

***Ektaphelenchoides winteri* n. sp.**
(Nematoda : Ektaphelenchidae) from wood fly larvae
***Xylodiplosis* sp. (Diptera : Cecidomyidae)**

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Accepted for publication 27 September 1994.

Summary – *Ektaphelenchoides winteri* n. sp., adults and juveniles, were found attached by their stylet to the integument of wood-fly larvae (*Xylodiplosis* sp.) that had emerged from freshly cut timber. Males have prominent spicules in which the apex to rostrum distance is greater than from the rostrum to the distal tip; the tail has an offset terminal, tubular shaped, mucro 4-11 µm long. Males are 540-764 µm long and females 993-1350 µm long; their stylet is without basal knobs, 19-26 µm long. The nematode intestine contents, particularly in the posterior intestinal sac of the female, were pink in colour corresponding with the pink haemolymph of the fly larvae.

Résumé – *Ektaphelenchoides winteri* n. sp. (Nematoda : Ektaphelenchidae) provenant de larves de *Xylodiplosis* sp. (Diptera : Cecidomyidae) – Des adultes et des juvéniles d'*Ektaphelenchoides winteri* n. sp. ont été récoltés, attachés par leur stylet au tégument de larves de *Xylodiplosis* sp. venant de sortir de troncs d'arbres fraîchement coupés. Chez les mâles, les spicules sont saillants et la distance apex-rostre est plus longue que la distance rostre-extrémité postérieure; l'extrémité de la queue est pourvue d'un mucron tubulaire, bien détaché, long de 4-11 µm. Les mâles mesurent 540-764 µm et les femelles 993-1350 µm; le stylet, dépourvu de boutons basaux, est long de 19-26 µm. Le contenu intestinal des nématodes – et particulièrement celui du sac intestinal postérieur de la femelle – est de couleur rose ce qui correspond à celle de l'hoemolymph des larves de *Xylodiplosis* sp.

Key-words : Nematoda, *Ektaphelenchoides*, *Xylodiplosis*, Tarsonemid mite.

An ektaphelenchid nematode was noticed attached to the larvae of a wood fly, *Xylodiplosis* sp. (Diptera : Cecidomyidae), emerging from the vessels of logs freshly cut from an oak tree (*Quercus robur* L.). The nematodes protruded from the fly larvae giving them a "porcupine" appearance (Fig. 1 E). Ektaphelenchid nematodes are widely associated with bark beetles (Hunt, 1993) but this is the first record of an ektaphelenchid nematode attached to an insect larva; it is described here as *Ektaphelenchoides winteri* n. sp.

Materials and methods

Xylodiplosis larvae, with nematodes attached, were sent to Rothamsted in water or in moist paper tissue. The nematodes remained attached to some larvae which were put directly into hot lactophenol. Other fly larvae and nematodes associated with them were killed and fixed in glass cavity blocks by adding hot (99 °C) 5 % formalin, TAF or F.P. 4 : 1 fixative, each plus 2 % glycerol; the fixative was allowed to evaporate over several weeks until the specimens were left in glycerol, in which they were mounted. Some nematodes fixed in 5 % formalin were transferred to hot lactophenol in which they were mounted. Observations with the scanning electron microscope (SEM) were attempted on specimens fixed

as above, post-fixed in OsO₄, dehydrated to 100 % ethanol and then critical point dried using CO₂, and also on specimens processed to glycerol. Details of the above fixatives/methods are given in Southey (1986). Specimens freshly fixed and mounted in TAF were photographed using a Zeiss Universal Photomicroscope with differential interference contrast illumination. Measurements were made of specimens mounted in lactophenol or glycerol.

Ektaphelenchoides winteri * n. sp.

(Figs 1, 2)

MEASUREMENTS

See Table 1.

DESCRIPTION

Male : Lip region continuous with, or slightly narrower than, body contour with rounded sides and flattened anteriorly 7-8 µm wide by 3-4 µm high. When viewed with the SEM the lip region has 5-6 very fine transverse annulations which are not visible with the light micro-

* Named after Mr T. G. Winter who found specimens attached to *Xylodiplosis* larvae.

Table 1. Morphometrics of *Ektaphelenchoides winteri* n. sp. (measurements in μm).

	Holotype male	Paratype males	Paratype females
n	1	20	20
Stylet	20	19.1 \pm 0.22 (19-22)	22.4 \pm 0.37 (19-26)
Excretory pore distance from lip region	80	81 \pm 1.3 72-91	93 \pm 1.2 (85-102)
L	709	653 \pm 12.8 (541-764)	1211 \pm 28.1 (993-1460)
a	31	29 \pm 0.6 (23-33)	31 \pm 0.6 (25-35)
b	9.0	8.8 \pm 0.14 (7.8-9.7)	12.8 \pm 0.5 (9.0-16.6)
b'	4.4	3.9 \pm 0.06 (3.4-4.4)	8.3 \pm 0.31 (5.9-11.0)
c	13.6	15.1 \pm 0.28 (12.8-17.8)	
c'	2.1	2.3 \pm 0.06 (1.9-2.7)	
G1 or T	56	59 \pm 1.2 (51-71)	62 \pm 1.0 (54-70)
V			80 \pm 0.4 (78-85)
Post vulval "sac" in vulval body width			0.5 \pm 0.03 (0.4-0.8)
Vulva to "tail" tip (VT) in vulval body widths			7.4 \pm 0.14 (6.5-8.3)
Intestine end/VT %			67 \pm 1.3 (53-76)
Spicules ADT *	25	25 \pm 0.3 (23-27)	
Spicules A-T *	23	22 \pm 0.3 (20-24)	
Spicules A-R *	14	13 \pm 0.3 (11-15)	
Spicules R-T *	9	9.5 \pm 0.13 (9-11)	
Tail mucro	11	7.7 \pm 0.45 (4-11)	

* ADT = apex to distal tip along dorsal limb; A-T = straight line apex to distal tip; A-R = apex to rostrum; R-T = rostrum to distal tip; b' = body length \div distance from anterior end to posterior end of oesophageal glands.

scope. The lip region is divided longitudinally by depressions into six sectors; the lateral sectors are slightly narrower than the others with oval-shaped amphid apertures; the sublateral sectors each have a prominent cephalic papilla on their outer margins. A labial disc was not present. The body is curled ventrally when killed by heat, the tail end curled ventrally upon itself. The body cuticle has fine transverse annulations slightly less than 1 μm wide which are interrupted by the rather narrow lateral field which has three incisures. Stylet 19-22 μm long without basal knobs, the anterior cone is slightly

shorter than the shaft and is somewhat expanded anteriorly and thickened posteriorly at its junction with the shaft. Male and female specimens detached from fly larvae had a characteristic ring around the base of the protruding cone. Median oesophageal bulb prominent, oval to squarish with refractive thickenings just posterior to the centre. Well developed oesophageal glands overlap the intestine dorsally and extend just over twice the distance from the lip region to the base of the median bulb. Excretory pore usually within half a body width behind the median bulb, however in some specimens put directly into hot lactophenol it appeared just anterior to the median bulb. The multinucleate testis extends forward to just over half the body length. Small, rounded sperm in the *vas deferens* 2-3 μm in diameter. Tail dorsally convex, ventrally concave, about 2.5 cloacal body widths long, bluntly conoid with a rounded to bluntly conoid terminus bearing a prominent tubular mucro usually about 8 μm long. A prominent pair of subventral papillae present about half way along the tail plus a precloacal subventral pair opposite the spicule rostrum. Spicules well developed with a prominent, elongated and rounded apex just over half the length from the apex to distal tip; rostrum conical, moderately developed; the distal dorsal and ventral limbs converging distally to form a bluntly conical terminus. Spicule shape somewhat variable, sometimes those of the same pair differ.

Female: Body length about twice that of the males; body slightly curved ventrally when killed by heat. Lip region slightly more offset from body than in the male, stylet and oesophagus similar to the male. Intestine with dense contents coloured pink the same hue as the haemolymph of the insect host. The intestine appears to end in a blind sac at about two thirds the distance from the vulva to the posterior end; no rectum or anus seen in most specimens. However, in three specimens, out of many put directly into hot lactophenol, there was a cuticular indentation suggesting a vestigial anus occurring at 63-65 % of the distance from the vulva to the terminus, indicating a tail region 4.3 to 5 anal body widths long. Vulva at 78-85 % of the body length, a transverse slit somewhat subventral, vulval lips inconspicuous. Uterus and oviduct not well differentiated often containing sperm throughout. Uterine eggs very large (95-107 \times 20-23 μm) i.e. about five times as long as wide whereas deposited eggs were somewhat fatter (91-110 \times 29-32 μm). Ovary multinucleate throughout much of its length, extending past the oesophageal glands and sometimes reaching the median bulb but not seen reflexed. The uterine tissue extends posterior to the vulva for about half a vulval-body width but there is no distinct post-vulval sac. Posterior part of the body about seven vulval-body widths long with a conical terminus which may be sharply pointed or end in a narrow, finely rounded, tip.

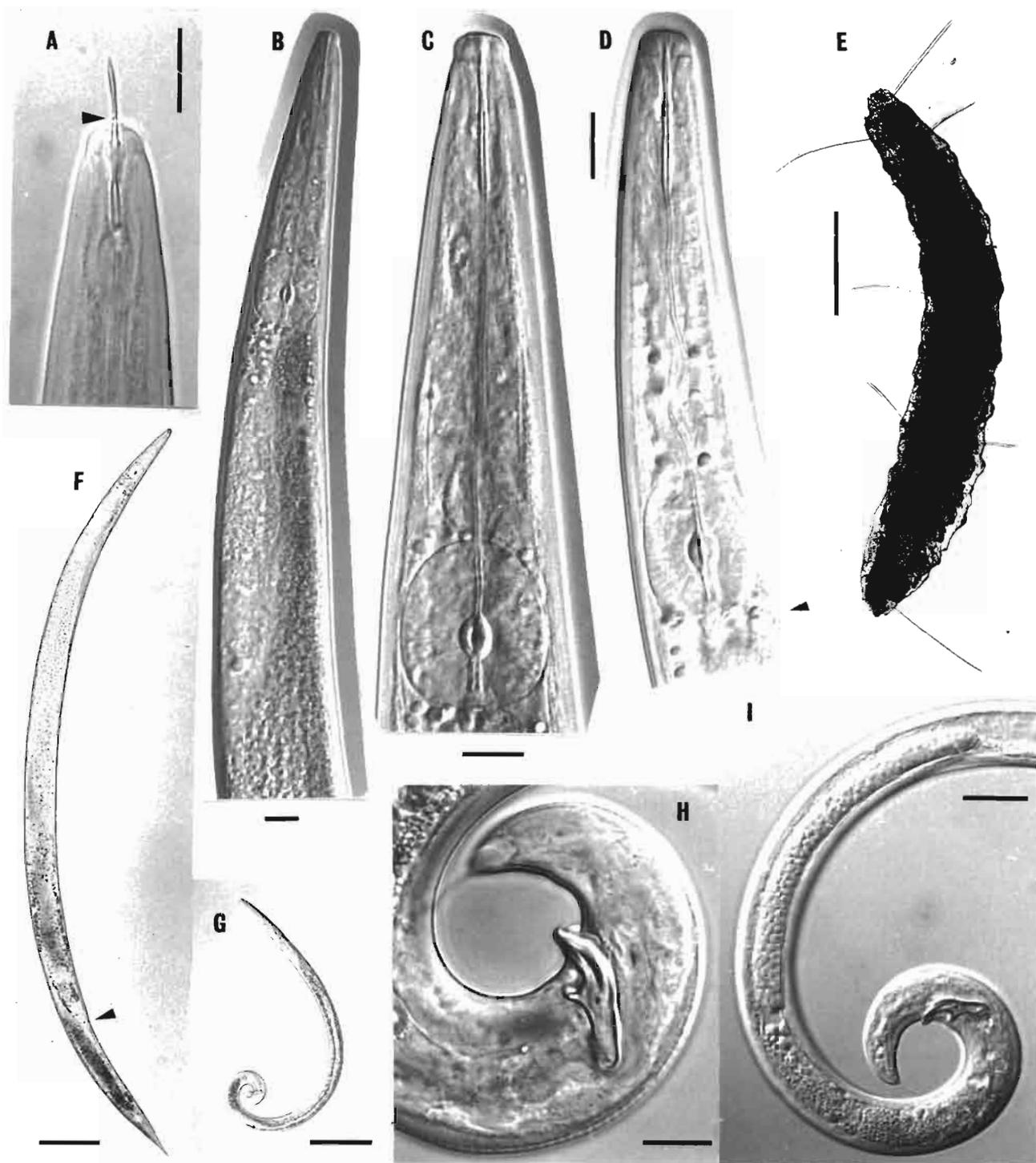
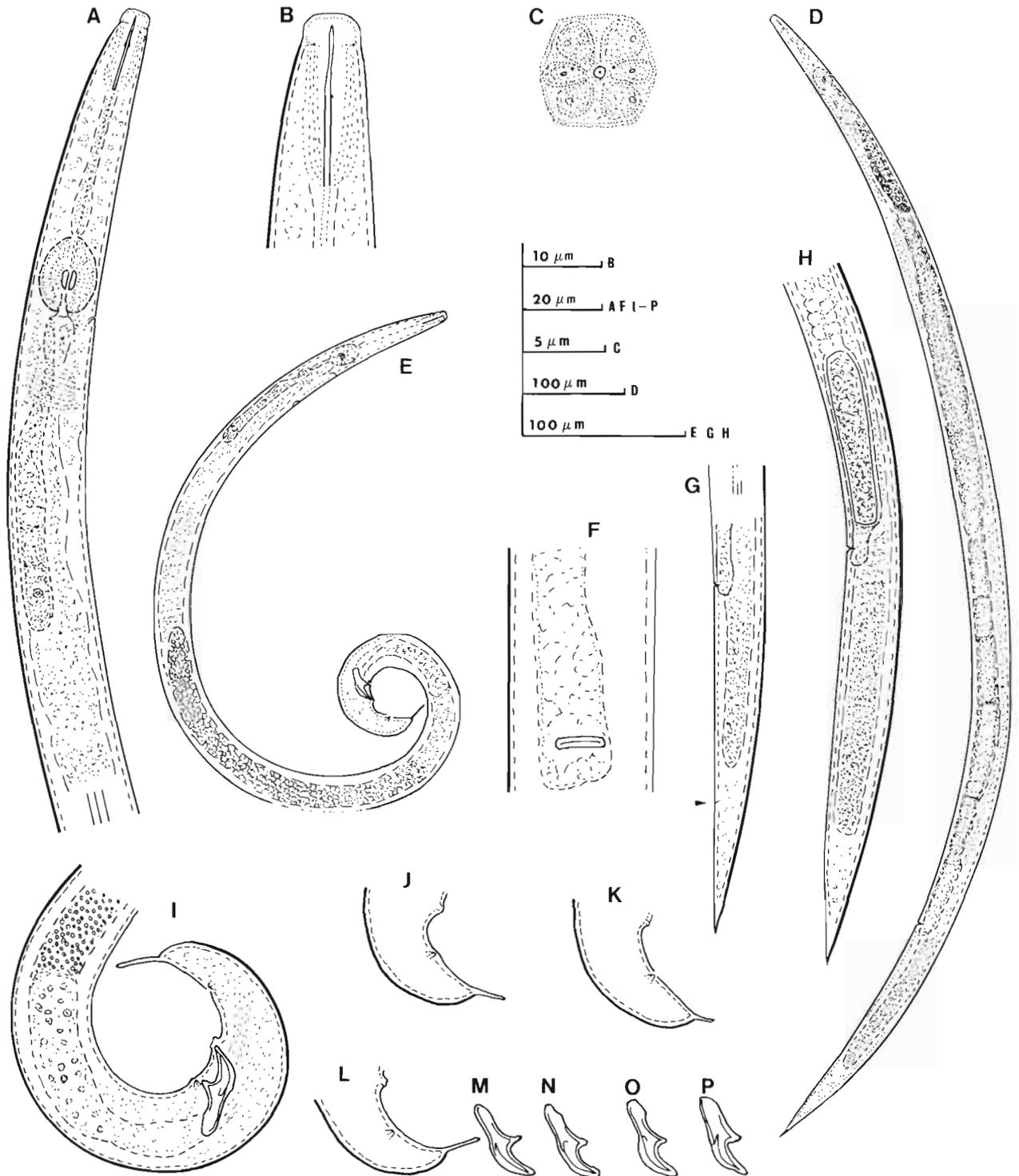


Fig. 1. *Ektaphelenchoides winteri* n. sp. *A* : Female head with protruded stylet as detached from fly larva, attachment "ring" arrowed; *B* : Female - oesophageal region; *C* : Female - anterior end; *D* : Male - anterior end, excretory pore arrowed; *E* : *Xylodiplosis* sp. fly larva with *E. winteri* attached; *F* : Female, vulva arrowed; *G* : Male; *H* : Male tail; *I* : Male - reproductive tract (Scale bars : *A-D, H* = 10 μ m; *E* = 1 mm; *F-G* = 100 μ m; *I* = 20 μ m).



10 μ m B
 20 μ m A F I - P
 5 μ m C
 100 μ m D
 100 μ m E G H

Fig. 2. *Ektaphelenchoides winteri* n. sp. **A** : Male oesophageal region - lateral; **B** : Male head region; **C** : Female - en face; **D** : Female; **E** : Male; **F**. Vulval region - ventral view; **G** : Female - posterior end, vestigial anus arrowed; **H** : Female - posterior end with uterine egg; **I** : Male - posterior end; **J-L** : Variation in male tail shape; **M-P** : Variation in spicule shape.

Juveniles : Specimens ranging in body length from 200-570 μm were also found attached to the integument of the fly larvae. They curled ventrally into a "C" shape when killed by heat. The lip region, stylet and oesophagus is similar to adults; the stylet of even the smallest specimens measured 15-16 μm long. The posterior end of the body tapered to a cone shape similar to the female. Moulting specimens shed the anterior cone part of the stylet with the moulted cuticle.

TYPE HABITAT AND LOCALITY

Attached to larvae of *Xylodiplosis* sp. (Diptera : Cecidomyiidae) emerging from logs of oak, *Quercus robur* L., cut from trees at Crickley Hill, Gloucestershire, England. National Grid Reference No. SO 930163.

TYPE SPECIMENS

Holotype (male) on slide no. 86 K/1/1 and paratype males and females on slides Nos. 86 K/1/2-36 in the Nematology slide collection of the Entomology and Nematology Department, Rothamsted Experimental Station. Paratype slides also deposited with the USDA Nematode Collection, Beltsville, Maryland 20705, USA and the Muséum National d'Histoire Naturelle, Paris, France.

DIAGNOSIS AND RELATIONSHIPS

Ektaphelenchoides winteri n. sp. is characterised by the male having spicules with a prominent, elongated, rounded apex and its tail with a prominent tubular mucro. The male and female both have three lateral incisures. The long female (1.0 to 1.5 mm) has a vulva at about 80 % of the body length without a distinct post-vulval sac; the posterior end of the body has a conical terminus.

E. winteri n. sp. is placed in the genus *Ektaphelenchoides* Baujard, 1984 because of its non expanded lip region, stylet without basal knobs, female lacking a distinct anus and rectum, an intestine ending in a blind diverticulum and male tail with an elongated mucro. It is differentiated in Table 2 from the four known species of *Ektaphelenchoides* described by Baujard (1984). The spicules of *E. winteri* n. sp. with the elongated apex are somewhat similar to *Ektaphelenchus macrobulbosus* Rühm, 1956 but the latter does not have an elongated, offset, tail mucro, the spicule tip is curved dorsally and the female is much shorter, L under 800 μm vs above 993 μm in *E. winteri* n. sp. As noted by Loof and Hooper (1993) some species of *Seinura*, in which the anus is indistinct, are close to *Ektaphelenchoides*. However, *E. winteri* n. sp. is readily distinguished from all *Seinura* spp. by the spicules having a longer apex to rostrum distance than rostrum to distal tip distance and also by the characteristic, offset, tubular mucro on the male tail.

REMARKS

The infested *Xylodiplosis* fly larvae emerged from freshly cut oak logs that had been stored for a few days in

Table 2. Characters differentiating species of *Ektaphelenchoides* from *E. winteri* n. sp.

<i>E. attenuata</i> (Massey, 1974) Baujard, 1984	Adults without lateral incisures. Female tail more filiform; post-vulval sac more distinct; vulva anterior : V = 61-63 vs 78-85. Male tail with a filamentous terminus and spicules with shorter apex.
<i>E. compsi</i> Baujard, 1984	Adults without lateral incisures. Female tail ventrally curved and sharply pointed; post-vulval sac 1.6-4.6 vulval body widths long. Male tail with two pairs of subventral papillae posteriorly; spicules with shorter apex.
<i>E. musae</i> Baujard, 1984	Female without lateral incisures, tail more filiform, vulva anterior : V = 67 (64-69) vs 80 (78-85). Males unknown.
<i>E. pini</i> (Massey, 1966) Baujard, 1984	Female tail more filiform, post-vulval sac more distinct; vulva anterior : V = 70 (67-74) vs 80 (78-85). Male tail more conical with spike-shaped mucro; spicules with shorter apex.

a polythene bag. These fly larvae had a characteristic pink colouration and the nematode intestine, particularly at its posterior end in females, had the same pink colour as the insect haemolymph. Adults and juveniles of *E. winteri* n. sp. were firmly attached by the stylet which penetrated the insects' integument. Some specimens remained attached when infested fly larvae were put directly into hot lactophenol. Nematodes subsequently detached from the insect had a characteristic ring around the stylet just anterior to the oral aperture suggesting some form of adhesive attachment. Also attached to the fly larvae were tarsonemid mites, *Ununguitarsonemus* sp. Attempts were made at the type locality in two successive years to obtain more wood fly larvae and their pupae and adults but none were found. Rühm (1956) suggested that *Ektaphelenchus* spp. derived nourishment from insect associates but this is the first record of actual feeding by any ektaphelenchid nematode. Their attachment to the wood fly larvae is similar to ectoparasitism recorded for the aphelenchid genera *Acugutturus*, *Noctuidonema* and *Vampyronema* (see Hunt, 1993).

Acknowledgements

Mr T. G. Winter of the Forestry Commission, Forest Research Station, Alice Holt Lodge, Surrey, England noticed the *Xylodiplosis* larvae attacked by nematodes and kindly sent the specimens to the author. The larvae were confirmed as *Xylodiplosis* sp. by Dr K. M. Harris, CAB International Institute of Entomology (I.I.E.), London. The tarsonemid mites were identified by Dr D. Macfarlane also of I.I.E. Dr D. J. Hunt, CAB International Institute of parasitology, St Albans, England, helped in the classification of the nematode. Mrs Janet Rowe conducted SEM studies and made the prints for Fig. 1.

References

- BAUJARD, P. (1984). Remarques sur la sous-famille des Ektaphelenchinae Paramonov, 1964 et proposition d'*Ektaphelenchoides* n. gen. (Nematode : Aphelenchoididae). *Revue Nématol.*, 7 : 147-171.
- HUNT, D. J. (1993). *Aphelenchida, Longidoridae and Trichodoridae : their systematics and bionomics*. CAB International, Wallingford, England, 352 p.
- LOOF, P. A. A. & HOOPER, D. J. (1993). Redescription of *Seinura demani* (T. Goodey, 1928) J. B. Goodey, 1960 (Nematoda : Seinuridae) and designation of a neotype. *Fundam. appl. Nematol.*, 16 : 163-169.
- RÜHM, W. (1956). Die Nematoden der Ipiden. *Parasit. Schrreihe*, 6 : 1-435.
- SOUTHEY, J. F. (Ed.) (1986). *Laboratory methods for work with plant and soil nematodes*. London, H.M.S.O., M.A.F.F. Reference Book 402, 202 p.