

## A Lungworm Nematode from Amphibian Hosts at Aswan Governorate, Egypt

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**Abstract:** Females of *Rhabdias* species were obtained from the lungs of the maculated toad *Bufo regularis* in Aswan, Egypt. The parasitized protandrous hermaphroditic female was described in details and was compared with the previously described species from Egypt. The present specimen was characterized by possessing a swelling cuticle at the anterior end, which extended to the posterior end; having four lips and two teeth; small buccal capsule; short oesophagus and large egg size. The present authors preferred to stop the identification of the parasite at the generic level for more studies on its life cycle to obtain males. Also, it was noticed that a lot of new species of *Rhabdias*, were described during this decade all over the world based on minor characters as shape and width of oesophagus in relation to intestine; a character which was found to be affected and changed by ageing. Currently, authors suggested that those species should be revised on basis of the life cycle and on the male characters as well as females' characters.

**Key words:** *Rhabdias* · *Bufo* · Female · Parthenogenetic · Protandrous · Aswan

### INTRODUCTION

Family Rhabdiasidae includes about 70 nominal species and possessing the alternation of two generations (heterogony) in their life histories. One generation is hermaphroditic and inhabits the lungs of amphibians and some reptiles. The other one is gonochoristic and dwells in the hosts' faeces. The comprehensive information on the development of *Rhabdias bufonis* (Schrank, 1788) Stiles and Hassall, 1905 is expanded. In this species, eggs of parasitic hermaphrodites pass from the host lungs into the intestine, accumulate in colon and, thereafter, are cast out of the host organism in faeces. The free-living stages develop in the droppings. Larvae from the eggs of hermaphrodites reach maturity as males and females of gonochoristic generation. The latter give rise to the hermaphroditic generation larvae which develop in droppings up to the third stage. At this stage the larvae become infective and are able to continue development only after the penetration into the host [1-2]. Fifty-nine *Rhabdias* species have been described; thirteen of them were distributed on Ethiopian, Nearctic, Neotropical, Oriental and Palearctic realms were described in recent years [3-4]. There are few studies dealing with parasites of amphibians and reptiles in Egypt [5-8]. During this study a species of *Rhabdias* was collected from endemic toad from Aswan as part of a biodiversity inventory of parasites of amphibians.

### MATERIALS AND METHODS

850 toads were collected by hand and then deposited in herpetological bags for transport. Animals were killed. Each host was subsequently necropsied and the organs were examined for helminths using a stereoscope. Lung worms were initially placed in saline (0.65%); afterwards they were killed by immersion in hot 70% ethanol and stored in 70% ethanol containing 5% glycerin. Nematodes were cleared with glycerin in temporary preparations. Drawings were made with the aid of a drawing tube. Measurements were given in micrometers unless otherwise stated. Minimum and maximum limits were given followed by mean value in parentheses. Some photomicrographs were taken from unstained fixed specimens. Ault females were deposited in the Department of Zoology, Faculty of Science, Aswan and the Department of Parasitology, Faculty of Medicine, Assiut, Egypt.

### RESULTS

The present species was collected from the lungs of the maculated toad *Bufo regularis*. All worms were only females. The percentage of infection was 25.9% (220 of 850 specimens) and the worm burden was 1-6 per host.

**Females (Based on 30 Gravid Worms) (Fig. 1-2 and**

**Photos A-C):** Living worm was brownish or black in colour and elongated in shape. The body measured 5.2-12.5 mm (8.8 mm) long and 0.2-0.7 mm (0.5 mm) wide at midbody. Anterior end was blunted and posterior end was tapered. Body cuticle inflated. Cuticle at anterior end was forming distinct swelling (alae), abrupt on anterior surface and gradually declining posteriorly, covering the whole body. The mouth opening was surrounded by four developed lips (two subdorsal and two subventral) and two lateral teeth. Each lip was provided with a small single papilla on the top. The mouth opening led to a cup-like buccal capsule which measured from 0.01-0.032 mm (0.025 mm) long. It opened into a muscular oesophagus (the corpus) which measured 0.25-0.5 mm (0.3 mm) in length; the posterior part of the oesophagus was slightly swollen forming a club-shape. (Fig. 1; Photo A). There was a pair of cervical glands which extended beside from the posterior extremity of buccal capsule till a little behind the nerve ring. They measured 0.07-0.1 mm (0.08 mm) in length. Nerve ring was encircling oesophagus just posterior to its muscular portion, at 0.09-0.11 mm (0.1 mm) far from the anterior end of the worm. Excretory pore situated at level of oesophagus midlength. Excretory duct thin and short, almost straight or slightly curved ventrally. Two excretory glands prominent, subventral in position, equal in size, much shorter than oesophagus, their posterior ends situated behind oesophageal-intestinal junction, at 0.225-0.4 mm (0.3 mm) from the anterior extremity. Anterior end of intestine was wider than oesophageal bulb. Intestinal lumen was narrow in anterior part, gradually widening posteriorly. Pre-rectal muscular sphincter was obvious. Rectum short, straight, lined with thick cuticle. Intestine was black in colour due to its contents. Genital system was amphidelphic with equal limbs. Ovaries were almost straight, with proximal ends overlapping level of vulva. Two uteri were joined and opened into oviduct. The uteri were located anterior and posterior to the vulva, which was located postequatorial. Vulva was at 3.75-5.5 mm from the anterior end and 3.5-4.95 mm from the posterior end. The uterus was filled with large number of eggs. Each egg was oval in shape, measured 0.1 - 0.12×0.06 - 0.08 mm (0.115×0.07 mm) in length and width respectively (Photo C). Some of eggs were containing fully developed larvae. Anus was located at 0.23 - 0.4 mm (0.3 mm) from the posterior end. The tail was pointed and measured 0.23-0.4 mm (0.3 mm) from the anus. Three pairs of papillae were located posterior to the anal opening. (Fig. 2, Photo B). Post-anal ventral inflation of body wall present in all specimens.

**DISCUSSION**

The present species was parasitic on the lung of the amphibian host, *Bufo regularis*. Only females were collected from the lung of their hosts, where the worms were protandrous hermaphrodite, which have a parthenogenetic mode of reproduction [9-10]. Members of the genus *Rhabdias* Stiles and Hassall, 1905 are widely distributed lung parasites of amphibians and reptiles. Twenty three species with type species *R. bufonis* (Schrank, 1788) Stiles and Hassall, 1905 were reported by Yamaguti [9]. The facultative nature of the parasite *R. bufonis* with full description of free living generation was described by Baylis and Doubney [11]. The morphology of the buccal extremity of the parasitic generation in Rhabdiasidae is poorly known, although it forms an important basis for separation of species and parasitic generation [12]. The differentiation of *Rhabdias* spp. is often fairly complicated due to the morphological uniformity of species and absence of males in parasitic generation [12-13]. Among the metric characters, buccal capsule dimensions and egg size appear to be stable regardless of individual body size. Usage of esophageal length and width in different regions as well as tail length and body width for species differentiation is problematic because these parameters depend on overall body size, which varied considerably in the adult parasitic generation of all *Rhabdias* species. Relative lengths of esophagus and tail can be useful as additional characters for species differentiation, particularly if these characters are examined in relationship with body length [14]. This approach was used for comparison of five species parasitic in amphibians and in some cases the graphical approach was effective for differentiation among two or more species with overlapping values of body length and relative length of esophagus and tail [14]. The relative length of the esophagus proved to be less variable than that of the tail and thus more appropriate for differentiation between *Rhabdias* of amphibians [12, 15]. The present authors noticed that minored criteria such as anterior position of nerve ring in relation to oesophagus anterior end, width of intestine in relation to the oesophageal bulb [15] were used in species differentiation, which in controversy with previous work [12, 14]. Regarding the criterion of the intestinal width in relation to oesophageal bulb, it was noticed that the corpus, metacarpus (oesophagus) and intestine of *Strongyloides ratti* was strikingly atrophied in both free living and parasitized stage due to ageing [16]. Although, the present authors noticed a lot of new species of

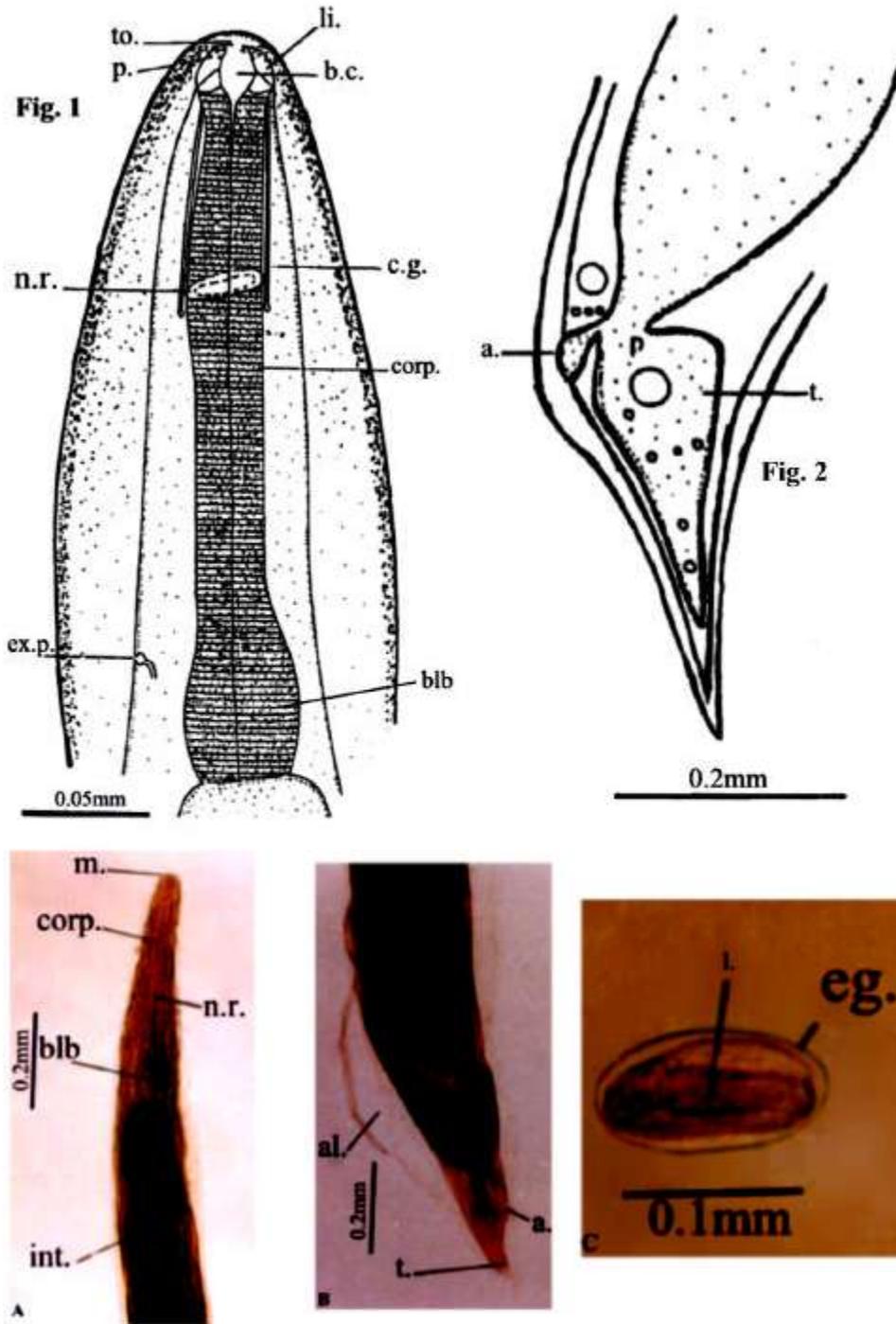


Fig. 1: Camera Lucida drawings of the anterior part of body showing its structure of it; b.c. buccal capsule; p. papilla; li. Lip; to. tooth; corp. corpus; blb bulb; c.g. cephalic gland; exc.p. excretory pore; a. anus; t. tail; al. alae.

Fig. 2: Camera Lucida drawings of the posterior part of body showing its structure  
Photomicrographs of:

- A- Anterior part of the body, m. mouth.
- B- Posterior part of the body.
- C- Magnified egg (eg.); showing the first larva (l.)

Table 1: Comparison between *Rhabdias egyptia* and the present species

Character	<i>Rhabdias egyptia</i>	Present material
Length	5.5-13.6(9.2)	5.2-12.5(8.8)
Width	0.2-0.6(0.54)	0.2-0.7(0.5)
Buccal capsule (Length)	0.01-0.038 ( 0.029 )	0.01-0.032 (0.025)
Teeth	Present	Present
Oesophagus (Length)	0.3-0.6 (0.54)	0.25-0.5 (0.3)
Vulva	Equatorial	Post-Equatorial
Tail length distance	0.24-0.38(0.3)	0.23-0.4(0.3)
Egg-size	0.07-0.1×0.03-0.05	0.1-0.12×0.06-0.08

*Rhabdias* have been erected during this decade (*Rhabdias tobagoensis* Moravec and Kaiser, 1995; *Rhabdias tarichae* Kuzmin, Tkach and Snyder, 2003; *Rhabdias kongmongthaensis* Kuzmin, Tkach and Vaughan, 2005; *Rhabdias alabialis* Kuzmin, Tkach and Brooks, 2005; *Rhabdias bakeri* Tkach, Kusmin and pulis, 2006; *Rhabdias leonae* Martínez-Salazar, 2006; *Rhabdias nicaraguensis* Bursey, Goldberg and Vitt, 2007; *Rhabdias pseudosphaerocephala* Kuzmin, Tkach and Brooks, 2007; *Rhabdias kuzmini* Martínez-Salazar and Leo'n-Re`Gagnon, 2007; *Rhabdias manantlanensis* Martínez-Salazar, 2008) on the bases of the previously mentioned minor criteria. So, it is worthy to mention that more studies on the free living stages of these species are needed to evaluate the identity of them by describing the unparasitized males before erection of any new species and the description of parasitized females only are not enough for creating new species.

From Egypt, only three *Rhabdias* sp. were recorded: *Rhabdias* sp. [5], *Rhabdias bufonis* [6-7] and *Rhabdias egyptia* which described as a new species from lungs of *Bufo regularis* in Sohag Governorate [8]. The latter species was differed from all described species in having conspicuous alae covering the whole body and two lateral teeth on the mouth opening. So, the present material may be confirm the identity of *Rhabdias egyptia* but studies of life cycle of both species are required to confirm their identities. Differences in Table 1 could be attributed to ageing of females [16-17]. On other hand, the position of the vulva in present species was postequatorial. *R. bufonis* (Schrank, 1788) Stiles and Hassall, 1905 was differed from the present material by absence of dilated oesophageal muscular portion, anterior cuticular inflation and by less anterior position of nerve ring in relation to oesophagus anterior end.

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