

***Howardula apioni* sp. n. (Allantonematidae: Nematoda), a Parasite of *Apion carduorum* Kirby (Curculionidae: Coleoptera) in Southern France**

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ABSTRACT: *Howardula apioni* sp. n. (Allantonematidae: Tylenchida) is described as a parasite of the weevil, *Apion carduorum* Kirby (Curculionidae: Coleoptera) in Southern France. The host is a pest of artichoke and the eggs, larvae, and pupae occur in the stems of this plant. Third-stage juvenile nematodes leave the adult beetle and enter the plant tissues where they molt twice and mate. The infective stage females enter the beetle larvae and are carried through the pupae and into the adult host. Most nematode development occurs during the diapause of the adult beetle. The rate of infection varied from 2-13% over an 8-year period.

Entomogenous nematodes of the genus *Howardula* have been recorded from insects belonging to the orders Diptera, Coleoptera, and Thysanoptera (Poinar, 1975). The effect of some *Howardula* species on their hosts has given them the rank of biological control agents that could be produced under laboratory conditions (Poinar, 1979).

During an investigation of the biology of the weevil, *Apion carduorum* in Southern France, a species of *Howardula* was found to be one of the most common enemies of this insect. Since *A. carduorum* develops on young artichoke plants (*Cynara scolymus* L.) which are extensively cultivated in certain areas along the Mediterranean, it is regarded as a pest. Thus any natural means of control would be of interest from a practical standpoint.

The present paper describes this nematode parasite, outlines its biology, and compares it with previously described members of *Howardula*.

Materials and Methods

In order to obtain the free-living stages of the nematode, infected adults of *Apion carduorum* Kirby were collected from artichoke plants from November to April and placed on stems of the same plant in laboratory cages. During their routine feeding and ovipositional behavior, they liberated nematode juveniles from their alimentary tract which matured to males and infective stage females in the plant tissues. These free-living stages were removed after macerating the stems in water and were heat killed in hot (80°C) water. The mature parasitic females and other stages found in the homocoel of beetles were removed and killed in hot (80°C) 0.9% saline. All specimens were then fixed in a mixture of formol (4%), acetic acid (3%), picric acid (0.2%), and glycerine (1%) and processed to glycerin by the Seinhorst evaporation method. All measurements were made with specimens fixed in the above manner.

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Results

SYSTEMATICS: The species of *Howardula* found parasitizing *A. carduorum* was determined as being new to science and a description follows below. In the quantitative portion of the description, the value following the character represents the average whereas the numbers in parentheses represent the range of the character. All measurements are given in micrometers unless otherwise specified.

Howardula apioni sp. n.

(Figs. 1, 2)

Tylenchida (Filipjev): Allantonematidae (Pereira), *Howardula* Cobb.

FIRST-STAGE JUVENILE ($N = 10$), (Figs. 1A, H): Elongate, length, 275 (240–340); head rounded, bearing a faint tylenchoid stylet approximately $7 \mu\text{m}$ in length; pharyngeal lumen slightly cuticularized from the base of the stylet to the junction with the subventral gland openings; nerve ring, excretory pore, and anus visible; genital primordium composed of 1 large medial and 2 smaller polar cells; tail rounded.

SECOND-STAGE JUVENILE ($N = 10$), (Fig. 1B): Elongate form with head and tail rounded; length, 420 (380–500); genital primordium with many cells; sexual differentiation occurs at the end of the stage.

THIRD-STAGE JUVENILE ($N = 10$), (Figs. 1D, E, I): Length nearly equal to that of adult female, 660 (590–710); male, 670 (620–730); gonad well-developed; head and tail rounded. This stage leaves the host and molts twice in succession in plant tissues to reach the adult stage.

The fourth-stage juveniles were very similar to those of the preceding stage and molted quickly to the adult stage.

FREE-LIVING FEMALE (Figs. 1E, F, G): Slightly longer and more slender than the 3rd-stage juvenile that leaves the host; usually retains the 2 thin cuticles of the 3rd- and 4th-stage juveniles; body straight or curved ventrally when heat killed; cuticle with fine transverse striations approximately $1 \mu\text{m}$ apart, head rounded, without a cephalic constriction; lips, amphids, and stylet guide not visible; stylet well-developed, with a large lumen, anterior part conical with a ventral opening, posterior part cylindrical with a diameter of $1 \mu\text{m}$, slightly less than the anterior portion; the dorsal “knob” is slightly anterior to the 2 subventral; pharyngeal lumen wide, strongly cuticularized after the stylet but becoming difficult to follow after reaching the subventral gland openings; dorsal gland opening very minute and distinct only in living material; all 3 pharyngeal glands contain distinct nuclei, approximately $20 \mu\text{m}$ from each other; ovary composed of several cells, oviduct elongate, lined with small cells; sometimes both ovary and oviduct are reflexed (rare in Allantonematidae); uterus small in nonmated forms, but greatly extended after copulation; vagina leads anteriorly before joining the uterus at the point of a small constriction; vulva faintly visible, in posterior $\frac{1}{3}$ of body; lacking lips; anus present, tail tip rounded.

QUANTITATIVE CHARACTERS ($N = 10$): Length, 683 (615–770); greatest width, 20 (17–23); length of stylet, 13 (12–14); distance from head to dorsal pharyngeal gland opening, 52 (45–57); nerve ring, 98 (93–104); hemizonid, 104 (100–110); excretory pore, 73 (68–78). Distance from vulva to anus, 22 (18–27); length

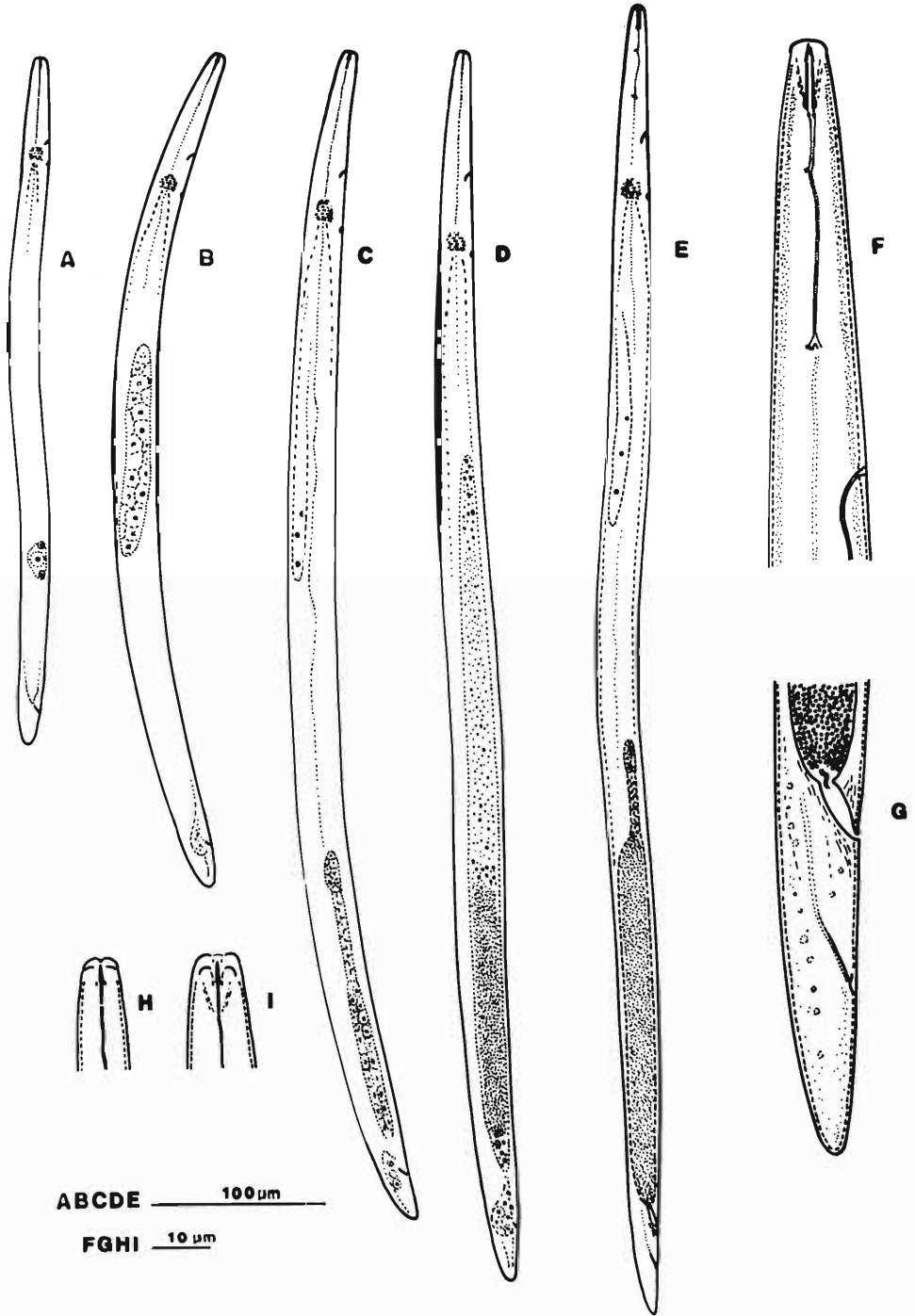


Figure 1. *Howardula apioni* sp. n. A. First-stage juvenile. B. Second-stage juvenile. C. Third-stage juvenile female. D. Third-stage juvenile male. E. Free-living infective stage female. F. Anterior part of infective stage female. G. Posterior of infective stage female. H. Head of first-stage juvenile. I. Head of third-stage juvenile female.

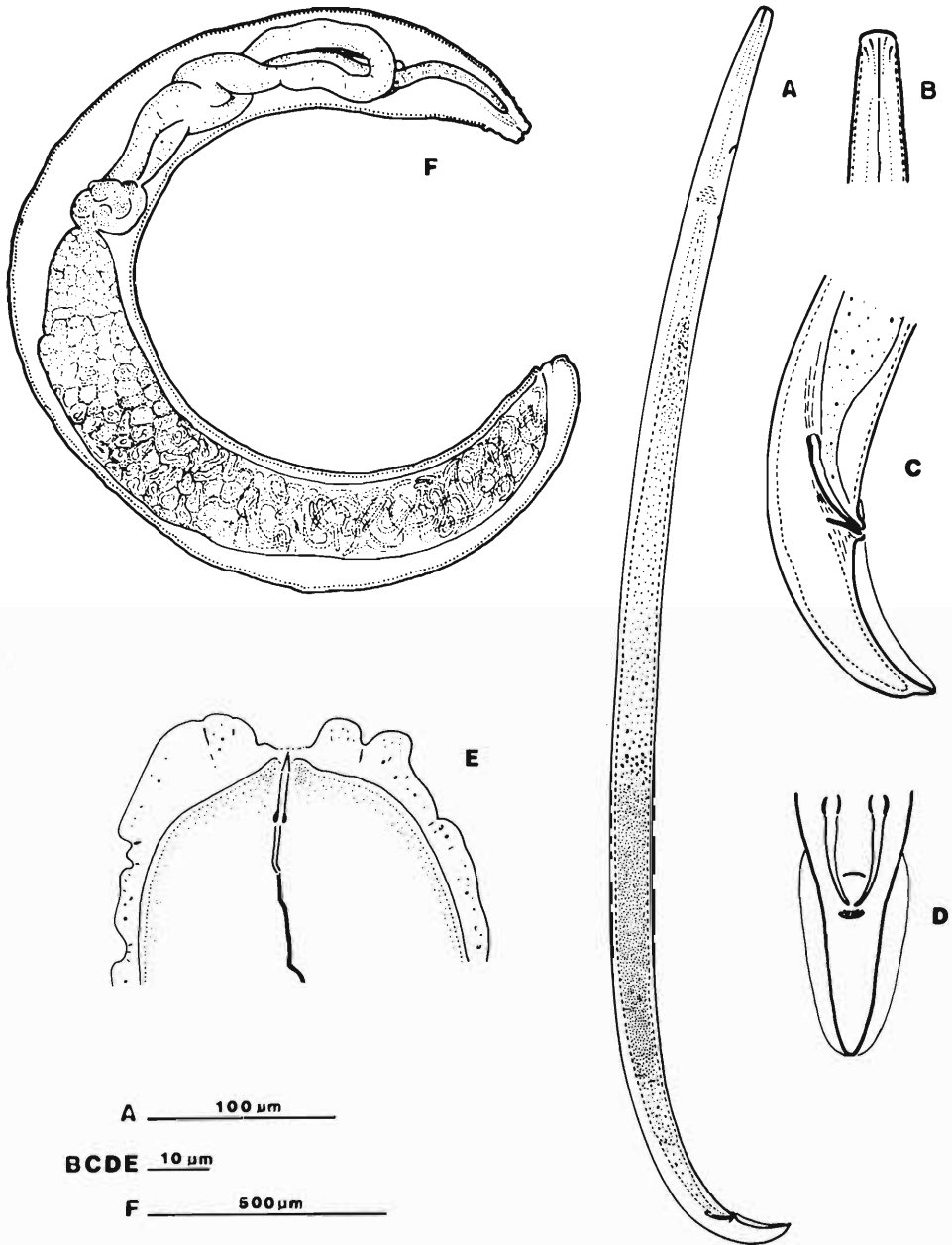


Figure 2. *Howardula apioni* sp. n. A. Free-living male. B. Head of male. C. Lateral view of male tail. D. Ventral view of male tail. E. Head of mature parasitic female. F. Mature parasitic female.

of tail, 31 (24–34); $a = 35$ (29–41); $b = 2.46$ (2.03–3.35); $c = 22.4$ (19.2–26.8); ratio of length to distance from head to hemizonid, 6.55 (6.15–7.10).

MALES (Figs. 2A, B, C, D): Cuticle smooth; body straight or curved ventrally after heat-killing; lateral cords distinct, about $4 \mu\text{m}$ wide; head flatter than in female; stylet degenerate, $9\text{--}10 \mu\text{m}$; pharyngeal lumen indistinct; pharyngeal

glands not visible; gonad well-developed, tip reflexed; diameter of the spermatozoa, ± 1.5 ; spicules paired, separate, equal, slightly curved; gubernaculum small, curved backwards; bursa peloderan, open; tail curved, sometimes terminated with a minute mucron.

QUANTITATIVE CHARACTERS ($N = 10$): Length, 712 (650–750); greatest width, 20 (16–24); distance from head to nerve ring, 93 (84–89); hemizonid, 111 (99–117); excretory pore, 78 (73–85); length of tail, 32 (30–42); length of gonad, 456 (350–522); length of spicules, 21 (19–26); length of gubernaculum, 7 (6–8); ratio of total length to distance from head to hemizonid, 6.5 (6.1–6.7); ratio of total length to length of gonad, 1.6 (1.4–2.0); $a = 37$ (31–43); $c = 19.9$ (16.4–22.0).

MATURE PARASITIC FEMALE ($N = 8$) (Figs. 2E, F): Length, 2.477 (2.040–2.860) mm; greatest width, 222 (172–286) μm ; cylindrical or sausage-shaped, fixed striations every 2–3 μm , but often smooth near the head and tail; head rounded with oral opening often indented; stylet similar to that of the infective stage female; pharyngeal lumen visible just to the beginning of the subventral pharyngeal glands; excretory pore, hemizonid, and nerve ring not distinct; pharynx and intestine regressive; anus present; gonad well-developed, reflexed several times, the tip often reaching the stylet; spermatheca indistinct; gravid female containing eggs and hatched juveniles (ovoviviparous); vulva distinct in posterior $\frac{1}{3}$ of body, without lips; tail rounded, variable in form.

TYPE HOST: *Apion carduorum* Kirby (Curculionidae: Coleoptera).

TYPE LOCALITY: Opio, Alpes-Maritimes, France.

TYPE SPECIMENS: Holotype (free-living female) and allotype (male) deposited at the Laboratoire des Vers, Museum National d'Histoire Naturelle, Paris.

DIAGNOSIS: There are presently 13 species described in the genus *Howardula*. The presence of a bursa separates *H. apioni* from *H. aoronymphium* Welch (1959), *H. medecassa* Remillet and Van Waerebeke (1975), *H. acris* Remillet and Van Waerebeke (1976), and *H. benigna* Cobb (1921). The species *H. dubium* Christie (1938) and *H. phyllotreta* Oldham (1933) are known only from females, which differ in size from the present species. The gravid females of both *H. acarinarum* Wachek (1955) and *H. husseyi* Richardson et al. (1977) are oviparous and the former possesses a spermatheca, whereas the latter has a protruding stylet region. The positions of the gland openings are different from those of *H. apioni* in the infective-stage females of *H. truncati* Remillet and Van Waerebeke (1975) and *H. husseyi* Richardson et al. (1977).

The infective-stage females of *H. oscinella* Goodey (1930) lack knobs on their stylets and the spicules and gubernaculum are almost half the length of those of *H. apioni*.

The lengths of the infective stage females of *H. dominicki* Elsey (1977) and *H. colaspidis* Elsey (1979) are smaller than the present species and the stylets are longer (20–21 vs. 12–13). The gravid female of *H. aptini* (Sharga, 1932) is swollen and not sausage shaped like *H. apioni*.

BIOLOGICAL OBSERVATIONS: Third-stage juveniles of *H. apioni* penetrate the intestinal wall and emerge from the anus of adult *Apion* weevils feeding, mating, or ovipositing on the stems of artichoke plants. The nematodes enter the plant tissue, molt twice to reach the adult stage and mate. This period of maturation is completed in the laboratory in 1–2 weeks at 20–22°C. The infective-stage females then search out and penetrate the larval stages of the host. Probably the

first or second stage of *Apion* is preferred yet under laboratory conditions, all three larval stages as well as the pupal stage of the host were infected. In nature, all stages of the insect except the adult remain in the plant tissues. It is after the adult weevils leave the field for their diapause when the mature females of *Howardula* begin their development. At the end of its diapause, the adult weevil usually contained a range of juvenile stages.

Over a period of 8 years, the rate of parasitism varied between 2 and 13%, depending on the time and place of sampling.

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