermediate hosts. Because *Hedruris* larvae present precocity in their development and reach immature adulthood in their intermediate host (isopods), fish can acquire the nematodes when feeding on infected isopods (Moravec 1998). Thus, the fish should be considered facultative hosts. Consequently, our record of *Hedruris orestiae* in *Hydromedusa tectifera* is the first from chelonians, which should be considered suitable definitive hosts of this species.

Table 2. Selected characteristics of South America species of *Hedruris* with 18 cloacal papillae.

	H. basilichtensis **Mateo, 1971**	***H. moniezi*** **Ibanez & Córdova, 1976**	***H. orestiae*** **Moniez, 1889**	***H. orestiae*** **Moniez, 1889**
Host	***Basilichthys bonariensis***	***Telmatobius peruvianus***	***Orestias luteus***	***Hydromedusa tectifera***
Male	—	—	—	*n* = 5
Length (mm)	6.00–9.80	5.46–7.02	7.15–8.45	7.38 (5.70–9.20)
Width	210–270	195–200	136–195	192 (120–220)
Esophagus (mm)	1.11–1.56	1.05–1.40	1.00–1.34	1.36 (1.15–1.75)
Excretory pore	330–420	400–460	337–350	458 (40–530)
Cervical papillae	210–280	210–250	199–210	244 (210–310)
Spicule length	175–193	279–300	178–194	170–210
Papillae pattern*	18:0:0:18	18:0:0:18	18:0:0:18	18:0:0:18
Female	—	—	—	*n* = 3
Length (mm)	4.60–9.36	7.15–7.34	8.64–10.14	6.93 (5.70–8.60)
Width	210–350	152–202	197–251	270 (250–310)
Esophagus (mm)	1.03–1.85	1.35–1.42	1.41–1.73	1.63 (1.50–1.80)
Excretory pore	360–460	467–600	376–381	480 (450–510)
Cervical papillae	220–300	272–330	225–235	263 (210–290)
Vulva†	—	450–510	650–870	560 (530–600)
Eggs	40 × 15	42 × 15	31 × 15	20 × 16
Distribution	Titicaca Lake, Peru	Titicaca Lake, Peru	Titicaca Lake, Peru	Buenos Aires, Argentina
Reference	Mateo (1971)	Ibanez and Córdova (1976)	Moniez (1889)	Present study

*Total number of papillae : precloacal: adcloacal: postcloacal + anterior cloacal lip.
† From posterior end.

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