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Rhabditis myriophila sp. n. (Rhabditidae: Rhabditida), Associated with the Millipede, *Oxidis gracilis* (Polydesmida: Diplopoda)

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ABSTRACT: A new species, *Rhabditis myriophila* (Rhabditidae: Rhabditida), is described as an associate of the garden millipede, *Oxidis gracilis* (Polydesmida: Diplopoda). This hermaphrodite can be distinguished from other members of the genus *Rhabditis* by the structure of the spicules and bursa and the size of the stoma, metacorpus, tail, and rectum. The dauer juveniles of *R. myriophila* enter the alimentary tract and body cavity of the host and can develop to fourth-stage juveniles in both locations. Reproduction only occurs when the millipede dies and becomes colonized by bacteria.

During routine dissections of the garden millipede, *Oxidis gracilis,* one species of rhabditoid nematode was consistently found inside the gut lumen, Malpighian tubules and hemocoel of the host. This nematode matured in living millipedes but only reproduced on dead hosts. It could be cultured on nutrient agar plates containing bacteria isolated from dead or dying millipedes.

The nematode was an unknown species of *Rhabditis* and is described below, along with observations on the nature of its association with *O. gracilis.*

Materials and Methods

Millipedes, *Oxidis gracilis* (Koch) (Polydesmida: Paradoxosomatidae), were collected and sent to the author by Gary Phipps of the Monrovia Nursery in Azusa, California. They were maintained in a plastic shoe box containing sterile damp soil and lettuce as a food source. Live millipedes were washed and dissected for rhabditoid nematode associates. During dissections, the entire alimentary tract was first removed by severing the head and then pulling on the posterior 2 segments. The removed gut and remaining body column were examined separately for associated nematodes. The numbers, stages, and location of nematodes were noted. These collected nematodes, together with reproducing stages recovered from millipedes that had died in the container, were placed on nutrient agar plates for later systematic studies.

All nematodes recovered from both living and dead millipedes belonged to the same species and were protandrous hermaphrodites. When they were cultivated at room temperature (20°C) on nutrient agar plates with a mixed population of bacteria, males appeared at a ratio of 250 hermaphrodites to 1 male.

When the colonies were placed at a higher temperature (32°C), males appeared at a ratio of about 100 hermaphrodites to 1 male. It is presumed that the higher temperature increased the rate of nondisjunction of the sex chromosome. In order to obtain a still higher ratio of males for taxonomic studies, 3 males obtained from the heat-treated plates were removed and placed with a single hermaphrodite on a nutrient agar plate at 20°C. The males mated with the hermaphrodite and the resultant F_1 population consisted of a 1:1 ratio (equal males and hermaphrodites).

Nematodes were fixed in TAF and processed to glycerin for identification and characterization.

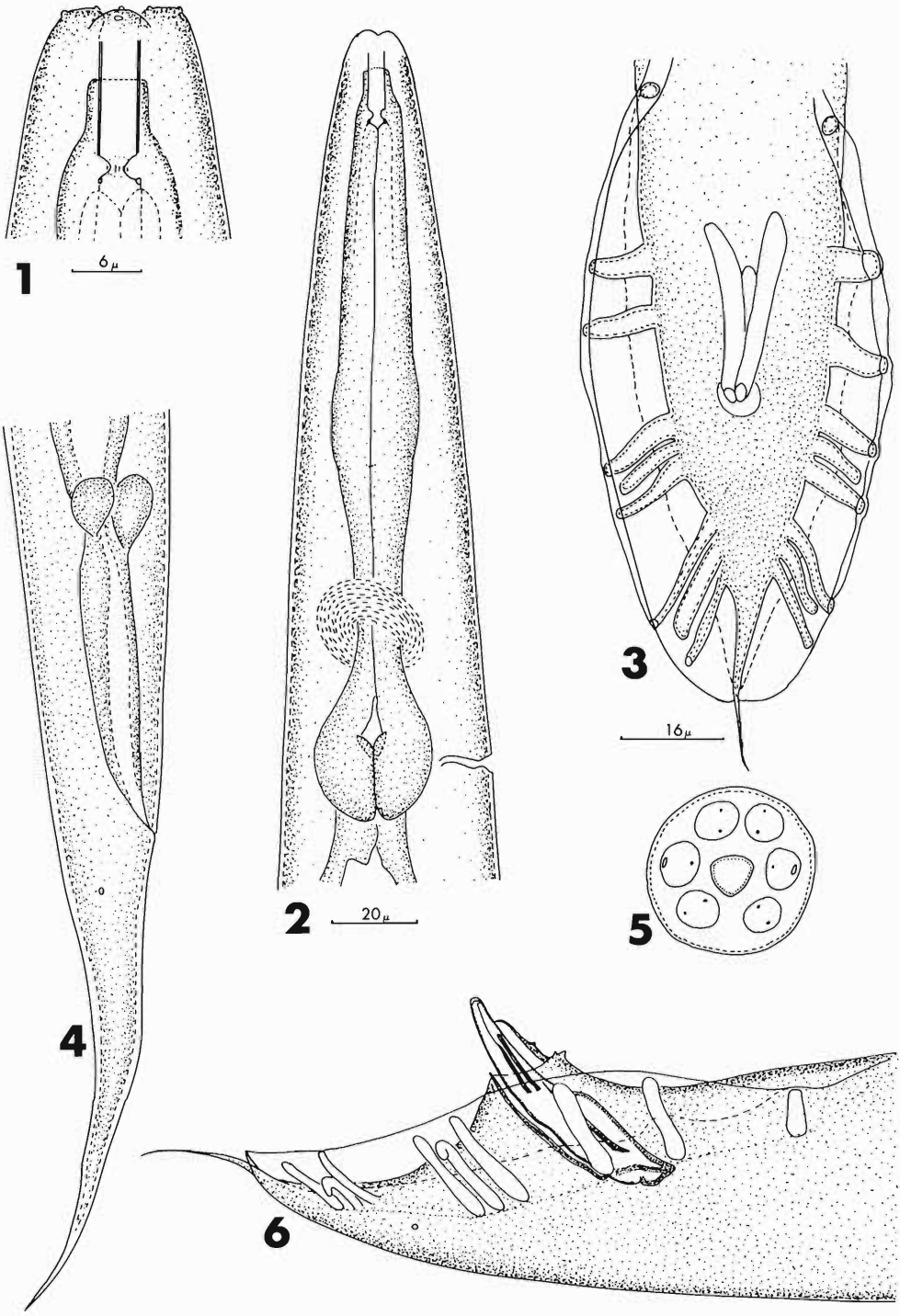
Results

The nematodes removed from both dead and living specimens of *O. gracilis* belonged to the same unknown species and are described below. All measurements are given in micrometers unless otherwise specified. The number following the character is the average value and the figures in parentheses represent the range of the characters.

Rhabditis myriophila sp. n. (Figs. 1-6)

Rhabditida Chitwood, 1933; Rhabditina Chitwood, 1933; Rhabditoidea (Örley, 1880) Travassos, 1920; Rhabditidae Örley, 1880; *Rhabditis* Dujardin, 1845 as defined by Andrássy (1983).

GENERAL CHARACTERISTICS: Cuticle smooth, with fine longitudinal striations. Head continuous with neck. Lips closed, partially fused, each containing 1 inner labial papilla and another outer labial papilla except for lateral lips that contain elliptical-shaped amphidial openings. Stoma cylindrical, distinct, cheilorhabdions not noticeably cuticularized, metarhabdions isomorphic, each bearing 2–3 minute tubercles, pharyngeal collar present. Corpus cylindrical, slightly enlarged to form a median bulb; isthmus surrounded by nerve ring. Basal bulb large, with well-formed valve plates. Excretory pore in adults opening at the vicinity of the basal bulb; phasmids distinct.



Figures 1-6. ***Rhabditis myriophila.*** **1. Lateral view of hermaphrodite head. 2. Lateral view of hermaphrodite pharyngeal region. 3. Ventral view of male tail. 4. Lateral view of hermaphrodite tail (mag. same as Fig. 2). 5. En face view of male (mag. same as Fig. 1). 6. Lateral view of male tail (mag. same as Fig. 3).**

HERMAPHRODITES ($N = 10$): Length, 1.32 (1.20–1.50) mm; greatest width, 62 (57–70); length of stoma, 20 (18–21); width of stoma, 3.2; head width, 10 (9–13); distance from head to excretory pore, 179 (165–190); distance from head to nerve ring, 139 (126–146); length of pharynx, 185 (174–193); length of tail, 117 (108–135); width at anus 25 (22–28); % vulva, 50 (49–51); length of eggs (in situ), 60 (56–72); width of eggs (in situ), 32 (25–40); reproductive system amphidelphic, ovaries reflexed; length of rectum, 79 (56–96); tail conical, tapering to a fine point. a = 19.1–21.3; b = 6.8–7.7; c = 2.2–3.5. Ratio of length of rectum to anal width, 2.2–3.5; ratio of length of tail to anal width, 4.2–5.3.

MALES ($N = 10$): Length, 1.27 (0.83–1.47) mm; greatest width, 63 (38–80); length of stoma, 17 (16–19); width of stoma, 3.2; head width, 11 (9–13); distance from head to excretory pore, 198 (149–229); distance from head to nerve ring, 150 (117–165); length of pharynx 187 (161–200); reflexion of testis, 191 (101–228); length of tail, 65 (56–72); body width at cloacal opening, 39 (32–45). Bursa open, leptoderan (Andrássy [1983] describes this condition as pseudopeloderan because the portion of the tail that protrudes outside of the bursa is small and fragile, measuring 17 (12–19) in length). The bursa is 105 (84–117) long and composed of a double membrane which encloses the bursal papillae. A shorter membrane or fold reaches slightly less than half way up the bursal papillae (represented by a dotted line in Fig. 3). Nine pairs of bursal papillae are present, 3 pairs precloacal and 6 pairs postcloacal. The 3 precloacal pairs are separate, although pair numbers 2 and 3 are closer together than pairs 1 and 2. Pairs 4, 5, and 6 form 1 group and pairs 7, 8, and 9 form the second postcloacal group. The tips of the fifth and eighth pairs are turned outwards. The spicules are paired and separate. They are slightly curved and have a triangular head and a rounded tip. Three longitudinal ribs occur on the outer surface of each spicule. The length and width of the spicules are 47 (32–54) and 10 (8–11), respectively. The gubernaculum is dorsal-ventrally flattened and follows the contour of the spicules. The proximal tip is curved upward. The length and width of the gubernaculum are 28 (19–32) and 0.87 (0.64–1.20), respectively. a = 18.4–21.9; b = 5.16–7.36; c = 14.8–20.4.

DAUER JUVENILES ($N = 6$): Length, 564 (504–611); greatest width, 23 (19–26); distance from the head to the excretory pore, 107 (97–114); distance from the head to the nerve ring, 89 (83–96); length of pharynx, 129 (120–136); length of tail, 78 (75–80); width at anus, 15 (14–16). This stage is the third-stage juvenile enclosed in a second-stage cuticle that surrounds the nematode like a sheath.

TYPE LOCALITY: The Monrovia Nursery in Azusa, California.

TYPE LOCATION: Found in the intestine and body cavity of the garden millipede, *Oxidis gracilis*. This species was originally introduced into North America from Southeast Asia.

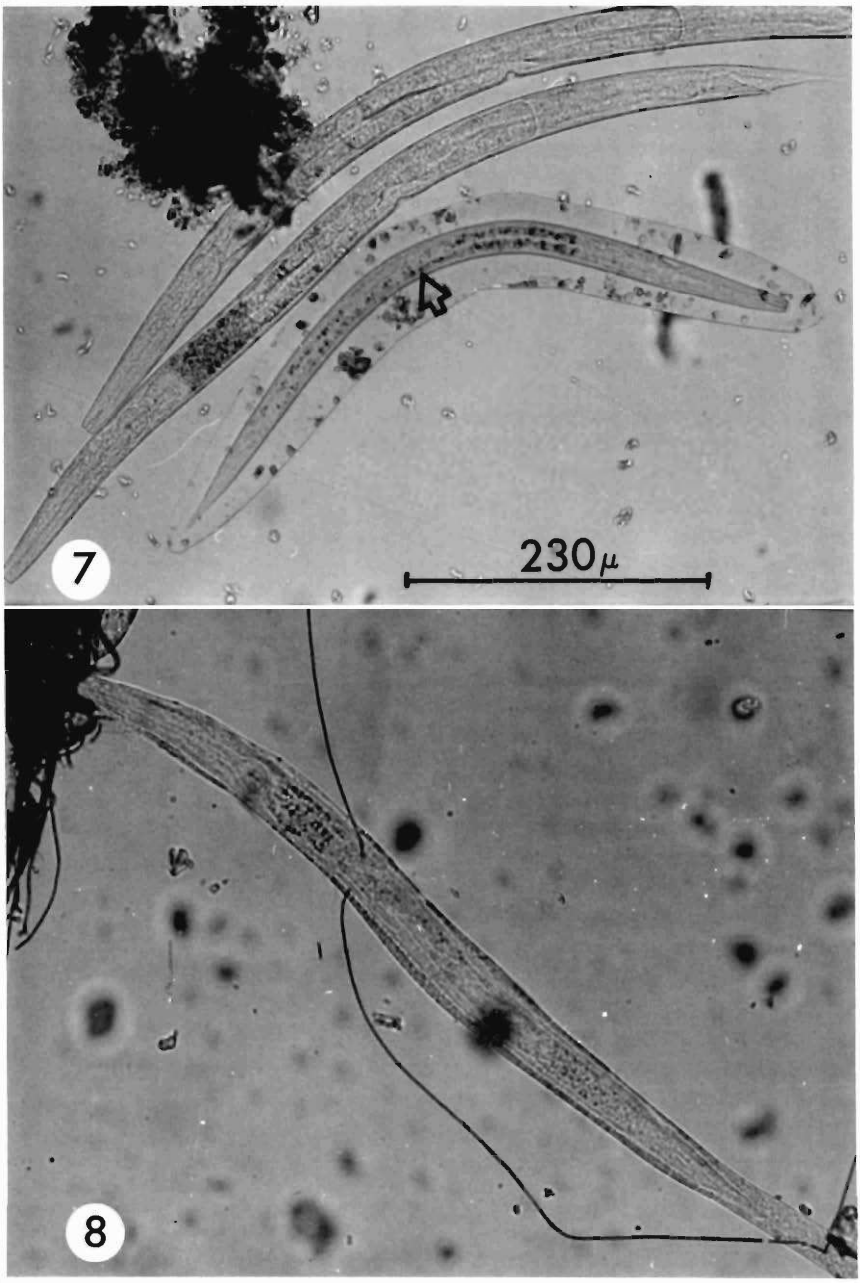
TYPE SPECIMEN: Holotype (female) and allotype (male) deposited in the nematology collection at the University of California, Davis.

DIAGNOSIS: The well-developed stoma and open bursa places *R. myriophila* in the genus *Rhabditis* sensu Andrássy, 1983. The pseudopeloderan bursa, three pairs of precloacal bursal papillae, long stoma (1.5 or more times longer than the head diameter), long female tail (between 4 and 8 anal body diameters long), and female rectum between 2.2 and 3.5 anal body diameters long separate this species from all existing members of *Rhabditis* except *R. wohlgemuthi* Völk, 1950. However, the latter species possesses a more distinct median pharyngeal bulb than in *R. myriophila*, a shorter pharyngeal collar covering half or less of the stoma (always more than half in *R. myriophila*), a shorter stoma length (2.5 times longer than wide) than *R. myriophila* (5.0–6.0 times longer than wide), and has all bursal papillae reaching the rim of the bursa (the fifth and eighth bursal papillae clearly do not reach the rim in *R. myriophila*). In addition, *R. wohlgemuthi* is an amphimictic species whereas *R. myriophila* is hermaphroditic.

HOST RELATIONSHIP: The dauer juveniles of *R. myriophila* enter the alimentary tract and body cavity of developing millipedes. Inside the gut and body cavity the dauers may exsheath and develop to fourth-stage hermaphrodites (Fig. 7). They remain very active, and may enter the Malpighian tubules of the millipede (Fig. 8). These fourth-stage juveniles never complete the final molt to adults in association with a living millipede. Further development into egg-laying hermaphrodites occurs only when the host dies and the body is colonized by bacteria.

Discussion

The association between *R. myriophila* and the millipede, *O. gracilis*, is similar to that of



Figures 7, 8. ***Rhabditis myriophila*** **in association with** ***Oxidis gracilis*****. 7. Juvenile nematodes removed from the millipede's alimentary tract. Arrow shows dauer juvenile. The other 2 are fourth-stage juveniles. 8. A fourth-stage juvenile inside a Malpighian tubule of the millipede.**

Rhabditis pellio (Schneider) and earthworms (Poinar and Thomas, 1975). The dauer stages of both nematodes enter the host's external openings (the excretory system with *R. pellio* and alimentary tract with *R. myriophila*) and body cavity, but they are unable to multiply until the host succumbs and is invaded by bacteria. Studies showed that the dauer juveniles of *R. myriophila* develop only to fourth-stage juveniles in the host's hemolymph. Bacteria are apparently required as

a food source. Encapsulated juveniles of *R. myriophila* were sometimes found in the body cavity of living millipedes. These dead nematodes appeared to be subjected to the same defense reactions reported earlier in this host against entomophagous rhabditoid nematodes (Poinar and Thomas, 1985). However, most nematodes found in the body cavity of *O. gracilis* were not encapsulated; thus the millipedes' defense system may be less efficient than the brown bodies formed in earthworms against *R. pellio* as reported by Poinar and Hess (1977). Similar to other autotokous rhabditids, *R. myriophila* is a protandric hermaphrodite, with males occurring rarely. In the hermaphrodite, sperm are produced in the fourth-stage juvenile and ova are produced after the final molt. Both gametes are produced in the syngonic gonad, the ovotestis.

Acknowledgments

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