Description of *Rhabditis (Rhabditoides) regina* n. sp. (Nematoda : Rhabditidae) from the body cavity of beetle larvae in Guatemala

Franz SCHULTE and George O. POINAR, Jr.

Institut für Zoologie der FU Berlin, AG Evolutionsbiologie, Königin-Luise-Strasse 1-3, 1000 Berlin 33, Bundesrepublik Deutschland, and University of California, Berkeley, CA 94720, USA.

SUMMARY

Rhabditis regina n. sp., a new species isolated from the body cavity of June beetle larvae, is described. The species fits in the subgenus *Rhabditoides* Goodey, 1929, where it is closest to *R. inermiformis* Osche, 1952. However, clear differences in the morphology of males (degree of spicules' fusion and bursa velum reduction, arrangement of bursal papillae), females (tail end dome-shaped *vs* long-conical) and in the behaviour of dauer juveniles (J3), make the species easy to recognize.

RÉSUMÉ

Description de Rhabditis (Rhabditoides) regina *n. sp. provenant du coelome de larves de coléoptères du Guatémala*

Une nouvelle espèce, *Rhabditis regina* n. sp., isolée du coelome de larves de coléoptères du Guatémala, est décrite. Cette espèce se classe aisément dans le sous-genre *Rhabditoides* où elle est proche de *R. inermiformis* Osche, 1952. Cette espèce se reconnaît toutefois aisément grâce à de nettes différences dans la morphologie des mâles (étendue de la fusion des spicules et de la réduction du volume de la bourse, disposition des papilles de la bourse) et des femelles (extrémité de la queue en ogive *vs* queue conique-allongée), ainsi que dans le comportement des Dauerlarven (J3).

Entomophagous nematodes (Heterorhabditidae spp., Steinernematidae spp.) associated with specific bacteria attract growing interest because of their potential value as biological control agents against different agricultural pest arthropods.

Improved mass production and application methods will eventually establish these nematodes as a common tool of pest management (Poinar, 1986).

Microbiotrophic species of the genus *Rhabditis* Dujardin, 1845, in contrast, have only been reported exceptionally as killing invertebrate hosts (Schulte, 1990). Thus their potential as control agents remains to be elucidated.

Nematodes belonging to the genus *Rhabditis* isolated from scarabaeid beetle larvae in Quetzaltenango, Guatemala, by Marcelo Velásquez were sent, through the kind efforts of Wayne T. Williams, to the junior author for further investigation. It proved to be a hitherto unknown species with uncommon morphological and ecological characters. This new species is described below as *Rhabditis (Rhabditoides) regina* n. sp.

The nematodes were grown on pure 2 % agar plates with little pieces of raw meat (to augment bacteria) at 20 °C. For morphological studies, specimens were taken randomly from the culture plates over a period of about 6 weeks.

Rhabditis (Rhabditoides) regina n. sp.

(Figs 1, 2)

MEASUREMENTS

See Table 1.

DESCRIPTION

Adults : White, mid-sized nematodes (1.0-2.0 mm), appearance quite clumsy. Cuticle about 1.0 µm thick, bearing a pattern of tiny dots arranged densely in transverse and longitudinal rows. Six lips, not (female) or hardly (male) set off from the rest of the body, bearing two minutes papillae each. Cheilorhabdions not cuticularized. Stoma lumen three-edged prismatic; stoma collapsing easily under the covering glass. Each metarhabdion with three little warts or teeth. Pharyngeal collar present; the respective part of stoma enveloped by pharyngeal collar shows fine transverse markings, arranged densely. Pharynx with median bulb. Nerve ring surrounds isthmus in its middle or its posterior half. Cervical pore apparently connected with two large cervical cells. Deirids not visible. Lateral canals reaching the anal or cloacal region resp. in both sexes.

Table 1

Measurements (in µm)
of *Rhabditis (Rhabditoides) regina* n. sp.*

	Males	*Females*	*Dauer juveniles*
n	25	25	25
L	1 460 (1 035-1 620)	1 850 (1 100-2 115)	626 (572-707)
V		60 (51-70)	
a	16.4-21.2 (18.8)	10.3-19.8 (15.3)	17.6-26.1 (22.5)
b	4.0-6.0 (5.2)	3.5-7.6 (4.7)	3.6-4.5 (4.0)
c	15.5-29.5 (20.0)	19.8-29.3 (24.5)	7.7-11.2 (9.0)
Body diam.	69 (63-99)	102 (68-153)	32 (24-34)
Stoma length	19 (15-20)	24 (18-25)	19 (16-20)
Tail length**	71 (54-90)	58 (41-72)	70 (63-81)
Gonad length***	940 (702-1 170)	814 (360-1 512)	94 (81-108)
Spicules	56 (50-63)		
Gubernaculum	34 (27-36)		

* Specimens maintained at 20 °C on 2 % agar with pieces of raw meat then heat relaxed in tap water; ** J3 tail in the dauer juveniles; *** Flexure to cloaca (male); anterior to posterior flexure (female); genital primordium (dauer juvenile).

Male : Pharyngeal collar covering 50-88 (64) % of stoma length. Corpus length corresponding to 49-60 (55) % or pharynx length. Diameter of posterior pharynx bulb 29-40 (39) µm. Stoma 5 µm broad, its walls somewhat differing in length. Cervical (" excretory ") pore opening at 171-243 (228) µm from anterior body end, i.e. at 77-92 (87) % of pharynx length. Diameter of posterior pharynx bulb 29-40 (39) µm. Single reflexed testis corresponding to 50-73 (64) % of body length. Flexure in 40 % ventral, in 60 % dorsal (n = 63), whereas it seems to be a constant direction in other rhabditid species. Reflexed part of testis 126-252 (189) µm long, corresponding to 17-31 (22) % of length of unflexed part. Usually two (sometimes only one, sometimes even three or four) pseudo-coelomocytes lying next to the gonad bending, 8-18 (11) µm in diameter. Two large ejaculatory glands (like in most species of the subgenus *Pelodera*) lying ventral to the testis, 189-324 (289) µm long, differing in length up to 31 µm. Diameter of sperm cells 6 µm; cells of mid-testis tissue with stored granules in their periphery. Open bursa of leptoderan type, ten pairs of papillae of different length arranged radially (formula : 1 + 2/2 + 2 + 1 +2). Papillae nos. 1, 2, 3 short, in a precloacal position on the ventral side of the body. The anterior precloacal inconspicuous pair of papillae in a distance of 56-99 (90) µm from the second one. Bursa velum narrow, rudimentary, held by clublike papillae nos. 5 and 8, which are the only ones reaching the edge of the bursa velum; the remaining papillae (nos. 4, 6, 7, 9, 10) stand in a ventral position. Size of papillae nos. 6/7 and nos. 9/10 resp. nearly identical. Tail end only occasionally protruding beyond the bursa 3-7 (5) µm. Spicules yellow-brownish, width at the capitula 18-34 (32) µm. Although seemingly joined in their distal half, the spicules can be separated by gently pressing the covering glass; these spicules therefore belong to the non-fused (" free ") type. No differences in length between the spicules of a single specimen were measured. Gubernaculum strongly curved, 27-36 (34) µm long, its length corresponding to 46-58 (52) % of entire spicule length; a thin membrane seems to be tightened over the curve.

Female : Stoma 6 µm broad with the walls somewhat differing in length. Pharyngeal collar enveloping 52-68 (59) % of entire stoma length. Corpus length corresponding to 48-60 (57) % of pharynx length; posterior (" valvular ") bulb of pharynx 33-45 (40) µm in diameter. Cervical pore opening at 171-243 (220) µm from anterior body end, i.e. 62-86 (71) % of length of pharynx. Two large cells visible in the vicinity of the cervical pore. Gonads amphidelphic with dorsally reflexed ovaries. Length of anterior branch (vulva to bending) 162-774 (413) µm, that of posterior one 180-720 (410) µm. Unflexed part of reproductive tract (anterior and posterior branch) 33-73 (50) % of total body length. Anterior flexure reaching the vulva region in most of the examined specimens. Each uterus followed by a set of eight sphincter cells, and a voluminous *receptaculum seminis*. Ovoviviparous; numbers of eggs found segmenting in the uteri depending on the age of the female : younger females (up to 5 days) with 35-65 (48), older ones (more than 5 days) with 1-6 (3) eggs (see below). Dimensions of eggs 54-72 (70) × 36 µm. Diameter or pseudo-coelomocytes (one or two) laying at the posterior gonad bending 9-13 (13) µm. Tail end dome-shaped, 41-72 (58) µm long; cuticle only slightly thickened at the base of the spine (Fig. 2 E, F). Tail spine 9-18 (17) µm long, corresponding to 18-36 (25) % of total tail length. Phasmids opening close [16-27 (19) µm] to base of tail spine.

Dauer juveniles : Lips not set off, stoma relatively long and narrow (3 µm). Corpus occupying 57-66 (59) % of pharynx length, cervical pore at 117-136 (132) µm from

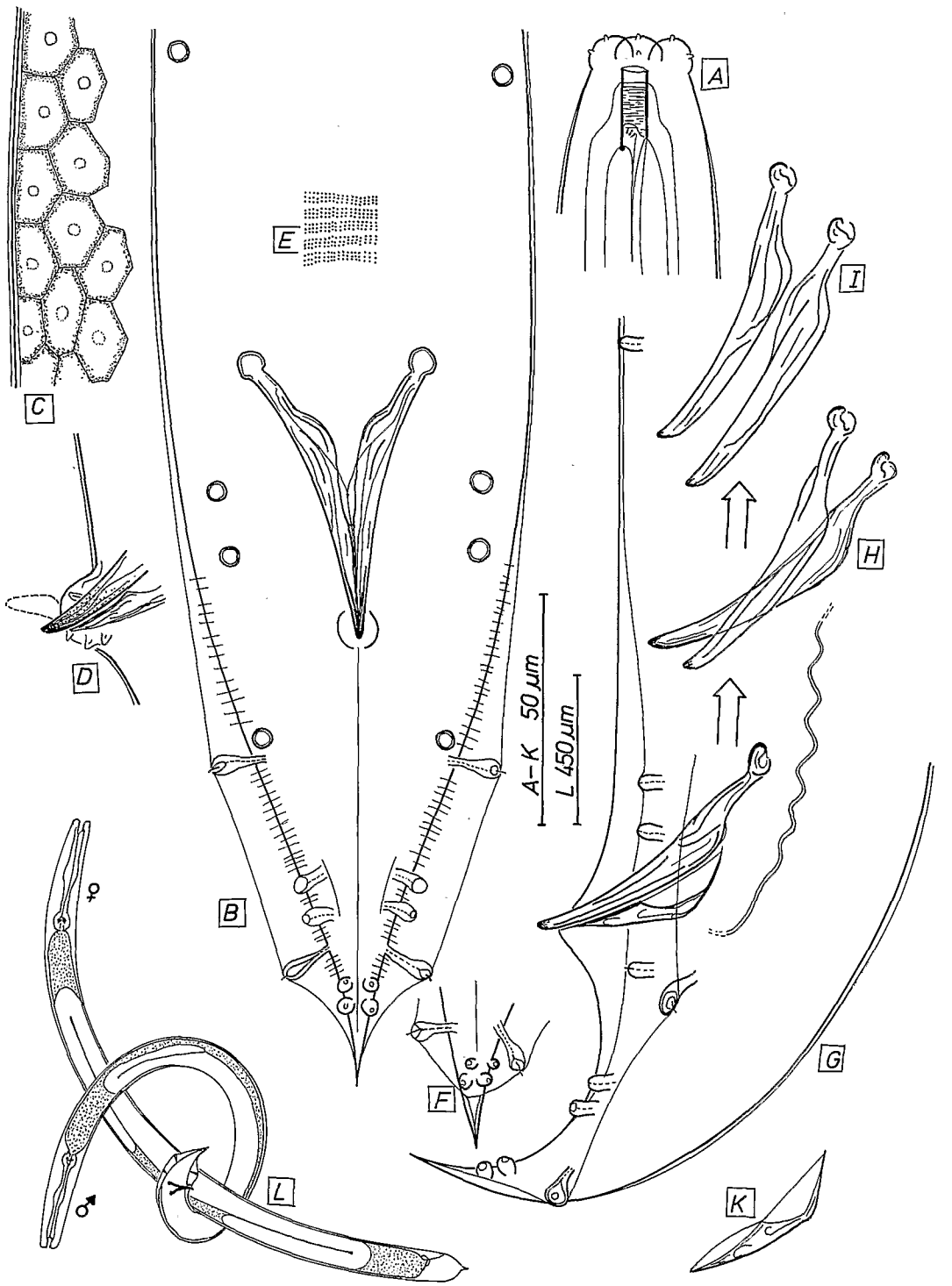

Fig. 1. *Rhabditis (Rhabditoides) regina* n. sp. Male. A : Anterior region, lateral; B : Bursa and spicules, ventral; C : Tissue of testis, flexure region; D : Cloacal region, lateral in heat relaxation; E : Cuticle structure, midbody region; F : Tail end, ventral, protruding the bursa; G : Bursa and spicules, lateral; H. I : Artificial separating of spicules; K : gubernaculum, lateral; L : Pair in " spiral " type copulation.

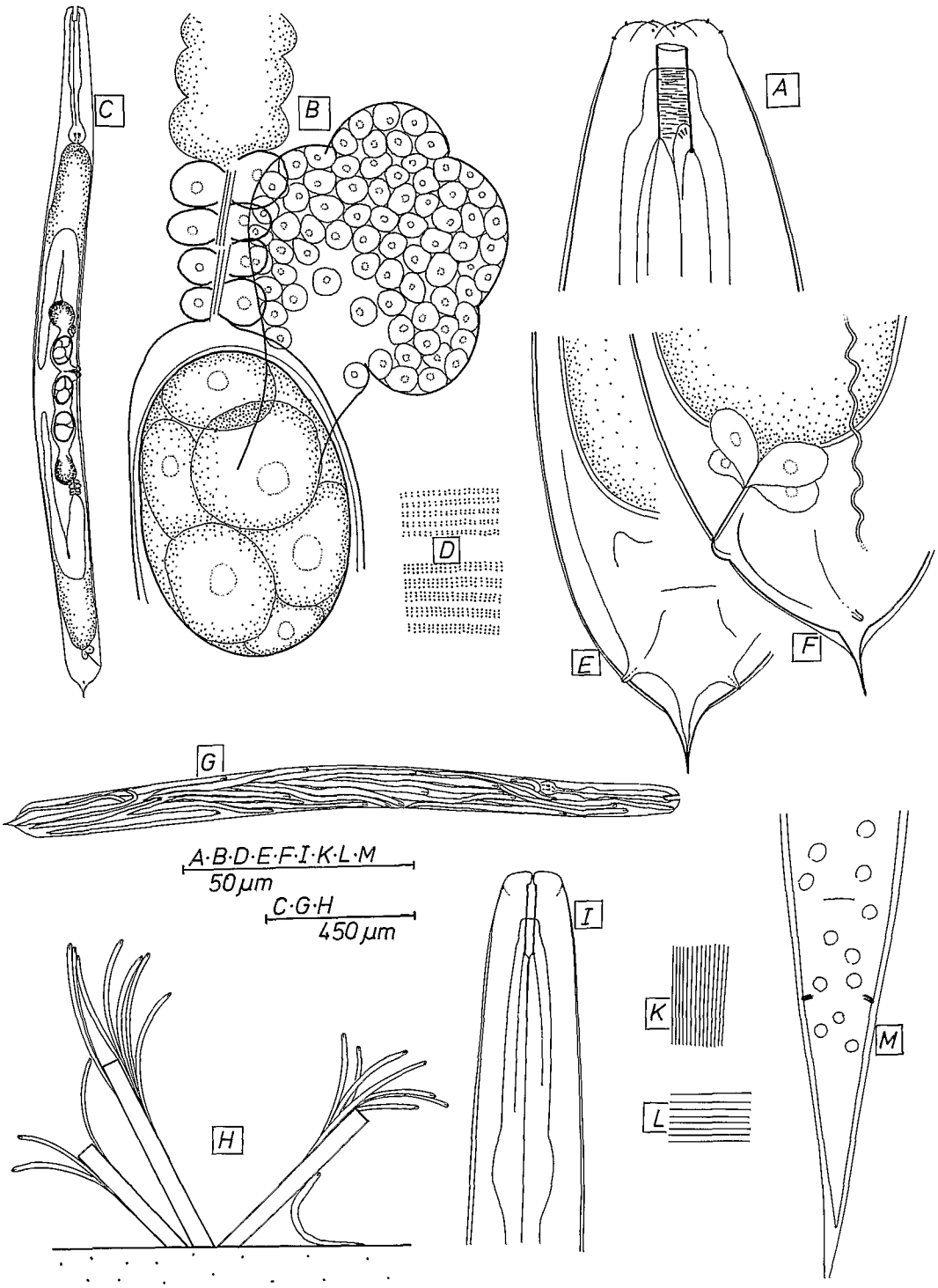

Fig. 2. *Rhabditis (Rhabditoides) regina* n. sp. A-F : Female. — A : Anterior region, ventral; B : Ending of anterior uterus, with a single egg in segmentation, sphincter consisting of eight cells and the voluminous *receptaculum seminis* filled with sperms; C : Female in toto, lateral, with three eggs in its uteri; D : Cuticle structure; E : Tail end ventral; F : Tail end lateral; G : Female *in toto* filled with hatched juveniles. — H-L : Dauer juvenile. — H : Dauer juveniles " waving " on the tip of projections in a culture; I : Anterior region, lateral; K : Structure of J2-cuticle; L : Structure of J3-cuticle; M : Tail end, ventral.

anterior body extremity. J2-cuticle still kept and enveloping the entire body, striped longitudinally. Actual J3-cuticle with fine transverse striations width of ten annules 14-16 µm. Lateral field 6 µm broad, two central lines present. Tail ending in a sharp tip; phasmids inconspicous, opening at 45-50 (47) µm, i.e. 55-79 (67) % of entire tail length.

Type locality

The laboratory culture on which the description is based was originally grown from developmental stages removed from the body cavity of a June beetle larva (Scarabaeidae : Coleoptera) in the vicinity of Quetzaltenango, Guatemala in the spring of 1989 by Marcelo Velásquez.

Type specimens

Holotype male, allotype female, paratype males, females and juveniles in the collection of Museum für Naturkunde der Humboldt Universität zu Berlin, Zoologisches Museum. Berlin, GDR. Other paratypes including males, females and immatures deposited in the following collections : Nematode collection, University of California, Davis, USA; Laboratoire des Vers. Muséum national d'Histoire naturelle, Paris, France; and in the collection of W. Sudhaus, Institut für Zoologie der FU, Berlin.

Diagnosis

Rhabditidae, subgenus *Rhabditoides* Goodey, 1929. Bisexual, copulation following the " spiral " type. Pharynx collar covering more than 50 % of stoma. Tail end in the female dome-shaped. Vulva in the midbody region, ovaries amphidelphic with voluminous *receptacula seminis*. Matricidal hatching frequent. Male characterized by an open bursa of leptoderan type; bursa velum reduced to a narrow hem. Ten bursal papillae present, three in a precloacal position. Papilla no. 1 set off by a big gap from the rest. Spicules not fused, although difficult to separate. Dauer juveniles aggregating and exhibiting " waving " behaviour.

Relationships

The combination of characters as : long pharyngeal collar; three distinct metastomatal teeth; vulva in midbody region; open leptoderan bursa with ten radially arranged papillae and a rudimentary velum; long nonfused spicules, place *R. regina* n. sp. in the subgenus *Rhabditoides* of *Rhabditis*. Further species of this monophyletic group (Sudhaus, 1976) are : *R. frugicola* (Goodey, 1942), *R. hanuskai* (Kokordák, 1969), *R. helversenorum* Sudhaus, 1974, *R. inermis* (Schneider, 1866), *R. inermiformis* Osche, 1952 and *R. longispina* Reiter, 1928. Andrássy (1984) arranges these species in three different genera (*Rhabditoides; Rhitis* Andrássy, 1983; *Rhabditella* [Cobb, 1929] Chitwood, 1933), which does not seem to be justified by the above mentioned arguments. Most of the species in *Rhabditoides* are distinctly different from *R. regina* n. sp. in the arrangement of bursal papillae, the shape of tail end and the length and shape of spicules. The closest relative to *R. regina* n. sp. is *R. inermiformis*, considering the shape of spicules (which, however, are truly free in *R. inermiformis*), the degree of bursa velum reduction and the arrangement of the ten bursal papillae. Clear differences lay in the shape of the female tail (dome-shaped *vs* long-conical) and distinct *receptacula seminis* in the female of *R. regina* n. sp. Males of both species differ in the shape of the gubernaculum (more curved in *R. regina* n. sp.), and in the seeming fusion of spicula in *R. regina* n. sp. *vs* truly free ones in *R. inermiformis*.

" Waving " behaviour was not observed in the dauer juveniles of *R. inermiformis*, and this species has never been found in association with insects or other invertebrates (Osche, 1952).

Notes on biology and life cycle

Dauer juveniles put on a fresh substrate (agar supplied with a sufficient amount of bacteria) leave their J2-covering through a scratch on the front side. Males reach adulthood within 24 h, females up to 6 h later. At this point, the nematodes can easily be seen copulating : The male bends its tail end around the female in order to find the vulvar region (Fig. 1 L). Sperms are transferred in high numbers and stored in the voluminous *receptacula seminis* (Fig. 2 B). Only a very small mucus vulva plug is deposited by the male. After two days in culture, copulating pairs can only occasionally be found, although males are actively moving around searching for females still unfertilized. It seems that a female of *R. regina* n. sp. copulates only once during its life span. Only the first batch of eggs is laid in the early segmentation stage. After four days in culture most of the females are visible inside the agar with numerous larvae hatched in their uteri (Fig. 2 G). Hatching larvae will eventually kill the female, which thus becomes a container for its offspring. While this phenomenon was exceptionally observed in many microbotrophic rhabditids (Sudhaus, 1976), it seems to be a normal part in the life cycle of the new species. Juveniles developing inside the females body will become dauers. Dauer juveniles are activated by vibration, e.g. handling the Petri dish, then aggregating on dry projections and waving with the foreparts of their bodies in high numbers (Fig. 2 H). This behaviour is commonly found in rhabditid nematodes as an adaptation of making contact with a carrier arthropod (" phoresis "; Sudhaus, 1976). It is also reported from parasitic nematode larvae (*Ancylostoma* spp., *Strongyloides* spp.) searching for a new warmblooded host (Fülleborn, 1932). The dauer juveniles of

R. regina n. sp. are able to tolerate desiccation at room temperature for at least 8 days. Studies performed by Marcelo Velásquez in Quetzaltenango, Guatemala, showed that *R. regina* n. sp. attacked June beetle larvae (gen. sp.) as well as pupae of the Mediterranean fruit fly *Ceratitis capitata* (Wiedemann) (Tephritidae : Diptera) and three other fruit fly larvae (W. T. Williams, *in litt.*, July 25th, 1989).

The dauer juveniles of *R. regina* n. sp. were placed on moistened filter paper in standard deep Petri dishes together with larvae of the wax moth [*Galleria melonella* (L.) (Galleriidae : Lepidoptera)] (n = 20) and white grubs (Scarabaeidae : Coleoptera), (n = 20), respectively. After 70 days, 30-40 % of each insect species had died. Developing stages of *R. regina* n. sp. were found inside and outside of the cadavers where they were apparently feeding on bacteria. It is not known yet if the nematode juveniles are carrying specific (symbiotic) bacteria. Further studies are necessary to see if and how the dauer stages of this nematode are able to bring about the mortality of selective insect species.

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