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# New species of *Filaria* (Nematoda: Filariidae) in the Lesser Grison *Galictis cuja* (Molina) (Carnivora: Mustelidae) from northern Patagonia, Argentina and comments about the South American species of the genus

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**Abstract:** *Filaria lorenzo* n. sp. is described in the Lesser Grison, *Galictis cuja* from northern Patagonia, Argentina. The new species can be differentiated from the eight species of *Filaria* from the Old World by the shape of the sclerotized preesophageal ring. The four remaining species of the genus parasitize American Mephitidae and/or Mustelidae. *Filaria carvalhoi* from Brazil and *F. texensis* from USA lack a preesophageal ring, whereas *F. taxideae* from USA and *F. conepati* from Argentina share with our specimens a preesophageal ring mostly mushroom shaped. Our specimens differ from *F. taxideae* by lacking lateral alae, by a different ornamentation of the female tail and by the male lacking adcloacal papillae. The new species also differs from *F. conepati* by the shape of the preesophageal ring and by the shape of the eggs. The indirect examination of the types of *F. conepati* and *F. carvalhoi*, corroborated the existence of differences between these two species, allowing us to refute their synonymy, as proposed by some authors. Our results extend the taxonomy of *Filaria* to 13 species and comprise the first report of a filarioid nematode for an Argentinean mustelid, and the first report for Patagonia.

**Key words:** Argentina, *Filaria*, Lesser Grison, Mustelidae, Patagonia, parasites.

## INTRODUCTION

The Lesser Grison, *Galictis cuja* (Molina) (Carnivora: Mustelidae) is distributed from southern Peru, western Bolivia and E and S Brazil to Paraguay, Uruguay, Argentina and central and southern Chile (Yensen & Tarifa 2003). In Argentina, it reaches the southern end of Santa Cruz province (Prevosti & Travaini 2005, Chébez et al. 2014). The Lesser Grison occupies a wide variety of habitats, including shrub and herbaceous steppes, pastures, scrublands, savannahs, open forests, forests and humid forests and marine coasts from the sea level up to 4200 masl (Larivière & Jennings 2009).

Helminthes parasites of the Lesser Grison reported from Argentina are very scarce: only *Aonchotheca putorii* (Rudolphi, 1819) López-Neyra 1947 (Nematoda: Capillariidae) from Entre Ríos province (Moleón et al. 2015) was recorded. Other parasites from this host were reported in Paraguay and Brazil: the digenean *Platynosomum illiciens* (Braun, 1901) (Dicrocoeliidae), the nematodes *Dioctophyme renale* (Goeze, 1782) (Dioctophymatidae), *Molineus elegans* (Travassos, 1921) (Trichostrongylidae), *Physaloptera* sp. (Physalopteridae),

*Strongyloides* sp. (Strongyloididae), *Lagochilascaris* sp. (Ascaridae), *Gnathostoma* sp. (Gnathostomatidae), *Cruzia* sp. (Kathlaniidae), *Dirofilaria* sp. (Onchocercidae), *Crenosoma brasiliense* Vieira, Pereira, Lima, Neto, Gonçalves & Luque, 2012 (Metastrongylidae), Oxyuridae gen. et sp., Trichostrongylidae gen. et sp. and the acanthocephalan *Pachysentis gethi* (Machado, 1950) (Oligacanthorhynchidae) (Vieira et al. 2008, Corrêa et al. 2016).

The genus *Filaria* Mueller, 1787 includes large parasites of subcutaneous tissues of mammals. The taxonomy of this nematode has been subjected to disputes among parasitologists with several species of *Filaria* reported from different host species (Otranto et al. 2007). *Filaria martis* Gmelin, 1790 was fixed by Stiles (1907) as the type-species, and the genus comprises at present 12 valid species (Table I), of which the last four were described by Chabaud & Mohammad (1989).

In South America, *Filaria patersoni* Mazza, 1928 was reported from Argentina but it was then transferred to *Litomosoides* Chandler, 1931 by Chitwood (1933). This species is a parasite of the peritoneal cavity of *Holochilus brasiliensis* Desmarest (Rodentia: Cricetidae) (Mazza 1928, Chitwood 1933, Gomes et al. 2003) and was recently redescribed by Notarnicola et al. (2010). *Filaria kitti* Seldmeier, 1931 was reported, without an adequate description, from the rodent *Myocastor coypus* (Molina) imported into Europe probably of Argentinean origin. The original description of the parasite was barely enlarged by Grieder (1937, 1938) but there are no accompanying illustrations and no reference to type material is made. *Filaria serpicula* Molin, 1858 was described from Brazil in *Carollia brevicauda* Wied-Neuwied (=*Phyllostoma brevicaudum*), and *Sturnira lilium* Geoffroy (=*Phyllostoma spiculatum*) (Chiroptera: Phyllostomidae) but it was transferred by Guerrero et al. (2002) to the genus *Litomosoides* (Molin 1858, Guerrero et al. 2002). *Filaria conepati* Schuurmans-Stekhoven, 1952 was described in *Conepatus chinga* (Molina) (Carnivora: Mephitidae) from Formosa province in Argentina (Schuurmans-Stekhoven 1952) although it was later considered by some authors as a synonym of *Filaria carvalhoi* Freitas & Lent, 1937, a species described in the same host from Brazil (Anderson 1960, Chabaud & Mohammad 1989).

In this paper we describe a new species of *Filaria* from the subcutaneous tissue of *Galictis cuja* from Patagonia using light and scanning electron microscopy. In addition, the taxonomy of *F. conepati* and *F. carvalhoi* is clarified by reexamining the original type specimens.

## MATERIALS AND METHODS

Four specimens of Lesser Grison road-killed were collected, dissected and examined for helminths: two males near the city of Sierra Grande on National Route N°3 (41° 29.3'S; 65° 21.2'W), Río Negro province; and two females from Provincial Route N°2 between El Desempeño and Istmo Ameghino (42° 28.5'S; 64° 41.4'W), Chubut province, Argentina. The corpses were kept frozen and then necropsied. The skin of the specimens was completely removed and the nematodes found in the subcutaneous tissues were removed. Some nematodes were fixed in 4% formalin/distillated water and preserved and stored in 70% ethanol. Specimens designated for morphological study were cleared in lactoglycerol for examination under light microscope (Leica DM500 and/or Leica DM2500) (Leica, Wetzlar, Germany). Measurements of body length, given in millimeters (mm), were taken under a stereoscope microscope (Leica Wild M3Z with drawing attachment Wild 308700). Other measurements (as range, followed by

**Table I. Species of *Filaria*, their hosts (Order: Family), site of infection and geographical distribution.**

| Species | Hosts | Site | Distribution | References |
|---|---|---|---|---|
| *Filaria martis* Gmelin, 1790 | Carnivora: Mustelidae, Felidae | ST | Europe, Africa, Mexico | Caballero (1948); Anderson (1960); Tadros (1964); Chabaud & Mohammad (1989); Otranto et al. (2007); Torres et al. (2016) |
| *Filaria carvalhoi* Freitas & Lent, 1937 | Carnivora: Mephitidae | ST | Brazil | Freitas and Lent (1937); Chandler (1947); Chabaud & Mohammad (1989) |
| *Filaria hyracis* (Ortlepp, 1937) | Hyracoidea: Procaviidae | SM and F | South Africa | Ortlepp (1937); Chabaud and Rousselot (1956); Chabaud & Mohammad (1989) |
| *Filaria texensis* Chandler, 1947 | Carnivora: Mephitidae | S | USA | Chandler (1947); Chabaud & Mohammad (1989) |
| *Filaria conepati* Schuurmans-Stekhoven, 1952 | Carnivora: Mephitidae | N, E | Argentina | Schuurmans-Stekhoven (1952); Chabaud & Mohammad (1989) |
| *Filaria cephalophi* Chabaud & Rousselot, 1956 | Cetartiodactyla: Bovidae | ST | Gabonese Republic | Chabaud and Rousselot (1956); Chabaud et al. (1978); Chabaud & Mohammad (1989) |
| *Filaria russelli* Tadros, 1964 | Carnivora: Felidae | ST | Kenya | Tadros (1964); Chabaud & Mohammad (1989) |
| *Filaria taxideae* Keppner, 1969 | Carnivora: Mustelidae, Mephitidae | ST | USA | Keppner (1969); Chabaud & Mohammad (1989) |
| *Filaria bakerhugoti* Chabaud & Mohammad, 1989 | Rodentia: Hystricidae | ST | South Africa | Chabaud & Mohammad (1989) |
| *Filaria latala* Chabaud & Mohammad, 1989 | Carnivora: Felidae | NM | South Africa | Chabaud & Mohammad (1989) |
| *Filaria melis* Chabaud & Mohammad, 1989 | Carnivora: Mustelidae | ST | Irak | Chabaud & Mohammad (1989) |
| *Filaria versterae* Chabaud & Mohammad, 1989 | Rodentia: Pedetidae | NM | South Africa | Chabaud & Mohammad (1989) |
| *Filaria lorenzo* n. sp. | Carnivora: Mustelidae | ST | Argentina | This study |

**Abbreviations: E, ear; F, fascia; N, neck; NM, not mentioned; S, skin; SM, superficial muscle; ST, subcutaneous tissues.**

mean in parentheses) are given in micrometers (μm) and were calculated with software of Leica DM500. Photographs of mounted specimens were taken with a Leica ICC50W camera with software connected to the microscope. Specimens intended for scanning electron microscopy study (SEM) were dehydrated in ethanol, dried by the critical point technique, coated with gold and examined in a Jeol 6360 LV microscope (Jeol, Tokyo, Japan). Photographs of the type material of *Filaria conepati* were requested to the Helminthological Collection

of the Instituto de Invertebrados, Fundación Miguel Lillo (FML-II), Tucumán province, Argentina (FML- 6/VIII/47-Lote 202). Photographs of the type material of *Filaria carvalhoi* were requested to the Helminthological Collection of Instituto Oswaldo Cruz (CHIOC), Rio de Janeiro, Brazil (CHIOC 9511 a-d, 9512 paratypes). Type specimens of *Filaria lorenzo* n. sp. were deposited in the Helminthological Collection of the Museo de La Plata (MLP-He), La Plata, Argentina. Host specimens were deposited in the Colección Ecológica de Vertebrados de Ecosistemas Continentales (CEVEC) of the Instituto Patagónico para el Estudio de los Ecosistemas Continentales- CONICET, Puerto Madryn, Chubut province. The classification of the nematodes above the genus level follows Anderson & Bain (1977) and Gibbons (2010).

## RESULTS

Superfamily Filarioidea Weinland, 1858

**Family Filariidae (Weinland, 1858) Cobbold, 1879**

**Subfamily Filariinae Weinland, 1858**

**Genus *Filaria* Mueller, 1787**

**Description**

***Filaria lorenzo* n. sp.** (Figs. 1-2)

Based on 2 entire males and 4 broken females from Río Negro province, plus 1 entire female from Península Valdés, Chubut province.

***General.*** Adult specimens whitish in life. Body long and slender, anterior end blunt. Lateral alae absent. Anterior end with four submedian cephalic papillae, two amphids and four small internal labial papillae surrounding the mouth (Figs. 1a, 2a). Buccal cavity reduced, tubular with thin walls, surrounded at base by thick, refractory preesophageal ring, mushroom shaped, embedded in anterior end of muscular esophagus (Figs. 1b, 1g). Esophagus divided into short anterior muscular part and long glandular part; junction between two parts often not discerned. Deirids spinous, close to anterior extremity.

***Male holotype***. Body length 65.5 mm. Maximum width 302. Muscular part of esophagus 570 long by 76 wide; glandular part more than 1,222 long by 112 wide, posterior end indistinct. Preesophageal ring 22 long by 23 wide (Fig. 1g). Nerve ring 102, deirids 162 from anterior end (Fig. 1g). Excretory pore not discerned. Posterior extremity somewhat attenuated with long and narrow caudal alae. Caudal alae 4,055 long by 37 wide (Fig. 1i). Cloacal opening 221 from posterior end. Eight pairs caudal papillae. Two pairs precloacal, six pairs postcloacal (Fig. 1i). Phasmids not observed. Spicules markedly dissimilar in size and morphology. Right spicule 134 long, curved ventrally (Fig. 1h). Left spicule 696 long, consisting of long tubular calomus, 314 long by 13 wide, and broad, membranous lamina; lamina 316 long, 8 wide; membrane 194 long by 26 maximum wide (Fig. 1j). Gubernaculum present (Figs. 1h, 1i).

***Male paratype*** (n=1). Body length 68 mm. Maximum width 331. Muscular and glandular parts of esophagus not discerned. Preesophageal ring 26 long by 24 wide. Nerve ring 68, deirids 123 from anterior end. Excretory pore not discerned. Caudal alae 5,552 long by 46 wide (Fig. 1i). Cloacal opening 137 from posterior end. Right spicule 147 long, curved ventrally. Left spicule 570 long, calomus 238 long by 17 wide, and broad, membranous lamina; lamina 316 long, 6 wide; membrane 252 long by 24 maximum wide. Gubernaculum present.

***Female*** (n = 4, broken at different levels. Four proximal fragments 118-152 mm in length, maximum width 404-440 (421). Muscular part of esophagus 651-907 (820, n=4) long by 44-121 (75, n=4) wide; glandular part 1,490-2,109 (n=2) long by 95-195 (150, n=4) wide, posterior end

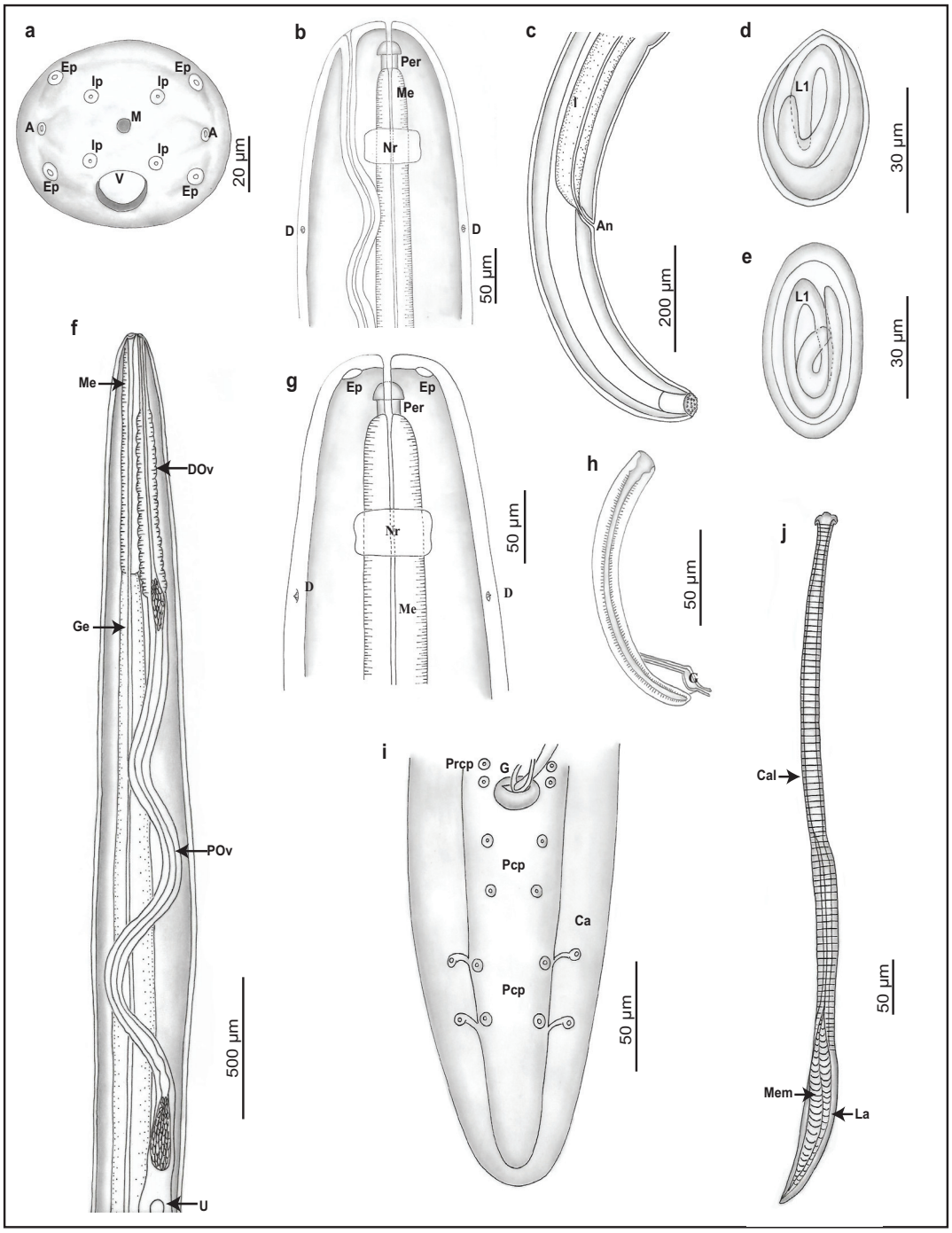


**Figure 1.** Line drawings of *Filaria lorenzo* n. sp. (Nematoda: Filariidae) in the Lesser Grison *Galictis cuja* (Molina) from Patagonia, Argentina. Scale values are given beside each bar. **(a)** Female head, apical view. **(b)** Detail of anterior end of female, left lateral view. **(c)** Posterior end of female, right lateral view. **(d)** Embryonated egg in uterus. **(e)** Embryonated egg in ovejector. **(f)** Anterior end of female, right lateral view. **(g)** Detail of anterior end of male, ventral view. **(h)** Right spicule, lateral view. **(i)** Posterior end of male, ventral view. **(j)** Left spicule, lateral view. **Abbreviations:** A, amphid; An, anus; Ca, caudal ala; Cal, calomus; D, deirid; DOv, distal ovejector; Ep, external papilla (cephalic); G, gubernaculum; Ge, glandular esophagus; I, intestine; Ip, internal papilla (labial); L1, First stage larva; La, lamina; M, mouth; Me, muscular esophagus; Mem, membrane; Nr, nerve ring; Per, pre-esophageal ring; POv, proximal ovejector; Prcp, pre-cloacal papillae; Pcp, post-cloacal papillae; U, uterus; V, vulva.

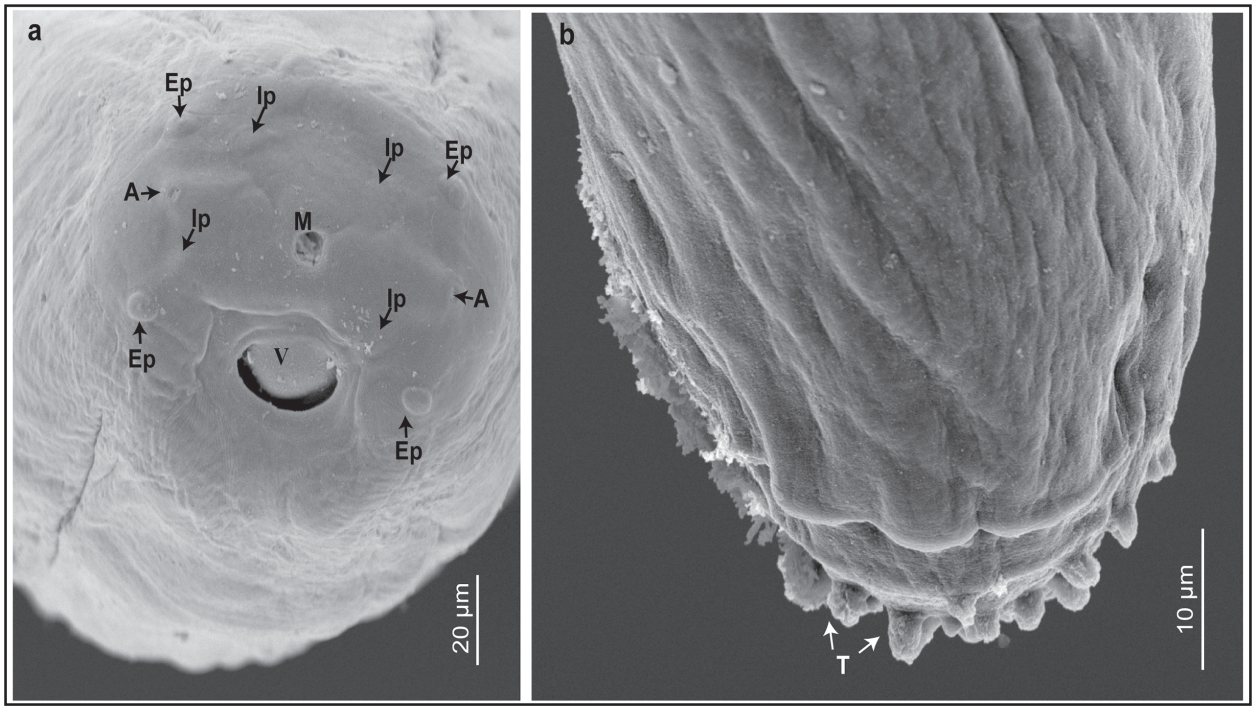


**Figure 2. Scanning electron micrographs of *Filaria lorenzo* n. sp. (Nematoda: Filariidae) in the Lesser Grison *Galictis cuja* (Molina) from Patagonia, Argentina. Scale values are given beside each bar. (a) Female head, subapical view. (b) Posterior end of female, detail of tubercles. Abbreviations: A, amphid; Ep, external papilla (cephalic); Ip, internal papilla (labial); M, mouth; T, tubercles; V, vulva.**

indistinct. Preesophageal ring 20-27 (24, n=3) long by 22-23 (22, n=3) wide (Fig. 1b). Nerve ring 81-108 (92, n=4), and deirids 139-211 (178, n=3) respectively from anterior end. Excretory pore not discerned. Vulva subapical, just ventral to oral opening. Ovejector 1,805-3,171 (2,556, n=4) long, divided into two distinct parts. Distal ovejector with strong muscular walls and lined with cuticle, 795-851 (811, n=4) long, its diameter decreasing gradually to the apex in the last 250-300 micrometers. Proximal ovejector longer 1,007-2,319 (1,745, n=4) long, passing into a short chamber and then into a didelphic uterus (Fig. 1f). Eggs 38-56 (47) long by 21-39 (31) wide (n=19) with first larval stage inside (Figs. 1d; in uterus 1e; in ovejector). Two distal fragments 142, 86 in length, maximum width 423, 364. Tail 322, 484 long, with blunt end bearing apically 15-20 small rounded tubercles (Fig. 1c). Phasmids not observed.

***Female*** (n = 1, whole specimen from Chubut province). Body length 116 mm. Maximum width 327. Muscular part of esophagus 728 long by 59 wide; glandular part more than 1,500 long by 155 wide, posterior end indistinct. Preesophageal ring 21 long by 23 wide. Nerve ring and deirids 95 and 99 from anterior end, respectively. Excretory pore not discerned. Ovejector 2,096 long, distal part 799 long, proximal part 1,296 long. Eggs 36-47 (42) long by 18-28 (23) wide (n=14). Tail 308 long, with 18 small rounded tubercles. Phasmids not observed.

## Taxonomic summary

***Type host:*** *Galictis cuja* (Molina, 1782) (Carnivora: Mustelidae).

***Type locality:*** Proximities of Sierra Grande (41° 29.3'S; 65° 21.2'W), Río Negro province, Argentina.

***Other locality:*** Proximities of Istmo Ameghino (42° 28.5'S; 64° 41.4'W), Península Valdés, Chubut province, Argentina.

***Site of infection:*** Subcutaneous tissue of hind legs.

***Prevalence and intensity of infection:*** One out of two Lesser Grisons from Río Negro infected with two males and four females. One out of two Lesser Grisons from Chubut infected with one female.

***Type specimens:*** Holotype male, MLP-He 7737; paratype male, paratypes female (2 anterior fragments and 1 posterior fragment, and two heads and two tails in SEM samples), MLP-He 7738 and, voucher female, MLP-He 7739.

***Host specimens deposited:*** CEVEC-1099 and CEVEC-1105.

***Etymology.*** The specific epithet is dedicated to Rafael S. Lorenzo, Park Ranger of the Administración de Parques Nacionales of Argentina, who works at the Reserva Natural de la Defensa Punta Buenos Aires at Península Valdés and collected the Lesser Grison specimens.

ZooBank access: http://zoobank.org/References/61D3017D-A30F-4ACE-AB47-CE4D54587541

**Remarks**

These specimens belong to the family Filariidae, subfamily Filariinae which are large parasites of subcutaneous tissues of mammals, with the vulva markedly anterior to nerve ring; oral opening not surrounded by cuticularized spines, esophagus long and divided, tail long, spicules markedly different in size and structure, and male with numerous caudal papillae. Within this family these specimens can be assigned to the genus *Filaria* which is characterized by a reduced buccal cavity, a cuticular ring on the anterior end of the esophagus, and the vulva besides the oral opening (Anderson & Bain 1977).

At present, it is considered that the genus comprises 12 valid species inhabiting carnivores, rodents, artiodactyls and hyracoids. Within the Carnivora, Mustelidae and Mephitidae are the main hosts, and in a much lesser degree Felidae (Table I) (e.g., Ortlepp 1937, Freitas & Lent 1937, Chandler 1947, Chabaud & Rousselot 1956, Anderson 1960, Tadros 1964, Keppner 1969, Chabaud et al. 1978, Chabaud & Mohammad 1989, Otranto et al. 2007, Torres et al. 2016). No new species have been described since 1989. Some authors (e.g., Caballero 1948, Anderson 1960) preferred to consider the species published until 1960 as variations of the type species. However Chabaud & Mohammad (1989) rather upheld the idea of the plurality of species, supported by the examination of a great amount of material from different hosts; at the time that stated that one of the main characters for differentiating species is the morphology of the preesophageal ring.

Based on this character, our specimens, which possess a mushroom shaped preesophageal ring, can be easily differentiated from the eight species found in the Old World: *Filaria martis*, *Filaria hyracis* (Ortlepp, 1937), *Filaria cephalophi* Chabaud & Rousselot, 1956, *Filaria russelli* Tadros, 1964, *Filaria bakerhugoti* Chabaud & Mohammad, 1989, *Filaria latala* Chabaud & Mohammad, 1989, *Filaria melis* Chabaud & Mohammad, 1989 and *Filaria versterae* Chabaud & Mohammad, 1989. The four remaining species are *Filaria texensis* Chandler, 1947 and *Filaria taxideae* Keppner, 1969 from USA, *F. carvalhoi* from Brazil, and *F. conepati* from Argentina, all parasites of Mephitidae and/or Mustelidae. *Filaria texensis* was considered as synonym of *F. carvalhoi* by Anderson (1960), whereas *F. conepati* was also proposed as synonym of *F. carvalhoi* by Anderson (1960) and Chabaud & Mohammad (1989).

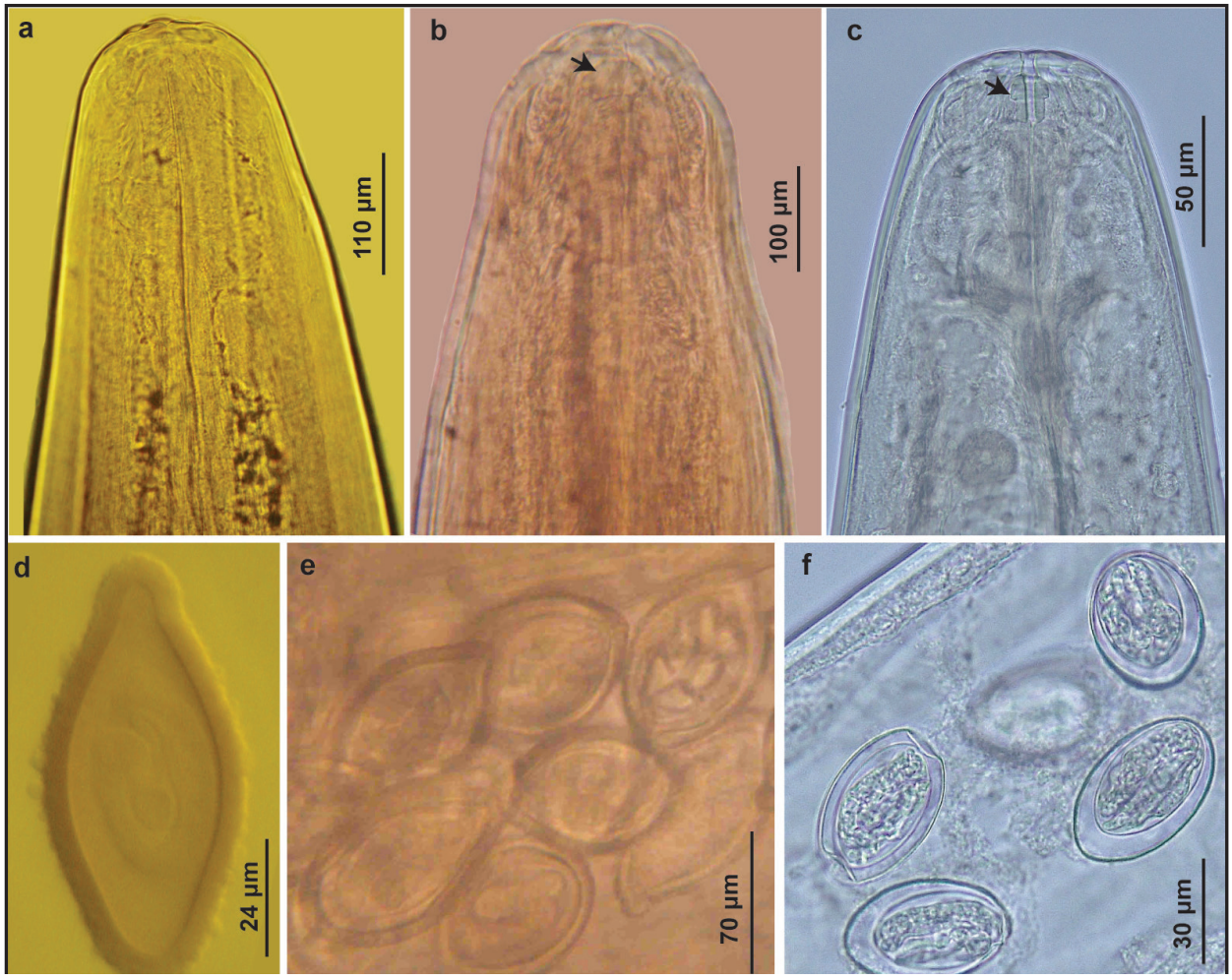


**Figure 3. Photographs comparing anterior ends and shape of eggs of South American species. (a, d) *Filaria carvalhoi* Freitas and & Lent, 1937, no preesophageal ring, eggs lemon shaped and thick shelled. (b, e) *Filaria conepati* Schuurmans-Stekhoven, 1952, preesophageal ring (indicated by arrow), with anterior part elliptical and posterior one shorter and stout, eggs lemon shaped and thick shelled. (c, f) *Filaria lorenzo* n. sp., preesophageal ring (indicated by arrow) with distal part semispherical and proximal one cylindrical, of about same length , eggs elliptical and thin shelled.**

A preesophageal ring is not mentioned nor illustrated in the descriptions of *Filaria carvalhoi* and *F. texensis,* differentiating these two species from the specimens studied herein but also from the remaining species of *Filaria*. The lack of a preesophageal ring in *F. carvalhoi* could be confirmed herein through the observation of photographs taken on the type material (Fig. 3a). *Filaria taxideae* shares with our specimens the presence of a preesophageal ring. It strongly resembles the new species by the shape of the ring, but differs from it by having a cervical cuticular inflation and a pair of lateral alae (absent in the new species); by the ornamentation of the female tail, consisting in an apical double tip plus 8-10 spaced tubercles (*vs*. 15-20 subequal tubercles), and by having a pair of adcloacal papillae (absent in our specimens).

Our specimens also differ from the species described as *F. conepati*. The indirect observation (through photographs) of types of *F. conepati*

revealed the presence of a preesophageal ring (Fig. 3b), with anterior part elliptical and posterior one shorter and stout. It is worth noting that such structure was not mentioned nor illustrated in the original description. The shape of the ring of *F. conepati* is different from that of our specimens (composed of an anterior semispherical portion followed by a posterior cylindrical portion of about the same length). Additional differences lie in the shape of the eggs, which in *F. conepati* are lemon shaped and thick shelled, whereas in our specimens the eggs are elliptical and thin shelled (Fig. 3e, 3f). Finally, the males of the new species were characterized by only eight pairs of caudal papillae, differing from the remaining species in the genus which usually possess nine pairs. The above pointed differences with respect to the existing species in the genus lead us to consider our specimens as belonging to a new species which is named *Filaria lorenzo* n. sp.

## DISCUSSION

Our results extend the taxonomy of *Filaria* and comprise the first report of a species of the genus in Patagonia, and the first report of a filarioid nematode for a mustelid in Argentina. The examination, although indirect, of the types of *F. conepati* and *F. carvalhoi* allows us to corroborate the existence of differences between these two species: notably, the absence of a preesophageal ring in *F. carvalhoi* and the presence of such a structure in *F. conepati*. This is an important difference allowing us to refute the synonymy of *F. carvalhoi* and *F. conepati* proposed by Anderson (1960) and Chabaud & Mohammad (1989).

According to Chabaud & Mohammad (1989) the more significant morphological characters in differentiating species of *Filaria* are: the morphology of the preesophagal ring, the caudal extremity of the female, the caudal alae and symmetry of caudal papillae in the male, and the morphology of the eggs. However, it has to be noted that females with two different morphologies of tail tip (e.g. smooth or mamillated) were repeatedly observed in *F. martis* (Chandler 1947, Anderson 1960, Otranto et al. 2007) as well as in *F. texensis* and *F. carvalhoi* (Chandler 1947, Anderson 1960), which means that the value of the female caudal end as a specific character should be relativized.

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## Author contributions

Estefanía Bagnato: processed the samples, studied and described the nematodes, wrote the manuscript, made the drawings and plates. Romina L. D´Agostino: samples collection and processing. Daniel E. Udrizar Sauthier: samples collection. M. Celina Digiani: Helped in studying the nematodes, writing the manuscript, managed the request of type material, supervised the work.