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Morphology of amoeboid cells in the uterus of *Steinernema* species (Rhabditida: Steinernematidae)

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Summary. Amoeboid cells of different morphology and behavior were observed in the lumen of the uterus of giant females of *Steinernema* spp. Cells from 13 described species (*S. affine, S. arenarium, S. bicornutum, S. carpocapsae, S. ceratophorum, S. cubanum, S. glaseri, S. feltiae, S. intermedium, S. karii, S. kraussei, S. longicaudatum* and *S. riobrave*) and 6 undescribed species (British isolates C1 and B3, Sri-Lankan isolates SSL1 and SSL2 and Japanese isolates MY1 and MY5) were studied. These amoeboid cells are similar in their the morphology to the spermatozoon in the nematode *Caenorhabditis elegans*, lending support to the suggestion that they are also spermatozoa. Morphologically similar and related species of steinernematids are characterized by having similar structure and behaviour of their associated amoeboid cells.
Key words: *Steinernema* spp., taxonomy, in *uteri* structures, amoeboid cells, rhabditid spermatozoon.

Entomopathogenic nematodes of the family Steinernematidae have potential as biocontrol agents. Information on their biogeography is limited, but surveys have produced numerous isolates from many parts of the world (Hominick *et al.*, 1996). A morphological approach does not always provide reliable identification of new isolates, therefore molecular methods have been successfully implemented to elucidate their taxonomy (Reid *et al.*, 1997). Despite the success of molecular methods, adequate morphological keys are still essential (Hominick *et al.*, 1997). Distinct morphological details of sperm cells are considered a promising source of taxonomic characters for aquatic nematodes (Riemann, 1983) and for trichodorids (Decraemer, 1995). Therefore, amoeboid cells with different morphology and behavior in the lumen of the uterus of giant females of *Steinernema* were examined to determine whether differences in the morphology of these cells can be used in steinernematid taxonomy.

MATERIAL AND METHODS

Gravid females of the first generation were picked by a needle from dissected *Galleria mellonella* larvae which previosly had each been infected with a single steinernematid species. The females were examined live under high magnification (mainly x100 - oil immersion objective) using an Olympus microscope. Infective stages were obtained from the voucher collection of living steinernematid nematodes maintained at CABI Bioscience and the identity of the nematodes had been confirmed by molecular taxonomy (Reid *et al.*, 1997). Individual giant females were placed in physiological saline (0.9% NaCl) under a cover glass on supports. The orientation of the females was changed by gentle movements of the cover glass to obtain the optimal perspective of the distal part of the uterus and oviduct. Live cells in the lumen of the uterus were examined, measured and photographed within a few minutes of dissection, as prolonged exposure to saline resulted in a cessation of amoeboid movement and in changes in cell shape. A minimum of 10 individual females were examined for each species. All drawings were made from photographs.

A molecular database for over 500 isolates of entomopathogenic nematodes is maintained at the CABI Bioscience UK Centre (Egham) and allows an assessment of variation both between and within species. All isolates have been characterized by the same molecular methods used to characterize the named species (Reid *et al.*, 1997). The voucher collection contains 28 steinernematids which are new to science. Some of these were examined during the present study, e.g. SSL1 and SSL2 from Sri Lanka, C1 and B3 from the UK and MY1 and MY5 from Japan. The SSL1, SSL2 and C1 cultures were part

of the phylogenetic analysis conducted by Reid *et al.* (1997), the RFLP patterns for two B3 isolates are in Miduturi *et al.* (1996) and patterns for MY1 and MY5 are in Yoshida *et al.*, (1998).

RESULTS

In the descriptions below, the abbreviation AC is used for amoeboid cells. The function of these bodies is not known although it is likely that they are spermatozoa. These cells exhibit considerable morphological diversity, both quantitative and qualitative. One end of the cell is usually rigid, hemispherical, and contains a nucleus-like structure and the other end produces pseudopodia, which are usually directed towards the oocytes appearing from the oviduct. The end with pseudopodia is referred as the "anterior end of AC", and the hemispherical end as the "posterior end of AC".

The terms globule, conglomerate and vesicle are used in the descriptions defined here as:

Globules - spherical particles of 1-3 μm diameter (e.g. Fig. 1B) situated on the AC surface (mainly on its posterior end) in many steinernematid species.

Conglomerates - found in several steinernematid species with large ACs. A much larger amoeboid cell can usually be observed simultaneously with ACs of a normal size. Several nuclei are discernible inside the cytoplasm of the conglomerates, which are considered to have formed from the merging of several ACs.

Vesicles - the outer membrane of an AC or conglomerate is highly mobile, producing invaginations and even autonomous structures, designated vesicles, from fragments of the outer membrane. Naturally, globules covering the AC or conglomerate surface are situated on the inner surface of vesicles and invaginations.

Described steinernematid species:

The following citations with full authority follow the recommendations of Hominick *et al* (1997).

1. *S. affine* (Bovien, 1937) Wouts, Mracek, Gerdin & Bedding, 1982. Separate AC and chains of up to twelve ACs in length (usually 2-4) were observed in the uteri (Fig. 1A). The distal part of the uterus in females with numerous eggs, or the area near the vulva in some females without eggs, were tightly packed with numerous ACs. Diameter of AC posterior part 10-12 μm, length from 16 to 24 μm. No globules on AC surface. Conglomerates were not observed.

2. *S. arenarium* (Artykhovsky, 1967) Wouts, Mracek, Gerdin & Bedding, 1982. Up to 20 separate large ACs can be seen in the uterus (Fig.1B). Posterior end of AC is 30 μm diameter, surface of AC posterior end is covered with scattered globules of 1.5 μm diameter containing refractive particles; total length of AC around 40 to 50 μm. Anterior end of AC compact, no more than 20-30 μm long, but in some ACs pseudopodia can be very long, up to 45 μm. Smaller, elongated ACs can be seen between eggs with posterior end 10 x 10 μm in size, and with small pseudopodia on the anterior end. Conglomerates were not observed.

3. *S. bicornutum* Tallosi, Peters & Ehlers, 1995. Only 5-6 ACs with a length of 50 μm and diameter of 20-40 μm were observed (Fig. 1C). Posterior end of AC hemispherical, with tightly placed globules of 2 μm diameter on the surface. Vesicles inside the AC cytoplasm with 2-20 globules on the inner surface. Conglomerates were not observed.

4. *S. carpocapsae* (Weiser, 1955) Wouts, Mracek, Gerdin & Bedding, 1982. Up to 24 large, separate ACs were observed in females of the strain"All" (Fig. 1D). Posterior end of AC with a diameter of 30-35 μm; length of AC about 40-50 μm. Flattened globules of 2 μm diameter present on the posterior end of AC. Conglomerates were not observed.

5. *S. ceratophorum* Heng, Reid & Hunt, 1997. From 3 to 8 large ACs in the uterus (Fig. 1E). Posterior end of AC with a diameter of 25-35 μm. Numerous 1.5 μm diameter globules containing refractive particle were present on the AC surface. Conglomerates were not observed.

6. *S. cubanum* Mracek, Hernandez & Boemare, 1994. Few ACs present (Figs.1F & 2D). ACs elliptical, without pronounced pseudopodia, 20-22 x 25-30 μm. Globules containing refractive particles are distributed over entire AC surface. In some females, amoeboid conglomerates with a diameter of 70-100 μm can be seen, and these completely occupy the lumen of the gonad tube. Usually eggs are pushed through such conglomerates. Numerous vesicles with globules on the inner surface can be seen inside such conglomerates.

7. *S. glaseri* (Steiner, 1929) Wouts, Mracek, Gerdin & Bedding, 1982. Few ACs present (Figs. 1G & 2A-C). Each AC is elliptical, usually without pronounced pseudopodia, usually 20 x 30 μm. Globules containing refractive particles observed on the AC surface. Amoeboid conglomerates of 80-140 μm diameter present, completely sealing the gonad lumen. Vesicles with globules on inner surface are present in the cytoplasm of the conglomerates (Figs. 1G & 2C).

8. *S. feltiae* (Filipjev, 1934) Wouts, Mracek, Gerdin & Bedding, 1982. Numerous chains of up to 16 ACs in length occur frequently in the uterus of the A1 isolate. Usually distal parts of the uterus contain more than 1000 ACs per gonad branch. Each

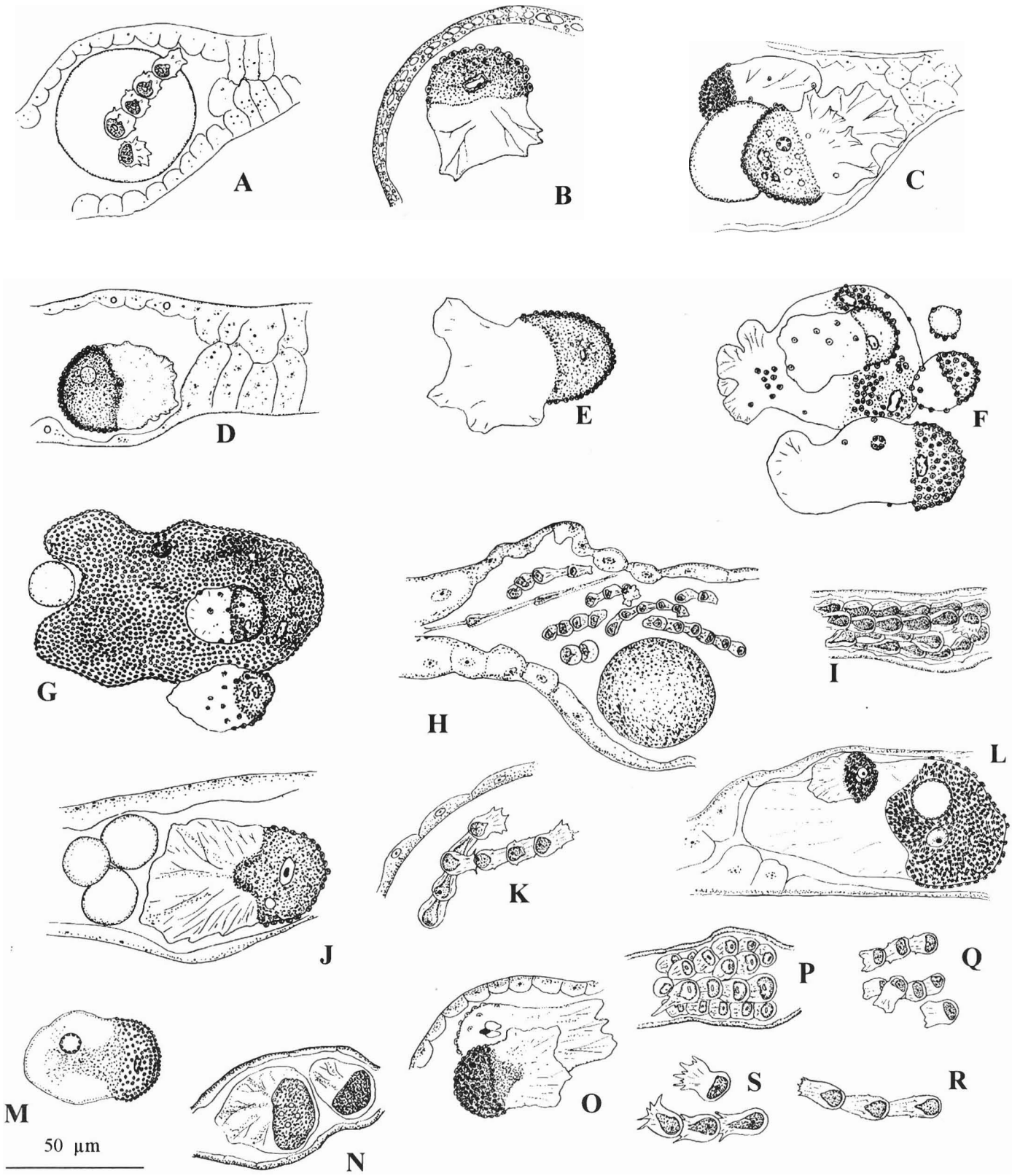

Fig. 1. Morphology of amoeboid cells in uteri of first generation steinernematid females. A: *S. affine*; B: *S. arenarium*; C: *S. bicornutum*; D: *S. carpocapsae*; E: *S. ceratophorum*; F: *S. cubanum*; G: *S. glaseri*; H: *S. feltiae*; I: *S. intermedium*; J: *S. karii*; K: *S. kraussei*; L: *S. longicaudum*; M: *S. riobrave*; N: Sri-Lankan isolate SSL1; O: Sri-Lankan isolate SSL2; P: British isolate C1; Q: British isolate B3; R: Japanese isolate MY1; S: Japanese isolate MY5.

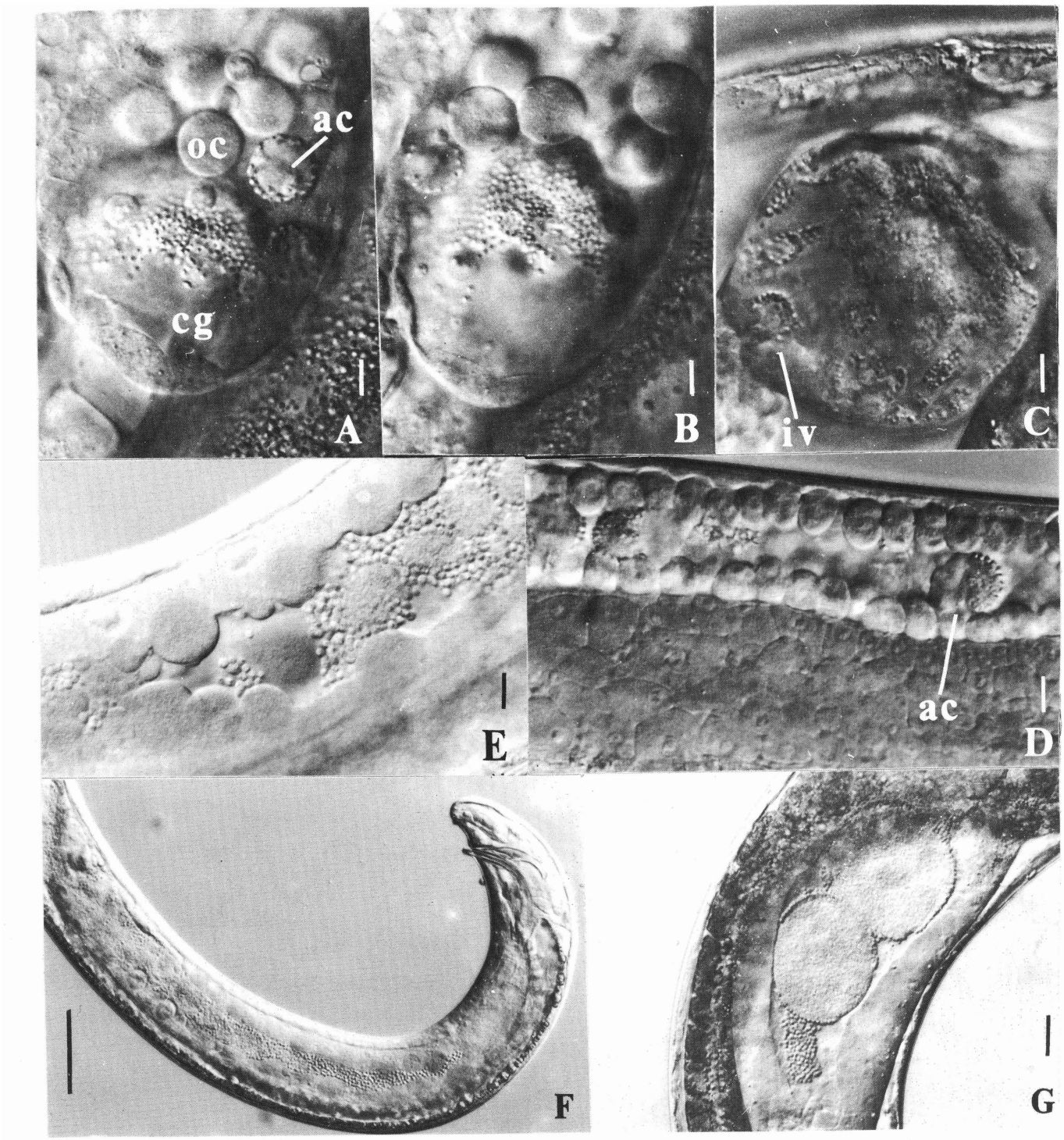

Fig. 2. Cells in the gonad tubes of adult steinernematids. A: Comparative size of oocyte, separate amoeboid cell and amoeboid conglomerate in uterus of *S. glaseri* (surface view), ac - separate amoeboid cell; oc - oocyte; cg - conglomerate; B: Distribution of globules on amoeboid conglomerate of *S. glaseri*; C: Conglomerate of AC in *S. glaseri* female (inner optical section), iv - inner vesicle with the globules on inner surface; D: the uterus lumen in a young *S.cubanum* female ac - separate amoeboid cell wandering in the tube; E: The lumen of vas deferens in *S. glaseri* male; F: General appearance of posterior end of a *S. glaseri* male; G: Proboscis-like agglomeration of smaller cells and big cells with homogeneous content in the vas deferens of a *Steinernema* sp. isolate from Costa-Rica. Scale bars: A-E, G - 10 μm, F - 50 μm.

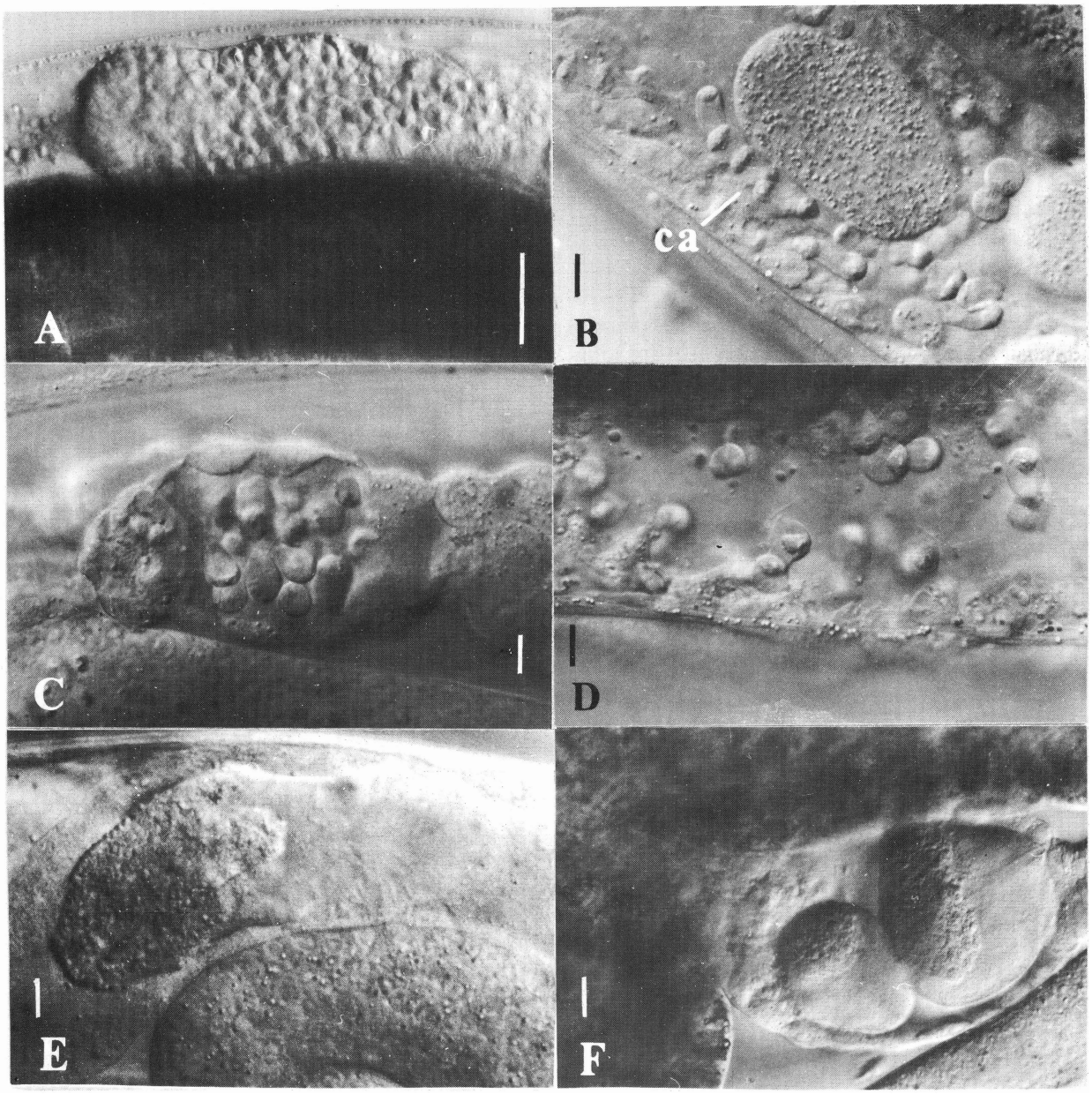

Fig. 3. Different types of amoeboid cells in steinernematid nematodes. A: Appearance of distal part of uterus in a freshly fertilized 1st generation female of *S. feltiae*; B: Chains of amoeboid cells in the uterus of a *S. feltiae*, ca - chains of amoeboid cells; C: Chains of amoeboid cells in the uterus of British isolate B3; D: Chains of amoeboid cells in the uterus of a *S. kraussei*; E: Giant amoeboid cell of *S. karii*; F: Giant amoeboid cells of Sri-Lankan isolate SSL1. Scale bars: A - 50 μm, B-F - 10 μm.

AC is 8-14 μm long, with a posterior end of 6-8 μm diameter (Figs. 1H & 3A, B). No globules on the surface. Waves of constrictions move through the chain surface from the AC with free anterior part to the posterior AC in the chain. No differences were found between the continental strain A1 of *S. feltiae* and the British isolate A2 (Reid *et al.*, 1997). Chains of 4-14 ACs were observed in the uteri of the A2 isolate. Diameter of each AC about 5-7 μm, with a length around 7-12 μm. Conglomerates were not observed.

9. *S. intermedium* (Poinar, 1985) Mamiya, 1988. Separate ACs and chains of up to 14 ACs in length were observed (Fig. 1I). Usually distal parts of the uterus were tightly packed with ACs with approximately 1000-1500 per gonad branch. Posterior end of AC with diameter of 7-8 μm, and length from 12 to 18 μm. Conglomerates were not observed.

10. *S. karii* Waturu, Hunt & Reid, 1997. From 3 to 5 ACs with single large pseudopodium were observed in the uterus of each female (Figs. 1J & 3E). AC length 70-120 μm, diameter of posterior end 40-50 μm. Numerous unmoving 1.5-2 μm globules with refractive particle on posterior end of AC. Two or three streams of flowing cytoplasm can be seen inside pseudopodium.

11. *S. kraussei* (Steiner, 1923) Travassos, 1927. ACs were observed mainly in chains of 3-8 cells, each with 6-9 μm diameter at the posterior end and 8-16 μm in length (Figs. 1K & 3D). Nucleus inside hemispherical part of some ACs has a 1-2 μm long projection protruding along the cell axis. Conglomerates were not observed.

12. *S. longicaudum* Shen & Wang, 1992. Few ACs present, each with a 30 μm diameter of the posterior end (Fig. 1L). Conglomerates of 70-130 x 50-60 μm diameter were also seen in this species. The AC surface bears numerous globules containing refractive bodies inside. Vesicles with globules on the inner surface can be seen inside conglomerates. The posterior part of ACs is filled with rod-like granules.

13. *S. riobrave* Cabanillas, Poinar & Raulston, 1994. Few ACs present, each with a 25-30 μm diameter of the posterior end (Fig. 1M). Globules without refractive particles densely cover the posterior end of ACs. Vesicles with globules on the inner surface can be seen in the cytoplasm. Conglomerates were not observed.

Unidentified steinernematid isolates:

14. **Sri Lankan isolate SSL1.** Only two unequal ACs occupy the distal part of the uterus (Figs. 1N & 3F). Usually, one AC measures about 65 x 50 μm and the other 50 x 30 μm. AC surface covered with very flat globules without refractive bodies inside. Pseudopodia usually compact, looking like small protuberances of a single large pseudopodium. Cytoplasm of AC with small granules measuring 0.5 μm in diameter at the anterior end and small rod-like granules at the posterior end. Conglomerates were not observed.

15. Sri Lankan isolate SSL2. Only 2-5 ACs per uterus were discernible (Fig. 1O). Posterior end of AC 25 μm in diameter; usually with single pseudopodium. Globules with refractive particles cover the posterior part of AC. Conglomerates were not observed.

16. UK isolate C1. ACs united in chains of 3-8 cells were observed in the uteri. Each AC is 13-22 μm long with a diameter of 11-14 μm at the posterior end (Fig. 1P). Sometimes very long (16-17 μm) pseudopodia are formed by the anterior AC in the chain. Conglomerates were not observed.

17. UK isolate B3. Only very short chains of 2-3 ACs were found in the uteri. Each AC is 6-8 μm wide and 10-15 μm long (Figs. 1Q & 3C). Conglomerates were not observed.

18. Japanese isolate MY1. ACs united in chains of 3-12 cells. Each AC is 12-20 μm long, with a 7-9 μm diameter at the posterior end. Pseudopodial movements discernible as waves running over the surface of the entire chain (Fig. 1R). Conglomerates were not observed.

19. Japanese isolate MY5. Several hundred ACs observed in uteri. ACs united in chains of about 10 cells. Each cell characterized by measurements similar to MY1: 10-20 μm long with the posterior end 6-9 μm in diameter (Fig. 1S). Conglomerates were not observed.

DISCUSSION

The amoeboid cells described here can be easily seen in gravid steinernematid females. Only females of the first generation were studied, but examination of *S. carpocapsae, S. feltiae, S. karii* and several other species revealed ACs of the same morphology in females of the second generation as compared to the first generation. First generation females are recommended for study as they have more voluminous gonad tubes, and the AC structure can be easily observed.

Amoeboid cells united in chains were reported for steinernematids by Bovien (1937), who described them as spermatozoa. The morphology of the spermatozoon in the nematode *Caenorhabditis elegans* has been described (Ward *et al.*, 1982) and is qualitatively similar to the morphology of the ACs observed in the present study, lending support to the suggestion that they are also spermatozoa. One end of the *C. elegans* spermatozoon is hemispherical and contains the nucleus and also contains numerous mitochondria when pseudopodia are produced from the opposite end of the cell. The *C. elegans* spermatozoon is 5-6 μm long and 4 μm wide and thus is smaller than any amoeboid cell observed in the uteri of steinernematid females. Apart from their tendency to unite in chains, the amoeboid cells observed in *S. feltiae* most closely resemble the spermatozoa of *C. elegans*. In *S. feltiae,* the number of ACs in the distal part of the uterus is large enough to be compared with the number of oocytes emerging from the oviduct. It also seems that sufficient numbers of these amoeboid cells for fertilization can be found in *S. affine, S. intermedium, S. kraussei,* and in the isolates B3, C1, MY1 and MY5. However, in the steinerne-

matid species with large ACs, the total number of cells is much lower and does not correspond to the number of oocytes emerging from the ovary. It is also not apparent how the amoeboid conglomerates of *S. cubanum* or *S. glaseri* can participate in fertilization, as very large zygotes would have to be formed if the cytoplasm fuses. No such zygotes or eggs were observed in the uteri. Therefore, with several steinernematid species, the function of the ACs described here is not obvious. However, two types of cells can be observed inside the *vas deferens* of males in steinernematids with giant amoeboid cells. Thus, in the *vas deferens* of *S. glaseri* males, large cells with a homogenous content can be found in the lumen (Fig. 2 E,F). Also, more numerous smaller cells containing a refractile particle can be seen in the lumen and these small cells are similar in size to the globules on the surface of *S. glaseri* ACs in the uterus. In other species such as *S. carpocapsae* and *S. ceratophorum,* it can be observed that these smaller cells are produced by bigger cells with a homogenous content in the growth zone of the testis. The smaller cells in some species lose contact with the bigger cell close to the cloacal opening and appear to be injected into the female together with the large cell. Such cells have been seen in a *Steinernema* sp. from Costa Rica (an isolate presumed close to *S. carpocapsae*) in which detached smaller cells produced a proboscis-like agglomeration in front of the row of large, homogenous cells in the *vas deferens*.(Fig. 2G). The small cells from the *vas deferens* resemble the globules on the AC surface in both number and size while the large cells with a homogenous content may become the main body of future ACs.

Despite the unknown function of the amoeboid cells, differences in their shape and behaviour can be used in *Steinernema* taxonomy. Steinernematid species belong to clusters when assessed by molecular taxonomy (Reid *et al.*, 1997) and species within clusters can be characterized by similarities of their amoeboid cells. Thus, the two clustered species *S. glaseri* and *S. cubanum,* are characterized by having ACs of a similar shape (comparing individual ACs), and by the presence of conglomerates with numerous globules flowing over the surface. The ability to produce conglomerates was also noted for *S. longicaudum* which is of unknown taxonomic affiliation. An ability of the ACs to unite as chains was observed for the cluster of species comprising *S. intermedium, S. affine* and *Steinernema* sp. C1, and for *S. kraussei, Steinernema* sp. B3 and *S. feltiae*. The size of individual ACs and the length of chains are similar in the 3 species: *S. affine, S. intermedium* and *Steinernema* sp. C1. Also, *S. kraussei* is nearly identical in its AC structure to the related B3 isolate (Reid, unpublished), but can be distinguished from its nearest relative, *S. feltiae,* by the shorter chains of ACs and larger individual cells.

An investigation of AC morphology should be made with each newly discovered isolate. Large ACs were found in the uterus of the Sri Lankan isolate SSL2 and the morphology of these ACs resembles that of *S. bicornutum* or *S. carpocapsae*. It was found that genetically SSL2 belongs to that cluster of species (Reid *et al.*, 1997). The Japanese isolates MY1 and MY5 are characterized by having chains of ACs, and according to the AC size these undescribed isolates probably belong to the "*feltiae*" or "*kraussei*" group. Such features of AC morphology as the shape of cytoplasmatic inclusions, presence of vesicles in the cytoplasm and mobility of globules on the surface can be used to describe AC diversity in steinernematids, and will probably be useful additional characters for steinernematid taxonomy (Hominick *et al.*, 1997).

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Spiridonov S.E., Hominick W.M., Briscoe B.R. Морфология амебоидных клеток из маток видов *Steinernema* (Rhabditida: Steinernematidae).

Резюме. Дается описание морфологии и поведения амебоидных клеток, обнаруженных в просвете маток гигантских самок 13 валидных (*S. affine, S. arenarium, S. bicornutum, S. carpocapsae, S. ceratophorum, S. cubanum, S. glaseri, S. feltiae, S. intermedium, S. karii, S. kraussei, S. longicaudatum* и *S. riobrave*) и 6 неописанных (изоляты C1 и B3 из Великобритании, SSL1 и SSL2 из Шри-Ланки, MY1 и MY5 из Японии) видов *Steinernema.* По своим морфологическим особенностям строения амебоидные клетки штейнернематид сходны со сперматозоидами нематоды *Caenorhabditis elegans*, что подтверждает предположение о том, что данные клетки являются спермиями. Близкие виды штейнернематид имеют сходное строение и поведение амебоидных клеток.