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***Rhabdias bufonis* (Rhabdiasidae) from the lung of the African common toad, *Amietophrynus regularis* (Bufonidae) in Egypt. New data on the basis of light and scanning electron microscopic study**

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Background and Aims. *Rhabdias bufonis* (Rhabdiasidae) is one of the highly pathogenic nematode parasites infecting the lung of amphibians. The present study provides the morphological description of this nematode isolated from the lung of the African common toad, *Amietophrynus regularis* collected from its natural habitat; the damp, moist fields and gardens at Giza governorate, Egypt. The description was based on the data obtained from light and scanning electron microscopic examination. **Methods.** Forty specimens of the examined animals were collected during the period from March to August 2017. At necropsy, the toads were dissected ventrally from the cloacae region to the anterior end and all organs were examined searching for helminthes using a stereomicroscope. **Results.** Of 40 examined specimens 14 (35%) were found to harbor a large number of this parasite. All the recovered worms were females and measuring 3.22-9.86 mm long and 0.09-0.048 mm wide at mid body. The anterior end was blunted while the posterior one was tapered. The body was covered by a delicate inflation of the cuticle strongly folded on its surface. **Conclusions.** The SEM study presented new details regarding the cephalic end of this nematode which was not identified in the previous studies, of them, a slit-like mouth surrounded by two pairs of lateral papillae and two amphids. Also, three pairs of cuticular inflation supporting the area around mouth opening were observed

1 ***Rhabdias bufonis* (Rhabdiasidae) from the lung of the African common toad,**
2 ***Amietophrynus regularis* (Bufonidae) in Egypt. New data on the basis of light**
3 **and scanning electron microscopic study**

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11 **ABSTRACT**

12

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14 parasites infecting the lung of amphibians. The present study provides the morphological description of
15 this nematode isolated from the lung of the African common toad, *Amietophrynus regularis* collected
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27 papillae and two amphids. Also, three pairs of cuticular inflation supporting the area around mouth
28 opening were observed.

29 **Key words:** *Rhabdias bufonis*, Nematoda, *Amietophrynus regularis*, Bufonidae, cuticular inflation,
30 papillae, morphology, morphometry

31 **INTRODUCTION**

32 Studies on the helminth fauna of amphibians have received much attention in the recent years (Dusen &
33 Oguz, 2010; Akani et al., 2011; Santos et al., 2013). The recent interest in amphibian parasites stems from
34 the declining in amphibian populations (Johnson et al., 2001; Ibrahim, 2008; Iyaji et al., 2015). Nematodes

35of the genus *Rhabdias* (Stiles & Hassall, 1905) are a large group of lung-dwelling parasites of amphibians
36and reptiles. So far, more than 80 nominal species of *Rhabdias* have been described in the Lissamphibia
37and Squamata (Kuzmin & Tkach, 2014; Kuzmin, Preezz & Junker, 2015). The life cycles of these
38nematodes alternate between parasitic and dioecious free-living generations. Parasitic individuals are
39protandrous hermaphrodites parasitizing the lungs of amphibians and reptiles (Anderson, 2000; Kuzmin,
402013; Tkach, Kuzmin & Snyder, 2014). Of about 40 species of *Rhabdias* parasitizing anuran hosts, eight
41species of them of four families have been reported from Afrotropical region (Junker et al., 2010). In
42Egypt, three different *Rhabdias* species were reported from amphibian hosts, *Rhabdias bufonis* (Moravec,
43Barus & Rysavy, 1987), *Rhabdias aegyptiaca* (Elgarhy & Garo, 2006) and *Rhabdias* sp. (left unnamed)
44from the lungs of maculated toad *Bufo regularis* at Aswan governorate (Saad, Khalifa & Mostafa., 2009).
45Due to their similarity in the morphological characteristics such as the labial structures, the position of
46vulva , the tail shape and absence of males in parasitic generations, the differentiation between species
47belonging to the genus *Rhabdias* became complicated (Tkach, Kuzmin & Pulis, 2006; Kuzmin , Tkach &
48Brooks, 2007; Saad, Khalifa & Mostafa., 2009). However, the morphology of the anterior end of some
49*Rhabdias* species yields some characters appropriate for the species diagnostics (Kuzmin, Tkach &
50Vaughan, 2005). Several authors see. (Tkach, Kuzmin & Pulis, 2006; Kuzmin , Tkach & Brooks, 2007;
51Martinez-Salazar , Leo'n & Olea, 2009) suggested that, additional taxonomical tools like; molecular
52biology techniques, scanning electron microscopy, host specificity and geographic distribution are
53required to diagnose species of this genus. The African common toad, *Amietophrynus regularis* (Reuss,
541833) is widespread in the Sub-Saharan Africa, with its range extending to the oases in Algeria and
55Libya as well as to northern Nilotic Egypt (Frost, 2014). According to Rodel (2000) and Ibrahim (2008)
56the prey of *Amietophrynus regularis* often includes beetles, ants, bugs, insects, grubs, slugs, worms and
57other invertebrates. The more passive hunting toads apparently select their prey by means of its size and
58activity. The present study provides new morphological details for the nematode *Rhabdias bufonis*
59isolated from the lungs of the African common toad, *Amietophrynus regularis* by light and scanning
60electron microscopy.

61 MATERIALS & METHODS

62 During the period from March to August 2017, forty specimens of the African common toad,
63 *Amietophrynus regularis* were collected by hand or noose from its natural habitat; the damp, moist fields
64 and gardens at Giza governorate, Egypt. Animals were subjected to euthanasia using 20% benzocaine gel
65 (Anbesol, Pfizer, Inc., New York, New York). Each specimen was subsequently necropsied and all

66organs were examined searching for helminthes using a stereomicroscope. Where necessary, All animal
67procedures was carried out according to the regulatory laws regarding experimental Animal Ethics
68Committee, Faculty of Science, Beni-Suef University, Beni-Suef, Egypt (Ethical Approval Number:
692015/10).The encountered nematodes from the lungs were heat fixed in 10% neutral buffered formalin for
7015 min and then preserved in 70% ethanol in 5% glycerol solution to avoid sudden drying. Finally,
71samples were transferred to lactophenol for clearance. The prepared samples were examined using
72differential interference contrast (DIC) light microscopy with digital image analysis system (analysis auto
735.0). Drawings were made with the aid of a camera lucida. Measurements are in millimeters unless
74otherwise stated. For scanning electron microscopy, samples were fixed in 4% glutaraldehyde in 0.1 M
75sodium cacodylate buffer, then washed in the same buffer, and dehydrated in alcohol series. Samples
76were then processed in a critical point drier “Bomer-900” with freon 13, sputter-coated with gold-
77palladium in a Technics Hummer V, and finally examined with an Etec Autoscan at 20 kV Jeol scanning
78electron microscope.

79RESULTS

80*Rhabdias bufonis* (Schrank, 1788)

81**Diagnosis** (based on 28 gravid specimens) (Figs.1-9)

82Light microscopic examination revealed that all the recovered parasites were gravid females. They
83possessed a medium sized and elongated body, measured 3.22-9.86 mm long and 0.09-0.48 mm wide at
84mid body. The anterior end was blunted while the posterior one was tapered. The body covered by a
85delicate inflation of the cuticle strongly folded on its surface as indicated by and scanning electron
86microscopy. A simple, slit like oral opening was found at the anterior extremity, its lipless edges bordered
87with tiny cuticular elevations, which radiate outwards from the opening. Two pairs of lateral cephalic
88papillae were located around the oral opening and two amphids were observed. Buccal capsule was cup-
89shaped; measured 0.013-0.041 mm in length. Esophagus throughout muscular being expanded at its
90posterior end, measured 0.27-0.63 mm in length and its maximum width 0.25-0.5 mm (0.3 mm). Nerve
91ring was 0.168-0.240 from the anterior end. Anterior end of intestine was wider than esophageal bulb.
92Reproductive system was amphidephlic. Ovaries were almost straight. The uteri were located anterior and
93posterior to the vulva. Vulva was nearly equatorial 1.47-5.98 from the anterior end. Uterus filled with
94large number of eggs. Eggs were oval in shape, measured 0.120-0.132×0.039-0.812 mm in size and some
95of them were with fully developed larvae inside. The tail was comparatively short, conical and sharply
96pointed. Tail length was 0.131-0.435 mm long.

97Taxonomic Summary

98Type species: *Rhabdias bufonis* (Schrank, 1788)

99Type-host: *Amietophrynus regularis* (Family: Bufonidae)

100Site of infection: Lungs

101Type locality: Giza governorate, Egypt

102Prevalence: 14 out of 40 (35%) hosts were infected.

103Deposition: Permanent slides gravid females were deposited at the parasitology division, Zoology
104department museum, Faculty of Science, Cairo University, Egypt.

105DISCUSSION

106The differentiation among species belonging to the genus *Rhabdias* (Stiles & Hassall, 1905) is often
107complicated due to their high morphological uniformity (Chu, 1936; Baker, 1978; Tkach, Kuzmin &
108Pulis, 2006; Kuzmin, Tkach & Brooks, 2007). Within the species of *Rhabdias*, the shape of cephalic end
109represents an important character for the differentiation between species and genera of the family
110Rhabdiasidae (Railliet, 1915) and yields characters suitable for species diagnostics (Kuzmin, Tkach &
111Vaughan, 2005). Genus *Rhabdias* can be divided into three groups based on the morphology of their head
112end according to Baker (1978): species without lips, species with six lips and species with two lateral
113pseudolabia. Three different *Rhabdias* species were reported from amphibian hosts belonging to family
114Bufonidae, represented by a single genus, *Amietophrynus* (formerly included in *Bufo*), in the Afrotropical
115region. These were *R. bufonis* (Moravec, Barus & Rysavy, 1987), *R. picardiae* (Junker et al., 2010) and *R.*
116*africanus* (Kuzmin, 2001). By comparing the recovered parasite with different species of the same genus
117previously recorded in the Afrotropical region, it was found that the present species is morphometrically
118more or less different from the comparable species and the only similar species was *Rhabdias bufonis*
119described previously from the same host by Moravec, Barus & Rysavy (1987). Both species resemble
120each other by possessing similar head structure (absence of lips, presence of small submedian mouth
121papillae); the intestinal apex is broader than the esophageal base and the equatorial position of vulva.
122*Rhabdias bufonis* recorded in the present study has a longer body (9.86 mm vs. 8.35 mm), a shorter
123oesophagus (270-630 vs. 690-790) and a longer buccal capsule (13-41mm vs. 8-10 mm) than *R.*
124*picardiae* and differ from *R. africanus* in the body size and the absence of two lateral pseudolabia. The
125present form, differs from the description of *Rh. bufonis* given by Travassos (1930) and Hartwich (1972)
126in the body length (3.22-9.86 vs. 9-12) and in the size of buccal capsule which is of diagnostic
127importance (Lhermitte- Vallarino et al., 2008, Junker et al., 2010). The current study introduced new

128morphological characteristics for the nematode *Rhabdias bufonis* isolated from the lung of *Amietophrynus*
129*regularis* based on scanning electron microscopy. One of the most important features of *Rhabdias bufonis*
130is the cephalic end structure which has overlooked by previous studies. In Egypt, Only three different
131species of the genus *Rhabdias* have been described based on the light microscopic studies and these
132species are: *Rhabdias bufonis* (Moravec, Barus& Rysavy, 1987), *Rhabdias aegyptiaca* (El Garhy & Garo,
1332006) and *Rhabdias* sp. (Saad, Khalifa & Mostafa, 2009). The parasite recovered in the present study
134differs from *Rhabdias aegyptiaca* where the later possesses six lips with unique arrangement around
135mouth opening, cuticle lined pores on its lateral surface and the size of the buccal capsule which is of
136diagnostic importance (Lhermitte-Vallarino et al., 2008; Junker et al., 2010). Also, it differs from
137*Rhabdias* sp., where it has four developed lips and two lateral teeth on the mouth opening, the cuticular
138swelling that covers the whole body and the post-equatorial position of vulva. *Rhabdias* species that were
139recovred from a bufonid member with a sub-Saharan distribution in Egypt were compared in Table (1).

140CONCLUSION

141According to the current study that depended on light and scanning electron microscopy, it is concluded
142that there are new data were obtained and recorded for the nematode, *Rhabdias bufonis* infecting the
143African common toad which is the second study reported for this parasite in Egypt representing an
144important reference for upcoming studies.

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Explanation of figures

256 **Fig.1: (A-D)** Light micrographs of *Rhabdias bufonis*, (A) Females, left and right lateral views;
257 (B) the anterior part, OE esophagus; (C) The uterine region filled with eggs (EG); (D) Tail end.
258 **(E-H)** Scanning electron micrographs of the parasite, (E, F) Apical views of the cephalic end, PA
259 symbolized for the cephalic papillae, CI for the three pairs of cuticular inflation around mouth
260 (MO) and (AM) for amphids; (G) Cuticular inflation (CI); (H) Posterior end of female, TS
261 symbolized for the transvers striation of cuticle.
262 **Fig. 2.** Line drawings of *Rhabdias bufonis* female paratype: (A) the anterior part of the body,
263 lateral view; (B) Apical view of the cephalic region, PA symbolized for the cephalic papillae, CI

264 for the three pairs of cuticular inflation around mouth and AM for amphids; (C) Loop of anterior
265 genital tube; (D) Tail end, lateral view.

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Figure 1

Figs. 1: Light micrographs and Scanning electron micrographs of *Rhabdias bufonis*,

(A-D) Light micrographs of *Rhabdias bufonis*, (A) Females, left and right lateral views; (B) the anterior part, OE esophagus; (C) The uterine region filled with eggs (EG); (D) Tail end. **(E-H)** Scanning electron micrographs of the parasite, (E, F) Apical views of the cephalic end, PA symbolized for the cephalic papillae, CI for the three pairs of cuticular inflation around mouth (MO) and (AM) for amphids; (G) Cuticular inflation (CI); (H) Posterior end of female, TS symbolized for the transvers striation of cuticle.

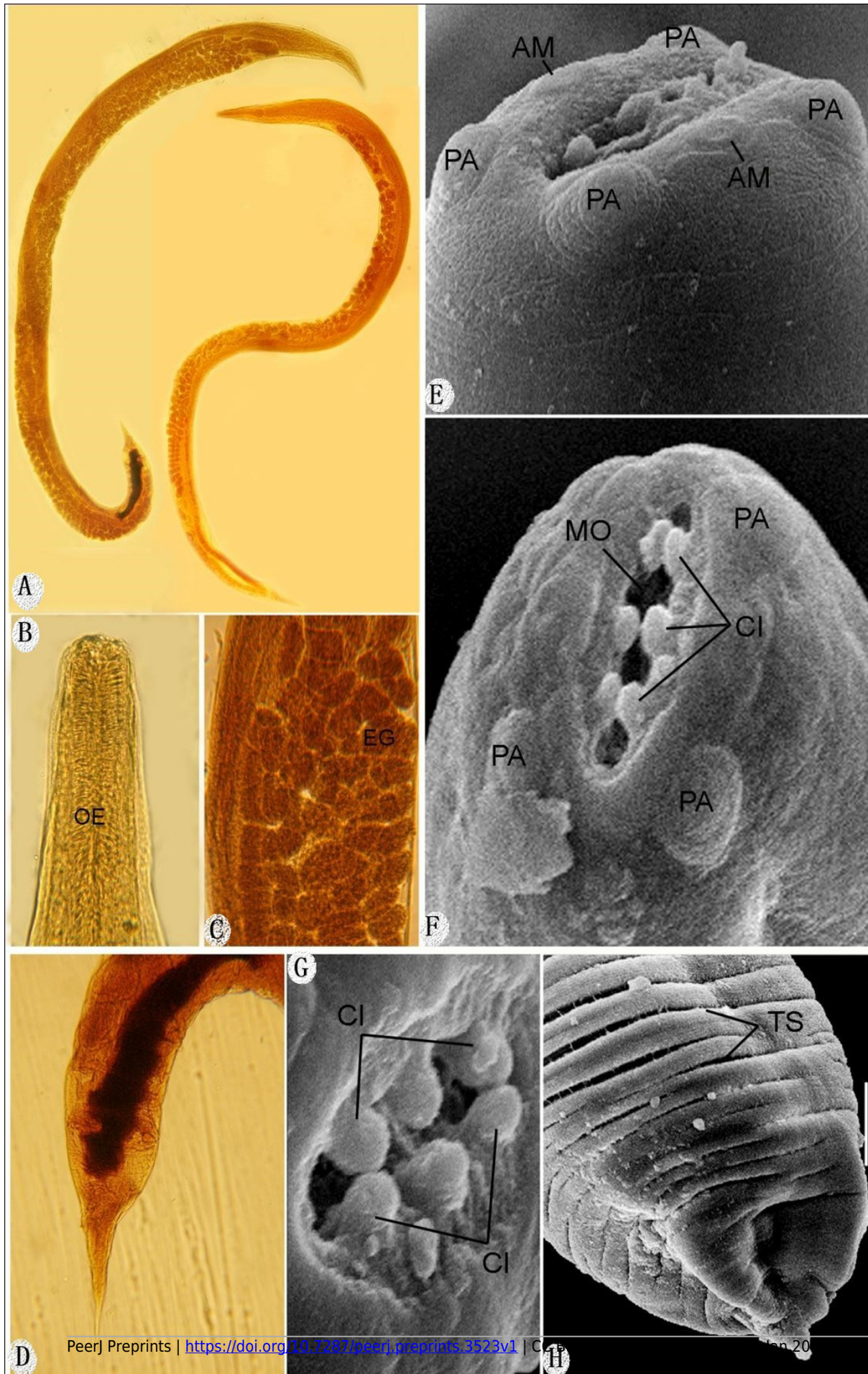


Figure 2

Fig. 2. Line drawings of *Rhabdias bufonis* female paratype

(A) the anterior part of the body, lateral view; (B) Apical view of the cephalic region, PA symbolized for the cephalic papillae, CI for the three pairs of cuticular inflation around mouth and AM for amphids; (C) Loop of anterior genital tube; (D) Tail end, lateral view.

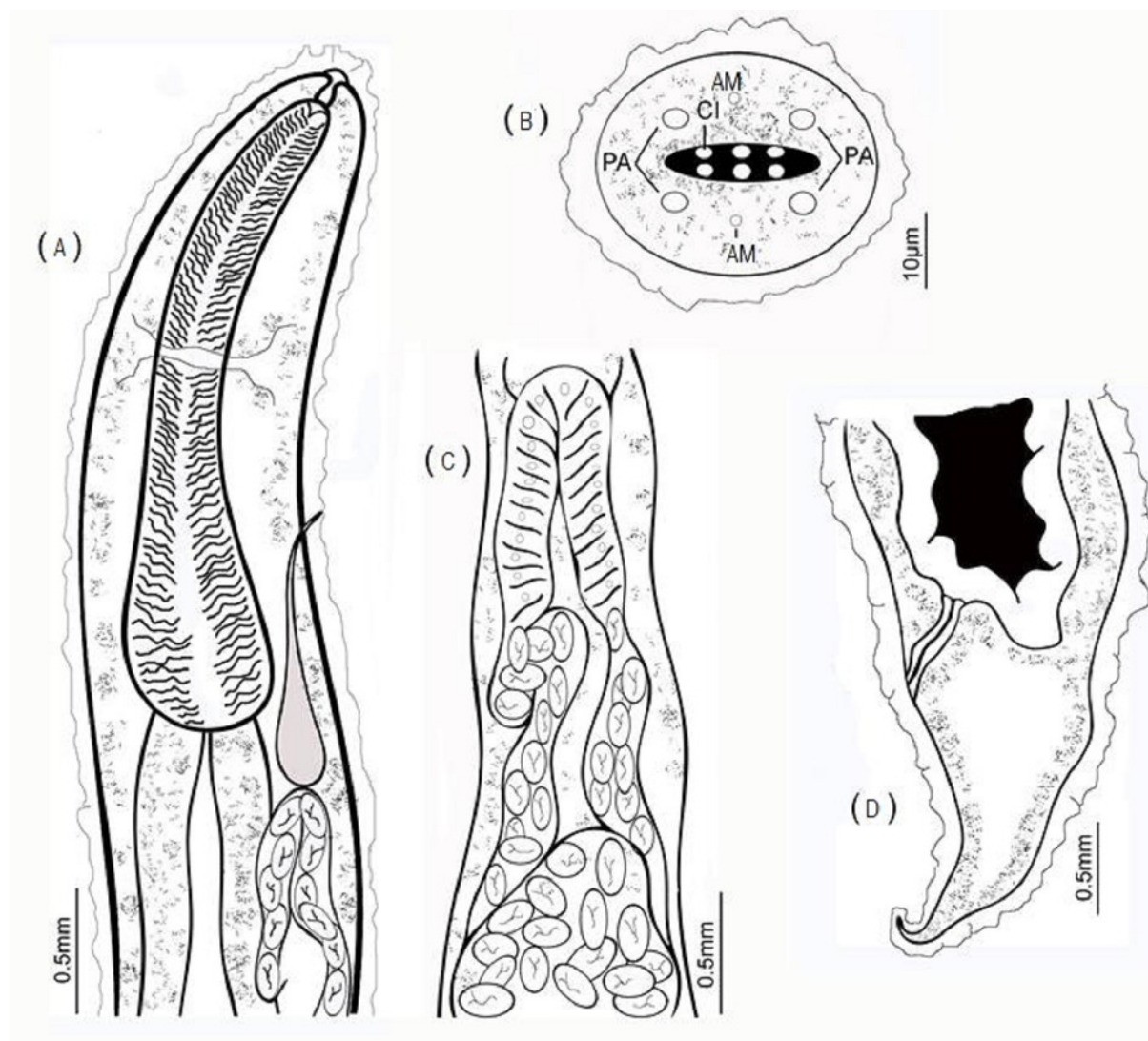


Table 1 (on next page)

Table 1: Comparative measurements between the present species and members of the genus *Rhabdias* previously reported in Egypt, measurements in mm, otherwise stated.

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Parasite	<i>Rhabdias bufonis</i> (Moravec <i>et al.</i> 1987)	<i>Rhabdias aegyptiaca</i> Elgarhy, and Garo, 2006	<i>Rhabdias sp</i> Saad <i>et al.</i> (2009)	<i>Rhabdias bufoni</i> present study
Length	2.99-13.02	8-10	5.2-12.5	3.22-9.86
Width	0.136-0.476	0.3-0.5	0.2-0.7	0.09-0.48
Buccal capsule (length)	0.015	-	0.01-0.032	0.013-0.041
Teeth	Absent	Absent	Two lateral teeth	Absent
Esophagus (length)	0.288-0.510	550µm	0.25-0.5 (0.3)	0.27-0.63
Vulva	Equatorial	Equatorial	Post-Equatorial	Equatorial
Tail	0.144-0.420	170-200µm	0.23-0.4(0.3)	0.131-0.435
Egg size	0.117-0.144× 0.051-0.72	66µm in length	0.1-0.12×0.06-0.08	0.120-0.132× 0.039-0.812

Table 1: Comparative measurements between the present species and members of the genus *Rhabdias* previously reported in Egypt, measurements in mm, otherwise stated.