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REDESCRIPTION OF *PHYSALOPTERA LIOPHIS* VICENTE & SANTOS, 1974 (NEMATODA: PHYSALOPTERIDAE) A PARASITE OF ANURA FROM PAMPA BIOMA, BRAZIL

REDESCRIPCIÓN DE *PHYSALOPTERA LIOPHIS* VICENTE & SANTOS, 1974 (NEMATODA: PHYSALOPTERIDAE) UN PARÁSITO DE ANURA DEL BIOMA PAMPA, BRASIL

Marco Antonio Afonso Coimbra[1], Carolina Silveira Mascarenhas[2]* & Gertrud Müller[3]

[1] Núcleo de Reabilitação da Fauna Silvestre e Centro de Triagem de Animais Silvestres, Universidade Federal de Pelotas (NURFS-CETAS/UFPel), Campus Universitário, s/n., Capão do Leão, CEP 96160-000, RS, Brazil. coimbra.nurfs@gmail.com
[2] Instituto Federal Sul-rio-grandense (IFSul), Campus Pelotas, Praça Vinte de Setembro, 455, Centro, Pelotas, CEP 96015-360, RS, Brazil.
[3] Independent researcher. Pelotas, Brazil. gertrudmuller40@gmail.com
* Corresponding author: phrybio@hotmail.com

Marco Antonio A. Coimbra: https://orcid.org/0000-0003-3902-706X
Carolina Silveira Mascarenhas: https://orcid.org/0000-0002-7045-0926
Gertrud Müller: https://orcid.org/0000-0002-2213-6721

ABSTRACT

Physaloptera liophis Vicente & Santos, 1974 was described as a parasite of snakes in southeastern Brazil; however, some morphological aspects need updating. In the study of helminths associated with *Rhinella dorbignyi* (Duméril & Bibron, 1841) (Anura: Bufonidae) in the south of the country, specimens of *P. liophis* were found and used for the redescription presented by this paper. Thirteen out of 100 anurans under analysis were parasitized by *P. liophis*. Redescription provides new information on the number of papillae of males and morphological characteristics of the reproductive system of females.

Keywords: Bufonidae – Dorbigny's Toad – Nematoda – *Physaloptera* – *Rhinella dorbignyi* – Southern Brazil

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RESUMEN

Physaloptera liophis Vicente & Santos, 1974 fue descrito como un parásito de serpiente en el sudeste de Brasil; sin embargo, algunos aspectos morfológicos necesitan actualización. En el estudio de los helmintos asociados a *Rhinella dorbignyi* (Duméril & Bibron, 1841) (Anura: Bufonidae) en el sur del país, fueron encontrados especímenes de *P. liophis* y utilizados para la redescripción presentada por este trabajo. Trece de los 100 anuros analizados fueron parasitados por *P. liophis*. La redescripción aporta nueva información sobre el número de papilas de los machos y sobre las características morfológicas del aparato reproductor de las hembras.

Palabras clave: Bufonidae – Nematoda – *Physaloptera* – *Rhinella dorbignyi* – Sapito de jardin de D'Orbigny– Sur de Brasil

INTRODUCTION

Physaloptera Rudolphi, 1819 species parasitize the gastrointestinal tract of vertebrates, mainly in the stomach of mammals, birds, reptiles and, rarely, amphibians and fish (Anderson, 2000; Pereira *et al.,* 2012). The genus has more than 105 named species distributed in all continents (Ortlepp, 1922; Ortlepp, 1937; Baker, 1987; Pereira *et al.*, 2012; Pereira *et al.,* 2014; Luiz *et al.,* 2015; Maldonado Jr. *et al.,* 2019; Matias *et al.,* 2020, Alves *et al.*, 2022).

In Brazil, eight species have been recorded in reptiles (Pereira *et al.,* 2014; Matias *et al.,* 2020). *Physaloptera liophis* Vicente & Santos, 1974 was described as parasite of the snake *Erythrolamprus miliaris* Linnaeus, 1758 (=*Liophis miliaris*) (Dipsadidae) in Rio de Janeiro state, in southeastern Brazil (Vicente & Santos, 1974), being also recorded in *Bothrops neuwiedi* Wagler, 1824 (Gouveia *et al.*, 2012) and in *Erythrolamprus viridis* (Günther, 1862) (Quirino *et al.*, 2018) in Minas Gerais and Ceará, respectively.

Physaloptera larvae have been frequently recorded in several amphibian species (González & Hamann, 2007; Hamann *et al.*, 2013a; Campião *et al.*, 2014; Velarde-Aguilar *et al.,* 2014; Aguiar *et al.*, 2015; Toledo et al., 2015; Santos *et al.*, 2016; Campião *et al.*, 2016; Lins *et al.*, 2017; Toledo *et al.*, 2017ab). However, only three species have been recorded worldwide; two were described in anurans while one was described in lizards. *Physaloptera amphibia* Linstow, 1899 was described in *Limnonectes macrodon* (Duméril & Bibron, 1841) (= *Rana macrodon*) in the Philippines and *Physaloptera tigrinae* Ali & Farooqui, 1969 in *Hoplobatrachus tigerinus* (Daudin, 1802) (= *Rana tigrina*) in India (Baker, 1987; Pereira *et al.,* 2012). Baker (1987) also listed four Ranidae species as hosts of *P. amphibia* in Europe, but he cast doubt on the records. Regarding *P. tigrinae*, there are no records in the literature other than the species description in 1969, which is cited by Baker (1987), and Pereira *et al.* (2012). *Physaloptera retusa* (Rudolphi, 1819) was described in Squamata (Ortlepp, 1922), and recorded in northern Brazil parasitizing *Rhinella granulosa* (Spxi, 1824) (=*Bufo granulosus*), and *Rhinella margaritifera* (Laurenti, 1768) (=*Bufo thyfonius*), and *Physaloptera* sp. in *Rhinella marina* (Linnaeus, 1758) (= *Bufo marinus*) in Amazonas state (Gonçalves *et al.*, 2002).

In a study of helminths associated with *Rhinella dorbignyi* (Duméril & Bibron, 1841) (= *Rhinella fernandezae*) (Anura: Bufonidae) in the southern of Brazil, specimens of *P. liophis* were found and used for the redescription presented by this paper, i.e., it redescribes some characteristics previously described by Vicente & Santos (1974). *Rhinella dorbignyi* occurs in Argentina, Uruguay, and southern Brazil and Paraguay (Frost, 2024), where it inhabits grasslands, wetlands, agroecosystems and areas close to human households (Loebmann, 2005; Maneyro *et al.,* 2017). In *R. dorbignyi* species of Nematoda, Digenea, Cestoda and Acanthocephala have been reported in Paraguay (Lent *et al.*, 1946; Smales, 2007), Uruguay (Lent & Freitas, 1948), Brazil (Santos & Amato, 2010; Henzel *et al.*, 2020; Coimbra *et al.*, 2023), and Argentina (González & Hamann, 2007; Hamann *et al.*, 2013a, Hamann *et al.*, 2013b; Draghi *et al.*, 2020). Before Coimbra *et al.* (2023) (material used in this study), there were only records of *Physaloptera* larvae parasitizing this buffonid (González & Hamann 2007; Hamann *et al.* 2013a). Therefore, this study redescribes *P. liophis* associated with *Rhinella dorbignyi* from Pampa biome, southern Brazil.

MATERIAL AND METHODS

A hundred specimens of *R. dorbignyi* were collected in Pelotas (31°46'38.0"S - 52°13'57.2"W) and Capão do Leão (31°48'5.79"S - 52°24'53.39"W), Rio Grande do Sul (RS), southern Brazil, from August 2017 to October 2020. Anurans were manually collected and individually taken to the Laboratório de Parasitologia de Animais Silvestres (LAPASIL/UFPel), where they were weighed and measured.

Fifty-four anurans were necropsied after freezing while the others were either examined immediately after death or refrigerated for no more than 24 hours. During the necropsy, all organs were individualized and examined. Nematodes were fixed in AFA (ethanol 70°GL - 93 parts; formalin 37% - 5 parts; glacial acetic acid - 2 parts), preserved in glycerinated ethanol (5% glycerin in 70°GL ethanol), and clarified in Amann's lactophenol (Amato & Amato, 2010).

Three male specimens were prepared for scanning electron microscopy (SEM) analysis at the Central Laboratory of Microscopy and Microanalysis of the Pontificia Universidade Católica do Rio Grande do Sul (LabCEMM/PUCRS), Brazil. Specimens preserved in 70% ethanol was dehydrated in 90% ethanol for 20 minutes and then in 100% ethanol for 20 minutes. Subsequently, the critical point procedure was performed in a BALZERS CPD30 equipment for final drying of the sample. In this process liquid CO_2 is injected into the chamber to make the replacements until the ethanol is completely removed. Next, the samples were metallized with Au. The metallization was done in a Quorum Q 150R ES plus metallizer in order to make the samples conductive to be visualized on the SEM. The images were taken on an SEM-FEG from FEI, model Inspect F50.

Measures (mean, standard deviation and range) are expressed as micrometers (µm), unless otherwise indicated. Minimum and maximum values (range) are shown between parentheses. Parasitological indices were calculated in agreement with Bush *et al.* (1997). Photomicrographs were prepared on an Olympus BX 41 microscope with a camera system and plates were made with Adobe Photoshop CS5. Vouchers were deposited in the "Coleção Helmintológica do Instituto Oswaldo Cruz" (CHIOC), Rio de Janeiro, Brazil, and in the "Coleção de Helmintos do Laboratório de Parasitologia de Animais Silvestres" at the Universidade Federal de Pelotas (CHLAPASIL-UFPel), RS, Brazil. Images of holotype, allotype and paratype (CHIOC 31034a-c) from the Coleção Helmintológica at the Instituto Oswaldo Cruz were examined.

Ethic aspects: The study was licensed by the Instituto Chico Mendes de Conservação da Biodiversidade (ICMBio no. 47397) and approved by the Ethics Committee on Animal Experimentation (CEEA/UFPel no.1859/2015). Euthanasia was performed in agreement with Resolution no. 1000/2012 issued by the Conselho Federal de Medicina Veterinária (CFMV, 2012).

RESULTS

Redescription

***Physaloptera liophis* Vicente & Santos,1974** (Figs. 1 – 6)

Host: *Rhinella dorbignyi* (Duméril & Bibron, 1841), Dorbigny´s Toad.

Site of infection: stomach.

Locality: Laranjal, Pelotas (31°46'38.0"S - 52°13'57.2"W), and UFPel Campus, Capão do Leão (31°48'5.79"S - 52°24'53.39"W), Rio Grande do Sul, Brazil.

Prevalence and mean intensity of infection: 13%; 13.69 helminths/host (1-89 nematodes).

Specimens deposited: CHLAPASIL-UFPel (904-918), and CHIOC (39171, 39172).

Description based on 15 specimens: Body filiform with anterior extremity more tapered than posterior extremity. Cuticle with transverse striations forming well marked annulations on the first and last third of the body. Cuticle at the anterior end has dilatations that form the cephalic collar. Oral opening surrounded by two well-developed lateral pseudolips, convex and semicircular in shape. Each pseudolip has a pair of cephalic papillae dorsoventrally located, a lateral small amphids, and a well-developed triangular tooth. Absence of buccal capsule. Deirids located at the same level. Excretory pore just below the deirids. Long esophagus divided into a muscular anterior part and a shorter glandular posterior part. Nervous ring surrounding the muscular esophagus.

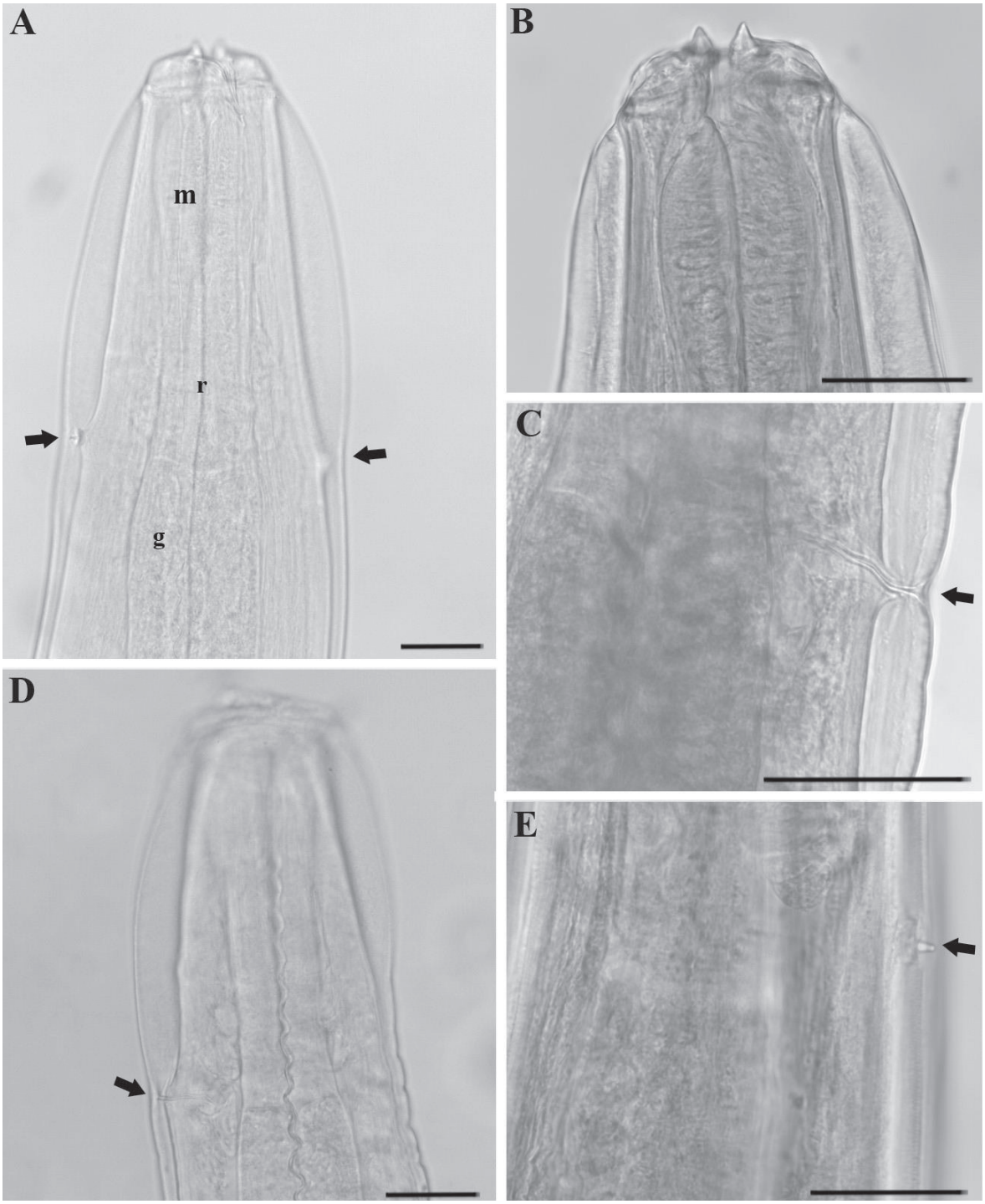

Figure 1. *Physaloptera liophis* Vicente & Santos, 1974 a parasite of *Rhinella dorbignyi* (Duméril & Bibron, 1841) (Anura: Bufonidae) in southern Brazil. **A-B**: Dorsoventral view of the anterior extremity (g - glandular esophagus; r - nerve ring; m - muscular esophagus; arrows indicate the deirids) (bar – 60µm). **C-D**: Lateral view, the arrow indicates the excretory pore located near the junction of the glandular and muscular esophagus (bar – 60µm). **E**: Dorsoventral view, the arrow indicates the deirid (bar – 60µm).

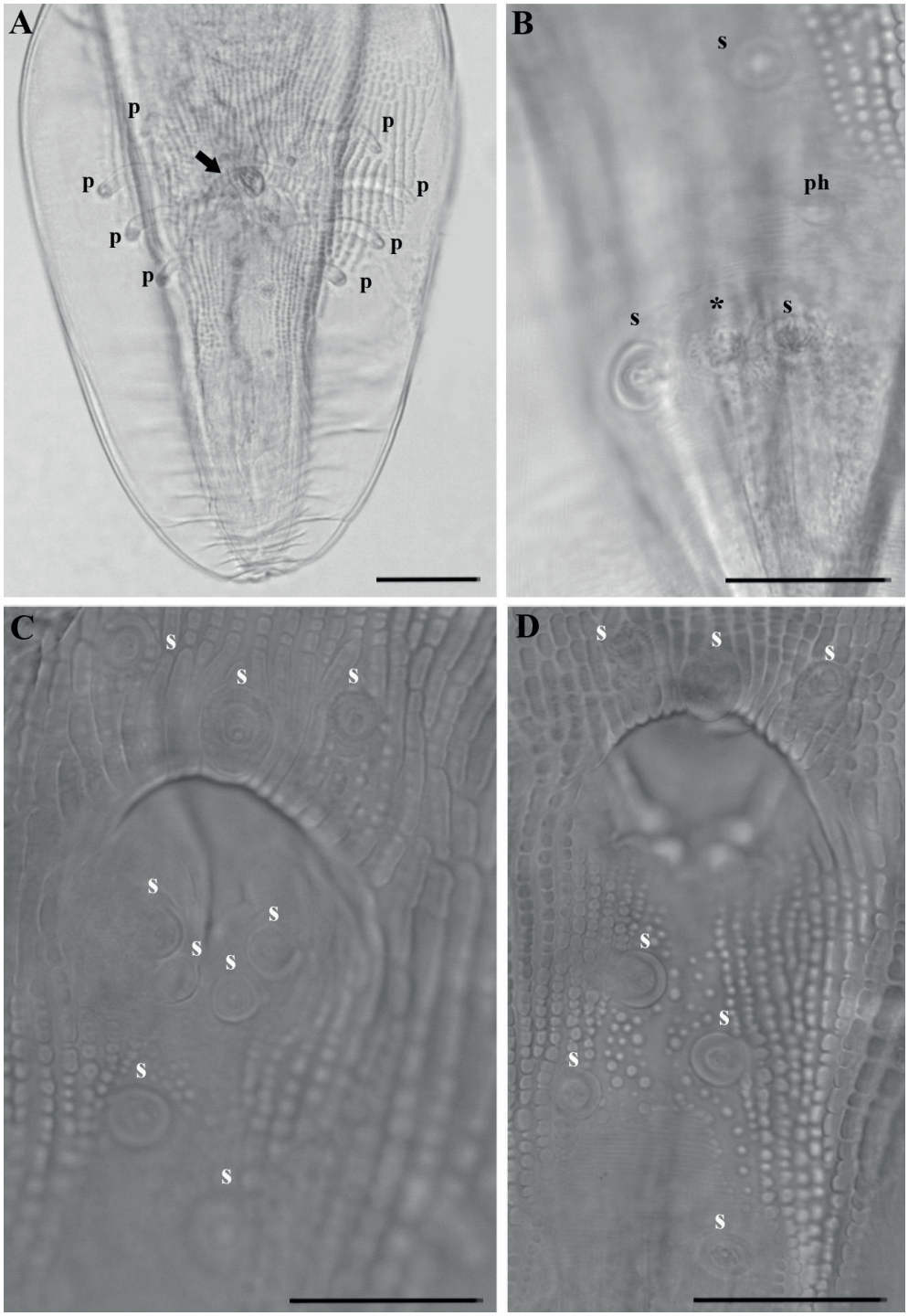

Figure 2. Ventral view of the tail of a *Physaloptera liophis* Vicente & Santos, 1974 male parasite of *Rhinella dorbignyi* (Duméril & Bibron, 1841) (Anura: Bufonidae) in southern Brazil. **A**: General view of the tail, emphasis is given to the position of the four pairs of pedunculated papillae (p) and the tip of the left spicule exiting through the cloaca (arrow) (bar – 75µm). **B**: Detail of phasmids (ph), between the two last pairs of sessile papillae (s), and the dome-shape protuberance (asterisk) between the papillae of the last pair (bar – 75µm). **C – D:** Detail of the sessile papillae (s) (bar – 50µm).

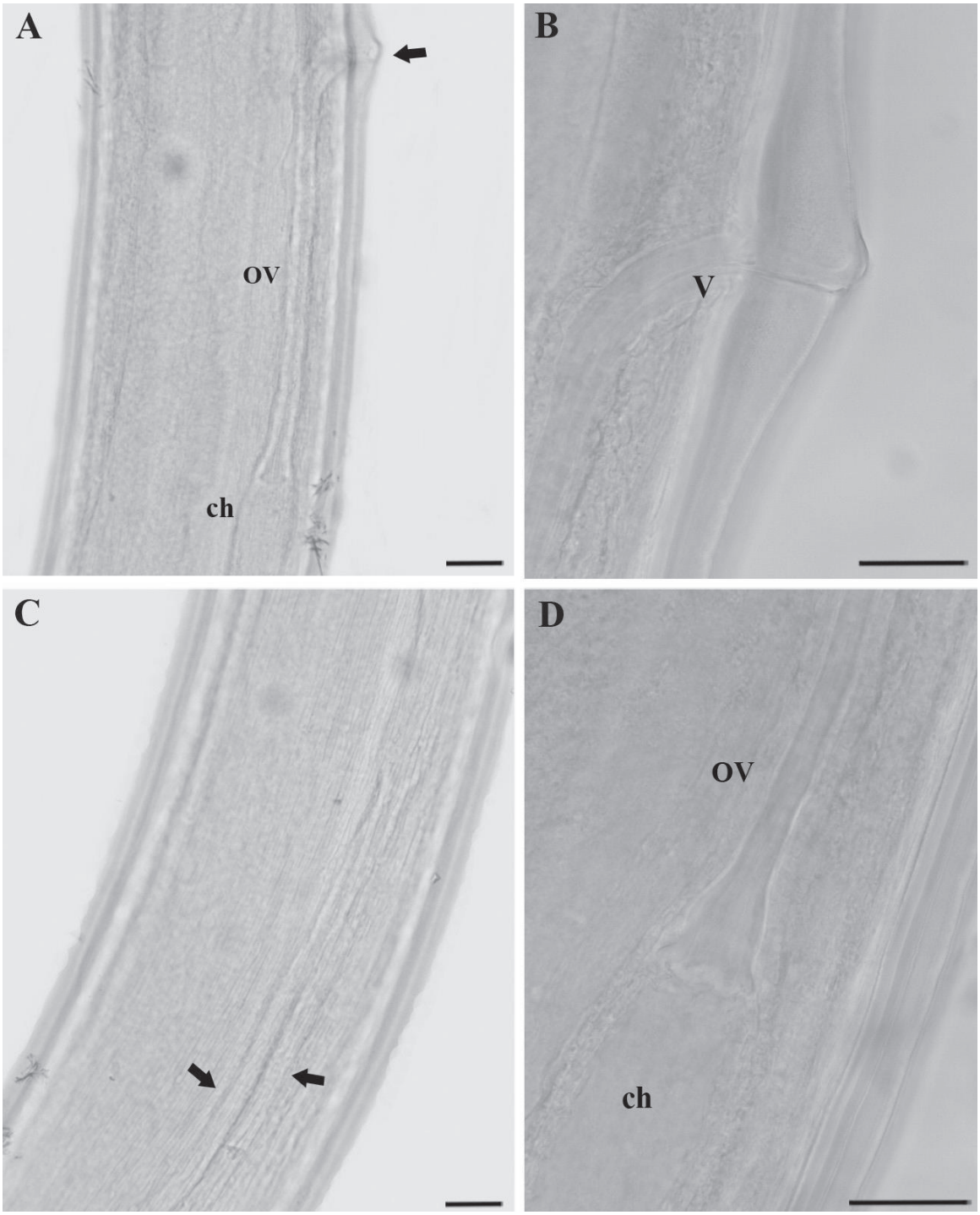

Figure 3. Lateral view of the middle third of a *Physaloptera liophis* Vicente & Santos, 1974 female parasite of *Rhinella dorbignyi* (Duméril & Bibron, 1841) (Anura: Bufonidae) in southern Brazil. **A**: Detail of vulvar opening (arrow), ovojector (ov) and beginning of egg chamber (ch) (bar – 100µm). **B**: Detail of the vulvar opening and muscular vagina (v) (bar– 25µm). **C:** Detail of the didelph uterus (arrows) directed towards the posterior body region (bar – 75µm). **D:** Detail of the junction of the ovojector (ov) and egg chamber (ch) (bar – 100µm).

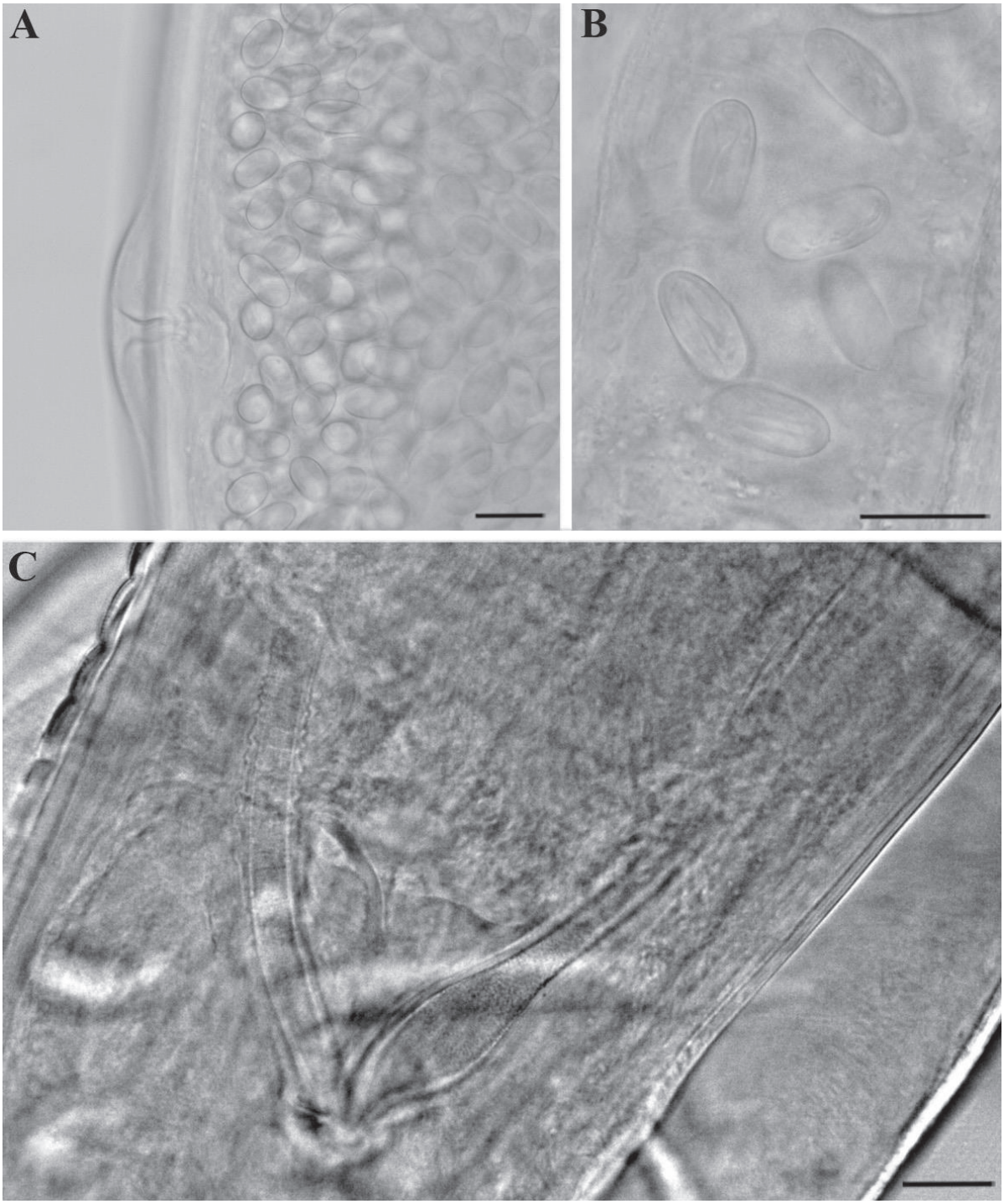

Figure 4. *Physaloptera liophis* Vicente & Santos, 1974 a parasite of *Rhinella dorbignyi* (Duméril & Bibron, 1841) (Anura: Bufonidae) in southern Brazil. **A**: Detail of the vulvar opening and eggs in uterus, lateral view (bar – 55µm). **B**: Embryonated eggs (bar – 55µm). **C**: Spicules of the male (bar – 33µm).

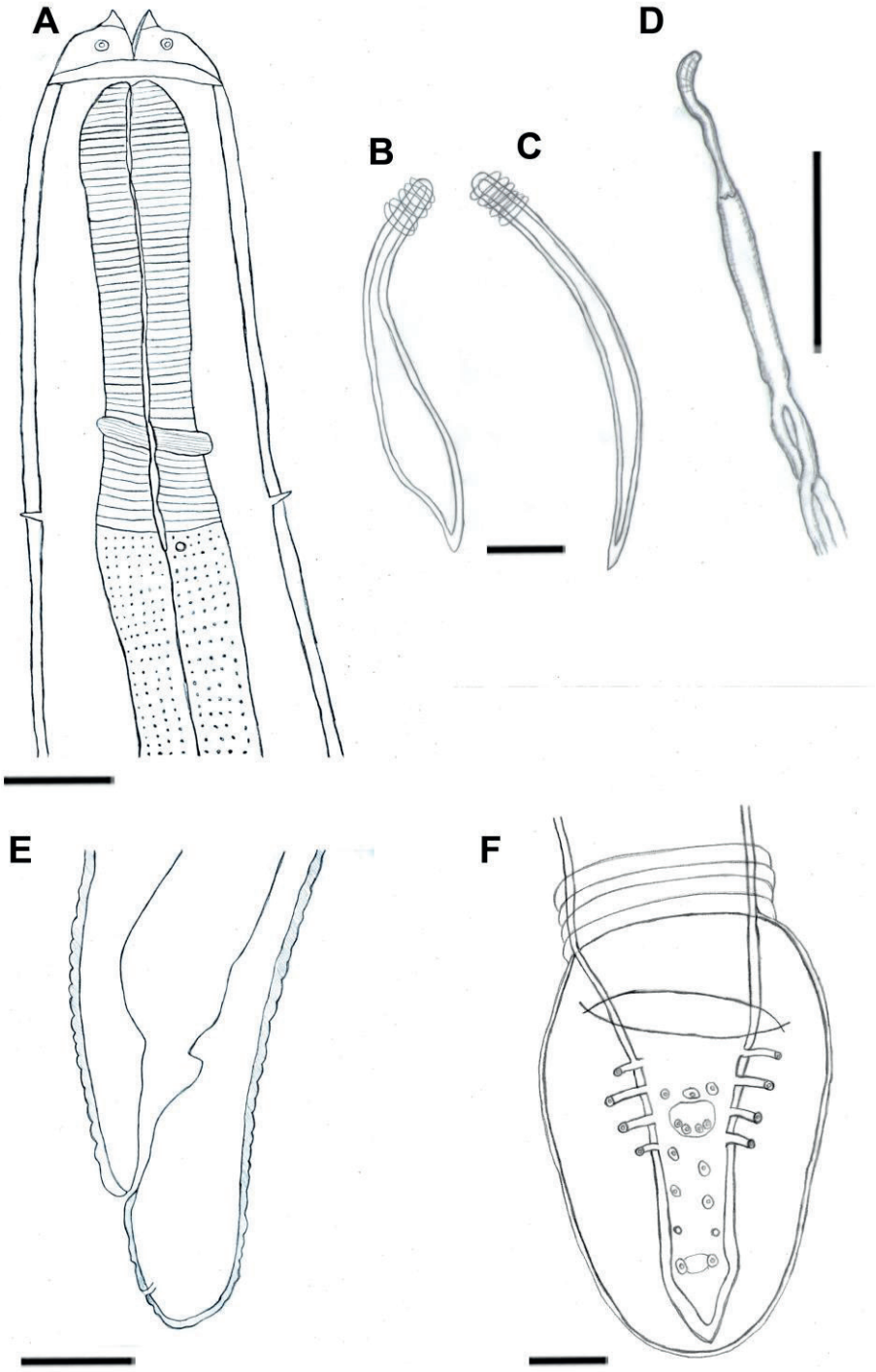

Figure 5. *Physaloptera liophis* Vicente & Santos, 1974 a parasite of *Rhinella dorbignyi* (Duméril & Bibron, 1841) (Anura: Bufonidae) in southern Brazil. **A:** Dorsoventral view of the anterior extremity (bar – 60µm). **B:** Left spicule (bar – 35µm). **C:** Right spicule (bar – 35µm). **D:** Genital tract from vagina to uterus didelph (bar– 520µm). **E:** Lateral view of the posterior extremity of the female (bar – 250µm). **F:** Ventral view of the tail of male (bar – 75µm).

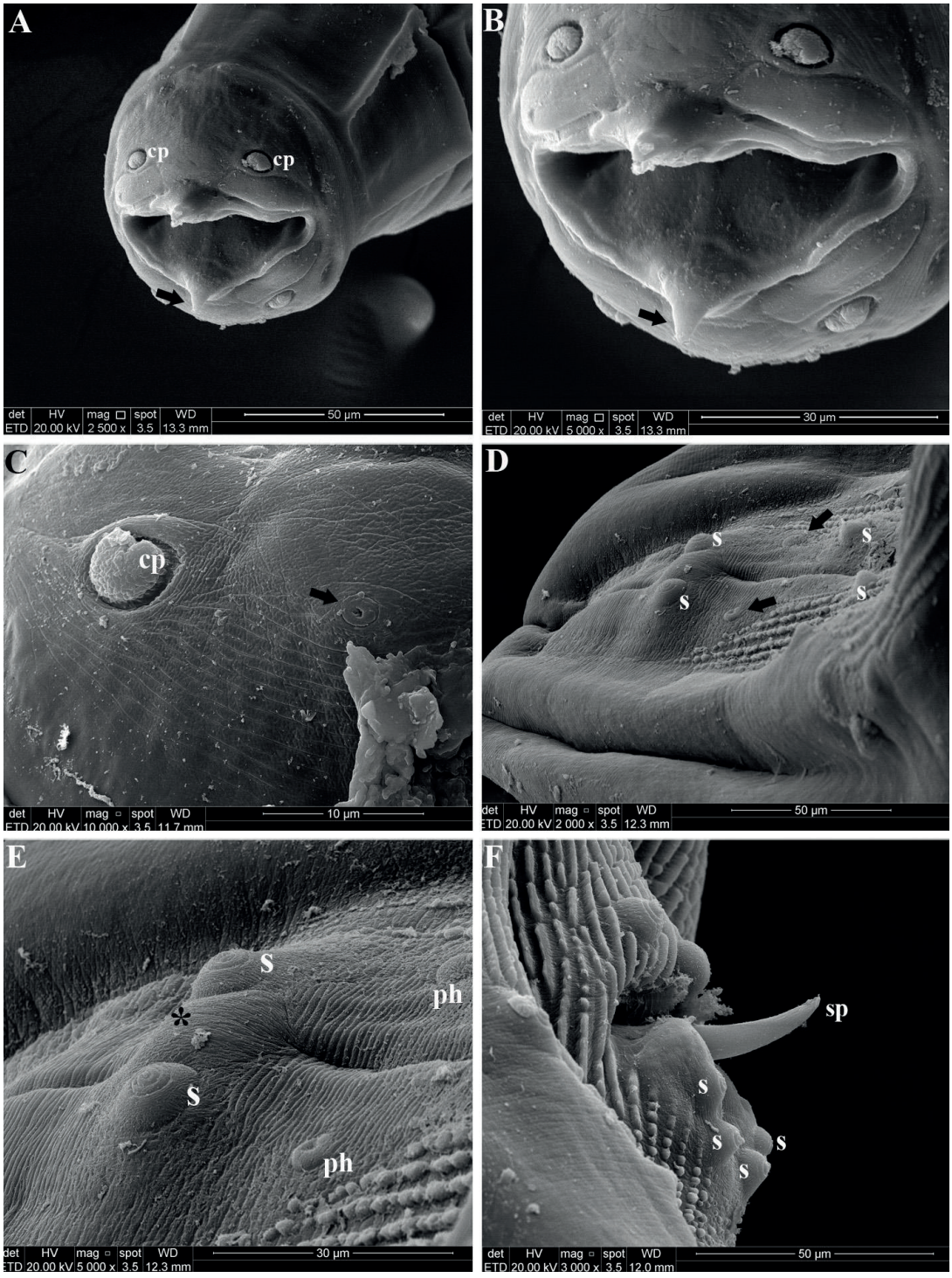

Figure 6. *Physaloptera liophis* Vicente & Santos, 1974 male parasite of *Rhinella dorbignyi* (Duméril & Bibron, 1841) (Anura: Bufonidae) in southern Brazil. **A - B:** Anterior extremity (cp - cephalic papillae; arrow indicate the triangular tooth. **C:** Detail of cephalic papillae (cp) and amphid (arrow). **D:** Posterior extremity (s - sessile papillae; phasmids (arrow). **E:** Detail of the 5th pair of sessile papillae (s), phasmids (ph) and dome-shaped protuberance (asterisk). **F:** Detail of the mammiliform sessile papillae (s) on the lower margin of the cloacal opening, and of the tip of the right spicule (sp).

Male based on seven specimens: overall body length 7.61 ± 1.11 (6.20–9.50) mm; width (at the level of the esophagus-intestine junction) 334 ± 46.50 (280–410). Distance from nerve ring to anterior extremity of the body 238.93 ± 24.57 (202.50–282.50). Distance from excretory pore to anterior extremity 317 ± 26.28 (300–360). Distance from deirid to anterior extremity 276 ± 35.52 (240–330). Esophagus length 1935 ± 273.5 (1550–2330), representing 25.43% ± 2.57 % (21.47–29.64%) of total body length. Muscular esophagus 267 ± 17.99 (240–290) long, and 61 ± 9.00 (50–70) width; glandular esophagus 1668 ± 259.69 (1300–2050) long, and 120 ± 16.33 (100–140) width. Curved tail with well-developed two caudal alae fused posteriorly. Caudal ala 714 ± 89.97 (610–850) long, right portion 116 ± 24.40 (80–150) wide, and left portion 111 ± 32.88 (50–150) wide. Ventral face of alae ornamented with small tubercles arranged in longitudinal rows, less evident near posterior extremity of tail. Twenty-one caudal papillae: 4 pedunculated pairs, 6 sessile pairs and 1 large precloacal unpaired papillae. Pedunculated papillae: 2 subventral precloacal pairs, 1 pair aligned with the cloaca and 1 postcloacal pair. Precloacal sessile papillae: 1 small median pair on the same line followed by 1 large unpaired papilla near the cloacal opening. Post-cloacal sessile papillae: 2 pairs of median mammiliform papillae on the lower margin of the cloacal opening; 3rd pair just below the cloaca, followed by the 4th pair, both paired diagonally (in some specimens, pairs are aligned); 5th pair apart from the others and aligned near the tip of the tail. Between the papillae of the 5th pair, there is a dome-shape protuberance. Spicules of different shape, and sub-equal size, slightly sclerotized, surrounded by a small membranous sheath. Spear-shaped left spicule, with rounded base and widening in the second half, ending in a thin tip, 181.43 ± 24.70 (162.5–220) long. Needle-shaped right spicule with rounded base and fine tip, 172.1 ± 10.04 (155–187.5) long. Distance from cloaca to tail end 387 ± 62.91 (320–500). Gubernacle absent. A pair of phasmids is located between the 4th and 5th pairs of postcloacal sessile papillae.

Female based on eight specimens (six non-gravid females and two gravid with few eggs): overall body length 7.79 ± 1.32 (6.00–9.75) mm; width (at the level of the esophagus-intestine junction) 357.5 ± 39.55 (290–400). Distance from nerve ring to anterior extremity of the body 232.50 ± 21.51 (187.5–255). Distance from excretory pore to anterior extremity 362.5 ± 41.32 (290–410). Distance from deirides to anterior extremity 315.63 ± 31.42 (267.5–370). Esophagus length 1805.63 ± 320.75 (1310 – 2285), representing 23.47% ± 3.87% (15.73–27.50%) of the total body length. Muscular esophagus 290 ± 41.40 (230–360) long, and 67.50 ± 8.86 (50–80) width; glandular esophagus 1515.63 ± 306.24 (980–1925) long, and 125 ± 27.26 (90–150) width. Vulvar opening located in the middle third of the body, distant 3277.14 ± 657.57 (2450–4125) from anterior extremity (seven specimens were measured). Vulvar lips not very prominent. Muscular short vagina 72.50 ± 20.54 (50–100) (five specimens were measured), directed posteriorly followed by muscular ovojector of 420.83 ± 81.83 (337.50–500) (three specimens were measured); egg chamber 563.33 ± 79.67 (470–660) long, posteriorly directed didelph uterus, with the two uterine loops beginning after the egg chamber, and no common trunk. Smooth-shelled eggs 40 (40–40) long and 22.5 (22.5–22.50) width (three eggs were measured), fully developed, containing larvae. Posterior end rounded; lateral phasmidial pores evident 93.75 ± 18.90 (62.5–112.50) distant from tail tip. Tail length 221.50 ± 26.96 (200–280).

Remarks: Specimens of *P. liophis* that parasitize the anuran *R. dorbignyi* are proportionally smaller than those described while parasitizing the snake *E. miliaris* (Table 1). Vicente & Santos (1974) described the presence of 23 caudal papillae in *P. liophis* male; however, the penultimate pair of post-cloacal papillae are redescribed by this study as phasmids. Furthermore, the authors did not mention dome-shape protuberance between the last pair of post-cloacal papillae. Such characteristics were observed in the holotype (CHIOC 31034a) and also in the specimens collected from *R. dorbignyi*. Redescription of the female provides information on the morphology and morphometry of the vagina, ovojector and egg chamber, which were not described by Vicente & Santos (1974), possibly because they examined only a female, which was considered oviparous. Females collected from anurans by this study exhibited larval eggs.

Regarding *Physaloptera* species described in amphibians, *P. amphibia* and *P. trigrinae*, there is no information on the uterus morphology (Ortlepp, 1922; Pereira *et al.*, 2012). However, the *P. liophis* male differs from the *P. amphibia* one, since the latter has 16 caudal papillae (Ortlepp, 1922) while the former has 21 papillae. *Physaloptera liophis* could not be compared with *P. trigrinae*, since the species description could not be accessed; it was listed by Baker (1987) and Pereira *et al.* (2012) and there no other records of the species after its description in 1969 were found.

Table 1. Morphometry of *Physaloptera liophis* as parasites of snakes and anuran in Brazil**.**

	Vicente & Santos (1974)	**This study**
	Host: *Erythrolamprus miliaris* Linnaeus (Serpentes)	**Host:** *Rhinella dorbignyi* (Duméril & Bibron) (Anura)
	Locality: Volta Redonda, Rio de Janeiro	**Locality**: Pelotas and Capão do Leão, Rio Grande do Sul
Male		
Body length (mm)	12.18 – 13.8	6.2 – 9.5
Body width	500 – 520	280 – 410
Length of muscular esophagus	310 – 350	240 – 290
Length of glandular esophagus	1860 – 2270	1300 – 2050
Distance of the deirids from the anterior extremity	340 – 380	240 – 330
Distance of the nerve ring from the anterior extremity	260	202.5 – 282.5
Spicules length	250 – 260	162.5 – 220 (left) 155 – 187.5 (right)
Female		
Body length (mm)	12.78	6.0 – 9.75
Body width	510	290 – 400
Length of muscular esophagus	360	230 – 360
Length of glandular esophagus	2380	980 – 1925
Distance of the deirids from the anterior extremity	390	267.5 – 370
Distance of the nerve ring from the anterior extremity	310	187.5 – 255
Distance from vulva to anterior end	7070	2450 – 4125
Egg length x width	50 x 20	40 x 22.5
Tail length	390	200 – 280

Among the species recorded in reptiles in Brazil, *P. liophis* differs from *P. retusa*, *P. obtusissima* Molin, 1860, *P. lutzi* Cristofaro, Guimarães & Rodrigues, 1976, *P. tupinambae* Pereira, Alves, Rocha, Lima & Luque, 2012, *P. bainae* Pereira, Alves, Rocha, Lima & Luque, 2014 and *P. nordestina* Matias, Moraes & Ávila, 2020 due to the position of the vulvar opening, which is located near the anus in *P. lutzi*, and in the first third of the body in the other species. However, in *P. liophis*, the vulva is located in the middle third of the body. *Physaloptera liophis* and *P. bonnei* Ortlepp, 1922 have the same number of caudal papillae, but differ in the shape and size of the spicules, which measure 455µm in *P. bonnei*. The female of *P. bonnei* has a vagina, egg chamber, and the bifurcation of the uterus directed forward, unlike *P. liophis*. In addition, *P. bonnei* has two pairs of teeth on each pseudolabial (the outer is conical and obtuse, whereas the inner is membranous and tripartite), unlike *P. liophis* which has only one pair of triangular teeth.

DISCUSSION

Even though transmission and development of some *Physaloptera* species in mammals have been intensively studied, there is limited information on species in reptiles. Physalopterinae species are usually found firmly adhered to the gastric mucosa of their definitive hosts; however, studies of species that occur in mammals suggest that the helminths do not feed on their mucosa but on contents found in their stomach, a fact that influences development of the third-stage larvae into adult forms. Infection of the definitive hosts involves prey-predator interactions, since

vertebrates become infected by ingesting arthropods (e. g., cockroaches and crickets) that act as intermediate hosts in which the infective third-stage larvae develop (Anderson, 2000).

The role of anurans in the life cycle of *Physaloptera* species is not sufficiently known. There are several records of larval forms parasitizing these vertebrates in the Neotropics (González & Hamann, 2007; Hamann *et al.*, 2013a; Campião *et al.*, 2014; Velarde-Aguilar *et al.*, 2014; Aguiar *et al.*, 2015; Toledo *et al.*, 2015; Santos *et al.*, 2016; Campião *et al.*, 2016; Lins *et al.*, 2017), suggesting that anurans may act as paratenic hosts which transmit infective forms through the trophic chain. On the other hand, the few records of adult forms of *Physaloptera* in anurans suggest that these parasites, throughout their evolution, have not had the same success of infection and establishment in these vertebrates, by comparison with mammals, birds, and reptiles. Four *Physaloptera* species have been recorded as stomach parasites of anurans; two of them have been described in reptiles, *P. retusa* and *P. liophis*, and recorded in anurans belonging to the *Rhinella* Fitzinger (Ortlepp, 1922; Cristofaro *et al.*, 1976; Baker, 1987; Gonçalves *et al.*, 2002; Pereira *et al.*, 2012; Pereira *et al.*, 2014; Coimbra *et al.*, 2023; present study). González *et al.* (2021) reported that anurans can be definitive hosts of *Physaloptera venancioi* Lent, Freitas & Proença, 1946; however, this species, described in *Rhinella diptycha* (Cope, 1862) (= *Bufo paracnemis*) in Paraguay (Lent *et al.*, 1946), was transferred to *Physalopteroides* Wu & Liu, 1940 (Bursey & Goldberg, 1994).

In general, the association between *Physaloptera* species (adults and/or larvae) and anurans belonging to *Rhinella* is characterized by low infection indices (Gonçalves *et al.*, 2002; González & Hamann, 2007; Hamann *et al.*, 2013a; Toledo *et al.*, 2017ab; Teles *et al.*, 2018; Coimbra *et al.*, 2023) possibly reflecting the diet of these anurans. Several species of *Rhinella*, such as *R. dorbignyi*, use ants as their main food resource, and may also consume other arthropods (e.g., coleopterans, isopterans, arachnids, hemipterans, dipterans, cockroaches, orthopterans and lepidopterans), which are usually little important in the diet of species that belong to this genus (Sabagh *et al.*, 2008; Quiroga *et al.*, 2009; Batista *et al.*, 2011; Maragno *et al.*, 2011; Piatti *et al.*, 2011; Isacch & Barg, 2002; Maia-Carneiro *et al.*, 2013; Oliveira *et al.*, 2014). Studies of the feeding ecology of *R. dorbignyi* were carried out in Argentina, where the species was found to consume mainly Formicidae (Isacch & Barg, 2002; Da Rosa *et al.*, 2002; Duré *et al.*, 2009; Peltzer *et al.*, 2010). The helminth fauna of *R. dorbignyi* is well known in its area of occurrence, since studies have been developed in Paraguay (Lent *et al.*, 1946; Smales, 2007), Uruguay (Lent & Freitas, 1948), Brazil (Santos & Amato, 2010; Henzel *et al.*, 2020; Coimbra *et al.*, 2023), and Argentina (González & Hamann, 2007; Hamann *et al.*, 2013a; Hamann *et al.*, 2013b; Draghi *et al.*, 2020). González & Hamann (2007) and Hamann *et al.* (2013a) recorded *Physaloptera* larvae, whose prevalence ranged from 3.1% (2/65) to 4% (1/25) and mean intensity of infection was one to six helminths/host. Therefore, the feeding preference of *Rhinella* species for ants supports the hypothesis that infections by *Physaloptera* species are infrequent or occasional.

This study introduced new information on the morphology of males and females of *P. liophis*, a parasite of anurans in the Pampa biome, and provided ecological information for future studies on this anuran species.

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Author contributions: CRediT (Contributor Roles Taxonomy)

MAAC = Marco Antonio Afonso Coimbra
CSM = Carolina Silveira Mascarenhas
GM = Gertrud Müller

Conceptualization: MAAC, CSM
Data curation: MAAC, CSM
Formal Analysis: MAAC, CSM, GM
Funding acquisition: GM
Investigation: MAAC, CSM
Methodology: MAAC, CSM

Project administration: CSM
Resources: MAAC, CSM, GM
Software: CSM
Supervision: GM
Validation: MAAC, CSM, GM
Visualization: MAAC, CSM, GM
Writing – original draft: MAAC, CSM
Writing – review & editing: MAAC, CSM, GM

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