

male with two pairs of papillae and no bursa. Spicules small, gubernaculum without projection. Tail in both sexes elongate, filiform.

TYPE AND ONLY SPECIES

Aenigmenchus floreanae gen. n., sp. n.

RELATIONSHIPS

The new genus exhibits a curious mixture of resemblances to various groups, as well as some peculiarities of its own. Firstly, the presence of a prodelphic, monodelphic female reproductive system and the shape of spicules and gubernaculum are reminiscent of Cephaloboidea (Rhabditida). Within this superfamily, representatives of the family Ostellidae also lack valves in the basal bulb, and species of the genus *Drilocephalobus* Coomans & Goodey, 1965 even have a similar external appearance of the lip region, with fused lips and six labial papillae at the margins of a small perioral disc (cf. Fig. 2 A, B, D in De Ley & Coomans, 1990). Another representative, *Alirhabditis* Suryawanshi, 1971 (Alirhabditidae), is known to have a narrow lip region, filiform tail and males with only six distinct genital papillae (cf. Fig. 1 A, I-L in Suryawanshi, 1971; it has not yet been studied with SEM). Furthermore, although no Cephaloboidea were hitherto described with valvate median bulb, this condition does in fact occur in at least one genus (see note added in proof).

Aenigmenchus also resembles the genus *Diplogasteroides* de Man, 1912 (Diplogasterida: Diplogasteroididae), with which it shares a slender body, pharynx and tail, and a prodelphic, monodelphic female gonad with postvulval sac. However, *Diplogasteroides* has fairly large amphids, a distinctly tubular stoma and males with four to eight pairs of genital papillae (Andrássy, 1984).

A second diplogasterid to be considered is *Tylopharynx* de Man, 1876 (Tylopharyngidae). The type and only species of this genus, *T. foetida* (Bütschli, 1874) Goffart, 1930, has a filiform tail and a stylet-like stoma armature with two large dorsosublateral knobs and a smaller ventral knob. The "conus" of this "stylet" actually consists of two teeth surrounded by a thin sheath, the "shaft" is laterally flattened and surrounded by a rather complex arrangement of protractor muscles, and it is unclear whether the entire structure is homologous to the tylenchid stylet or not (De Ley *et al.*, 1993). Compared to *Aenigmenchus*, *T. foetida* has a much larger "stylet" (it is 14-15 μm long in ten specimens from Gent, Belgium), a larger body ($L = 0.6-1.3$ mm in Andr ssy, 1984), much larger amphidial apertures facing anteriorly, a diplogasterid pharynx that is much less slender, a didelphic female reproductive system with the ovaries containing many oocytes and reaching the point of flexure of each branch, nine pairs of male genital papillae, and much slenderer, longer and more arcuate spicules (22-33 μm in Andr ssy, 1984; 32-42 μm in seven specimens from Gent) that have the

fenestra located apically on an offset, rounded manubrium.

Finally, the new genus shows a marked resemblance in overall body shape and proportions to members of the family Tylenchidae within the order Tylenchida, being impossible to distinguish from these under the stereomicroscope. The isthmus and bulbus of Tylenchidae are also similar, their (true) stylet can be quite small, and some species also have ovaries with very few oocytes. In addition, the green intestinal globules found in some *A. floreanae* specimens suggest that it feeds on soil algae, presumably like many Tylenchidae. Once again, however, there are also numerous differences: Tylenchidae have a true stylet with one dorsal and two ventrosublateral knobs, a muscular corpus with circular lumen, outstretched male and female gonads, bursate male tail, genital papillae grouped around cloacal aperture, and spicules with apical fenestrae.

Three features of *A. floreanae* have to our knowledge not yet been reported in any free-living secernentean: the extremely small amphidial apertures, the presence of only four genital papillae in the male, and the peculiar reflexed prodelphic female gonad with a proportionately long uterus-oviduct and a postvulval ovary. The ovary must undoubtedly be capable of stronger development than what we found in our material, and perhaps the long prevulval genital canal serves to accommodate several eggs. However, as we did not find gravid specimens, the functional variability of the female reproductive system remains to be ascertained. Two other peculiarities are the apparent absence of deirids and phasmids.

The puzzling web of resemblances between *Aenigmenchus* and known Secernentea makes its allocation very difficult. Two basic alternatives present themselves: spicule shape suggests affinity with Cephaloboidea, while pharynx structure points towards inclusion in Diplogasterida. These hypotheses could be reconciled by the assumption that our new genus forms a link between cephalobids and diplogasterids, but this is quite unlikely in view of other known taxa. Thus, the ancestry of diplogasterids can clearly be traced back to Rhabditina through the families Diplogasteroididae and Pseudodiplogasteroididae, and the ancestry of Rhabditina probably lies within Cephalobina (cf. Andr ssy, 1984). One would therefore have to assume that Diplogasterida are polyphyletic for *Aenigmenchus* to fit in as a second link between cephalobids and diplogasterids. In the absence of any supporting evidence, we reject this possibility and must consequently also exclude one of either hypothesis of relationship for our new genus.

Despite the typically cephalobid spicules, we assume that *Aenigmenchus* is not close to Cephaloboidea, on the basis of two characters: its amuscular posterior pharynx and its female reproductive system without offset spermatheca. These differences are given great weight, because Cephaloboidea are a very diverse group in nearly every respect except in the constant presence of an

offset spermatheca and muscular isthmus and bulbus. Secondary evidence in support of our assumption is provided by two items : *i*) the position of the sperm cluster (when occurring) relative to the gonoduct flexure and the very short ovary suggest that *Aenigmenchus* lacks the typical short oviduct found at the flexure in between uterus and ovary of Cephaloboidea; *ii*) all known Cephaloboidea have a much more strongly annulated cuticle than in the new genus.

It could also be argued that, while *Aenigmenchus* is not a true cephalobid, it might in fact belong to the probable sister group of Cephaloboidea within Rhabditida, i.e. the superfamily Panagrolaimoidea, in which the cuticle is less strongly annulated, the offset spermatheca is usually absent (not always : De Ley, unpubl.), and a valvate median bulb occasionally occurs. Nevertheless, like cephalobids all known Panagrolaimoidea have a muscular bulbus and isthmus, and an ovary extending up to the gonad flexure. Also, Panagrolaimoidea usually have differently shaped spicules, and no known representative has a narrow lip region, low number of genital papillae and filiform tail approaching that of *Aenigmenchus*. We therefore reject this possibility too.

Instead, we place *Aenigmenchus* in the order Diplogasterida, superfamily Diplogasteroidea on the basis of its pharynx structure. We assume that the cephalobid spicule shape of *Aenigmenchus* either represents a plesiomorphy or a homoplasy : the fundamental resemblance with Cephaloboidea lies in the ventrally offset manubrium with dorso-subapical fenestra, and in comparison with the range of spicule shapes found in diplogasterids it seems more probable that evolution could yield a misleading pattern in these respects rather than in the pharynx and female reproductive system.

Following the most recent classification proposed for this group (Andrássy, 1984), which relies heavily on stoma structure, we allocate the genus to the family Tylopharyngidae. This allocation remains tentative because of the numerous differences with *Tylopharynx* and the uncertain anterior internal organization of *A. floreanae*. If correct it would imply that the Tylopharyngidae are (or were) much more diverse than hitherto known. The ambiguous stoma structure of the new genus is particularly tantalizing because it does not allow us to determine whether the similarities with Tylenchidae are either convergent, artefactual or reflections of true phylogenetic affinity. Less ambiguous evidence will require living specimens for ultrastructural or biochemical studies, or else must await the discovery of related new forms.

***Aenigmenchus floreanae* gen. n., sp. n.**
(Figs 1-4)

MEASUREMENTS

See Table 1.

Table 1. Measurements (in μm) of *Aenigmenchus floreanae* gen. n., sp. n.

	Holotype (female)	Holotype and paratypes (eight females)	Allotype (male)	Paratypes (three males)
L	509	534 \pm 31 (472-573)	471	502-560
L'	337	352 \pm 25 (302-388)	309	336-376
Body width	12	12 \pm 1.4 (10-15)	9.5	10-12.5
Pharynx length	96	100 \pm 3 (95-104)	94	96-100
Tail length	172	182 \pm 10 (170-196)	162	166-198
Anal body width	6.5	6.5-7	7	7-8
a	42	46 \pm 6 (36-55)	50	45-50
b	5.3	5.3 \pm 0.2 (5.0-5.7)	5.0	5.1-5.8
c	3.0	2.9 \pm 0.1 (2.8-3.2)	2.9	2.8-3.1
c'	26	27 \pm 1.7 (24-30)	231	22-25
Stylet	3	2.5-3	3	2.5-3
Corpus	46	48 \pm 2 (45-50)	44	48-50
Isthmus	36	40 \pm 3 (36-44)	36	36-46
Bulbus	12	11 \pm 1.5 (8-13)	10	10
Nerve ring	61	62 \pm 3 (56-68)	68	60-65
Excretory pore	64	64 \pm 3 (59-69)	66	63-67
N.r. (% pharynx)	63	62 \pm 3 (59-68)	72	61-65
E.p. (% pharynx)	67	65 \pm 2 (62-69)	70	64-67
V	44	41-44	-	-
V'	66	65 \pm 2 (62-66)	-	-
G/T	21.4	20 \pm 1.8 (16-22)	25	23-27
Vagina/spicules	4.5	3-4.5	11	10-13
Rectum/gubernaculum	12	13 \pm 2.0 (11-17)	4.5	4-5

DESCRIPTION

Adults : Body ventrally coiled or irregularly curved. Cuticle less than 1 μm thick, with very fine annuli that are 0.6-0.7 μm wide at mid-body ($n = 3$) and usually indistinguishable with light microscope. Cuticle becom-

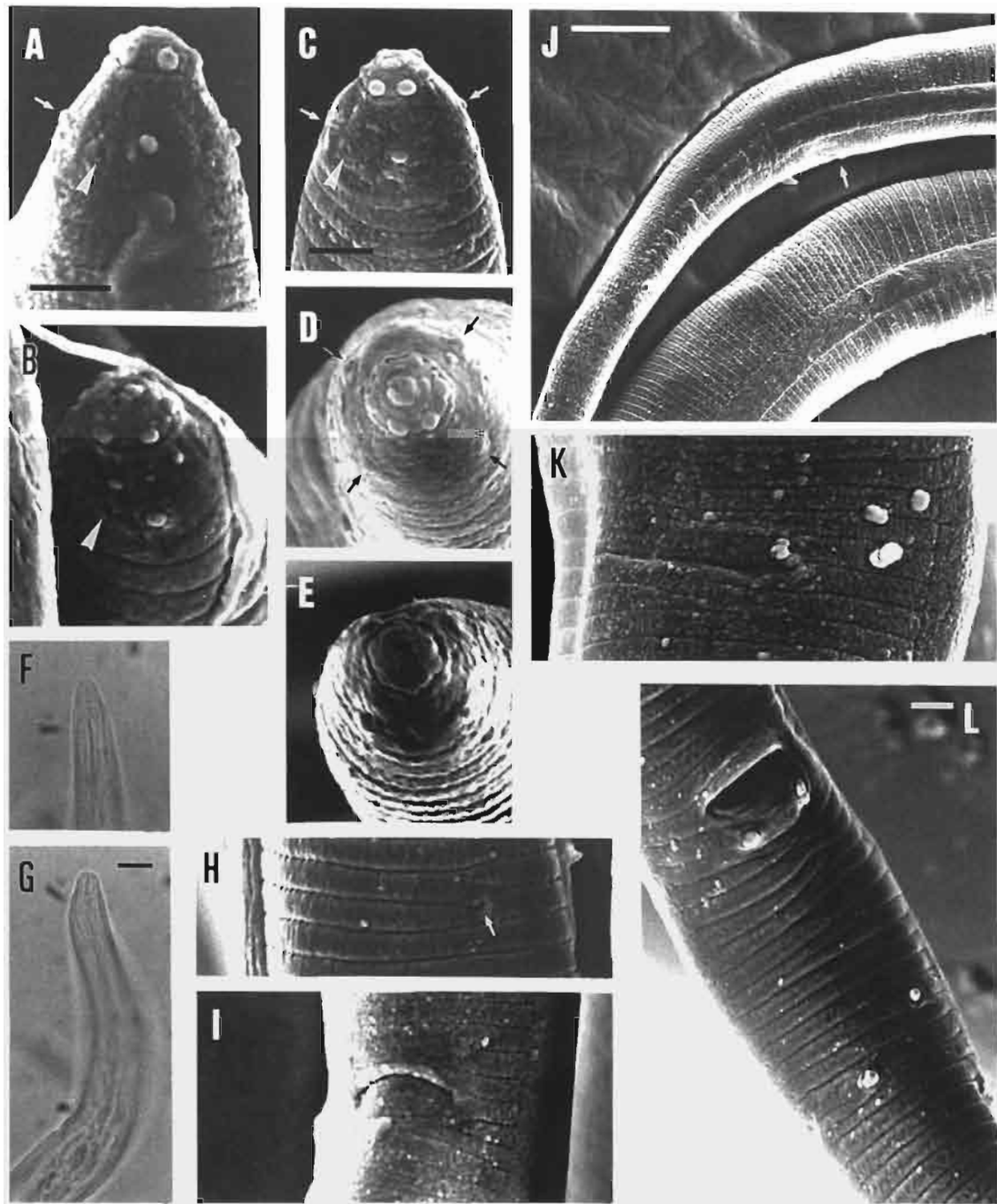
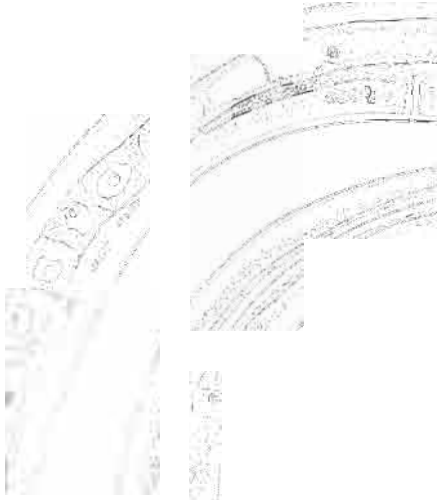


Fig. 1. *Aenigmenchus floreanae* gen. n., sp. n. A-E : Lip region (arrowheads point at amphids, arrows at less clear cephalic papillae; B, E : males, A, C, D : females); F : Female anterior end under light microscope (cf. Fig. 4 A); G : Male anterior end and metacarpus under light microscope (cf. Fig. 4 C); H : Excretory pore (arrow); I : Anus; J : Lateral field on body and in anal region (arrow points at anus); K : Vulva; L : Cloacal aperture and genital papillae. (Scale bar equals 5 μ m in G, J and 1 μ m in A, C, L. B is at scale of A; D, E, H, I, K are at scale of C; F is at scale of G.)



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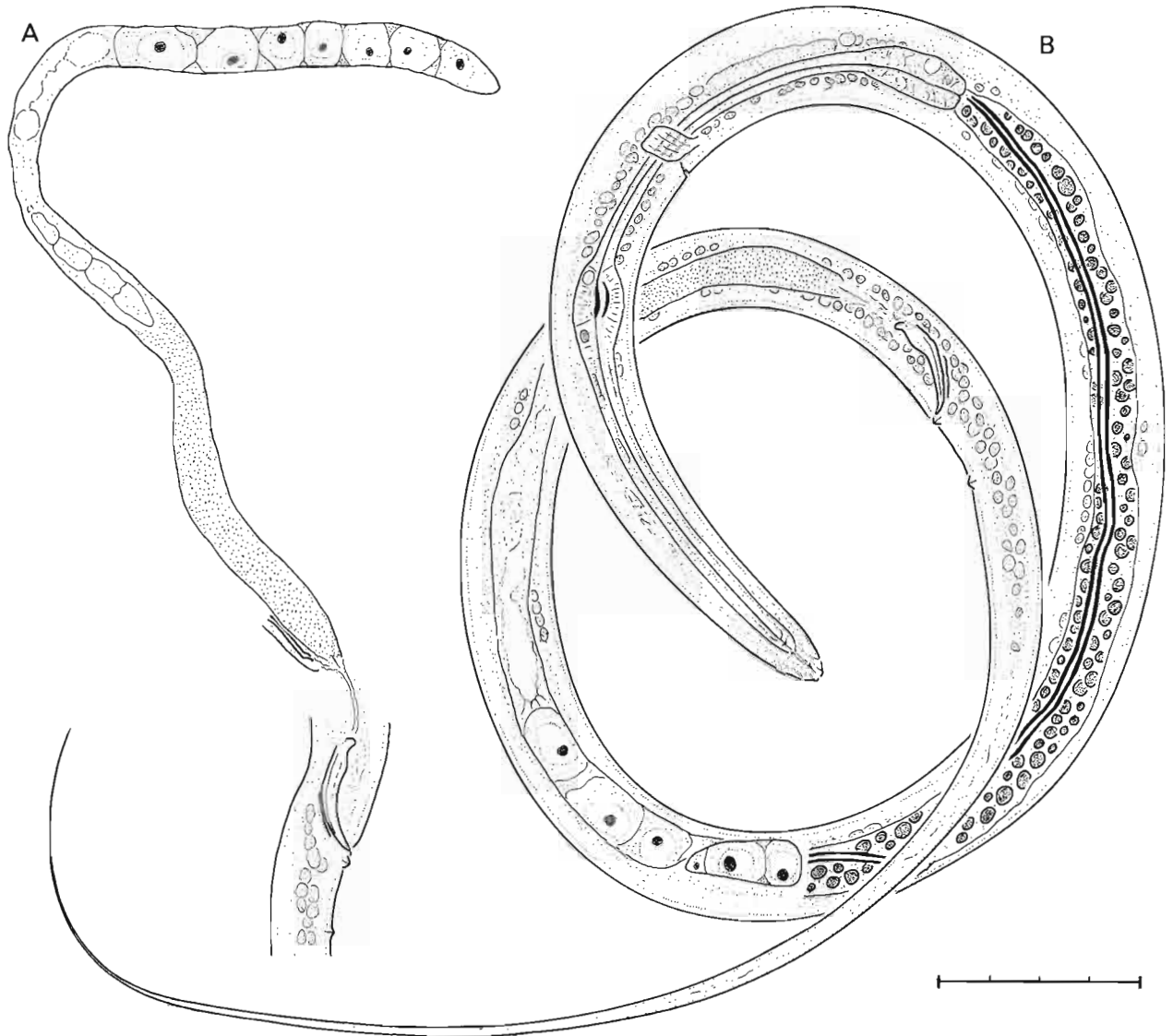


Fig. 3. *Aenigmenchus floreanae* gen. n., sp. n. *A*: Male reproductive system; *B*: allotype male. (Bar = 25 μ m.)

one (slightly fainter) midventrally. Small, hyaline globules are usually faintly visible just posterior to the knobs, or are perhaps part of the knobs (compare Fig. 4 A₅ with 3 B-D). "Stylet" surrounded by a very thin hyaline sheath that is posteriorly radially divided into three sectors and could represent stoma lining anteriorly and protractors posteriorly. Pharynx lumen always showing two discontinuities at respectively 0.5-1 μ m and 1.5-2 μ m posterior to the "stylet", of which the anterior one could correspond with the dorsal gland opening, while the posterior one probably represents a point where the lumen changes in transverse shape (not clear in *en face*). Procorpus very slender, 42 ± 2 (39-45) μ m long and only 2.5-3 μ m wide, with triradiate lumen (exact transverse shape not clear) and weakly

muscular walls. Metacarpus swollen, 7-8 μ m long and 4.5-5.5 μ m wide, clearly muscular and containing 3.5-4.5 μ m long crescentic valves. Whole corpus 1.3 ± 0.1 (1.1-1.5) times as long as isthmus; the latter extremely slender, only 1.5-2 μ m wide, not muscular. Basal bulb pyriform, not overlapping intestine, 8-13 μ m wide and 4-5.5 μ m wide, without muscles or valves. Excretory pore inconspicuous, located opposite nerve ring, i.e. at anterior third of isthmus. An elongate cell or cell-mass (excretory cell?) is occasionally visible dorsally of the isthmus posterior to the nerve ring. Deirids not seen. No separate cardia distinguishable between pharynx and intestine. Intestine with narrow lumen demarcated by refractive lining, its cells anteriorly packed with refractive globules that are colourless in most specimens, but green

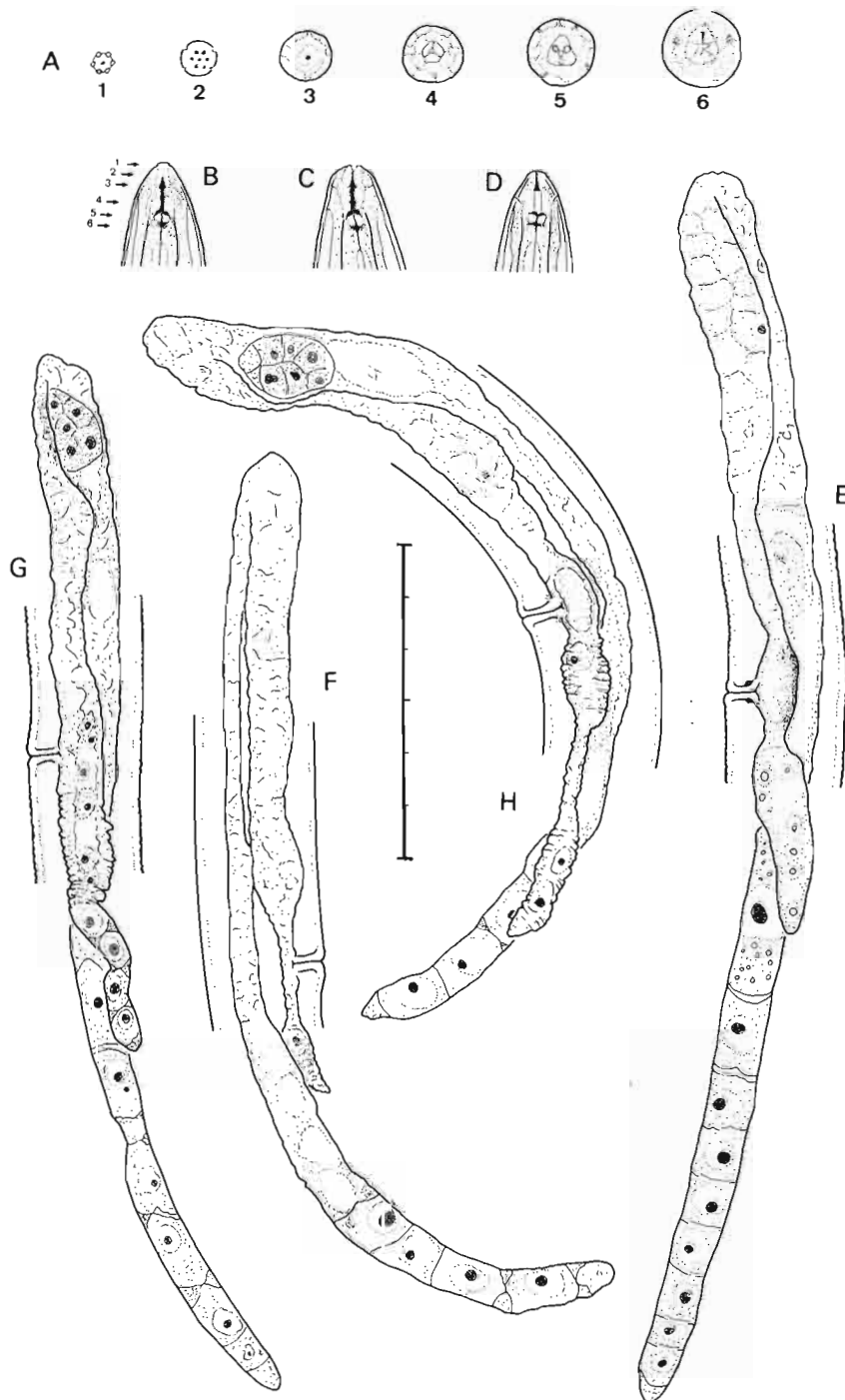


Fig. 4. *Aenigmenchus floreanae* gen. n., sp. n. *A* : Optical sections of anterior end in en face view; *B-D* : Anterior end in lateral view (*B* : Female, *C* : Male, *D* : Juvenile); *E-G* : Female reproductive system. (Scale bar equals 20 μm for *A-D* and 30 μm for *E-G*.)

in others ($n = 5$). Tail elongate-filiform, longer than one third of the total body length. Phasmids not seen.

Female : Vulva an inconspicuous, transverse slit located at about two-fifths of the body length (Fig. 1 K).

Vagina short and straight, extending over one-third to two-fifths of the vulva body width. Posterior reproductive branch present but reduced, consisting of a 26 ± 8 (13-35) μm long sac that sometimes seems to end in a

rudimentary ovary containing up to six nuclei, but more probably derives its ovary-like appearance from the presence of a few sperm cells. Anterior reproductive branch reflexed at roughly one-third of its length, consisting mostly of oviduct and uterus (separation between the two not clear) and terminating posterior to the vulva in a relatively short, straight ovary that contains two to nine oocytes in single file. One third of all females with a compact, globular sperm mass lying close to the flexure and apparently being located in the dorsal part of the anterior branch. No distinctly offset or delineated spermatheca visible, no females with eggs. Both branches of the reproductive system on right side of intestine, with the anterior branch reflexed in such a way that its ovary lies to the right of the posterior branch. Rectum anteriorly directed, 1.9 ± 0.3 (1.6-2.4) anal body widths long. Anal aperture a short arcuate slit.

Male : Testis single, containing four to seven spermatocytes in single file and lying outstretched or with the terminal spermatocyte(s) reflexed. One male apparently with four sperm cells in *vesicula seminalis*. *Vas deferens* characteristically finely granulated. Spicules small and slender, with offset, ventrally directed manubrium carrying a dorso-subapical fenestra. Gubernaculum short, lineate in lateral view. Only two pairs of minute genital papillae present, one pair located just posterior to the cloacal aperture and the other pair at slightly more than an anal body width posterior to the first.

TYPE LOCALITY AND HABITAT

Sampling locality 13 : Highland of Isla Floreana (altitude ca 350 m), Galápagos Archipelago, Ecuador; humid soil around roots of grasses in secondary forest with Guava, *Zanthoxylum*, *Citrus* sp., ferns, herbs.

TYPE SPECIMENS

Holotype female (N° 3713), allotype male (N° 3714) with 7 females, 13 males and 27 juvenile paratypes kept

on slides in the Nematode Collection of the Instituut voor Dierkunde, Gent, Belgium. Paratypes distributed as follows : 1 female, 3 males and 2 juveniles in the Collection Nationale de Nématodes, Muséum National d'Histoire Naturelle, Paris, France; 1 female, 2 males and 2 juveniles in the Nematode Collection, International Institute of Parasitology, St. Albans, UK; 2 females and 2 males in the USDA Nematode Collection, Beltsville, Maryland, USA; 2 females, 1 male and 1 juvenile in the Nematode Collection of Argentina, Córdoba, Argentina; 1 female, 2 males and 4 juveniles in the Waite Institute of Nematology Collection, Glen Osmond, Australia; and 1 female, 1 male and 1 juvenile in the National Nematode Collection of India, New Delhi, India.

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Note added in proof

Following the acceptance of our paper, a description has been published of the new cephalobid genus *Medibulla* Siddiqi, 1993 (Siddiqi, M. R. 1993. Nematodes of tropical rainforest : 2. Five new genera and eight new species of cephalobids. *Afro-Asian J. Nematol.*, 3 : 212-225). It has a pharynx with valvate median bulb and non-muscular basal bulb. Because of numerous differences in other respects, we still assume that *Aenigmenchus* n. gen. is not related to this or other Cephalobidina.