

A NEW SPECIES OF THE GENUS *HETERORHABDITIS* POINAR, 1976 (NEMATODA: HETERORHABDITIDAE) PARASITIZING *GRAPHOGNATHUS* SP. LARVAE (COLEOPTERA: CURCULIONIDAE) FROM ARGENTINA

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ABSTRACT: *Heterorhabditis argentinensis* n. sp., an entomogenous nematode isolated from *Graphognathus larvae* (Coleoptera: Curculionidae) from Rafaela (Province of Santa Fe), Argentina, is described and illustrated. This species is mainly distinguished from other members of the same genus by the size of the males and hermaphroditic females, the shape and size of the male's spicules and gubernaculum, and the arrangement of the genital papillae, as well as by the body width and tail length of the third larval stage (infective juveniles).

KEY WORDS: Nematoda, Heterorhabditidae, *Heterorhabditis argentinensis* n. sp., *Graphognathus* sp., Coleoptera, Curculionidae, Argentina.

INTRODUCTION

During a survey of diseased weevils, conducted in Rafaela (Province of Santa Fe), Argentina, in November of 1990, the author came across a field containing several dying dark-purple coloured curculionid larvae (*Graphognathus* sp.)

Further observations revealed that the white fringed beetles were parasitized by a nematode belonging to an undescribed species of the genus *Heterorhabditis* Poinar, 1976 [Rhabditoidea (Oerley, 1880): Heterorhabditidae Poinar, 1975], named as *Heterorhabditis argentinensis* n. sp., which is described and illustrated below.

MATERIAL AND METHODS

Heterorhabditis argentinensis n. sp. was isolated from white fringed weevils (*Graphognathus* sp.) from alfalfa fields in the area of Rafaela (Argentina).

Infective juveniles (third larval stage) of this nematode were recovered from host cadavers, and were reared *in vivo* with *Galleria mellonella larvae* (Greater wax moth). The infected insects, which died after the second day of infection, were maintained at 25° C and dissected on the 3-4 day to recover the first generation hermaphroditic females, and on the 5-6 day to obtain males and amphimictic females of second generation. Infective juveniles left the host cadavers between the 10-12 day after initial exposure.

For morphological studies the nematodes were killed in hot (60° C) Ringer's fixed in TAF and processed to glycerin using evaporation method (POINAR, 1975 a).

Measurements and drawings were done with a Zeiss Microscope with camera lucida and a drawing tube.

RESULTS

Heterorhabditis argentinensis n. sp.

Holotype: male isolated from the hemocoel of *Galleria mellonella larvae* (Lepidoptera: Galleridae), derived

from the original population isolated from *Graphognathus* sp. larvae (Coleoptera: Curculionidae); slide No. HE-001, deposited in the collection of the Center of Parasites and Vectors Studies (CEPAVE), La Plata, Argentina.

Allotype: hermaphroditic female, first generation; same data as holotype; slide No. HE-010, collection of the Center of Parasites and Vectors Studies (CEPAVE), La Plata, Argentina.

Paratypes: males, and first generation females; same data as holotype; ten males and ten females in TAF (HE-100-120), collection of the Center of Parasites and Vectors Studies (CEPAVE), La Plata, Argentina.

Type Host: *Graphognathus* sp. larvae (Coleoptera: Curculionidae).

Type Locality: Rafaela, province of Santa Fe, Argentina.

Etymology: from the source country, Argentina.

Description

Adults (Fig. 1, 2) with a smooth cuticle; lateral fields not observed. Head slightly truncated. Six distinct protruding lips surrounding the mouth (in fixed specimens they are withdrawn or pointed toward the center of the mouth). Six small labial papillae located at the tip of each lip. Six cephalic papillae. Small amphids located in the lateral lips near the level of the labial papillae. Phasmids inconspicuous. Cheilorhabdions represented as slightly refractile areas just below the lips, and anterior to the oesophagus. Metarhabdions bear a small tooth, telorhabdions not visible. Oesophagus without metacorpus. An isthmus present as well as a basal bulb which lack valvae. Nerve ring surrounding the isthmus. Excretory pore usually posterior to basal bulb. Intestine composed of few giant cells.

Males

With single reflexed testis. Spicules paired, separated

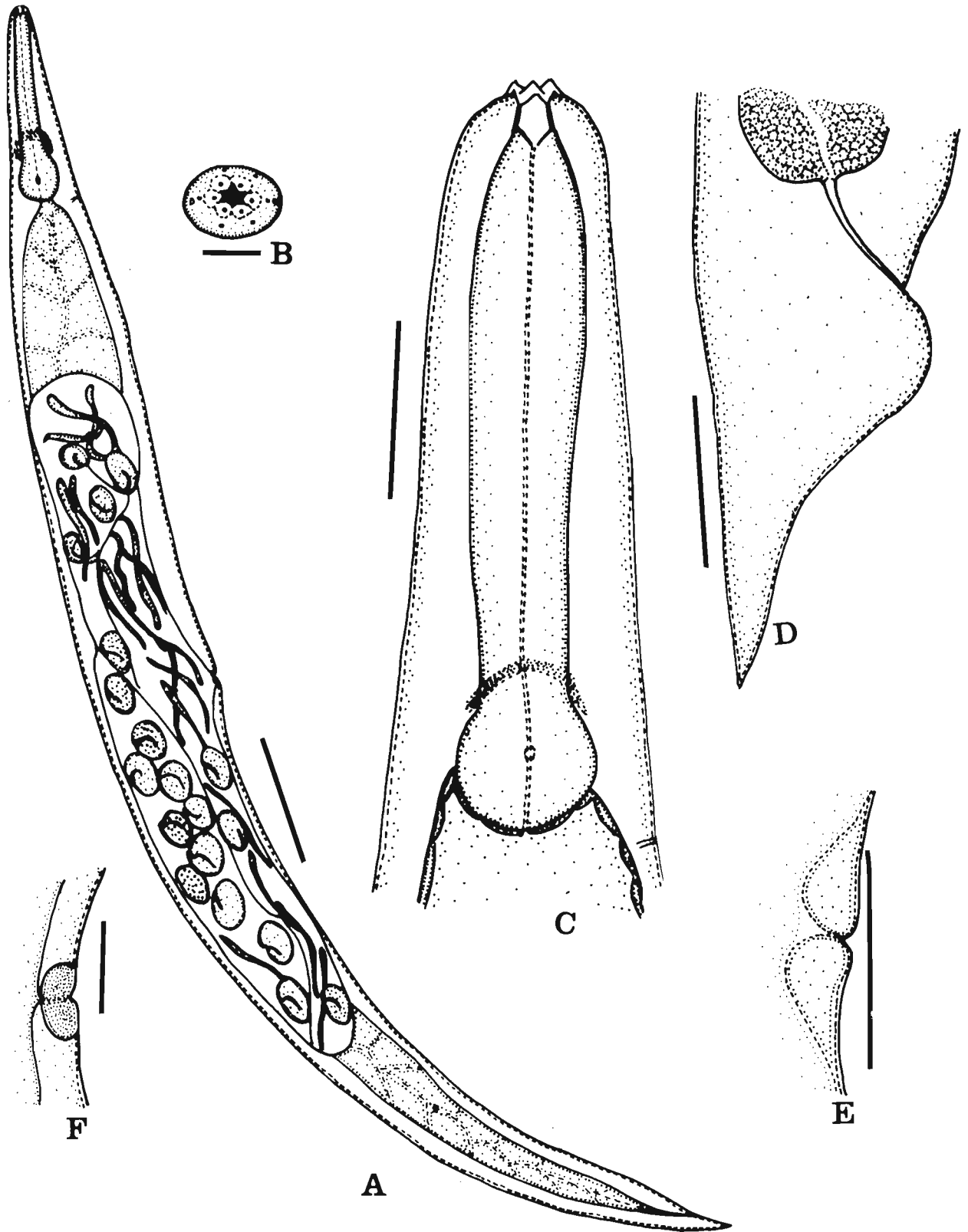


Fig. 1.— Females of *Heterorhabditis argentinensis* n. sp.: A) mature amphimictic female; B) «en face» view of amphimictic female; C) lateral view of oesophageal region of mature hermaphroditic female; D) tail of mature hermaphroditic female; E) lateral view of vulva of a mature amphimictic female; F) lateral view of vulvar region of a mature amphimictic female. Scale bars: A: 100 μ m; B: 10 μ m; C, D, E, F: 50 μ m.

and arcuate. Capitulum longer than broad, somewhat angular and well set off from the shaft which contains a single rib. Gubernaculum single, wide and fusiform, half the length of the spicules. Nine pairs of genital papillae: 3 pairs are preanal and 6 postanal. Numbering from anterior to posterior: pair 1 is small and isolated from pairs 2 and 3; these are adjacent to the spicules. The latter 6 pairs are arranged as follows: pair 4 is separated from pairs 5 and 6 which are close to each other; then a set of three pairs of papillae. Usually pairs 8 and 9 are branched, and in this case the branches do not reach the rim of the bursa. The arrangement of the genital papillae can be summarized as follows: 1 2 () 1 2 3. Bursa peloderan.

Measures: Based on the measurement of $n=25$ specimens. Total length 1,00-2,00 mm (1,50 mm); greatest width 42-70 μm (56 μm); stoma length 3,5-6,0 μm (5,0); stoma width 2,5-5,0 μm (4,0); distance from head to nerve ring 64-82 μm (72); distance from head to excretory pore 145-170 μm (157); oesophagus length 103-120 μm (113); testicle reflexion 100-194 μm (133); tail length 28-49 μm (37); width at anus 21-30 μm (24); spicules length 42-49 μm (46); gubernaculum length 20-26 μm (23).

Females

Paired ovaries, whose reflexed portion extends past the vulvar opening. Uterus and spermatheca located ventrally between vulva and ovarian reflexion. Hermaphroditic females with vulvar lips slightly protruded. Tail pointed, usually with postanal swelling. Amphimictic females (second generation females) resemble the large hermaphroditic females (first generation), in most qualitative characteristics. The vulva appears to be non-functional and it is not protruded, often surrounded by a hardened deposit or plug. Anal region slightly protruded.

Hermaphroditic female: Based on the measurement of $n=25$ specimens. Total length 5,00-7,50 mm (6,50 mm); greatest width 250-575 μm (360 μm); stoma length 10-16 μm (13); stoma width 6-12 μm (10); distance from head to nerve ring 132-196 μm (160); distance from head to excretory pore 250-340 μm (294); oesophagus length 235-300 μm (274); % vulva 40-50 (44,50); tail length 100-140 μm (118); width at anus 70-120 μm (86).

Amphimictic female: Based on the measurement of $n=25$ specimens. Total length: 2,00-3,50 mm (3,00 mm); greatest width 90-180 μm (130 μm); stoma length 7-12 μm (9,50); stoma width 5-10 μm (8); distance from head to nerve ring 88-140 μm (114); distance from head to excretory pore 105-240 μm (203); oesophagus length 162-200 μm (180); % vulva 42-48 (45); tail length 75-108 μm (93); width at anus 33-55 μm (45).

Third larval stage (infective juvenile)

Cuticle with delicate longitudinal striations. Mouth and anus are closed. Six labial papillae. Lip region transparent. Oesophagus and intestine collapsed. Tail pointed. Excretory pore located at the level of the isthmus. Body of

third larval stage covered with the enclosing second stage cuticle, which is lost after they leave the host cadaver.

Measures: Based on the measurement of $n=25$ specimens. Total length 610-710 μm (657 μm); greatest width 24-38 μm (31); distance from head to nerve ring 82-116 μm (95); distance from head to excretory pore 68-122 μm (107); oesophagus length 101-150 μm (132); tail length 70-105 μm (84).

Taxonomic considerations

There are only 4 species adequately described in the genus *Heterorhabditis*: *H. bacteriophora* Poinar, 1975, which is the type species; *H. megidis* Poinar, Jackson et Klein, 1987; *H. zealandica* Poinar, 1990, which has been recently considered as a synonym of the New Zealand population of *H. heliothidis sensu* Wouts, 1979; (POINAR, 1990); and *H. indicus* Poinar, Karunakar et David, 1992.

Other recorded heterorhabditid species, including *H. hambletoni* (Pereira, 1937) from Brazil (PEREIRA, 1937), which is the first report of a heterorhabditid nematode in South America, and the *Heterorhabditis* population isolated from Argentina (DOUCET, 1985), are still too incompletely described to assign them to any species, and they should be considered up to now as *nomina dubia*.

Heterorhabditis argentinensis n. sp. is characterized by the large size of the adult males and first generation females.

First generation females possess a large stoma (13 μm) and oesophagus (274 μm). The vulvar lips are slightly protruded compared with the other species. The tail length is also larger than the already described species (118 μm).

The males' spicules are remarkable arcuate, the capitulum is longer than broad. The shaft has a single rib and a conspicuous rostrum. The gubernaculum is single and fusiform in lateral view. The bursa is open, peloderan and with 9 pairs of genital papillae arranged as follows: 1 2 () 1 2 3.

Infective juveniles are wider than the other 4 species (31 μm); and the tail length is shorter (84 μm).

H. argentinensis n. sp. differ from all the described species in the colour they induce to their hosts when they release the symbiotic bacteria in the hosts' hemocoel (deep purple), whereas for *H. bacteriophora* it is reddish, for *H. zealandica*, greenish yellow and for *H. megidis*, deep red. This should probably indicate a difference between the symbiotic bacteria they carry in their intestine.

DISCUSSION

Heterorhabditis argentinensis n. sp. as a member of the genus *Heterorhabditis* shares its general morphology with the already-described species of this genus. But it can be distinguished from them as follows:

—from *H. bacteriophora*, *H. megidis*, *H. zealandica* and *H. indicus* in the body size of the adult males and hermaphroditic females (1,50 mm and 6,5 mm, respective-

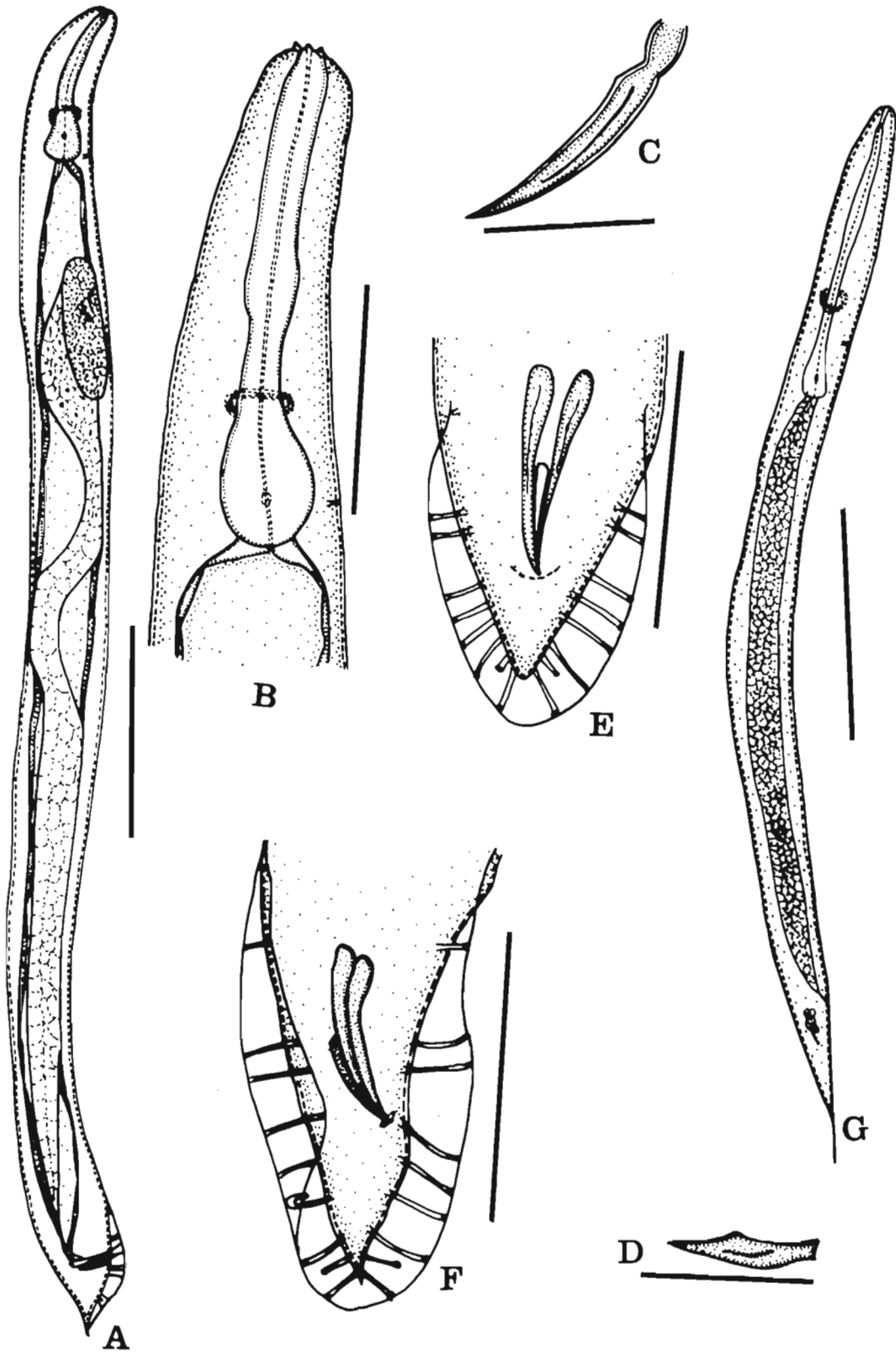


Fig. 2.— Male and third larval stage of *Heterorhabditis argentinensis* n. sp.: A) mature male *in toto*; B) lateral view of oesophageal region; C) spicule, in lateral view; D) gubernaculum, in lateral view; E) tail, in ventral view; F) tail, in lateral view. G) third larval stage (infective juvenile). Scale bars: A, F, G: 100 μ m; B, E: 50 μ m; C, D: 25 μ m.

- ly), and arrangement of the male genital papillae;
- hermaphroditic females of *H. argentinensis* n. sp. differ from *H. bacteriophora* in the stoma length (13 μm vs 1,8 μm); this is also observed within males (5 μm vs 1,3 μm);
- the shape of the spicules and gubernaculum is another significant difference between the new species and that already described;
- it can be separated from *H. megidis* by the number of cephalic papillae (single cephalic papillae vs double cephalic papillae in each submedial lip), and the shape of the bursa (peloderan vs leptoderan or pseudoleptoderan);
- H. argentinensis* n. sp. differs from *H. indicus* in the length of the infective juveniles (*H. indicus* possess the shortest known infective juveniles, by the aspect of the bursal rays (pairs 8 and 9), which are atrophied in *H. indicus*; and in the lack of an intestinal pouch that is present in *H. indicus*;
- infective juveniles of *H. argentinensis* n. sp. differ from *H. zealandica*, *H. bacteriophora*, *H. megidis* and *H. indicus* in the body width; the present species is the widest of them; also the tail length is shorter than in the other species.

Considering the aforesaid characteristics, it is concluded that this heterorhabditid nematode belongs to a new species for which we propose the name of *Heterorhabditis argentinensis* n. sp.

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