

# Description of *Pristionchus stercoraris* sp. n. and first report of *Tylopharynx foetida* from India (Nematoda: Diplogastrina)

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*Pristionchus stercoraris* sp. n. and *Tylopharynx foetida* (Bütschli, 1874) Goffart, 1930 of the suborder Diplogastrina are described and illustrated in detail. Both the species were isolated from farmyard manure collected from Baramulla, Jammu & Kashmir, India. *P. stercoraris* sp. n. is characterized by faint annulations on cuticle, steno- as well as eury stomatous types of stoma, thin-walled cheilostom, long elongate cardia, slender spicules with slightly anteriorly curved distal tip, a boat-shaped gubernaculum with a medial dorsal convex; proximally with an anteriorly directed needle-like extension and distally with a sleeve and eight pairs of genital papillae. Measurements and descriptions of our specimens of *T. foetida* agree well with that of Bütschli (1874). However, slight differences were observed in the length of stoma, tail, anal body diameter, anterior pharynx, and in the position of excretory pore. No species of both *Pristionchus* and *Tylopharynx* have so far been reported from India.

**Key words:** Descriptions, farmyard manure, first report, new species, *Pristionchus*, taxonomy, *Tylopharynx*

## INTRODUCTION

Andrássy (1984) placed *Pristionchus* Kreis, 1932 under Neodiplogastridae and *Tylopharynx* de Man, 1876 under Tylopharyngidae. Fedorko and Stanuszek (1971) described *P. uniformis* as a facultative parasite of *Leptinotarsa decemlineata* and *Melolontha melolontha* from Poland. Sommer *et al.* (1996) described *P. pacificus* after which this species has been established as a satellite organism in evolutionary developmental biology (Eizinger *et al.*, 1999; Sommer, 2000). Herrmann *et al.* (2006) showed that some *Pristionchus* sp. are often associated with scarab beetles and the Colorado potato beetle. Kanzaki *et al.* (2012) also described *P. fissidentatus* from Nepal and La Re´union Island and *P. elegans* from Japan.

The controversy regarding the origin and relationships of the genus *Tylopharynx* (Andrássy 1984; Maggenti, 1963; 1983; Poinar, 1983; Siddiqi, 1980) was resolved when De Ley *et al.* (1993), based on detailed observations of the ultrastructure of the stoma of *T. foetida*, suggested that there is no strong affinity between the stoma of *Tylopharynx* and the stylet of Tylenchida. Wu *et al.* (2001) described *T. clariamphida* and also redescribed *T. foetida* (Bütschli, 1874) Goffart, 1930. Fürst von Lieven (2002) considered *Tylopharynx* de Man, 1876 to be a sister taxon of the genus *Neodiplogaster* Cobb, 1924. These two genera share a narrow and elongated cylindrical stegostom extending to the periradial struts which possess a subdorsal apodeme as a synapomorphic character. Sudhaus and Fürst von Lieven (2003) accepted 27 species in *Pristionchus* and two in *Tylopharynx*.

In the present paper, a new species, *P. stercoraris* sp. n. and a known species, *T. foetida* (Bütschli, 1874) Goffart, 1930 under the suborder Diplogastrina are described and illustrated in detail. *P. stercoraris* sp.

n. and *T. foetida* were isolated from farmyard manure collected from Baramulla, Jammu and Kashmir. No species of *Pristionchus* and *Tylopharynx* have so far been reported from India. The terminology used in the text to describe the parts of stoma is of De Ley *et al.* (1995) and of other structural details of Fürst von Lieven and Sudhaus (2000).

## MATERIAL AND METHODS

The nematodes were extracted by modified Cobb's sieving and decantation and modified Baermann's funnel techniques. The nematodes thus extracted were killed and fixed in hot FA (4:1) for 24h and then transferred to glycerin-alcohol (5parts:95parts 30% alcohol) for slow dehydration in a desiccator containing fused calcium chloride. Dehydrated specimens were mounted in anhydrous glycerin on glass slides and sealed with wax. All observations, drawing and photographs were made on an Olympus BX 50 DIC microscope.

## RESULTS

### Description

***Pristionchus stercoraris* sp. n.** (Figs 1 & 2)

*Measurements:* See Table I.

*Female:* Body straight to slightly arcuate on fixation. Cuticle with faint transverse annulations. Lip region round, continuous. Lips six, each bearing a small papilla. Amphidial openings small, oval, at the level of anterior end of the dorsal tooth. Stoma spacious, 8–9  $\mu\text{m}$  long. Cheilostom represented by a thin-walled arch. Gymnostom anisotropic, dorsal wall shorter than subventrals. Stegostom anisomorphic, dorsal wall with a large claw-like tooth, right subventral wall with a retrose triangular tooth and left subventral with a serrated ridge. Anterior pharynx muscular, 57–65% of the pharyngeal length. Median bulb muscular, oval-shaped with strong valve-plates. Isthmus long expanding posteriorly to a globose, glandular basal bulb. Nerve ring located at middle of the isthmus, at 65–79% of pharyngeal length from anterior end. Hemizonid at 79–83% of total pharynx length from anterior end. Excretory pore just posterior to hemizonid, opposite basal bulb. Cardia large elongate. Intestinal cells large with prominent nuclei.

Reproductive system amphidelphic. Anterior genital branch on right and posterior on left side of intestine. Ovaries reflexed, tip of ovaries extending beyond vulva. Oviduct long, narrow. Spermatheca oval, generally continuous with uterus, containing sperms usually. Uterus divisible into a long glandular and a short muscular part. Vulva transverse. Rectum 1.1–1.4 times anal body diameter. Phasmids 1.2–1.7 anal body diameters posterior to anus. Tail acute, long, tapering gradually from anus to the terminus.

*Male:* Body J-shaped when heat-relaxed. Stoma as in females. Anterior end similar to that of females except for four additional cephalic setae. Testis single and reflexed ventrally. Spicules slender, arcuate, cephalated, 1.5–1.6 anal body diameters long, with distal tip slightly curved anteriorly. Gubernaculum boat-shaped with a medial dorsal convex, proximally with an anteriorly directed needle-like extension and distally with a sleeve, 48–50% of the spicules length. Genital papillae eight pairs; two pairs precloacal and six pairs postcloacal. Genital papillae formula: v1, v3d/v4, ad, phasmids, (v5, v6, v7), pd. GP v1 beyond the range of spicules when retracted, GP v2 absent. GP v4 just posterior to cloaca. Phasmids posterior to ad. GP v7 and pd almost at the same level. Tail divisible into two parts, a short conoid and a long filamentous part.

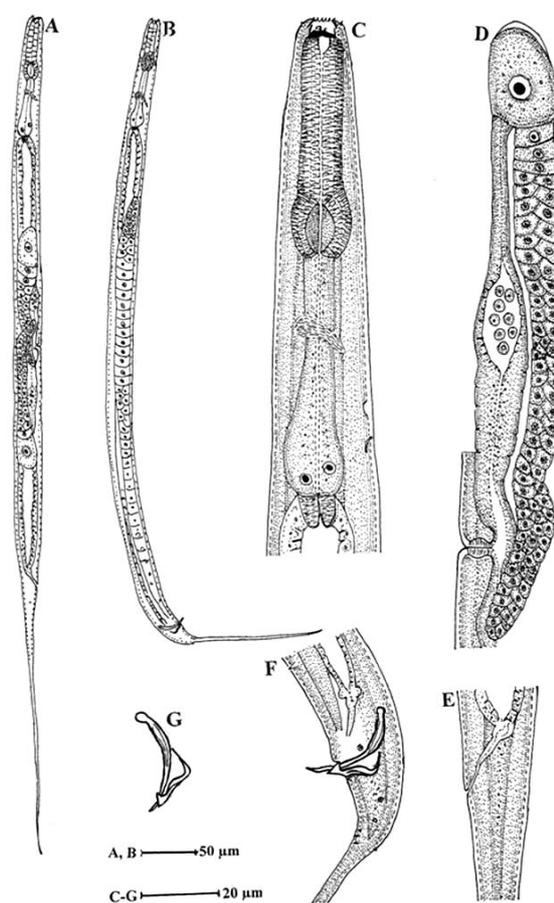
*Diagnosis and relationships:* The new species *Pristionchus stercoraris* sp. n. is characterized by faint transverse annulations on cuticle, long isthmus, elongate cardia, slender spicules with a slightly anteriorly curved distal tip, a boat-shaped gubernaculum with a medial dorsal convex; proximally with an anteriorly directed needle-like extension and distally with a sleeve and eight pairs of genital papillae.

The new species resembles *P. lheritieri* (Maupas, 1919) Paramonov, 1952. However, the new species differs from *P. lheritieri* in having smaller spicules (29–30  $\mu\text{m}$  long *vs* 35–50  $\mu\text{m}$  long), shape of gubernaculum (boat-shaped, a sharp anteriorly directed needle-like extension proximally *vs* proximally rounded), in the number and arrangement of genital papillae (eight pairs: two precloacal, six postcloacal *vs* nine pairs: three precloacal, six postcloacal), more slender body ( $a = 28.9\text{--}33.2$  *vs* 16.0–17.0 in females and 25.6–30.3 *vs* 13.0–20.0 in males), slightly longer tail in males (4.2–4.4 *vs* 5.0–9.0), greater  $c'$  value (13.8–19.0 *vs* 6.0–10.0 in females and 7.9–8.4 *vs* 3.0–4.0 in males) and in the slightly anterior position of vulva ( $V = 39\text{--}41\%$  *vs* 44–50%).

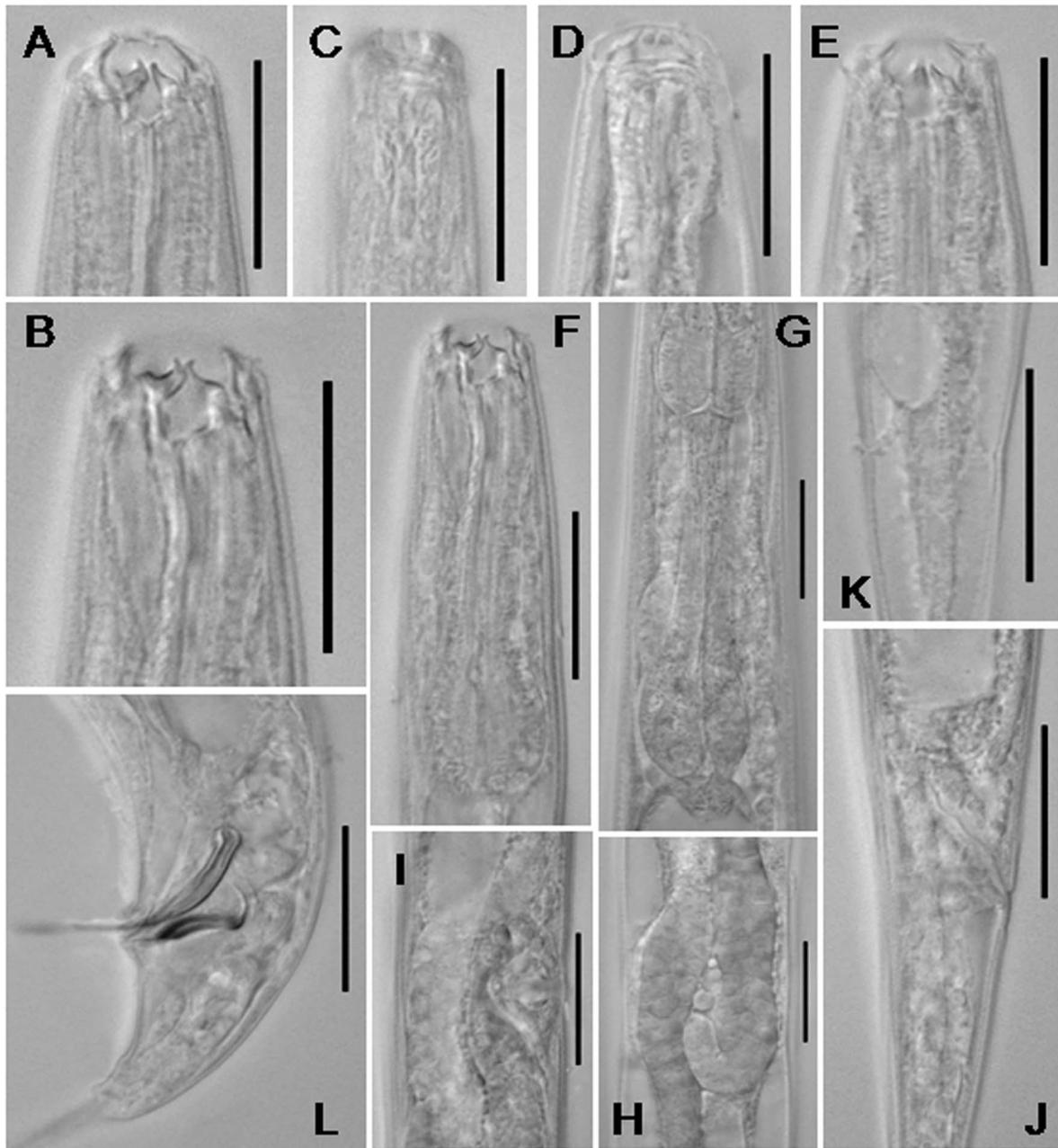
*Type habitat and locality*: Farmyard manure collected from Baramulla, Jammu and Kashmir, India.

*Type material*: Holotype female on slide *Pristionchus stercoraris* sp. n./5; paratype females and males on slides *P. stercoraris* sp. n./1–4 deposited in the nematode collection of Department of Zoology, Aligarh Muslim University, Aligarh, India.

*Etymology*: The name of the species is derived from the Latin word '*stercore*' meaning manure from which it was collected.



**FIGURE 1.** *Pristionchus stercoraris* sp. n. A: Entire female; B: Entire male; C: Pharyngeal region; D: Female reproductive tract (Anterior); E: Female posterior region; F: Male posterior region; G: Spicules & Gubernaculum.



**FIGURE 2.** *Pristionchus stercoraris* sp. n. (Scale bars = 20µm). A & B: Anterior region showing stoma; C & D: Anterior region showing cheilorhabdions; E: Anterior region showing amphids (dorsoventral); F: Anterior pharynx; G: Median bulb, isthmus, basal bulb & cardia; H: Ovaries showing overlap; I: Vulval region; J: Female posterior region; K: Female posterior region showing phasmids (dorsoventral); L: Male posterior region showing spicules & gubernaculum.

***Tylopharynx foetida*** (Bütschli, 1874) Goffart, 1930 (Figs 3 & 4)

*Measurements:* See Table II.

*Female:* Body almost straight upon fixation. Cuticle faintly annulated, bearing shallow longitudinal striae. Lip region appears smooth, continuous with the body contour or separated from the body by a slight depression. Lips six, amalgamated, each bearing a minute labial papilla. Amphidial fovea rarely distinct in lateral view, goblet-shaped. Amphidial apertures slit-like, just below the anterior end, more or less obscure in lateral view. Stoma 11–13  $\mu\text{m}$  long. Chielostom and gymnostom very short. Stegostom long, narrow, tube-like, anteriorly with a dorsal tooth and a right subventral tooth and posteriorly with two subdorsal knobs at base. Knobs 1.5–2.0  $\mu\text{m}$  in diameter. Anterior pharynx cylindrical, muscular, 55–58% of total pharyngeal length. Median bulb measuring 12–15  $\mu\text{m}$  in length and 9–11  $\mu\text{m}$  in width with crescentic valves. Isthmus narrow, expanding into a glandular basal bulb. Nerve ring surrounding isthmus at 71–106  $\mu\text{m}$  from anterior end. Hemizonid at 75–83% of the total pharyngeal length from anterior end. Excretory pore located posterior to hemizonid, opposite to the basal bulb. Cardia small but distinct. Intestine with wide lumen.

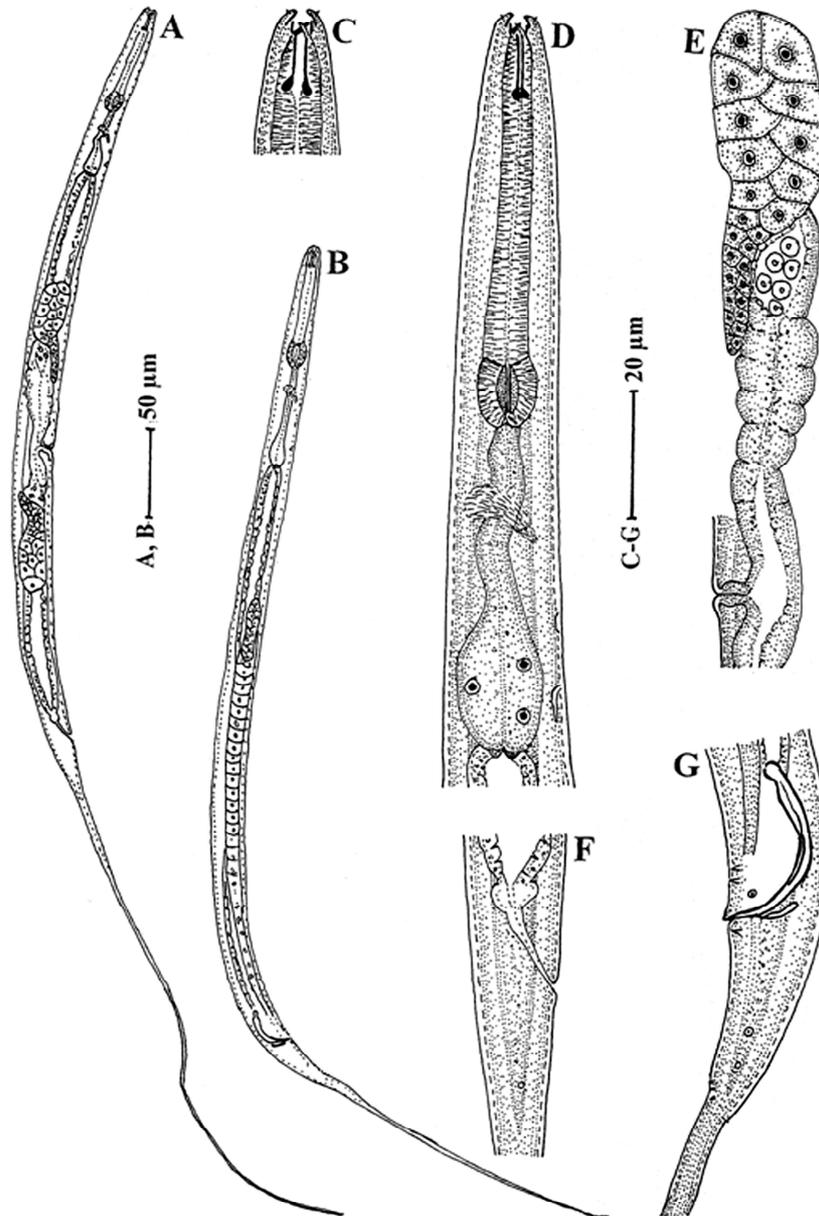
Reproductive system amphidelphic, with antidromously reflexed ovaries. Anterior gonad on the right side and posterior gonad on the left side of intestine in all females. Oocytes arranged in two or more rows. Oviduct short and narrow. Spermatheca slightly demarcated from uterus by a constriction. Uterus divisible into a large glandular and a narrow muscular part. Vulva small, pore-like. Vulva-anus distance 8–10 vulval body diameters long. Rectum 1.0–1.3 anal body diameters long. Tail long, filiform, 1.2–1.5 times vulva-anus distance long. Phasmids conspicuous, 1.2–1.7 times anal body diameter posterior to anus.

*Male:* Body slightly ventrally curved in posterior region. Lip region with four cephalic sensilla, in addition to the six labial sensilla. Testis single, flexure on right side of intestine. Spicules arcuate, 1.9–2.0 times anal body diameter long, proximal end cephalated and distal end with a pointed digitate terminus. Gubernaculum simple, thin, 35.7–37.9% of spicules length. Nine pairs of genital papillae: three pairs precloacal, six pairs postcloacal. Genital papillae formula: (v1, v2), v3d / v4, ad, ph, (v5, v6, v7), pd. GP v2 lies adjacent to GP v1. GP v3d just above or at the level of upper cloacal lip. GP v1, v2 and v3d within the range of spicules. GP v4 slightly posterior to the cloaca. GP v5, v6, v7 grouped together posterior to phasmids. Phasmids located 1.2–1.3 anal body diameters from anus. Bursa absent. Rectum 1.2–1.3 anal body diameters long. Tail divisible in two parts; anterior short, conoid and posterior long, filamentous.

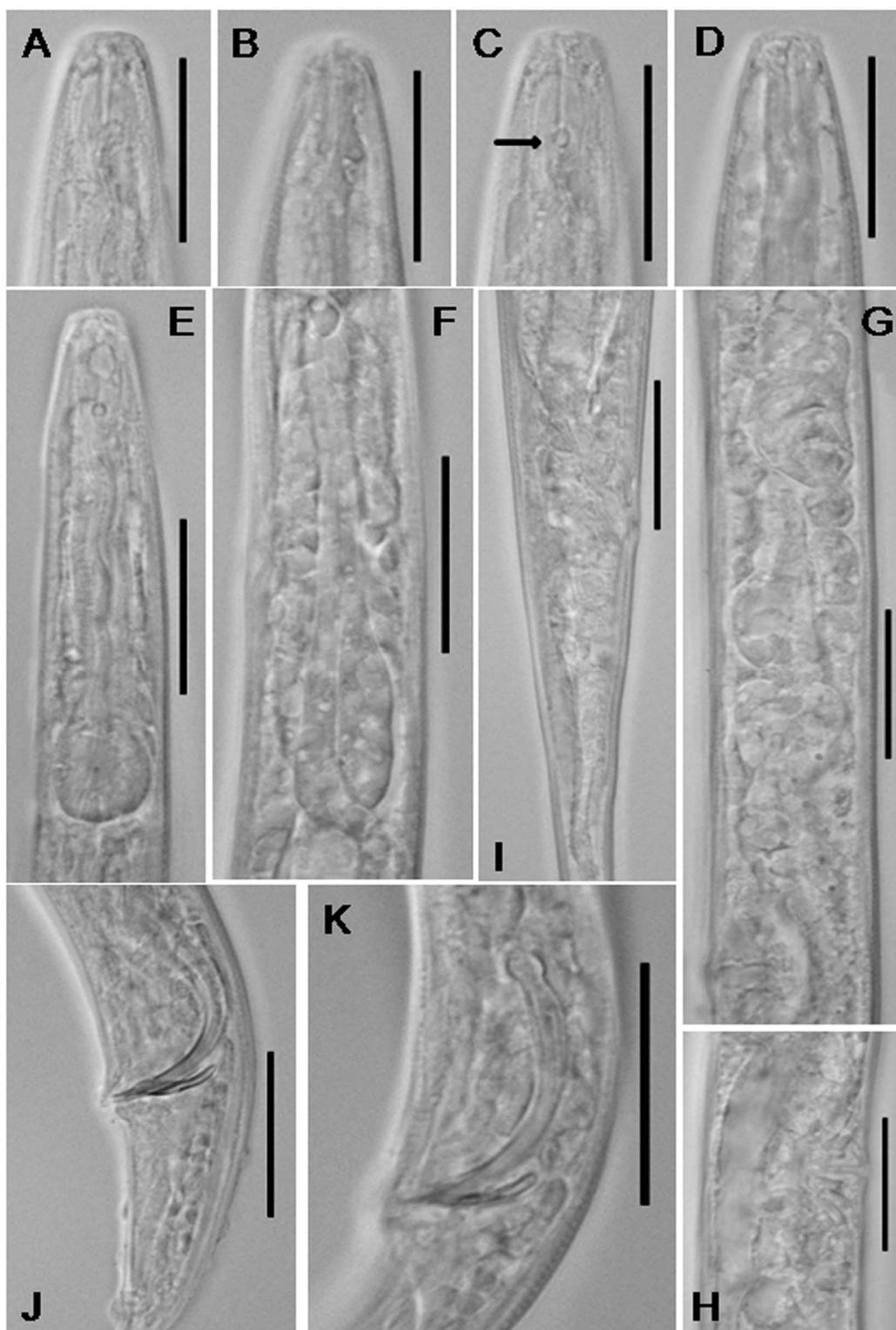
*Habitat and locality:* Farmyard manure collected from Baramulla, Jammu and Kashmir, India.

*Voucher specimens:* Six females and three males on slides *Tylopharynx foetida*/1–4; deposited in the nematode collection of Department of Zoology, Aligarh Muslim University, Aligarh, India.

*Remarks:* Measurements and descriptions of our specimens agree well with that of Bütschli (1874). However, differences were found in having slightly shorter stoma (11–13  $\mu\text{m}$  vs 14–15  $\mu\text{m}$ ), position of excretory pore (opposite to basal bulb vs opposite to isthmus), slightly smaller anterior pharynx (59–70  $\mu\text{m}$  vs 72–81  $\mu\text{m}$ ), shorter anal body diameter ( $c' = 18.2\text{--}20.4$  vs 9.8–15.0), and longer tail ( $c = 2.7\text{--}3.0$  vs 3.4–4.8). So far, there are only two species representing the genus *viz.*, *T. foetida* (Bütschli, 1874) Goffart, 1930 and *T. clariamphida* Wu *et al.*, 2001. *T. foetida* has been reported from different parts of Europe and *T. clariamphida* was collected from China. Besides, no species of the genus *Pristionchus* has so far been reported from the country. This is the first time that both the genera are being reported from India.



**FIGURE 3.** *Tylopharynx foetida* A: Entire female; B: Entire male; C: Anterior end; D: Pharyngeal region; E: Female reproductive tract (Anterior); F: Female posterior region; G: Male posterior region.



**FIGURE 4.** *Tylopharynx foetida*. (Scale bars = 20  $\mu$ m). A–D: Anterior region showing stoma, arrow showing subdorsal knobs; E: Anterior pharynx; F: Posterior pharynx; G: Female reproductive tract (anterior branch); H: Vulval region; I: Female posterior region; J & K: Male posterior region showing spicules & gubernaculum.

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