

Original paper

Rhabdias elegans (Nematoda: Rhabdiasidae) in the toad, *Rhinella arenarum* (Hensel, 1867) from Argentina

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ABSTRACT. This study describes the morphology of *Rhabdias elegans* Gutierrez, 1945, isolated from the lungs of the toad *Rhinella arenarum* (Hensel, 1867) from Lomas de Tafi Tucumán, Argentina. This is the first record of *R. elegans* in *R. arenarum* from northwestern Argentina as previously described *Rhabdias truncata* is a *species inquirendae*. The distribution of *R. elegans* is extended since it was previously recorded in Buenos Aires, Chaco and Corrientes Provinces, Argentina. Our light microscopical study of morphological details of *R. elegans* revealed a few previously unreported structures in the apical region including position of the excretory pore and lateral sessile papillae in the tail. In addition, morphometric variability was observed on comparing present specimens and those described in previous studies. Our study made it possible to validate the occurrence of three *Rhabdias* species occurring in Argentina: *R. elegans*, *R. füelleborni* and *R. cf. sphaerocephala*. The information presented in this study represents an important contribution to the parasitological knowledge of *R. arenarum* from northwestern Argentina.

Keywords: *Rhinella arenarum*, lung, nematode, *Rhabdias elegans*, Argentina

Introduction

The genus *Rhabdias* Stiles et Hassall, 1905, lungworms commonly found in amphibian and some reptilian hosts, are cosmopolitan in distribution [1]. The infection is produced by penetration into the skin [2], orally or potentially through vectors [3]. *Rhabdias* sp. have a complex life cycle that alternates from a free-living generation on the soil to another that is a parasite in the lungs of amphibians and reptiles [3,4]. In South America, 12 species of *Rhabdias* were reported parasitizing amphibians: *R. androgyna* Kloss, 1971; *R. breviensis* Nascimento et al. [5]; *R. elegans* Gutierrez, 1945; *R. füelleborni* Travassos, 1926; *R. galactonoti* Kuzmin et al. [1]; *R. hermafrodita* Kloss, 1971; *R. mucronata* Schuurmans-Stekhoven, 1952; *R. paraensis* Santos et al. [6]; *R. pseudosphaerocephala* Kuzmin et al. [1]; *R. spha-*

erocephala Goodey, 1924; *R. stenocephala* Kuzmin et al. [1] and *R. truncata* Schuurmans-Stekhoven, 1952. Of those, the following are known from Argentina: *R. elegans*, *R. pseudosphaerocephala*, *R. truncata*, *R. mucronata*, *R. füelleborni*, and *R. cf. sphaerocephala* Schuurmans-Stekhoven 1952 [7–9]. *Rhabdias elegans* and *R. pseudosphaerocephala* in *Rhinella major* (Müller and Hellmich 1936) from Chaco Province [10] also *R. elegans* in *Rhinella arenarum* from Buenos Aires and Salta Provinces [11,12], *Rhabdias* aff. *sphaerocephala* in *Rhinella fernandezae* from Corrientes Province [8], *Rhabdias mucronata* in *Leptodactylus ocellatus* (L.) from Corrientes Province, *R. truncata* in *Telmatobius laticeps* Laurent, 1977 from Tucumán Province [7] and *Rhabdias füelleborni* Travassos, 1926 in *Rhinella schneideri* and *Scinax acuminata* from Corrientes Province [13].

However, *R. mucronata* and *R. truncata* are *species inquirendae* that are poorly described and difficult to recognize, especially since only female and young individuals are known [9]. It should be noted that Oliveira da Silva et al. [9] mention *R. pseudosphaerocephala* from Chaco province, Argentina. However, according to the literature review carried out in this study, this record is erroneous since the correct reference is Hamann and González [10] (and not Hamann and González 2017). Hamann and González [10] recorded only *R. elegans* parasite of *R. major* and not *R. pseudosphaerocephala*. Therefore, if *R. truncata* and *R. mucronata* are *species inquirendae*, and *R. pseudosphaerocephala* is an incorrect record, the three species valid for Argentina are: *R. elegans*, *R. füelleborni* and *R. cf. sphaerocephala*.

On the other hand, amphibians are particularly susceptible to habitat alterations since they are small, poorly tolerant of desiccation and have a low dispersal capacity; in addition to requiring both aquatic and terrestrial environments to survive [14]. Bufonidae comprises about 49 genera and 592 species distributed around the world, of which about 15% (89 species) belong to the *Rhinella* genus [15]. *Rhinella arenarum* (= *Bufo arenarum*) is a toad with a very wide distribution that lives in arid or humid environments. In Argentina it extends from the North of Jujuy Province to the Chubut River, near the Patagonian coast. To the West it reaches the borders of southern Neuquén, approaching the Limay River. On the cordilleran slopes reaches to 1500 m a.s.l. at the Mendoza latitude, higher in the northern most provinces, as La Rioja or Catamarca [16]. Their diet includes Araneae, Coleoptera, Diptera, Hymenoptera, and Orthoptera [17]. It is considered as a non-threatened species [18].

The purpose of this article is to describe for the first time *Rhabdias elegans* in *Rhinella arenarum* from Tucumán Province as *R. truncata* is *inquirendae species*. The distribution is extended since it was previously recorded in Buenos Aires, Chaco and Corrientes Provinces. It should be noted that structures were observed that were not seen in the original description of *R. elegans*.

Materials and Methods

The study area is located Northeast of San Miguel de Tucumán city, on the Eastern slope of San Javier saws. It corresponds Pedemonte located between 400–700 m a.s.l. [19]. In the lowest zone of

the Yungas, there is a warm and humid climate influenced by the Chaco region [20]. Vegetation corresponds to the “Mountain Jungles” or “Yunga” [21]. It is one of the ecosystems with the greatest biodiversity and fragility north-western Argentina [22].

Three adult (2 females, 1 male), weight (68.2–145 g); snout-vent (8.5–10.5 cm) of *Rhinella arenarum* were collected by hand at Lomas de Tafí (26°47'10.1"S; 65°14'0.0"W; 495 m a.s.l.), Tafí Viejo Department, Tucumán Province, Argentina, in March 31, 2019 were examined for helminths. They were fixed in neutral buffered 10% formalin before preservation in 70% alcohol. The body cavity was opened by a longitudinal incision from vent to throat, and the lungs and digestive tract were removed, opened, and searched for helminths under a dissecting microscope. The liver, gall bladder, reproductive system, and body cavity were also examined. Nematodes were cleared in lactophenol, and examined with a light microscope. Drawing were made using a camera lucida. Measurements are given in millimetres unless otherwise indicated and are presented as mean SD followed by range in parentheses. Nematodes and toads were deposited in the Colección Helminológica (FML-N-FML# 07780 y CH-N-FML#07781) and Colección Herpetológica (FML#30524 at 30527) Fundación Miguel Lillo, Miguel Lillo 251, (4000) San Miguel de Tucumán, Argentina, respectively.

The toads with which the work was performed, was manipulated taking into account the international protocols “Guide for the Care and Use of Laboratory Animals of the National Institutes of Health” [23].

Results

The analysis of lungs of *R. arenarum* indicated the presence of 100 gravid female nematodes, which were identified as *R. elegans*.

Family Rhabdiasidae Railliet, 1915

Genus *Rhabdias* Stiles & Hassall, 1905

Rhabdias elegans Gutierrez, 1945 (Fig. 1)

Measurements based on 10 gravid specimens. Body cuticle irregularly inflated along entire body, inflation more pronounced in cephalic and tail regions (Fig. 1A). Body length of parthenogenetic female 6.00 ± 0.70 (5.00–7.00). Maximum width 0.40 ± 0.04 (0.33–0.48). Anterior end rounded, posterior end tapered. Oral opening without defined lips and with pair of small amphids (Fig. 1B). Short buccal cavity cup shaped, round in apical view; 0.01

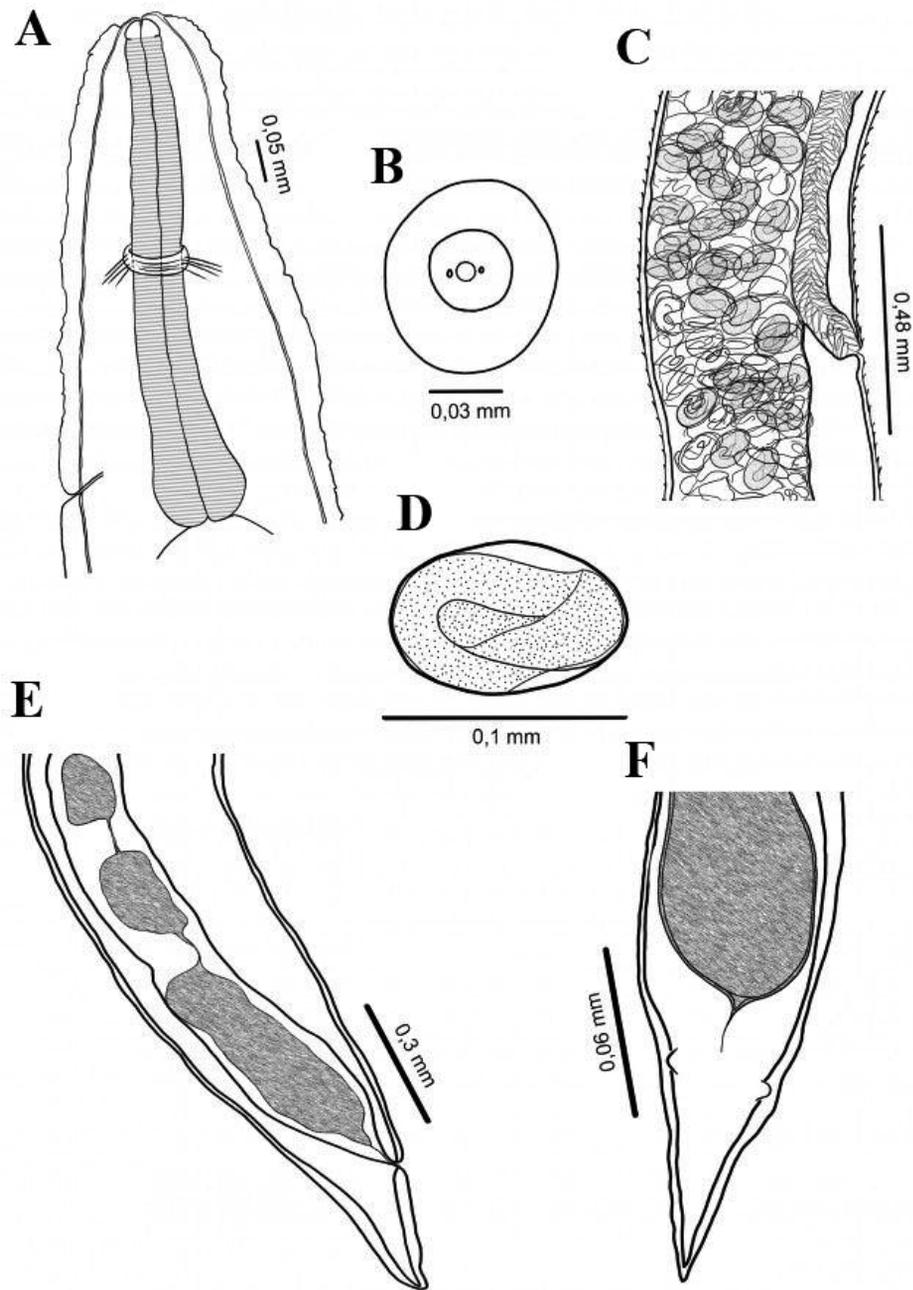


Figure 1. *Rhabdias elegans*

A: female, anterior end, lateral view; B: female, apical view; C: female, vulva, lateral view; D: egg; E: female, posterior end, lateral view; F: female, posterior end, ventral view.

± 0.003 (0.01–0.02) length, 0.02 ± 0.003 (0.01–0.02) width. Claviform oesophagus 0.35 ± 0.03 (0.31–0.40) length, maximum width 0.05 ± 0.006 (0.04–0.06) at posterior end. Nerve ring and excretory pore 0.17 ± 0.03 (0.10–0.20) and 0.31 ± 0.008 (0.30–0.32) from anterior end, respectively. Excretory duct short, excretory glands indistinct. Nerve ring and excretory pore difficult to distinguish (Fig. 1A). Width of intestine at oesophagus intestinal junction 0.045 ± 0.007

(0.03–0.05). Intestine filled with brown or black contents. Slightly post-equatorial position of the vulva, 2.78 ± 0.42 (2.36–3.20) from posterior end, distinct nonsalient lips (Fig. 1C). Amphidelphic ovaries begin about body midpoint with occasional short overlap. Ovaries straight, lying along intestine. Oviducts fold back upon themselves. Uteri wide, thin walled, filled with numerous eggs; egg shell thin, smooth, hyaline; larvated eggs near vulva 0.10 ± 0.01 (0.065–0.10) length 0.05 ± 0.005 (0.04–0.05)

Table 1. Selected characteristics of parthenogenetic females of *Rhabdias* species (mm)

	<i>Rhabdias elegans</i> Gutierrez, 1945 [11]	<i>Rhabdias füelleborni</i> Travassos, 1928 [13]	<i>Rhabdias füelleborni</i> Travassos, 1928 [11]	<i>Rhabdias elegans</i> present study
Host:	<i>Rhinella arenarum</i>	<i>Rhinella schneideri</i>	<i>Rhinella marina</i>	<i>Rhinella arenarum</i>
Locality:	Buenos Aires	Corrientes	Brazil	Tucumán
Total length	4.55-9.5	8.55-12.0	12.6-17	5-7
Maximum width	0.27-0.357	0.35-0.45	0.431-0.473	0.33-0.48
Oesophagus length	0.314-0.49	0.46-0.65	–	0.31-0.40
Oesophagus width	0.04-0.05	0.05-0.075	–	0.04-0.06
Length oral cavity	0.007	–	0.009	0.01-0.02
Width oral cavity	0.007	–	0.009	0.01-0.02
Tail	0.255-0.400	0.25-0.45	0.615-0.664	0.24-0.34
Vulva-PE	2.4-4.4	–	–	2.36-3.2
Nerve ring-AE	0.045-0.086	0.19-0.35	0.198	0.10-0.19
Length egg	0.091-0.112	0.097-0.16	–	0.065-0.1
Width egg	0.052-0.059	0.048-0.08	–	0.04-0.05
Excretory pore-AE	–	–	–	0.30-0.32
Oesophagus-intestinal junction	–	–	0.15-0.16	0.03-0.05

PE - posterior end; AE - anterior end

wide (Fig. 1D). Tail conical, 0.30 ± 0.03 (0.24–0.34) length, narrowing to sharp point (Fig. 1E). Tail supporting 1 pair of lateral sessile papillae located 0.24 ± 0.03 (0.20–0.27) posterior end (Fig. 1F).

Discussion

The females of *R. elegans* examined in this study are closely related to the original description of *R. elegans* by Gutierrez [11] (Table 1) when considering, accentuated expansions of the cuticle, especially in the anterior and posterior regions of body, slightly post-equatorial position of the vulva, body length, absence of lip structures, claviform oesophagus length, length and width of eggs similar, and numerous larvated eggs in the uterus. It should be noted that the excretory pore, apical region and lateral sessile papillae in tail were first described in this study. Likewise, it is compared with *R. füelleborni* because Gutierrez [11] observed that the external morphological characters of both species did not differ enough and according to our observations the isolated specimens of *R. arenarum* of Lomas de Tafi correspond to *R. elegans* and not to *R. füelleborni*. Also in table 1 it is compared to *R. füelleborni* according to González and Hamann [13]. It could be

that the specimens determined as *R. truncata* by Schuurmans-Stekhoven [7] correspond to *R. elegans* but we cannot verify it because the author did not deposit the material in any collection. For all the above, we affirm that the distribution of *R. elegans* in Argentina is extended to the Northwest (Tucumán), since previous studies mention it in the Northeast (Chaco and Corrientes) and centre (Buenos Aires) regions. The three valid species of *Rhabdias* in Argentina are: *R. elegans*, *R. füelleborni* and *R. cf. sphaerocephala*. The information presented in this study represents an important contribution to the parasitological knowledge in *R. arenarum* from north-western Argentina.

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