Materials and methods

Isolates R1.5 and V25A of *S. feltiae (=bibionis)* were isolated from soil samples collected in Reading (UK) and southern Norway respectively, using the *Galleria* trapping technique (Bedding & Akhurst, 1975). *S. carpocapsae* UK, was obtained from a laboratory culture at Reading University (originally isolated from sawfly larvae (Georgis & Hague, 1981)). The nematodes were cultured *in vivo* on late instar *Galleria* larvae.

For scanning electron microscopy first generation adult nematodes were dissected from previously infected *Galleria* larvae in 1/4 strength Ringer's buffer. They were rinsed several times in Ringer's before being fixed in 8 % gluteraldehyde (gluteraldehyde 25 % EM Grade, diluted in Ringer), for 2 h at room temperature. Five day old infective stage juveniles (J3) were rinsed in 0.05 % NaCl three times at 30 min intervals and fixed in 8 % gluteraldehyde made up with distilled water. The fixed nematodes were then washed three times in Ringer (or distilled water J3) and post-fixed in 1 % osmium tetroxide for 1 h. They were rinsed again, dehydrated at 15 min intervals through 30 %, 50 % and 70 % acetone and placed at 4 °C overnight in 70 % acetone.

Subsequent dehydration continued through 90 %, 95 %, 100 % and absolute dry acetone also at 15 min intervals. The nematodes were then critically point dried in a " Samdri-780 " critical point drier, mounted on stubs and finally sputter coated with gold. Twenty or more specimens were prepared for each isolate and stage examined. Photographs were taken of the head

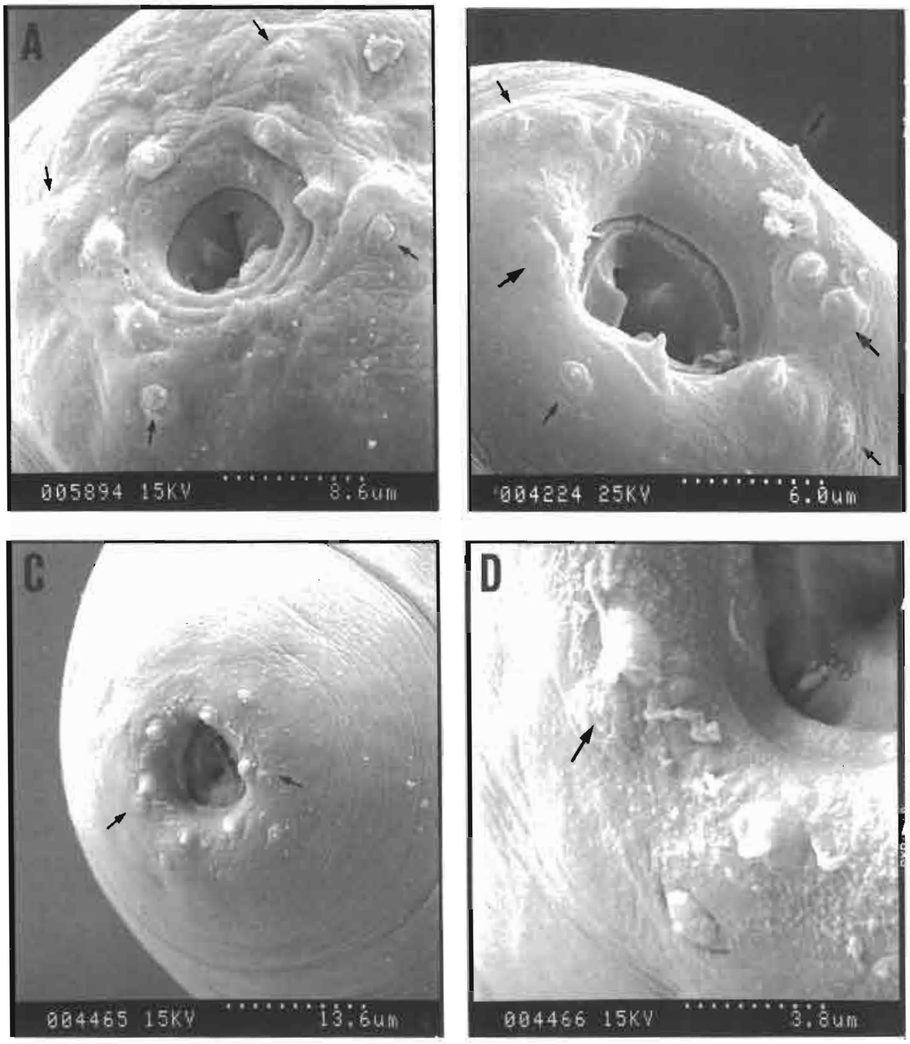

Fig. 1. Anterior region of adult female *Steinernema*. A : *S. carpocapsae* (UK), showing cephalic papillae (arrows), and six labial papillae; B : *S. feltiae* showing six labial and the usual four cephalic papillae as well as the additional cephalic papillae (arrows); C, D : *S. feltiae* having less developed additional cephalic papillae (arrows).

region using a Hitatchi S-570 scanning electron microscope at 15 or 25Kv.

Results

First generation *S. carpocapsae* females revealed a head sensilla pattern of six labial and four cephalic papillae on all specimens observed (Fig. 1 A). However, *S. feltiae* females appeared to be less consistent with respect to the number of cephalic papillae. In some specimens six cephalic papillae were observed, which were in most cases morphologically similar, i.e. " button like ". The two sublateral cephalic papillae were located close to the lateral labial papillae with the remaining four cephalic papillae in the usual laterodorsal or lateroventral position (Fig. 1 B). This phenomenon was not always easily observed, in some cases these additional cephalic papillae appeared morphologically similar to the usual four, whereas in others they appeared more like a bump in the cuticle (Fig. 1 C, D) but were always found in the same position, close to or at the base of the lateral labial papillae. No definite pore or slit-like amphids were observed on any of the females.

The males of both species clearly had six labial and four cephalic papillae, and amphids could be observed on good specimens (Fig. 2 A). These were in a similar position to the additional cephalic papillae found on the *S. feltiae* females.

Observations of the infective stage juveniles showed four distinct cephalic papillae, morphologically similar to the adult stage labial papillae, and the amphids were consistently very clear and larger, more pore-like than those found on the males. Labial papillae were not ob-

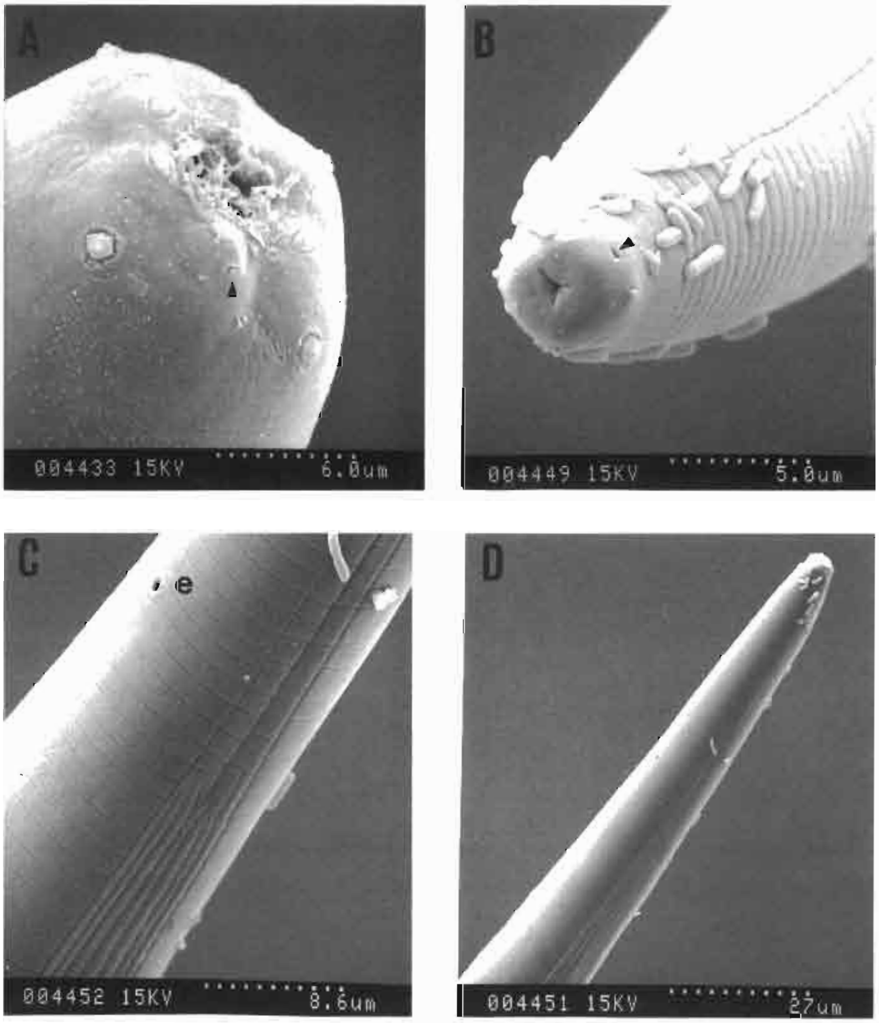

Fig. 2. Anterior region of male and the infective stage of *Steinernema*. A : Amphid (arrow) on a male *S. carpocapsae;* B : Pore-like amphid (arrow) on infective stage juvenile of *S. feltiae* (note also the four cephalic papillae; C, D : The beginning of the lateral field of *S. feltiae,* as well as the excretory pore (e).

served (Fig. 2 B). Eight lines in the lateral field was observed on some infective stage juveniles of *S. feltiae* specimens (Fig. 2 C, D). No lateral field was found on the adult stages.

Discussion

Observations on the head sensilla of *S. carpocapsae* and *S. feltiae* in this study showed two circles of papillae, an inner circle of six labial papillae and an outer circle of four, or apparently six, cephalic papillae for *S. feltiae*. The papillae in the two circles differed morphologicaly, the labial papillae protruding more from the base, whereas the cephalic papillae appeared flatter and " button-like ". The labial papillae were always more obvious than the cephalic papillae.

The presence of two additional cephalic papillae on *S. feltiae* has not been reported previously. In some cases they were not distinctly papillae-like, but were always located in the same position, suggesting that the number of cephalic papillae may be variable in the genus *Steinernema*. Doucet (1986) reported no cephalic papillae in the original description of *Steinernema rara* from Argentina, but a re-examination of this species by Poinar *et al.* (1986) showed the presence of the usual four cephalic papillae, although they were not particularly distinct. The presence of cephalic papillae therefore may either be difficult to observe and hence missed, or some may be indistinct and not counted. In the present study there appeared to be some variability in the number of cephalic papillae on the *S. feltiae* adult females, hence the presence or number of cephalic papillae will not only be due to the observers interpretation, but also to the variability of the cephalic papillae on the nematodes themselves.

Amphids were not observed on the female specimens, although on *S. feltiae* the additional cephalic papillae were located in the usual amphid position indicating that these cephalic papillae have not completely developed to amphids (amphids being derived from cephalic papillae). Figure 1 B clearly shows that one of the sublateral cephalic papillae is very similar to the usual four, whereas the other is less developed, perhaps indicating a transitional phase in which these cephalic papillae have not developed into amphids. It may be postulated that the females, in relation to their micro habitat in an insect cadaver, being surrounded by their nutritional requirements have no particular need for amphids, which are chemosensory (Coomans, 1979). In most descriptions of *Steinernema* species (adult forms) the presence of amphids are shown and located at the base of the lateral labial papillae (*eg.* Doucet & Doucet, 1990; Poinar, 1988), however their function may be questionable, since their development can be partial or apparently absent, based on the SEM observations of *S. feltiae* and *S. carpocapsae* in this study. Males on the other hand, on which small slit-like amphids were found, may have amphids to assist in finding females in order to mate.

The adult males from both species showed no variability in the number of head sensilla, both the inner circle of six labial papillae and the outer circle of four cephalic papillae being distinct and easily observed. Small slit-like amphids were quite distinct in most cases and located in a similar positon to the extra cephalic papillae seen on some of the *S. feltiae* females.

In contrast to the adult stages, the infective stage juveniles showed only one circle of four cephalic papillae, the labial papillae not being visible. Most striking were the relatively large pore-like amphids (Fig. 2 B). Infective juveniles are present in soil naturally, and in order to infect a suitable host they must first be able to sense its presence, it has been shown that infective stage juveniles are attracted to insect excretory products and carbondioxide (Schmidt & All, 1979; Gaugler *et al.*, 1980; Pye & Burman, 1981) which probably accounts for the clear presence of amphids.

The lateral field for the *S. feltiae* infective stage juveniles examined had eight lines, which is in accordance with Mráček and Bednarek (1991), for this species.

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