Notes on the cytogenetics of some South African *Xiphinema* species (Nematoda: Longidoridae)

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The cytogenetics of several South African species of *Xiphinema* were studied and compared with the existing data for this genus from Europe. The process of oogenesis appears to be very similar in all species studied, with little interspecific and intraspecific differences. The ovaries of the South African specimens are larger, with more oogonia and oocytes. The chromosomes are also relatively larger, but could not be counted in oocytes during diakinesis, which usually represents the best stage. Chromosome numbers were therefore determined in the oogonia, which yielded quite accurate and consistent results. The diploid number of 10 seems to be constant in all species studied, but differs from one species of the *X. americanum* group, for which a number of five has been reported. Polyploids were not encountered in any of the South African specimens.

Die sitogenetika van 'n aantal Suid-Afrikaanse spesies van *Xiphinema* is bestudeer en vergelyk met bestaande inligting vir hierdie genus vanuit Europa. Die proses van oögenese stem aansienlik ooreen in al die spesies tans bestudeer, en min interspesie- en intraspesievariasies is waargeneem. Die ovariums van die Suid-Afrikaanse eksemplare is groter, met meer oögoniums en oösiete. Die chromosome is ook relatief groter, maar tellings kon nie in oösiete gedurende diakinese, wat gewoonlik die beste stadium verteenwoordig, gedoen word nie. Chromosoomgetalle is gevolglik in oögoniums bepaal, wat taamlik akkurate en konsekwente resultate gelewer het. Die diploïede-getal van 10 blyk konstant te wees in al die spesies bestudeer, maar verskil van een spesie van die *X. americanum*-groep, waarvoor 'n getal van vyf aangemeld is. Poliploïedie is nie in enige Suid-Afrikaanse eksemplare aangetref nie.

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Although cytogenetic information could be of considerable value in the taxonomy of nematodes, less than 1% of the valid species have so far been studied cytogenetically. The economically important Tylenchida have been investigated more extensively (approximately 90 species), but information for the Dorylaimida is recent and limited to five of the approximately 190 genera (Triantaphyllou 1983). This is mainly due to certain difficulties (temperature and moisture sensitivity and relatively long life cycles) experienced in culturing and rearing members of the Dorylaimida (especially the Longidoridae) under artificial laboratory conditions (Dalmasso 1975). Of the approximately 170 species of *Xiphinema,* only three species viz. *X. diversicaudatum* (Micoletzky, 1927) Thorne, 1939, *X. index* Thorne & Allen, 1950 and *X. mediterraneum* Martelli & Lamberti, 1967 [= *X. pachtaicum* (Tulaganov, 1938) Kirjanova, 1951] have so far been studied to some extent under European conditions (Dalmasso & Younes 1969; Dalmasso & Younes 1970; Dalmasso 1970; Dalmasso 1975), but no information is yet available for any of the species of this genus from South Africa. The main purpose of this study, therefore, was to obtain preliminary cytogenetic information for *Xiphinema* under South African conditions, and to compare this with the data for the abovementioned species.

Material and methods

Fresh material of *X. brevicolle* Lordello & Da Costa, 1961, *X. diversicaudatum, X. index, X. elongatum* Schuurmans, Stekhoven & Theunissen, 1938 and *X. vanderlindei* Heyns, 1962 was extracted from soil and immediately processed for cytogenetic study according to a method developed for root-knot nematodes (Triantaphyllou 1985). The ovaries were studied and photographed with a Zeiss standard 18 microscope equipped with a photomicrographic camera. Chromosome numbers were determined by means of camera lucida drawings.

No results could be obtained for *X. brevicolle,* owing to the presence of numerous bacteria in the ovaries of this species, which prevented clear observation of the oogonia and oocytes (Figure 2A).

Results

No fundamental cytogenetic differences were noticed among species, and therefore the description below applies to all of the species studied (see Figure 1).

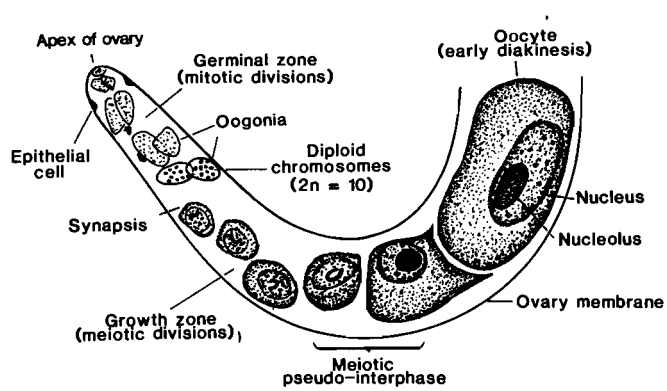

Figure 1 Schematic illustration of the process of oogenesis in the ovary of *Xiphinema*.

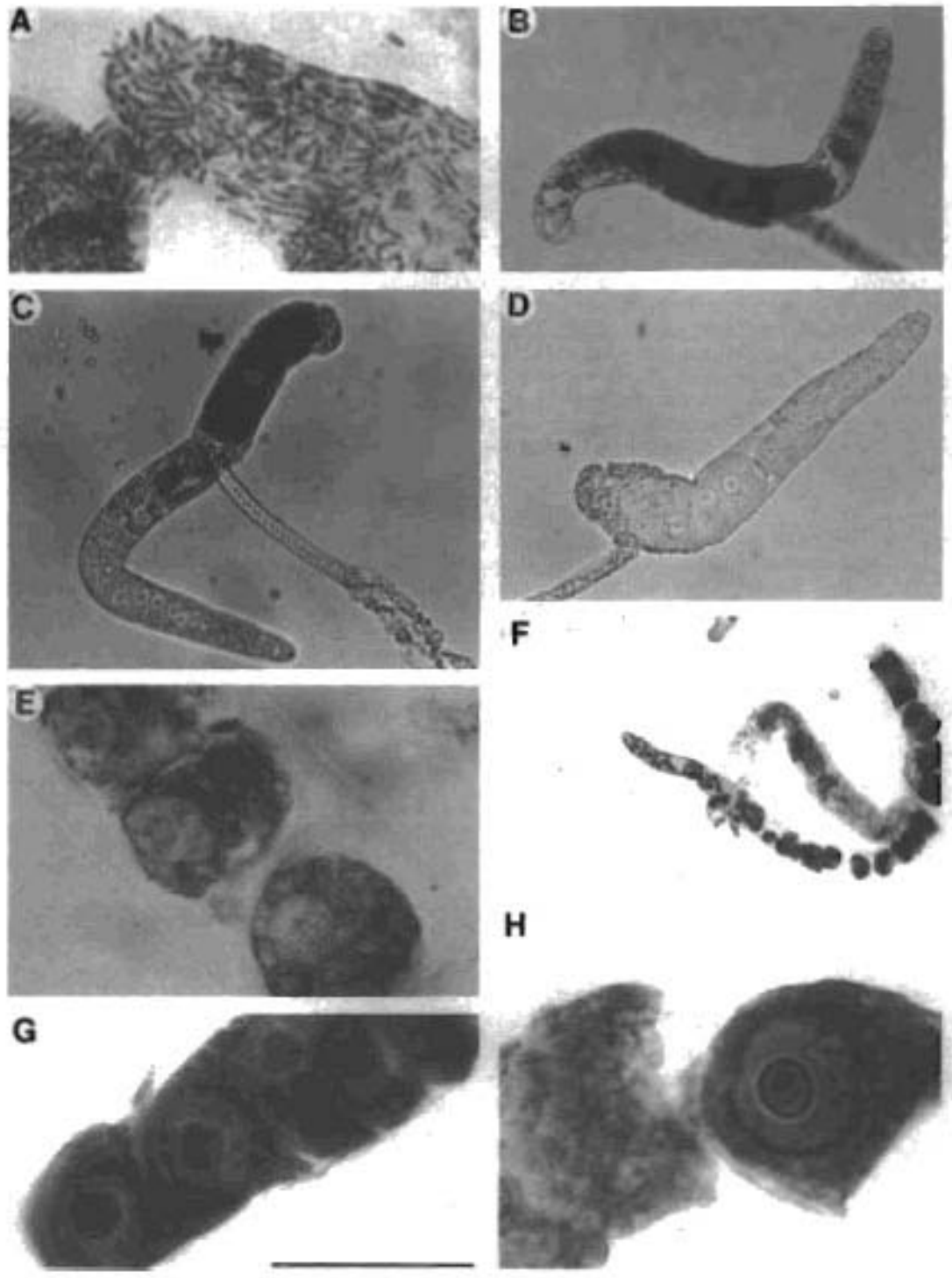

Figure 2 (A) Numerous bacteria in the ovary of *X. brevicolle*. (B–D) Ovary of *X. vanderlindei* (B), *X. index* (C) and *X. diversicaudatum* (D). (E) *X. elongatum* oocytes. (F) *X. index* ovary. (G–H) *X. index* oocytes. [Material dissected in saline (B–D); fixed and stained material (A, E–H). Bar length: A, E, G–H (20 μm); B–D (90 μm); F (150 μm).]

The germinal zone occupies from 15 to 20% of the relatively large ovary (Figure 2D, F), which is enveloped with a membrane (Figure 1E–G). Oogonia (30–45 in number) are arranged in two to four rows in the germinal zone (Figures 2D, F & 3G, H). Owing to their relatively large size, oocytes (12–15 in number) are mostly arranged in a single row in the growth zone (Figure 2D–G), and seem to be interlocked with each other (Figure 2G–H). They exhibit slight interspecific, and sometimes even intraspecific variation in shape (*cf.* Figure 2H with Figure 3A).

Oocytes in early to late diakinesis are present in the blind sac of the ovaries of *X. vanderlindei* (Figure 2B) and *X. index* (Figure 2C). However, no primary oocytes were seen in the ovaries of several specimens of *X. diversicaudatum* (Figure 2D). Conspicuous nucleoli with thick membranes were observed in the nuclei of oocytes during the pseudo-interphase (i.e. between synapsis and diakinesis) (Figures 1, 2G–H & 3A–B). Nuclei membranes and chromosomes were usually difficult to

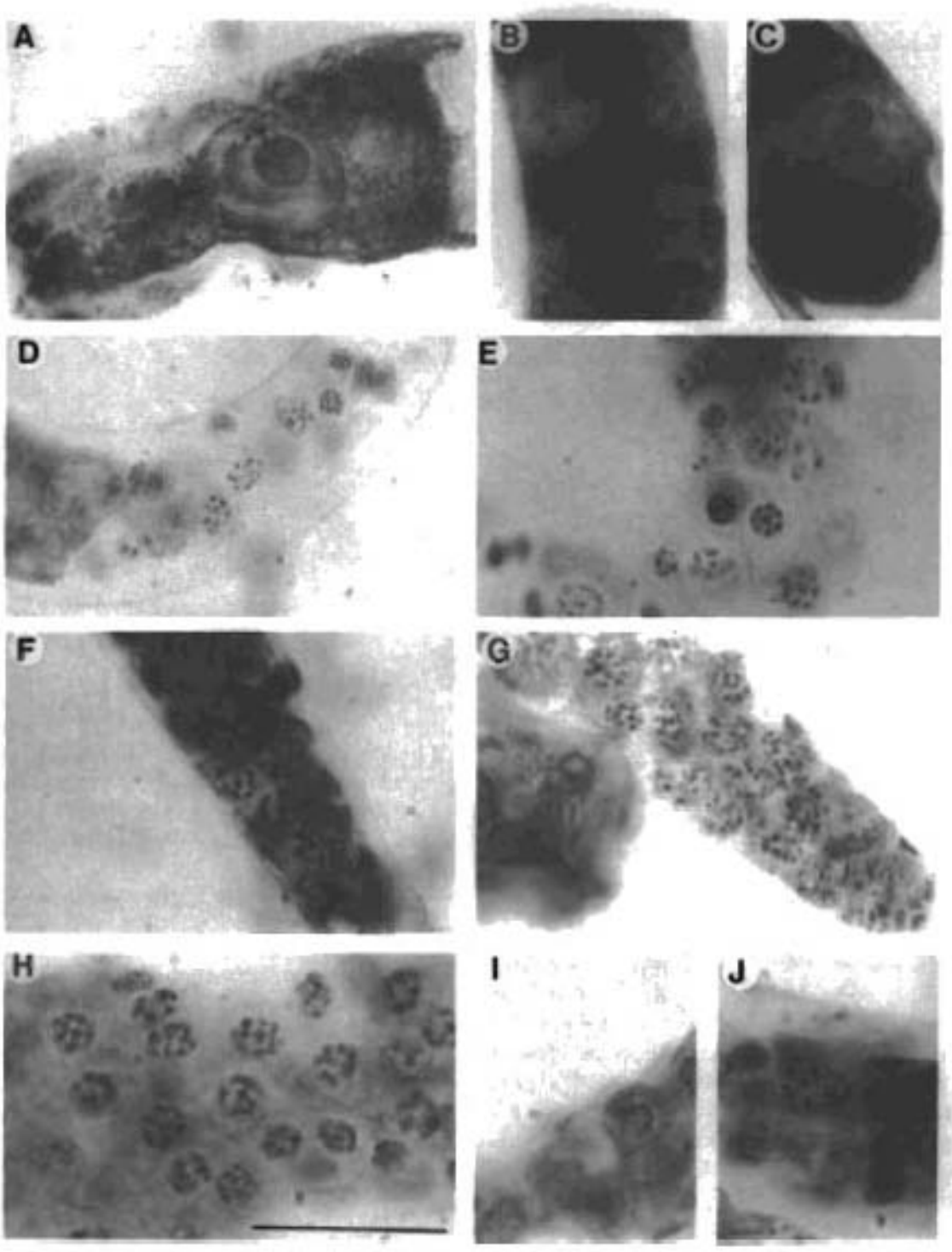

Figure 3 (A–C) Oocytes of *X. diversicaudatum* (A), *X. vanderlindei* (B) and *X. index* (C). (D–J) Oogonial chromosomes of *X. elongatum* (D–E), *X. index* (F–G), *X. diversicaudatum* (H–I) and *X. vanderlindei* (J). [Fixed and stained material (A–J). Bar length: A–C, E, H, J (20 µm); D, F, G, I (30 µm).]

distinguish during this phase, and in some specimens (viz. *X. index*), multiple nucleoli were present in the same nucleus (Figure 3C). Both the nuclei and the nucleoli differ somewhat in size between the different species (Table 1).

The chromosomes appear quite uniform in all the species and vary from round to elongate in shape (Figure 3D–J). They are usually randomly dispersed in the nuclei of the oogonia and oocytes (Figures 2E & 3G), but are sometimes arranged in a crescent (Figure 3I), and at the periphery of the oogonia (Figure 3E). Chromosomes were not clearly distinguishable in the oocytes (see Figures 2E, G–H & 3A–C), and therefore reliable chromosome numbers could not be determined during diakinesis. However, 10 chromosomes (2n = 10), varying from 0,7 to 0,8 µm in diameter (except in *X. diversicaudatum*), were counted during oogonial metaphase in all the *Xiphinema* species studied (Figure 3D–J).

Table 1 Comparative cytogenetics of some *Xiphinema* species from South Africa and Europe

Species	Germinal zone length	Oogonia number	Oocyte number	Oocyte nucleus diameter (µm)	Oocyte nucleolus diameter (µm)	Chromosome diameter (µm)	Chromosome number
X. diversicaudatum [a]	Relatively short (10% of ovary)	10 - 30	10 – 12	–	–	< 0,5	10 bivalents during diakinesis
X. diversicaudatum [b]	Relatively long (15 – 20% of ovary)	30 – 45	12 – 15	12 – 16	5 – 8	< 0,7	2n = 10 during oogonial metaphase
X. index [a]	Relatively short (10% of ovary)	10 – 30	10 – 12	–	–	0,5	10 bivalents during diakinesis [n = 20 (tetraploid)]
X. index [b]	Relatively long (15 – 20% of ovary)	30 – 45	12 – 15	17 – 30	6 – 8	0,7 – 0,8	2n = 10 during oogonial metaphase
X. elongatum [b]	Relatively long (15 – 20% of ovary)	30 – 45	12 – 15	10 – 25	4 – 6	0,7 – 0,8	2n = 10 during oogonial metaphase
X. vanderlindei [b]	Relatively long (15 – 20% of ovary)	30 – 45	12 – 15	12 – 20	5 – 7	0,7 – 0,8	2n = 10 during oogonial metaphase
X. mediterraneum [a] (= *X. pachtaicum*)	10 – 20 µm	10 – 30	3 – 5	–	–	–	5 bivalents during diakinesis [n = 10 (diploid)]

[a] Specimens from Europe (see Dalmasso 1975); [b] specimens from South Africa.

Discussion and conclusions

Cytogenetic information obtained during this study for the endemic species *X. vanderlindei,* as well as South African specimens of *X. elongatum, X. diversicaudatum* and *X. index,* largely agrees with the existing data for this genus from Europe. Little interspecific variation was noticed in the South African species, except for some differences in the size of the oocyte nucleus and nucleolus. However, a comparison of the South Africa species with those from Europe, revealed some differences that need to be pointed out (Table 1).

The germinal zone of the South African species seems to occupy a greater portion (15–20% versus 10%) of the relatively larger ovaries, and a greater number of oogonia (30–45 versus 10–30) and oocytes (12–15 versus 10–12) were observed in the ovaries of these species. This might suggest the existence of more favourable environmental conditions (i.e. food, moisture, temperature, etc.) at the time of sampling, which would result in a higher fecundity in the South African species. Primary oocytes were absent in the ovaries of some species studied (viz. *X. diversicaudatum* – Figure 2D). This was probably caused by the termination of the meiotic processes during oogenesis, a phenomenon usually induced in the longidorids by the absence of an adequate food source (viz. host plants).

Diakinesis and the meiotic anaphase usually represent the best stage for the determination of chromosome numbers in nematodes (Triantaphyllou 1979). However, oocytes often fail to take up stain for quite some time just before diakinesis, owing to the presence of large nuclei and nucleoli (Triantaphyllou 1971). This phenomenon could explain why chromosome numbers could not be determined in the oocytes of the species studied. Furthermore, the multiple nucleoli observed in the nuclei of some specimens of *X. index,* often occur in nematodes and represent the start of a growth period (i.e. late pachytene or early diplotene) (Triantaphyllou 1971).

The 10 chromosomes counted in the oogonia of the South African species, correspond with the results obtained for all the European species of this genus, except *X. mediterraneum* (Dalmasso & Younes 1970) (Table 1). However, this differs from the basic haploid chromosome number of six reported for *Xiphinema* (Triantaphyllou 1983), but corresponds with the number of five determined by Dalmasso & Younes (1970) for this genus. The tetraploids (n = 20) found by Dalmasso & Younes (1969) in some populations of *X. index,* were not observed in any specimens of *X. index* from South Africa.

The chromosomes of *X. vanderlindei* and South African specimens of *X. elongatum* and *X. index* are somewhat larger (0,7–0,8 µm in diameter) than those in their European counterparts (≤ 0,5 µm in diameter). This could possibly be explained by the difficulty normally encountered when measuring the exceptionally small chromosomes of nematodes, or else to the use of propionic orcein as a stain during this study, which has previously been found to induce slight chromosome swelling (Dyer 1979; Triantaphyllou 1979). However, this did not apply in the case of *X. diversicaudatum,* where our measurements agree with those from Europe. Feulgen stain was used in this study, but initially did not give satisfactory results, contrary to those of Dalmasso & Younes (1969). This was the reason for the eventual use of propionic orcein (which gave better results) as a chromosome stain in the present study.

Acknowledgements

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