

## REDESCRIPTIONS OF THE NEMATODES *LITOMOSOIDES PATERSONI* (MAZZA, 1928) (ONCHOCERCIDAE) AND *STILESTRONGYLUS STILESI* FREITAS, LENT, AND ALMEIDA, 1937 (HELIGMONELLIDAE) PARASITES OF *HOLOCHILUS CHACARIUS* (RODENTIA, CRICETIDAE) FROM SALTA, ARGENTINA

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**ABSTRACT:** Two nematode species are redescribed from the type host species *Holochilus chacarius* Thomas (Rodentia, Cricetidae, Sigmodontinae) and from the type locality of 1 of them, i.e., Ingenio San Martín de Tabacal, Salta Province, Argentina. Rodents were deposited at the Colección Mamíferos Lillo, Tucumán, Argentina. *Litomosoides patersoni* (Mazza, 1928) (Onchocercidae) possesses a buccal capsule with irregular external walls, a buccal cavity smooth, becoming thinner near the oral opening, a complete set of head papillae, 3–6 pairs of cloacal papillae, and the “*sigmodontis*” type of spicules. Filarioids were found in 3 of 17 examined hosts. *Stilestrongylus stilesi* Freitas, Lent, and Almeida, 1937 (Heligmonellidae), whose description was based on male specimens, was found in all 17 of the examined hosts. Here, we describe the female and the synlophe of both sexes. Females are characterized by a short uterus with less than 25 eggs, short ovejector, short and conical tail, and the posterior extremity strongly invaginated in a cuticular expansion usually harboring 1 to several eggs. The synlophe is characterized by 29–31 sub-equal cuticular ridges at the mid-body, with single (in males) or double (in females) axis of orientation of the ridges. The present work validates and enlarges the original descriptions of both species and assigns the specimens from *L. patersoni*, recovered from the type locality and the type host species, as neotypes.

In 1928, Mazza described a new filarioid species, *Filaria patersoni*, from several chacoan marsh rats, *Holochilus chacarius* Thomas, 1906 (originally regarded as *H. vulpinus* Brants, 1827) from the Ingenio San Martín de Tabacal, a sugar cane plantation in Salta Province, Argentina (Mazza, 1928). The systematic position of this species was complex and synonymized several times (Vogel and Gabaldon, 1932; Chitwood, 1933; Vaz, 1934; Esslinger, 1973; Bain et al., 1989). It was considered as *Vestibulosetaria patersoni* (Mazza, 1928) by Vogel and Gabaldon (1932) within a set of filarioid specimens parasitizing *Rattus norvegicus* (Berkenhout, 1769) from Caracas, Venezuela (Vogel and Gabaldon, 1932) and *Sigmodon hispidus* (Say and Ord, 1825) from Jalisco and Michoacan, Mexico (Ochoterena and Caballero, 1932); as *Litomosoides patersoni* (Mazza, 1928) by Chitwood (1933), who validated the genus *Litomosoides* Chandler, 1931; and as *L. carinii* (Travassos, 1919) by Vaz (1934) and Esslinger (1973) within a set of filarioids from *Nectomys squamipes* (Brants, 1827) from Sao Pablo, Brazil (Vaz, 1934), and *Melanomys caliginosus* (Tomes, 1860) from Valle, Colombia (Esslinger, 1973). Bain et al. (1989) reexamined the material of most of these sets, with the exception of that from Mazza, which is lost, and clarified the systematic position from *L. carinii* and *L. sigmodontis* Chandler, 1931; they also created a new species based on the material of Vaz (1934), i.e., *L. kohnae* (Vaz, 1934), and considered *L. patersoni* a valid species.

In 1934, Mazza sent some specimens of the chacoan marsh rat to Dr. F. Werneck of the Instituto Oswaldo Cruz, Brazil, who kindly gave the intestines to Dr J. F. T. Freitas. Years later, Freitas et al. (1937) described a new genus and species of heligmonellid nematode from the intestines of these hosts, i.e., *Stilestrongylus stilesi* Freitas, Lent, and Almeida, 1937, plus 3 other new species, i.e., *Hassalstrongylus argentinus* (Freitas, Lent,

and Almeida, 1937) (= *Longistriata argentina*), *Hassalstrongylus mazzai* (Freitas, Lent, and Almeida, 1937) (= *Heligmonoides mazzai*), and *Longistriata fortuita* Freitas, Lent, and Almeida, 1937 (Heligmosomoidea); all were reported from a single *H. chacarius* (syn. *Holochilus balnearum* Thomas, 1906) from Salta.

The original descriptions of most of these nematodes are incomplete, particularly the Heligmosomoidea, for which the publications prior to 1964 do not include the synlophe; additionally, females were typically poorly described. Currently, *Stilestrongylus* includes 24 species, and the synlophe of 21 of them is known, but that of the nominal species, *S. stilesi*, remains undescribed. The type material of *S. stilesi* is available from the Helminthological Collection of the Instituto Oswaldo Cruz (CHIOC), Rio de Janeiro, Brazil. However, its state of preservation precludes making a correct redescription of the species (M. C. Durette-Desset, pers. comm.). In contrast, specimens from *L. patersoni* were not deposited in any collection.

In the present paper, we provide redescriptions of *L. patersoni* and *S. stilesi*, based on specimens collected in *H. chacarius* from the Ingenio San Martín de Tabacal, and designate neotypes for the filarioid species, considering that the type material is lost.

### MATERIALS AND METHODS

Seventeen *H. chacarius* Thomas, deposited in the Colección Mamíferos Lillo (CML), Tucumán, Argentina, were examined for parasites. They were captured in the Ingenio San Martín de Tabacal, Departamento Orán, Salta Province, in August and September 1990. Rodents were fixed in 10% formalin and stored in 70% ethanol.

Filarial worms were recovered from the abdominal cavity and heligmonellids from the small intestine. Nematodes were preserved in 70% ethanol. A transverse section posterior to the vulva was made in a filarioid female, and microfilariae were isolated (Notarnicola et al., 2000). Synlophe was studied, following the method of Durette-Desset (1985). Measurements are given in micrometers, except otherwise stated, as the range followed by the mean in parentheses. Classification used above the family Heligmonellidae level follows Durette-Desset and Chabaud (1993). The nomenclature and synonymy of the host species follows Wilson and Reeder (2005). Parasites were deposited in the Helminthological Collections of the Museo de La Plata, La Plata, Argentina (CHMLP), and of the Muséum national d’Histoire naturelle, Paris, France (MNHN).

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## RESULTS

### REDESCRIPTIONS

#### *Litomosoides patersoni* (Mazza, 1928)

(Figs. 1–21; Tables I, II)

**General:** Males about 2.5 times shorter than females. Cephalic extremity attenuated. Mouth small. Four minute cephalic papillae placed in rectangle stretched laterally; 4 labial papillae surround oral opening (Fig. 4); amphids lateral, not salient. Buccal capsule embedded in esophagus, with irregular external walls (Figs. 2, 3); buccal cavity smooth, becoming thinner near oral opening. Esophagus muscular, slightly glandular posteriorly. Tail attenuated. Measurements given in Tables I and II.

**Males (based on 4 specimens):** Posterior region coiled. Left spicule with handle shorter than blade; blade constituted by membranous folded ala and terminal filament (Fig. 14). Right spicule with poorly cuticularized heel (Fig. 15). Number of cloacal papillae variable, from 3 to 6 pairs. One male with 1 pair of adcloacal, 1 pair of lateral postcloacal papillae, and 1 papilla in median ventral line (Fig. 18); other male with 1 precloacal pair, 1 adcloacal pair, and 4 postcloacal pairs (Figs. 19–21). Area rugosa composed of transverse ridges of small longitudinal crests, extending through coiled region (Figs. 16, 17).

**Females (based on 4 specimens):** Vulva far away from esophagus-intestine junction. Vagina globular, ovejector muscular directed posteriad (Fig. 1). Small divergent phasmids. In cross section, at mid-body, lateral chords flattened and expanded laterally; internal cuticular ridges square-shaped (Fig. 5).

**Microfilariae:** Body fusiform. Stout microfilariae. Anterior extremity with small visible hook, tail attenuated with nuclei near the tip tail. Sheath not visible (Fig. 9). Measurements based on uterine microfilariae from female 2:  $35 \times 6 \mu\text{m}$ ;  $44 \times 6 \mu\text{m}$ .

#### Taxonomic summary

**Host:** Three *H. chacarius* Thomas (Rodentia, Cricetidae) deposited at Colección Mamíferos Lillo, numbers CML 5810, CML 5813, CML 5825.

**Material studied:** Three males, 4 female anterior extremities, and 1 posterior extremity deposited at CHMLP, numbers 5995 and 6007; 1 male deposited at MNHN, number 591 MQ.

**Site of infection:** Body cavity, between the intestinal mesentery and testis. In host CML 5810, 1 male in small intestine.

**Locality:** Ingenio San Martín de Tabacal ( $23^{\circ}16'S$ ,  $64^{\circ}15'W$ ), lote Milagros, Departamento Orán, Salta, Argentina.

**Prevalence and mean intensity:** Three of 17 hosts infected (17.6%); 2.6 (1–5) worms per host.

#### Remarks

*Litomosoides patersoni* belongs to the “*sigmodontis*” group of species (Bain et al., 1989; Notarnicola et al., 2000; Bain et al., 2003) based on the morphological characteristics of both spicules. The left spicule has a handle shorter than the blade and the blade is divided in anterior membranous folded ala, with a terminal filament; heel of right spicule not heavily cuticularized. Mazza (1928) reported smaller length for the spicules; moreover, Figure 4 in his paper clearly shows the “*sigmodontis*” type of spicules. One of the males described herein possesses a similar arrangement of the cloacal papillae as stated by Mazza (1928), but others display a greater number of papillae (see Figs. 18–21).

Microfilariae are shorter and more stout compared with other species of *Litomosoides*, which was also reported by Mazza (1928) for microfilariae from blood smears.

*Litomosoides patersoni* can be differentiated from *L. navonae* Notarnicola, 2005, a parasite of *H. chacarius* from Chaco and Formosa Provinces, Argentina, by the shape of the buccal capsule, the number and disposition of the cloacal and the head papillae (Notarnicola, 2005). The 4 postcloacal papillae in *L. patersoni* are mostly symmetrical, while in *L. navonae* there are 5–6 asymmetric papillae. Moreover, the microfilariae in *L. patersoni* are shorter ( $35\text{--}44 \times 6 \mu\text{m}$  vs.  $70 \times 3.8 \mu\text{m}$ ) and the sheaths are not visible as in *L. navonae* (Notarnicola, 2005). Moreover, *L. patersoni* can be differentiated from other species of *Litomosoides* from cricetid rodents because of the shape of the buccal capsule, the stout microfilariae, and the complete presence of the head papillae.

#### *Stilestrongylus stilesi* Freitas, Lent, and Almeida, 1937

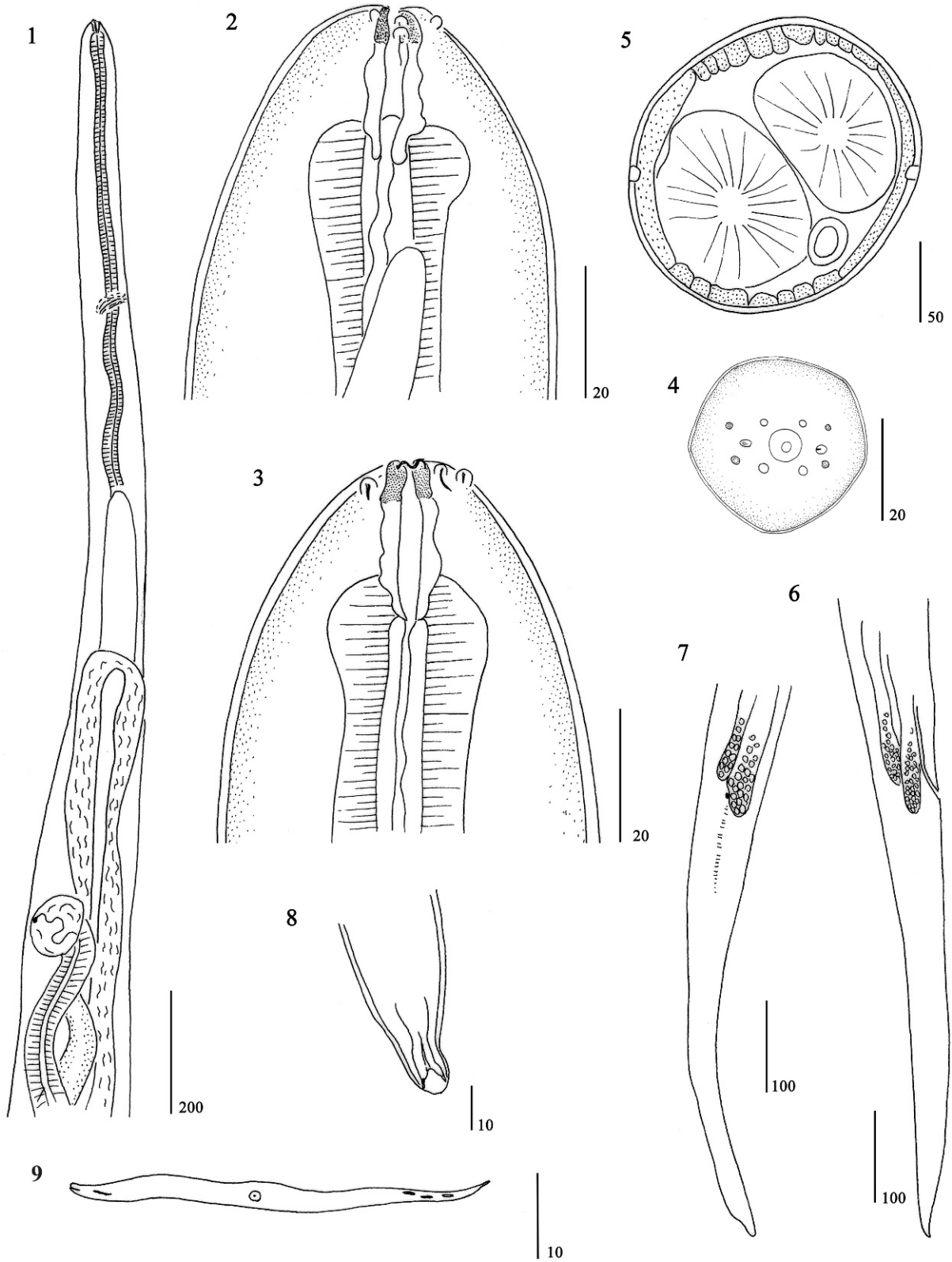
(Figs. 22–34)

**General:** Medium-sized nematodes, usually coiled sinistrally along ventral side, in different degrees. Coiling varying from tightly and completely coiled in up to 6 spirals, partly coiled with up to 3 spirals in anterior half, loosely coiled with 1–2 spirals at anterior end, or uncoiled. Excretory pore within 74–81% of esophagus length in males; 65–78% in females. Deirids small, situated at level of excretory pore or slightly posterior (Fig. 22). Ratio uterus length/body length 10–20%. Cephalic vesicle present. In apical view, triangular buccal opening surrounded by thin ring. Two amphids, 6 externo-labial papillae, and 4 submedian cephalic papillae (Fig. 23).

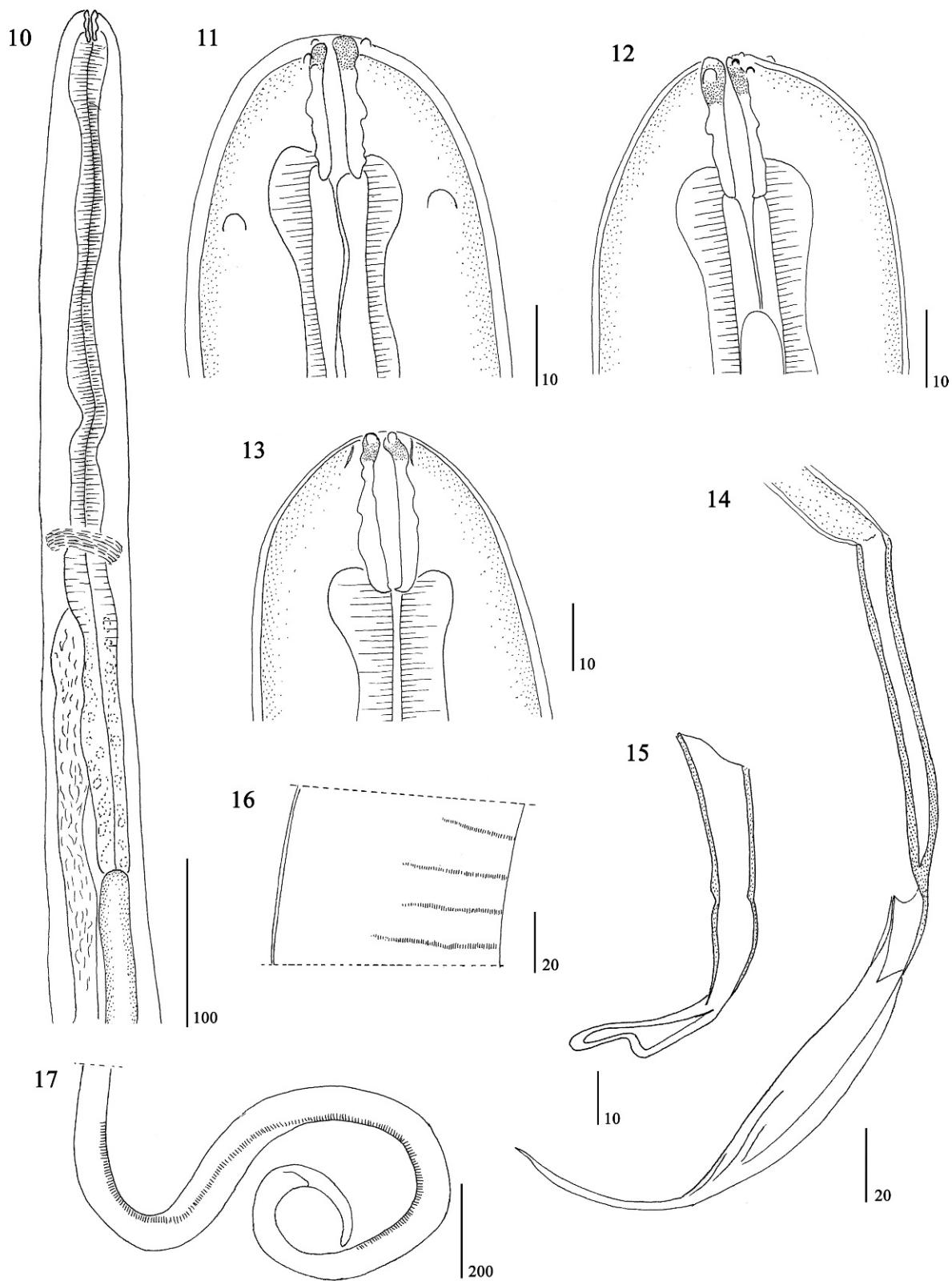
**Synlophe (2 males and 2 females):** In both sexes, cuticle with longitudinal, uninterrupted ridges appearing mainly on left side posterior to cephalic vesicle, disappearing just anterior to caudal bursa in males and extending up to end of terminal cuticular dilatation in female. At level of esophagus-intestinal junction: 24 ridges in male, 25 in female (Fig. 24); 29–31 ridges in both sexes at mid-body; unequal in size, ridges on ventral right quadrant, smaller. In female, double axis of orientation, right axis inclined at  $60^{\circ}$  to sagittal axis, left axis sub-frontal. In males, right axis inclined at  $63^{\circ}$ , left axis at  $54^{\circ}$  to sagittal axis (Figs. 25, 27). Within distal third of body length; in male, 29 ridges at  $150 \mu\text{m}$  anterior to caudal bursa, in female, 26 ridges at mid-length of uterus (Figs. 26–28); ridges sub-equal in size in both sexes. Most ridges with similar orientation than at mid-body.

**Males (20 specimens, except otherwise stated):** 2.62–3.73 (3.12) mm long and 80–130 (100) wide at mid-body. Cephalic vesicle 50–62 (57) long and 22–32 (28) wide. Nerve ring not observed. Excretory pore situated at 200–270 (228) from apex ( $n = 9$ ). Deirids, when observed ( $n = 4$ ), at same level than excretory pore. Esophagus 260–310 (263) long ( $n = 10$ ).

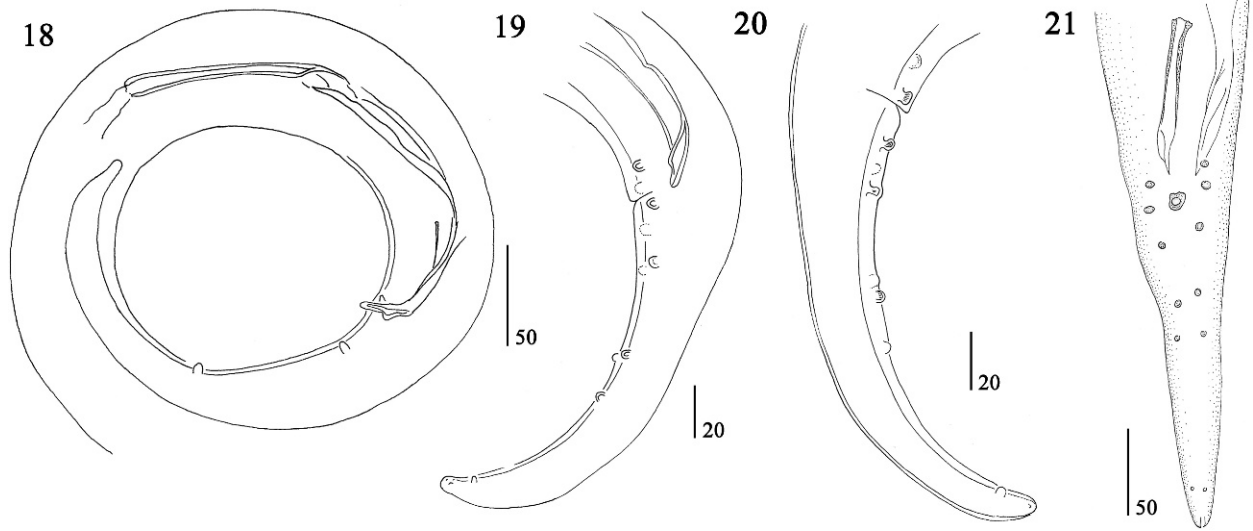
Caudal bursa asymmetrical, with right lobe more developed than left (Figs. 29, 30). Prebursal papillae not observed. Right lobe: pattern of type 2–3; rays 2 and 3 joined proximally, diverging at half of their length, stout and of similar length; rays 4–6 with long common trunk; ray 5 stout, with strongly reinforced margins; rays 4 and 6 thinner, divergent from ray 5 at distal and proximal third of its length, respectively (Fig. 29). Left lobe: pattern of type 2-2-1; rays 2 and 3 joined proximally, diverging at distal third of their length, stout and of similar length; rays 4 and



FIGURES 1–9. *Litomosoides patersoni* (Mazza, 1928) female. (1) Anterior extremity. (2–4) Cephalic end, lateral, median, and apical views from female 2. (5) Transverse section posterior to the vulva. (6, 7) Tail, lateral and ventral views. (8) Tip of tail with phasmids. (9) Uterine microfilaria.



FIGURES 10–17. *Litomosoides patersoni* (Mazza, 1928) male. (10) Anterior extremity. (11, 12) Cephalic end, lateral and median views from male 1. (13) Cephalic end from male 2. (14, 15) Left and right spicule, lateral view from male 1. (16) Area rugosa at mid-length. (17) Posterior extremity showing the extension of the area rugosa.



FIGURES 18–21. *Litomosoides patersoni* (Mazza, 1928) male. (18) Posterior end showing the spicules and papillae from male 1. (19–21) Tail from male 2, lateral views from left and right sides and ventral view, respectively.

TABLE I. Measurements of males of *Litomosoides patersoni* (Mazza, 1928).

Measurements	CML5813			CML5810
	M1	M2	M3	M4*
Body length	19.305 mm	15.85 mm	17.3 mm	12 mm
Maximum width	120	120	135	85
Buccal capsule (L × W)	18 × 7.5	22 × 8	19 × 7	17 × 9
Esophagus length	510	580	540	425
Nerve ring	340	360	340	–
Tail length	275	180	146	195
Left spicule	246	–	247	265
Handle/lamina	100/146	–	107/140	115/150
Right spicule	80	87	84	80
Area rugosa length	1,150	1,740	1,500	400
Area rugosa beginning at 500–1700 from tip of tail	550–1,700	760–2,500	770–2,270	260–660

\* Specimen found in the small intestine of the host.

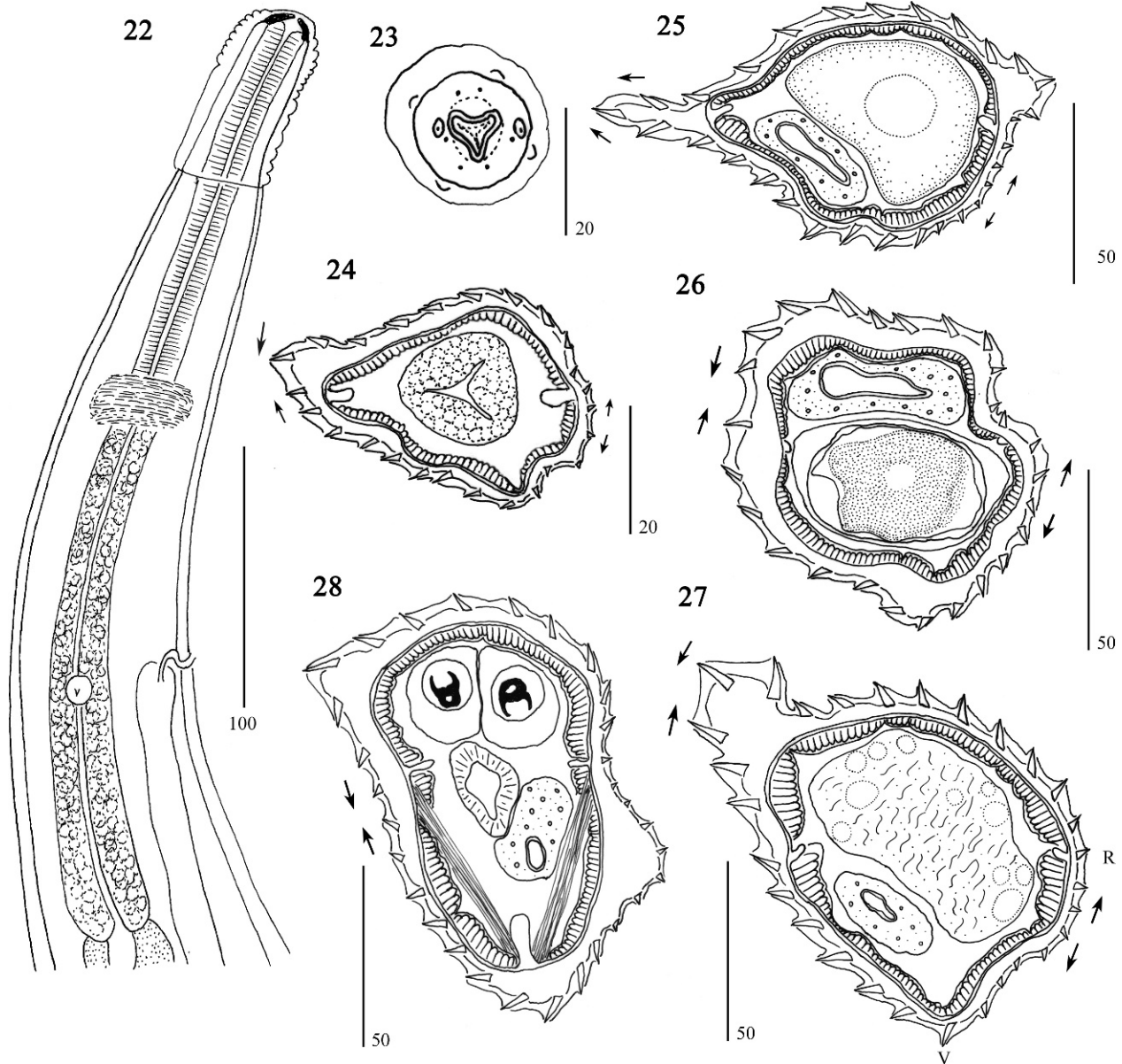
TABLE II. Measurements of females of *Litomosoides patersoni* (Mazza, 1928).

Measurements	CML5813		CML5825	
	F1 ae + Tail	F2 ae	F3*	F4 ae
Length of pieces	28.71 + 15.345 mm	–	~31.6 mm	–
Maximum width	250	300	160	260
Width at vulva	170	200	150	175
Buccal capsule (L × W)	24 × 9	24 × 9	26 × 9	26.5 × 10
Esophagus length	780	650	700	690
Nerve ring	490	260	470	600
Tail length	500	–	–	–
Vulva to apex	1,520	2,050	1,350	1,600
Vulva to e-i junction†	720	1,400	610	1,000

ae, anterior extremity.

\* Female with the tail broken.

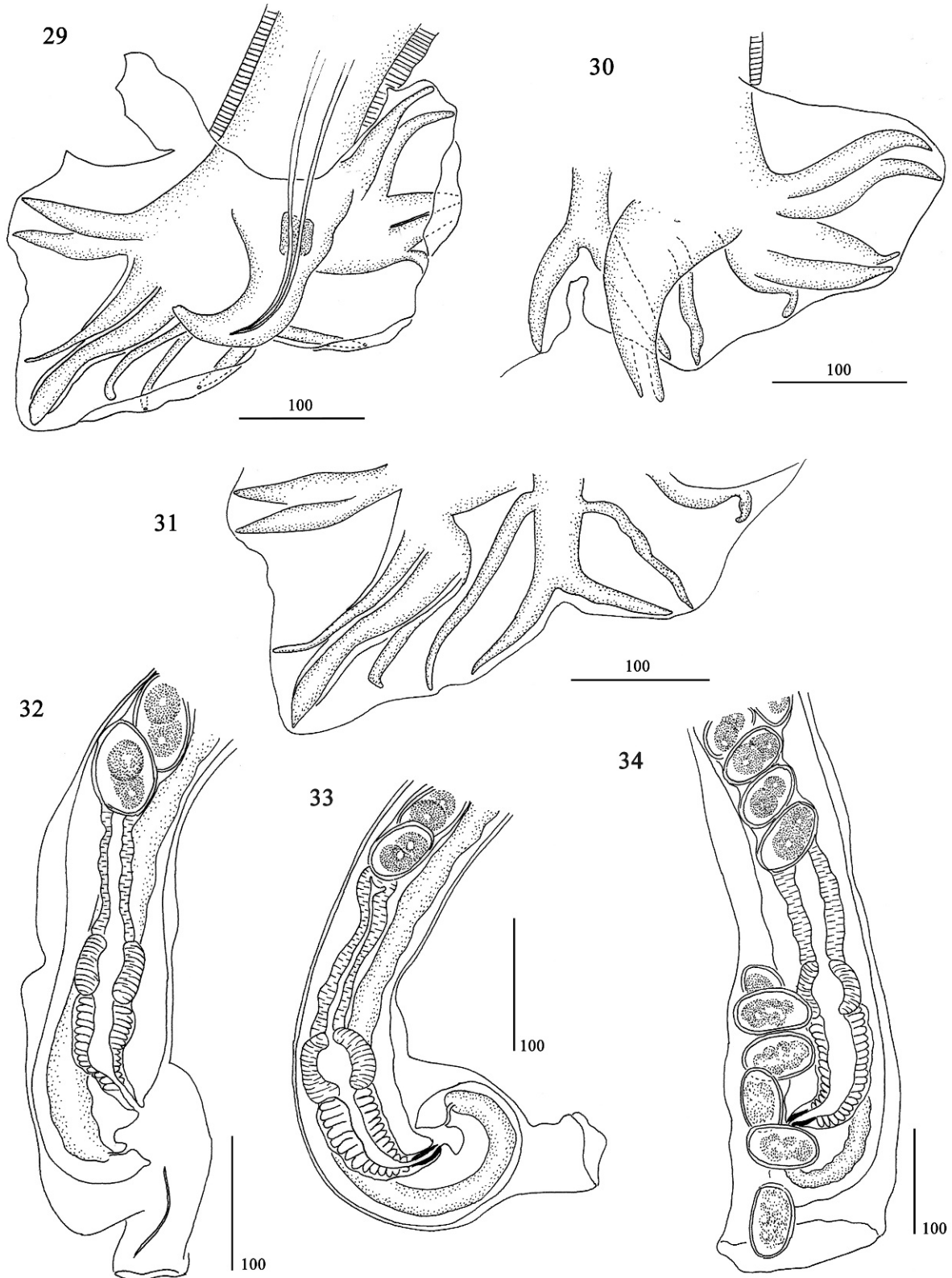
† Distance of the vulva to the esophagus-intestine junction.



FIGURES 22–28. *Stilestrongylus stilesi* Freitas, Lent and Almeida, 1937. (22) Female, anterior extremity, right lateral view. (23) Female, head, apical view. (24–28) Transverse sections of the body. (24) Female at esophageal level (220  $\mu\text{m}$  from apex). (25) Female, at mid-body (53% of body length). (26) Female at mid-length of uterus (400  $\mu\text{m}$  from posterior end). (27) Male at mid-body (50% of body length). (28) Male at 150  $\mu\text{m}$  anterior to caudal bursa. Abbreviations: R, right; V, ventral.

5 divergent at extremities; ray 6 short and thin, diverging from common trunk at same level than the group formed by rays 2–3 (Fig. 30). Rays 8 thin, arising asymmetrically from proximal third of dorsal ray. Dorsal ray divided at about distal third into 2 branches. These branches are not bifurcated at their distal end, indicating that rays 9 and 10 probably are merged (Fig. 31). Genital cone well developed, markedly conical, 100–140 (122) long by 50–60 (58) wide at base (n = 10). Papillae on genital cone not observed. Spicules subequal, alate, ending in simple, pointed tips. Length of spicules 740–970 (865), representing 23.3–33% of body length. Gubernaculum quadrangular, 20–50 (36) long and 15–30 (23) wide (Fig. 29).

*Females* (14 specimens, except otherwise stated): 3.08–5.10 (3.83) mm long and 80–120 (98) wide at mid-body. Cephalic vesicle 55–70 (60) long and 25–35 (29) wide. Nerve ring, excretory pore, and deirids situated at 125–160 (141) (n = 5), 190–260 (231) (n = 7), and 200–270 (240) (n = 4) from apex, respectively. Esophagus 280–400 (325) long (n = 10). Monodelphic. Vulva situated at 65–70 from caudal extremity (n = 7). Vagina vera 25–35 (31) long (n = 8), vestibule 55–90 (68) long (n = 11), sphincter 40–50 (44) long and 35–55 (46) wide, infundibulum 60–120 (82) (n = 11). Uterus 400–750 (560) long, taking up 9.8–20.5% (14.8%) of body length, containing 7–24 (16) eggs. Eggs at 2–8 blastomeres stage, 55–80 long and 30–45 wide. Tail 20 (n = 5) long. Posterior



FIGURES 29–34. *Stilestrongylus stilesi* Freitas, Lent and Almeida, 1937. (29–31) Male, caudal bursa. (29) Entire bursa, ventral view. (30) Detail of left lobe, ventral view (spicules omitted). (31) Detail of dorsal lobe, ventral view. (32–34) Female, posterior extremity in 3 different specimens. (32, 33) Right lateral view. (34) Left lateral view, showing eggs within cuticular dilatation.

extremity, from vestibular level, invaginated into a cuticular dilatation, 155–310 (187) long. Seven of 20 females harbored 1–8 eggs within this cuticular dilatation (Figs. 32–34).

### Taxonomic summary

*Host:* Seventeen *H. chacarius* Thomas (Rodentia, Cricetidae) deposited at Colección Mamíferos Lillo (numbers CML 5810–5826).

*Material studied:* Males and females deposited as CHMLP numbers 5993, 5994, 5996–6006, 6008, 6009, and 6096; males and females deposited at MNHN (numbers 592 MQ to 594 MQ).

*Site of infection:* Small intestine.

*Locality:* Ingenio San Martín de Tabacal (23°16'S, 64°15'W), lote Milagros, Departamento Orán, Salta, Argentina.

*Prevalence and mean intensity:* Seventeen hosts examined (100% infected); 108 (4–826) worms per host.

### Remarks

Freitas et al. (1937) based their description of *S. stilesi*, the nominal species, on an unspecified number of males, plus 1 female in the same work. The illustrations of the male (caudal bursa, spicules, and gubernaculum) made by Freitas et al. (1937) are very accurate, allowing confirmation of our specimens as *S. stilesi*. The preservation of type material, available from CHIOC, precludes a redescription of the species because some specimens are mounted on slides and others preserved in ethanol are too fragile to permit a description of the synlophe (M. C. Durette-Desset, pers. comm.). The finding of voucher specimens parasitizing the same host species, and probably from the same locality, permitted us to make a complete description of *S. stilesi*.

*Stilestrongylus* was originally defined based mainly on the characters of the caudal bursa, which is markedly asymmetrical (Freitas et al., 1937). However, Durette-Desset (1971) redefined the genus based on 5 species with known synlophe (*Stilestrongylus barusi* Durette-Desset, 1970; *S. dessetae* Yoyotte-Vado, 1972; *S. freitasi* Durette-Desset, 1968; *S. inexpectatus* Durette-Desset and Tchérakoff, 1969; and *S. renaudae* Durette-Desset, 1970) and 4 species with unknown synlophe (*S. stilesi*, *S. aculeata* [Travassos, 1918], *S. eta* [Travassos, 1937], and *S. riberoi* [Travassos, 1937]). She established *Stilestrongylus* as having a strongly asymmetrical caudal bursa, a hypertrophied genital cone, and synlophe with more than 24 cuticular ridges that are small and sub-equal, with axis of orientation of ridges from right ventral to left dorsal quadrant. Since the synlophe observed in the present specimens is coincident with the described characters, this redescription also validates the redefinition of the genus by Durette-Desset (1971).

In the material studied herein, some internal structures of the anterior extremity are difficult to observe; in females, the caudal structures are frequently hidden by the folds of the posterior cuticular dilatation or the presence of eggs within it. The inclination of the left axis of orientation differs between males and females, but this should not be interpreted as a specific character. The inclination of the axis on the left side is difficult to evaluate, due to the opacity of the cuticle (orientation of ridges unclear) and the frequent deformation of the cuticle on this side of the body. Despite these minor constraints, the most important characters can be observed and interpreted for both sexes. We clarify in this work the asymmetry of the caudal bursa in having

the right lobe more developed than the left one, in contrast to the affirmation by Freitas et al. (1937) where the left lobe is the most developed.

### DISCUSSION

Both species, *L. patersoni* and *S. stilesi*, are redescribed herein with new morphological data. Currently, specimens of the chacoan marsh rat distributed in the Yungas of Salta are referred to *H. chacarius* Thomas, 1906 (Cirignoli et al., 2006). Mazza (1928) misidentified the specimens from Salta, which he assigned to *H. vulpinus*, a synonym of *H. brasiliensis* (Desmarest, 1819) distributed in eastern Argentina (Pardiñas and Galliari, 1998a, 1998b). However, Freitas et al. (1937) were correct in that the hosts were *H. balnearum*, which is a subspecies of *H. chacarius* (Cirignoli et al., 2006).

The site of infection for *Litomosoides* spp. is typically the thoracic and/or abdominal cavity of the host. The present finding of 1 male of *L. patersoni* in the intestine is unusual. However, other authors have reported living filarioids in the intestine of their host, i.e., *Litomosia filaria* (van Beneden) in bats (Desportes, 1946). Considering that this male worm was smaller than the other collected in the body cavity, the intestine could be a transitory organ in the migratory route.

Since the type specimens of *L. patersoni* have been lost, we assigned the specimens from this survey (recovered from the type locality and the type host species) as neotypes, according with the Articles 75.3.4, 75.3.5, and 75.3.6 from the International Code of Zoological Nomenclature (ICZN, 1999). For the heligmosomoid *S. stilesi*, we validated and completed the description from Freitas et al. (1937) and provided voucher specimens.

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