

# Morphology of the reproductive system and structure of sperm cells in some species of the genus *Paratrichodoros* with uncommon males (Nematoda : Diphtherophorina)

Wilfrieda DECRAEMER and Eliseo CHAVES

Koninklijk Belgisch Instituut voor Natuurwetenschappen, Vautierstraat 29, 1040 Brussel, Belgium and Instituto Nacional de Tecnologia Agropecuaria, Casilla Correo 276, 7620 Balcarce, Argentina.

## SUMMARY

In *Paratrichodoros minor* and in other trichodorid species where males are rare, the reproductive system was studied with special attention to the structure of sperm cells. In *P. minor* both spermatids or sperms can be found in the uterus of females. In other species the sperm cells vary in shape from filliform with small nucleus (*P. teres*), to small cells with minute globular nucleus (*P. nanus*), and to elongated sperm cells with well developed sausage-shaped nucleus (*P. porosus*).

## RÉSUMÉ

Morphologie du système génital et structure des spermatozoïdes chez certaines espèces à mâles peu fréquents du genre *Paratrichodoros*

Les auteurs ont étudié le système reproducteur de *Paratrichodoros minor* et d'autres Trichodorides dont les mâles sont rares, en portant attention à la structure des spermatozoïdes. Chez *P. minor*, spermatides et spermatozoïdes sont présents dans l'utérus des femelles. Chez les autres espèces, les spermatozoïdes, soit ressemblent à des cellules filiformes à petit noyau (*P. teres*), soit ont une petite taille et un minuscule noyau globulaire (*P. nanus*), soit sont allongés, bien développés, avec un noyau bacilliforme (*P. porosus*).

In *Paratrichodoros minor* (Colbran, 1956) Siddiqi, 1974 (= *Trichodoros christiei* Allen, 1957), males are usually rare. Bird and Mai (1968) observed only twelve males among thousands of females; Chaves (1984), however, found a population from Santa Fe with a ratio of males to females of 1:3.

*P. minor* is considered to reproduce parthenogenetically (Rohde & Jenkins, 1957). In species that are predominantly parthenogenetic an adverse environment can increase the frequency of the normally rare males (Poinar & Hansen, 1983). Morphometric and allometric characters of *P. minor* are not only influenced by its host species but also by the geographical origin of a population (Bird & Mai, 1968). Evidence for physiological races was also supplied by Ayala, Allen and Noffsinger (1970).

In their overall review on sexual reproductive modifications in nematodes Poinar and Hansen (1983) summarized that the mechanism of the origine of the occasional male among parthenogens is not clear.

Nothing is known about the cytology of the rare males that occur among parthenogenetic populations in Trichodoridae. Since the structure of sperm cells can be an additional diagnostic feature in the identification, es-

pecially of closely related species, we studied the reproductive system with special attention to the presence and structure of sperm cells in those trichodorid species where males are rare: *P. teres* (Hooper, 1962) Siddiqi, 1974, *P. allius* (Jensen, 1963) Siddiqi, 1974, *P. porosus* (Allen, 1957) Siddiqi, 1974, *P. nanus* (Allen, 1957) Siddiqi, 1974. Also species where, at present, males were not found, are included in this investigation: *P. acutus* (Bird, 1967) Siddiqi, 1974, *P. tansaniensis* Siddiqi, 1974, *P. renifer* Siddiqi, 1974.

## Material and methods

*P. minor* was found in the following samples from the province Santa Fe, Argentina, all from fields with egg plants and collected by E. Chaves: no. 25: Helvecia (1 fem.); no. 26: Helvecia (11 fem., 9 mal., 2 juv.); no. 27: Santa Rosa de Calchines (1 fem., 1 mal.); no. 28: Campo del Medio (20 fem., 1 mal., 2 juv.); no. 29: Cayasta (4 fem., 2 mal.).

Specimens were mounted in anhydrous glycerine on Cobb slides. They were studied under a light microscope Reichert Polyvar, using Nomarski differential interference contrast.

*Nominal species studied from other collections :*

— *P. porosus* : 4 males paratype, slide UCNC 3 a; 7 mal., 5 fem. paratype slide UCNC 1802; 17 fem. UCNC slides 3g1, 3m, 3k from University of California Nematode Collection, Davis (UCNC). 5 fem., 1 mal., 2 juv. slides 12540, 12545 from Navorsingsinstituut vir Plantbeskerming, Pretoria. 9 fem., 3 juv. from University of Tennessee Nematode Collection.

— *P. minor* : 4 fem. paratype (*T. christiei*) slide UCNC 41; 30 fem., 1 mal. slides UCNC 5k, 51, A, B, C. 6 fem., 8 juv. paratype. 2 mal. from Nematode collection of the Agricultural Research Laboratories, Indooroopilly, Queensland, Australia. 2 fem., 3 juv. from the University of Florida, Nematode collection, Gainesville. 7 fem., 1 mal., 2 juv. slides 13680, 17938 from Navorsingsinstituut vir Plantbeskerming, Pretoria.

— *P. tansaniensis* : 1 fem. paratype WT 1772-1773. 17 mal. slides 191 A/7/12, 13, 23, 24, 27, 28 from Nematode collection Rothamsted Experimental Station, Harpenden, England.

— *P. renifer* : 5 fem. paratype slide WT 1774-1776 from Nematode collection of Wageningen, The Netherlands (WT). 2 fem. paratype slide UCNC 1404. 24 fem., 1 juv. slide DNST 3/191, 3, 4, 6 from Nematode collection Biologische Bundesanstalt für Land und Forstwirtschaft, Münster (DNST).

— *P. tansaniensis* : 1 fem. paratype WT 1772-1773. 4 fem. slide 3477 from Nematode collection Pretoria.

— *P. allius* : 1 mal., 9 fem., slide UCNC 860; 16 fem. slides UCNC 16 a, b, c.

— *P. nanus* : 1 fem. paratype slide UCNC 10 a; 1 fem., UCNC 10 g. 1 fem. slide WT 521.

— *P. acutus* : 4 fem. paratype slide T 524 p; 8 fem. slide T 525 p from Nematode Collection Beltsville. 4 fem., 3 juv. slide 3/13/1 DNST.

**Results**

*PARATRICHODORUS MINOR* (Figs 1 & 2)

Of the five samples from different localities in Santa Fe, four had populations with males and females. Apart from some minor morphometric variability, no morphological differences were found in and among the populations, except for the reproductive system. In two (nos. 26, 29) out of four samples with bisexual populations, we observed in each two groups of males and females based upon the structure of the sperm cells, present respectively in the *vesicula seminalis* and in the uterus.

*Group 1*

Males and females with globular sperm cells (spermatids) with a 1.5-2 µm nucleus (Figs 1 A-B, 2 A-D).

*Males* : The testis is monorchic, the germ or spermatogonial cells are produced from a single apical cell. The germinal zone and growth zone are rather short and are followed by the *vesicula seminalis*. The latter is filled with small globular sperm cells with distinct refractive nucleus (spermatids) (Fig. 2 A-B, arrows). This zone is followed by several (glandular) zones of the *vas deferens* : an anterior zone with small cells with a minute refractive nucleus (Fig. 1 Aa), followed by slightly larger cells with a small nucleus (Fig. 1 Ab), continuing in a more translucent zone with slightly turgescient cells (Fig. 1 Ac) and followed by a more or less finely granular, slender zone, forming the posterior half of the genital system (Fig. 1 Ad). The different zones of the genital system could be distinguished in several specimens.

*Females* : The same globular spermatids found in the *vesicula seminalis* of the male were found throughout the uterus (Figs 1 B; 2 C-D).

*Remark* : The globular spermatids were also observed in male and female specimens from Tavango, South Africa.

*Group 2.*

Males and females with thread-like sperms (Figs 2 E-F; 1 C-E).

*Males* : Testis as in group 1. *Vesicula seminalis* filled with small (3 µm) thread-like sperm cells. This zone is followed by the *vas deferens*, more or less similarly differentiated as in the above described specimens. In one specimen a few globular spermatids could be observed just anterior to the thread-like sperms (Fig. 1 E).

*Females* : The thread-like sperms were found throughout the uterus. No mixture of both types of sperm cells (globular or thread-like) was ever found in the uterus (Figs 1 C; 2 D).

*Remark* : Apparently thread-like sperms in females of *P. minor* were drawn by Roca and Lamberti (1984) and Vermeulen and Heyns (1983) who described them as unknown structures in the uterus.

OTHER *PARATRICHODORUS* SPECIES

— In specimens of *P. renifer* from several localities in Germany and in type specimens only three females possessed minute more or less thread-like structures in the uterus, however they cannot be identified for certain as sperms. More similar thread-like structures as in *P. minor* were observed in the uterus of females of *P. acutus* from Madeira, not in the type specimens. In *P. tansaniensis* small globular sperms were observed in the uterus (Fig. 1 I).

— Type specimens of *P. allius* and other specimens collected by Jensen from the type locality were rather poor internally. No sperm could be observed in the

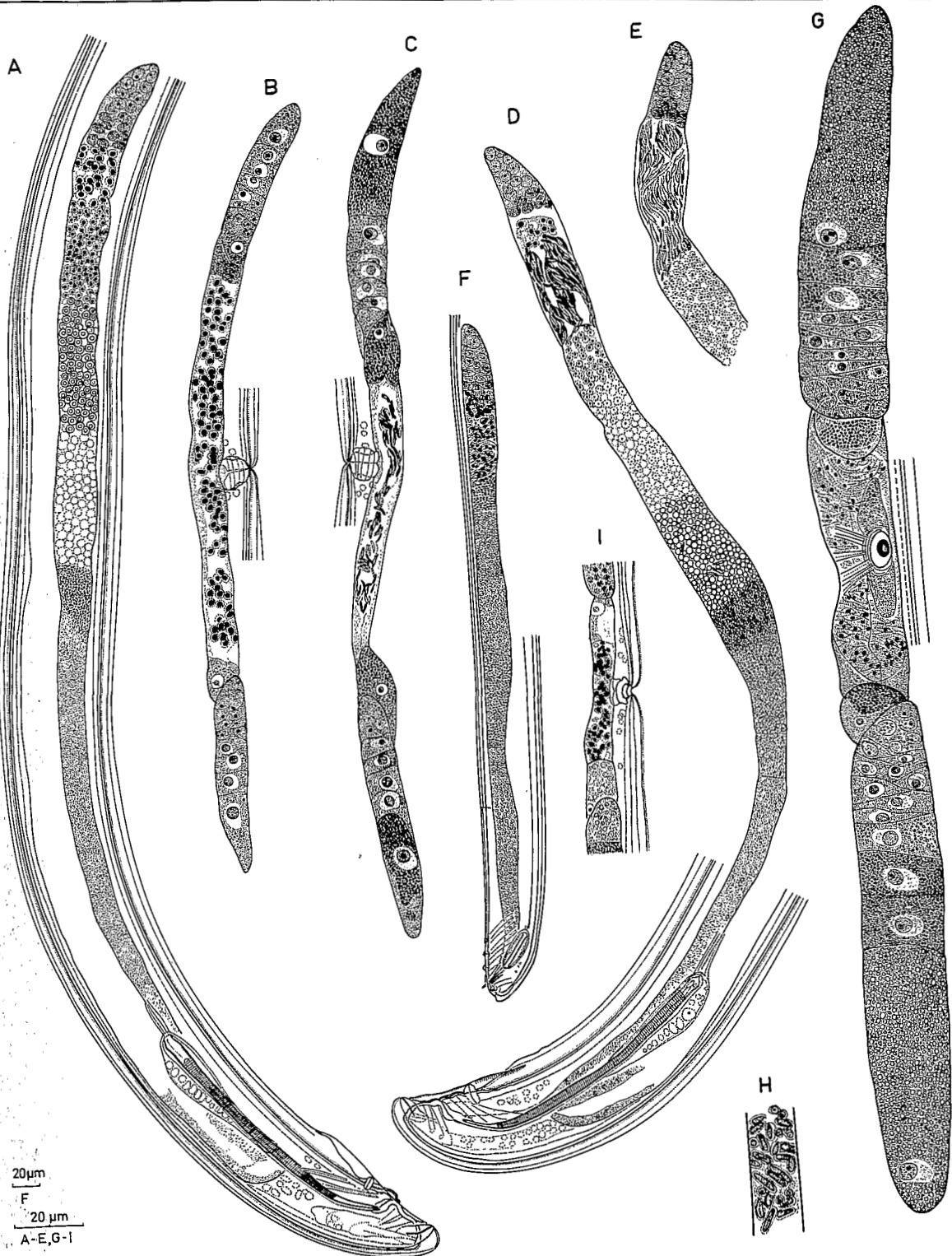


Fig. 1. *Paratrichodorus minor*. A : Male reproductive system and tail region, group 1; B : Female reproductive system, group 1; C : Female reproductive system, group 2; D : Male reproductive system, group 2; E : Male reproductive system, anterior part, group 2. — *P. teres*. F : Male reproductive system and tail region; G : Female reproductive system. — *P. porosus*. H : Sperm cells in *vas deferens*. — *P. tansaniensis*. I : Female reproductive system, uterus region.

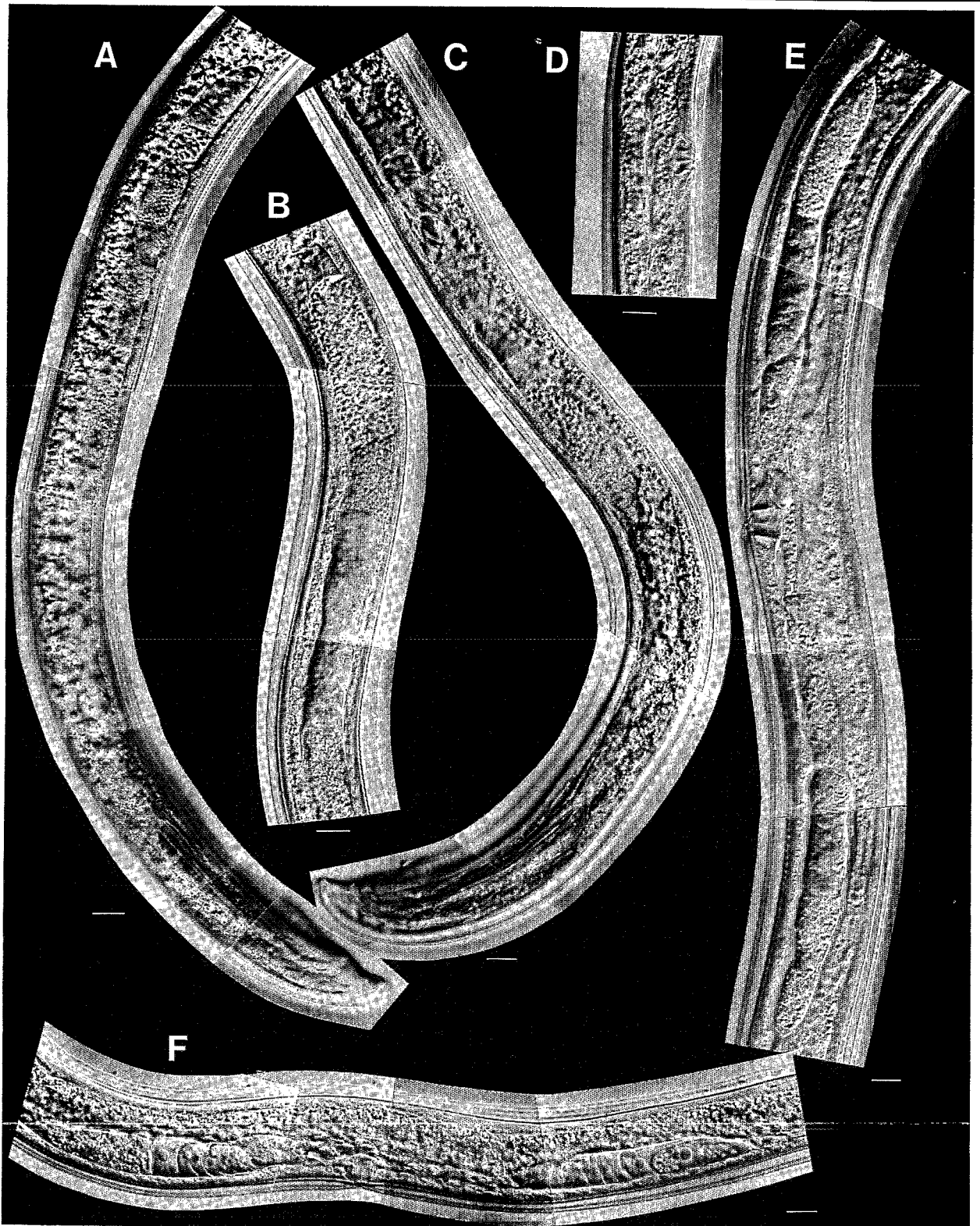


Fig. 2. *Paratrichodorus minor*, group 1. A : Male reproductive system and tail; B : Anterior part of male reproductive system in another specimen; C : Female reproductive system; D : Part of female reproductive system, uterus region in another specimen. — *P. minor*, group 2; E : Female reproductive system; F : Male reproductive system and tail (bars = 10  $\mu$ m).

uterus of female specimens. The only male specimens available apparently possesses small sperm cells with a small globular nucleus; the latter were found in the uterus of a female specimen (see Fig. 16 of original description).

— In *P. porosus* males are rare; they are only known from the type locality. Sperm cells are well developed, elongated structures 3.5 by 10 µm with a 8.5 µm long sausage-shaped nucleus (Fig. 1 H); the sperms were only detected in paratype females.

— A study of paratype females of *P. nanus* revealed the presence in the uterus of small sperms with minute globular nucleus, although no males were found at the type locality. However, males are known from a population from vine soils in Tunisia; they had a female : male ratio of 4:1 (Siddiqi, 1963).

— In *P. teres*, males are rare. Kuiper and Loof (1961) found only seventeen males along several thousands of females. However, populations exists where males occur in large numbers. The reproductive system in *P. teres* males has a short germinal zone and a short growth zone, followed by the *vesicula seminalis* filled with small threath-shaped sperm cells with small rounded nucleus (Fig. 1 F). In some female specimens the small sperm cells can be found throughout the uterus (Fig. 1 G).

## Discussion

In *P. minor* two stages of sperm cells (spermatids and sperms) can be found in the uterus of females. Since, apart from a single male specimen we never observed a mixture of both types of sperm cells, we presume that spermiogenesis must occur as a whole and suddenly, and that this process can take place as well in the *vesicula seminalis* of the male as in the uterus of the female.

Although reproduction in *P. minor* is considered parthenogenetic (Rohde & Jenkins, 1957), treath-like sperms can be found in the uterus of females from populations where males are rare or apparently absent as in some paratype specimens from Florida, females from Daretow NSW, specimens from different localities in Australia and Florida. These sperm cells are apparently non-fertile.

A possible explanation for the insemination of globular spermatids in females from populations from Santa Fe and Tavango, South Africa might be that this phenomenon only occurs in populations where males are more numerous. However, here too no evidence of fertilization could be found.

Loof (1965) found that *P. teres* reproduces parthenogenetically and he considered the male specimens as "Residualmännchen". Loof (1965, 1975) considered the testis in *P. teres* as degenerate and without sperm. However, we believe the testis not to be degenerate; the *vas deferens* contains smaller, narrow elongated sperm

cells with threathlike appearance when packed but with a distinct small nucleus. They are different from the usually well developed sperm cells in other trichodorids, and are apparently non-functional. They seem only to occur in species which rarely have males.

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## REFERENCES

- AYALA, A., ALLEN, M. W. & NOFFSINGER, E. M. (1970). Host range, biology, and factors affecting survival and reproduction of the stubby root nematode. *J. Agric. Univ. P. Rico*, 64 : 341-369.
- BIRD, G. W. & MAI, W. F. (1965). Plant species in relation to morphometric variations of the New York population of *Trichodorus christiei*. *Nematologica*, 11 : 34 [Abstr.].
- BIRD, G. W. & MAI, W. F. (1968). Morphometric and allometric variations of *Trichodorus christiei*. *Nematologica* (1967), 13 : 617-632.
- CHAVES, E. (1984). *Observations on plant parasitic nematodes from Argentina*. Ph. D. Dissert., Rijksuniv. Gent, 106 p.
- KUIPER, K. & LOOF, P. A. A. (1962). *Trichodorus flevensis* n. sp. (Nematoda : Enoplida), a plant nematode from a new polder soil. *Versl. PI Ziekt. Dienst Wageningen*, 136 : 193-200.
- LOOF, P. A. A. (1965). *Trichodorus anemones* n. sp. with a note on *T. teres* Hooper, 1962. *Versl. PI Ziekt. Dienst Wageningen (Jaarboek 1964)*, 142 : 132-136.
- LOOF, P. A. A. (1975). Taxonomy of Trichodoridae. In : Lamberti, F., Taylor, C.E. & Seinhorst, J. W. (Eds). *Nematode Vectors of Plant Viruses*. London, Plenum Press : 103-127.
- POINAR, G. O. & HANSEN, E. (1983). Sex and reproductive modifications in nematodes. *Helminthol. Abstr., Ser. B*, 52 : 145-163.
- ROCA, F. & LAMBERTI, F. (1984). Trichodorids (Nematoda) from Italy. *Nematol. medit.*, 12, 95-118.
- ROHDE, R. A. & JENKINS, W. R. (1957). Host range of a species of *Trichodorus* and its host-parasite relationships on tomato. *Phytopathology*, 47 : 295-298.
- SIDDIQI, M. R. (1963). *Trichodorus* spp. (Nematoda : Trichodoridae) from Tunisia and Nicaragua. *Nematologica*, 9 : 69-75.
- VERMEULEN, W. J. J. & HEYNS, J. (1983). Studies on Trichodoridae (Nematoda : Dorylaimida) from South Africa. *Phytophylactica*, 15 : 17-34.

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