

Description of a New Species of the Genus *Tylopharynx* (Nemata: Diplogasteroidea), *T. clariamphida* sp. n., with a Redescription of *T. foetida* (Bütschli, 1874) Goffart, 1930

JIHUA WU,¹ PAUL DE LEY,² AND YANLING LIANG³

Abstract: *Tylopharynx clariamphida* sp. n. is described from muddy sand collected in Anhui Province, China. It can be distinguished from *T. foetida* (Bütschli, 1874), the type and only species of the genus, by numerous characters: having 24 to 26 prominent and clearly separated longitudinal ridges, a higher lip region with no hint of a cephalic framework, more prominent amphidial foveae in lateral view, wider and more posteriorly located amphidial apertures, smaller basal knobs of stoma, longer metacarpus, more enlarged phasmids, shorter spicules with shorter digitate terminus, shorter reflexed part of testis, and thicker gubernaculum with more angular shape. For comparison, an expanded description is given for *T. foetida* from Belgium, and SEM photographs of both species are provided.

Key words: Belgium, China, Diplogasteroidea, morphology, taxonomy, *Tylopharynx*.

The genus *Tylopharynx* has attracted attention ever since it was erected. It figures prominently in the long-lasting controversy over the origins and relationships of Tylenchida (Andrássy, 1984; Maggenti, 1963, 1983; Poinar, 1983; Siddiqi, 1980), especially because of the apparent similarity of its stoma armature to the tylenchid stylet. De Ley et al. (1993) reviewed this discussion and suggested that there is no strong affinity between the stoma of *Tylopharynx* and the stylet of Tylenchida, based on detailed observations of the ultrastructure of the stoma structure of *T. foetida*. Furst von Lieven and Sudhaus (2000) confirmed that *Tylopharynx* and *Mononchooides* are closely related and that the right ventrosulateral tooth was acquired in the ancestral line leading to both genera.

In the classification of Andr ssy (1984), the only available system that is both comprehensive and relatively recent, *Tylopharynx* was placed as monospecific and sole genus in the family Tylopharyngidae. Subsequently, De Ley et al. (1994) also allocated their monospecific new genus *Aenigmenchus* to this family, although they emphasized that *Tylopharynx* and *Aenigmenchus* differ in many respects. In this paper, we present a new species of *Tylopharynx* that is clearly related to, yet easily distinguishable from, the type and only known species *T. foetida* (Bütschli, 1874) Goffart, 1930. While studying this new species, we found some previously ignored features of *T. foetida* in need of redescription or more detailed illustration. These characters included longitudinal ridges, amphids, and shape of spicules. We

therefore provide a detailed description of both species.

MATERIALS AND METHODS

A muddy sand sample was collected from a small ditch with flowing water in Anhui Province of China and fixed in 4% formaldehyde. Specimens of *T. clariamphida* sp. n. were extracted by flotation in Ludox TM (Somerfield and Warwick, 1996) and slowly dehydrated to anhydrous glycerol. Sandy soil was also collected from an aviary with pigeons in the garden of the second author in Landegem, Belgium. Live *T. foetida* were extracted using an extraction tray (Hooper, 1990), fixed with hot 4% formaldehyde: 1% glycerol, and transferred to anhydrous glycerol using a modification of the rapid method of Seinhorst (1962). Measurements and drawings were obtained from specimens mounted in anhydrous glycerol.

For Scanning Electron Microscopy (SEM), one female of *T. clariamphida* and five females plus five males of *T. foetida* were rehydrated via an ethanol series, sonicated, freeze-dried, mounted on stubs, and sputter-coated. Observations and electromicrographs were made with a JEOL LSM-840 (Japan).

SYSTEMATICS

Tylopharynx clariamphida sp. n. (Figs. 1–3)

Holotype male: L = 933 μ m; L' = 632 μ m; a = 51; b = 6.3; c = 3.1; c' = 20

Paratype males (n = 3): L = 898 (847–949) μ m; L' = 633 (582–699) μ m; a = 44 (36–50); b = 5.9 (5.5–6.2); c = 3.4 (3.2–3.8); c' = 18 (17–21)

Paratype females (n = 8): L = 1,257 (901–1,610) μ m; L' = 851 (644–1,045) μ m; a = 48 (43–51); b = 7.7 (6.4–9.2); c = 3.2 (2.9–3.8); c' = 30 (23–36); V% = 36 (34–40); V' % = 54 (50–57)

Females: Body almost straight after fixation. Cuticle 0.7–0.9 μ m thick, annulated, and bearing prominent longitudinal ridges. Annules 0.9–1.1 μ m wide. Longitu-

Received for publication 24 May 2000.

¹ Ministry of Education Key Laboratory for Biodiversity Science and Ecological Engineering, and Institute of Biodiversity Science, School of Life Sciences, Fudan University, Shanghai 200433, China.

² Vakgroep Biologie, Universiteit Gent, Ledeganckstraat 35, B-9000 Gent, Belgium. Current address: Department of Nematology, University of California — Riverside, Riverside, CA 92521.

³ Institute of Hydrobiology, Chinese Academy of Sciences, Wuhan, Hubei 430072, China.

The study was funded in part by the Donghu Experimental Station of Lake Ecosystem, CAS, by the State Key Basic Research and Development Plan (G2000046800) and by the key projects of the Chinese Academy of Sciences KZ951-A1-102 and KZ951-B1-104. The authors thank R. Van Driessche for technical assistance with SEM.

This paper was edited by R. T. Robbins.

E-mail: jihuwu@263.net

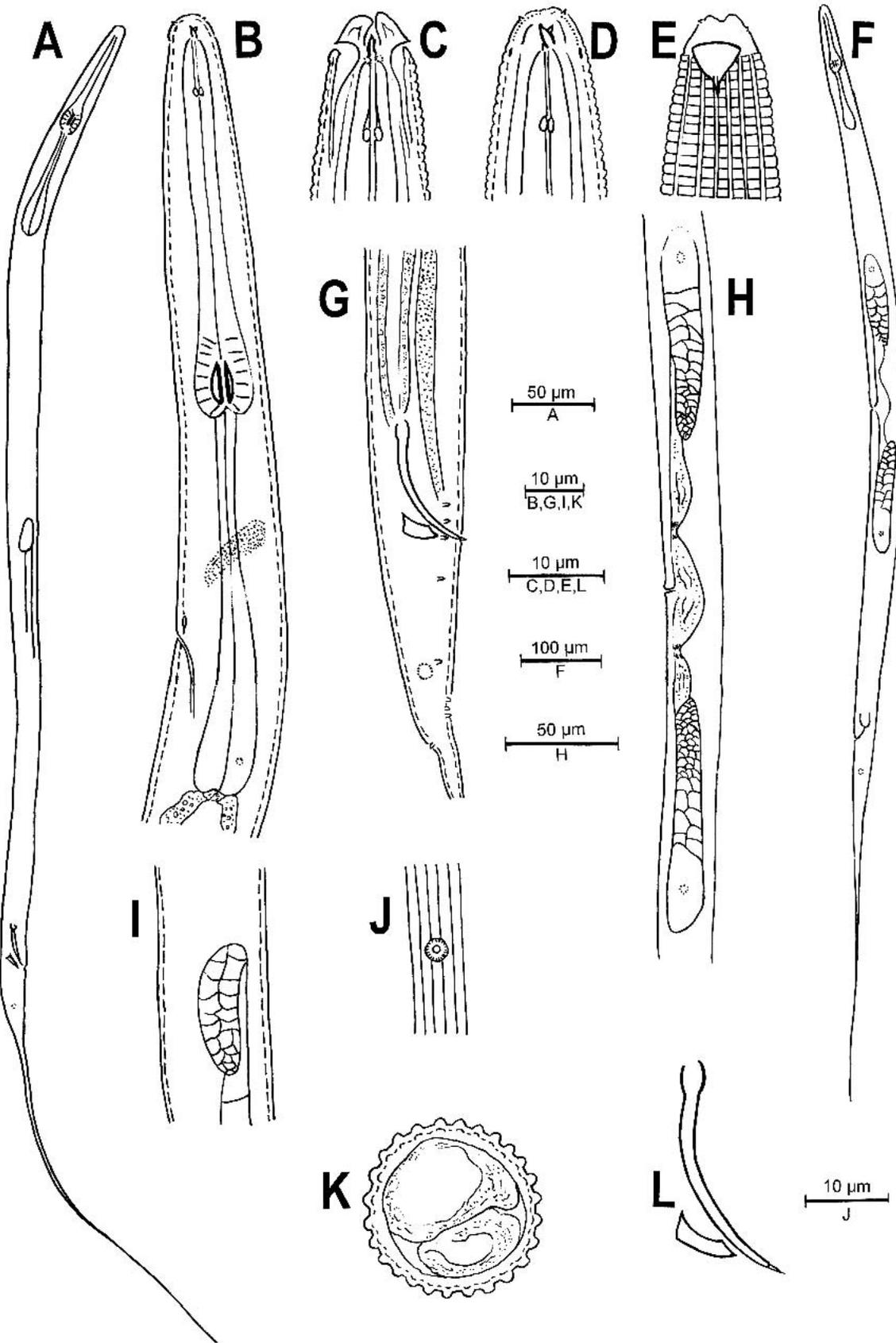


FIG. 1. *Tylopharynx clariamphida* sp. n. A) Entire male. B) Anterior region. C) Head region, ventral view. D) Head region, left-lateral view. E) Head region, lateral view showing amphid. F) Entire female. G) Cloacal region of male. H) Female reproductive system. I) Anterior reflexed part of testis in male, right-lateral view. J) Vulva, ventral view. K) Cross section showing longitudinal ridges. L) Spicule and gubernaculum.

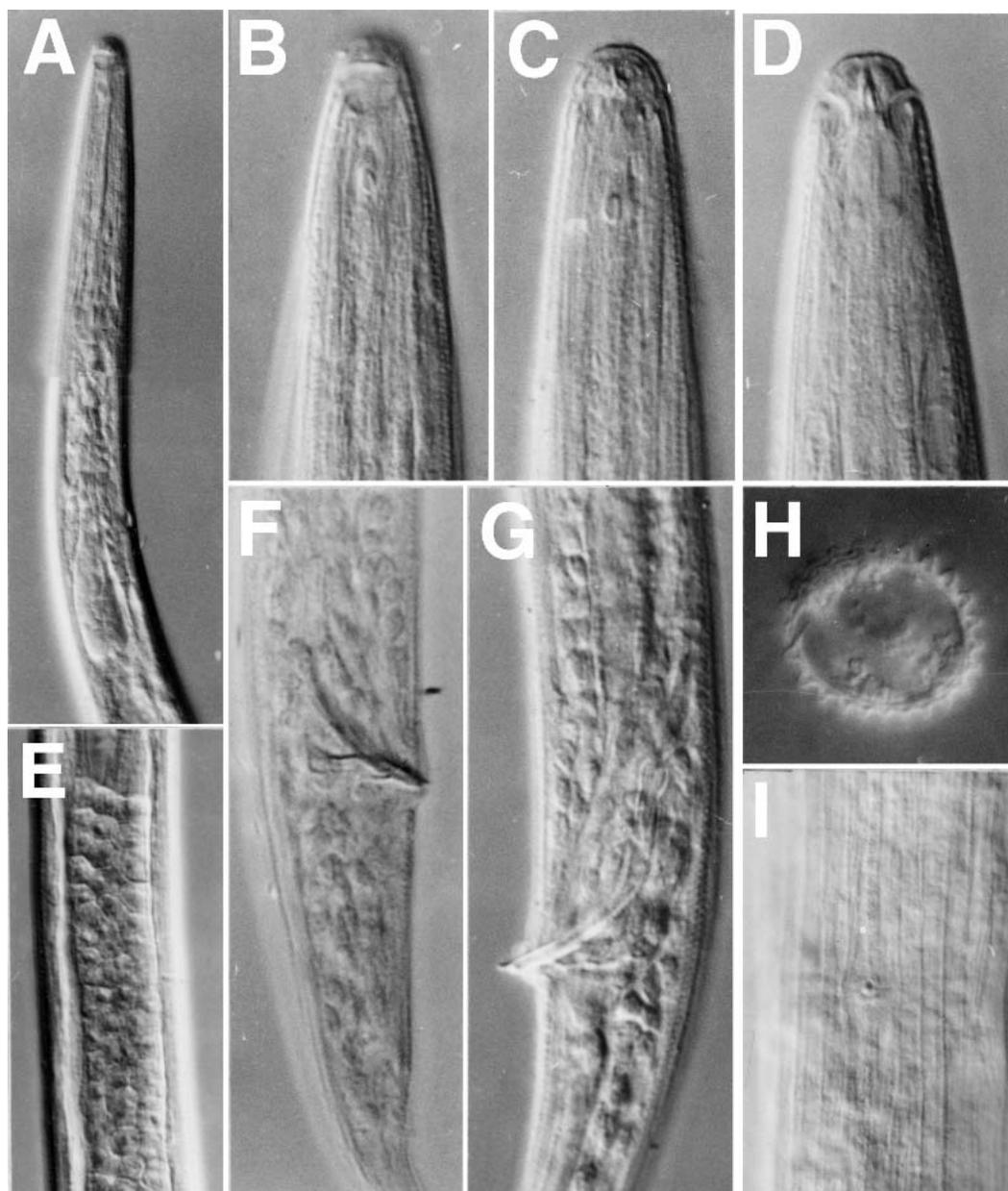


FIG. 2. *Tylopharynx clariamphida* sp. n. A) Anterior region. B) Head region, lateral view showing amphid. C) Head region, left-lateral view. D) Head region, ventral view. E) Part of testis showing spermatozoa. F) Cloacal region of male showing gubernaculum. G) Cloacal region of male showing spicule. H) Cross section showing longitudinal ridges. I) Vulva, ventral view.

dinal ridges 26 in number at mid-body region (counted on cross-sections, $n = 3$), ridges 1.0–1.5 μm high and 1.3–1.5 μm wide, separated by 1.5 to 2.6- μm -wide longitudinal grooves; 24 in number in procorpus region and at anal region ($n = 4$), ridges 0.8–1.1 μm high and 0.7–1.1 μm wide, separated by 0.6 to 1.0- μm -wide longitudinal grooves. Lateral field inconspicuous. Lip region separated from the rest of the body by a slight depression, more or less rounded, 4.2–5.3 μm high. Six lips amalgamated, each bearing a small conical labial sensillum. Amphidial fovea distinct in lateral view, stirrup-shaped. Amphidial aperture slit-like, located at the base of lip region, 4.5–6.3 μm wide, slightly shorter than corresponding body width.

Stoma 12–14 μm long, anteriorly with a dorsal tooth

and a right ventrosublateral tooth followed by a narrow, 9 to 11- μm -long tube with two subdorsal knobs at its base. Knobs 0.7–1.1 μm in diam. $\frac{1}{12}$ – $\frac{1}{13}$ of corresponding body width. Pharynx diplogasteroid: procorpus cylindrical, muscular, 40–48 μm long, widening toward a prominent median bulb. Median bulb measuring 16–18 by 10–12 μm , with crescentic longitudinal valves. Distance from anterior end to the end of median bulb equals 72–83 μm . Isthmus narrow, expanding into a valveless basal bulb, length of entire postcorpus 62–92 μm . Nucleus of dorsal pharyngeal gland distinct, at 3.5–5.6 μm from the base of the pharynx. Nerve ring surrounding isthmus at 88–105 μm from anterior end. Excretory orifice located opposite posterior half of isthmus, i.e., at 110–137 μm from anterior end. Hemizonid

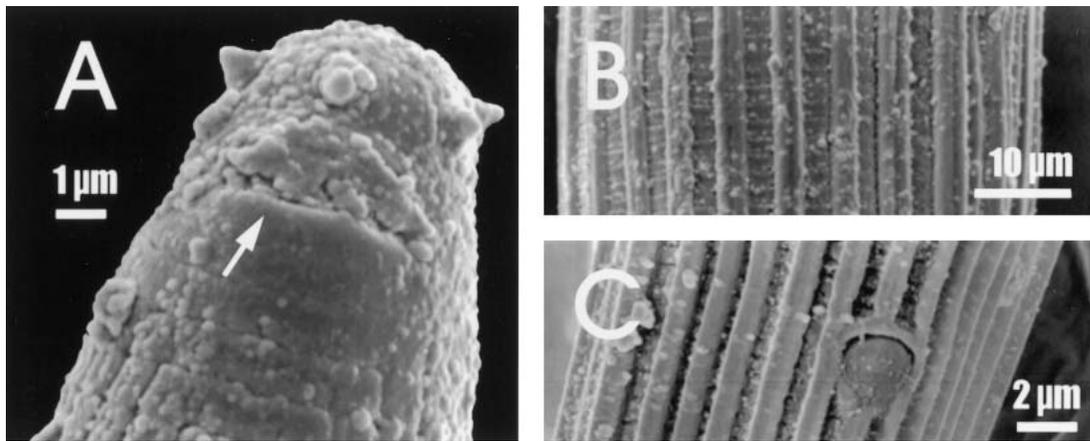


FIG. 3. *Tylopharynx clariamphida* sp. n.—female. A) Lip region (arrow points at partly covered amphidial aperture). B) Cuticle near mid-body. C) Anus.

anterior to excretory orifice. Deirids detected only in three females, 14–18 μm posterior to excretory orifice. Cardia small. Rectum 18–21 μm long, 1.2–1.9 times anal body width. Tail conical with a long, filiform terminus. Phasmids enlarged, scutellum-like, at 30–49 μm posterior to anus.

Female reproductive system didelphic, amphidelphic, with antidromously reflexed ovaries. Anterior gonad on the right side and posterior gonad on the left side of intestine in all females. Sphincter present at junction of oviduct and uterus. Vagina extending $\frac{1}{4}$ – $\frac{1}{3}$ of vulval body width. Vulva a small pore or short longitudinal slit, vulval region bulging slightly.

Males: Similar to females in most respects. Body straight after fixation except for the ventrally curved cloacal region. Phasmids located at 23–26 μm from anus. Testis single, lying on the left side of intestine (in holotype male and two paratype males) or on the ventral side of intestine (in one paratype male), reflexed dorsad or laterad. Length of reflexed part 1.1–1.4 times as long as corresponding body width. Spermatozoa with nucleus, rounded, 4.0–5.5 μm in diam. Spicules arcuate, 24–25 μm long, cephalated by constriction, distal end articulate with a digitate terminus of 1.5–2.0 μm . Gubernaculum 8.5–9.5 μm long, 2.1–2.5 μm thick. Nine pairs of caudal papillae present, typically arranged. Five pairs ventrolateral, of which three pairs located anterior to cloacal opening and two pairs posterior to it. Three subventral pairs grouped close together posterior to phasmids. One pair subdorsal, posterior to subventral group. Bursa absent.

Type locality and habitat

Collected in June 1996 from mud in a small ditch from Guichi city (30°6'N, 117°5'E), Anhui Province, China.

Type specimens

Holotype male (slide AHN96003a) with three paratype males and eight paratype females (slides AHN96003b–e) deposited in the Specimen Room of In-

vertebrates, Institute of Hydrobiology, Chinese Academy of Sciences, Wuhan, Hubei Province, China. One paratype female (slide 3994) deposited in the collection of the Biology Department, Universiteit Gent, Belgium.

Etymology: The specific epithet is derived from the Latin adjective “clarus” and refers to the distinct amphids of this new species.

Diagnosis and relationships: *Tylopharynx clariamphida* sp. n. is close to *T. foetida* (Bütschli, 1874) in the structure of stoma but can be distinguished by numerous characters, e.g., 24–26 prominent and clearly separated longitudinal ridges, a higher lip region with no hint of a cephalic framework, more prominent amphidial foveae in lateral view, wider and more posteriorly located amphidial apertures, smaller basal knobs, longer metacarpus, larger phasmids, shorter and less strongly curved spicules with shorter digitate terminus, shorter reflexed part of testis, and thicker gubernaculum with more angular shape.

In *T. foetida*, the cuticle bears longitudinal striae that demarcate 41–50 inconspicuous and contiguous longitudinal ridges; lip region is 3.0–4.0 μm high and appears to have a weak cephalic framework; amphidial fovea is indistinct in lateral view; amphidial aperture is inconspicuous because it faces forward, lies very close to the stomatal opening, and is only 1.8–2.1 μm wide; basal knobs of the stoma are 1.5–2.5 μm in diam.; the metacarpus is 12–14 μm long; phasmids are slit-shaped; the spicule is 30–40 μm long and its digitate terminus 3.5–5.0 μm long; the reflexed part of testis is 2.3–3.1 times as long as the corresponding body width, and the gubernaculum is only 1.0–1.5 μm thick and therefore much thinner in lateral aspect.

Tylopharynx foetida (Bütschli, 1874) Goffart, 1930 (Figs. 4–7)

Males ($n = 7$): L = 741 (626–881) μm ; L' = 580 (472–690) μm ; a = 32 (24–38); b = 5.8 (5.1–6.8); c = 5.3 (3.3–11); c' = 10 (3.7–14)

Females ($n = 10$): $L = 837$ (731–910) μm ; $L' = 624$ (533–703) μm ; $a = 30$ (26–35); $b = 6.1$ (5.5–6.8); $c = 4.0$ (3.4–4.8); $c' = 12$ (9.8–15); $V\% = 44$ (40–45); $V'\% = 58$ (55–62)

Females: Body almost straight after fixation. Cuticle 0.5–1.0 μm thick, faintly annulated, and bearing shallow longitudinal striae. Annules 1.1–1.4 μm wide. Longitudinal striae dividing cuticle into 41–46 contiguous longitudinal ridges at midbody (counted on cross-sections and including two wings of each lateral field, $n = 4$), ridges about 0.5 μm high and 1.5–2.0 μm wide, except for the wings of each lateral field, which can be up to 1 μm high and 3 μm wide. Lip region appears

smooth, separated from the rest of the body by a slight depression, more or less truncate, 3.0–3.5 μm high. Somatic muscle attachments at base of lip region clearly refringent, creating the appearance of a moderately developed cephalic framework. Six lips amalgamated, each bearing a small labial sensillum. Amphidial fovea rarely distinct in lateral view, goblet-shaped. Amphidial apertures slit-like, located adjacent to the mouth opening, more or less obscure in lateral view.

Mouth opening slit-shaped with folded rims. Stoma 14–15 μm long, anteriorly with a dorsal tooth and a right ventrosublateral tooth followed by a narrow, 9 to

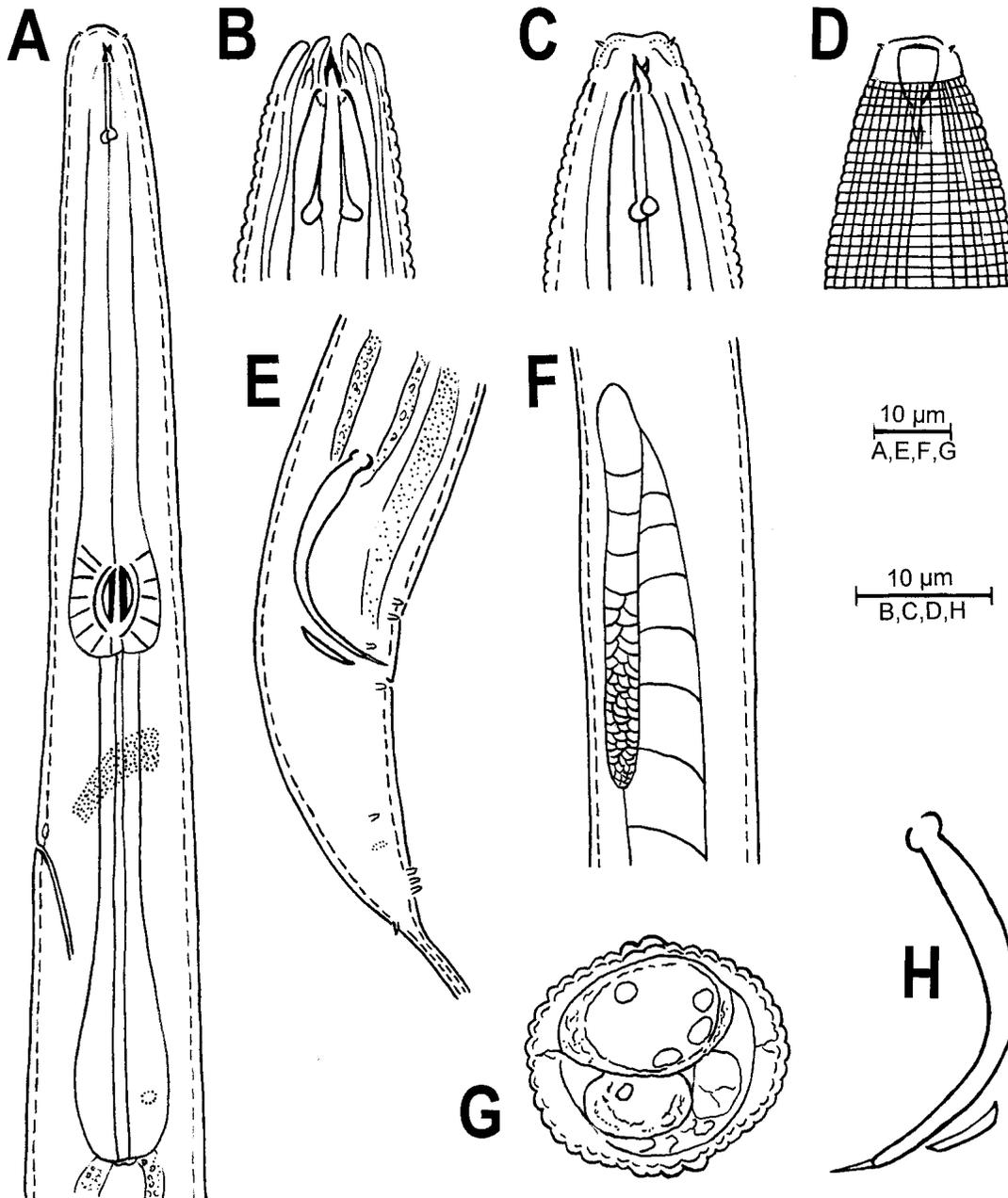


FIG. 4. *Tylopharynx foetida* (Bütschli, 1874) Goffart, 1930. A) Anterior region. B) Head region, ventral view. C) Head region, left-lateral view. D) Head region, lateral view showing amphid. E) Cloacal region of male. F) Anterior reflexed part of testis, right-lateral view. G) Cross section showing longitudinal ridges. H) Spicule and gubernaculum.

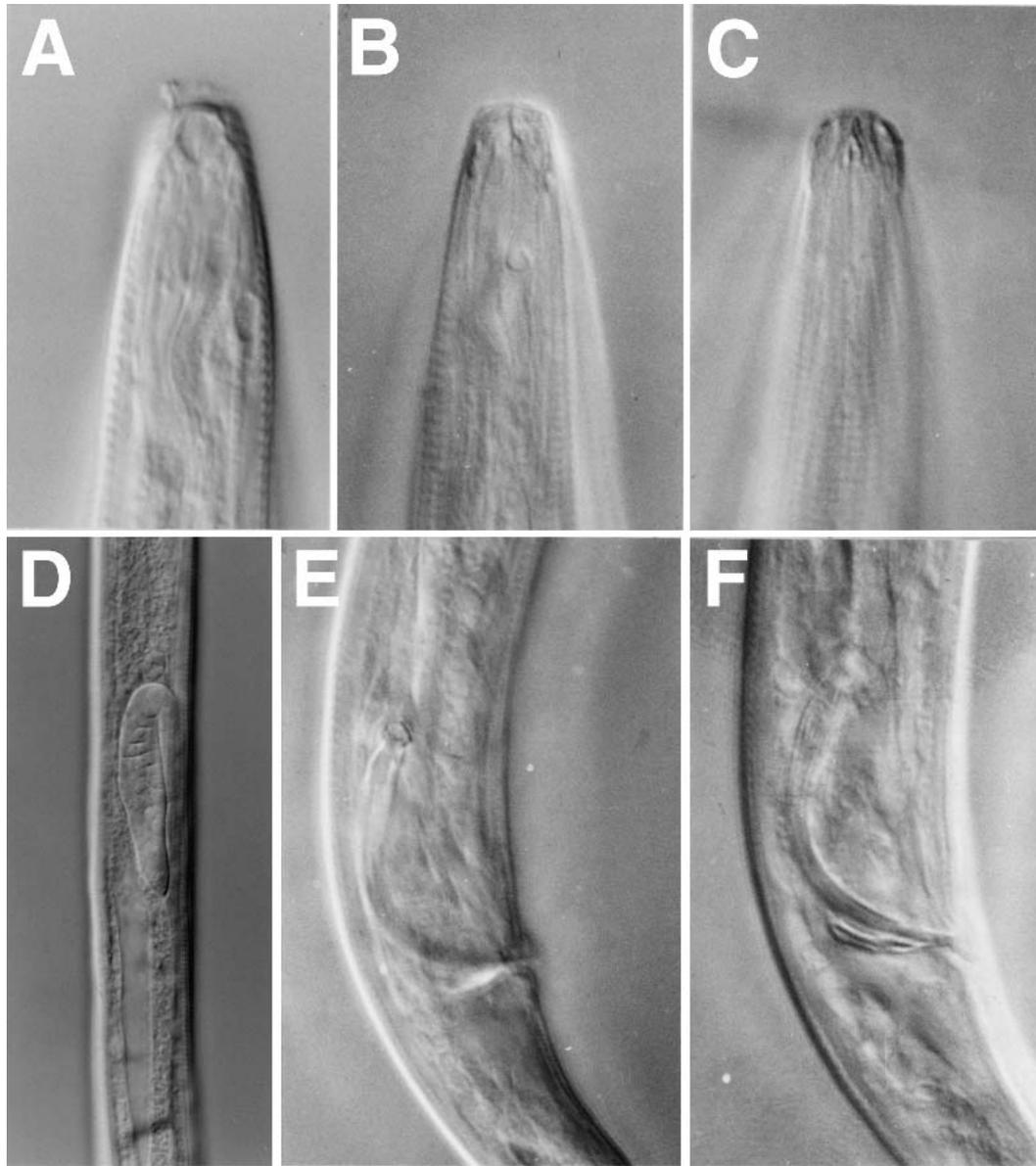


FIG. 5. *Tylopharynx foetida* (Bütschli, 1874) Goffart, 1930—male. A) Head region, lateral view showing amphid. B) Head region, left-lateral view. C) Head region, ventral view. D) Anterior reflexed part of testis, right-lateral view. E) Cloacal region showing proximal part of spicule. F) Cloacal region showing gubernaculum.

11- μ m-long tube with two subdorsal knobs at its base. Knobs 1.5–2.0 μ m in diam., $\frac{1}{6}$ – $\frac{1}{8}$ of corresponding body width. Pharynx diplogasteroid: procorpus cylindrical, muscular, 41–49 μ m long, widening toward a prominent median bulb. Median bulb measuring 12–16 by 12–14 μ m, with crescentic longitudinal valves. Distance from anterior end to the end of median bulb equals 72–81 μ m. Isthmus narrow, expanding into a valveless basal bulb with slight ventral overlap of the intestine. Length of entire postcorpus 59–65 μ m. Nucleus of dorsal pharyngeal gland distinct, at 5–10 μ m from the base of the pharynx. Nerve ring surrounding isthmus at 83–92 μ m from anterior end. Excretory orifice located near posterior half of isthmus, i.e., at 96–107 μ m from anterior end. Hemizonid just anterior to

excretory orifice. Deirids small but distinct, 0–12 μ m posterior to excretory orifice. Cardia indistinct. Intestine in nearly all specimens densely packed with spherical nucleate bodies resembling spore-like structures and measuring 2.5–5.0 μ m in diam. Rectum 15–22 μ m long, 0.9–1.4 times as long as anal body width. Tail conical, tapering smoothly to a long, filiform terminus that is often broken or knotted. Phasmids enlarged, 1.0 to 1.5- μ m-wide oblique or transverse slits, at 17–29 μ m posterior to anus.

Female reproductive system didelphic, amphidelphic, with antidromously reflexed ovaries. Anterior gonad on the right side and posterior gonad on the left side of intestine in all females. Sphincter present at junction of oviduct and uterus. Vagina extending $\frac{1}{4}$ – $\frac{2}{5}$

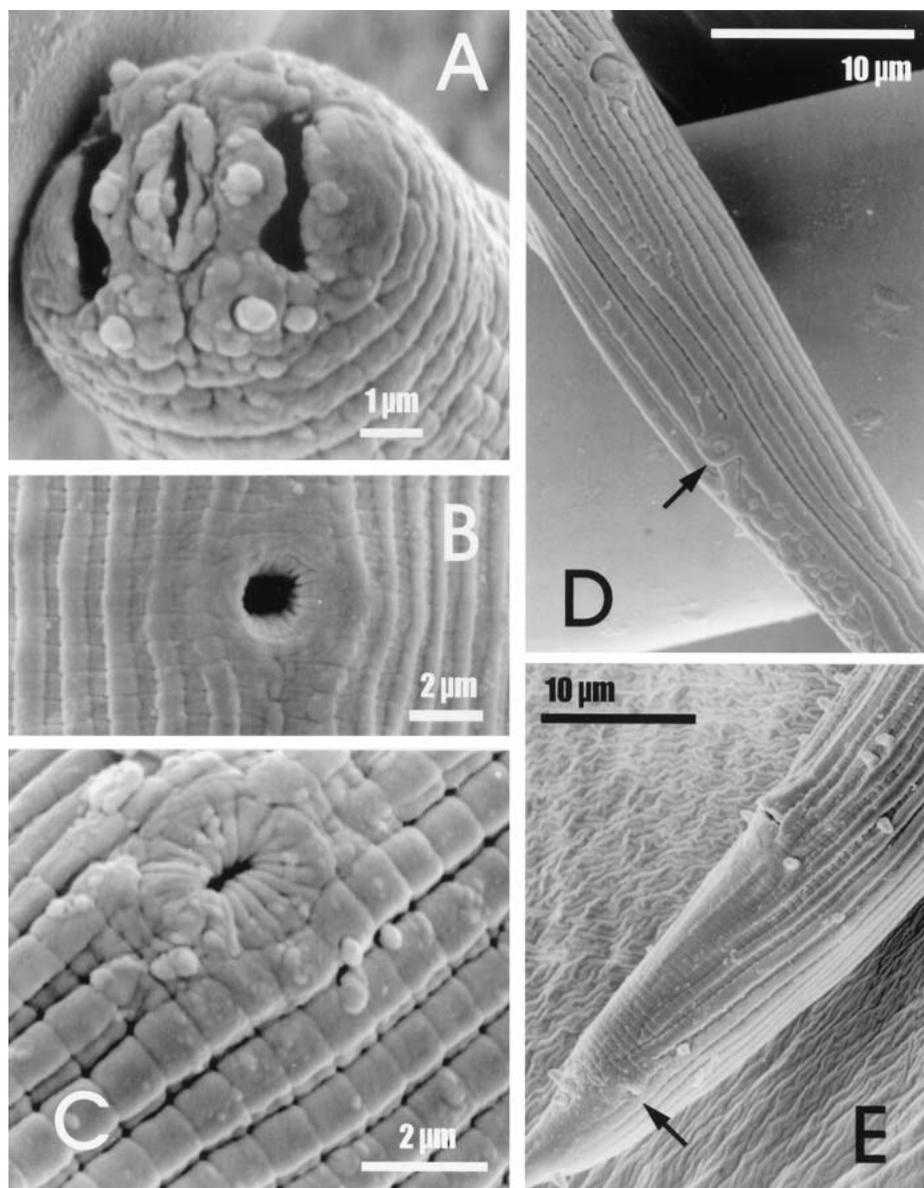


FIG. 6. *Tylopharynx foetida* (Bütschli, 1874) Goffart, 1930—male and female. A) Lip region of male. B) Pore-shaped open vulva. C) Slit-shaped closed vulva. D) Anterior part of female tail (arrow points at phasmid). E) Anterior part of male tail (arrow points at phasmid).

of vulval body width. Vulva a small pore or short longitudinal slit, vulval region bulging slightly.

Males: Similar to females in most respects. Body straight after fixation except for the ventrally curved cloacal region, with 42–50 longitudinal striae at mid-body ($n = 4$). Lip region with four cephalic sensilla, in addition to the six labial sensilla also found in the females. Phasmids located 19–25 μm from anus, positioned ventrosublaterally rather than midlaterally. Testis single, reflexed laterad or dorsad, located on right side of intestine (in all seven males). Reflexed part 2.3–3.1 times as long as corresponding body width. Spermatozoa with nucleus, rounded, 3.5–5.0 μm in diam. Spicules arcuate, 30–40 μm long, cephalated by constriction, distal end articulate with a digitate terminus of 3.5–5.0 μm . Gubernaculum 7.0–10.0 μm long, 1.0–

1.5 μm thick. Nine pairs of caudal papillae, arranged as follows: two pairs ventrosublateral, of which one is anterior to the cloacal opening and one anterior to phasmids. Three pairs ventrolateral, of which two pairs are located anterior to the cloacal opening and one pair level with or just posterior to it. Three subventral pairs grouped close together posterior to phasmids. One pair subdorsal, posterior to subventral group. Bursa absent.

Locality and habitat

Collected in May 1999 from soil in a pigeon aviary at Kasteellaan 21 in Landegem, Belgium.

Reference specimens

Six males and six females deposited in the Specimen Room of Invertebrates, Institute of Hydrobiology, Chi-

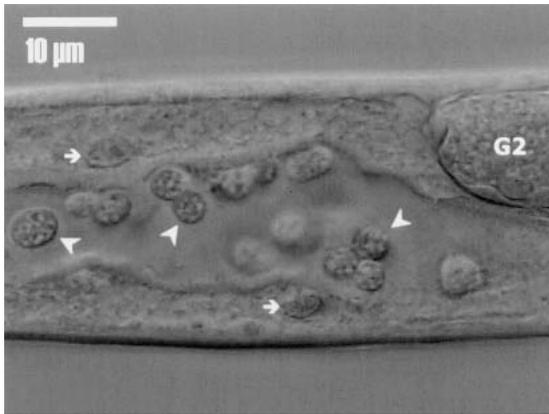


FIG. 7. *Tylopharynx foetida* (Bütschli, 1874) Goffart, 1930. Intestinal lumen near the posterior reproductive branch of a female, showing spore-like structures (arrowheads), nuclei of intestinal cells (arrows), and the tip of the largest oocyte in the posterior ovary (G2).

nese Academy of Sciences, Wuhan, Hubei Province, China. One male and five females kept in the collection of the second author in the Biology Department, Universiteit Gent, Belgium.

Remarks:

Although longitudinal striae were not previously reported for *T. foetida*, we do not doubt that our population truly represents this species in view of the inconspicuous nature of the striae, the general agreement with previous descriptions in other respects (cf. Goodey, 1929; Goodey, 1963), and the relative proximity of the sampling site to the type locality. The presence of anteriorly facing amphids in *T. foetida* was previously reported in De Ley et al. (1993) and De Ley et al. (1994) but not illustrated.

In discussing the ultrastructure of the buccal cavity of *T. foetida*, De Ley et al. (1993) speculated that this species might have highly specialized feeding habits but provided no clues as to the nature of its food source(s). Furst von Lieven and Sudhaus (2000) revealed that the buccal structures of *T. foetida* are used to tear apart the

cell walls of fungal hyphae and to suck out their contents. Our observations on live and fixed specimens from Landegem revealed the presence of small spore-like structures in the intestine (Fig. 7), suggesting that fungal spores could also be an important food source of *T. foetida*.

LITERATURE CITED

Andrássy, I. 1984. Klasse Nematoda (Ordnungen Monhysterida, Desmoscolecida, Araeolaimida, Chromadorida, Rhabditida). Stuttgart: Gustav Fischer Verlag.

De Ley, P., A. Coomans, and E. Geraert. 1994. Terrestrial nematodes of the Galápagos archipelago. 3. *Aenigmenchus floveanae* gen.n., sp.n., a puzzling new secernentean (Diplogasterida: Tylopharyngidae?). *Fundamental and Applied Nematology* 17:339–346.

De Ley, P., A. T. De Grisse, M. C. Van de Velde, and A. Coomans. 1993. Ultrastructure of the stoma in *Tylopharynx*. *Mededelingen Fakulteit Landbouwwetenschappen Universiteit Gent* 58:763–777.

Furst von Lieven, A., and W. Sudhaus. 2000. Comparative and functional morphology of the buccal cavity of Diplogastrina (Nematoda) and a first outline of the phylogeny of this taxon. *Journal of Zoological Systematics and Evolutionary Research* 38:37–63.

Goodey, J. B. 1963. Speculations on the identity of the parts of the Tylenchid spear. *Nematologica* 9:468–470.

Goodey, T. 1929. On some new and little-known free-living nematodes. *Journal of Helminthology* 7:27–62.

Hooper, D. J. 1990. Extraction and processing of plant and soil nematodes. Pp. 45–68 in M. Luc, R. A. Sikora, and J. Bridge, eds. *Plant-parasitic nematodes in subtropical and tropical agriculture*. Wallingford, UK: CAB International.

Maggenti, A. R. 1963. Comparative morphology in nemic phylogeny. Pp. 273–282 in E. D. Dougherty, ed. *The lower Metazoa—comparative biology and phylogeny*. Berkeley: University of California Press.

Maggenti, A. R. 1983. Nematode higher classification as influenced by species and family concepts. Pp. 25–40 in A. R. Stone, H. M. Platt, and L. F. Khalil, eds. *Concepts in nematode systematics*. London: Academic Press.

Poinar, G. O. 1983. *The natural history of nematodes*. New Jersey: Prentice-Hall.

Seinhorst, J. W. 1962. On the killing, fixation, and transferring to glycerin of nematodes. *Nematologica* 8:29–32.

Siddiqi, M. R. 1980. The origin and phylogeny of the nematode order Tylenchida Thorne, 1949 and Aphelenchida n. ord. *Helminthological Abstracts Series B* 49:143–170.

Somerfield, P. J., and R. M. Warwick. 1996. *Meiofauna in marine pollution monitoring programmes. A laboratory manual*. Suffolk: Ministry of Agriculture, Fisheries and Food.