The mycetophagous and entomophagous stages of *Iotonchium californicum* n. sp. (Iotonchiidae : Tylenchida)

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SUMMARY

Iotonchium californicum n. sp. is described from western North America. The adult stages of this species include a mycetophagous female (hexatyloid form), a male and infective stage female (*Iotonchium* forms) and a mature entomophagous female (allantonematid form). The mycetophagous stages of *I. californicum* n. sp. feed on the gilled mushroom, *Agrocybe praecox* (Bolbitiaceae, Agaricales) while the entomophagous stages parasitize the fungus gnat, *Mycetophila fungorum* (Mycetophilidae, Diptera). This is the first record of an *Iotonchium* from the Nearctic region and the first time the insect host of an *Iotonchium* has been found. Fossil evidence suggests that such an *Iotonchium*-gnat association has existed for some 25-40 million years.

RÉSUMÉ

Les stades mycétophage et entomophage de Iotonchium californicum *n. sp. (Iotonchiidae : Tylenchida)*

Iotonchium californicum n. sp., provenant de la région ouest de l'Amérique du Nord, est décrit. Les stades adultes de cette espèce comprennent : *i)* une femelle mycétophage (forme hexatyloïde); *ii)* un mâle et une femelle infestante (formes *Iotonchium*); *iii)* une femelle mature entomophage (forme allantonematide). Les stades mycétophages de *I. californicum* n. sp. se nourrissent sur le champignon à lamelles *Agrocybe praecox* (Bolbitiaceae, Agaricales) tandis que les stades entomophages parasitent le moucheron des champignons *Mycetophila fungorum* (Mycetophilidae, Diptera). Il s'agit de la première signalisation d'un *Iotonchium* dans la région néarctique et c'est la première fois qu'un insecte hôte est observé pour un *Iotonchium*. Des observations faites sur des fossiles laissent penser qu'une telle association *Iotonchium*-moucheron existe depuis 25 à 40 millions d'années.

During an investigation of insects occurring in mushrooms, the author discovered a new species of *Iotonchium* in northern California. Laboratory and field studies showed that this *Iotonchium* possessed both mycetophagous and entomophagous stages in its life cycle. The present paper provides a description of the new species and illustrates the *Iotonchium*, hexatyloid and allantonematid forms of the normal life cycle. Fossil evidence suggests that this nematode-gnat relationship has existed for at least 25-40 million years.

Materials and methods

Iotonchium infested mushrooms were collected in Albany (Alameda Co.), California during February and March, 1990. The mushroom host was identified by Harry D. Thiers as *Agrocybe praecox* (Fr.) Fayod (Bolbitiaceae, Agaricales). Cultures of *A. praecox* were made by removing portions of the pileus and stipe, sterilizing them for 30 seconds in a 3 % chlorox solution and placing them on the surface of sterile malt agar plates (5 g malt extract; 1.5 g agar; 100 ml distilled water). Mycetophagous stages of *Iotonchium* were removed from field collected *A. praecox* and placed directly on malt agar plates containing cultures of *A. praecox.*

The insect host of *Iotonchium* was identified as *Mycetophila fungorum* (DeGeer) (Mycetophilidae : Diptera) by Paul H. Arnaud, Jr. Parasitized adult flies containing various stages of *Iotonchium* were collected by placing field collected infested *A. praecox* in quart jars with a screen top. The mature larvae of *M. fungorum* would leave the mushrooms, pupate in the bottom of the jar and emerge several weeks later as adults. For taxonomic studies, the nematodes were killed in hot water (55 °C), fixed in TAF and processed to glycerin. All examinations were conducted with a Nikon optiphot microscope equipped with differential interference contrast.

Over 15 000 pieces of Dominican amber were examined before one containing a parasitized mycetophilid fly was located. Amber from the Dominican Republic has been estimated to range from Lower Miocene to

Upper Eocene in age (25-40 million years) and is known to contain examples of insect parasitism by allantonematid nematodes (Poinar, 1984).

***Iotonchium californicum* n. sp.**

(Figs 1-4; 6-8)

MEASUREMENTS

Mycetophagous female (n = 12) (Figs 3 A; 4 B; 6 C; 7 A; 10) : L = 1.2 (0.9-1.5) mm; greatest width = 50 (33-60) µm; distance from head to nerve ring = 90 (75-111) µm; distance from head to hemizonid = 112 (90-140) µm; distance from head to excretory pore = 106 (99-120) µm; distance from head to base of pharynx = 142 (135-150) µm; distance from head to tip of ovary = 129 (93-195) µm; distance from tail tip to vulva = 169 (150-210) µm; tail length = 103 (90-117) µm; tail diameter = 29 (24-39) µm; stylet length = 10 (9-11) µm; egg length = 114 (108-126) µm; egg diameter = 34 (33-40) µm; V = 86 (84-88); a = 24 (18-29).

Infective stage female (n = 12) (Figs 1; 4 A, E; 6 A, B; 7 D; 8 A, B) : L = 1.7 (1.5-2.0) mm; greatest width = 29 (24-33) µm; distance from head to nerve ring = 126 (114-135) µm; distance from head to hemizonid = 149 (138-159) µm; distance from head to excretory pore = 156 (138-168) µm; distance from head to base of pharyngeal glands = 274 (228-324) µm; distance from head to tip of ovary = 911 (753-1 050) µm; tail length = 123 (93-162) µm; tail diameter = 17 (15-21) µm; distance from tail tip to vulva = 248 (198-285) µm; stylet length = 20 (18-21) µm; V = 86 (85-87); a = 61 (47-76).

Male (n = 10) (Figs 2 B; 4 D, F, H, I; 8 A, B) : L = 1.1 (0.8-1.3) mm; greatest width = 33 (30-36); distance from head to nerve ring = 112 (99-123); distance from head to hemizonid = 138 (120-156) µm; distance from head to excretory pore = 142 (99-163) µm; distance from head to gonad tip = 502 (330-615) µm; tail length = 60 (53-63) µm; tail diameter = 25 (24-31) µm; length bursa = 158 (129-210) µm; spicule length = 28 (24-31) µm; greatest width of spicule = 6 (4-7) µm; length bursa = 158 (129-210) µm; spicule length = 28 (27-38).

Entomophagous female (n = 16) (Figs 3 B; 4 C; 7 C) : L = 2.0 (1.1-2.7) mm; greatest width = 153 (126-183) µm; distance from head to excretory pore = 160 (135-225) µm; length tail tip = 21 (9-30) µm; V = 95 (89-97); stylet length = 20 (18-21) µm; distance from head to gonad = 122 (51-243) µm; distance uterine cells extrude from vulva = 111 (90-150) µm; egg length = 60 (48-75) µm; egg diameter = 29 (27-39) µm; a = 12 (8-16).

Third stage juvenile from insect host (n = 6) (Figs 2 A; 6 D) : L = 504 (450-570) µm; greatest width = 22 (21-24) µm; stylet length = 8 (7-9) µm; distance from head to excretory pore = 73 (52-86) µm; distance from head to nerve ring = 80 (57-93) µm; tail length = 48 (38-53) µm.

DESCRIPTION

Mycetophagous female (Fig. 3 A) : Long and wide nematodes; cuticle smooth (very fine transverse annulations can sometimes be seen under oil immersion); lateral fields inconspicuous; head not set off from body; cephalic framework divided into eight sectors at surface level (twelve sectors at other levels); head with six inner labial papillae, ten outer papillae and paired amphids; stylet with distinct anterior cone portion, well developed shaft with two (sometimes three) guiding rings and three basal knobs (sometimes bifid or asymmetrical) extended latero-posteriorly; dorsal gland outlet opens near stylet base; subventral gland outlet opens within two stylet lengths behind stylet base; pharyngeal glands distinct, overlapping cylindrical non-muscular pharynx and anterior portion of intestine; pharyngeal-intestinal junction located posterior to nerve ring and excretory pore; hemizonid located just anterior to excretory pore; deirids located just posterior to nerve ring; ovary outstretched; uterus with only a single large egg at any one time; after oviposition, the uterine walls buckle and appear lumpy; oviparous; post-uterine sac absent; vulva a wide transverse slit; tail elongate-conoid, pointed at tip.

Infective stage female (Fig. 1) : Long and slender, usually lying straight and responding only to tactile stimuli; cuticle with fine transverse annulations; lateral fields distinct, not areolated, normally composed of two ridges from 5-10 µm apart depending upon location; lateral lines extend from vicinity of hemizonid nearly to tail tip; head not offset; cephalic framework divided into eight sectors at surface level; head with six inner labial and ten outer papillae; amphids distinct; stylet long and slender, shaft and cone portions not distinguishable, basal knobs absent but thickenings usually present; dorsal gland outlet within one stylet length behind stylet base; subventral gland outlet within two stylet lengths behind stylet base; pharynx cylindroid, non-muscular; pharynx enters intestine posterior to nerve ring; pharyngeal glands elongated, with equal-sized nuclei; deirids located at level of hemizonid; ovary outstretched, oviduct short, thick walled; uterus extensive (packet with spermatozoa in fertilized females); vagina short; vulva a transverse slit; vulvar opening covered with anterior cuticular flap; ventromedial papillae behind vulva absent however numerous hypodermal supplements occur in the tail region; postvulval uterine sac absent; tail elongate-conoid, with a pointed tip.

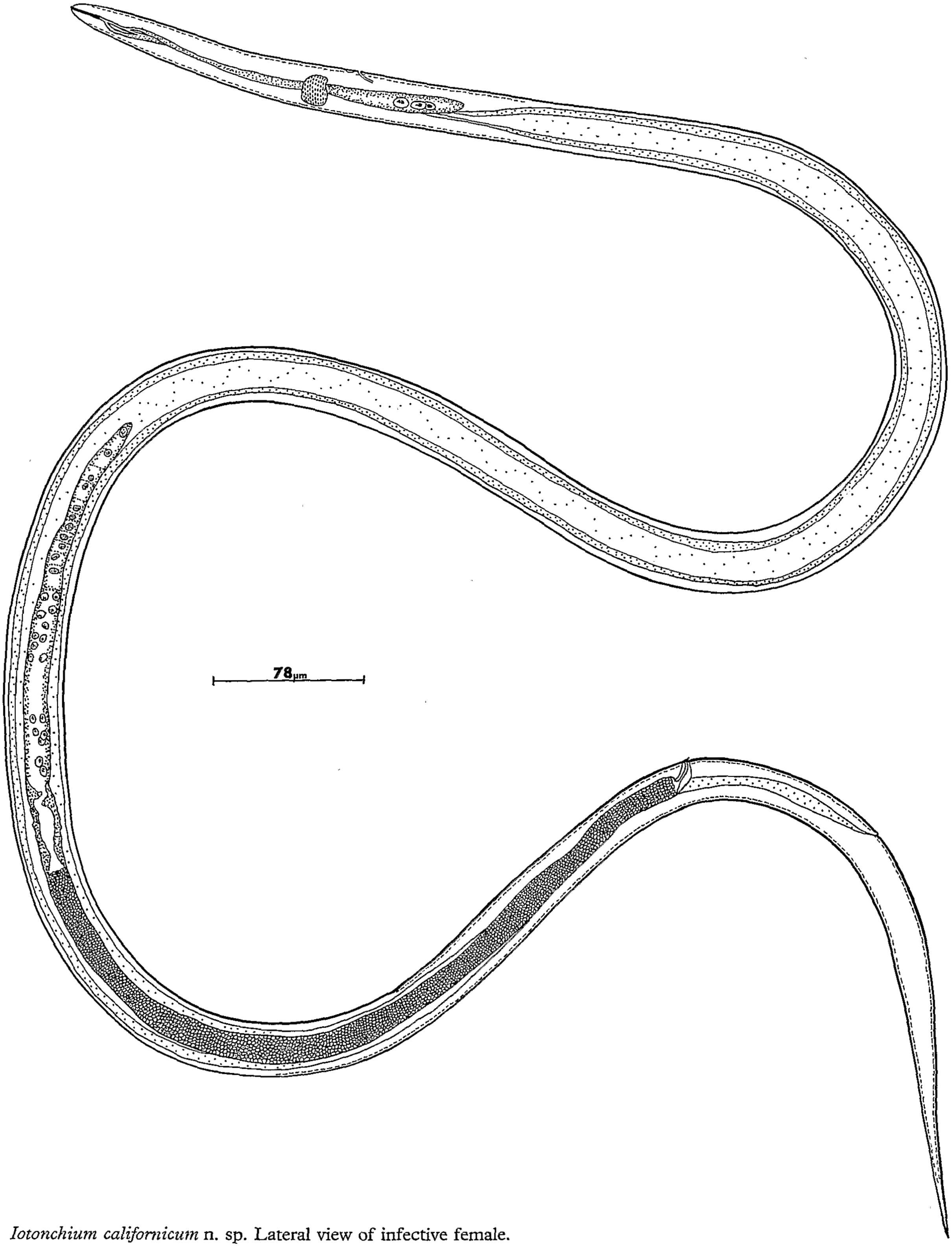

Fig. 1. *Iotonchium californicum* n. sp. Lateral view of infective female.

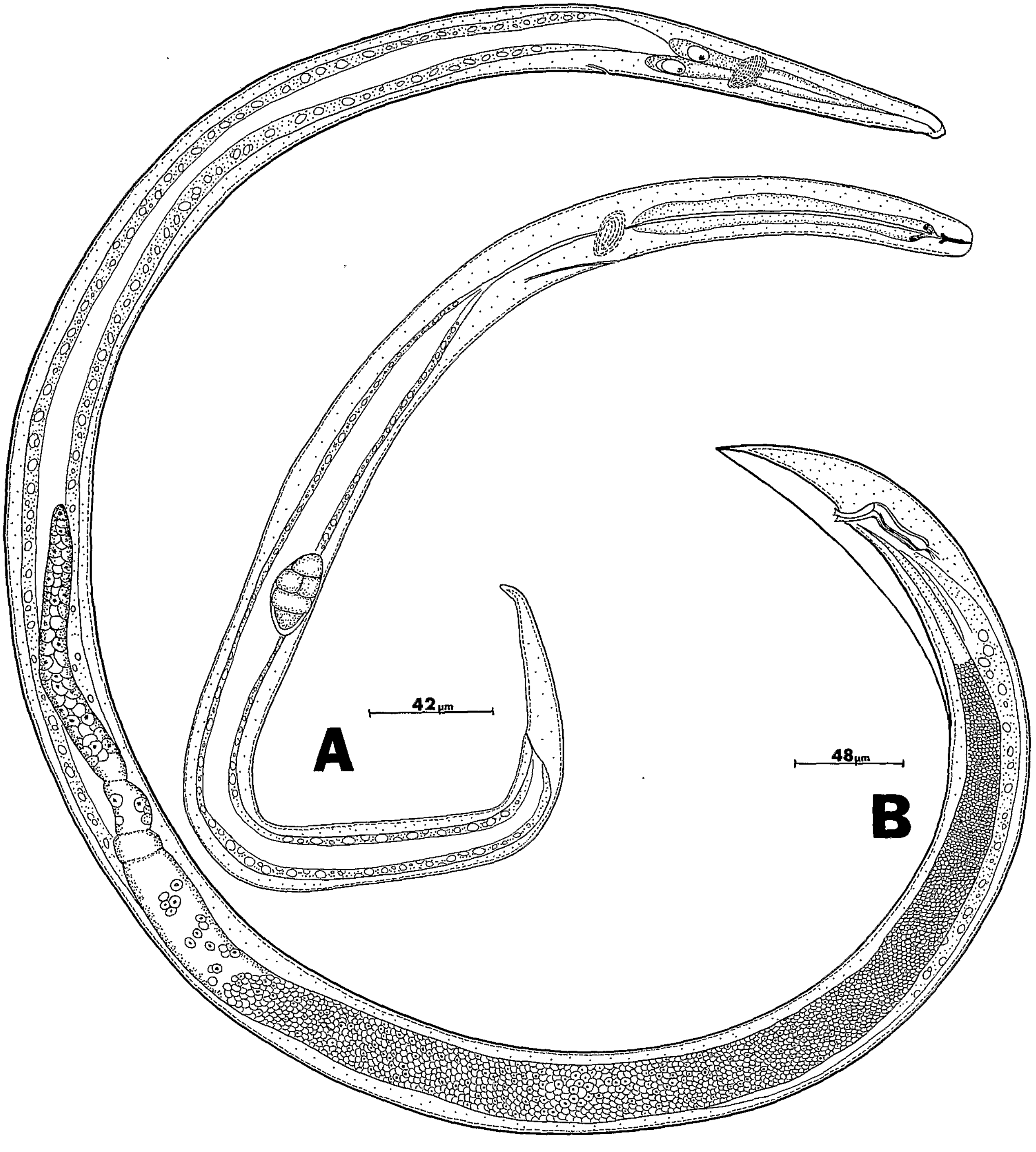

Fig. 2. *Iotonchium californicum* n. sp. A : Lateral view of third stage juvenile from the hemocoel of a fungus gnat; B : Lateral view of male.

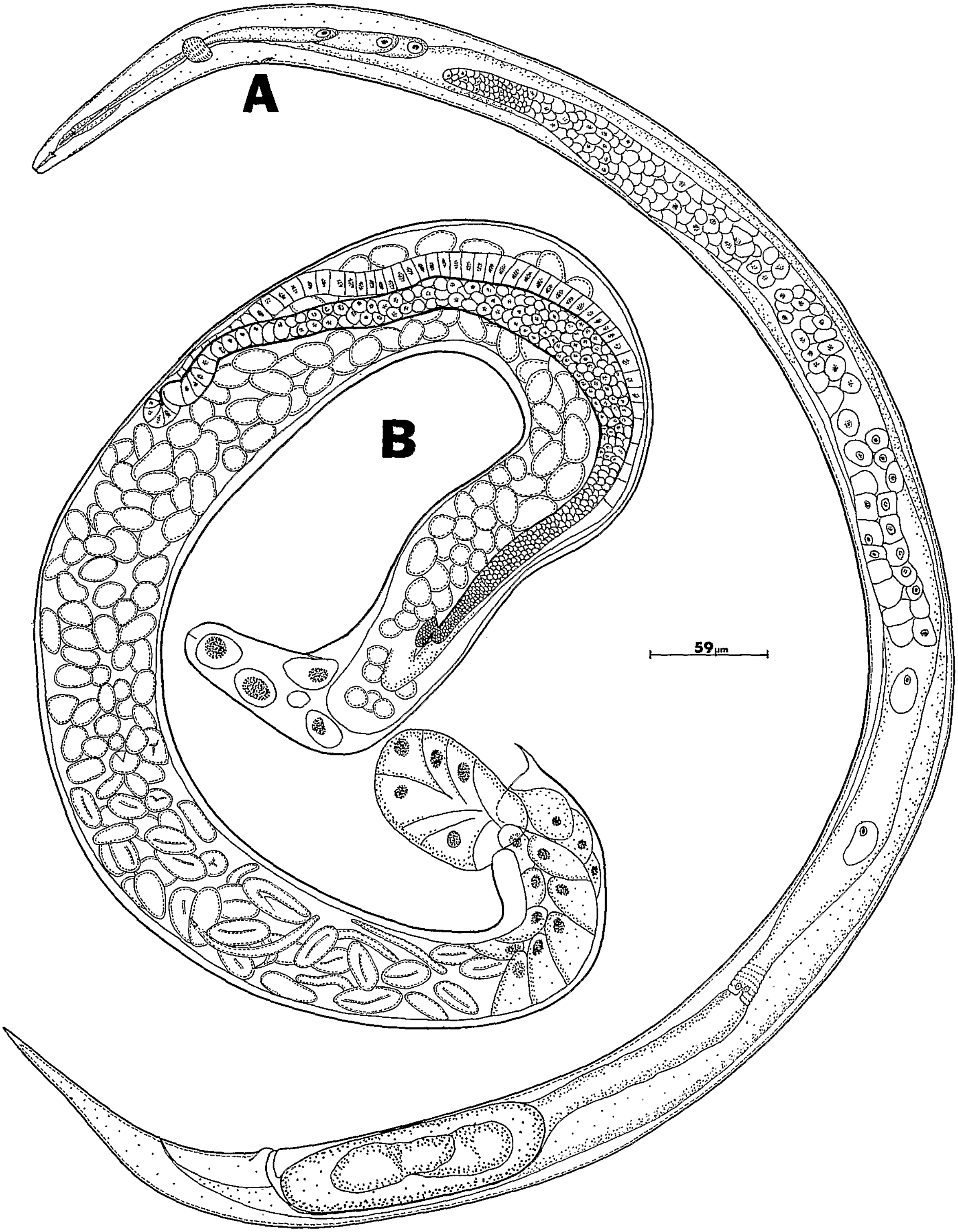

Fig. 3. *Iotonchium californicum* n. sp. A : Lateral view of mycetophagous female ; B : Lateral view of mature entomophagous female.

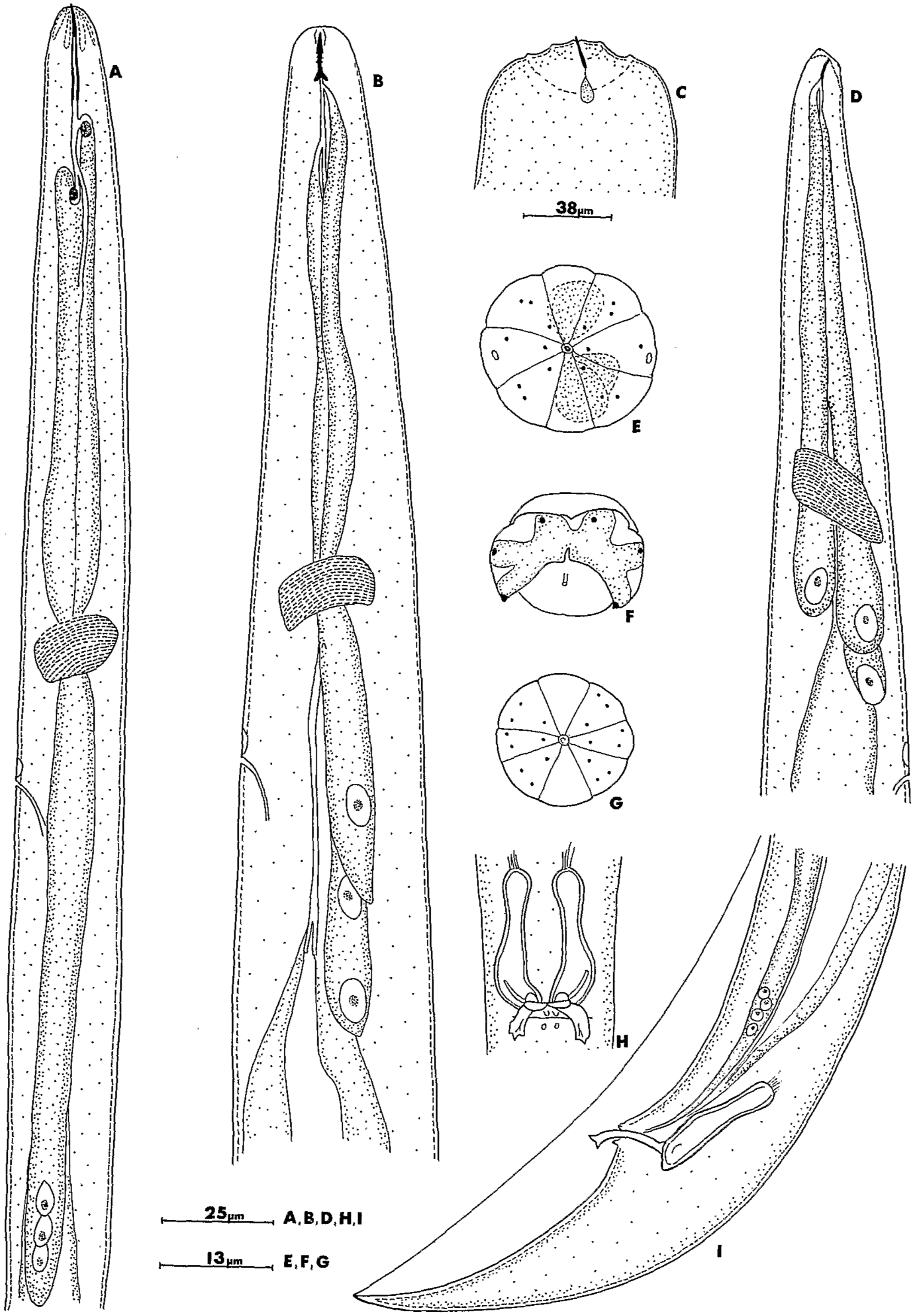

Fig. 4. *Iotonchium californicum* n. sp. A : Lateral view of head region of infective female; B : Lateral view of head region of mycetophagous female; C : Lateral view of head of mature entomophagous female; D : Lateral view of head region of male; E : En face view of infective stage female; F : En face view of male; G : En face view of mycetophagous female; H : Ventral view of spicules; I : Lateral view of spicules.

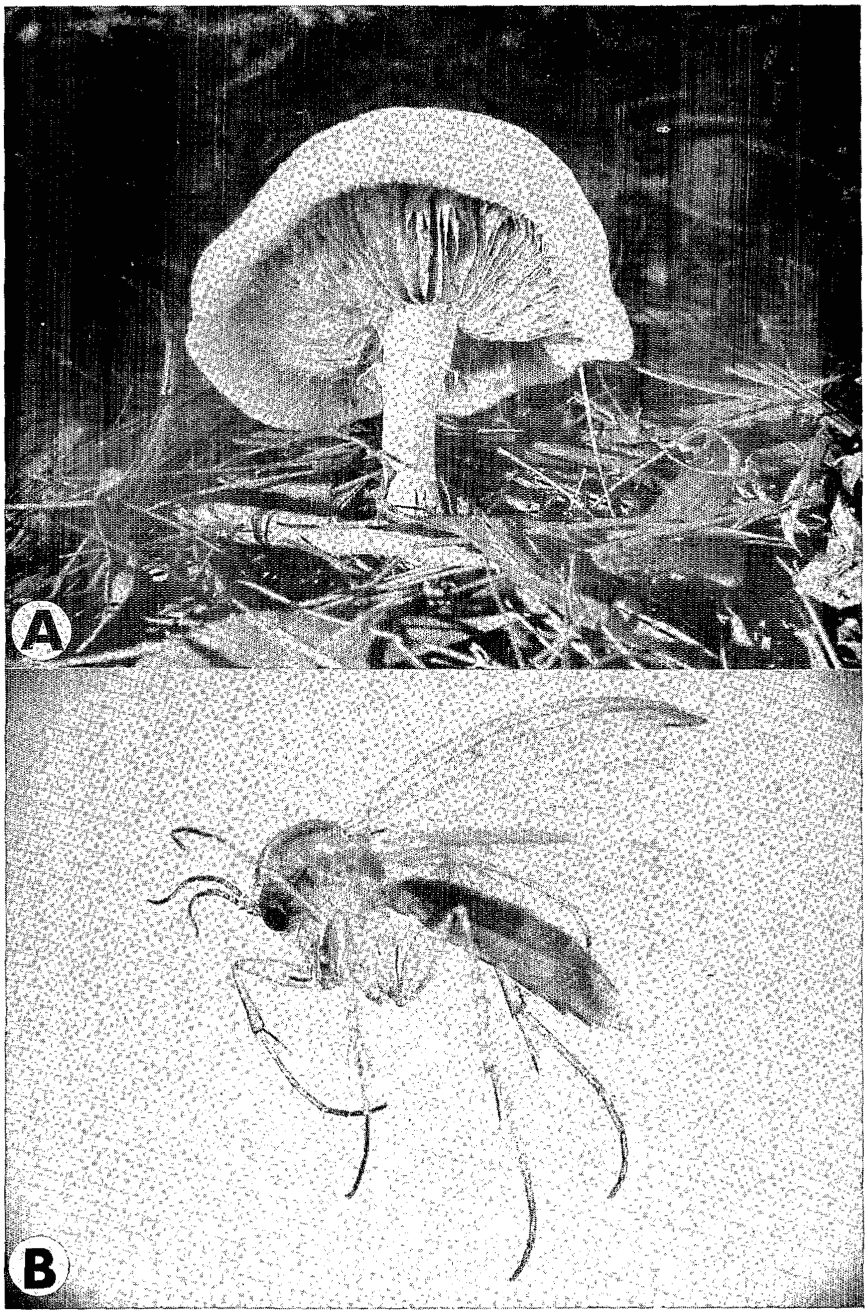

Fig. 5. A : Fungal host *(Agrocybe praecox)* of *Iotonchium californicum* n. sp.; B : Insect host *(Mycetophila fungorum)* of *Iotonchium californicum* n. sp.

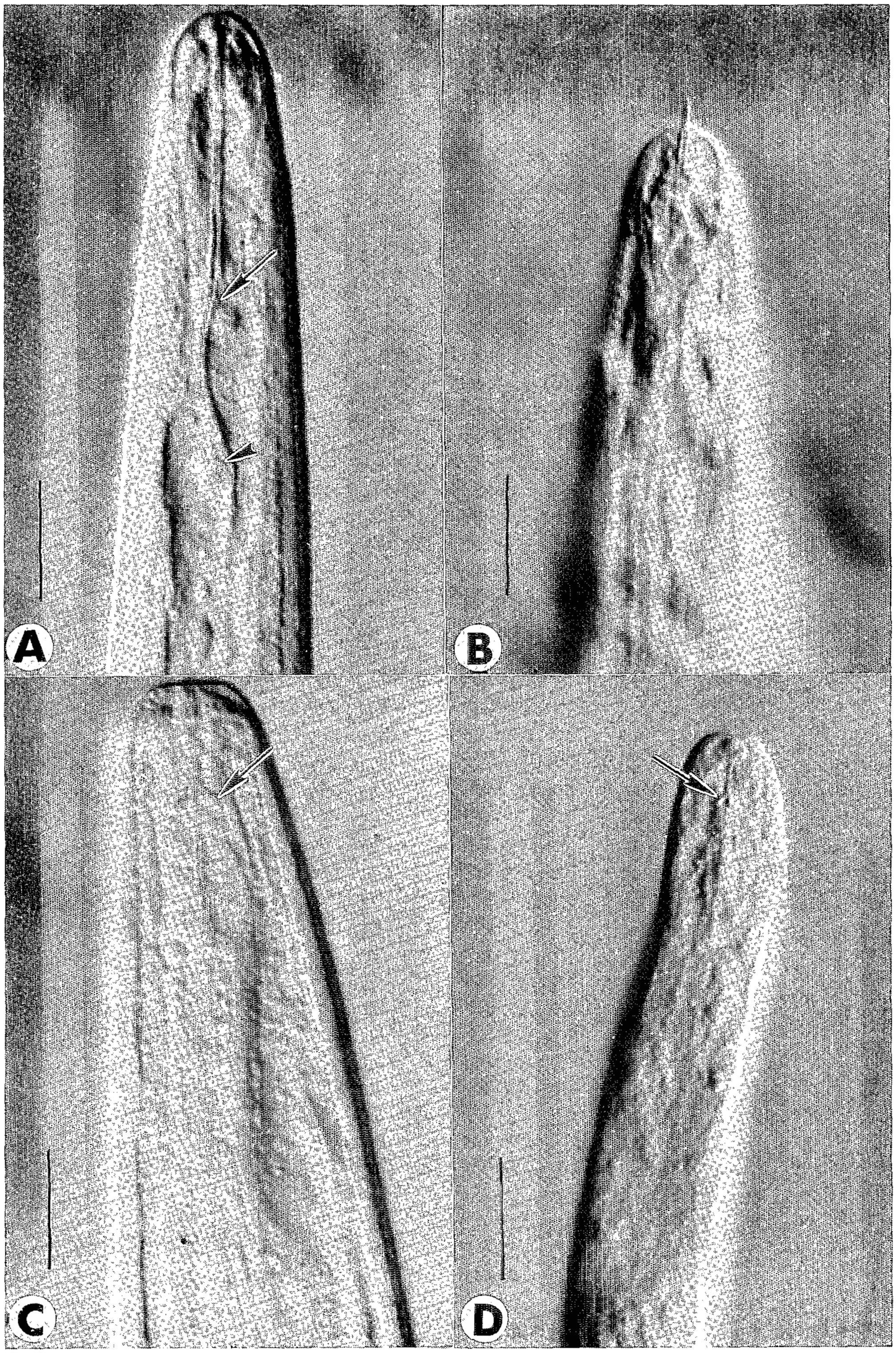

Fig. 6. *Iotonchium californicum* n. sp. A : Lateral view of head of infective female (arrow shows dorsal and arrow head sub-ventral gland openings); B : Head of infective female showing stylet partially exerted; C : Lateral view of head of mycetophagous female (arrow shows basal stylet knob); D : Lateral view of head of third stage juvenile from insect hemocoel (arrow shows basal stylet knob). *(Bars equivalent : A = 10 µm; B = 10 µm; C = 10 µm; D = 16 µm).*

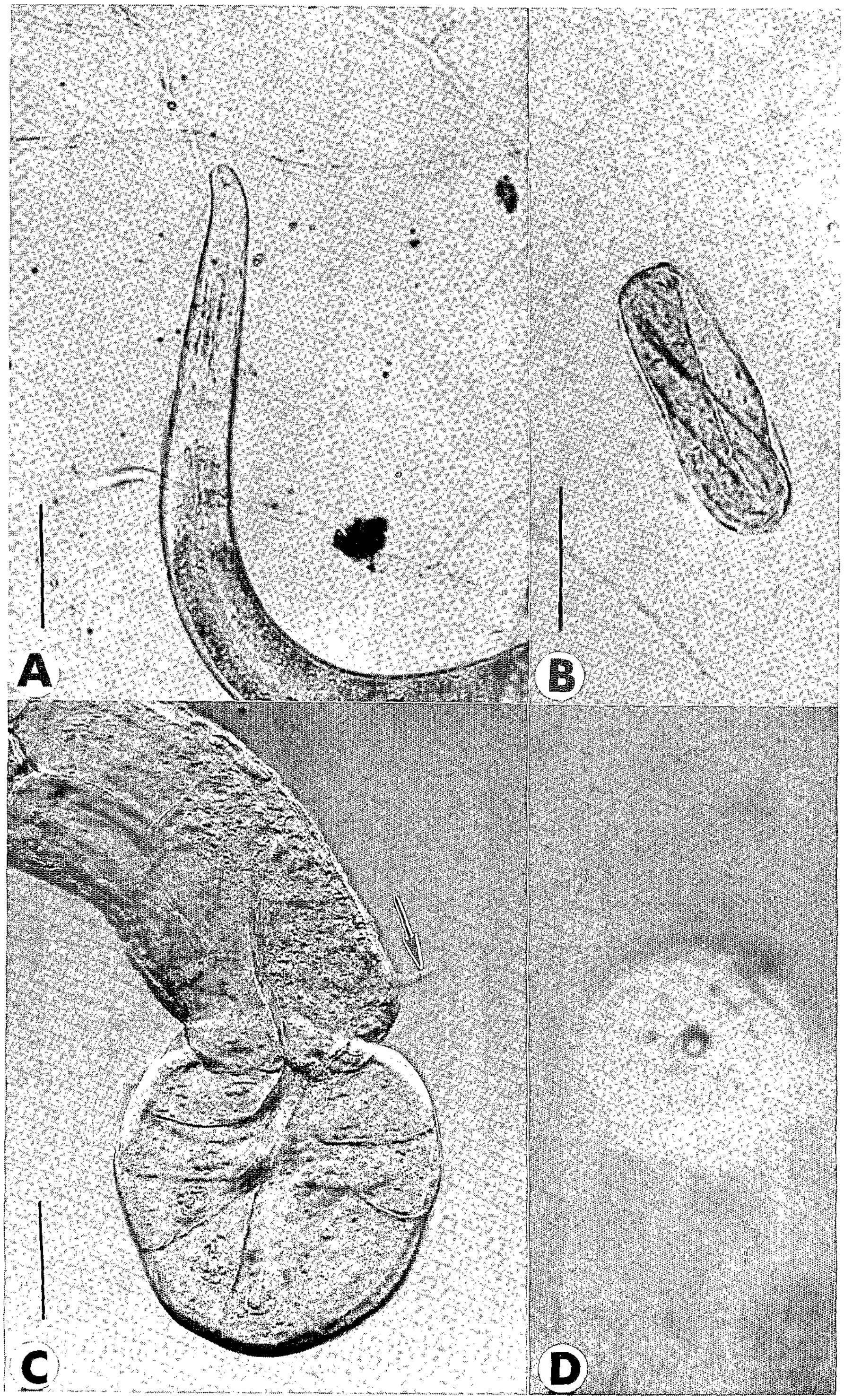

Fig. 7. *Iotonchium californicum* n. sp. A : Mycetophagous female with hyphae of *Agrocybe praecox* on malt agar plate; B : Developing egg of mycetophagous female in malt agar plate; C : Posterior portion of mature entomophagous female showing extruded uterine cells (arrow shows tip of tail); D : « En face » view of infective stage female (note octo-radiate pattern). *(Bars equivalent : A = 63 µm; B = 60 µm; C = 46 µm).*

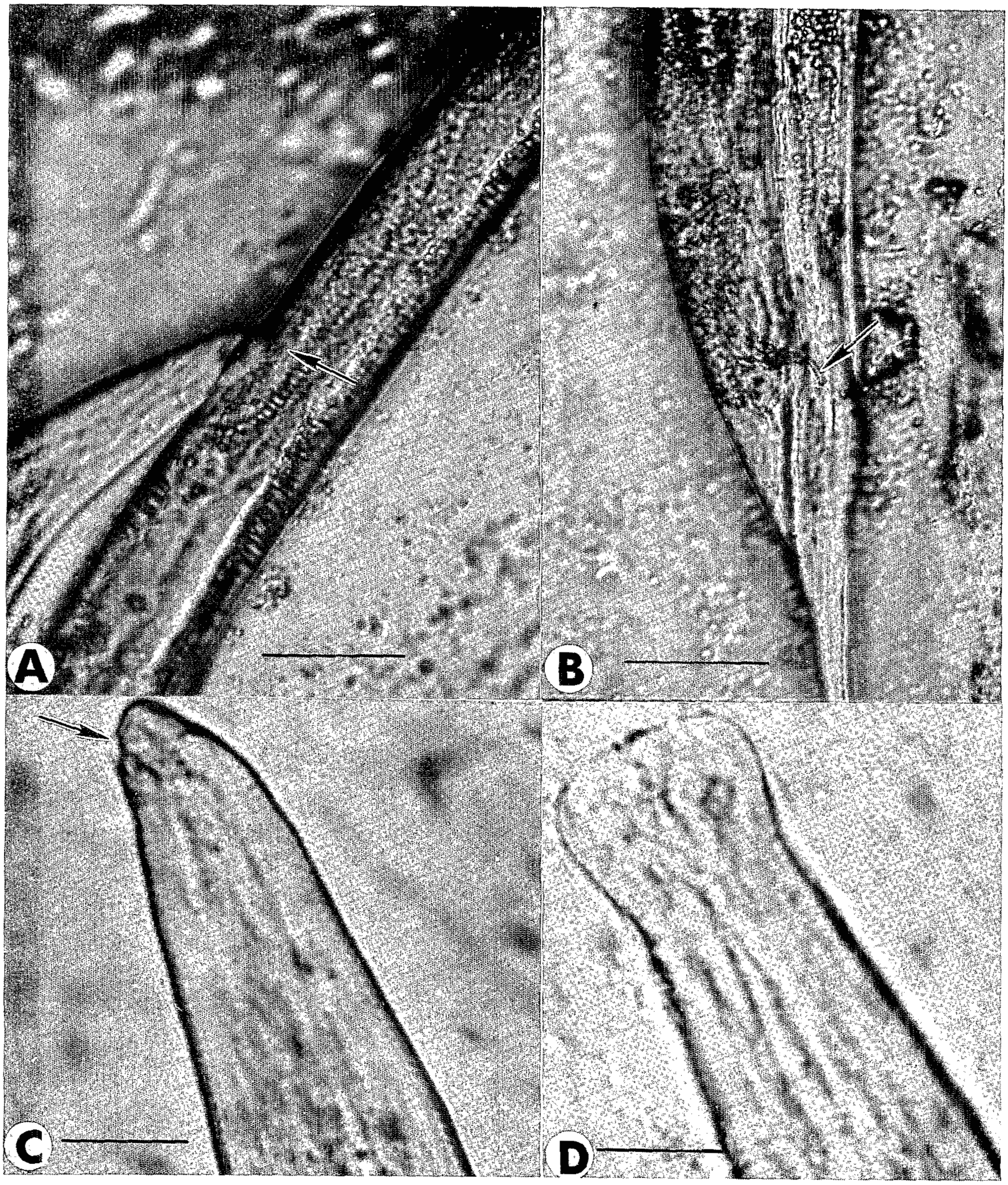

Fig. 8. *Iotonchium californicum* n. sp.; A : Head of male rubbing against body surface of female during mating (arrow shows lateral-subventral lip lobe of male appressed to body of female); B : Position of male and female during mating (arrow shows spicule extension inside female); C : Lateral view of asymmetrical head of male (arrow shows left lateral-subventral lip lobe); D : Dorsal view of male showing expanded head. *(Bars equivalent : A = 29 µm; B = 29 µm; C = 10 µm; D = 10 µm).*

Male (Fig. 2 B) : Shorter, but slightly wider than infective stage female; body curved ventrally upon relaxation; cuticle with faint transverse annulations, especially noticeable in cloacal area; lateral fields distinct, paired as in infective female; head asymmetrical, composed of a medial dorsal lobe containing two subdorsal lips, each with a distinct papilla; each lateral lip has combined with the adjacent subventral lip to form a lateral-ventral lobe; each latero-ventral lobe contains a pair of distinct labial papillae; mouth opens ventrally at base of head lobes; stylet inconspicuous, pharyngeal glands present; deirids occur in the region of the hemizonid just anterior to excretory pore; testis outstretched; vas deferens filled with small spermatozoa; bursa elongate, normally peloderan but in some cases leptoderan, edge smooth, lacking any incisions; spicules L-shaped, composed of two sectors, a spicule proper and an extension, both of which can occur within the cloacal chamber although the tips of the spicule extensions are generally exserted; spicule proper or proximal sector of spicule with an indistinct capitulum and a shaft expanded at base; spicule extension or distal sector of spicule with a proximal swelling connected to the base of the shaft of the spicule proper and an arm which curves posteriorly at an angle of 30-40°; the distal portion of this arm contains two conspicuous teeth-like projections, a third blunt tipped outer projection may also occur; a pair of papillae occur just anterior to the cloacal opening and a second pair of papillae occur just posterior to the cloacal opening; cloacal area often protruding from body surface; gubernaculum and bursal papillae absent; tail elongate-conoid, with pointed tip.

Mature insect parasitic female (Fig. 3 B) : Length similar to or slightly larger than that of the infective female; cuticle smooth, lateral lines inconspicuous, stylet present but inconspicuous; inner labial and outer papillae present; pharynx and pharyngeal glands inconspicuous; tail tip retains shape found in infective female; ovary extended and contorted within body cavity; ovoviviparous, with eggs in various stages of development and hatched first stage juveniles in the uterus; posterior uterine cells expanded into a probable food-absorbing organ and extruded through vulva into the body cavity of the fly host; eggs and juveniles exit through a canal in the center of the extruded uterine cells.

Third stage mycetophagous juveniles from insect host (Fig. 2 A) : Cuticle with faint transverse, striations; head not offset, stylet distinct, with two guiding rings and projecting basal knobs typical of mycetophagous female; dorsal gland outlet opening immediately behind stylet knobs; ventral gland outlet opening within two stylet lengths behind basal knobs; nerve ring, hemizonid and excretory pore distinct; pharynx joins intestine well posterior to excretory pore; gonad anlage composed of five to ten cells; intestinal cells filled with globules, some appear to contain spore-like bodies; tail bluntly pointed.

TYPE HOST

Fungus gnat, *Mycetophila fungorum* (DeGeer) (Mycetophilidae : Diptera); mycetophagous stages feeding on the Spring Agrocybe (*Agrocybe praecox* [Fr.] Fayod [Bolbitiaceae : Agaricales]).

TYPE LOCALITY

Under pine (*Pinus radiata* Don.) in a park in Albany (Alameda County), California, USA.

TYPE SPECIMENS

Holotype (male) and *allotype* (infective female) deposited at the Nematology collection, Department of Nematology, Davis, CA, USA.; *paratypes* deposited in the collection of the Laboratoire des Vers, Muséum national d'Histoire naturelle, Paris.

DIAGNOSIS AND RELATIONSHIPS

(*Iotonchium* Cobb, 1920; Iotonchiidae Goodey, 1953; Iotonchioidea Goodey, 1953 [Siddiqi, 1985]; Hexatylina Siddiqi, 1980). *Iotonchium californicum* n. sp. possesses four adult stages in its life cycle; mycetophagous female, infective stage female, male and mature entomophagous female. The mycetophagous females possess morphological features characteristic of previously described *Hexatylus* species. The infective stage females are characterized by their overall length, their stylet length and structure, the presence of three pharyngeal glands with distinct gland outlets and a general structure typical of members of the Allantonematidae. The males are unique in possessing an asymmetrical head and spicules with an extension. The entomophagous females are ovoviviparous and characteristically evert their posterior uterine cells through the vulvar opening.

In comparing *I. californicum* n. sp. with previously described species of *Iotonchium*, the mature entomophagous female and the mycetophagous female will be excluded from the discussion since they have not been considered in previous descriptions. The length of the infective stage females of *I. californicum* n. sp. (1500-2040 µm) differ from those of *I. fungorum* (Bütschli) (2770-3770 µm), *I. bifurcatum* Goodey (1200-1510 µm) and *I. cephalostrictum* Meyl (974-1135 µm). The length of the male separates *I. californicum* (810-1 230 µm) from *I. cephalostrictum* (538-683 µm) and the ratio of the length of the spicule extension divided by the length of the spicule proper separates *I. californicum* n. sp. (0.46) from *I. fungorum* (1.0) and *I. bifurcatum* (0.33).

The absence of a ventromedian papilla behind the vulva of the infective stage female separates *I. californicum* n. sp. from *I. bifurcatum* and *I. macrospiculatum* (Meyl). The shape of the spicules and extension separate *I. californicum* n. sp. from *I. fungorum*, *I. imperfectum* (Bütschli), *I. cephalostrictum* and *I. macrospiculatum*.

The presence of a pair of precloacal genital papillae as well as a pair of postcloacal genital papillae separates *I. californicum* n. sp. from all previously described species except *I. mycophilum* in which no males were described. From this latter species, *I. californicum* n. sp. differs in the longer stylet length of the infective stage females (18-21 µm in *I. californicum* n. sp. and 16-17 µm in *I. mycophilum*).

LIFE CYCLE

Young mycetophagous females of *I. californicum* n. sp. removed from the stipes of field collected infested mushrooms developed on agar cultures of *A. praecox*. These females matured on the fungal hyphae and deposited eggs (Fig. 7 B) which hatched in 2 days (22 °C) and produced another generation of mycetophagous females. Since no males were present and sperm could not be observed in the reproductive tract of the mycetophagous females, it is concluded that reproduction was by parthenogenesis. When cultures become old or contaminated, males and infective stage females were formed. It was only possible to determine whether a juvenile would develop into a mycetophagous female, male or infective stage female at the third stage, when internal changes in the reproductive system could be detected. However, with forms destined to become males or infective stage females, the mycetophagous type stylet was retained even in the pre-adult stage.

Mating of the males and infective stage females occurred in the agar cultures and is described later. After mating, the infective stage females entered the body cavity of a mature larva or pupa of *M. fungorum*. In nature, the mature fly larvae enter the soil beneath the mushroom remains and pupate. This is probably when infection occurs since the males and infective stages are formed only after the hyphal tissue begins to decompose. Entry into the host is by direct penetration of the insect's cuticle as observed on agar plates. The adult flies emerge from 2-3 weeks after pupation, depending on temperature. By this time, the nematode has matured, everted her uterine cells out through the vulva and produced a number of eggs, some of which hatch inside her uterus. The first stage juveniles range from 130-140 µm and occur inside the female uterus. Juveniles in the insect hemocoel fell into two size categories (presumably the second and third stage) based on overall length, 350-370 µm and 450-570 µm. All of these juveniles possessed stylets similar in shape to those of the mycetophagous females.

The juvenile nematodes invaded all tissues of the fly host, including the reproductive system. When infected adult flies attempted to oviposit, they released third stage juvenile nematodes. These nematodes initiated the mycetophagous phase of the life cycle when they were placed on fungal plates. The complete life cycle as determined from the present studies is represented in Fig. 10.

In nature, non-parasitized female flies deposit eggs on the cap and stipe of *A. praecox* soon after it has become full grown. The larvae develop quickly and finish their development within 10 days, the normal life expectancy of a mature *A. praecox*. Pupation occurs in the soil and adult flies emerge in about 2 weeks. At the time of emergence, parasitized flies contained fully developed female *I. californicum*. The adult flies survived for about 2 weeks, at which time the juvenile nematodes matured to mycetophagous stages inside the host's hemocoel.

MATING BEHAVIOR

The mating behavior of the males is unique for nematodes and illustrates the function of the peculiar male head. As observed on agar plates, the female lies outstretched while the male crawls on top of her, aligning his body in the same polarity as hers. He then arches the anterior portion of his body and brings the ventral portion of his head directly on the ventral surface of the female (Fig. 8 A). The lateral-subventral lobes of the male partially surround the female body. During the process of spicular penetration the male swings its head in rapid vibrational motion back and forth over the ventral surface of the female. Perhaps this motion serves to calm the female or keep her still while mating occurs which may last several minutes. During mating, the spicule extensions are inserted into the vagina, while the spicule proper remains in the male cloaca (Fig. 8 B). Some observations indicate that traumatic insemination may occur in *I. californicum* n. sp. Although viewing was difficult, spicular penetration into the vulva did not always appear to occur in mating individuals. Also, sperm-like bodies were occasionally observed in the body cavity of mated females. In addition, many of the females contained " hypodermal extensions " in the tail region that could represent areas where spicular penetration occurred. Further verification is needed to confirm this point but it would explain the unusual caressing behavior of the male during mating.

EFFECT ON HOST

Thus far only adult *M. fungorum* have been found parasitized by *I. californicum* n. sp. in nature although laboratory studies showed that infective stage females could enter fly larvae and pupae. In parasitized adult flies (both male and female) the gonads are either greatly reduced or completely atrophied. Parasitized male flies attempt to mate with females but have not been seen to deposit nematodes from their genital openings. However, female flies do attempt to oviposit and in so doing, deposit juveniles of *I. californicum* n. sp. Although the mature entomophagous nematodes were interwoven with host tracheoles and covered with host lipid cells, no host defense response was noted.

FOSSIL EVIDENCE OF *IOTONCHIUM*

During the examination of amber from the Dominican Republic, the author discovered a piece containing a single female Mycetophilidae parasitized by nematodes (Fig. 9 A). Juvenile nematodes could be seen in the body cavity of the fossil fly as well as in the amber surrounding the fly. Measurements of nematodes (n = 12) that were free in the amber (Fig. 9 B) showed them to have a length of 233 (208-296) µm and a width of 13 (12-16) µm.

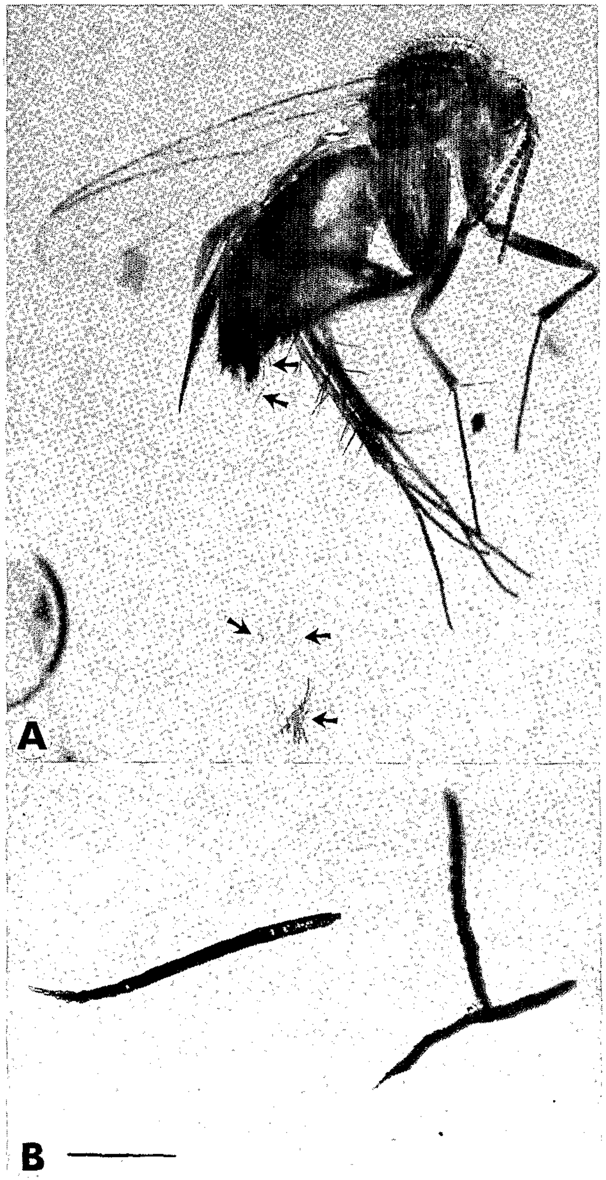

Fig. 9. Evidence of nematode parasitism of Mycetophilidae in Dominican amber. A : Fossil fungus gnat with parasitic juvenile nematodes (arrows); B : Detail of parasitic juvenile nematodes. *(Bar equivalent : B = 77 µm).*

Discussion

The present paper records for the first time the occurrence of *Iotonchium* in the Nearctic Region and describes the first complete life cycle of an *Iotonchium* species.

Following the recent treatment of the Tylenchida by Siddiqi (1986), the various stages of *I. californicum* n. sp. include characteristics of three separate nematode superfamilies. The mycetophagous females of *I. californicum* n. sp. are similar to those of *Hexatylus* in the Neotylenchoidea; the male and infective stage female would be classified as *Iotonchium* in the Iotonchioidea and the mature entomogenous female in the insect hemocoel would be placed in the Allantonematidae of the Sphaerularoidea. Siddiqi (1986) erected the genus *Fungiotonchium* for members of the original genus *Iotonchium* which possessed an anterior vulvar lip flap, a large ventromedian papilla behind the vulva and the distal portion of the spicule extensions permanently protruding. The present species possesses a vulvar lip flat, lacks a ventromedian postvulvar papilla and possesses spicule extensions that may or may not be protruding. The present author feels that the genus *Fungiotonchium* was erected prematurely before sufficient observations had been made on the existing species of *Iotonchium* and considers it a synonym of the latter genus.

For purposes of discussion, the stages in the life cycle of *I. californicum* n. sp. can be separated into a hexatyloid form (mycetophagous female), an *Iotonchium* form (male and infective stage female) and an allantonematid form (mature entomophagous female) (Fig. 10). It should be noted that the infective stage female could belong in the Allantonematidae as well as in the Iotonchiidae. The hexatyloid female form and *Iotonchium* male form of *I. fungorum* were originally described by Bütschli (1873). When T. Goodey (1953) discovered the male and female *Iotonchium* forms of *I. fungorum*, he discredited the *Hexatylus* female of Bütschli as being related to the *Iotonchium* male. T. Goodey (1953) stated " ... the nematode depicted in his [Bütschli's] Fig. 11 A is not the female of *Iotonchium fungorum*... it is fruitless to speculate as to the identity of the female shown by Bütschli beyond suggesting that it probably belongs to the writers [Bütschli] *Hexatylus* or to a closely related genus " Later, J. B. Goodey (1956) noted that the pre-adult female of *I. cephalostrictum* possessed a *Hexatylus* type of spear, however did not discuss the matter further. The only one who found all three of the adult forms in a mushroom was Bütschli (1873), who described the mycetophagous female, the male and infective stage female of *Iotonchium fungorum*. However, the infective female (his Fig. 67) was described under a separate section of his work and considered an unknown " peculiar " form; thus it was ignored by subsequent workers. All following descriptions of *Iotonchium* species were based on the infective stage female and (or) males.

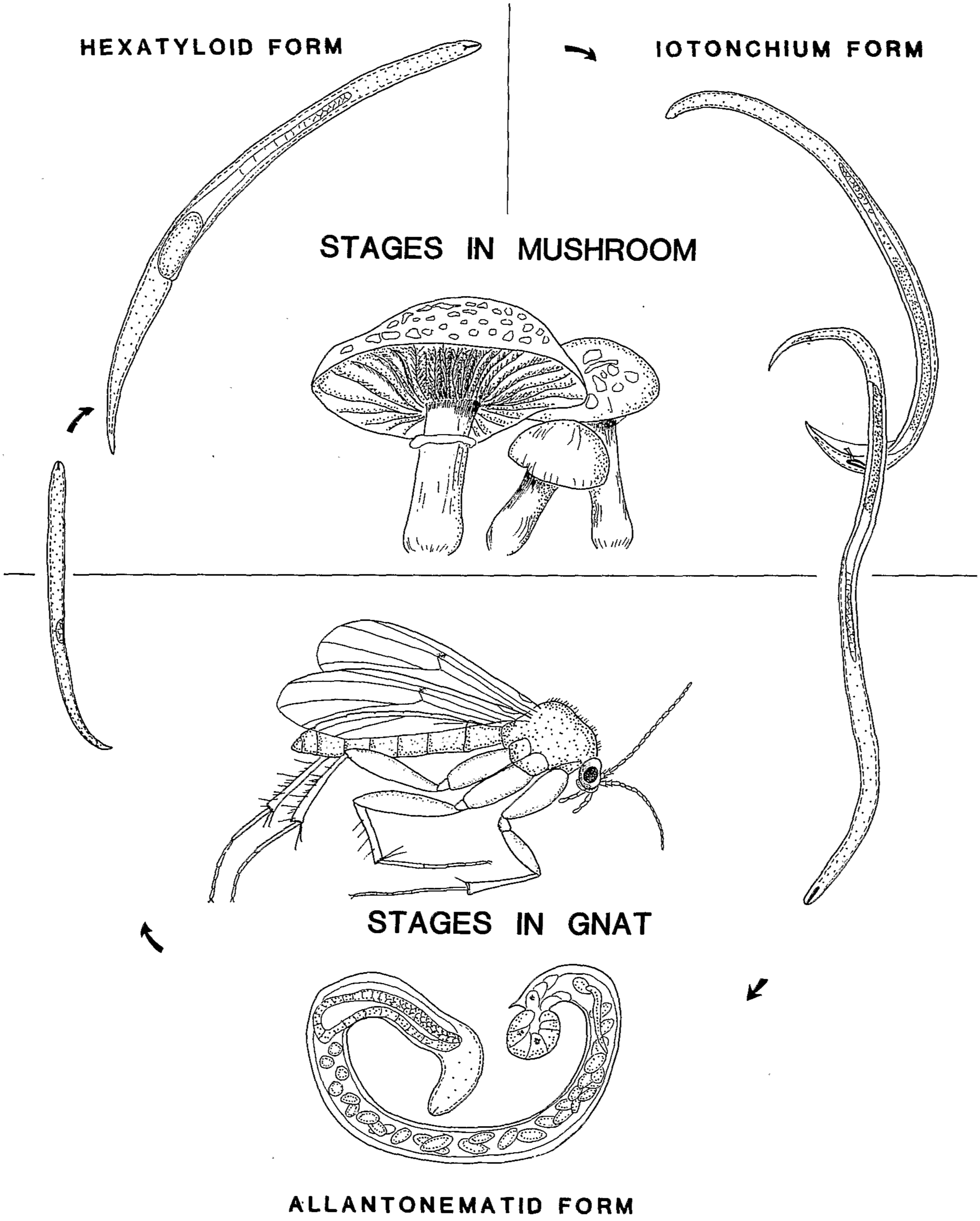

Fig. 10. Schematic presentation of the life cycle of *Iotonchium californicum* n. sp.

The mature entomophagous female of an *Iotonchium* has never previously been described, therefore it is not known whether the behavior in *I. californicum* n. sp. of everting the posterior uterine cells through the vulvar opening is common to other *Iotonchium* species. This behavior is found in several other genera of insect parasitic Sphaerularoidea and is not considered here to indicate affinity but is rather a secondary character which arises in various groups when conditions favor it.

The structure of the pharyngeal glands and position of the dorsal and subventral gland ducts in the infective stage female most closely resembles representatives of the Allantonematidae. In that family, members of the genus *Sphaerulariopsis* Wachek evert their complete uterus into the host's body cavity (the author does not concur with Siddiqi, 1986, in synonymizing *Sphaerulariopsis* with *Prothallonema* Christie). In the case of *I. californicum* n. sp., the mature female everts only the lower uterine cells, similar to the condition found in *Tripius* Chitwood, which however has only two pharyngeal glands in the infective stage female (Poinar, 1965).

It is possible that all previously described *Hexatylus* spp. which are autotokous are mycetophagous females of *Iotonchium* or related invertebrate-parasitic genera. The two nematodes described by Christie (1938) as *Hexatylus intermedius* and *Prothallonema dubium* are undoubtedly different stages of the same species and Siddiqi (1986) proposed the new combination *Prothallonema intermedium* (Christie) for this species. This species, which differs morphologically from *Iotonchium* spp. may have a similar life cycle, however. It is also probable that previously described Palearctic species of *Iotonchium* have an entomophagous stage in fungus gnats and a cycle similar to *I. californicum.*

The life cycle of *I. californicum* n. sp. shows similarities to that of *Deladenus siricidicola* as illustrated by Bedding (1972). However, significant differences do occur. Whereas the mycetophagous stages of *D. siricidicola* consist of both males and females, only parthenogenetic females occur in *I. californicum* n. sp. Sirex woodwasps (Siricidae : Hymenoptera) are the insect hosts of *D. siricidicola* whereas fungus gnats are hosts of *I. californicum* n. sp.

With *D. siricidicola,* the woodwasp deposits nematodes in pine trees along with spores of a symbiotic fungus of the genus *Amylostereum* (Family Stereaceae, Order Aphyllophorales, Class Hymenomycetes, Subdivision Basidiomycetina). With *I. californicum* n. sp., adult mushroom gnats deposit nematodes on gilled mushrooms belonging to the genus *Agrocybe* (and possibly other genera) (Family Bolbitiaceae, Order Agaricales, Class Hymenomycetes, Subdivision Basidiomycetina).

Whereas infectives of *D. siricidicola* take a relatively long time to mature and produce young since siricid larvae are long lived (many months), the development of *I. californicum* n. sp. must be rapid since the entire host cycle is only about a month. Morphological modifications of the males related to mating and of the mature entomophagous females (extrusion of the uterine cells) are unique characters of *I. californicum* n. sp. not known to occur in *Deladenus* species.

The head skeleton morphology of *I. californicum* n. sp. is complex. On the surface, eight sectors can be distinguished in the mycetophagous female, infective stage female and pre-adult male. However, twelve sectors can be distinguished beneath the surface. These findings compare with those of Shepherd Clark and Hooper (1983) who reported a twelve sectored pattern for *Hexatylus viviparus.* It is likely that the four small sublateral muscle sectors noted by Shepherd, Clark and Hooper (1983) on *H. viviparus* were visible only on a sub-surface plane in *I. californicum* n. sp.

It is curious that the present report of *I. californicum* n. sp. attacking *M. fungorum* and the fossil nematodes attacking an undescribed mycetophilid in Dominican amber constitute the only known cases of nematode parasitism of members of the family Mycetophilidae. The size of the fossil juvenile nematodes (L = 208-296 µm) differ from those of juvenile stages of *I. californicum* n. sp. as reported here. It is not possible at this time to state whether the fossil forms belong to an extinct species of *Iotonchium* or some other group of allantonematid or sphaerulariid that has not yet been discovered or has become extinct. However, on the basis of the size and shape of the fossil juvenile nematodes and their host affinity, the author believes that they probably represent an *Iotonchium* species, thus establishing a relationship between an insect family and nematode genus that has survived some 25-40 million years.

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