

RESEARCH NOTE

First report of *Schulzia travassosi* (Nematoda, Trichostrongylina, Molineoidea) for amphibians of the Chaco region in Argentina and proposal of *Oswaldocruzia melanostictusi* nov. comb.

Cynthia E. González* and Monika I. Hamann

Centro de Ecología Aplicada del Litoral, Consejo Nacional de Investigaciones Científicas y Técnicas, Ruta Provincial Número 5, km 2,5, 3400, Corrientes, Argentina

Abstract

This is the first record of the nematode parasite *Schulzia travassosi* (Trichostrongylina, Molineidae) in two Argentinean amphibians, *Leptodactylus bufonius* and *Rhinella major*, collected in two localities of the Chaco region, Las Lomitas (Formosa Province) and Taco Pozo (Chaco Province). The species was observed using light and scanning electron microscopy (SEM) and compared with previous studies of Paraguayan specimens. Regarding metric characters, the measurements found in Argentinean specimens generally did not differ from those of Paraguayan specimens; some differences were observed in the total length of males and females (some longer and some shorter than Paraguayan specimens), length of the oesophagus (in almost all specimens shorter than in Paraguayan ones) and position of the vulva (nearer to posterior end in Argentinean specimens). The branches and sphincter of ovejector are slightly longer than in Paraguayan female specimens. In males, ray 4 is almost the same length as rays 2, 3 and 5, 6. Cephalic end, cephalic vesicle, *synlophe* and vulvar depression were viewed under SEM. *Schulzia melanostictusi* Chopra, Shing and Kumar, 1986 is reassigned as *Oswaldocruzia melanostictusi* (Chopra, Shing and Kumar, 1986) nov. comb.

Keywords

Schulzia travassosi, Nematoda, *Leptodactylus bufonius*, *Rhinella major*, Chaco region, Argentina

In 1937, Travassos established the genus *Schulzia* but did not specify precisely the reasons for separating this genus from *Oswaldocruzia* Travassos, 1917; he only stated that he re-described *O. subventricosa* Travassos, 1925, which happens to be a well-characterized species, and established for these specimens a new genus, *Schulzia*.

Durette-Desset *et al.* (1985) studied Paraguayan specimens of genus *Schulzia* and observed that these were identical to the description given by Travassos (1925) for specimens collected in Brazil under the name *Oswaldocruzia subventricosa*. Therefore, these authors proposed the name *Schulzia travassosi* Durette-Desset, Baker and Vaucher, 1985 for Paraguayan

specimens whose description agreed with the specimens of Travassos (1925), and also appointed this species as type species of the genus *Schulzia*.

In the same work, Durette-Desset *et al.* (1985) redefined the genus *Schulzia* and listed for this genus the following characteristics: *synlophe* with numerous undulations; rays 2 to 6 evenly spaced; gubernaculum absent. Females with particular ovejector, the vestibule consists of 3 strongly muscularised branches, the first is always situated as a prolongation of *vagina vera*. Embryonated eggs in the second half of the uterine branches. Caudal spine absent in females. Parasites of Neotropical amphibians.

*Corresponding author: cynthyaelizabethg@hotmail.com

Table I. Comparative morphometrics of *Schulzia travassosi* from different amphibian hosts from Argentina and Paraguay

	Durette Desset <i>et al.</i> (1985)	Present study	Present study	Present study	Present study
Host	<i>Rhinella major</i> (= <i>Bufo g. major</i>), <i>Leptodactylus</i> <i>bufonius</i>	<i>Rhinella</i> <i>major</i>	<i>Rhinella</i> <i>major</i>	<i>Leptodactylus</i> <i>bufonius</i>	<i>Leptodactylus</i> <i>bufonius</i>
Locality	Concepción, Paraguay	Concepción del Bermejo, Chaco	Las Lomitas, Formosa	Las Lomitas, Formosa	Taco Pozo, Chaco
Females (n)		9	9	2	2
Total length	16.4mm	12.6 ± 1.1mm (11.4–15.0)	15.8 ± 1.4mm (14.0–17.8)	11.6 ± 1.2mm (10.7–12.4)	14.0 ± 3.9mm (11.2–16.8)
Width	175	145.2 ± 15.4 (128.8–177.1)	134.9 ± 24.3 (105–163)	125.3 ± 1.62 (124.2–126.5)	180 ± 28.3 (160–200)
Oesophagus length	437	393.0 ± 23.8 (356.5–427.8)	415.0 ± 18.3 (390–450)	362.2 ± 30.9 (340.4–384.1)	352.5 ± 109.6 (275–430)
Oesophagus wide[‡]	–	57.52 ± 5.87 (50.6–71.3)	71.9 ± 6.0 (62–80)	60.95 ± 4.87 (57.5–64.4)	62.5 ± 17.7 (50–75)
Nerve ring[†]	203	189.2 ± 17.8 (161–207)	213.3 ± 19.9 (195–260)	189.75 ± 1.62 (188.6–190.9)	207.5 ± 10.6 (200–215)
Excretory pore[†]	481	399.0 ± 9.75 (384.1–414)	467.9 ± 26.6 (425–500)	442.7 ± 47.16 (409.4–476.1)	445.0 ± 35.4 (420–470)
Length of cephalic vesicle	103	97.2 ± 4.5 (92–103.5)	101.9 ± 7.8 (90–112)	90.85 ± 1.62 (89.7–92)	82.0 ± 14.1 (72–92)
Wide of cephalic vesicle	63	57.0 ± 2.0 (55–59)	57.3 ± 5.6 (50–65)	49.0 ± 1.4 (48–50)	52.5 ± 0.7 (52–53)
Vulva[*]	6.8mm	5.1 ± 0.6mm (4.3–6.1)	6.1 ± 0.6mm (5.2–6.7)	4.4 ± 0.4mm (4.1–4.7)	5.6 ± 1.2mm (4.7–6.5)
Length of eggs	–	92.1 ± 3.4 (87.4–96.6)	95.1 ± 2.4 (92–100)	81.65 ± 1.62 (80.5–82.8) [‡]	94.5 ± 6.4 (90–99)
Width of eggs	–	54.1 ± 4.6 (50–62.1)	52.2 ± 1.6 (50–56)	49.45 ± 4.87 (46–52.9) [‡]	52.5 ± 0.7 (52–53)
Anus[*]	228	181.4 ± 19.2 (151.8–211.6)	180.3 ± 23.5 (155–220)	174.8 ± 9.7 (167.9–181.7)	166 ± 18.4 (153–179)
Males (n)		9	6		
Total length	7.3mm	5.7 ± 0.3mm (5.2–6.2)	7.5 ± 0.5mm (6.6–8.1)		
Wide	120	107.2 ± 6.3 (96.6–119.6)	109.4 ± 5.94 (100–115)		
Oesophagus length	387	355.2 ± 18.9 (322–379.5)	376.8 ± 14.6 (355–400)		
Oesophagus wide[‡]	–	50.0 ± 4.6 (42–57.5)	63.5 ± 4.92 (56–69)		
Nerve ring[†]	187	162.7 ± 11.1 (142.6–174.8)	200.8 ± 7.35 (195–215)		
Excretory pore[†]	425	357.7 ± 25.7 (322–395.6)	425.5 ± 37.98 (375–465)		
Cephalic vesicle length	97	85.6 ± 6.8 (73.6–92)	97.3 ± 7.2 (85–105)		
Cephalic vesicle wide	56	55.3 ± 3.0 (52–58)	49.0 ± 3.6 (44–52)		
Spicules length	159	152.3 ± 14.3 (138–174.8)	148.3 ± 9.9 (139–165)		

†from anterior end; *from posterior end; ‡morulate eggs; †at the widest posterior part

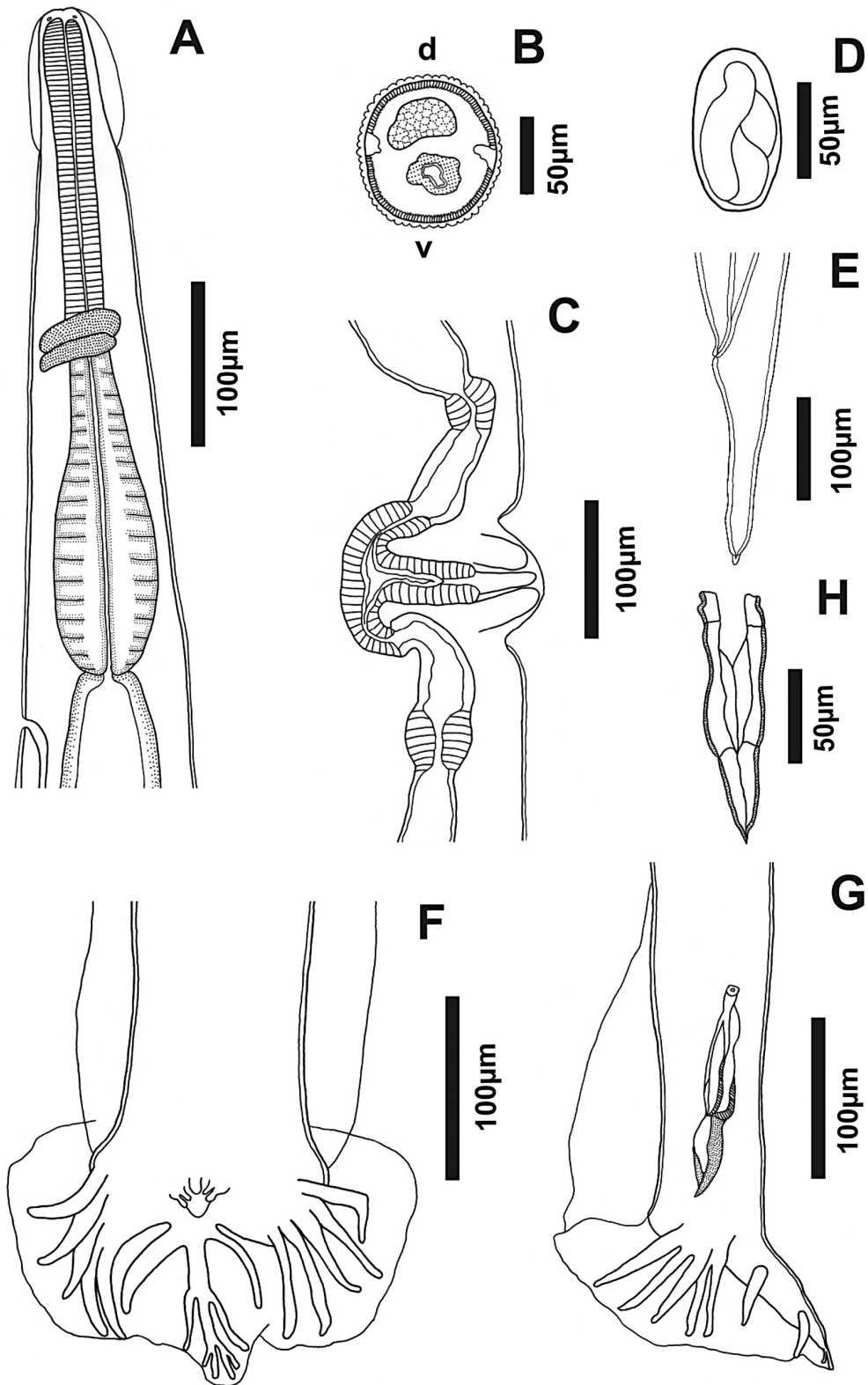


Fig. 1. *Schulzia travassosi* Durette-Desset, Baker and Vaucher, 1985 from Argentinean amphibians, *Leptodactylus bufonius* and *Rhinella major*, from Chaco region. **A** – Anterior end of female, lateral view; **B** – Transversal section of body of male at mid-body; **C** – Ovejector, lateral view; **D** – Egg; **E** – Posterior end of female, lateral view; **F** – Posterior end of male, ventral view; **G** – Posterior end of male, lateral view; **H** – Spicules, ventral view. Abbreviations: v: ventral side, d: dorsal side

In 1986, Chopra *et al.* described a species of *Schulzia* found outside the Neotropics, *S. melanostictusi* Chopra, Singh and Kumar, 1986. However, these authors did not take into account the work of Durette-Desset *et al.* (1985) and described this species comparing their specimens with the description of Travassos (1937) of the species *Schulzia subventricosa*, which had been later redescribed as *Maciela subventricosa* by Durette-Desset *et al.* (1985).

The aim of the present study is to provide morphological details of *S. travassosi* on the basis of specimens recovered from two species of anurans captured in the Chaco region, Argentina, and to reassess the generic allocation of *Schulzia melanostictusi*.

Ten adult specimens of *Leptodactylus bufonius* Boulenger, 1894 (Leptodactylidae), three of them from Taco Pozo, Chaco Province (25°36'54"S, 63°15'54"W), and 7 from Las Lomitas, Formosa Province (24°42'26"S, 60°35'40"W) were collected on October 2011 and May 2014, respectively. Fifty-eight adult specimens of *Rhinella major* (Müller and Hellmich, 1936) (Bufonidae), 27 of them from Las Lomitas, Formosa Province, and 31 from Concepción del Bermejo, Chaco Province (26°36'S, 60°57'W) were collected between November 2011 and November 2012. Frogs were transported live to the laboratory and killed using a chloroform solution (CHCl₃). At necropsy, hosts were sexed and the alimentary canal, lungs, liver, kidneys, urinary bladder, musculature, and integument examined for parasites by dissection. Nematodes were observed *in vivo*, counted, and killed in hot distilled water and preserved in 70% ethanol, cleared in glycerine, and examined as temporary mounts. Drawings were made with the aid of a drawing tube. Nematodes were dehydrated in an ethanol series, dried using the critical point technique, coated with gold, and examined with a Jeol 5800LV scanning electron microscope (SEM). The synlophe was studied following the method of Durette-Desset (1985). Measurements are presented in μm , unless otherwise stated, as mean \pm SD values followed by minimum and maximum values in parentheses. Voucher specimens were deposited in the Helminthological Collection of the Centro de Ecología Aplicada del Litoral (CECOAL), Corrientes, Argentina, with accession numbers: CECOAL 12010018 (1 female, 1 male from *R. major* from Concepción del Bermejo), CECOAL 12110825 (1 female, 1 male from *R. major* from Las Lomitas), CECOAL 14050705 (1 female from *L. bufonius* from Las Lomitas), CECOAL 11101912 (1 female from *L. bufonius* from Taco Pozo). Amphibians were deposited in the Herpetology Collection of Centro de Ecología Aplicada del Litoral, with accession number LHC-CECOAL 5062 *Leptodactylus bufonius*; LHC-CECOAL 5081 *Rhinella major*. The approval for using animals in this research was given by the Secretary of Natural Resources, Department of Fauna and Protected Areas from Production Ministry of the Chaco and Formosa provinces, Argentina.

Forty five specimens of *S. travassosi* were found in the small intestine and in the gallbladder of examined specimens (*R. major*, Las Lomitas, n = 18; *R. major*, Concepción del Bermejo, n = 23; *L. bufonius*, Las Lomitas, n = 2; *L. bufonius*,

Taco Pozo, n = 2). Measurements from the present study and those provided by previous authors are presented in Table I. Morphological details, observed with light microscopy and SEM, are shown in Figures 1 and 2.

General: Slender nematodes with marked sexual dimorphism, females are twice as long as males. Excretory pore and deirids located at the same level, close to end of oesophagus or slightly posterior. Small deirids. Claviform oesophagus (Fig. 1A). Cephalic vesicle with transverse striations approximately 2 μm wide, in some specimens the striations become narrower at the end of the cephalic vesicle (Fig. 2B).

Head: Lips and buccal capsule absent; rounded triangular opening; presence of a small dorso-esophageal tooth; 6 cephalic papillae and two amphids; buccal opening 8 μm (Fig. 2A).

Synlophe: in males and females *synlophe* beginning just posterior to cephalic vesicle (Fig. 2B), in females extending until after anus, approximately in the middle of the tail (Fig. 2E), in males ridges extending to caudal bursa; ridges are more marked in dorsal view and gradually disappear in ventral and lateral view (Fig. 2H-J). Number of ridges: 64 at mid-body of females, and 56 at mid-body of males (Fig. 1B). Besides the longitudinal ridges, the cuticle surface presents narrow transverse striations between ridges, approximately 2.5 μm wide in females and 2 μm wide in males (Fig. 2C, D).

Female: vulva postequatorial, located in a depression and covered by two button-shaped cuticular structures (Fig. 2F-G). *Vagina vera* 51.2 \pm 6.3 (45–60) long, directed perpendicularly to vulvar opening. It presents two diverticula before vestibule. Ovejector Y-shaped (Fig. 1C). Vestibule muscularised, composed of 3 branches of approximately the same length 73 \pm 6.7 (65–80); unpaired branch situated as extension of *vagina vera*. Sphincters 78.6 \pm 10.9 (65–90). Infundibula 38 \pm 5.7 (32–45) by 44.3 \pm 4.3 (40–50). Anterior and posterior uterine branches with eggs. Embryonate eggs (Fig. 1D). Thin tail without caudal tip, but with a cuticular projection, 8.3 \pm 1.0 (7–9) (Fig. 1E).

Male: caudal bursa of type 2-1-2. Ray 4 almost the same length as rays 2, 3 and 5, 6 (Fig. 1F, G). Ray 8 arising at the root of dorsal ray, but shorter than the latter. Ray 9 reaching edge of the dorsal ray, separating from it before its division. Dorsal ray divided into two branches, one external with the phasmids, and one internal (rays 10), almost reaching edge of caudal bursa. Genital cone with papillae zero on anterior lip surrounded by numerous small cuticular protuberances. Spicules subequal, alate, divided into two branches: short internal branch and long external branch (Fig. 1H).

Schulzia travassosi has been previously reported from *Rhinella crucifer* (Wied-Neuwied, 1821) (= *Bufo crucifer*), *Rhinella icterica* (Spix, 1824) (= *Chaunus ictericus*), and *Proceratophys appendiculata* (Günther, 1873) from Brazil (Travassos 1925, Boquimpani-Freitas *et al.* 2001, Lux Hoppe *et al.* 2008), and *Rhinella granulosa* (Spix, 1824) (= *Bufo granulosa*) and *Leptodactylus bufonius* from Paraguay (Durette-Desset *et al.* 1985). Other species of this genus found in amphibians and reptiles are *S. chiribita* Durette-Desset, Florindez and Morales, 2000

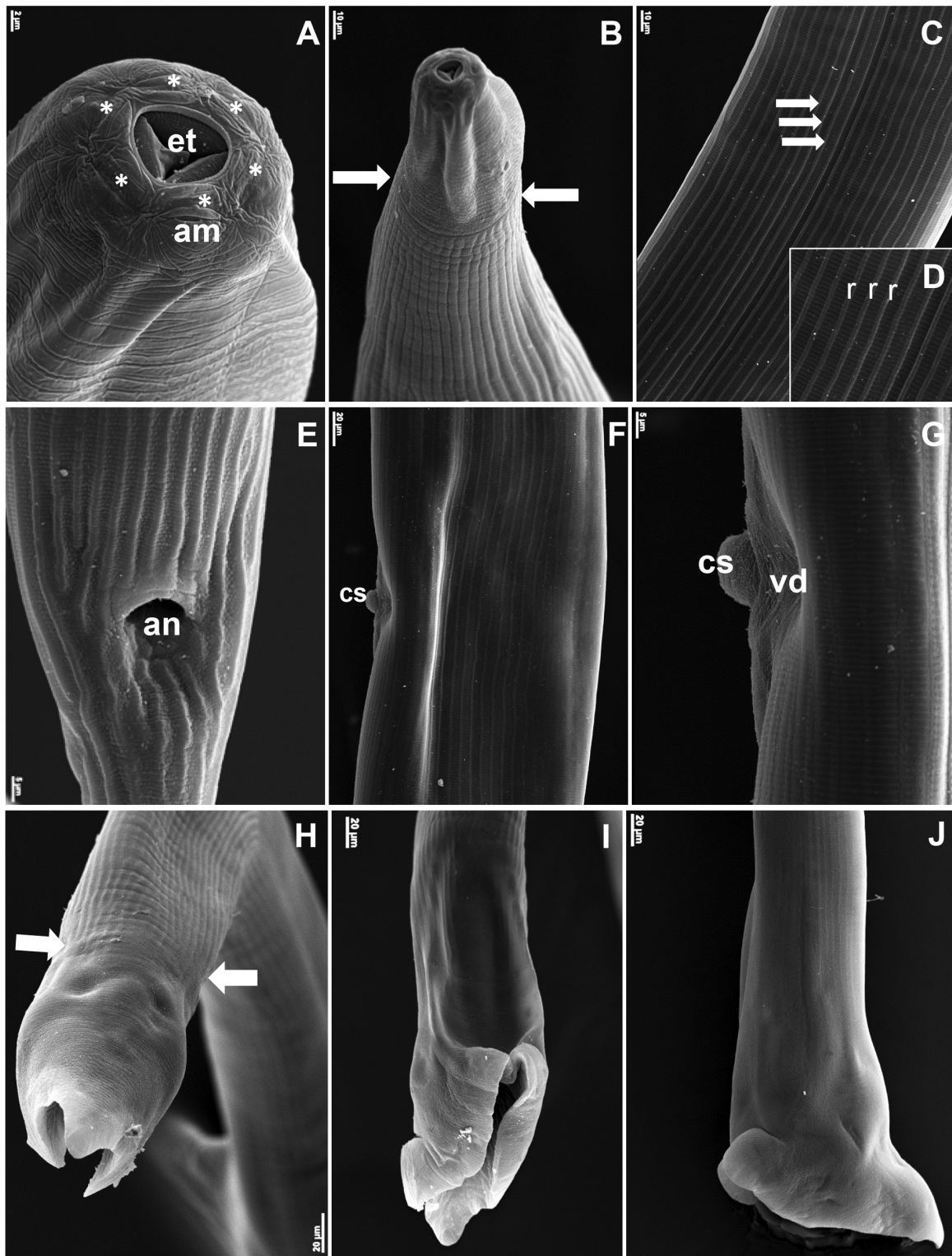


Fig. 2. *Schulzia travassosi* Durette-Desset, Baker and Vaucher, 1985 from Argentinean amphibians, *Leptodactylus bufonius* and *Rhinella major*, from Chaco region. **A** – Cephalic end of female, apical view; **B** – Anterior end of female, dorsolateral view; **C** – Disposition of ridges at the middle of body in females; **D** – Details of ridges and transverse cuticular striations between ridges; **E** – Posterior end of female, ventral view; **F** – Female, lateral view at the level of vulva; **G** – Detail of vulva, lateral view; **H** – Posterior end of male, dorsal view; **I** – Posterior end of male, ventral view; **J** – Posterior end of male, lateral view. Abbreviations: am: amphid; asterisk: cephalic papillae; et: dorso-esophageal tooth; r: ridges; an: anus; cs: cuticular structures of vulva; vd: vulvar depression. Arrows in each figure indicate: finish of cephalic vesicle and beginning of ridges (Fig. B); the ridges (Fig. C); end of ridges in males at level of the caudal bursa, dorsal view (Fig. H)

Table II. Nematode parasites found in *Leptodactylus bufonius* and *Rhinella major* from Chaco region, Argentina

Host species	Nematode species	Reference
<i>Leptodactylus bufonius</i>	<i>Aplectana</i> sp.	González and Hamann (2006a); Hamann <i>et al.</i> (2012).
	<i>Aplectana hylambatis</i> (Baylis, 1927) Travassos, 1931	González and Hamann (2006a); Hamann <i>et al.</i> (2012).
	<i>Cosmocerca parva</i> Travassos, 1925	González and Hamann (2006a); Hamann <i>et al.</i> (2012).
	<i>Cosmocerca podicipinus</i> Baker and Vaucher, 1984	González and Hamann (2006a); Hamann <i>et al.</i> (2012).
	<i>Ortleppascaris</i> sp.	González and Hamann (2006a); Hamann <i>et al.</i> (2012).
	<i>Oswaldocruzia</i> sp.	González and Hamann (2006a).
	<i>Physaloptera</i> sp.	González and Hamann (2006a).
	<i>Rhabdias elegans</i> Gutiérrez, 1945	González and Hamann (2006a); Hamann <i>et al.</i> (2012).
	<i>Schrankiana chacoensis</i> González and Hamann, 2014	González and Hamann (2014).
<i>Rhinella major</i>	<i>Schulzia travassosi</i>	This study
	<i>Aplectana delirae</i> (Fabio, 1971) Baker, 1980	González and Hamann (2006b).
	<i>Cosmocerca parva</i>	González and Hamann (2006b).
	<i>Cosmocerca podicipinus</i>	Mordeglia and Digiani (1998); González and Hamann (2006b).
	<i>Physaloptera</i> sp.	González and Hamann (2006b).
	<i>Schulzia travassosi</i>	This study

found in *Leptodactylus rhodonotus* (Günther, 1868) from Peru, *S. usu* Lent and Portes Santos, 1989 in *Atelopus oxyrhynchus* Boulenger, 1903 from Venezuela, and *S. ptychoglossi* Bursey, Goldberg and Telford Jr., 2006 in *Ptychoglossus festae* (Peracca, 1896) from Panama (Lent and Portes Santos 1989, Durette-Desset *et al.* 2000, Bursey *et al.* 2006).

Our *S. travassosi* specimens closely resemble the Paraguayan specimens, but we found some differences. Among the morphometric characters of females, the total length of specimens collected in *R. major* from Concepción del Bermejo and in *L. bufonius* from Las Lomitas was shorter than in specimens described by Durette-Desset *et al.* (1985) (11.4–15.0mm and 10.7–12.4mm, respectively vs. 16.4mm). The length of the oesophagus of all females studied here was shorter than in *S. travassosi* from Paraguay (all of them shorter than 437, see Table I). In some specimens from Concepción del Bermejo, the excretory pore was situated before the end of the oesophagus, while in the rest, the excretory pore was situated slightly posterior to the end of the oesophagus. In all cases, the vulva and anus were located closer to the posterior end than in those studied by Durette-Desset *et al.* (1985) (see Table I). Regarding the ovejector, it shows three branches mentioned in the original description; however, these branches are slightly longer than those of the specimens studied by Durette-Desset *et al.* (1985) (65–80 vs. 50). The same occurs with the sphincter (65–90 vs. 30). Regarding males morphology, the specimens collected in *R. major* from Concepción del Bermejo were shorter than those of Durette-Desset *et al.* (1985) (5.2–6.2 vs. 7.3mm) while specimens collected from the same host but in Las Lomitas were longer (6.6–8.1 vs. 7.3mm). In the description of Durette-Desset *et al.* (1985) the ray 4 is considerably shorter than the other rays (2, 3 and 5, 6);

in the specimens analyzed here, ray 4 presented almost the same length as the other four.

Intraspecific variation in adult nematode parasites has been attributed to different factors, such as size, age, physical condition, metabolic rate, and food supply of the host at the time of the entrance and growth of the parasite, in addition to climate or locality (Chitwood 1957, Levsen and Berland 2002).

Here, we provide the first record of the genus *Schulzia* in two species of amphibians from Argentina as well as the description of new features for both sexes of this nematode parasite (e.g. cuticular details viewed with SEM as *synlophe*, cephalic vesicle, vulvar opening; measurements of branches and sphincter of ovejector; size of eggs).

Schulzia melanostictusi was originally described from specimens taken from the intestine of *Duttaphrynus melanostictus* (= *Bufo melanostictus* (Schneider, 1799) from India (Chopra *et al.* 1986). This species can be assigned to *Oswaldocruzia* since the illustrations and short description of Chopra *et al.* (1986) established that in males, the dorsal lobe is not well differentiated and has a triangular shape, the ventrolateral (ray 2) and lateroventral (ray 3) rays are parallel, and the spicules are trifurcated distally; in females the ovejector is a T-shaped with a vestibule that does not present three muscularized branches, the tail has a caudal spine, and eggs are present in the morula stage.

Up to the moment, the nematofauna of *L. bufonius* from Argentina comprises nine species, and the nematofauna of *R. major* comprises four species (Table II). With this work we add a new genus of nematode parasite for Argentinean amphibians. Currently, the distribution of genus *Schulzia* comprises six countries: Venezuela, Peru, Brazil, Panama, Paraguay and Argentina.

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