

Terrestrial nematodes of the Galápagos archipelago. 6. *Punchaulus gemellensis*, a new genus and species of Aphelenchina (Tylenchida)

Paul DE LEY and August COOMANS

Instituut voor Dierkunde, Universiteit Gent, Ledeganckstraat 35, 9000 Gent, Belgium.

Accepted for publication 28 February 1995.

Summary – *Punchaulus gemellensis* gen. n., sp. n. is described from Los Gemelos, Isla Santa Cruz, Galápagos, Ecuador. It is characterized by short and plump body ($L = 300 - 404 \mu\text{m}$, $a = 14-19$); large excretory pore located near the base of the procorpus; nearly spherical median bulb; a non-muscular, $19-22 \mu\text{m}$ long, isthmus; monodelphic female reproductive system without post-vulval sac; spicules with well-developed rostrum and ventral limb extending beyond dorsal limb; and conical tail ending in a truncate, finely fimbriated stub.

Résumé – *Nématodes terrestres de l'archipel des Galapagos. 6. Punchaulus gemellensis, nouveau genre et nouvelle espèce d'Aphelenchina (Tylenchida)* – *Punchaulus gemellensis* n. gen., n. sp. provenant de Los Gemelos, Ile de Santa Cruz, Galapagos, Équateur, est décrit. Il est caractérisé par un corps court et trapu ($L = 300-404 \mu\text{m}$; $a = 14-19$), un pore excréteur large et localisé au niveau de la base du procorpus, un bulbe médian presque sphérique, un isthme non musculéux long de $19-22 \mu\text{m}$, un système reproducteur femelle monodelphe sans sac postvulvaire, des spicules à rostre prononcé et à limbe ventral plus long que le limbe dorsal, une queue conique à terminus tronqué et finement fimbrié.

Key-words : Galápagos, Nematoda, *Punchaulus* n. gen., SEM, taxonomy.

This paper proposes a new genus and species of Aphelenchina, which combines characters of several known families. It was found in two soil samples from the Galápagos Islands collected on February 12, 1988 (see De Ley *et al.*, 1993 for full list of sampled sites). One female and three juveniles of the new genus were submitted to critical point drying and sputter-coated for observation with a JEOL LSM-840 scanning electron microscope (SEM). Four females and four males of *Schistonchus caprifici* (Gasparini, 1864) Fuchs, 1937 were also examined with SEM to compare labial morphology.

Punchaulus * gen. n.

DIAGNOSIS

Aphelenchina : Aphelenchoididae? Body small and plump. Lip region low, cap-like, without annuli but with radial striae and a small labial disc. Stylet with rounded basal knobs. Excretory pore opposite base of procorpus or at junction of procorpus and metacarpus. Pharynx with gland bulb overlapping intestine and a true non-muscular isthmus about $20 \mu\text{m}$ long. Vulva at almost three-quarters of body. Postvulval sac rudimentary. No gubernaculum. Spicules with well-developed rostrum

and with ventral limb extending beyond dorsal limb. Tail in both sexes conical, (almost) straight, with a truncate tip carrying a fimbriated coronet.

TYPE AND ONLY SPECIES

Punchaulus gemellensis * gen. n., sp. n.

DIFFERENTIAL DIAGNOSIS

The new genus shares the presence of a distinct pharyngeal isthmus with *Aphelenchus* (Aphelenchidae) and *Paraphelenchus* (Paraphelenchidae), but differs from these two genera mainly in the absence of a bursa and presence of thorn-shaped spicules. The latter two characters are typically found in Aphelenchoididae, but as far as we could determine, *P. gemellensis* differs from all genera in this family in its $19-22 \mu\text{m}$ long isthmus. It must be noted, however, that many published descriptions and drawings do not resolve the structure of the posterior pharynx clearly, and that the term « isthmus » is sometimes used differently.

Thus, the long « isthmus » described in *Tylaphelenchus yamani* Raski & Valenzuela, 1988 actually consists mostly of the very narrow anterior end of the intestine, and only for a very short part of truly pharyngeal tissue (cf. Fig. 4 A, G in Raski and Valenzuela, 1988; con-

* Derived from « Punchau », the name of the main Inca sun idol, in reference to the highly refringent, spherical median bulb of the new genus.

* The specific epithet is a latinized adjective referring to Los Gemelos, the twin craters near which the species was found.

firmed by study of two paratypes). Similarly, *Schistonchus caprifici* (Gasparrini, 1864) Fuchs, 1937 has a rudimentary pharyngeal isthmus that hardly separates the median bulb from the narrow intestinal lumen (see Fig. 15 B, C in Thorne, 1961), as confirmed by our observations on specimens kindly provided by Dr. Vovlas (and contrary to Fig. 5 B, C in Vovlas *et al.*, 1992).

Apart from the isthmus, our new genus differs from all known Aphelenchoididae in one or more other respects. In the genus groups distinguished and diagnosed by Baujard (1988), *Punchaulus* is closest to *Laimaphelenchus* Fuchs, 1937 and *Tylaphelenchus* Rühm, 1956. It differs from all *Laimaphelenchus* species reviewed by Hirling (1982) in that these have more slender body ($a = 22-25$), excretory pore posterior to the metacarpus, spicules with proportionately less developed rostrum and more developed dorsal limb, and tail tip with either four fimbriated caudal stubs or three to four cuticular spikelets.

Our specimens resemble *Tylaphelenchus yamani* in having a lip region with radial striae instead of annulation (cf. Fig. 5 A, D, G in Raski & Valenzuela, 1988). A similar lip morphology was found by Hooper and Clark (1980) in *Aphelenchoides helicosoma* Maslen, 1979, a species that probably belongs in *Tylaphelenchus*. However, unlike our specimens both species have the high lip region typical of *Tylaphelenchus* as well as a prominent labial disc.

Absence of annuli and presence of radial striae on the lip region is also characteristic of *Schistonchus* species (Fig. 3; Lloyd & Davies, unpubl.), but again these differ from *P. gemellensis* in having a much more prominent labial disc. *Schistonchus* is furthermore characterized by a long stylet (16-28 μm) with proportionately long and strongly sclerotized conus (cf. Thorne, 1961; Hunt, 1993; Lloyd & Davies, unpubl.).

According to the reviews of aphelenchoid genera by Nickle (1970) and Nickle and Hooper (1991), the spicules of our species are similar to those of *Parasitaphelenchus* Fuchs, 1929, *Rhadinaphelenchus* Goodey, 1960 and *Huntaphelenchoides* Nickle, 1970 in their relatively short dorsal limb. The usefulness of spicule shape in generic diagnoses of Aphelenchoididae is doubtful, as illustrated by the variation in shapes found in *Bursaphelenchus* (cf. Fig. 13 in Yin *et al.*, 1988). Nevertheless, for the sake of completeness we must differentiate our genus from the three mentioned above.

Free-living adults of *Parasitaphelenchus* do not agree with our species in having a long, slender body. Reviewing species descriptions we found $L = 660-1170 \mu\text{m}$, $a = 41-75$), a more posterior vulva ($V = 85-90\%$), a well-developed postvulval uterine sac, an acute or subacute tail tip or tail mucro, and males with terminal bursa. The only known species of *Rhadinaphelenchus*, *R. cocophilus* (Cobb, 1919) Goodey, 1960, is quite unlike our animals in many respects. Thus, the labial region is annulated and high with complex labial disc

(Giblin-Davis *et al.*, 1989), the body is long and slender ($L = 0.84-1.18 \text{ mm}$, $a = 71-179$) and the postvulval uterine sac is very long (Goodey, 1960). The type species of *Huntaphelenchoides*, *H. fungivorus* Franklin & Hooper, 1962, differs from our species in having a larger and more slender body ($L = 0.57-1.16 \text{ mm}$, $a = 28-45$), an excretory pore posterior to the metacarpus, a well-developed postvulval uterine sac, a subacute male tail with a small bursa and a narrowly rounded, elongated female tail. Both *Huntaphelenchoides* and *Rhadinaphelenchus* were synonymized with *Bursaphelenchus* Fuchs, 1937 by Giblin and Kaya (1983) and Baujard (1989), respectively.

Five other genera in Nickle (1970) resemble our genus in having a very prominent rostrum on the spicules: *Cryptaphelenchus* Fuchs, 1937, *Paraseimura* Timm, 1960, *Praecocilenchus* Poinar, 1969, *Schistonchus* Cobb, 1927 and *Roveaphelenchus* Nickle, 1970. However, all five genera have spicules with dorsal limb extending to or beyond the ventral limb. *Cryptaphelenchus* further contrasts with our genus i.e. in having an acute or subacute tail tip, and in the absence of rectum and anus in the females. *Paraseimura* contains only one species, *P. musicolus* Timm, 1960, which supposedly has a stylet with «jointed» conus (although this is not evident on Fig. 68 in Nickle, 1970) and a very long, filiform tail. Free-living adults of *Praecocilenchus* have stylet without knobs, excretory pore posterior to metacarpus, subacute to acute tail tip, and males with swollen lip region, thin-walled stylet and somewhat reduced bulb. *Schistonchus* was discussed above. *Roveaphelenchus jonesi* Nickle, 1970, the type species of its genus, has free-living adults with a stylet devoid of knobs, an excretory pore posterior to the metacarpus, females with reduced ovary and subcylindrical tail terminating in four points, and males with strongly curved, blunt tail.

Finally, the recently proposed genera *Noctuidonema* Remillet & Silvain, 1988 and *Vampyronema* Hunt, 1993 resemble our genus in having a short, plump body, an excretory pore anterior to the metacarpus, no postvulval uterine sac, and spicules with very well-developed rostrum. However, both genera have an extremely long stylet (resp. 106-185 μm and 53-69 μm in Hunt, 1993), excretory pore closer to lip region than to metacarpus, rounded to subdigitate tail, and spicules with dorsal limb extending well beyond ventral limb.

RELATIONSHIPS

We follow the classification of Nickle and Hooper (1991), and refer to their work for references to the families and subfamilies discussed below. Hunt (1993) raised several of the subfamilies in Nickle and Hooper (1991) to families.

In view of the differences noted, and especially the particular combination of pharynx structure and male characters, a new genus is proposed for our species. This new genus is quite difficult to place with respect to

known families and genera, as it resembles different groups in different respects. It is perhaps related to *Cryptaphelenchus* and *Vampyronema*, placed by Nickle and Hooper (1991) in Aphelenchoididae: Ektaphelenchinae and Entaphelenchidae: Acugutturinae, respectively. Presence of rectum and anus in the females of our new genus preclude their placement in Ektaphelenchinae, and because the stylet is not elongate, placement in Acugutturinae is also not justified (cf. diagnoses in Nickle and Hooper, 1991 and key in Hunt, 1993).

Several other subfamilies and families are also clearly excluded. Aphelenchoididae: Bursaphelenchinae have males with a small terminal bursa, while Aphelenchoididae: Rhadinaphelenchinae contain only the completely different genus *Rhadinaphelenchus* (see above), and Seinuridae have elongated to filiform tails. Finally, it cannot be excluded that the new genus has a zooparasitic stage that we did not encounter in our soil samples, but in the absence of any positive evidence for such a stage we cannot place it in Entaphelenchidae or Entaphelenchinae.

The distinct isthmus with overlapping gland bulb would suggest that *Punchaulus* belongs in Paraphelenchidae, but this is contradicted by the male characters, which undermine the distinctions between Paraphelenchidae and Aphelenchoididae. In all, we tentatively place our new genus in Aphelenchoididae, by default of convincing alternatives rather than because of good agreement with current family diagnosis.

Punchaulus gemellensis gen. n., sp. n.

(Figs. 1-3)

MEASUREMENTS

See Table 1.

DESCRIPTION

Adults: Body short and squat, weakly ventrally curved. One fully developed female still encapsulated in intact fourth-stage cuticle. Cuticle 1.0 μm thick or less, with more or less inconspicuous annuli 0.8-1.1 μm wide at mid-body ($n = 4$) and numerous fine longitudinal striae that are only visible with SEM (Fig. 1 A-D, G-I). Lateral field extending from slightly anterior to metacarpus level to tail tip, with three incisures; under light microscope the outer two incisures are much more distinct than the central one.

Lip region offset by a slight constriction, with cap-like profile, 6.5-7.5 μm and 2.5 μm high. Lip sectors low, rounded-trapezoid in optical section, basally well offset from each other, but apically flattened and confluent. Externally, they lack annulation and are separated by shallow radial incisures (Fig. 1 A-C). Anterior sensilla not visible with light microscope or with SEM, except perhaps inner labial sensilla, which are possibly grouped immediately around the mouth opening (Fig. 1 A, C). A

Table 1. Measurements in μm of *Punchaulus gemellensis* gen. n., sp. n. from Isla Santa Cruz (allotype from sample 5, rest from sample 4).

	Holotype ♀	Allotype ♂	Holotype and paratypes (5 ♀♀)	Paratype ♂
L	380	356	336 ± 31 (300-380)	404
Body width	27	19	23 ± 3 (19-27)	21
Neck length*	68	73	64 ± 3 (60-68)	67
Pharynx (with glands)	97	108	95 ± 3 (89-98)	115
Tail length	27	31	26 ± 3 (22-29)	36
Anal body width	11.5	14	10-12.5	12
a	14	19	14-16	19
b	5.6	4.9	5.3 ± 0.3 (4.7-5.6)	6.0
b'	3.9	3.3	3.5 ± 0.3 (3.1-3.9)	3.5
c	14	11.5	12-14	11
c'	2.3	2.2	2.2-2.5	3.0
Stylet length	13	12	12 ± 1 (10-14)	12
Anterior end to procorpus base	32	38	30 ± 2 (28-32)	32
Bulb length	13.5	13.5	12.5-13.5	13
Bulb width	12	11	11-12.5	11
Gland lobe	39	41	40 ± 4 (34-45)	55
Excretory pore	29	31	31 ± 2 (29-34)	25.5
Nerve ring	53	58	52 ± 2 (50-54)	56
Excretory pore (% neck)	50	42	49 ± 2 (45-52)	38
Nerve ring (% neck)	79	79	81 ± 3 (79-87)	84
V (%) / T (%)	72	63	72-74	58
G (%) / spicule length	58	21	41-58	22
vagina	6.5	-	6-7	-
rectum	15	-	14 ± 2 (11-16)	-

* : Measured from anterior end to base of pharyngeal isthmus. Ratio b was calculated from this value, ratio b' from pharynx length including glands.

small, weakly delineated labial disc appears to be present (Fig. 1 B).

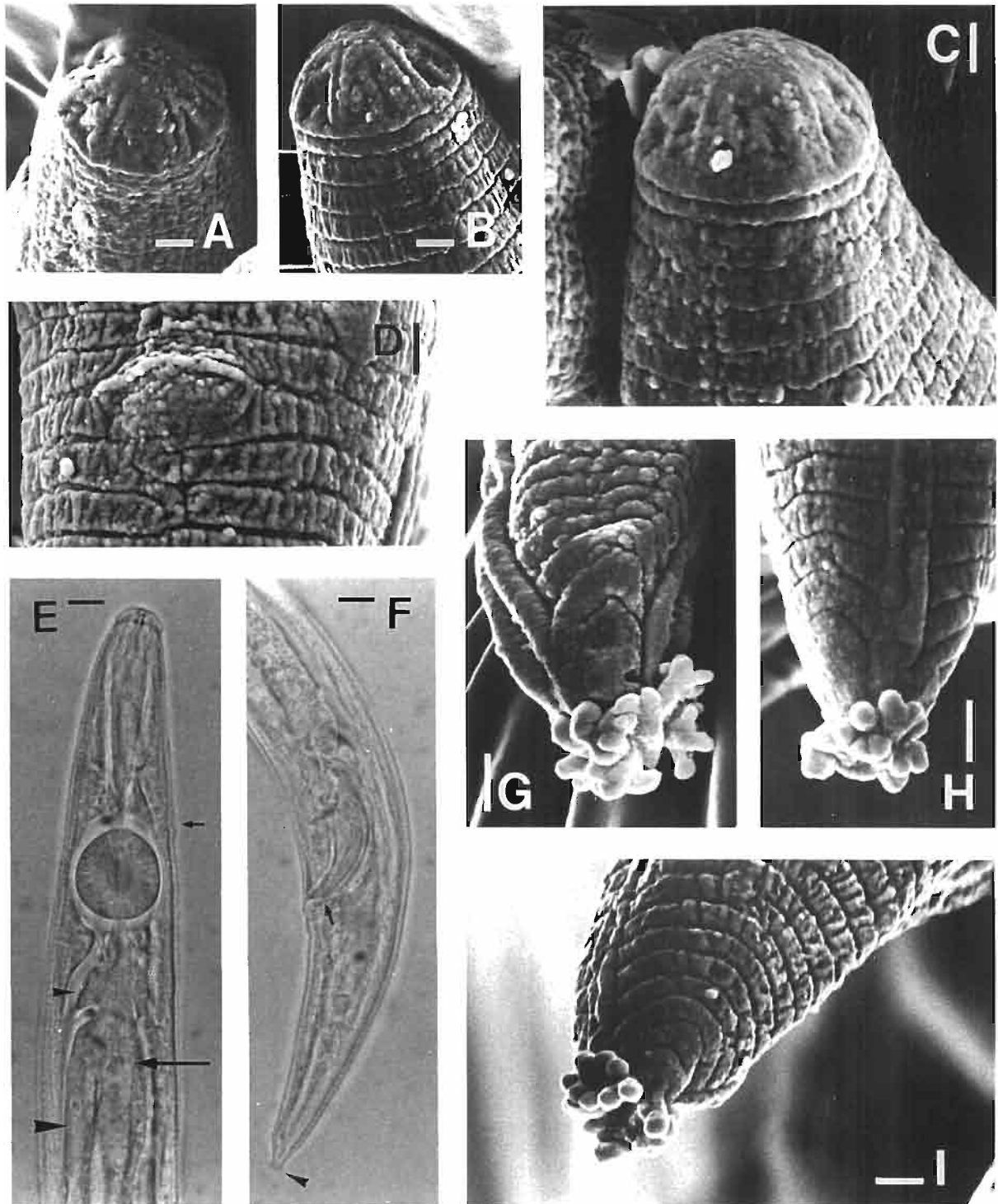


Fig. 1. *Punctaulus gemellensis* gen. n., sp. n. A-C: Lip region (juveniles); D: Anus; E: Neck region of female drawn in Fig. 2 B, focused on posterior end of isthmus (long arrow), part of gland lobe (large arrowhead) and nerve ring (small arrowhead) – excretory pore is out of focus (small arrow); F: Spicule and tail of paratype male (cf. Fig. 2 L), showing fimbriated terminal tail stub (arrowhead) and shortened dorsal limb of spicule (arrow); G-I: Tail tip (G is of female; H, I of juvenile). (Scale bars = 5 μ m in E, F and 1 μ m in A-D, G-I).



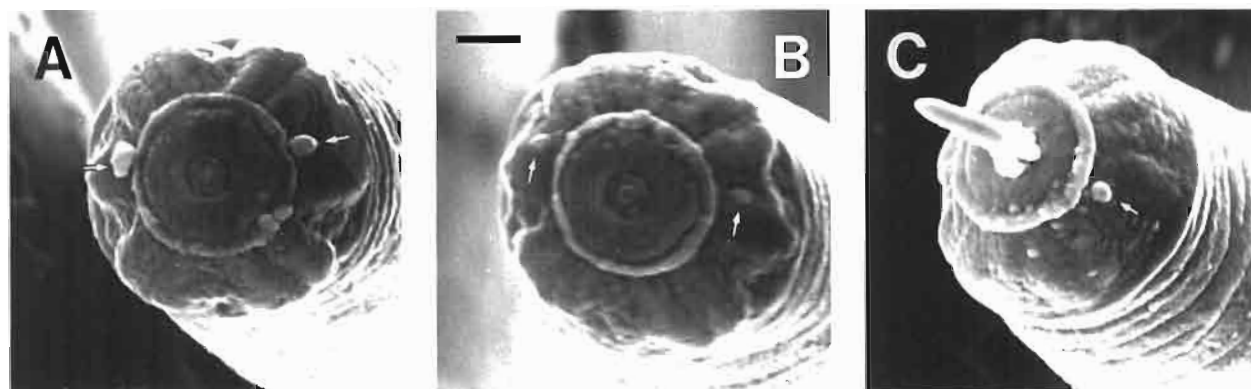


Fig. 3. *Schistonchus caprifici* (Gasparrini, 1864) Fuchs, 1937. A-C: Lip region (female in A, B; male in C). Arrows point at the probable locations of the amphids, which are apparently covered by a secretory plug. (Scale bar = 1 μm).

Mouth opening less than 1 μm wide. Walls of cheilos-tome refringent, finely drop-shaped in optical section. Stylet 1.25-2.0 times as long as the lip region is wide, its conus 4.5-6.5 μm long and fairly refringent, its shaft 5.5-7.5 μm long, weakly refringent and bearing relatively well-developed basal knobs. Stylet protractors distinct. Procorpus amuscular, tapering posteriorly, only 15-18 μm or 1.4-1.6 stylet lengths long (25 μm or 2.3 stylet lengths in allotype male, but this has somatic musculature detached anteriorly: Fig. 2 C). Metacorpul bulb muscular, almost perfectly spherical in shape, 12.5-13.5 μm long and 11-12.5 μm wide, very conspicuous under light microscope (Fig. 1 E). Bulb valves refringent, lenticular, slightly eccentric and 4.5-6 μm long. Isthmus not muscular, enveloped by tapering extensions of the long and slender intestinal wall: the intestinal lumen begins at 19-22 μm from base of bulb (Figs 1 E; 2 A-C). Pharyngeal gland lobe dorsally overlapping intestine, beginning 53-67 μm from anterior end or 10-16 μm from the bulb, 34-55 μm long, with three large gland cells and anteriorly with a few small cell-like components which merge with the intestinal envelope of the isthmus at 3-8 μm from the intestinal lumen.

Excretory pore 1-1.5 μm wide, with rather refringent walls, located 27-34 annuli from lip region ($n = 5$), i.e. anterior to the bulb or opposite the anterior end of the bulb. Excretory pore not seen with SEM, corresponding region always covered by detritus (secretion?). Nerve ring enveloping isthmus at 5-8 μm from bulb or 6-11 μm from intestinal lumen. Hemizonid not seen.

Tail tip constricted, ending in a single fimbriated stub (Figs 1 F-I; 2 F, G, K, L). Fimbriated coronet may or may not consist of two separate, flattened fringed rings (suggested by Fig. 1 H, I but not by Fig. 1 G).

Females: Vulva at nearly three quarters of body length. Postvulval uterine sac reduced to a 4-7 μm long tissue fold with a pair of posteriolateral support fibre bundles attached to the body wall ventrosublaterally (Fig. 2 D, E). Anterior branch of reproductive system

outstretched, with 19-33 μm long uterus, 16-20 μm long spermatheca and 72-151 μm long ovary. Uterus and spermatheca thin and poorly demarcated from each other in the two smallest females (Fig. 2 E), but thick-walled and very deeply offset in the other three females (Fig. 2 D). In the latter, the uterus wall is packed with minute, non-refringent granules whereas the spermatheca wall is filled with larger, more refringent granules and the spermatheca lumen is tightly packed with rounded sperm cells about 2-4 μm in diameter. Ovary with 14-23 oocytes in single file, reaching the nerve ring in holotype. Rectum 1.1-1.5 anal body widths long. Tail straight.

Males: Body ventrally curved in posterior region. Testis outstretched, reaching gland lobe in allotype. Spicules separate, proportionally large, with very prominent rostrum (spicules 7-7.5 μm wide at rostrum) and ventral limb extending beyond dorsal limb (tips of both limbs 2.5-3 μm apart; Figs 1 F; 2 K, L). Tail straight or almost straight, carrying three pairs of genital papillae. Bursa absent.

TYPE LOCALITY AND HABITAT

Sampling locality 4 (5 ♀♀, 1 ♂, 4 JJ): at 630 m altitude on N slope of Los Gemelos, Isla Santa Cruz, Galápagos, Ecuador. Clearing in *Scalesia* forest with grasses and sedges.

OTHER LOCALITY

Sampling locality 5 (1 ♂): at 630 m altitude on N slope of Los Gemelos, Isla Santa Cruz, Galápagos, Ecuador. *Scalesia* forest.

TYPE SPECIMENS

Holotype female, allotype male and one paratype female kept on slides 3818-3820 in the Nematode Collection of the Instituut voor Dierkunde, Universiteit Gent, Belgium; one male and female paratype deposited in the Collection Nationale de Nématodes, Muséum National d'Histoire Naturelle, Paris, France; one female paratype

deposited in the USDA Nematode Collection, Beltsville, Maryland, USDA.

Acknowledgements

We are grateful to Dr P. Baujard for many valuable comments and to Dr N. Vovlas and Dr P.A.A. Loof for providing us with requested reference material. We also wish to thank Dr J. Lloyd and Dr K. A. Davies for permission to consult and quote their forthcoming manuscript on *Schistonchus*. The first author is postdoctoral researcher with the National Fund for Scientific Research of Belgium.

References

- BAUJARD, P. (1988). Identification of aphelenchids. In: FORTUNER, R. (Ed.). *Nematode identification and expert system technology*. New York, USA, Plenum Press, 153-156.
- BAUJARD, P. (1989). Remarques sur les genres des sous-familles Bursaphelenchinae Paramonov, 1964 et Rhadinaphelenchinae Paramonov, 1964 (Nematoda: Aphelenchoididae). *Revue Nématol.*, 12 : 323-324.
- DE LEY, P., LOOF, P. A. A. & COOMANS, A. (1993). Terrestrial nematodes from the Galápagos Archipelago II: Redescription of *Aporcelaimellus obtusicaudatus* (Bastian, 1865) Altherr, 1968, with review of similar species and a nomenclature for the vagina in Dorylaimida (Nematoda). *Bull. Inst. R. Sci. nat. Belg., Biol.*, 63 : 13-34.
- GIBLIN, R. M. & KAYA, H. K. (1983). *Bursaphelenchoides seani* n. sp. (Nematoda: Aphelenchoididae) a phoretic associate of *Anthophora bomboidea stanfordiana* Cockerell, 1904 (Hymenoptera: Anthophoridae). *Revue Nématol.*, 6 : 39-50.
- GIBLIN-DAVIS, R. M., MUNDO-OCAMPO, M., BALDWIN, J. G., GERBER, K. & GRIFFITH, R. (1989). Observations on the morphology of the red ring nematode, *Rhadinaphelenchus cocophilus* (Nematoda: Aphelenchoididae). *Revue Nématol.*, 12 : 285-292.
- GOODEY, J. B. (1960). *Rhadinaphelenchus cocophilus* (Cobb, 1919) n. comb., the nematode associated with « red-ring » disease of coconut. *Nematologica*, 5 : 98-102.
- HIRLING, W. (1982). Eine Übersicht über die Arten der Nematodengattungen *Laimaphelenchus* Fuchs, 1937 und *Ruidosaphelenchus* Laumond und Carle, 1971 mit Bestimmungstabellen und drei neue *Laimaphelenchus*-Arten (Nematoda: Tylenchida). *Z. PflKrankh. PflSchutz*, 89 : 30-42.
- HOOPER, D. J. & CLARK, S. A. (1980). Scanning electron micrographs of the head region of some species of Aphelenchoidea (Aphelenchina: Nematoda). *Nematologica*, 6 : 47-56.
- HUNT, D. J. (1993). *Aphelenchida, Longidoridae and Trichodoridae: their systematics and bionomics*. Wallingford, UK, CAB International, 352 p.
- NICKLE, W. R. (1970). A taxonomic review of the genera of the Aphelenchoidea (Fuchs, 1937) Thorne, 1949 (Nematoda: Tylenchida). *J. Nematol.*, 2 : 375-392.
- NICKLE, W. R. & HOOPER, D. J. (1991). The Aphelenchina: bud, leaf and insect nematodes. In: Nickle, W. R. (Ed.) *Manual of agricultural nematology*. New York, USA, Marcel Dekker : 465-507.
- RASKI, D. J. & VALENZUELA, A. (1988). Descriptions of four new species of Criconematidae, male of *Ogma terrestris* (Tylenchida: Criconematoidea) and *Tylaphelenchus yamani* sp. n. (Aphelenchina: Aphelenchoididae). *Nematologica*, 33 (1987) : 149-166.
- THORNE, G. (1961). *Principles of nematology*. New York, USA, McGraw-Hill, 553 p.
- VOVLAS, N., INSERRA, R. N. & GRECO, N. (1992). *Schistonchus caprifici* parasitizing caprifig (*Ficus carica sylvestris*) florets and the relationship with its fig wasp (*Blastophaga psenes*) vector. *Nematologica*, 38 : 215-226.
- YIN, K., FANG, Y. & TARJAN, A. C. (1988). A key to species in the genus *Bursaphelenchus* with a description of *Bursaphelenchus hunanensis* sp. n. (Nematoda: Aphelenchoididae) found in pine wood in Hunan Province, China. *Proc. helminth. Soc. Wash.*, 55 : 1-11.