

***Howardula phyllotretae* (Tylenchida: Allantonematidae) - parasite of *Phyllotreta* flea beetles (Coleoptera: Chrysomelidae)**

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Summary. A description of the free-living stages of entomogenous nematode *H. phyllotretae* Oldham, 1933 is given for the first time. Data are presented on the prevalence and intensity of flea beetle infestation with these nematodes. Parasite induced changes in host reproductive system are described. An identification key for *Howardula* species is presented.

Key words: Tylenchida, Allantonematidae, *Howardula phyllotretae*, morphology, host-parasite relationships, key for *Howardula*.



An infestation of all flea beetles on cabbage (*Brassica oleracea* L. var. *capitata*), radish (*Raphanus sativus* L. var. *radicula* Pers.) and black radish (*Raphanus sativus* L. var. *sativus*) growing in the Moscow region with nematodes identified as *Howardula phyllotretae* was reported in the summer of 1990. All populations of flea beetles studied were infected and the invasion prevalence was between 20% and 80%. A study of the morphology and biological properties of the parasitic nematodes and their effect on the host, an important pest of cultivated crucifers, was carried out. A taxonomic description is given for *H. phyllotretae* specimens found for the first time in Russia.

MATERIALS AND METHODS

Estimations of flea beetle infestations were made during two vegetation seasons (May - September 1992 & 1993) 2-4 times each month. An experimental site at 2 hectares of white-headed cabbage was chosen for regular observations.

The number of flea beetles was estimated on cabbage heads along a diagonal transect across the field. Fifty randomly chosen beetles were dissected on each occasion. The length and the number of ovarioles were used to evaluate beetle gonad development. Also, the "total length of ovarioles" was calculated as a product of the length and total number of ovarioles.

Measurements of the gonads were performed on 10 uninfected beetles found in cohort with beetles of

different levels of invasion (from 1 to 7 parasitic female nematodes per host). The total length of ovarioles in uninfected individuals was considered to represent 100% gonad development. The percentage of gonad development was calculated for the cohort beetles with different levels of infestation.

Free-living stages of *H. phyllotretae* were obtained during the observation from the hosts cultivated on radish plants. High densities of hosts were cultured during two months on single radish plants covered with mesh screens. The mean value of flea beetle infestation was about 70%. The parasitic stages recovered through dissections and free-living stages found on radish roots were fixed in TAF, then mounted in glycerin. Description and drawings were prepared from living nematodes and from specimens on glycerin slides.

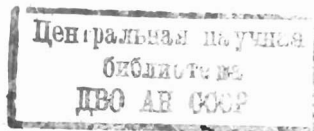
DESCRIPTION

***Howardula phyllotretae* Oldham, 1933 (Fig. 1)**

Free-living invasive females (n = 7): L = 584-665 (633) μ m; D = 14.3-17.1 (16.0) μ m; a = 34.2-44.8 (40.0); b = 28.6-35.9 (30.8); c = 14.4-15.8 (15.0); V = 90.0-90.9 (90.5)%; stylet = 14.6-19.6 (17.1) μ m; tail = 40.8-45.6 (43.2) μ m.

Free-living males (n=3): L = 527-570 (548) μ m; D = 17.1-19.9 (18.5) μ m; a = 28.6-31.0 (29.8); spicules = 11.4-14.6 (13.0) μ m; tail = 38-42 (40) μ m.

Parasitic females (n = 10): L = 1425-2080 (1661)



μm ; $a = 12-15$ (13); stylet = 12-14 (13) μm .

Free-living invasive female. Body slender, straight or slightly curved ventrally. Cuticle with fine annulation. Cephalic capsule not set-off. Lip region slightly protruded. Amphidial pores slit-like. Stylet distal end thickened, stylet lumen 1-1.5 μm wide, proximal end with rudimentary knobs. Visible sclerotization of oesophageal lumen 18-23 μm , dorsal gland orifice at 8-10 μm from that of subventral ones. Nerve ring at 74-78 μm and excretory pore at 42-46 μm from anterior end, both poorly visible. Oesophageal glands occupy 2/3 of body length and reach the ovary germinative zone. Vulva close to anus (distance 16-21 μm), with protruded lips. Tail with ventrally curved or straight mucron.

Free-living male. Body slender, straight with fine cuticular annulation. Lateral field of cuticle with two incisures. Lip region protruded, slightly off-set. Excretory pore 37-40 μm and nerve ring 8 μm from anterior end. Oesophagus with poorly conspicuous structure. Gonad length about 300 μm . Spicules arcuate, broaden to proximal end. Gubernaculum 3 μm long. Tail conical, tapering to the terminus, without bursa.

Parasitic female. Body cylindrical, elongated, ventrally curved or C-shaped. Head end hemispherical, slightly flattened. Stylet and deformed oesophageal lumen are conspicuous. Oesophagus with poorly conspicuous structure. Excretory pore 87-90 μm from anterior end. Ovary 2-3 times reflected. Postovarial orifice short, spermatheca elliptical, uterus filled with eggs and juveniles. Viviparous. Vulva subterminal, with slightly protruded lips. Anus poorly conspicuous, vulvar-anal distance 31-36 μm . Tail rounded, with numerous cuticular folds.

Addition to specific diagnosis. Free-living males and females vermiform. Female stylet with thickened distal end and rudiments of knobs at proximal end. Oesophageal glands occupy 2/3 body length and reach ovary germinative zone. Vulva with slightly protruding lips. Tail with short narrow terminus. Male without stylet, arcuate spicules broadening to proximal end. Gubernaculum present. No bursa.

Insect host and distribution. Originally found in Great Britain (Oldham, 1933), *Howardula phyllotretae* were found in two species of flea beetles: *Phyllotreta undulata* and *P. atra*. The survey of flea beetles in Moscow, Vladimir, Jaroslavl and Nizhni Novgorod regions of Central Russia revealed that all populations of beetles are infected with these nematodes.

Specimens. Slides of *H. phyllotretae* were deposited in the Institute of Parasitology, the Russian Academy of Sciences and in the German Nematode Collection in Münster.

Biological peculiarities. After copulation the fertilized free-living female IV stage juveniles enter the host coelom percutaneously. During this period the host larvae inhabit the rhizosphere of crucifer plants. At the time of host imago formation the IV stage juveniles moult and hibernate (Fig. 2). If infestation occurs early during the vegetative season, the invasive female produces juveniles which serve as the hibernating stage.

Intensity of flea beetle infestation from our observations was between 1 and 7 parasitic females per host (Fig. 3). Occasionally up to 10-11 parasitic females were recovered from a single host.

In spring the *Howardula phyllotretae* juveniles moult and accumulate in the host reproductive tract. During host oviposition the second-stage juveniles of *H. phyllotretae* leave the host. After two moults juveniles turn into juvenile males and females and copulate. The fertilized female invades new hosts.

As a highly specialized parasite *H. phyllotretae* is specifically adapted to the host life cycle. No external changes to the host morphology or behavior were observed.

At the observation site in the Moscow region infection prevalence was relatively stable during the two year observation period (20-40%). The percentage of infected beetles was similar in autumn (before hibernation) and in spring (after hibernation), i.e. no direct effect of infestation on the viability of hibernating beetles was observed. Nevertheless, changes in the host's reproductive system were obvious *viz.* the length and the number of ovarioles in the ovaries of infected beetles decreased. The rate of ovary development was affected by the infection intensity. Infection of the host with 1-3 parasitic females, commonly observed in 80% of the beetles, resulted in the ovaries being 20-25% smaller than in uninfected hosts. In beetles containing 4-5 parasitic nematodes the ovaries were 60% smaller (Fig. 4). With the infection intensity equal to six and more nematodes the ovaries were completely degenerate and nonfunctional (Fig. 5).

Taxonomical remarks on the *Howardula Cobb, 1921*

The single significant taxonomic feature used in the diagnosis of the genus *Howardula* is the absence of a stylet in the free-living male (a single exception is *H. apioni* which has a degenerated 10 μm long stylet).

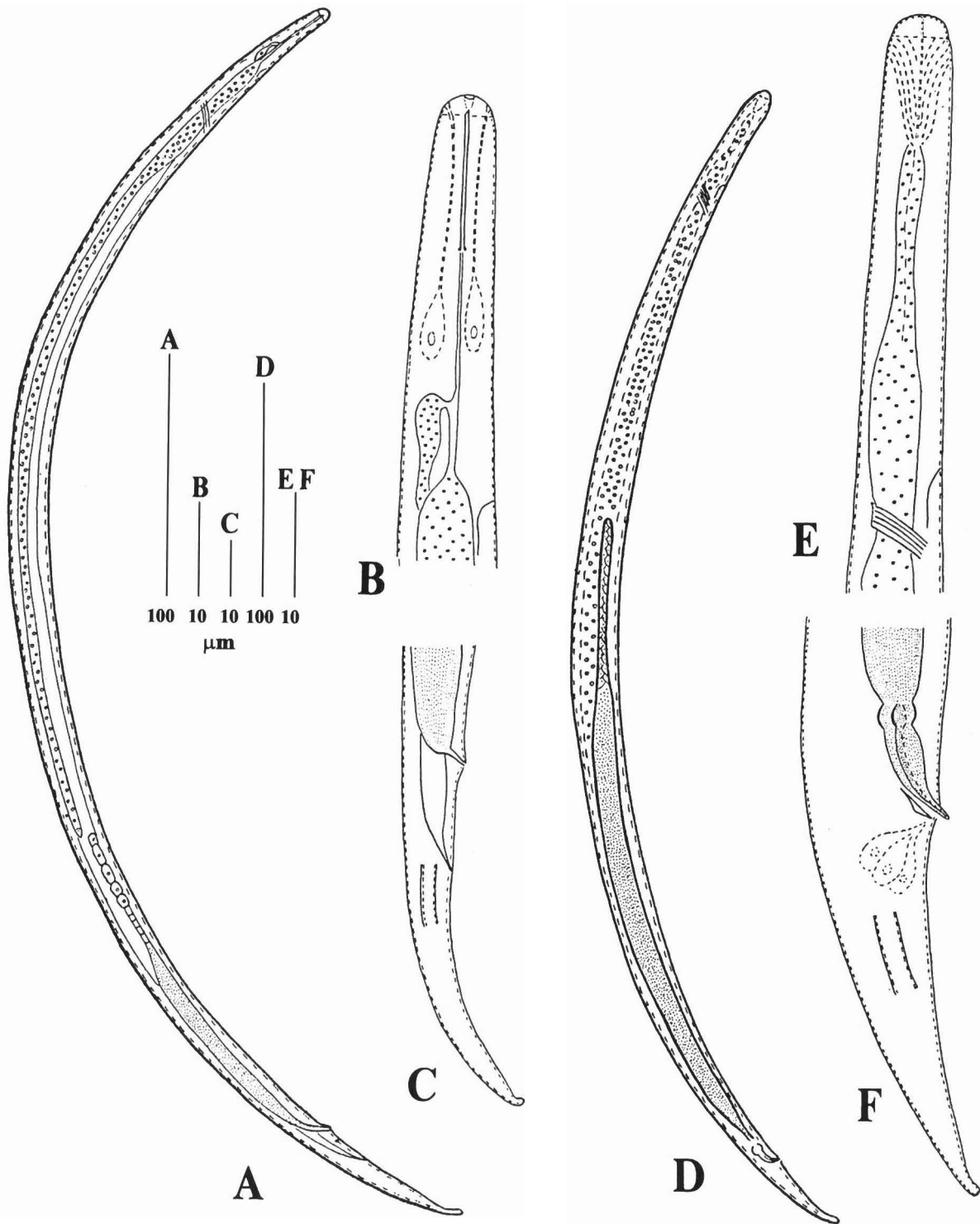


Fig. 1. *Howardula phyllotretae*, Central Russian population. A: Free-living female, total view; B: Anterior end of free-living female; C: Posterior end of free-living female; D: Free-living male, total view; E: Anterior end of free-living male; F: Posterior end of free-living male.

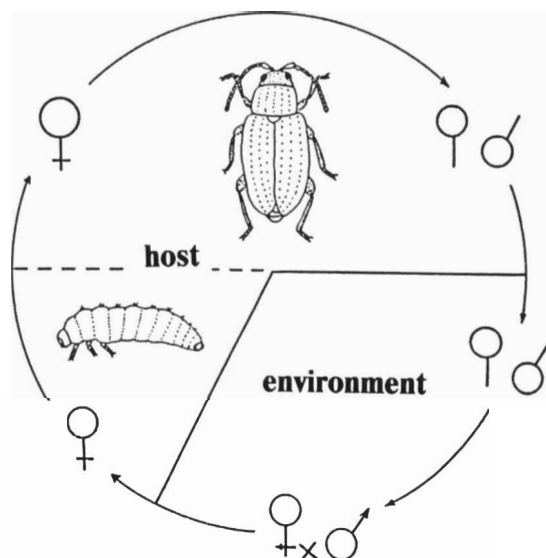


Fig. 2. Life cycle of *Howardula phyllotretae*.

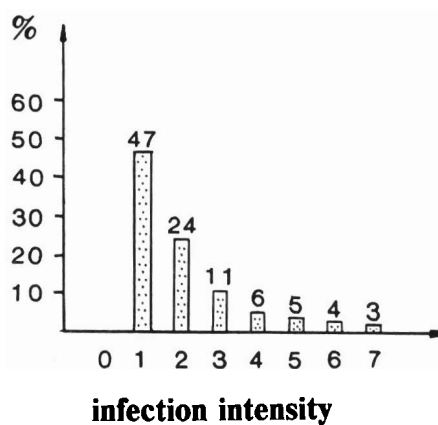


Fig. 3. The percentage of hosts with different levels of infection intensity.

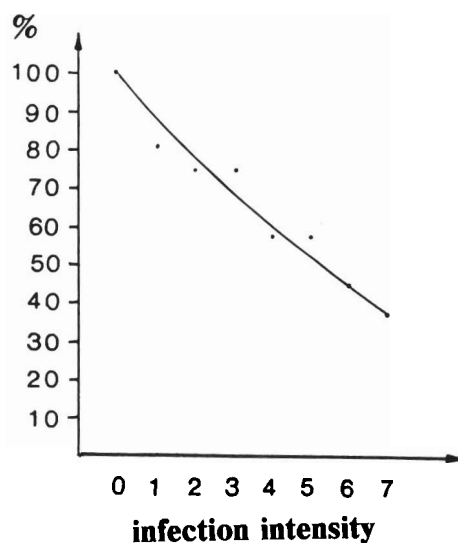


Fig. 4. Dependence of development of ovaries in *Howardula phyllotretae* from the infection intensity.

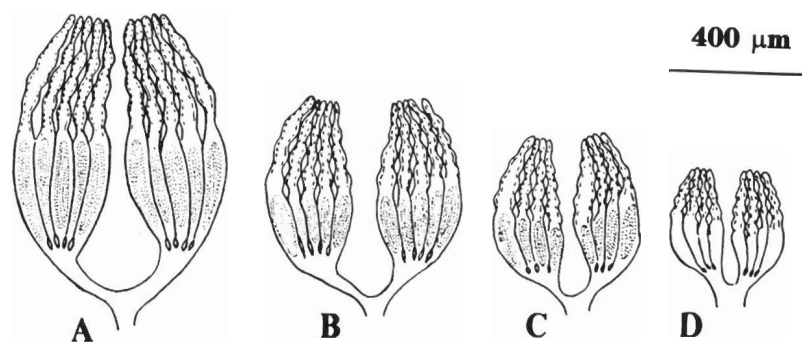


Fig. 5. Ovaries of *Howardula phyllotretae* under different intensity of infection. A: Uninfected beetles; B: Ovaries of beetles with 2-3 parasitic females; C: Ovaries of beetles with 4-5 parasitic females; D: Ovaries of beetles with 6-7 parasitic females.

The first revision of the genus *Howardula* was presented by Wachek (1955) and resulted in 5 species being considered as valid representatives of this taxon. Four species described by Warren (1941) from gamasid mites of the genera *Cosmolaelaps*, *Hypoaspis*, *Haemogamasus*, *Euryparasitus*) were only conditionally ascribed to *Howardula*.

In the revision by Slobodyanyuk (1984) only 14 species from 21 described as representatives of *Howardula* were left in this genus with 8 of these having conditional status. Four species described by Warren were transferred to *insertae sedis*, and three species (*H. prima*, *H. stenoloba*, *H. dubium*) to *species inquirendae*.

Fifteen species were included in *Howardula* by Siddiqi (1986), though species described by Warren also being considered as *species inquirendae*.

Remillet and Laumond (1991) ascribed 16 species to *Howardula*, and Warren's species were also considered as *species inquirendae*.

In addition to these species from Acarina several poorly described species by Rubzov (1981) from fleas, *H. prima* and *H. stenoloba* should be also considered as *species inquirendae*. The description of *H. albopunctata* Yatham & Rao, 1980 was not available for this study, therefore, a total of 12 species in *Howardula* are considered here as being valid.

Key for the identification of *Howardula* species

1. Parasitic female viviparous 2
 - Parasitic female oviparous 11
2. Free-living male with bursa 3
 - Free-living male without bursa 8
3. Adanal or subterminal bursa in male 4
 - Peloderan bursa 5
4. Adanal bursa. Parasitic female length more than 3.5 mm. Parasites of chrysomelid beetles *Diabrotica* and *Acalymma* *H. benigna* Cobb, 1921
 - Subterminal bursa. Parasitic female length less than 1.6 mm. Parasites of *Oscinella frit* (Chloropidae; Diptera) *H. oscinella* Goodey, 1930
5. Bursa anterior margin posterior to the anus. Parasites of chrysomelids 6
 - Bursa anterior margin anterior to the anus. Parasites of Nitidulidae and Curculionidae 7
6. Parasitic female length more than 2.5 mm. Body straight. Parasite of *Colaspis brunnea* (Chrysomelidae, Coleoptera) *H. colaspidis* Elsey, 1979

- Parasitic female length less than 2.5 mm. Body C-shaped. Parasite of *Epitrix hirtipennis* (Chrysomelidae, Coleoptera) *H. dominicki* Elsey, 1977

7. Male tail without mucron. Parasitic female with large body diameter ($a = 5.0-7.4$). Parasite of *Carpophilus truncatus* (Nitidulidae) *H. truncati* Remillet & Waerebeke, 1975

- Male tail with small mucron. Parasitic female cylindrical, elongated ($a = 10-12$). Parasite of *Apion cardiorum* (Curculionidae) *H. apioni* Poinar, Laumond & Bonifassi, 1980

8. Gubernaculum absent. Parasite of *Drosophila* flies (Drosophilidae) *H. aoronymphia* (Welch, 1959)

- Gubernaculum present 9

9. Excretory pore in free-living stages at level of or posterior to the nerve ring. Male tail with characteristic spike-like appendage. Parasite of Hydrophilidae beetles *H. acris* Remillet & Waerebeke, 1976

- Excretory pore in free-living stages anterior to the nerve ring. Male tail without appendage 10

10. Stylet length in free-living females 21.5-22.5 μm . Male tail rounded. Parasite of *Carpophilus* (Nitidulidae) *H. madecassa* Remillet & Waerebeke, 1975

- Stylet length in free-living females 14.7-19.6 μm . Male tail conical, tapered. Parasite of *Phyllotreta* beetles (Chrysomelidae) *H. phyllotretae* Oldham, 1933

11. Male without bursa. Parasite of *Copromyza* flees (Sphaeroceridae) *H. marginatis* Reddy & Rao, 1981

- Male with bursa. Parasite of *Megaselia* flies (Phoridae) *H. husseyi* Richardson, Hesling & Riding, 1977

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Захаренкова Н.Н. *Howardula phyllotretae* (Tylenchida: Allantonematidae) - паразит жуков-блошек рода *Phyllotreta* (Coleoptera: Chrysomelidae).

Резюме. Впервые дано описание свободноживущих стадий энтомопаразитических нематод *H. phyllotretae* (Oldham, 1933). Приводятся данные по экстенсивности и интенсивности заражения жуков этими нематодами; описываются изменения в половой системе хозяина, вызываемые паразитированием нематод. Предложен ключ для определения видов рода *Howardula*.
