Fundam. appl. Nematol., 1998, **21** (5), 547-552

Howardula neocosmis sp.n. parasitizing North American *Drosophila* (Diptera: Drosophilidae) with a listing of the species of *Howardula* Cobb, 1921 (Tylenchida: Allantonematidae)

George O. POINAR, JR.*, John JAENIKE** and DeWayne D. SHOEMAKER**

**Department of Entomology, Oregon State University, Corvallis, OR 97331, USA, and **Department of Biology, University of Rochester, Rochester, NY 14627, USA.*

Accepted for publication 27 March 1998.

Summary – *Howardula neocosmis* sp. n. (Tylenchida: Allantonematidae) is described as a parasite of *Drosophila acutilabella* Stalker (Diptera: Drosophilidae) from Florida, USA and *D. suboccidentalis* Spencer from British Columbia, Canada. These two strains represent the first described *Howardula* from North American drosophilids. Notes on the biology of the parasite and a list to the species of *Howardula* Cobb are presented. © Orstom/Elsevier, Paris

Résumé – **Howardula neocosmis *sp. n. parasite de drosophiles nord-américaines (Diptera : Drosophilidae) et liste des espèces du genre* Howardula *(Tylenchida : Allantonematidae)*** – Description est donnée d'*Howardula neocosmis* sp. n. (Tylenchida : Allantonematidae) parasite de *Drosophila acutilabella* Stalker (Diptera : Drosophilidae) provenant de Floride, USA et de *D. suboccidentalis* Spencer provenant de Colombie Britannique, Canada. Ces deux souches représentent le premier *Howardula* décrit sur des drosophiles nord-américaines. Des notes sur la biologie de ce parasite et une liste des espèces du genre *Howardula* Cobb sont présentées. © Orstom/Elsevier, Paris

Keywords: Allantonematidae, *Drosophila*, *Howardula neocosmis* sp. n., insect nematode, parasitism.

Allantonematid nematode parasites of Drosophilidae were first reported by Gershenson in 1939 (see Poinar, 1975, for citations of nematodes from drosophilids). The first descriptions of members of this family attacking lesser fruit flies involved species of the genera *Howardula* Cobb, 1921 and *Parasitylenchus* Micoletzky, 1922 in England (Welch, 1959). In 1985, Montague and Jaenike discussed the presence of allantonematids parasitizing drosophilids in North America and subsequently Poinar *et al.* (1997) described *P. nearcticus* attacking *Drosophila recens* in New York state.

The present study describes the first species of *Howardula* parasitizing drosophilids in North America. From these studies and the report of drosophilid parasitism by allantonematids in Japan (Kimura & Toda, 1989), it is clear that allantonematid parasitism of drosophilids is widespread, demonstrating that these two nematode genera have formed species groups orientated towards infecting drosophilids as well as other acalyptrate Diptera.

Materials and methods

Adult *Drosophila acutilabella* Stalker and *D. suboccidentalis* Spencer were collected in Manasota Key, FL, USA and Peachland, BC, Canada, respectively, in 1997. Newly emerged parasitized flies were placed in culture vials containing instant *Drosophila* Medium (Carolina Biological Supply) plus a piece of commercial mushroom (*Agaricus bisporus*). They were transferred every 4 days to fresh food until they were 3 weeks old. At this age, two parasitized male flies were placed in a culture vial and two female flies from an uninfected culture were added to the same culture after 2 days. All cultures were maintained at 22 °C.

Living parasitized adult flies were dissected at different times to obtain nematodes at various stages of development. All dissections were made in Ringer's solution and the nematodes were heat killed (60 °C), fixed in TAF and processed to glycerin. All measurements were made on slide mounted fixed material, however certain morphological details, *i.e.*, stylet structure, pharyngeal gland morphology, excretory pore position, location of anus and vulva were best determined by observing living individuals.

Howardula neocosmis sp. n.

(Fig. 1)

MEASUREMENTS

Free-living vermiform female (n = 10): L = 416 (384-447) µm; greatest diameter = 16 (13-18) µm; head to middle of nerve ring = 69 (64-74) µm ; head to excretory pore = 80 (75-85) µm; stylet length = 11 (10-12) µm; tail length = 55 (52-56) µm; distance vulva to anus = 23 (22-26) µm ; head to dorsal gland orifice = 25 (22-26) µm; distance from dorsal gland

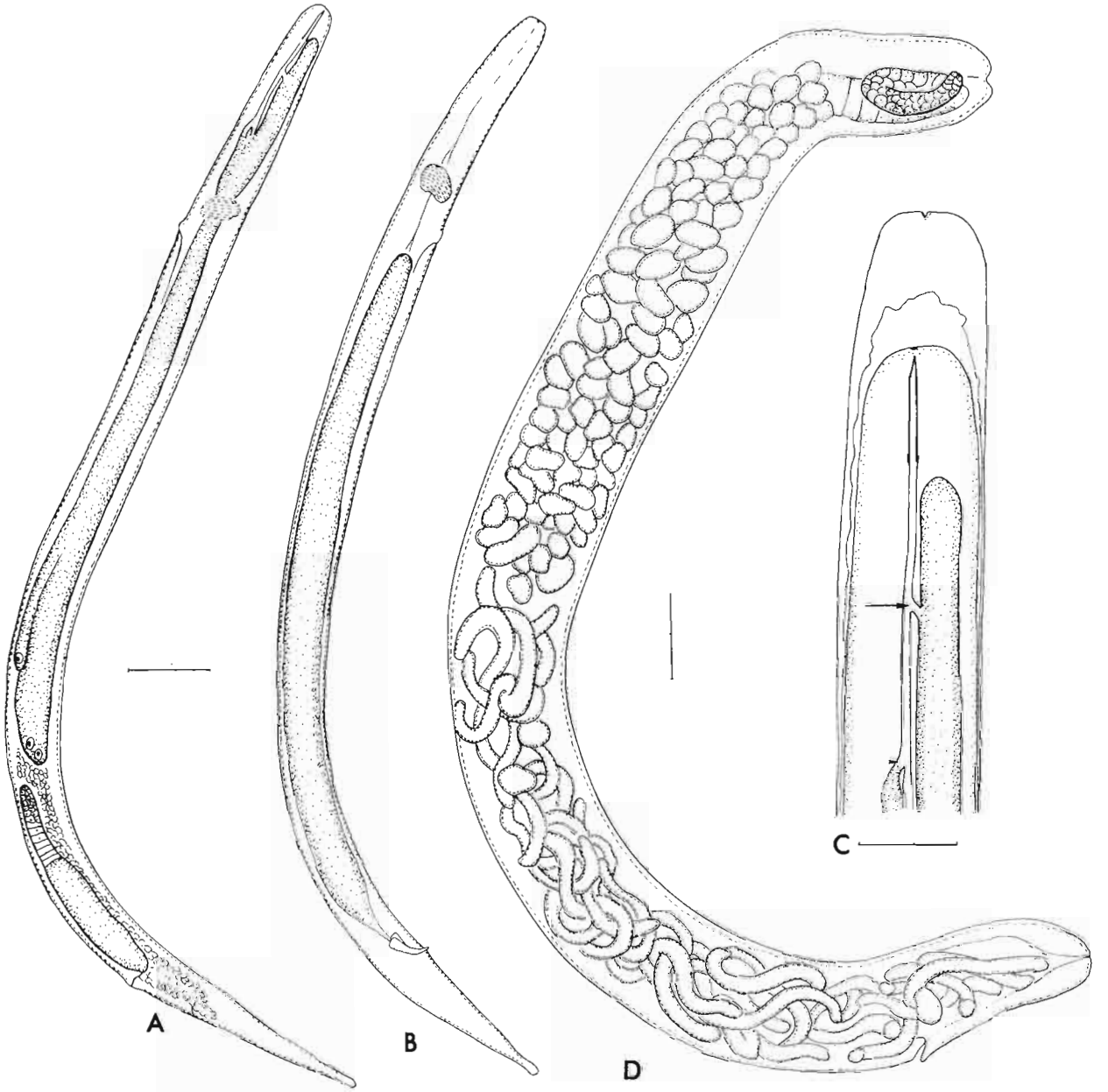

Fig. 1. Howardula neocosmis *sp.n. A: Free-living female; B: Free-living male; C: Head of free-living female with two surrounding cuticles (arrow points to opening of dorsal pharyngeal gland; arrowhead subventral gland opening); D: Parasitic female containing eggs and juveniles (Scale bars: A, B = 25 µm; C = 10 µm; D = 80 µm).*

orifice to ventral gland orifice = 15 (13-19) µm; head to tip of dorsal gland = 11 (9-15) µm ; V = 80 (78-82).

Parasitic female (n= 10): L = 1129 (960-2560) µm; greatest diameter = 125 (100-170) µm; vulva to tail tip = 152 (95-184) µm; stylet length = 9 (8-10) µm; eggs = 32 (20-43) × 20 (16-22) µm; V = 90 (87-93).

Free-living male (n = 10): L = 403 (360-435) µm; greatest diameter = 16 (14-18) µm; head to middle of nerve ring = 70 (56-75) µm; head to excretory pore =

84 (75-88) µm; tail length = 48 (44-57) µm; spicule length = 12 (11-15) µm; greatest width of spicule = 2.0 (1.9-3.0) µm.

DESCRIPTION

Free-living females: Slender body, originally enclosed in a thick, enclosing cuticle and later in the same outer thick cuticle but with an inner membranous cuticle as well. Head truncate, lip region not off-set. Stylet straight, with tip sloping toward ventral surface; stylet base slightly swollen, delineating stylet from remainder of chitinized pharyngeal tube. Distance from stylet base to dorsal gland orifice slightly greater than stylet length; distance from dorsal gland orifice to ventral gland orifice slightly longer than distance from stylet base to dorsal gland orifice; subventral glands extending posteriorally to tip of ovary. Dorsal gland extending only part of that distance; contents of the subventral glands appearing creamy white while that of the dorsal gland granular, indicating different components within. Excretory pore located posterior to the nerve ring and just posterior to the hemizonid. Lateral fields 3-4 µm wide with five striae in widest region. Vulva located on small protuberance. Vagina narrow, leading to a small uterus with no or very slight post vulvar sac; oviduct and ovary small, tip of ovary often reaching to the tip of the subventral pharyngeal glands. Tail slender, ending in a slightly swollen rounded tip.

Parasitic females: Varying in size depending on number per host (the more per host, the smaller the parasites). Cuticle thin except in tail and head region where often thick and wrinkled. Original stylet minus basal thickening portion present although this structure often obscured and shoved aside by the developing gonad (this explaining the size discrepancy between the stylets in the free-living and parasitic females). Lateral fields not discernible. Anus terminal. Vulva present although often obscured. After egg hatching, juveniles undergoing two molts in uterus. Ovoviviparous.

Males: Lacking a stylet and associated pharyngeal glands. Hemizonid prominent as swelling just above excretory pore. Lateral fields, 3-4 µm in width, present but striae faint. Spicules broad at base, with terminal fourth bent outward; bursa and gubernaculum absent. Tail elongate with faint rounded swelling at tip.

Remarks

The preceding account is based on the examination of living specimens which show morphological features much clearer than fixed material. The British Columbia strain of *H. neocosmis* sp. n. did not appear to possess any qualitative characters that separated it from the Florida strain. However, some of the measurements of the females of the British Columbia strain ranged higher than those of the Florida strain and are given below. There were no quantitative differences between the males of the two strains. In the British Columbia strain, those measurements that differed from the Florida strain in the free-living females were the length (458 [384-517] µm), the distance from the head to the middle of the nerve ring (84 [72-93] µm), the distance from the head to the excretory pore (93 [85-99] µm), the stylet length (12 [11-13] µm), and the distance from the dorsal gland orifice to the ventral gland orifice (18 [16-20] µm). The length of the parasitic females of the British Columbia strain ranged from 1920 to 3200 µm. Since at this time, clear characters separating these two populations could not be found, they will be referred to as the Florida and British Columbia strains of *H. neocosmis* sp. n.

TYPE HOST AND LOCALITY

Found in the hemocoel of *Drosophila acutilabella* Stalker collected from Manasota Key, FL, USA (Florida strain). Also found in *D. suboccidentalis* Spencer from Peachland, BC, Canada (British Columbia strain).

TYPE MATERIAL

Holotype: free-living female of the Florida strain (UCDNC 3651) - *Allotype*: male of the Florida strain (UCDNC 3652); deposited in the Nematology Collection, Department of Nematology, University of California, Davis. *Paratypes* in the collection of the Muséum National d'Histoire Naturelle, Paris, France, and in the author's collection.

DIAGNOSIS AND RELATIONSHIPS

The absence of a bursa and gubernaculum in *H. neocosmis* sp.n. separates this species from all of the other members of the genus with the exception of a group of three species which parasitize acalyptrate Muscomorpha, namely *H. marginatis* in Sphaeroceridae, *H. albopunctata* in Sepsidae and *H. aoronymphium* in Drosophilidae. The oviparous nature of the former and the long stylet of *H. albopunctatis* distinguish them from *H. neocosmis*. It is obvious that the new species is closest to the European drosophilid parasite *H. aoronymphium*. However, the following characters separate the two species: size of the spicules (11-15 in *H. neocosmis vs* 19-21 µm in *H. aoronymphium*), the distance from the head to the excretory pore in the male (80-88 in *H. neocosmis* sp.n. *vs* 98 µm in *H. aoronymphium*), the presence of basal thickenings on the stylet in *H. neocosmis* and their absence in *H. aoronymphium*, the presence of a stylet, anus and vulva in the parasitic female of *H. neocosmis* and their absence in *H. aoronymphium,* and the length of the stylet in the infective female (10-12 in

Table 1. *List of species of the genus* Howardula *Cobb, 1921 with indication of their host(s).*

Species	Hosts
Type species	
H. benigna Cobb, 1921	Coleoptera: Chrysomelidae
Valid species	
H. acarinorum Wachek,1955	Acarina
H. acris Remillet & Van Waerebeke, 1976	Coleoptera: Hydrophilidae
H. albopunctata Yatham & Rao, 1980	Diptera: Sepsidae
H. aoronymphium Welch,1959	Diptera: Drosophilidae
H. apioni Poinar, Laumond & Bonifassi, 1980	Coleoptera: Curculionidae
H. belgaumensis Raj & Reddy, 1989	Coleoptera: Chrysomelidae
H. colaspidi Elsey,1979	Coleoptera: Chrysomelidae
H. dominicki Elsey,1977	Coleoptera: Chrysomelidae
H. husseyi Richardson, Hesling & Riding, 1977	Diptera: Phoridae
H. madecassa Remillet & Van Waerebeke, 1975	Coleoptera: Nitidulidae
H. marginatis Reddy & Rao,1981	Diptera: Sphaeroceridae
H. mutilatus Devi, Rao & Reddy, 1991	Coleoptera: Mitidulidae
H. neocosmis sp. n.	Diptera: Drosophilidae
H. oscinellae Goodey, 1930	Diptera: Chloropidae
H. phyllotretae Oldham, 1933	Coleoptera: Chrysomelidae
H. saginata Rajashekar, Rao, Reddy & Reddy, 1995	Coleoptera: Chrysomelidae
H. truncata Remillet & Van Waerebeke,1975	Coleoptera: Nitidulidae
Species inquirendae	
H. claviger Warren, 1941	Acarina
H. cuneifer Warren, 1941	Acarina
H. hirsuta Warren, 1941	Acarina
H. terribilis Warren, 1941	Acarina
Species dubiae	
H. prima Rubtsov & Tshumakova, 1981	Siphonaptera
H. stenoloba Rubtsov & Tshumakova, 1981	Siphonaptera

H. neocosmis vs 14-15 [rarely 12] µm in *H. aoronymphium*.).

BIOLOGICAL OBSERVATONS

The life cycle of *H. neocosmis* sp.n. appears to be similar to those of other members of the genus. The fertilized free-living female enters a young host larva by penetrating the cuticle with the aid of its stylet and salivary glands. This was easily demonstrated when young fly larvae were placed in Petri plates with infective stage female nematodes. Up to ten nematodes were observed in a single fly larva and it appears that when penetration occurs in very young hosts, the latter may succumb to septicemia from bacteria entering the penetration wounds. By the time the infected host has emerged as an adult, the female nematodes have swollen into sausage-shaped parasites and are producing numerous eggs. These eggs hatch inside the uterus of the female and the juveniles develop and molt twice while still in this location (no evidence of a molt occurring within the egg was noted, although in one case, a molt occurred at the time the juvenile emerged from the egg). It appears that when the juveniles leave the uterus of the female and enter the host hemocoel, they have molted twice and are in the third stage. Thus the two molts seen in the free-living nematodes would account for the normal four molts in the nematodes life cycle. Exit from the host occurs from the genital and digestive tracts. Once in the environment, the nematodes molt twice to the adult stage, mate and the females search for a new host.

Species in the genus *Howardula*

There are at least 24 described species in the genus *Howardula* Cobb, 1921 (see Table 1). Four of these (*H. claviger*, *H. cuneifer*, *H. hirsuta*, and *H. terribilis*) were described by Warren (1941) only on the basis of histological sections made through the bodies of parasitized mites and are considered *species inquirendae* (Siddiqi, 1986). The two species described from fleas, *H. prima* and *H. stenoloba* by Rubtsov and Tshumakova (*in* Rubtsov, 1981) are based only on the structure of the parasitic female and we agree with Deunff (1984) that there is no evidence that these parasites belong to the genus *Howardula*. Therefore, they should be considered as *species dubiae*. The species *H. aptini* from Sharga (1932) and other *Howardula* spp. attacking thrips have been transferred by Siddiqi (1986) to the genus *Thripinema* . Thus, at this time, the host range of *Howardula* includes Coleoptera, Diptera and Acarina. Most diagnostic characters of members of this genus reside in the free-living males and females. These include the presence of a bursa and gubernaculum and size of the spicules in the male, the stylet length in the female and the overall body lengths as well as the distances from the head to the nerve ring and excretory pore in both sexes.

Discussion

As a group, the Allantonematids have a curious host range. While the great majority of described species parasitize holometabolous insects of the orders Coleoptera and Diptera, others attack mites, thrips and Hemiptera (Poinar, 1975). Fossil records of Allantonematids date back to the Tertiary, some 20-40 million years ago (Poinar, 1984, 1993; Poinar & Brodzinsky, 1986) and involve both dipteran (Drosophilidae) and coleopteran (Staphylinidae) hosts. It has been speculated that the Allantonematidae arose in the Carboniferous (Poinar, 1983), quite possibly with acarines or Hemiptera serving as the original hosts. It is not known when the two genera (*Howardula* and *Parasitylenchus*) initiated parasitic relationships with the Drosophilidae (as well as with other acalyptrate families of Diptera), but it may have occurred in the late Cretaceous or early Tertiary. The report by Gershenson (1939) listing a *Chondronema* Christie & Chitwood, 1931, infection of Drosophilidae is undoubtedly an error since this genus of parasites is only known to occur in members of the Passalidae (Coleoptera) and has now been transferred out of the Allantonematidae (Sidiqqi, 1986).

It is interesting that two allantonematid genera have adapted to the same family of hosts. While Welch (1959) reported that *P. diplogenus* and *H. aoronymphium* both occurred in separate host species in England, Poinar *et al.* (1997) noted that in New York state, *D. recens* was parasitized (even the same individuals) by both *Parasitylenchus* and *Howardula*.

Of the five reported Diptera parasitized by *Howardula* spp., all belong to the Brachycera and four are acalyptrate Muscomorpha. With the exception of *H. oscinellae* parasitizing a member of the Chloropidae, all of the *Howardula* parasitizing flies form a morphological group characterized by the loss of the gubernaculum.

Acknowledgments

We would like to thank R. Huey for collecting *Drosophila suboccidentalis* in British Columbia, Canada.

References

COBB, N.A. (1921). *Howardula benigna*, a nemic parasite of the cucumber-beetle (*Diabrotica*). *Science*, 54: 667-670.

DEUNFF, J. (1984). *Parasites de Siphonaptères*. U.E.R. Médicale et Pharmaceutique. Université de Rennes, France, 406 p.

DEVI, T.R., RAO, P.N. & REDDY, Y.N. (1991). A new nematode parasite *Howardula mutilatus* n. sp. of the maize kernel pest *Carpophilus mutilatus*. *Current Nematol.*, 2: 23-26.

ELSEY, K.D. (1977). *Howardula dominicki* n. sp. infesting the tobacco flea beetle in North Carolina. *J. Nematol.*, 9: 338-342.

ELSEY, K.D. (1979). *Howardula colaspidis* (Allantonematidae) n. sp. a new parasite of the grape *Colaspis* (Coleoptera: Chrysomelidae). *Nematologica*, 25: 54-61.

GERSHENSON, S. (1939). Parasitism in Drosophilidae. *Drosophila Inform. Serv.*, 11: 44.

GOODEY, T. (1930). One remarkable new nematode, *Tylenchinema oscinellae* gen. *et* sp. n., parasitic in the frit-fly, *Oscinella frit* L. attacking oats. *Phil. Trans. R. Soc. London*, 218: 315-343.

KIMURA, M.T. & TODA, M.T. (1989). Food preferences and nematode parasitism in mycophagous *Drosophila*. *Ecol. Res.*, 4: 209-218.

MONTAGUE, J.R. & JAENIKE, J. (1985). Nematode parasitism in natural populations of mycophagous drosophilids. *Ecology*, 66: 624-626.

OLDHAM, J.N. (1933). On *Howardula phyllotretae* n. sp., a nematode parasite of flea beetles (Chrysomelidae: Coleoptera), with some observations on its incidence. *J. Helminth.*, 11: 119-136.

POINAR, JR. G.O. (1975). *Entomogenous nematodes, a manual and host list of insect-nematode associations*. Leiden, The Netherlands, E.J. Brill, 317 p.

POINAR, JR. G.O. (1983). *The natural history of nematodes*. Prentice-Hall, Englewood Cliffs, New Jersey, USA, 323 p.

POINAR, JR. G.O. (1984). First fossil record of parasitism by insect parasitic Tylenchida (Allantonematidae: Nematoda). *J. Parasit.*, 70: 306-308.

POINAR, JR. G.O. (1993). Insects in amber. *Ann. Rev. Ent.*, 38: 145-159.

POINAR, JR. G.O. & BRODZINSKY, J. (1986). Fossil evidence of nematode (Tylenchida) parasitism in Staphylinidae (Coleoptera). *Nematologica*, 32: 353-355.

POINAR, JR. G.O., JAENIKE, J. & DOMBECK, I. (1997). *Parasitylenchus nearcticus* sp. n. (Tylenchidae: Allantonematidae) parasitizing *Drosophila* (Diptera: Drosophilidae) in North America. *Fundam. appl. Nematol.*, 20: 187-190.

POINAR, JR. G.O., LAUMOND, C. & BONIFASSI, E. (1980). *Howardula apioni* sp. n. (Allantonematidae: Nematoda), a parasite of *Apion carduorum* Kirby (Curculionidae: Coleoptera) in southern France. *Proc. helminth. Soc. Wash.*, 47: 218-223.

RAJ, O.K. & REDDY, Y.N. (1989). Parasitism of *Longitarsus belgaumensis* (Coleoptera: Chrysomelidae) by nematode of the genus *Howardula*. *Ind. J. Nematol.*, 19: 82-84.

RAJASHEKAR, A.V., RAO, P.N., REDDY, Y.N. & REDDY, V.B. (1995). A description of a new species (Nematoda: Allantomenatidae) of parasite *Howardula saginata* n. sp. from a polyphagous pest *Monopepta saginata* (Insects: Coleoptera). *Afro-Asian Nematol. Network*, 1: 27-35.

REDDY, Y.N. & RAO, P.N. (1981). Description of morphology, biology life history of a new species *Howardula marginatis* sp. n. from flies. *Riv. Parassit.*, 42: 127-135.

REMILLET, M. & VAN WAEREBEKE, D. (1975). Description et cycle biologique de *Howardula madecassa* n. sp. et *Howardula truncati* n. sp. (Nematoda: Sphaerulariidae) parasites de *Carpophilus* (Coleoptera: Nitidulidae). *Nematologica*, 21: 192-206.

REMILLET, M. & VAN WAEREBEKE, D. (1976). Description et cycle biologique de *Howardula acris* n. sp. (Nematoda: Sphaerulariidae) parasite d'Hydrophilidae (Coleoptera). *Cah. ORSTOM, Sér. Biol.*, 11: 219-224.

RICHARDSON, P.N., HESLING, J.J. & RIDING, I.L. (1977). Life cycle and description of *Howardula husseyi* n. sp. (Tylenchida: Allantonematidae), a nematode parasite of the mushroom phorid *Megaselia halterata* (Diptera: Phoridae). *Nematologica*, 23: 217-231.

RUBTSOV, I.A. (1981). [*Parasites and enemies of fleas.*] "Nauka", Leningrad, SSSR, 104 p.

SHARGA, U.S. (1932). A new nematode, *Tylenchus aptini* n. sp., parasite of Thysanoptera (Insects: *Aptinothrips rufus* Gmelin). *Parasitology*, 24: 268-279.

SIDDIQI, M.R. (1986). *Tylenchida. Parasites of plants and insects*. Farnham Royal, Slough, UK, Commonwealth Institute of Parasitology, 645 p.

WACHEK, F. (1955). Die entoparasitischen Tylenchiden. *Parasit. Schriftenr.*, 3: 1-119.

WARREN, E. (1941). On the occurrence of nematodes in the haemocoel of certain gamasid mites. *Ann. Natal Mus.*, 10: 79-95.

WELCH, H.E. (1959). Taxonomy, life cycle, development, and habits of two new species of Allantonematidae (Nematoda) parasitic in drosophilid flies. *Parasitology*, 49: 83-103.

YATHAM, N.R. & RAO, P.N. (1980). Description and biology of *Howardula albopunctata* sp. n. from the two species of *Sepsis* (Sepsidae: Diptera). *Proc. Ind. Acad. Parasit.*, 1: 42-46.