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MORPHOLOGY OF PARASITIC AND FREE-LIVING ADULTS OF *RHABDIAS RUBROVENOSA* (NEMATODA, RHABDIASIDAE)

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Morphology of Parasitic and Free-living Adults of *Rhabdias rubrovenosa* (Nematoda, Rhabdiasidae). Kuzmin Yu. I. — The description of parasitic hermaphrodites, free-living males and females of *Rhabdias rubrovenosa* (Schneider, 1866) Semenov, 1929 is presented. Parasitic adults of the species are characterized by short vestibulum, absence of esophastome, pre-equatorial position of vulva and atrophy of rectum. The last two characters are less distinct in young individuals. Morphology of free-living adults of the species is similar to that in corresponding stages of other species of the genus *Rhabdias*. Free-living females of *R. rubrovenosa* had not more than 2 eggs in the uteri; 1 or 2 larvae developed inside each female.

Key words: *Rhabdias*, morphology, parasitic generation, free-living generation.

Морфология взрослых стадий паразитического и свободноживущего поколений *Rhabdias rubrovenosa* (Nematoda, Rhabdiasidae). Кузьмин Ю. И. — Представлены описания паразитических гермафродитных особей, самцов и самок свободноживущего поколения *Rhabdias rubrovenosa* (Schneider, 1866) Semenov, 1929. Взрослые особи паразитического поколения характеризуются коротким вестибулумом, свободной от пищеводной ткани ротовой капсулой, преэкваториальным положением вульвы и атрофией ректума. Последние 2 признака менее выражены у молодых особей. Для свободноживущего поколения характерно строение типичное для соответствующих стадий других видов рода *Rhabdias*. В матках свободноживущих самок этого вида наблюдалось не более 2 яиц; 1 или 2 личинки развивались в каждой самке.

Ключевые слова: *Rhabdias*, морфология, паразитическое поколение, свободноживущее поколение.

Introduction

Rhabdias rubrovenosa (Schneider, 1866) Semenov, 1929 was described from the lungs of toad *Bufo cinereus* from Germany. Semenov (1929) included this species into the list of European species of the genus *Rhabdias* Stiles et Hassall, 1905. Mazurmovich (1951) found *R. rubrovenosa* in the lungs of European common toad (*Bufo bufo*), green toad (*B. viridis*) and common spadefoot (*Pelobates fuscus*) from vicinities of Kyiv and Kanev (Ukraine). The author considered this species to be quite rare and occasionally met. Hartwich (1975) stated the parasitic generation of *R. rubrovenosa* resembled that of *R. bufonis*. The distinguishing characters of *R. rubrovenosa*, as noted by Hartwich (1975), were the pre-equatorial position of vulva and atrophy of anus and rectum. In short description of free-living males and females of *R. rubrovenosa* given by Schneider (1866; cited after Hartwich, 1975) their general similarity to those in *R. bufonis* has been mentioned.

Studying nematodes of the genus *Rhabdias* I have found *R. rubrovenosa* in the material from *Bufo* spp. and *Bombina bombina* from Ukraine and South-West of Russia. The species appeared to be quite common parasite of *Bufo viridis* from these territories. The free-living generation of *R. rubrovenosa* was obtained in laboratory cultures. The aim of this paper is to give new description of parasitic and free-living generation adults of *R. rubrovenosa* on the original material.

Material and methods

Material stored in the Department of Parasitology of the Institute of Zoology of NAS of Ukraine was studied. It included 17 samples of *R. rubrovenosa* (more than 200 specimens of parasitic generation) from 15

localities. Thirty-three specimens from several samples including fresh material from *B. viridis* were measured.

Free-living stages of development of *R. rubrovenosa* were obtained in laboratory. The cultivating method proposed by Chu (1936) was accepted. Culturing was carried out at 18–20°C, in the feces of *B. viridis* naturally infected by *R. rubrovenosa*. The description of free-living generation was made on the material from 4 samples obtained from 3 separate cultures.

Material from the collection was fixed and stored in 4% formaldehyde in saline. Experimentally obtained material was fixed in hot mixture of 70° alcohol and 5% neutral formaldehyde in 2 : 1 ratio, with the addition of glycerol (not over than 10% of mixture).

The material was cleared in pure glycerol and studied under the light microscope "Zeiss-Axiolab".

Results

Parasitic adults (fig. 1; tabl. 1). Body elongated. Head end rounded, tail end tapered. Body cuticle inflated, especially in anterior part, and covered with irregular folds. Oral opening round. Each of six reduced cephalic lips bearing a small papilla on top. Vestibulum reduced; small hemispherical buccal capsule situated close to the oral opening. Esophageal tissue surrounding the bottom of buccal capsule. Esophagus club-shaped. Nerve ring surrounding esophagus near its middle. Excretory pore situated behind the level of nerve ring. Excretory duct short and straight. Excretory glands narrow, elongated, slightly widened and rounded in the posterior portion. Intestine wide, thick-walled in anterior part and thin-walled posteriorly. Rectum in gravid specimens thin, straight, sufficiently reduced. Posterior part of intestine blind and sac-like, filled with black content and often stretched into the tail region (fig. 1, *d*). The last feature occurring mainly in the largest (and probably the oldest) worms. In several cases specimens with rupture of intestine were noted. The black filling of posterior gut was dispersed in the body cavity of these individuals. Despite this the worms were live

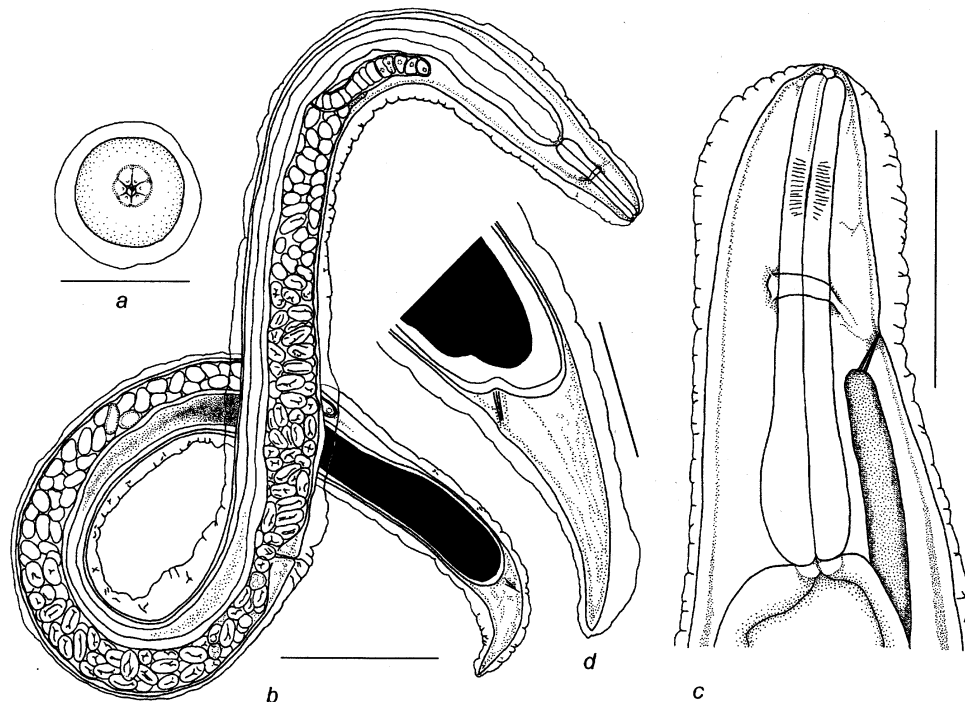


Fig. 1. Parasitic adult of *R. rubrovenosa*: *a* — head end, apical view; *b* — general view of worm; *c* — anterior part of the body, lateral view; *d* — tail end, lateral view. Scale: *a*, *c* — 0.1 mm; *b* — 0.5 mm; *d* — 0.2 mm.

Рис. 1. Взрослая особь паразитического поколения *R. rubrovenosa*: *a* — головной конец, апикально; *b* — общий вид; *c* — передняя часть тела, латерально; *d* — хвостовой конец, латерально. Масштаб: *a*, *c* — 0,1 мм; *b* — 0,5 мм; *d* — 0,2 мм.

Table 1. Morphometry of parasitic generation adults of *R. rubrovenosa* (33 specimens from *Bufo viridis*). Here and below all dimensions in micrometers

Таблица 1. Морфометрия взрослых особей паразитического поколения *R. rubrovenosa* (33 экз. из *Bufo viridis*). Здесь и далее все измерения в микрометрах

Characters	Aver.	Min.	Max.	SD	CV
Body length	7078	4000	11900	2367	33.44
Body width	265	158	398	58.13	21.97
Buccal capsule depth	7.7	6	10	1.015	13.19
Buccal capsule width	12.7	10	14	1.08	8.527
Esophagus length	355.6	307	423	31.61	8.889
The same, % to body length	5.45	3.11	7.72	1.369	25.14
Width of esophagus anterior end	33.3	30	40	2.73	8.206
Width of esophagus middle	37.1	32	44	3.083	8.3
Width of esophagus bulbus	61.6	54	78	5.869	9.531
Distance from anterior edge of esophagus to nerve ring	173.8	141	216	18.2	10.47
The same, % to esophagus length	48.91	41.5	54.76	3.52	7.197
Distance from anterior end to vulva	3180	2050	5600	1014	31.88
The same, % to body length	45.33	40.5	53.13	2.882	6.357
Tail length	315.4	208	498	77.31	24.51
The same, % to body length	4.70	3.28	7.43	1.218	25.89

and able to move and lay the eggs. Tail conical.

Genital system amphidelphic. Vulva pre-equatorial in gravid specimens, post-equatorial in younger individuals (fig. 2). Vulva lips reduced. Uteri large, thin-walled, filled with numerous eggs. Eggs located in region of vulva containing fully developed larvae. Both genital tubes reflexed in zone of oocytes. Ovaries narrow, slightly twisted, their proximal ends overlapping at level of vulva.

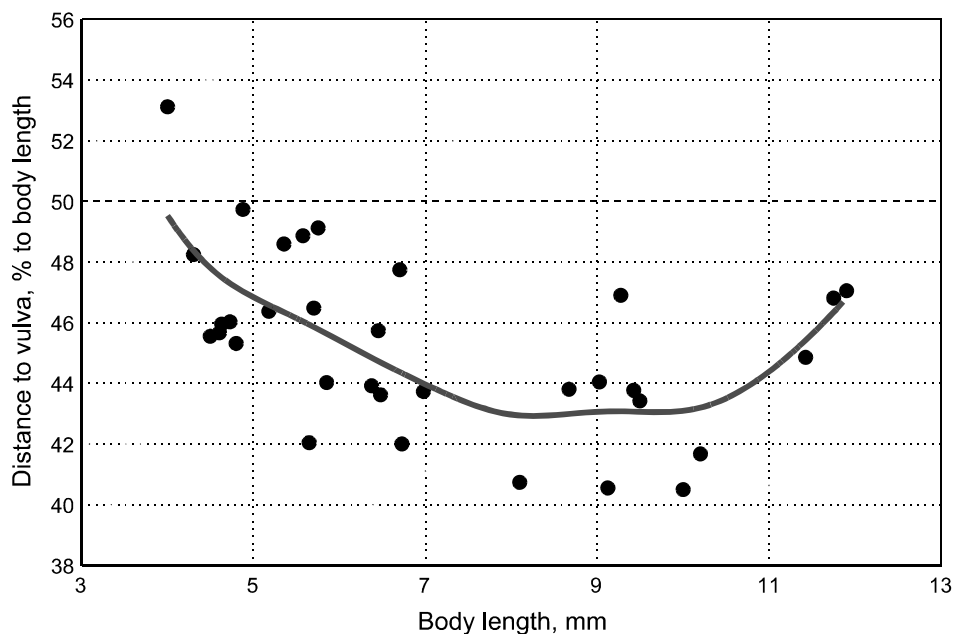


Fig. 2. Relationship of distance to vulva (% to body length) to body length in studied sample of parasitic adults of *R. rubrovenosa*. Scatterplot with the least squares line.

Рис. 2. Зависимость между расстоянием до вульвы (% от длины тела) и длиной тела в исследованной выборке взрослых особей паразитического поколения *R. rubrovenosa*.

Free-living males (fig. 2, *a, b*; tabl. 2). Body elongated, posterior part curved ventrally. Maximum body width behind its middle. Oral opening surrounded by six small lips. Stoma short, rhabditoid. Esophagus rhabditoid. Nerve ring surrounding the middle of isthmus. Cardial bulb wide, spherical. Valve in bulb present. Two pear-like subventral excretory cells situated behind the esophageal bulb. Intestine wide, thin-walled. Rectum short and thin. Tail conical, bearing a short cuticular needle on the tip. Seven pairs of small ventrolateral papillae (3 pairs preanal and 4 pairs postanal) joined by thin cuticular membrane forming reduced genital bursa.

Genital tube straight, widened in the anterior portion (spermatocyte growth zone). Sperm duct narrow. Two small lateral diverticuli present in the anterior portion of ejaculatory duct. Spicules equal, with cup-like capitulum and joined posterior ends. Gubernaculum inconspicuous.

Free-living females (fig. 3, *c-f*; tabl. 3). Body cuticle smooth and thin. Anterior part of body being of the same structure as that in males. Intestine wide, with prominent anterior proventriculus, thick-walled middle part and thin-walled posterior

Table 2. Morphometry of free-living males of *R. rubrovenosa* (20 specimens)

Таблица 2. Морфометрия самцов свободноживущего поколения *R. rubrovenosa* (20 экз.)

Characters	Aver.	Min.	Max.	SD	CV
Body length	530.8	498	556	16.26	3.064
Body width	31.6	30	34	1.392	4.404
Stoma length	6.1	6	8	0.447	7.331
Esophagus length	88.4	78	100	5.256	5.945
The same, % to body length	16.67	14.9	19.28	1.157	6.943
Esophagus bulbus width	13.9	12	14	0.447	3.217
Distance from anterior edge of esophagus to nerve ring	57.3	50	66	3.743	6.532
The same, % to esophagus length	64.85	58.7	71.11	2.566	3.957
Genital tube length	265.6	216	299	20.86	7.854
The same, % to body length	50.06	41.3	56.67	3.894	7.778
Tail length	24.1	22	26	1.518	6.3
The same, % to body length	4.54	4.21	4.89	0.205	4.52
Spicule length	30.7	28	32	1.174	3.825

Table 3. Morphometry of free-living females of *R. rubrovenosa* (25 specimens)

Таблица 3. Морфометрия самок свободноживущего поколения *R. rubrovenosa* (25 экз.)

Characters	Aver.	Min.	Max.	SD	CV
Body length	656.7	614	730	27.14	4.133
Body width	41.5	38	46	1.759	4.236
Stoma length	7.9	6	8	0.4	5.051
Esophagus length	115.4	106	126	4.379	3.793
The same, % to body length	17.6	16.3	19.21	0.736	4.182
Esophagus bulbus width	16.9	16	18	1.013	6.003
Distance from anterior edge of esophagus to nerve ring	71.3	66	74	2.151	3.018
The same, % to esophagus length	61.78	58.7	64.91	1.703	2.756
Distance from anterior ovary loop to vulva	167.7	141	199	12.9	7.696
Distance from vulva to posterior ovary loop	147.7	133	182	11.74	7.945
Distance between ovary loops	315.4	282	365	22.09	7.004
The same, % to body length	48.02	42.7	53.09	2.504	5.215
Distance from anterior end to vulva	384.1	357	415	16.86	4.389
The same, % to body length	58.5	55.1	61.25	1.345	2.299
Tail length	52.3	44	60	3.591	6.863
The same, % to body length	7.98	6.54	9.51	0.606	7.599
Number of eggs	1.32	0	2	0.557	42.18

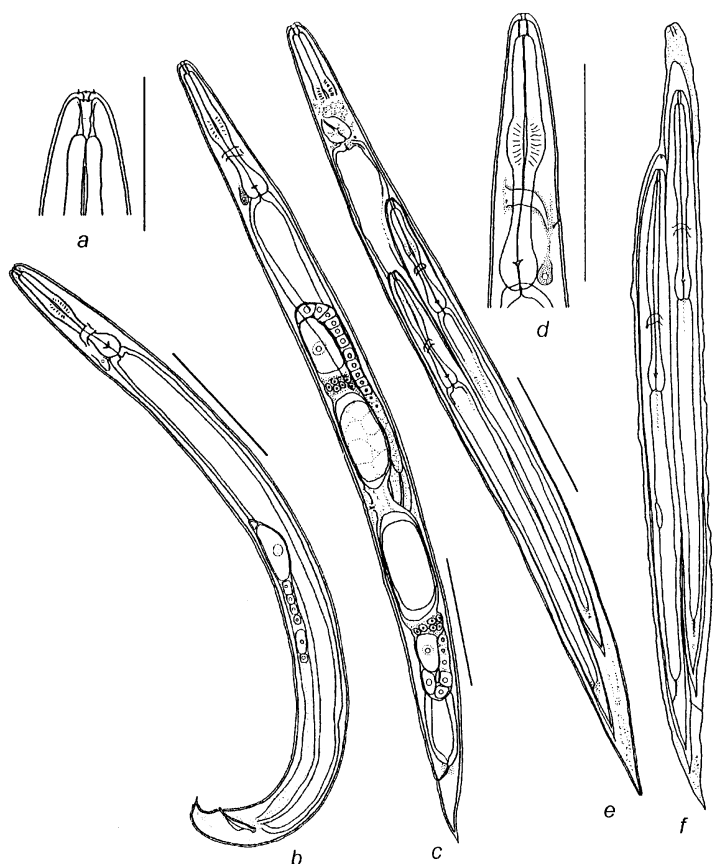


Fig. 3. Free-living adults of *R. rubrovenosa*: *a* — head end of male, lateral view; *b* — male, general view; *c* — gravid female, general view; *d* — same, anterior part of the body, lateral view; *e* — female with 2 newly hatched larvae; *f* — second-stage larvae inside the female's cuticle. Scale: *a* — 0.05 mm; *b-f* — 0.1 mm.

Рис. 3. Взрослые особи свободноживущего поколения *R. rubrovenosa*: *a* — головной конец самца, латерально; *b* — самец, общий вид; *c* — взрослая самка, общий вид; *d* — то же, передняя часть тела, латерально; *e* — самка с двумя личинками; *f* — личинки второй стадии внутри кутикулы самки. Масштаб: *a* — 0,05 мм; *b-f* — 0,1 мм.

part. Rectum thin and straight. Tail conical, elongated, sharply pointed.

Genital system amphidelphic, with approximately equal limbs. Uteri joined, sac-like, thin-walled. Seminal receptacles short. Ovaries narrow. Proximal ends of both ovaries situated at the level of vulva. Anterior genital limb located to the right, posterior one to the left of intestine. Vagina reduced, lips of vulva indistinct. Not more than one egg occurred in each uterus, thus maximum number of eggs developing in a female being 2. Eggs being at various stages of embryo development were never observed to be deposited and finally one or two larvae hatched inside each female. Larvae fed on mother's inner organs thus completely destroying its organism (fig. 3, *e*, *f*).

Discussion

Pre-equatorial position of vulva is not characteristic for species of the genus *Rhabdias* from amphibians, except *R. rubrovenosa*, and this character is obviously related with the atrophy of rectum in the latter species. The posterior body part of parasitic adults of the species grows more intensively than anterior one due to the

elongation of the posterior portion of intestine (fig. 2). Vulva was relocated posteriorly in the largest worms with ruptured intestine (fig. 2; the last three points on a graph).

The reduction of rectum was less conspicuous in young parasitic generation specimens, so this character should not be used by itself for differentiation of the species. Present investigation revealed two additional morphological characters to be appropriate for distinguishing *R. rubrovenosa* from the closely related *R. bufonis*. Specimens of *R. rubrovenosa* had the buccal capsule situated immediately behind the oral opening. Specimens of *R. bufonis*, in contrast, have comparatively elongated vestibulum. The posterior half of buccal capsule in *R. rubrovenosa* is not surrounded by esophageal tissue, whereas it is in *R. bufonis*.

The morphology of free-living generation adults of *R. rubrovenosa* is similar to that in other species of the genus *Rhabdias* that parasitize amphibians. The fecundity of free-living generation of *R. rubrovenosa* was, however, comparatively the lowest among the species studied. Females had not more than 2 eggs, and maximum 2 larvae developed in each female, whereas maximum 3 or 4 eggs were encountered in *R. ranae*, *R. americanus*, *R. sphaerocephala* and *R. bufonis* (Baker, 1979; personal observations). The free-living males of *R. rubrovenosa* had comparatively short genital tube with indistinct anterior reflexed portion noted in some other species of the genus.

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