ヒラタケ白こぶ病病原線虫Iotonchium ungulatumの昆虫寄生態雌線虫及び生活環の記載

誌名	日本線虫学会誌
ISSN	09196765
著者名	津田,格 二井,一禎
発行元	日本線虫学会
巻/号	30巻1-2号
掲載ページ	p. 1-7
発行年月	2000年12月

The Insect-parasitic Stage and Life Cycle of *Iotonchium ungulatum* (Tylenchida: Iotonchiidae), the Causal Agent of Gill-knot Disease of the Oyster Mushroom[1]

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Iotonchium ungulatum is the causal agent of gill-knot disease of the oyster mushroom, *Pleurotus ostreatus*. The insect-parasitic females of this nematode are described. The insect-parasitic female nematodes inhabit and lay many eggs in the haemocoel of the fungus gnat, *Rhymosia domestica*. Juvenile nematodes hatched from these eggs invade host gnats ovaries, and the nematodes are supposed to be deposited on the gills of the oyster mushroom where they generate knots and grow into the mycetophagous females. In the laboratory experiment, female gnats harbored significantly greater number of insect-parasitic female nematodes than did the male gnats.
Jpn. J. Nematol. 30 (1/2): 1-7 (2000).
Key words: gill-knot disease, insect-parasitic nematode, *Iotonchium ungulatum*, *Pleurotus ostreatus*, *Rhymosia domestica*.

The gill-knot disease generating many knots on the gills of the fruiting body of the oyster mushroom, *Pleurotus ostreatus*, has been reported from many places in western Japan and a tylenchid nematode, *Iotonchium ungulatum*, has been clarified as the causal agent of this disease (1, 6). Inside the knots, mycetophagous female nematodes inhabit and produce the progeny growing males and infective females (1, 6). The above three forms available from the fruiting body were described by AIHARA (1). *Iotonchium ungulatum* is considered to have a life cycle similar to that of *I. californicum* (2), which is vectored by a species of fungus gnat and possesses four adult forms; mycetophagous female, infective female, male, and insect-parasitic female. The parthenogenetic mycetophagous female nematodes of *I. californicum* inhabit in the fruiting body of the basidiomycetous fungus, *Agrocybe praecox*, and their progeny consists of males and infective females (2). Infective female nematodes copulate with males, then enter the body cavity of a matured larva or pupa of the fungus gnat, *Mycetophila fungorum* (2). In the haemocoel of the host gnat, the infective female nematode matures into insect-parasitic female everting her uterine cells out through the vulva (2). In the case of *I. ungulatum*, insect-parasitic stage was observed in the haemocoel of the host fungus gnat, *Rhymosia domestica* (6), but the morphological characters of that stage have not been described. The present paper describes the insect-parasitic stage and the total life cycle of *I. ungulatum*.

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MATERIALS AND METHODS

Nematode association with the fungus gnat

Fruiting bodies of *P. ostreatus* with gill-knot disease collected in the Botanical Garden of Kyoto University in Kyoto, Japan, were placed on a 3-4 cm thick layer of vermiculite in 500-ml wide-mouthed bottles covered with a screen net and kept at 15°C in moderate humidity. The matured larvae of the host fungus gnat, *R. domestica*, left the fruiting bodies, pupated in the vermiculite bed, and emerged 2-3 weeks later as adult gnats. Emerged individuals were dissected in 0.9% NaCl solution.

Insect-parasitic female nematodes obtained from the haemocoel of the gnat, and mycetophagous females, infective females and males from the diseased fruiting bodies were killed in hot water (55°C), fixed in TAF, and processed to glycerin (4) for morphometrical study.

To elucidate when infective females invade the host gnats, diseased fruiting bodies were kept on a vermiculite bed as above-mentioned in moderate humidity at ambient temperature. At various time after matured larvae left the decaying fruiting bodies, larvae and pupae were sampled and dissected in 0.9% NaCl solution to investigate nematode invasion.

Nematode inoculation on the fruiting body

Thousands of eggs (length = 85-122 μm, diameter = 42-57 μm) and juveniles (L = 0.30-0.44 mm) of the insect-parasitic nematodes were observed in the female gnats haemocoel. In some parasitized female gnats, their genital organs were partly occupied by the juvenile nematodes (L = 0.40-0.49 mm) (Fig. 1). These juveniles were presumably deposited on the gills of oyster mushroom by mock-oviposition of female gnats to enter into a mycetophagous life cycle. The juveniles were obtained by dissecting the gnats genital organs in distilled water. They were washed in distilled water ten times and used as inocula. The bottle-cultivated fruiting bodies of *P. ostreatus* was settled upside-down. Nematodes-water suspension was pipetted on the gills. After a while for drying up the water suspension, the fruiting body was set upright and incubated in moderate humidity at 15°C.

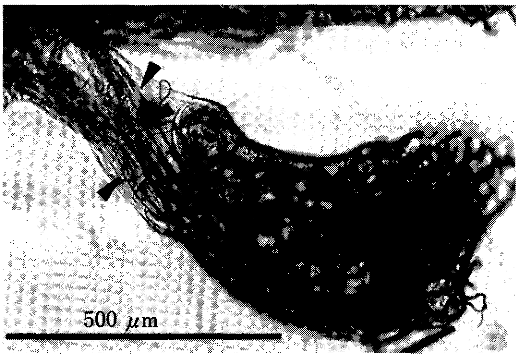

Fig. 1. Ovary of female host fungus gnat, *Rhymosia domestica*, invaded by juvenile nematodes. Arrow heads show juvenile nematodes.

Fig. 2. Knots generated on the gills of the bottle-cultivated fruiting bodies of *Pleurotus ostreatus*. Arrow heads show knots.

Table 1. Parasitized rate and number of insect-parasitic female nematodes in the haemocoel of *R. domestica*.

R. domestica	No. of *R. domestica* examined	No. of *R. domestica* with nematodes (% parasitized)	No. of insect-parasitic female nematodes/gnat (mean±SD)
Female	57	45(78.9)	9.4±9.1
Male	101	41(40.6)	2.9±7.3
Total	158	86(54.4)	5.2±8.6

RESULTS AND DISCUSSION

Nematode association with the fungus gnat

Table 1 shows the rate of parasitized gnats by nematodes and the mean number of insect-parasitic female nematodes per gnat. Female gnats harbored significantly greater number of nematodes than did the male gnats ($p<0.01$, MANN-WHITNEY U-test).

No nematodes were detected from 12 individuals of gnat larvae examined when the insects left the decaying fruiting body. On day 1 after making their cocoons in vermiculite bed, all of ten larvae examined were still at larval stage with faint brown color and loaded many infective female nematodes on their body surface. No nematodes were found inside their body. On day 4 after creeping into the vermiculite bed, 1 to 2 days after pupation, four out of six pupae examined harbored young insect-parasitic female nematodes. Further, eight out of ten pupae examined on day 5 were infected. No male nematodes were found in any of the gnats examined.

Nematodes inoculation upon the fruiting body

The formation of knots was confirmed on the gills of the fruiting body at a part of pipetted regions 3 days after inoculation of juvenile nematodes (Fig. 2). Mycetophagous female nematodes were recovered from inside the knots.

MEASUREMENTS

Insect-parasitic female (n=7, in the haemocoel of the gnat) (Figs. 3, 4): L = 4.90±0.38 (4.36-5.31) mm; greatest body width = 138.5±17.2 (116.1-167.9) μm; body width at vulva = 116.8±7.5 (108.9-126.8) μm; body width at anus = 75.3±11.0 (62.5-94.6) μm; distance from anterior end to gonad = 94.1±12.0 (78.5-114.3) μm; distance from vulva to anus = 317.3±33.1 (253.6-357.1) μm; distance from vulva to posterior end = 448.0±53.7 (367.9-507.1) μm; gonad length = 4.5±0.4 (3.7-4.9) mm; stylet length = 14.4±0.6 (13.4-15.1) μm; a = 35.7±3.9 (31.6-42.3); c = 39.7±10.4 (28.4-61.1); V = 90.9±0.7 (90.1-92.4).

Male (n = 25): L = 1.12±0.06 (0.94-1.28) mm; greatest body width = 34.1±3.0 (30.2-41.6) μm; length of proximal arm of spicule = 23.1±2.1 (19.8-26.7) μm; length of distal arm of spicule = 20.8±1.1 (18.8-22.8) μm; a = 33.0±2.9 (25.7-37.5); c = 17.8±1.2 (15.4-20.4).

Infective female (n = 27): L = 1.35±0.03 (1.29-1.42) mm; greatest body width = 31.0±1.1 (28.8-33.6) μm; body width at vulva = 24.6±0.9 (22.8-26.0) μm; body width at anus = 18.1±0.7 (16.8-19.6) μm; stylet length = 15.8±0.4 (14.9-16.8) μm; a = 43.5±1.9 (40.0-47.6); c = 12.9±0.7 (11.7-14.1); V = 85.5±1.1 (83.8-87.6).

Mycetophagous female (n = 10): L = 2.25±0.29 (1.64-2.65) mm; greatest body width = 105.8±10.8 (87.9-122.5) μm; body width at vulva = 59.2±2.7 (54.5-63.1) μm; body width at anus = 28.6±2.6 (24.8-33.4) μm; distance from anterior end to gonad = 57.9±17.8 (34.7-85.4) μm; distance from anterior end to intestine = 140.6±19.3 (101.5-173.3) μm; distance from vulva to anus = 29.1±6.8 (17.3-42.1) μm; distance from vulva to posterior end = 64.4±10.0 (44.6-76.7) μm; stylet length = 9.1±0.5 (8.4-10.0) μm; tail tip = 21.1±2.0 (18.8-25.7) μm; a=21.3±1.4 (18.7-22.7); c = 65.6±15.6 (41.5-89.3); V = 97.1±0.7 (95.6-98.1).

DESCRIPTION

Insect-parasitic female (Figs. 3, 4): Length greatly larger than that of infective female; body cylindrical, ventrally spirally coiled upon relaxation; cuticle smooth with fine transverse annulations, lateral fields areolated or inconspicuous; stylet small with distinct anterior corn portion, slightly curved shaft; cephalic region continuous, with four minute papillae (Fig. 4); pharyngeal glands present; orifice of dorsal gland close to stylet base; oesophagus and intestine inconspicuous; ovary outstretched and sometimes reflexed at the anterior part of the body; oviduct-terminus characteristically ball-shaped; oviparous, with spermatozoa and several eggs in the uterus; vagina short, thick-walled; postvulval uterine sac absent; tail cylindroid with rounded terminus.

REMARKS

The nematode population from Kyoto agrees morphologically and morphometrically with the original description of *I. ungulatum* (1) in every respect of mycetophagous female, infective female and male. This is the first description of insect-parasitic female of *I. ungulatum*. Two species in genus *Iotonchium, I. californicum* (2) and *I. cateniforme* (7), have so far been elucidated their complete life cycle having four adult forms. In the length of insect-parasitic female, *I. ungulatum* (4.4-5.3 mm) differs from *I. californicum* (1.1-2.7 mm) and *I. cateniforme* (1.9-2.5 mm). The difference in the "a" value between insect-parasitic female of *I. ungulatum* (32-42) and *I. californicum* (8-16) suggests that the former has grown both longitudinally and transversely, while the latter has swollen transversely from infective female (a = 47-76, L = 1.5-2.0).

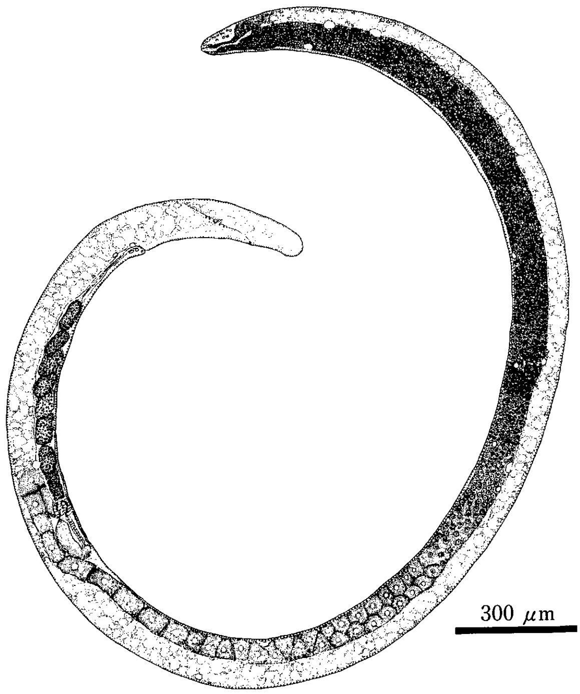

Fig. 3. Insect-parasitic female of *Iotonchium ungulatum*.

Fig. 4. Head region of insect-parasitic female nematode. Arrow heads show minute papillae.

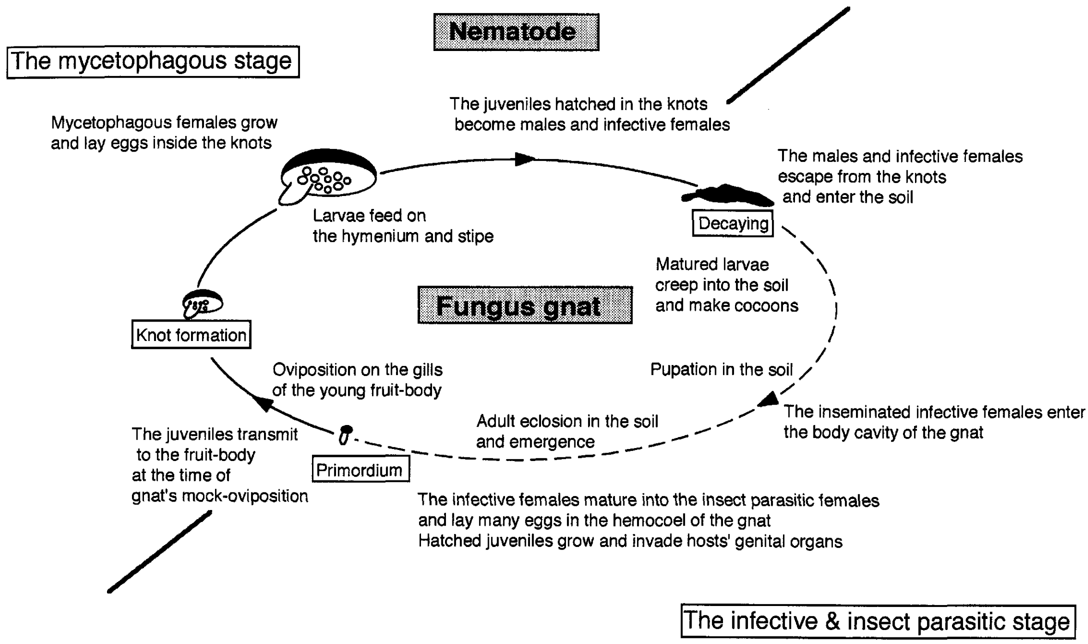

Fig. 5. Life cycles of *Iotonchium ungulatum* and the host fungus gnat, *Rhymosia domestica*.

The insect-parasitic female of *I. ungulatum* differs from those of other two species in having four minute papillae on the cephalic region. There is a prominent difference between these three species in their reproductive systems of the insect-parasitic females. While *I. californicum* is ovoviviparous with eggs and hatched first stage juveniles in the uterus, other two species are oviparous. The posterior uterine cells are extruded through vulva into the body cavity of the host in *I. californicum*, but the others do not have such character. The feature of everting posterior uterine cells protruding from the vulval opening is known in several other genera of insect-parasitic nematodes (3, 5). POINAR (1) mentioned that the feature of everting the uterine cells is rather a secondary character which arises in various groups when conditions favor it. This can be possible explanation of the morphological differences in the insect-parasitic stage between these species.

LIFE CYCLE

Iotonchium ungulatum, the causal agent of the gill-knot disease of *Pleurotus ostreatus*, seemed to have similar life cycle to *I. californicum* (1, 2, 6). The present results describe the insect-parasitic stage and thereby make up whole part of the nematode life cycle, particularly the succession of each stage. The complete life cycle is shown in Fig. 5. The progenies of the mycetophagous females, hatched in the knots, grow into males and infective females. They leave the fruiting body when the fruiting body begin to collapse, then get into the soil. By the time many infective females had been inseminated, mating may probably occur inside the knots.

At the pupation of the host fungus gnat, *Rhymosia domestica*, inseminated infective females enter the gnats' body cavity. Infective females are supposed to be the preadult fourth stage because several exuviae (length = 1.04-1.23 mm) were observed in the haemocoel of some host gnats (Fig. 6). After the invasion, they are supposed to moult and mature into insect-parasitic female

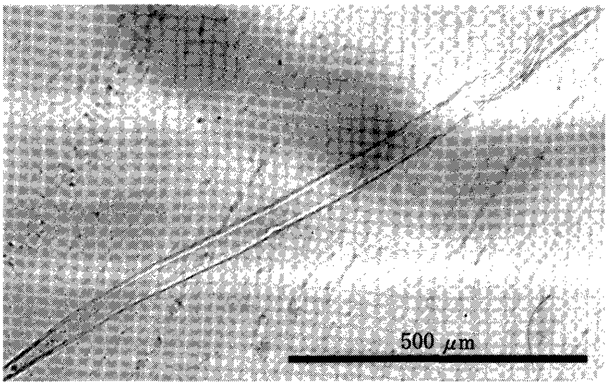

Fig. 6. Exuvia of infective female nematode in the haemocoel of *Rhymosia domestica*.

adults and lay many eggs. Juvenile nematodes hatched from these eggs invade host ovaries before the female gnats begin to oviposit, and the nematodes are deposited on the gills of the oyster mushroom by possible mock-oviposition of female gnats. They generate knots and undergo a mycetophagous life cycle.

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Accepted for publication: May 5, 1999.

和文摘要

ヒラタケ白こぶ病病原線虫 *Iotonchium ungulatum* の昆虫寄生態雌線虫及び生活環の記載

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ヒラタケ白こぶ病の病原線虫 *Iotonchium ungulatum* の昆虫寄生態雌線虫の形態、及び生活環を記載した。昆虫寄生態雌線虫は、キノコバエ科の一種ナミトモナガキノコバエ *Rhymosia domestica* の血体腔内に寄生し、そこで産卵する。病害子実体から羽化してきたキノコバエを解剖したところ、血体腔内で孵化した幼線虫のキノコバエ卵巣への侵入が観察された。キノコバエ卵巣から取り出した幼線虫をヒラタケ子実体に接種すると、ひだ上にこぶが生じた。このことから、キノコバエの産卵行動時に新しいヒラタケ子実体に産み付けられた幼線虫が菌食態雌線虫へ発育するものと考えられた。病害子実体から羽化してきたキノコバエが保持する寄生態雌線虫数は、雄キノコバエよりも雌キノコバエにおいて有意に多かった。