

REDISCOVERY AND NEW MORPHOLOGICAL DATA ON TWO *HASSALSTRONGYLUS* (NEMATODA: HELIGMONELLIDAE) COPARASITIC IN THE MARSH RAT *HOLOCHILUS CHACARIUS* (RODENTIA: CRICETIDAE) FROM ARGENTINA

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ABSTRACT: Two species of *Hassalstrongylus* Durette-Desset, 1971, coparasitic in *Holochilus chacarius* Thomas (Rodentia, Cricetidae) and not recorded since their original description in 1937, were newly found in their type host and locality. *Hassalstrongylus mazzai* (Freitas, Lent and Almeida, 1937) and *Hassalstrongylus argentinus* (Freitas, Lent and Almeida, 1937) were obtained from *Ho. chacarius* from 2 different populations: one from Salta Province (northwest Argentina) and another from Chaco Province (northeast Argentina). The species described as *Heligmonoides mazzai* Freitas, Lent and Almeida, 1937 had been transferred to *Hassalstrongylus* even though its synlophe had never been studied. We provide the first descriptions and illustrations of the synlophe of males and females of *Hassalstrongylus mazzai* and the female of *H. argentinus* and account for morphological and metrical variability. We confirm, through the study of the synlophe, the placement of *Hassalstrongylus mazzai* in the genus *Hassalstrongylus* and designate neotypes for the species because the type material deposited by the authors could not be found. Females of both species were morphologically very similar, and a principal components analysis (PCA) performed on some morphometrical characters showed that the body length, uterus length, and an unexpected character as the number of eggs were useful characters in the discrimination of both species.

Freitas, Lent, and Almeida (1937) described in a single individual of the chacoan marsh rat *Holochilus chacarius* Thomas (syn = *Holochilus balnearum*) (Cricetidae: Sigmodontinae) from Salta, Argentina, 4 species of heligmonellids: *Stilestrongylus stilesi* Freitas, Lent, and Almeida, *Longistriata fortuita* Freitas, Lent, and Almeida, *Longistriata argentina* Freitas, Lent, and Almeida, and *Heligmonoides mazzai* Freitas, Lent, and Almeida.

The first 3 species were redescribed and, mainly through the study of their synlophe, their generic attribution was either confirmed—the case of *S. stilesi* (Notarnicola et al., 2010)—or rectified. Digiani et al. (2013) created the genus *Mazzanema* Digiani, Notarnicola, and Paulos, 2013 to accommodate the species *L. fortuita*, for which neotypes were designated. Both *S. stilesi* and *Mazzanema fortuita* (Freitas, Lent, and Almeida) were redescribed from newly collected material from the type host and locality (Notarnicola et al., 2010; Digiani et al., 2013). The combination *Hassalstrongylus argentinus* (Freitas, Lent, and Almeida) was proposed by Durette-Desset (1971) for *L. argentina* after the description of the synlophe on 1 male type (Durette-Desset, 1968). Finally, *He. mazzai* was also transferred to *Hassalstrongylus* by Durette-Desset (1971) without studying its synlophe. This latter character remained unknown because the species was never restudied or reported since its original description.

We recently examined for parasites the viscera of several specimens of the marsh rat from different localities of northern Argentina, including the type locality of the 4 species described by Freitas et al. (1937). We found nematodes corresponding in most characters to those described as *Hassalstrongylus mazzai* (Freitas, Lent, and Almeida), which allowed the redescription of the species and the confirmation of its generic assignment. A complete redescription of *H. argentinus* is also provided. Additionally, a principal components analysis (PCA) was performed on morphometrical characters of the females with the aim of finding additional tools for discrimination between both species.

MATERIALS AND METHODS

Seventeen specimens of *Ho. chacarius* deposited at the Colección Mamíferos Lillo (CML), Tucumán, Argentina were kindly loaned by the curator R. Barquez and their viscera examined for parasites. The rodents were captured at Ingenio San Martín de Tabacal, Departamento Orán, Salta Province, Argentina in March, August, and September 1990; fixed in 10% formalin, and stored in 70% ethanol. Twelve specimens of *Ho. chacarius* were captured in Selvas del Río de Oro, Departamento Libertador General San Martín, Chaco Province, Argentina in July 2000 by J.N. They were deposited in the Colección de Mamíferos del Centro Nacional Patagónico (CNP), Puerto Madryn, Chubut, Argentina, and the Colección de Mamíferos of the Museo de la Plata, La Plata, Argentina (MLP).

The nematodes recovered were preserved in 70% ethanol. The synlophe was studied following the method of Durette-Desset (1985), and the description of the caudal bursa follows Durette-Desset and Digiani (2012). Measurements are given in micrometers (μm), except when otherwise stated, as the range followed by the mean in parentheses. SpL/BL and UtL/BL stand for the proportion (in percentage) of the spicule length on the body length and the uterus length on the body length, respectively. The classification used above the family level follows Durette-Desset and Chabaud (1993) and the taxonomy of the host species follows Gonçalves et al. (2015). The parasites were deposited in the Colección de Helmintos, Museo de La Plata, La Plata, Argentina (MLP-He). Type material of *Heligmonoides mazzai* was requested from the Helminthological Collection of the Instituto Oswaldo Cruz, Rio de Janeiro, Brazil (CHIOC).

As the differentiation of females of *H. mazzai* and *H. argentinus* was difficult, a multivariate morphometric analysis was conducted on 3 main characters that we considered could separate the species: body length (BL), uterus length (UtL), and egg numbers (Egg no.). To be confident the female specimens correspond to either *H. mazzai* or *H. argentinus*, we described and measured females from host specimens where males of the other species were absent or underrepresented. To test the normality of the data, we used the Shapiro-Wilk test (Shapiro and Wilk, 1965) as presented in PAST software (Hammer et al., 2001). Tests showed the non-normality of the data, so we used the nonparametric Mann-Whitney *U*-test to compare whether the medians of the characters from *H. mazzai* and *H. argentinus* samples were different. We executed a PCA using the covariance matrix for the 3 characters (BL, Egg no., and UtL) as variables. Variables were \log_{10} -transformed in order to standardize the data.

RESULTS

In both samples examined we found specimens corresponding to *H. mazzai* and *H. argentinus*, along with the 3 other species of heligmonellids described originally by Freitas et al. (1937).

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Hassalstrongylus mazzai showed a prevalence of 35.3% (6 out of 17) in the sample from Salta and 66.6% (8 out of 12) in the sample from Chaco. It was coparasitic with *S. stilesi*, *Mazzanema fortuita*, and *H. argentinus* in all the 14 hosts wherein it was found. *Hassalstrongylus argentinus* showed a prevalence of 70.6% (12 out of 17 hosts examined) in the sample from Salta and 100% (12 hosts examined) in the sample from Chaco. It was coparasitic with *S. stilesi* and *M. fortuita* in all 24 hosts wherein it was found and with *H. mazzai* in 14 out of 24.

REDESCRIPTION

Hassalstrongylus mazzai (Freitas, Lent, and Almeida, 1937)

= *Heligmonoides mazzai* Freitas, Lent, and Almeida, 1937

(Table I; Figs. 1–15)

General: Medium-sized, uncoiled nematodes. Cephalic vesicle present. Excretory pore and deirids at same level, at about 73–92% of esophagus length in males, within 59–71% in females. Nerve ring just anterior to excretory pore and deirids (Fig. 1). In apical view, rounded buccal opening surrounded by thick ring; 2 amphids, 4 externo-labial (2 dorsal, 2 ventral), and 4 cephalic papillae visible; lateral externo-labial papillae probably fused with amphids (Fig. 2).

Males (measurements correspond to 2 neotypes from the type locality, except as indicated): 2.97–3.06 mm long and 90 wide at mid-body. Cephalic vesicle 50–55 long and 28–30 wide. Nerve ring at 120–172 from apex. Excretory pore and deirids situated at the same level, 255–295 from apex. Esophagus 320–350 long.

Caudal bursa usually fully opened, slightly dissymmetrical, with right lobe more or less longer than left lobe and dorsal lobe reduced (Figs. 3, 4). Prebursal papillae visible. Lateral lobes with pattern of type 2-2-1 tending to 1-4. Rays 2 and 3 arising separately, rays 2 shorter and curved inwards, toward median line. Rays 4 and 5 diverging at extremity, rays 4 longer, strongly curved anteriorly and curly distally; rays 5 straight. Rays 4–5 on right lobe diverging more proximally than those on left lobe. Rays 6 diverging from common trunk of rays 2 to 6 at same level as rays 3. Rays 8 thin and long, transversely elongated, arising symmetrically from base of dorsal ray. Dorsal ray short, divided at its proximal third into 2 branches, each one bifurcated distally into 2 papillae, rays 9 (external) and rays 10 (internal) (Fig. 4). Genital cone bulbous, well developed, 32–40 long and 32–35 wide at base. Genital cone with strong internal sclerotization. Telamon triangular, 32–35 long and 20–33 wide. Paired papillae 7 visible on dorsal lip (Fig. 5a). On ventral lip, single projection forming a lappet, easily visible in lateral view (Fig. 5b). Spicules thin, subequal, alate, 185–190 long, ending in simple, pointed tips. SpL/BL 6.0–6.4%. Gubernaculum rather inconspicuous, ovoid to rhomboidal, 11–15 long and 13–25 wide, better observed in dorsal view (Fig. 5a).

Females (measurements correspond to 10 neotypes from the type locality, except as indicated): 3.75–5.55 (4.42) mm long and 90–120 (103) wide at mid-body. Cephalic vesicle 40–65 (57) long and 30–35 (33) wide. Nerve ring situated at 110–180 (157) (n = 9) from apex. Excretory pore and deirids, respectively, situated at 190–310 (356) and 182–310 (255) (n = 9) from apex. Esophagus 315–460 (399) long. Monodelphic. Vulva situated at 55–80 (69) (n = 6) from caudal extremity. Vagina vera 15–20 (18) long (n = 5), vestibule 80–130 (94) long (n = 8), sphincter 25–45 (39) long and 30–50 (45) wide, infundibulum 70–130 (103) (n = 8) (Fig. 6). Proximal part of uterus differentiated (Fig. 7), comparable to a spermatheca, 100–110 (108) (n = 4). Uterus 700–850 (782) long, containing 22–44 (35) eggs, 55–70 long and 30–38 wide. UtL/BL 15.1–21.3% (17.8%). Presence of distal cuticular inflation from level of infundibulum up to level of vulva. Posterior extremity usually not, or barely, invaginated into cuticular inflation (Fig. 6). Tail conical, 18–25 (21) long (n = 7), with 2 lateral cuticular expansions, without struts (Fig. 6).

Table I shows measurements of other males (not types) parasitizing *Ho. chacarius* from Salta province and males and females from the same host from Chaco province.

Synlophe (studied in 4 males and 6 females): In both sexes, cuticle bearing longitudinal, uninterrupted ridges with well developed struts. Ridges appearing gradually posterior to cephalic vesicle and mainly on the left side; disappearing just anterior to bursa in male and reaching distal cuticular inflation in females. Number of ridges: at esophago-intestinal

junction, 17 in male, 18 in female (Figs. 9, 10); at mid-body 19 in both sexes (Figs. 11, 12); within posterior third of BL 19 in male, 18–19 in female (Figs. 13–15). At mid-body: ridges unequal in size, with smallest ridges on ventral right quadrant. Largest ridges: dorsal ridge situated just above the right lateral cord and ridge in the middle of ventral right quadrant. Gradients in ridge size present: dorsally from right to left and ventrally from left to right, starting from the largest ridges. Axis of orientation of the ridges: in males, single axis inclined at about 60° to sagittal axis; in females, double axis of orientation: right axis at 62° and left axis at 74° to sagittal axis. In males, frequently some ridges on dorsal left quadrant oriented nearly perpendicular to body surface. In females, usually all ridges with orientation. Within distal third of BL: in male, ridges subequal in size, with little marked orientation (Fig. 13); in female, at level of proximal uterus, similar number, orientation and size of ridges as at mid-body (Fig. 14). At ovejector level: 18–16 ridges (number diminishing towards distal end), short and stout, subequal in size, with 2 pairs of lateral ridges slightly smaller, mostly perpendicular to body surface (Fig. 15). Most ridges disappear between vulva and anus, at the end of cuticular inflation.

Taxonomic summary

Material studied (host number in parentheses): 2 males and 1 posterior extremity, neotypes, 6923 (CML 5816), and 10 females, neotypes, MLP-He 3443-1 (CML 5549).

Other material deposited (host number in parentheses): 3 males MLP-He 6915 (CML 5825), 1 male MLP-He 6916 (CML 5824), 8 males, 4 females MLP-He 6917 (CNP 3955), 20 males, 8 females MLP-He 6918 (MLP 27.XI. 01.10), 116 males, 132 females MLP-He 6919 (CNP 3958).

Hosts: *Ho. chacarius* Thomas (Rodentia, Cricetidae) CML 5544, CML 5549, CML 5550, CML 5816, CML 5824, CML 5825, CNP 3954, CNP 3955, CNP 3956, CNP 3958, CNP 4615, MLP 27.XI. 01.10.

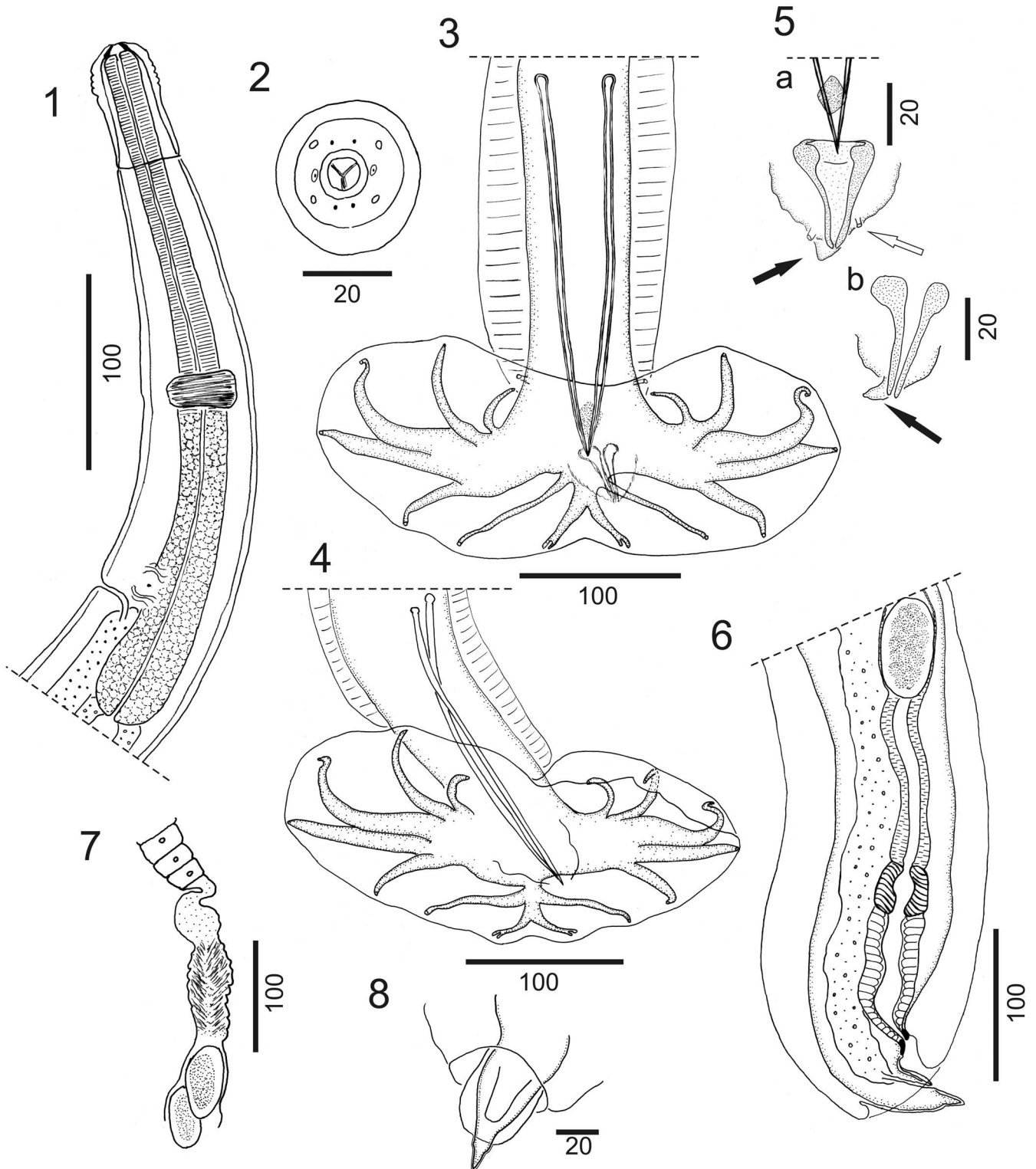
Site of infection: Small intestine.

Localities: Ingenio San Martín de Tabacal (23°16'S, 64°15'W), lote Milagros, Departamento Orán, Salta, Argentina (type locality); Selvas del Río de Oro (23°48'S, 58°58'W), Departamento Libertador General San Martín, Chaco, Argentina.

Remarks

Characters such as the synlophe with 19 unequal ridges with oblique axis of orientation, the presence of a cuticular dilatation in the dorsal left quadrant observed in transverse section, the caudal bursa slightly dissymmetrical, and the genital cone not hypertrophied, allow us to confirm the placement of the specimens in the genus *Hassalstrongylus* Durette-Desset, 1971. The specimens corresponded in most characters with the species described by Freitas et al. (1937) as *He. mazzai*. With respect to the females, the description provided by Freitas et al. (1937) was rather succinct, although only the anterior extremity was illustrated and, in addition, there was not certitude of the authors about the correspondence of males and females, which was expressly remarked upon by them. Anyway, the females observed in the present material do not contradict the description provided, and the measurements are largely coincident. With respect to the males, our specimens match the original description in all body measurements, spicules size and shape, gubernaculum and telamon shape, and shape and pattern of the caudal bursa, including the slight dissymmetry in the lobe size (right lobe larger) and in the divergence of rays 4–5 (more distally on left lobe). The unique difference observed lies in the extremity of left ray 4, which in our specimens is curly and strongly curved forwards (as in the right lobe) whereas in the original illustration it appears nearly straight.

Such dissymmetry of the rays 4 (one strongly curved, the other straight) is not at all frequent among members of *Hassalstrongylus*. The bursal dissymmetry in the Heligmonellinae takes place usually through the transversal (lateral) lengthening of one of the lobes (Durette-Desset and Digiani, 2012). Species of *Hassalstrongylus* show bursae dissymmetrical or slightly dissymmetrical. Examples of dissymmetrical are *Hassalstrongylus aduncus* (Chandler, 1932), *Hassalstrongylus chabaudi* Diaw, 1976, *Hassalstrongylus dollfusi* (Díaz-Ungria, 1963), *Hassalstrongylus echalieri* Diaw, 1976, *Hassalstrongylus epsilon* (Travassos, 1937), *Hassalstrongylus puntanus* Digiani and Durette-Desset, 2003 and *Hassalstrongylus schadi* (Durette-Desset, 1971). However, the possibility of examining several specimens reveals that the degree of enlargement of 1 lobe may vary intraspecifically, as observed in both species studied here.



FIGURES 1–8. *Hassalstrongylus mazzai* (Freitas, Lent, and Almeida, 1937). (1) Male, anterior extremity, left lateral view. (2) Female, head, apical view. (3–5) Male. (3,4) Two caudal bursae, ventral views. (5) Genital cone. (a) Dorsal view, showing telamon, gubernaculum, spicules, ventral lappet (black arrow), and papillae 7 (white arrow). (b) Left lateral view, showing lappet (black arrow) on ventral lip. (6–8) Female. (6) Posterior extremity, right lateral view. (7) Spermatheca. (8) Tail and end of cuticular inflation, ventral view. Scale bars in μm .

TABLE I. Measurements of *Hassalstrongylus mazzai* (voucher specimens) parasitizing *Holochilus chacarius* from Salta and Chaco provinces, Argentina.

Character	Salta sample		Chaco sample	
	♂ (n = 11)	♂ (n = 16)	♂ (n = 16)	♀ (n = 11)
Body length (in mm)	2.38–3.39 (3.11)	2.80–3.60 (3.30)	2.80–3.60 (3.30)	3.75–5.10 (4.30)
Body width	60–100 (79)	78–150 (107)	78–150 (107)	100–150 (120)
Cephalic vesicle long × wide	50–65 (56) × 25–32 (30)	57–70 (65) × 28–35 (33)	57–70 (65) × 28–35 (33)	55–72 (66) × 32–40 (36)
Esophagus length	280–360 (325)	350–385 (362)	350–385 (362)	380–450 (406)
Nerve ring*	125–190 (151)	160–215 (182) (n = 6)	160–215 (182) (n = 6)	150–200 (178) (n = 9)
Excretory pore*	215–270 (242)	265–310 (292) (n = 7)	265–310 (292) (n = 7)	250–305 (280)
Deirids*	230–270 (247)	265–325 (297)	265–325 (297)	250–320 (382)
Spicules length	170–225 (200)	175–220 (201)	175–220 (201)	—
SpL/BL ratio (%)	5.1–7.6 (6.4)	4.9–7.1 (6.1)	4.9–7.1 (6.1)	—
Genital cone long × wide	27–40 (33) × 30–40 (36) (n = 6)	40–45 (44) × 38–50 (44) (n = 6)	40–45 (44) × 38–50 (44) (n = 6)	—
Telamon long × wide	29–37 (34) × 22–28 (25) (n = 6)	25–38 (34) × 15–28 (24) (n = 5)	25–38 (34) × 15–28 (24) (n = 5)	—
Gubernaculum long × wide	12–22 (14) × 10–12 (11) (n = 5)	13–35 (21) × 8–27 (15) (n = 5)	13–35 (21) × 8–27 (15) (n = 5)	—
Vulva†	—	—	—	60–85 (68) (n = 6)
Vagina vera	—	—	—	10–18 (12) (n = 7)
Vestibule	—	—	—	70–95 (82)
Sphincter long × wide	—	—	—	30–35 (33) × 30–45 (39)
Infundibulum	—	—	—	70–125 (106)
Spermatheca	—	—	—	60–180 (101)
Uterus length	—	—	—	570–1,000 (863)
UtL/BL ratio (%)	—	—	—	15.2–22.9 (20)
Tail length	—	—	—	18–33 (27)
Number of eggs	—	—	—	18–43 (32)
Eggs length × width	—	—	—	60–80 × 35–42

* Distance from the anterior extremity.

† Distance from the caudal extremity.

In these species, the little lengthening of 1 lobe usually affects the point of divergence of rays 4–5, which becomes more proximal in the largest lobe. It does not affect the lateral pattern or the shape and curvature of the rays, which remain the same in both lobes. We may assume the dissymmetry of the extremities of the rays 4 in *He. mazzai* as a misinterpretation of the character in the original description. Indeed, if the left bursal margin was folded between rays 3 and 4, the distal extremity of ray 4 is possible to be overlooked, and this latter may appear as shorter and blunt. It is also to be noted that in the original description the bursa was not illustrated fully opened and the number of specimens studied was not specified.

Several facts lead us to identify our specimens as *He. mazzai* described by Freitas et al. (1937). The material comes from the type host species and type locality. Moreover, it was found in several individual hosts, together with the 3 other species (*S. stilesi*, *M. fortuita*, and *H. argentinus*) described as coparasitic by the same authors from only 1 individual, which suggests that the 4 species are frequent and characteristic of this rodent species. Metrical and morphological characters are largely coincident, with the exception of the distal extremity of left ray 4, commented above; but considering that such a dissymmetry of rays 4 is very unlikely, we may assume a misinterpretation of the character in the original description.

A comparison with the type material of *He. mazzai* deposited in the CHIOC was not possible. Only 2 vials were referred as containing specimens of *He. mazzai*: CHIOC no. 9058 with 2 males (types) and CHIOC no. 9064 with 1 female (not referred as type). However, the attempts to find the types in vial no. 9058 were unsuccessful, whereas vial no. 9064 contained 1 broken specimen (Dr. M. Knoff, Curator, pers. comm.). Thus, in the absence of type material which may serve as reference, we designate our specimens as neotypes for the species *H. mazzai*.

***Hassalstrongylus argentinus* (Freitas, Lent, and Almeida, 1937)**
= *Longistriata argentina* Freitas, Lent, and Almeida, 1937
(Table II; Figs. 16–29)

General: Large nematodes, normally uncoiled. Cephalic vesicle present. Excretory pore and deirids at same level, at about 64–89% of esophagus

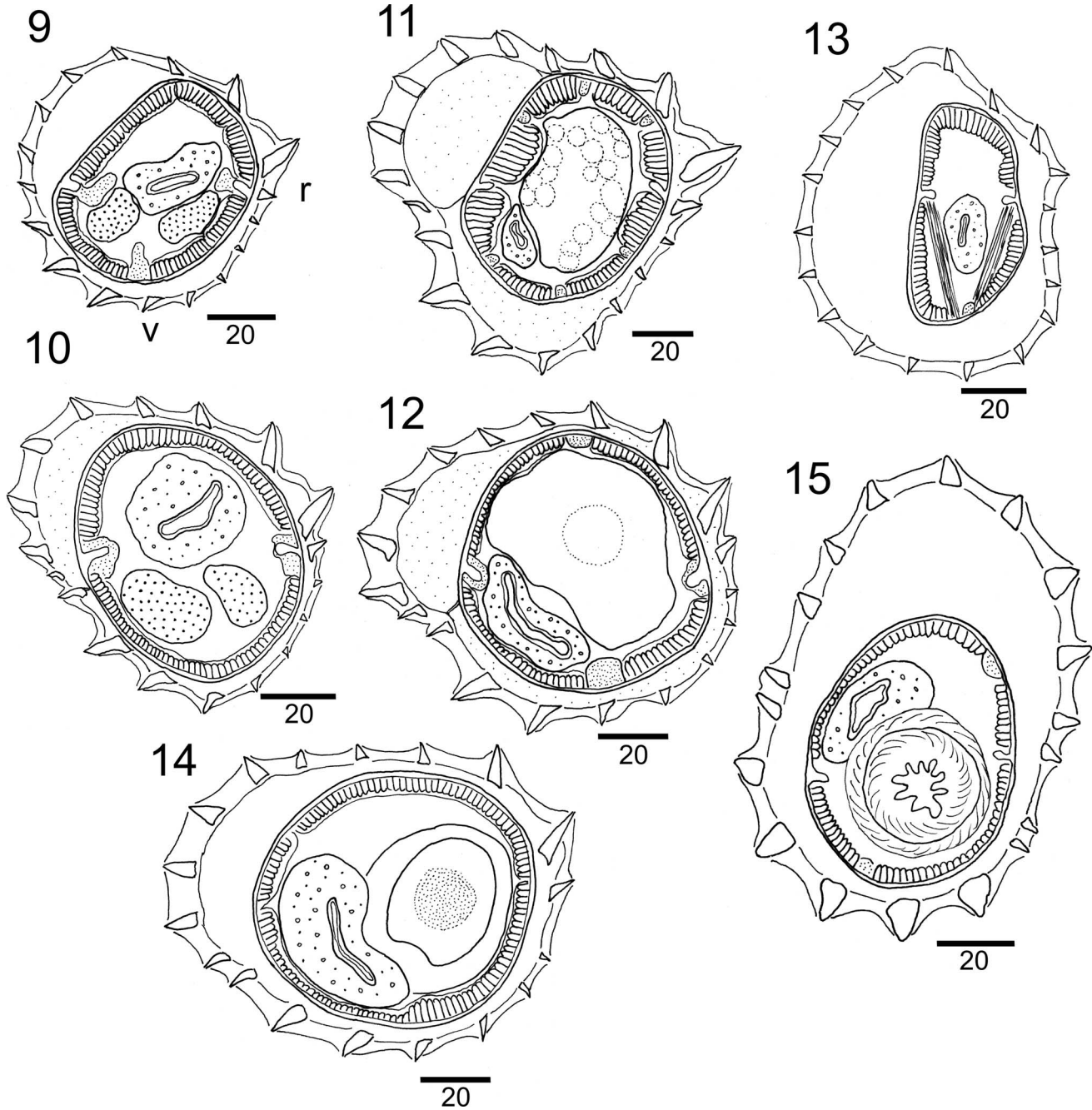
length in males, within 54–81% in females. Nerve ring just anterior to excretory pore and deirids (Fig. 16). In apical view, rounded buccal opening surrounded by thick ring; 2 amphids, 6 externo-labial papillae (2 dorsal, 2 ventral, 2 lateral), and 4 cephalic papillae visible (Fig. 17).

Males: Caudal bursa subsymmetrical or slightly dissymmetrical with right lobe larger and dorsal lobe developed, usually folded ventrally. Prebursal papillae visible (Fig. 18). Pattern of type 2-2-1 in both lobes. Rays 2 and 3 arising separately, rays 2 shorter and thinner than rays 3. Rays 4 and 5 diverging from mid-length, rays 4 longer and curved anteriorly. Rays 6 stout, diverging from common trunk of rays 2 to 6 at same level as rays 3. Rays 8 long, arising symmetrically from base of dorsal ray. Dorsal ray well developed, divided at mid-length into 2 branches, each one bifurcated distally into 2 papillae, rays 9 (external) and rays 10 (internal) (Fig. 18). Genital cone well developed, with strong internal sclerotization. Telamon triangular, with distal accessory piece supporting apically the ventral lip (Fig. 19). Paired papillae 7 observed on dorsal lip, papilla 0 not observed. Gubernaculum ovoid (Fig. 19). Spicules thick, subequal, with spicule tips simple, blunt, bent ventrally at right angle, better visible in lateral view (Fig. 20).

Females: Monodelphic (Fig. 21). Proximal part of uterus differentiated, interpreted by previous authors as a spermatheca (Fig. 22). Distal cuticular inflation from level of infundibulum up to level of vulva. Posterior extremity never completely invaginated into cuticular inflation. Tail conical (Figs. 21, 23).

Table II summarizes the measurements from both males and females parasitizing *Ho. chacarius* from Salta and Chaco provinces.

Synlophes (studied in 2 males and 2 females): In both sexes, cuticle bearing longitudinal, uninterrupted ridges with well developed struts, appearing gradually posterior to cephalic vesicle; disappearing just anterior to bursa in male and reaching distal cuticular inflation in females (Fig. 23). Number of ridges: at esophago-intestinal junction, 15 in male, 19 in female (Figs. 24, 25); at mid-body and within posterior third of BL: 20 in both sexes (Figs. 26, 27). At mid-body, ridges markedly unequal in size: 3 smallest ridges on ventral right quadrant; largest ridges: right dorsal ridge just above right lateral cord and 2 ridges on the ventral left quadrant (Figs. 26, 27); remaining ridges medium-sized. Gradient in ridge size



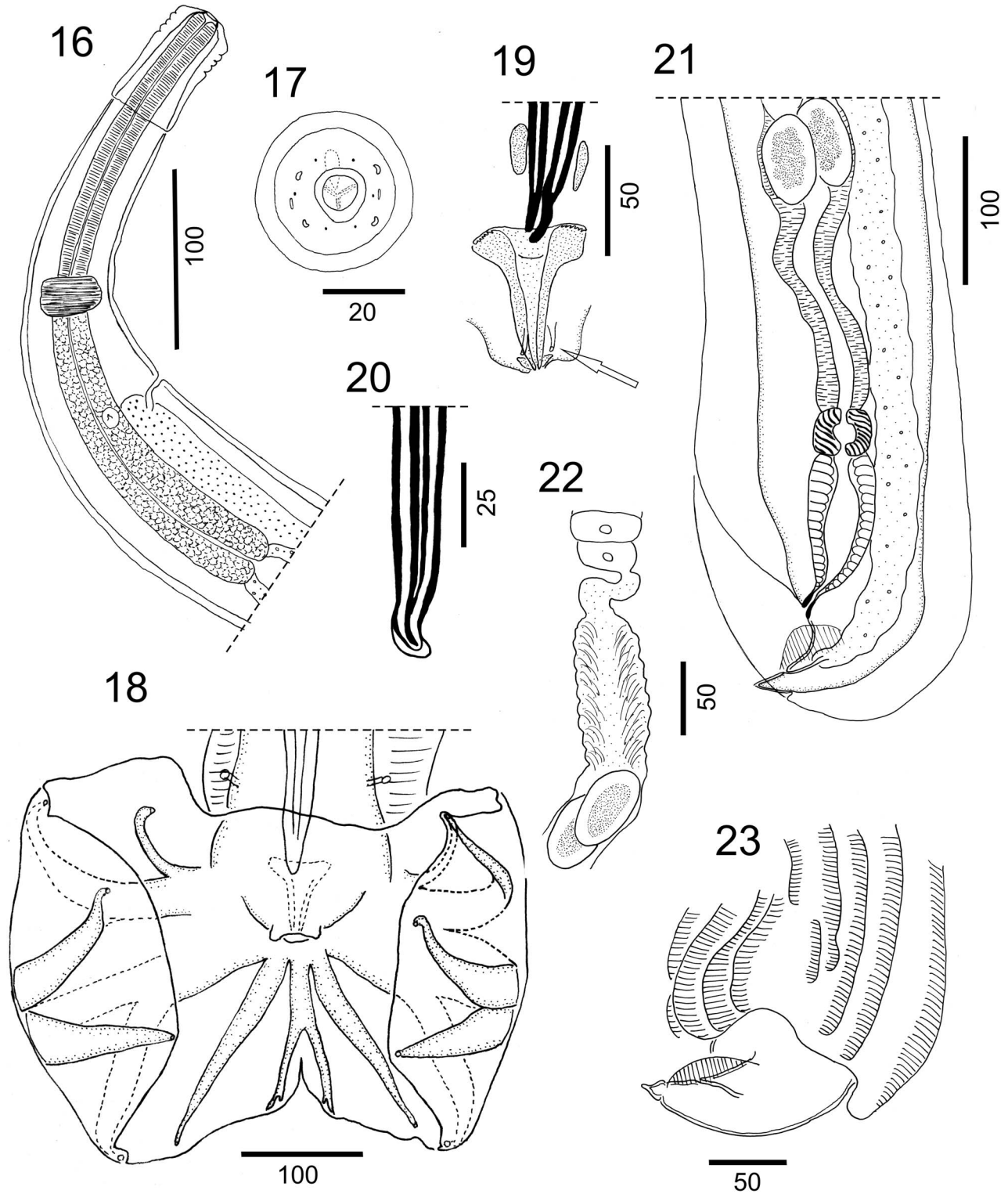
FIGURES 9–15. *Hassalstrongylus mazzai* (Freitas, Lent, and Almeida, 1937). Synlophe in transverse sections of the body. (9, 10) At level of esophago-intestinal junction. (9) Male, distal esophagus. (10) Female, proximal intestine. (11, 12) At mid-body. (11) Male. (12) Female. (13) Male, within distal third of BL. (14) Female, at level of proximal uterus. (15) Female, at ovejector level (vestibule). Abbreviations: r = right; v = ventral. All sections oriented as in Fig. 9. Scale bars in μm .

present dorsally, starting from the largest dorsal ridge to the left. Single axis of orientation of ridges inclined at about 70° to sagittal axis in both sexes. Ridges on dorsal left quadrant with less-marked orientation, nearly perpendicular to body surface. Within distal third of BL: in male, ridges subequal in size, with much-less marked orientation (Fig. 28); in female, at level of proximal uterus, same number, orientation, and size of ridges as at mid-body. At ovejector level: 19 ridges short and stout, mostly perpendicular to body surface, subequal in size, with paired lateral ridges slightly smaller (Fig. 29). Between vulva and anus, at the end of cuticular inflation: right ridges disappearing first, then left ridges, ventral ridges most developed and extending most distally (Fig. 23).

Taxonomic summary

Material deposited (host number in parentheses): 3 males, 2 females MLP-He 3441-2 (CML 5546), 5 males, 5 females MLP-He 6920 (CML 5817), 16 males, 10 females MLP-He 6921 (CNP 3955), 18 males, 45 females MLP-He 6922 (MLP 27.XI.01.10).

Hosts: *Ho. chacarius* Thomas (Rodentia, Cricetidae) CML 5544, CML 5546, CML 5549, CML 5550, CML 5552, CML 5553, CML 5810, CML 5816, CML 5817, CML 5822, CML 5824, CML 5825, CNP 735, CNP 3952, CNP 3954, CNP 3955, CNP 3956, CNP 3958, CNP 3980, CNP 4615, MLP 27.XI.01.10.



FIGURES 16–23. *Hassalstrongylus argentinus* (Freitas, Lent, and Almeida, 1937). (16) Male, anterior extremity, right lateral view. (17) Female, head, apical view. (18–20) Male. (18) Caudal bursa, ventral view. (19) Genital cone, dorsal view showing gubernaculum, spicules, telamon, and papillae 7 (white arrow). (20) Spicules tip, right lateral view. (21–23) Female. (21) Posterior extremity, left lateral view. (22) Spermatheca. (23) Tail and end of cuticular inflation, left lateral view. Scale bars in μm .

TABLE II. Measurements of male and female *Hassalstrongylus argentinus* parasitizing *Holochilus chacarius* from Salta and Chaco provinces, Argentina.

Character	Salta sample		Chaco sample	
	♂ (n = 13)	♀ (n = 13)	♂ (n = 16)	♀ (n = 7)
Body length (in mm)	4.00–5.20 (4.53)	4.90–6.15 (5.53)	4.60–5.80 (5.08)	5.00–7.95 (6.30)
Body width	110–150 (129)	130–210 (163)	90–200 (159)	150–240 (179)
Cephalic vesicle long × wide	50–70 (64) × 32–40 (35)	50–70 (65) × 35–50 (41)	60–80 (71) × 35–42 (40)	55–73 (64) × 42–50 (46)
Esophagus length	360–460 (400)	390–460 (425) (n = 8)	390–470 (428)	430–500 (474)
Nerve ring*	150–195 (180)	130–205 (179)	170–240 (204)	170–207 (185)
Excretory pore*	265–325 (299) (n = 10)	220–340 (296) (n = 10)	270–370 (329)	225–350 (288) (n = 5)
Deirids*	265–325 (305) (n = 7)	220–340 (303) (n = 8)	270–385 (322)	225–350 (302) (n = 5)
Spicules length	420–550 (483)	—	485–530 (481)	—
SpL/BL ratio (%)	9.4–12.9 (10.8)	—	8.6–10.9 (9.5)	—
Genital cone long × wide	45–70 (56) × 50–100 (70)	—	65–70 (68) × 30–52 (40) (n = 5)	—
Telamon long × wide	55–70 (63) × 32–60 (47)	—	55–75 (64) × 15–28 (n = 8)	—
Gubernaculum long × wide	18–38 (25) × 30–40 (34)	—	20–32 (25) × 30–38 (32) (n = 5)	—
Vulva†	—	50–75 (65) (n = 4)	—	60–100 (86) (n = 5)
Vagina vera	—	15–25 (20) (n = 5)	—	12–20 (15) (n = 5)
Vestibule	—	70–130 (98) (n = 8)	—	90–120 (104) (n = 5)
Sphincter long × wide	—	35–50 (42) × 45–55 (50) (n = 7)	—	30–40 (34) (n = 5) × 38–45 (42)
Infundibulum	—	120–180 (156)	—	100–150 (123) (n = 4)
Spermatheca	—	100–130 (107) (n = 8)	—	145–250 (185)
Uterus length	—	800–1,060 (914)	—	770–1,230 (968)
UtL/BL ratio (%)	—	13.7–20.0 (16.6)	—	11.3–17.1 (15.2)
Tail length	—	20–30 (24) (n = 6)	—	20–40 (27) (n = 5)
Number of eggs	—	56–88 (73)	—	50–95 (63)
Eggs length × width	—	50–70 × 25–40	—	50–70 × 32–50

* Distance from the anterior extremity.

† Distance from the caudal extremity.

Site of infection: Small intestine.

Localities: Ingenio San Martín de Tabacal (23°16'S, 64°15'W), lote Milagros, Departamento Orán, Salta, Argentina; Selvas del Río de Oro (23°48'S, 58°58'W), Departamento Libertador General San Martín, Chaco, Argentina.

Remarks

Our specimens correspond largely with the original description made by Freitas et al. (1937) and with the redescription, including the synlophe, made by Durette-Desset (1968) on 1 male type. Correspondence was found in body measurements of males and females, shape and pattern of the caudal bursa, and also on very specific characters such as the spicule shape, spicule tips, telamon, and presence of a conspicuous spermatheca in the females. In the synlophe, the relative size of the ridges and their orientation correspond to the description by Durette-Desset (1968), who reported 19 ridges in male and 21 in female at mid-body, assuming that the difference with the 20 ridges found in both sexes in our material may well correspond to slight variations in the body diameter. We complete the previous descriptions providing measurements of males and females from hosts of 2 different populations and illustrations of the synlophe at 3 different levels of the BL in both sexes.

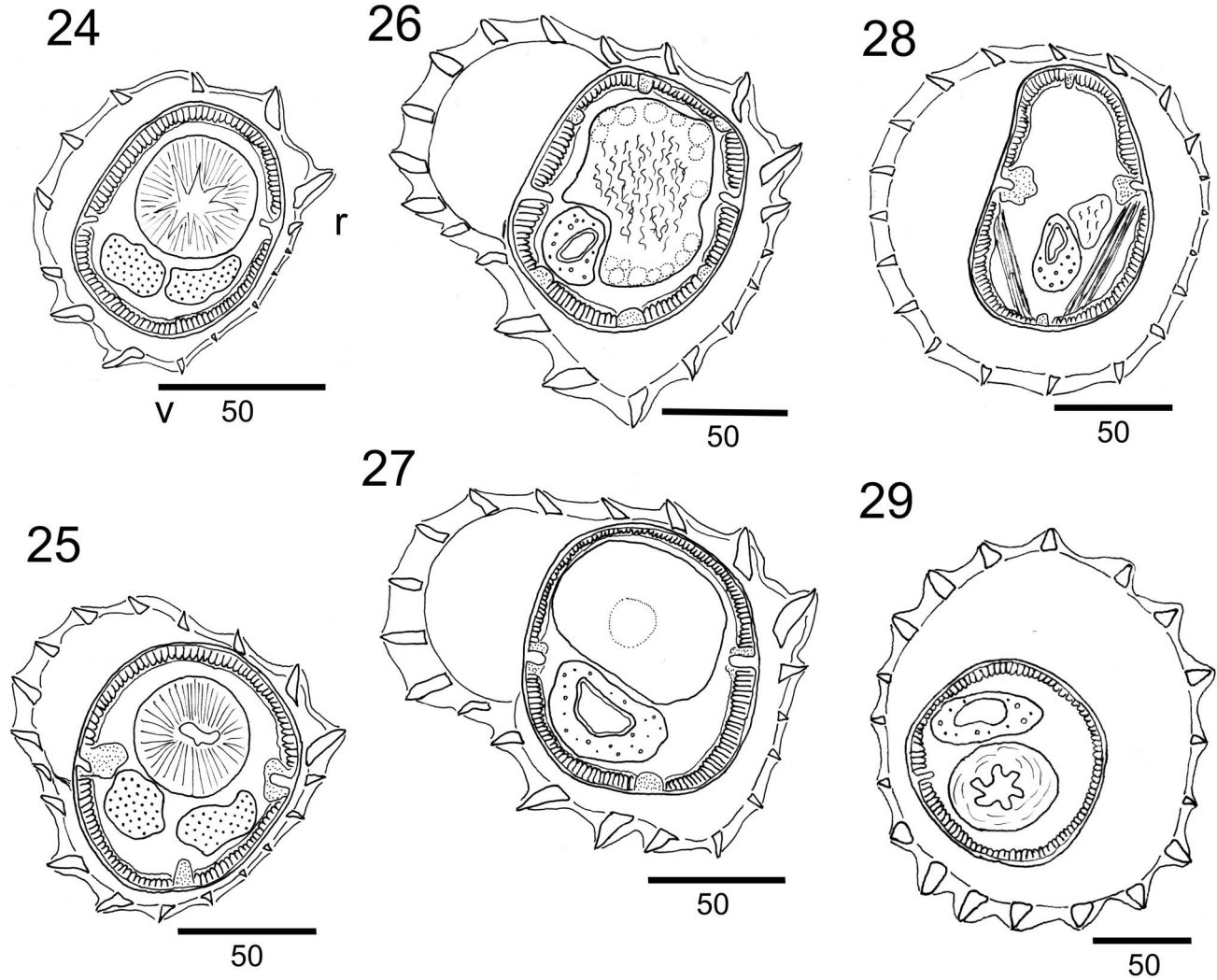
Multivariate analysis

Medians of the 3 selected female characters were statistically significant (LT [*mazzai* (n = 28) versus *argentinus* (n = 19)]: Mann–Whitney $U = 10.5$, $z = -5.5$, $P = 0.000$; Egg no. [*mazzai* (n = 28) versus *argentinus* (n = 19)]: Mann–Whitney $U = 8.5$, $z = -5.57$, $P = 0.000$; and UtL [*mazzai* (n = 28) versus *argentinus* (n = 19)]: Mann–Whitney $U = 109.5$, $z = -3.36$, $P = 0.0007$). The PCA showed that the first 2 principal components account for the 77.7 and 16.1% of the total variance, respectively. PCA loadings are shown in Table III. The loadings of this first component are all positive, confirming its correlation. This axis can discriminate smaller individuals (BL) and uterus lengths (UtL) and fewer numbers of eggs on the left. Thus, CP1 separates a group of females corresponding to *H.*

argentinus on the right and a group of females belonging to *H. mazzai* on the left (Fig. 30). We found no apparent biological explanation for the principal components 2 and 3.

DISCUSSION

Hassalstrongylus mazzai and *H. argentinus* frequently coexist in the same individual host and have synlophes almost identical. The body size, pattern and shape of caudal bursa, ratio SpL/BL, and the size and shape of spicules and genital cone easily differentiated males of both species. The females, on the contrary, did not show significant qualitative differences. This is not surprising since among the Heligmonellidae, the females are morphologically very homogeneous and rarely provide characters useful for classification below the family level. The synlophe is then an extraordinary tool allowing the matching of males and females in the case of coparasitic species (Durette-Desset, 1971, 1985; Digiani et al., 2013). However, synlophes having numerous or subequal ridges normally provide a lesser number of characters. Furthermore, they are more susceptible to variation in function of the body diameter (i.e., species of *Stilestrongylus* Freitas, Lent, and Almeida, 1937, *Guerrerostrongylus* Sutton and Durette-Desset, 1991, most *Hassalstrongylus*, among the Neotropical forms). Such homogeneity of the synlophes does make the specific discrimination much more difficult. For example, *Hassalstrongylus musculi* (Dikmans, 1935), *Hassalstrongylus forresteri* Durette-Desset, 1974, and *Hassalstrongylus lichtenfelsi* Durette-Desset, 1974 display synlophes with 22–25 similar ridges and are coparasitic in *Oryzomys palustris* (Harlan) from Florida. They are distinguished from each other based on a few characters of the males.



FIGURES 24–29. *Hassalstrongylus argentinus* (Freitas, Lent, and Almeida, 1937). Synlophe in transverse sections of the body. (24, 25) At level of esophago-intestinal junction. (24) Male, distal esophagus. (25) Female, proximal intestine. (26, 27) At mid-body. (26) Male. (27) Female. (28) Male, within distal third of BL. (29) Female, at ovejector level (vestibule). Abbreviations: r = right; v = ventral. All sections oriented as in Fig. 24. Scale bars in μm .

However, the females of the 3 species remain indistinguishable (Durette-Desset, 1972, 1974; Kinsella, 1988).

Roughly, the females of *H. mazzai* were smaller than those of *H. argentinus*, although their measurement ranges may eventually overlap (BL in *H. mazzai* 3.75–5.55 vs. 4.90–7.95 in *H. argentinus*). The unique character that rarely overlapped was the egg number; this was the case almost without exception <50 (mean = 32) in *H. mazzai* and ≥ 50 in *H. argentinus* (mean = 70), even including females of both species with similar BL and UtL/

BL ratio. This was confirmed in the PCA, where 2 groups could be clearly discriminated, proving that females from *H. mazzai* are smaller and possess fewer eggs than does *H. argentinus* (Fig. 30). The egg numbers seems then to be an unexpected but useful character in the differential diagnosis of females from both species.

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TABLE III. PCA loadings of the first 3 axes.

Character*	Axis 1	Axis 2	Axis 3
BL	0.6103	-0.2283	-0.7585
Egg no.	0.5855	-0.515	0.6261
UtL	0.5335	0.8262	0.1807

* BL = body length; UtL = uterus length.

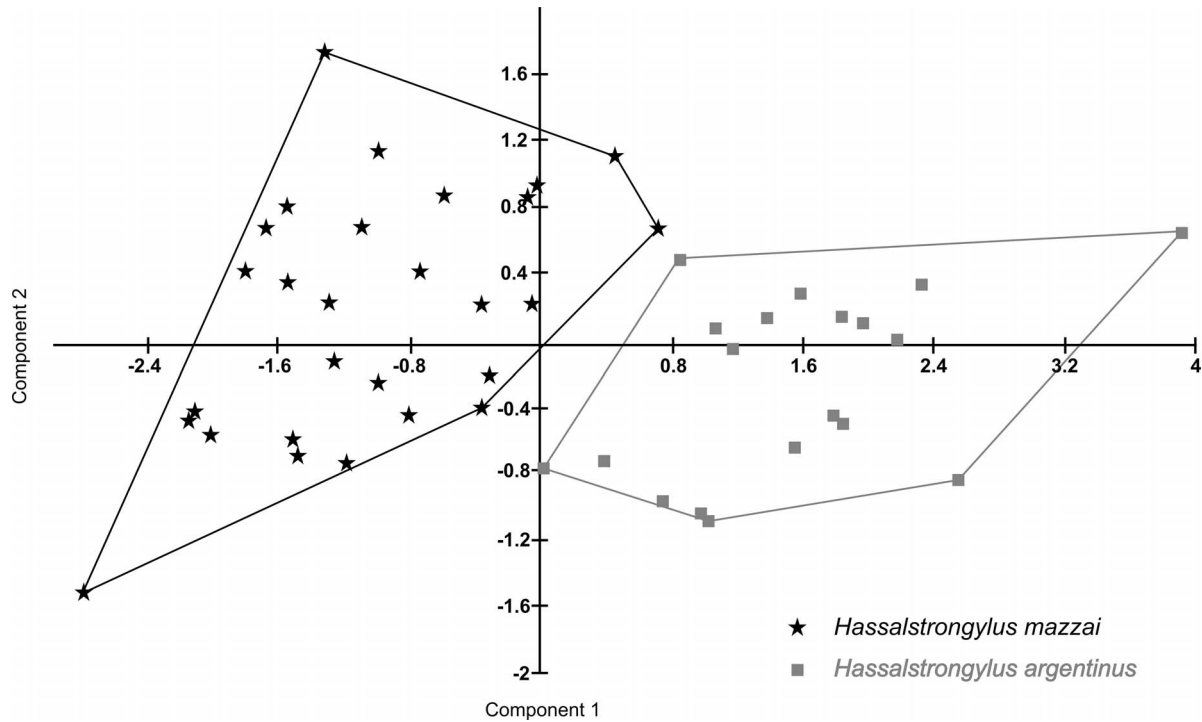


FIGURE 30. Principal components analysis of morphometrical characters of females from *Hassalstrongylus mazzai* and *Hassalstrongylus argentinus* parasitic in the marsh rat *Holochilus chacarius*. First and second components.

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