

## TWO NEW SPECIES OF *LITOMOSOIDES* (NEMATODA: FILARIOIDEA) IN SIGMODONTINES (RODENTIA: MURIDAE) FROM RIO DE LA PLATA MARSHLAND, ARGENTINA

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**ABSTRACT:** Two new species of coelomic filarioid belonging to *Litomosoides* are described from sigmodontine murids from the Rio de La Plata marshland, Argentina. *Litomosoides bonaerensis* n. sp., a parasite of *Oligoryzomys delticola*, belongs to the *carinii* group and is close to *L. silvai*, which differs by the head and tail papillae, buccal capsule and cavity, area rugosa, and morphology of the microfilaria. *Litomosoides oxymycteri* n. sp., from *Oxymycterus rufus*, belongs to the *sigmodontis* group. Differential diagnosis is based on the morphology of the buccal capsule, the head and tail papillae, and microfilaria. The ectoparasitic gamasid *Ornithonyssus bacoti*, in which several *Litomosoides* species develop, has been recovered from sigmodontines trapped in the study.

The Rio de La Plata marshland represents an ecotonal area between the subtropical and pampasic ecological zones of Argentina. This area constitutes the Galería Uruguayense woodland, with few subtropical features (Ringuelet, 1962). The mammalian fauna of the area is composed of numerous species of sigmodontine rodents. These rodents are frequent in stands of tall grass in marshes, rivers, and streams and in drier parts of marshy areas among clumps of pampas grass (*Cortaderia selloana*), away from the flood plain (Barlow, 1969).

Two species of filarioid nematodes have been found in the thoracic and abdominal cavities of sigmodontine rodents. They belong to the genus *Litomosoides* Chandler, 1931 (Onchocercinae), which is characterized by a long sclerotized buccal capsule (Anderson and Bain, 1976). Species of this genus have a large host spectrum, including bats, marsupials, and various groups of rodents, suggesting host switching (Bain et al., 1980; Bain and Phillip, 1991). Thirty species are now listed, half of which have been identified during the past 25 yr (Esslinger, 1973; Padilha and Faria, 1977; Bain et al., 1980, 1989; Muller, 1980; Gardner and Schmidt, 1986; Brant and Gardner, 1997; Moraes Neto et al., 1997). A few species are Nearctic, whereas others are Neotropical occurring in Bolivia, Colombia, Venezuela, Brazil, and Argentina. In Argentina only, *L. patersoni* (Mazza, 1928) has been reported from the sigmodontine *Holochilus vulpinus* by Mazza (1928).

### MATERIALS AND METHODS

In 1995, seasonal samples of murid rodents were collected from Hudson (34°45'S, 58°06'W) and Punta Lara (34°47'S, 58°01'W), in the province of Buenos Aires, Argentina. The rodents were captured alive in Kuns-Massoia traps baited with oiled bread, killed with ether, and frozen until their viscera were examined. The thoracic and abdominal cavities were checked for parasites. In 1998, blood samples were taken from live rodents. The described filarioids were recovered from *Oligoryzomys delticola* (Thomas, 1917), which is endemic to the Paraná Delta (Wilson and Reeder, 1993), and *Oxymycterus rufus* (Fischer, 1814), which has a large distribution in tall grass and strands of bunch grass in wet meadows and marshes in Uruguay, Brazil, and Argentina (Barlow, 1969).

Parasites were preserved in 70% ethanol and cleared in lactophenol for light microscopy. The lateral cuticular internal alae of the worms,

which reached almost to the anterior extremity, enable us to orient the head in lateral and median views; in apical view, the dorsal edge is recognized by the transverse Y-shaped section of the esophageal lumen. The nerve ring may be inconspicuous. In females, a cross section was made at the level of the vulva. Spicules of 1 or 2 males of each species were dissected to determine their morphological characteristics. Microfilariae were obtained from the uteri of fixed mature females. Blood samples from live-trapped hosts were fixed in 2% aqueous formalin. The blood was concentrated, and the sediment was stained with methylene blue and examined on slides. Illustrations were made with the aid of a drawing tube. Measurements are presented in the following order: holotype, male paratypes, allotype, and female paratypes. If more than 2 paratypes were examined, mean values and standard deviations are presented with ranges in parentheses. Measurements are given in micrometers, unless otherwise stated.

We examined specimens of *L. silvai* Padilha and Faria, 1977 collected by Moraes Neto et al. (1996) and *L. chagasfilhoi* Moraes Neto, Lanfredi and De Souza, 1997 (respective numbers 49 SE and 161 SE, Muséum National d'Histoire Naturelle [MNHN] collections, Paris).

### DESCRIPTION

#### *Litomosoides bonaerensis* n. sp.

(Figs. 1A–F, 2A–F, 3A–H)

**General description** (based on 11 females and 3 males): Onchocercidae (Leiper, 1911) Chabaud and Anderson, 1959, Onchocercinae (Leiper, 1911), *Litomosoides* Chandler, 1931. Cephalic extremity attenuated. Mouth small. Four externolabial papillae forming a rectangle stretched dorsoventrally, joined in sagittal plane, 2 smaller dorsal cephalic papillae, not far from amphids; amphidial canals conspicuous. Buccal cavity regularly tubular, asymmetrical, wider posteriorly in some female specimens. Buccal capsule with anterior segment transparent, the posterior parts strongly cuticularized; thickened ring at midlength with posterior edge directed backwards. Esophagus undivided or with glandular portion slightly differentiated.

**Male:** Width reduced anterior to thickened coiled region; area rugosa beginning anterior to cloaca, composed of transverse ridges of small longitudinal crests. Four or 5 pairs of postcloacal papillae along tail, papillae of third pair close to the median longitudinal ventral line; unpaired precloacal papilla; subterminal parallel phasmids. Left spicule with handle longer than lamina; lamina with sclerotized axis, 2 narrow alae, its distal part membranous (observed after dissection). Right spicule heavily sclerotized, distal 1/5 part with membranous edges; dorsal heel; terminal cap forming dorsal hook, with bifid membrane at top.

**Measurements:** Holotype: length 21 mm; width 150; buccal capsule 16 long and maximum diameter 10; esophagus 520 long; tail 180 long; left spicule 230 long with handle 140 long, right spicule 106 long; area rugosa 1,870 long, beginning 3,070 and extending to 1,200 from tip of tail, with ridges about 70 long, distance between 2 consecutive ridges about 7. Paratypes (n = 2): length 18.69–19 mm; width 147–170; buccal capsule 18–21 long and maximum diameter 6–10; nerve ring 160–240 from apex; esophagus 642 long; tail 132–150 long; left spicule 183–235 long with handle 114–145 long; right spicule 90–102 long;

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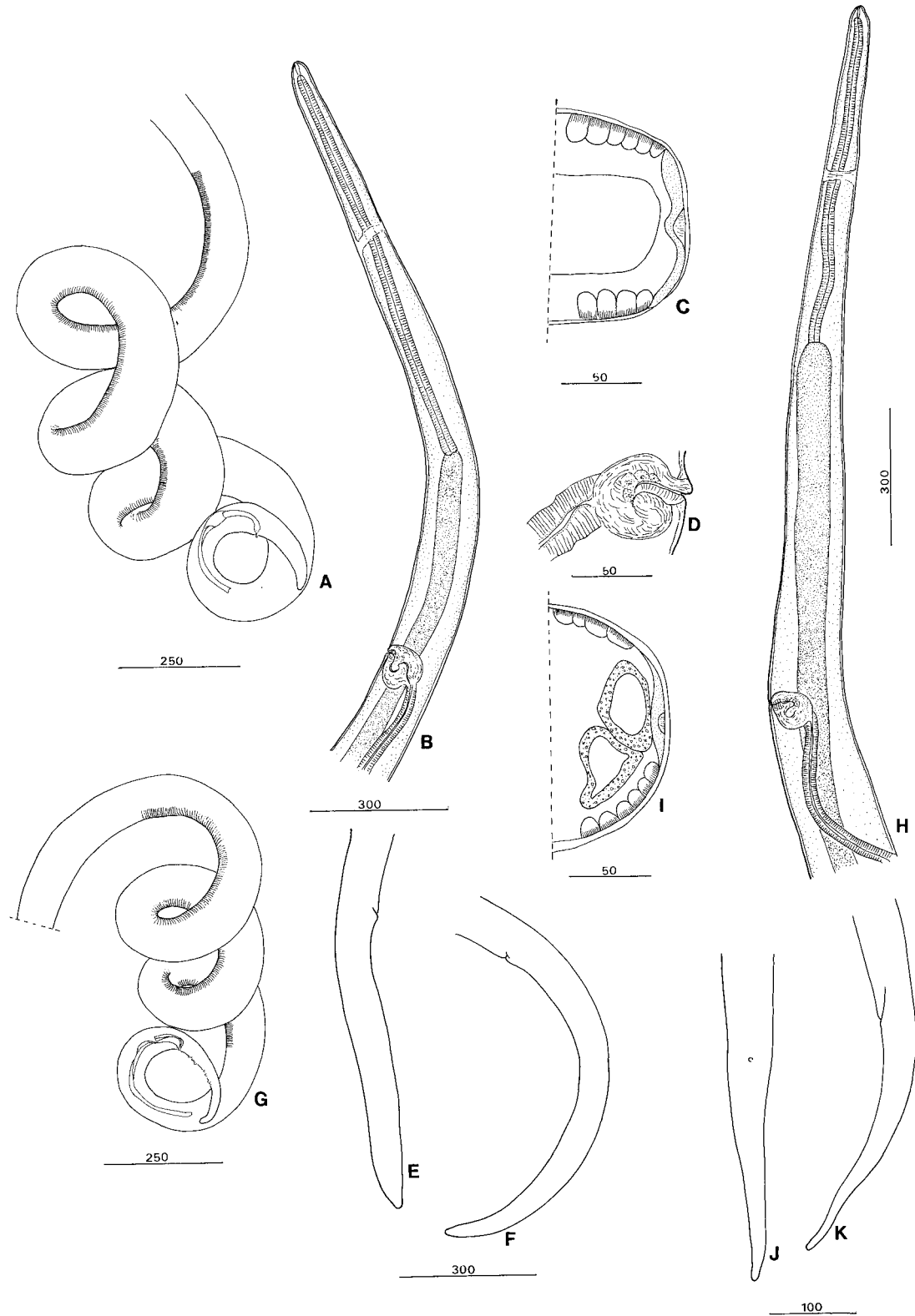


FIGURE 1. **A–F.** *Litomosoides bonaerensis* n. sp.; **G–K.** *Litomosoides oxymyteri* n. sp. **A.** Posterior region of male, lateral view. **B.** Anterior region of female, lateral view. **C.** Cross section at the level of the vulva. **D.** Vagina. **E, F.** Paratype and allotype female tails, lateral view. **G.** Posterior region of male. **H.** Anterior region of female, lateral view. **I.** Cross section at the level of the vulva. **J, K.** Paratype female tail, lateral and ventral views.

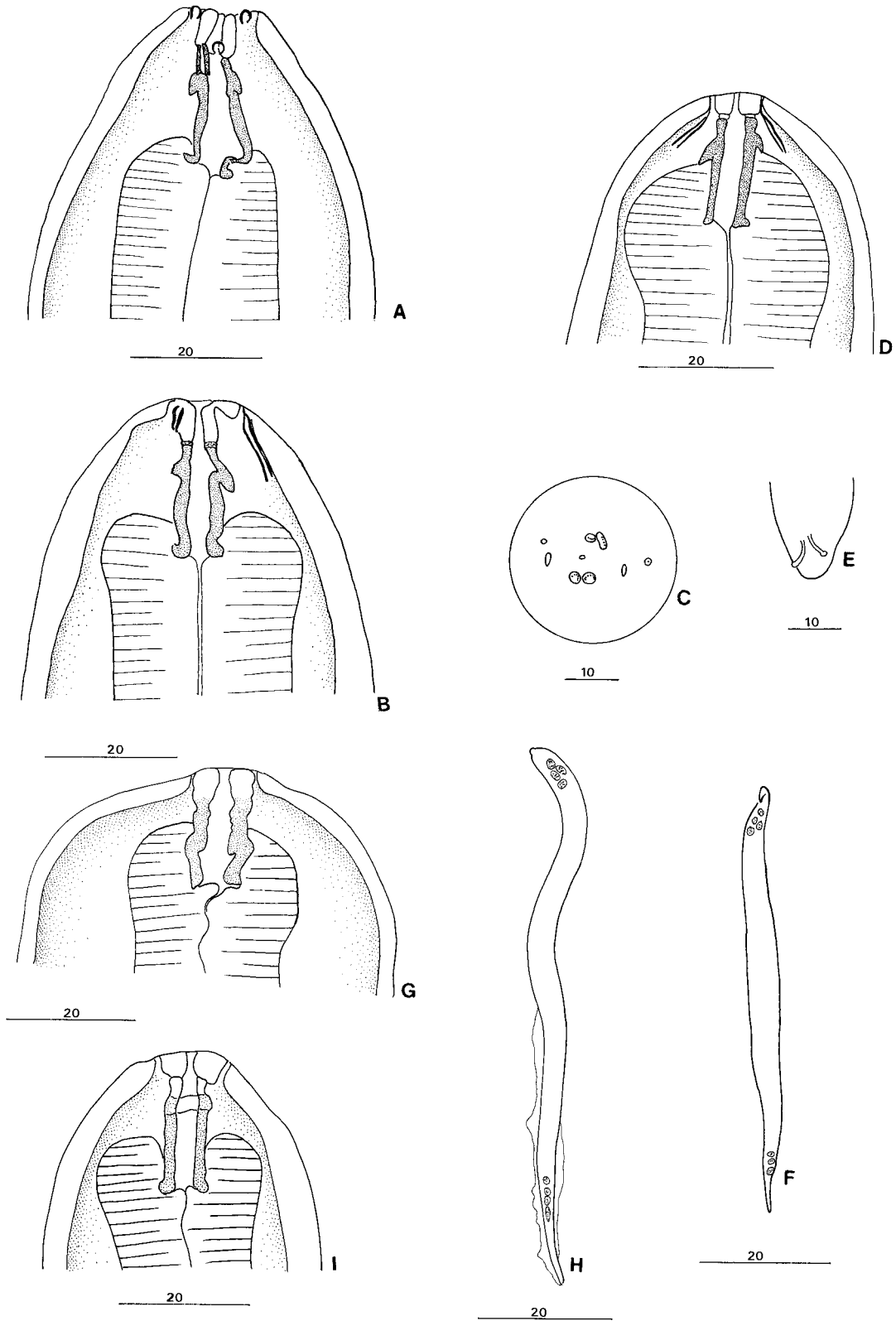


FIGURE 2. **A–F.** *Litomosoides bonaerensis* n. sp. **G, H.** *Litomosoides silvai* Padilha and Faria, 1977. **I.** *Litomosoides chagasfilhoi* Moraes Neto, Lanfredi and De Souza, 1997. **A–C.** Female head, lateral, median, and apical views. **D.** Buccal capsule of male, median view. **E.** Distal end of female tail. **F.** Uterine microfilaria. **G.** Buccal capsule of female, lateral view. **H.** Uterine microfilaria. **I.** Buccal capsule of male, median view.

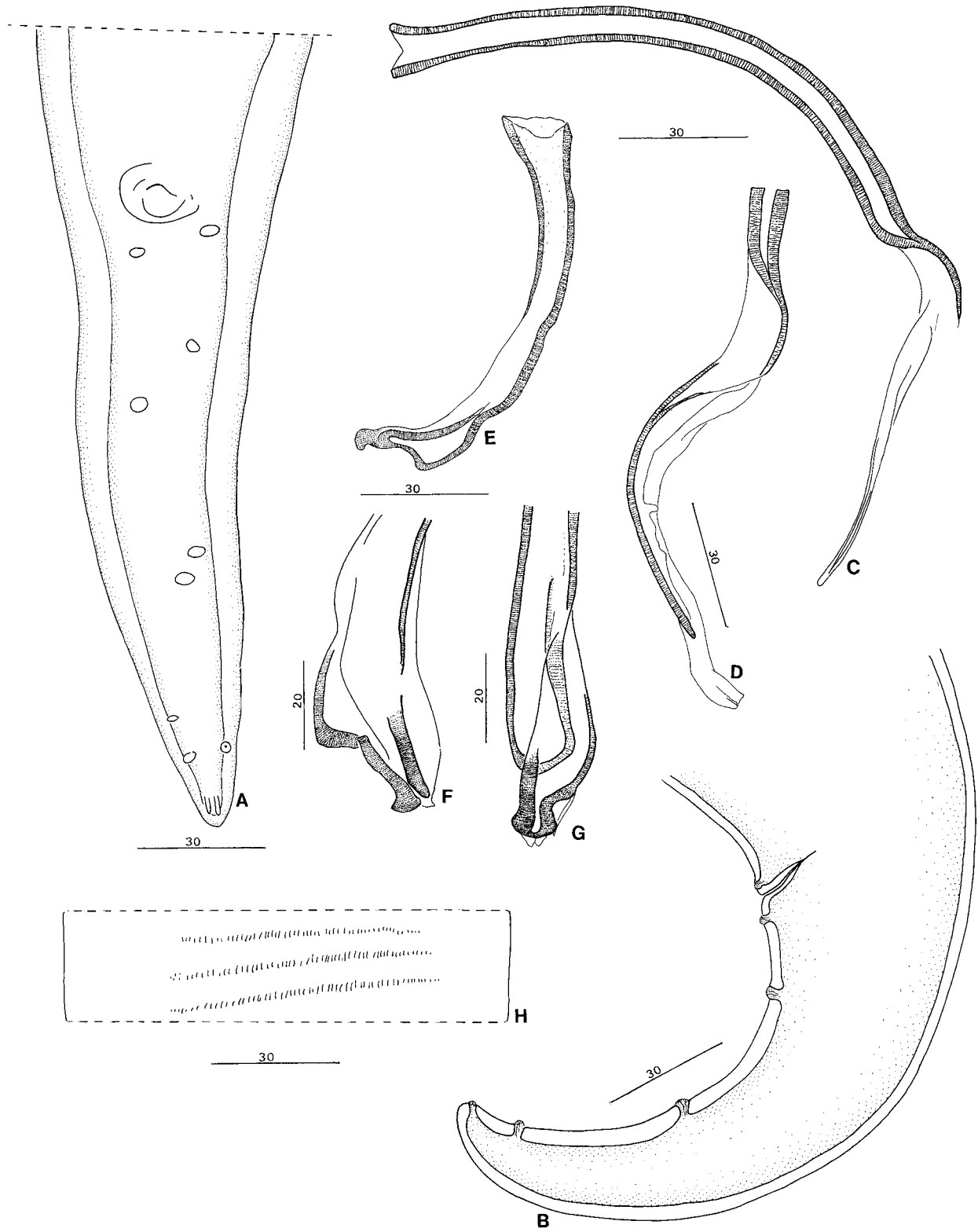


FIGURE 3. *Litomosoides bonaerensis* n. sp. male paratype. **A, B.** Male tail, ventral and lateral views. **C.** Left spicule. **D.** Left spicule, lamina after dissection. **E.** Right spicule, lateral view. **F, G.** Distal extremity in lateral and dorsal views, after dissection. **H.** Area rugosa at midlength, ventral view.

area rugosa 1,200–1,250 long, beginning 2,250–2,300 and extending to 1,000–1,100 from tip of tail.

**Female:** Vulva generally posterior to esophagus–intestine junction; vagina globular. Tail curved ventrally or straight, with subterminal, parallel or divergent phasmids. In cross section near level of vulva (Fig. 1C), lateral chords broad, internal cuticular ridges semicircular.

Measurements: allotype: length 74 mm; width 250; buccal capsule 23 long and external diameter 8; nerve ring 430 from apex; esophagus 980 long; vulva 1,500 from apex; tail 800 long. Paratypes (n = 10): length  $68.8 \pm 5.3$  (62–78) mm; width  $242 \pm 22.4$  (210–282); buccal capsule  $23.5 \pm 1.3$  (21–25) long and maximum diameter  $10 \pm 0.6$  (9.3–10.6); nerve ring  $481 \pm 99.4$  (315–649) from apex; esophagus  $716.3 \pm 124.8$  (591–943) long; vulva  $1,756.6 \pm 218.6$  (1,482–2,173) from apex; tail  $618.5 \pm 107$  (453–746) long.

**Microfilariae:** Sheath present, only observed on blood smears; body fusiform; salient cephalic hook; thin caudal extremity without nuclei. Uterine microfilariae from female allotype (n = 5):  $71 \pm 4.3$  (66–76) long; all specimens 3.5  $\mu$ m wide.

### Taxonomic summary

**Type host:** *Oligoryzomys delticola* (Thomas, 1917) (Muridae). Deposited at the Museo de La Plata, Argentina, Mammalogy Collection number 08.IV.97.77.

**Site of infection:** Abdominal cavity.

**Type locality:** Hudson (34°45'S, 58°06'W), Buenos Aires province, Argentina.

**Specimens deposited:** Holotype, allotype, 2 paratypes, MNHN collections, Paris 312 ES; 10 paratypes Museo de La Plata, Helminthological Collection, Argentina (CHMLP) 4610.

**Prevalence:** In 1995, 8/10 (80%) *O. delticola* from Hudson were parasitized.

**Etymology:** The specific name is derived from the name Buenos Aires province, where the hosts were collected.

### DIAGNOSIS

These specimens belong to the *carinii* group of *Litomosoides* as defined by Bain et al. (1989): right spicule sclerotized until its distal extremity and with a terminal cap; lamina of left spicule simple, without large membranous longitudinally folded anterior alae. Eight species, parasites of bats, marsupials, and rodents, belong to this group.

Four species, parasitic in bats, are distinct from *L. bonaerensis* n. sp.: *L. guiterasi* (Viguera, 1934), redescribed by Sandground (1934), Rego (1961), and Esslinger (1973), is smaller, with a tail about 3 times as short in both sexes (respectively, 121–130  $\mu$ m in females, 50–59  $\mu$ m in males) and truncated in female worms. It has 2 or 3 pairs of cloacal papillae, vulva at level of esophagus, shorter microfilariae with the last nucleus at tip of tail. *Litomosoides brasiliensis* Lins de Almeida, 1936 is larger (Rego, 1961; Esslinger, 1973), with 3 or 4 pairs of cloacal papillae aligned closely to the median longitudinal ventral line (Diaz-Ungria, 1963). The spicules are twice as long, the right spicule with a strong heel and a large cap forming a ventral subterminal hook in addition to the strong dorsal hook. The microfilaria is longer (mean length = 90  $\mu$ m; Esslinger, 1973) and more attenuated posteriorly. *Litomosoides molossi* Esslinger, 1973 is much smaller, except for the buccal capsule. The posterior region of the female has an ornamentation made up of minute lateral “papillae” and small caudal lappets. There are no cloacal papillae present along the male tail, and the microfilaria is longer (mean = 92  $\mu$ m), with the last nucleus at the tip of the tail. *Litomosoides chandleri* Esslinger, 1973 is similar to *L. molossi* in being small, having caudal lappets, having lateral ornamentation on the female, and having a microfilaria with a nucleated tail. In addition, it has a wider buccal cavity than *L. bonaerensis* n. sp., the vulva is at the level of the esophagus, there are 3 pairs of cloacal papillae, the heel of the right spicule is closer to the cap, and the tail of the microfilaria is not attenuated.

The single species parasitic in marsupials, *L. petteri* Bain, Petit and Berteaux, 1980, differs from *L. bonaerensis* n. sp. in having the vulva at level of the esophagus, 4 cephalic papillae symmetrically located, 3 pairs of cloacal papillae, and a longer membranous distal part on the left spicule (equal in length to the sclerotized anterior part of the lamina).

Three species parasitic in rodents differ from *L. bonaerensis* n. sp. *Litomosoides carinii* (Travassos, 1919), from *Sciurus* sp. (Sciuridae) in

Brazil, has a shorter buccal capsule (13–16  $\mu$ m) with a relatively wider buccal cavity. The coiled posterior part of the male is not inflated, and the area rugosa is narrower and the ridges are relatively more spaced out. *Litomosoides scotti* Forrester and Kinsella, 1977, from the sigmodontine *Oryzomys palustris* in Florida, has a shorter buccal capsule (13  $\mu$ m) with a larger ring located at midlength, protruding amphids, and thickenings of the cephalic cuticle (Bain et al., 1989). *Litomosoides silvai* Padilha and Faria, 1977, from the sigmodontine *Akodon arviculoides* in Rio de Janeiro, is the species most similar to our specimens. It has been redescribed from the same host species by Moraes Neto et al. (1996), and we examined some of these specimens. The buccal cavity is not regularly tubular but instead has 2 short enlargements. The buccal capsule has no distinct thick ring (Fig. 2G). The female tail is abruptly constricted anterior to its extremity, the coiled posterior region of the male is not inflated, and the area rugosa ridges are narrower. The membranous extremity of the left spicule is longer, as are the microfilariae (Fig. 2H).

Four *Litomosoides* species cannot be placed in the *carinii* group or in the *sigmodontis* group because of the absence of males. These species, parasites of bats, differ from *L. bonaerensis* n. sp. *Litomosoides artibeii* Esslinger, 1973 has a buccal capsule without a ring, a more posterior vulva, and a microfilaria with a longer, thinner posterior extremity and terminal nucleus. The microfilariae of *L. colombiensis* Esslinger, 1973 display a terminal elongated nucleus. Those of *L. caliensis* Esslinger, 1973 are short (53–65  $\mu$ m) with a round terminal nucleus. *Litomosoides* sp. Chitwood, 1938 has a different buccal capsule with thin regular walls.

*Litomosoides bonaerensis* n. sp. was also found in *Oligoryzomys* sp. from Punta Lara locality (prevalence of 1/6 individuals = 16.6%).

### *Litomosoides oxymycteri* n. sp.

(Figs. 1G–K, 4A–L)

**General description** (based on 13 females and 5 males): Cephalic extremity and papillae as presented in Figure 4C (2 cephalic papillae are ventral). Amphids salient. Buccal cavity tubular. Buccal capsule with anterior segment transparent, thickened ring at midlength with posterior edge pointed backwards. Esophagus undivided. Tail well attenuated in both sexes.

**Male:** Five pairs of cloacal papillae, pair 3 is more ventral than lateral and papillae of pair 4 joined on the median longitudinal ventral line; unpaired precloacal papilla. Small phasmids. Left spicule with handle as long as lamina, membranous longitudinally folded alae at anterior half of lamina; right spicule not heavily sclerotized, with membranous distal extremity supported by 2 thin parallel rods.

Measurements: Holotype: length 23.3 mm; width 160; buccal capsule 20 long and external diameter 8; nerve ring 360 from apex; esophagus 750 long; tail 240 long; left spicule 340 long; right spicule 90 long; area rugosa 1,500 long, beginning at 2,250 and extending to 750 from tip of tail, with ridges about 30 long, distance between 2 consecutive ridges about 15. Paratypes (n = 4): length  $19.3 \pm 3.3$  (15.6–23.6); width  $148 \pm 9$  (138–159); buccal capsule  $21.1 \pm 2$  (18–22.6) long and external diameter  $9.3 \pm 1.7$  (7–10.6); nerve ring  $372.4 \pm 30$  (351–394) from apex; esophagus  $733.4 \pm 186.8$  (532–980) long; left spicule  $293.4 \pm 43.5$  (231–327) long with the handle  $133.8 \pm 19.2$  (111–146) long; right spicule  $111.1 \pm 38.8$  (80–167) long; tail  $205.7 \pm 19.6$  (186–230) long; area rugosa  $1,635.5 \pm 39.6$  (1,599–1,678.9) long, beginning at  $2,371.2 \pm 98.8$  (2,239–2,476) and extending to  $739 \pm 76.2$  (640–800) from tip of tail.

**Female:** Allotype: length 70.5 mm; width 275; buccal capsule 21, with external diameter 8; esophagus 700 long; vulva 1,250 from apex; tail 295 long. Paratypes (n = 12): length  $64.4 \pm 9.8$  (43–70.8); width  $269.8 \pm 16.5$  (250–295); buccal capsule  $20.5 \pm 2$  (17–23.9) long; external diameter  $8.5 \pm 1$  (7–10.6); nerve ring  $450.1 \pm 33.4$  (400–508) from apex; esophagus  $826.6 \pm 138.8$  (518.7–1,023.3) long; vulva  $1,265.9 \pm 174.2$  (1,071.3–1,650) from apex; tail  $416.4 \pm 116.9$  (292.6–590) long.

**Microfilariae:** Sheathed; body fusiform; salient cephalic hook; attenuated anucleated posterior extremity. Measurements of uterine microfilariae from female allotype (n = 5): length  $91.2 \pm 12.7$  (75–103); width 3–5.

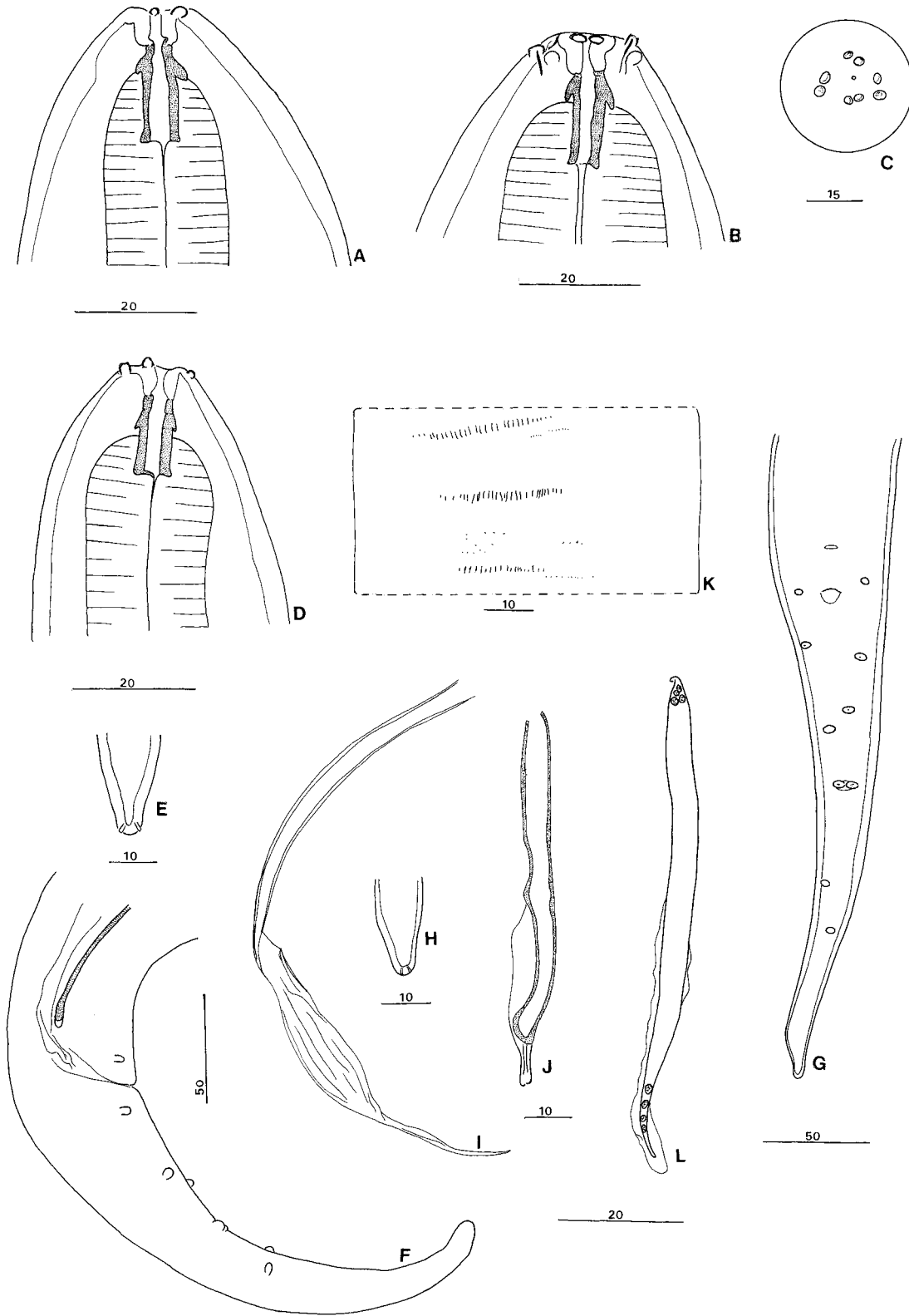


FIGURE 4. *Litomosoides oxymyteri* n. sp. paratypes. **A–C**. Female head, lateral, median, and apical views. **D**. Male head, lateral view. **E**. Caudal extremity of female, ventral view. **F, G**. Male tail, lateral and ventral views. **H**. Caudal extremity of male, ventral view. **I**. Left spicule, lateral view. **J**. Right spicule, dorsal view after dissection. **K**. Area rugosa at midlength, ventral view. **L**. Uterine microfilaria.

### Taxonomic summary

*Type host:* *Oxymycterus rufus* (Fischer, 1814).

*Site of infection:* Thoracic and abdominal cavity.

*Type locality:* Punta Lara (34°47'S, 58°01'W), Buenos Aires, Argentina.

*Specimens deposited:* Holotype, allotype, 2 paratypes, MNHN collections, Paris 313 ES; 16 paratypes Museo de La Plata, Helminthological collections, Argentina (MPHC) 4611.

*Prevalence:* In 1995, 12/15 (80%) *O. rufus* from Punta Lara were infected.

*Etymology:* Named in reference to the host.

### DIAGNOSIS

This material belongs to the *sigmodontis* group (Bain et al., 1989) based on the morphology of the right and left spicules. Fifteen species parasitic in bats, marsupials, and rodents belong to this group.

Four species parasitic in bats differ from *L. oxymycteri* n. sp. *Litomosoides hamletti* Sandground, 1934 has a shorter body, tail about 5 times as short in both sexes, and preesophageal vulva. *Litomosoides leonilavasquezae* Caballero, 1939 has a male tail half as long, a shorter left spicule (209–229 µm), and a right spicule with an elongated distal part (Caballero, 1944). *Litomosoides fosteri* Caballero, 1947 has a buccal capsule with 2 thickened rings, a shorter female body, and a male with a shorter tail and right spicule. *Litomosoides teshi* Esslinger, 1973 has a buccal capsule irregularly and asymmetrically thickened, and the sheath of the microfilaria is very tight posteriorly.

The unique species parasitic in marsupials, *L. barretti* Muller, 1980, differs from *L. oxymycteri* n. sp. in having no heel on the right spicule and its extremity attenuated, not "calciform or spatulate". It has only 4 head papillae and no pair of cloacal papillae joined on the median longitudinal ventral line, and the microfilaria is shorter.

Ten species parasitic in rodents differ from *L. oxymycteri* n. sp. *Litomosoides ctenomyos* Brant and Gardner, 1997, a parasite of Ctenomyidae, has a thick buccal capsule without a distinct ring and no head papillae. *Litomosoides hoplomyis* Esslinger, 1973, from Echimyidae has a body half as long with a more attenuated tail in both sexes and slightly shorter microfilaria. In sigmodontines, *L. legerae* Bain, Petit and Berteaux, 1980 has 4 externolabial papillae and 4 cephalic papillae symmetrically placed, salient amphids, and a thicker and irregular buccal capsule. *Litomosoides sigmodontis* Chandler, 1931 has a thicker and irregular buccal capsule. *Litomosoides galizai* Bain, Petit and Diagne, 1989 has a longer buccal capsule and a reduced number of head papillae, and the lateral chords are not broader at the level of the vulva. *Litomosoides chagasfilhoi* Moraes Neto, Lanfredi and De Souza, 1997 has a thinner ring (Fig. 2I) and slightly longer microfilaria. *Litomosoides esslingeri* (Esslinger, 1973) has a shorter microfilaria. These last 2 species display in cross section thin and narrow lateral chords. In addition, none of the last 5 species in which the male tail was studied from the ventral view display a cloacal pair of papillae joined on the median longitudinal ventral line. *Litomosoides kohnae* Bain, Petit and Diagne, 1989 has a buccal capsule with a ring that does not protrude backwards, a more posterior vulva, and smaller microfilariae (55–62 µm). In *L. patersoni* (Mazza, 1928) the female has a curved anterior extremity, a sharp pointed tail, and smaller spicules (125 and 50 µm); *L. circularis* (Linstow, 1899) has a longer right spicule and a shorter left spicule (190 and 260 µm).

The 4 species parasitic in bats and for which the male is unknown also differ from *L. oxymycteri* n. sp. in the morphology of the buccal capsule and the microfilaria.

*Litomosoides oxymycteri* n. sp. was also found in the abdominal and thoracic cavity of *Oxymycterus rufus* in Hudson (prevalence of 14/30 = 46.6%).

### DISCUSSION

The 2 groups of *Litomosoides* species, *carinii* and *sigmodontis*, are recognized in the sympatric sigmodontines *O. delticola* and *O. rufus*. The 2 new species of *Litomosoides* are morphologically evolved as shown by the particularities of the sensory apparatus. The cephalic papillae are reduced in number

(2 instead of 4), the labial papillae tend to join in the sagittal plane, and the caudal papillae tend to join on the median longitudinal ventral line, as observed in some other species (Diaz-Ungria, 1963; Bain et al., 1989).

The ectoparasitic gamasid *Ornithonyssus bacoti* has been shown to transmit, naturally or experimentally, several species of the *carinii* and *sigmodontis* groups (Forrester and Kinsella, 1973; Bain et al., 1980; Diagne et al., 1989). This gamasid is present on various species of murid rodents in Argentina (Lareschi, 1996; Lareschi and Mauri, 1998), and it could be a vector of the new filarioids, as suggested by its recovery from *O. delticola* and *O. rufus* trapped in this study (Lareschi, pers. comm.).

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