

# *Pterygorhabditis saprophila* sp. n. and *Bunonema flexistoma* sp. n. (Nematoda: Bunonematoidea) from India

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**Abstract.** Two new species belonging to superfamily Bunonematoidea are described and illustrated. *Pterygorhabditis saprophila* sp. n. is characterised by having 0.41-0.51 mm long and stout body; presence of heavily sclerotised transverse bars on cuticle; 5 fimbria-like projections in each lateral sector of lip region; absence of cuticular shield in anterior body region; presence of distinct vulval flaps; males with slender, distally fused spicules with elliptical capitula and an open, pseudopeloderan bursa with 9 pairs of genital papillae. *Bunonema flexistoma* sp. n. has 0.17-0.23 mm long body having 30-38 paired tubercles on right side, faint or obscure network between tubercle pairs; thick cuticularized cheilostom and metastegostom; dorsally arcuate mesostegostom and acutely pointed tail of 2-3 anal body diameters.

**Keywords.** *Bunonema flexistoma*, description, India, new species, *Pterygorhabditis saprophila*, taxonomy.

## INTRODUCTION

Bunonematids are free-living, terrestrial nematodes mostly found in moss, rotten wood, forest leaf litter, farmyard manure and organic debris. Micoletzky (1922) raised superfamily Bunonematoidea for the rhabditid species exhibiting asymmetry of lip region and prominent cuticular markings with warts or tubercles having a complex pattern of rods or network. Timm, 1957 proposed the genus *Pterygorhabditis* with *P. pakistanensis* as type species. Later *P. panopla* Bernard, 1979 and *P. hungarica* Andr ssy, 1982 were added to the genus. The genus *Bunonema* was erected with type species *B. richtersi* by J gerski ld in 1905. The other described species of the genus include *B. reticulatum* Richters, 1905; *B. multipapillatum* and *B. penardi* Stefanski, 1914; *B. hessi* Steiner, 1914; *B. steineri* Stefański, 1924; *B. ditlevseni* Micoletzky, 1925; *B. tuerkorum* Sachs, 1949; *B. franzi* Andr ssy, 1971 and *B. pustulatum* Andr ssy, 1986.

Little is known about the bunonematoid fauna of India, except for *Bunonema irregularis*, *B. minutum* and *Pterygorhabditis superbus* described by Huseni, Ahmad and Firdausi, 1997. A study of nematodes of cow dung and other organic manures in and around Aligarh, North India, yielded two new bunonematoid species belonging to genera *Pterygorhabditis* Timm, 1957 and *Bunonema* J gerski ld, 1905, which are described here. Manure samples were

processed by Cobb's (1918) sieving and decantation and modified Baerman's funnel techniques. Extracted nematodes were heat-killed and fixed in formalin-glycerol fixative, dehydrated by slow evaporation method (Seinhorst, 1959) and mounted in anhydrous glycerine on glass slides. The mounted nematodes were later measured by ocular micrometer and drawn using drawing tube attached to Olympus BX-51 DIC Microscope. LM photographs were taken using Olympus digital camera C3030.

## DESCRIPTIONS

### *Pterygorhabditis saprophila* sp. n.

(Figs 1, 2)

**Measurements.** See Table 1.

**Female:** Body 0.41-0.51 mm long, stout, tapering at both extremities. Cuticle with heavily sclerotised, thick, transverse bifid bars, forming flattened, hexagonal blocks arranged in six longitudinal rows. Bifid bars interlock with adjacent bars to form zigzag pattern longitudinally. Right side of body heavily annulated, annuli 2-2.5  $\mu$ m wide in different body regions. Cuticle on left side smooth with two faint longitudinal lines. Cuticular sheath not differentiated into lobed shields. Cuticular sheath markedly loose and swollen on ventral side extending from collar base to tail tip. Dorsal cuticular sheath

**Table 1.** Morphometric characteristics of *Pterygorhabditis saprophila* sp. n. Measurements are in  $\mu\text{m}$  and in the form: mean  $\pm$  standard deviation (range).

Character	Holotype	Paratype	Paratype
	♀	♀♀ (n=2)	♂♂ (n=3)
Body length	512	411 $\pm$ 1.41 (410-412)	369 $\pm$ 42.80 (342-420)
Body diameter	42	33 $\pm$ 1.41 (32-34)	30.33 $\pm$ 7.37 (22-36)
a	12.19	12.46 $\pm$ 0.57 (12.05-12.87)	13.00 $\pm$ 5.20 9.66-19.01)
b	4.61	4 $\pm$ 0.14 (3.9-4.10)	4.11 $\pm$ 0.15 (4.0-4.17)
c	9.66	9.70 $\pm$ 0.84 (9.1-10.3)	23.95 $\pm$ 1.33 (22.42-24.85)
c	3.31	3.80 $\pm$ 0.28 (3.60-4.09)	1.20 $\pm$ 0.17 (1.07-1.40)
V/T	54	53.51 $\pm$ 0.70 (53-54)	60.66 $\pm$ 1.52 (59-62)
G <sub>1</sub>	43	46.00 $\pm$ 1.00 (45-47)	
G <sub>2</sub>	40	43.51 $\pm$ 1.5 (42-45)	
Lip diameter	6	5.75 $\pm$ 0.25 (6.5-5.00)	5.8 $\pm$ 0.28 (5.5-6)
Lip height	5	4.75 $\pm$ 0.25 (4.5-5.00)	4.80 $\pm$ 0.28 (4.5-5)
Stoma length	22	17.50 $\pm$ 0.70 (17-18)	17.66 $\pm$ 1.15 (17-19)
Pharyngeal length	111	102 $\pm$ 2.80 (100-104)	88.33 $\pm$ 6.36 (82-97)
Nerve ring	67	69.5 $\pm$ 0.52 (69-70)	64.33 $\pm$ 9.29 (58-75)
Tail length	53	42.50 $\pm$ 3.53 (40-45)	15.33 $\pm$ 1.52 (14-17)
ABD	16	10.57 $\pm$ 0.70 (10-11)	12.60 $\pm$ 1.15 (12-14)
Spicules			56.33 $\pm$ 1.15 (55-57)
Gubernaculum			14.33 $\pm$ 0.57 (14-15)

extended from posterior of stomal base up to tail end. Lip region set-off; lips small, not distinct, provided with six minute inner labial sensilla, four long, setose cephalic sensilla and 5 fimbria-like projections arising from each lateral sector. Amphids indistinguishable. Stoma prismatic, rhabditoid type, about 1/5<sup>th</sup> pharyngeal length or 3-3.5 times labial diameter. Cheilostom conspicuous,

walls appearing rod-like; gymnostom cuticularized, with parallel walls, about 3-3.5 times cheilostomal length. Stegostom nearly as long as cheilostom, surrounded by pharyngeal collar; metastegostom with a fine denticle on each plate. Telostegostom obscure. Pharynx with swollen corpus, narrow isthmus and ovoid, valvate basal bulb 25 $\times$ 19  $\mu\text{m}$ . Corpus 58-60% of pharyngeal length, about 1.3-1.4 times longer than isthmus and basal bulb together. Body at pharyngeal end 5.5-5.7 times labial diameter. Nerve ring encircling isthmus at 67-69% of pharyngeal length. Hemizonid and excretory pore not visible. Cardia small, conoid, 4-4.5  $\mu\text{m}$  long. Intestinal cells large with prominent nuclei, boundary of intestinal lumen thin. Rectum 2-3 anal body diameters long, terminating into a tube formed by external cuticular sheath. Reproductive system didelphic, amphidelphic. Ovaries dorsally reflexed, with two rows of oocytes in germinal zone. Uterus muscular, clearly differentiated from crustaformeria. Eggs not observed in uteri. Vulva slit-like, slightly post-equatorial with distinct vulval flaps. Anterior genital branch 1.01-1.09 times longer than posterior branch. Vulva-anus distance 3-3.5 times tail length. Tail elongate uniformly tapering into a fine tip, usually dorsal curved.

**Male:** Body slender, almost straight upon fixation. Similar to females in general morphological characters. Testis single, ventrally reflexed; vas deferens a broad tube, without demarcation of seminal vesicle; ejaculatory glands absent. Spicules slender, distally fused, 3.9-4.7 times longer than cloacal body diameter with elliptical capitula. Gubernaculum small, distally pointed, spatula-shaped, about 1/3-1/4 of spicular length. Bursa open, pseudopeloderan type, leaving small tail spike free. Bursal papillae nine pairs with 4 precloacal, 1 adcloacal and 4 post cloacal pairs, spaced without definite configuration. Phasmids distinct, tubular, close to base of tail spike.

**Type habitat and locality:** Farmyard manure collected from Chherrat, Aligarh, Uttar Pradesh, India.

**Type specimens:** Holotype female on slide *Pterygorhabditis saprophila* sp. n. NOC/1 and paratype females and paratype males on slides *Pterygorhabditis saprophila* sp. n. NOC/2-6 deposited in the 'Nematode Collection' of Department of Zoology, Aligarh Muslim University, Aligarh. One paratype female and one paratype male on slide *Pterygorhabditis saprophila* sp. n. NON/7 deposited at the Laboratory of Nematology, Wageningen University and Research Center (WUR), 6700 ES, Wageningen, The Netherlands

**Differential diagnosis:** *Pterygorhabditis saprophila* sp. n. is characterised by a medium-sized body; presence of flattened, hexagonal sclerotised transverse bars forming six longitudinal rows; absence of cuticular shield in anterior body region; vulva with distinct vulval flaps; relatively shorter tail of 3-4 anal body diameters; distally fused spicules having elliptical capitula, open, pseudopeloderan

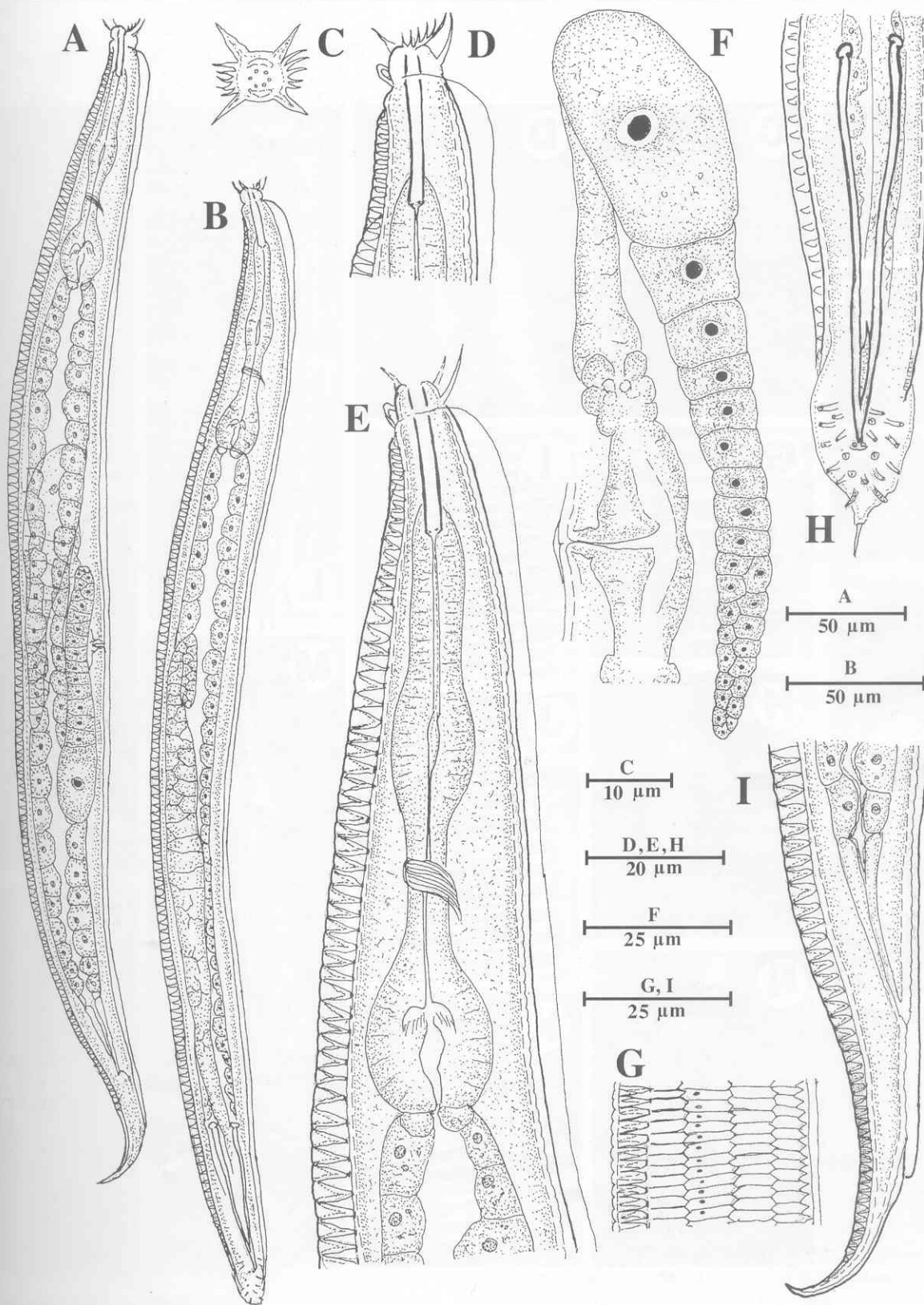


Fig. 1. *Pterygorhabditis saprophila* sp. n. A. Entire female; B. Entire male; C. En face view; D. Anterior end; E. Pharyngeal region; F. Female genital branch (anterior); G. Cuticular ornamentation; H. Male posterior end (ventral); I. Female posterior end (lateral).

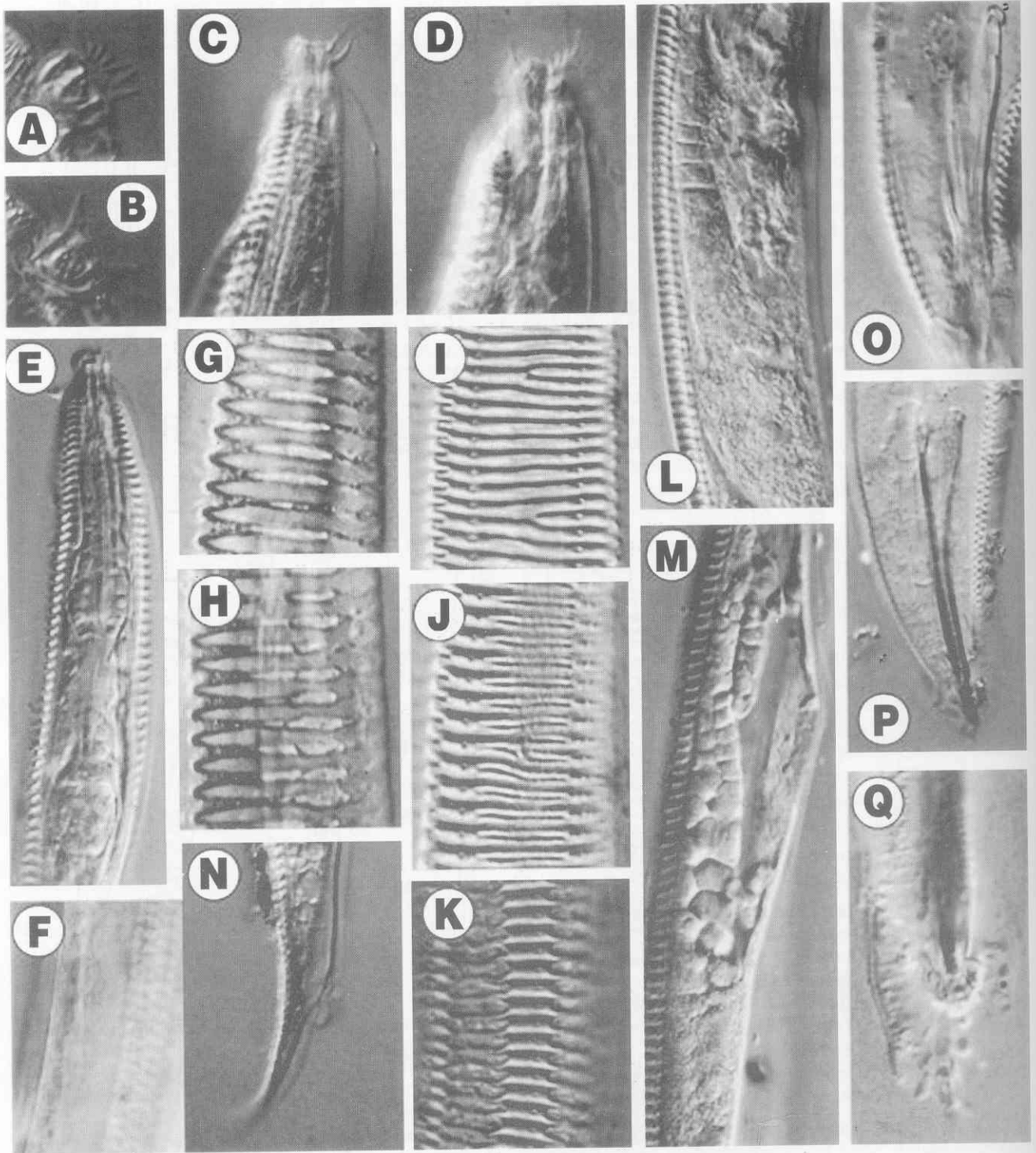


Fig. 2. *Pterygorhabditis saprophila* sp. n. A, B *En face* view; C, D Anterior ends; E. Pharyngeal region; F-K Types of cuticular ornamentation; L. Female genital branch; M. Part of male reproductive system; N. Female posterior end; O-Q Male posterior ends (ventral).



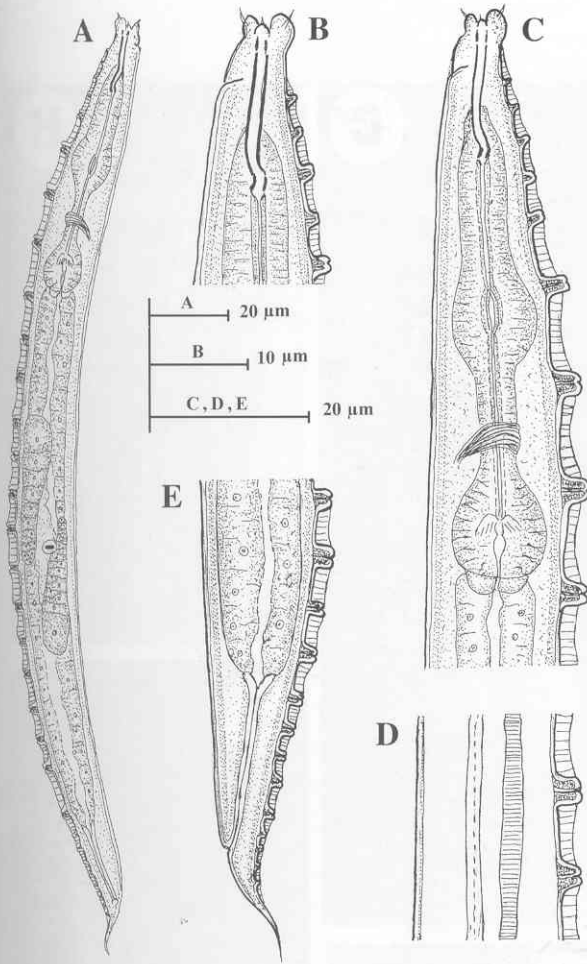


Fig. 3. *Bunonema flexistoma* sp. n. A. Entire female; B. Anterior end; C. Pharyngeal region; D. Tubercles and ridges; E. Female posterior end.

bursa and 9 pairs of genital papillae.

*Pterygorhabditis saprophila* sp. n. most closely resembles *P. superbus* Huseni, Ahmad and Firdausi, 1997 in most morphometric characteristics but differs in having lesser number of longitudinal lines on left side of body; larger intestinal cells with defined nuclei; presence of vulval flaps; relatively smaller rectum; pseudopeloderan bursa and greater number of bursal papillae with different configuration (4 longitudinal lines on left side of body; intestine without large cells; vulval flaps absent; rectum 3.6-5 anal body diameters; bursa peloderan and bursal papillae 8 pairs with four precloacal and four post-cloacal pairs in *P. superbus* Huseni, Ahmad and Firdausi, 1997).

*Pterygorhabditis saprophila* sp. n. also resembles *P. hungarica* Andrassy, 1982 but differs in having smaller 'a' value; dissimilar arrangement of labial sensilla; absence of cuticular shields in anterior body region; presence of vulval flaps; dissimilar shape of gubernaculum and lesser

number of genital papillae ( $a=13-16$ ; fimbriate projections absent in lateral sector of lips; 3 cuticular shields present in anterior body region; vulval flaps not reported; narrow attenuated gubernaculum and bursal papillae with 7 longer, lateral and 4 shorter, submedian pairs in *P. hungarica* Andrassy, 1982).

The new species further differs from *P. pakistanensis* Timm, 1957 in having smaller body; smaller 'V' value; dissimilar cuticular pattern formed by interlocked bars; swollen pharyngeal corpus; similar genital branches; smaller spicules and distally pointed gubernaculum ( $L=0.70-0.85$  mm;  $V=63-65\%$ ; sclerotized interlocked transverse bars forming diamond-shaped pattern; pharyngeal corpus cylindrical; anterior genital branch 3 times longer than posterior branch; spicules  $60-90\mu\text{m}$  long and gubernaculum with blunt distal end in *P. pakistanensis* Timm, 1957).

*Pterygorhabditis saprophila* sp. n. distinctly differs from *P. panopla* Bernard, 1979, in having smaller 'c'; absence of longitudinal cuticular ridges, cuticular shields in anterior body region and intestino-rectal sphincter; presence of cephalated, tubular spicules without internal bars and dissimilar arrangement of genital papillae ( $c=13-16$ ; cuticular ornamentation having strong longitudinal ridges with small triangular projections; one cuticular shield present in anterior body region; intestino-rectal sphincter present; spicules weakly cephalated with internal reinforcing bars and genital papillae 9 pairs with three adcloacal and six post-cloacal pairs in *P. panopla* Bernard, 1979).

**Etymology:** The name of the species is derived from its saprobic habitat i.e. farmyard manure.

### *Bunonema flexistoma* sp. n.

(Figs 3, 4)

**Measurements.** See Table 2.

**Female:** Body  $0.17-0.23$  mm long, plump, ventrally curved upon fixation, tapering at both extremities. Right side of body ornamented with 30-38 paired tubercles or warts. Each tubercle  $3-3.5\mu\text{m}$  high, provided with two internal cuticularized rods, regularly-spaced from another tubercle at  $12-13\mu\text{m}$  with 11-13 ribs in between. First pair located in region of gymnostom or 40% of stomal length and last pair in mid tail region. Five largest tubercles extended from procorpal swelling up to ovarian flexure. Two striated ridges existed mid laterally while three smooth ridges or 6 lines present in left side of body. Network between wart pairs obscure and indiscernible under LM. Lip region set-off from adjoining body. Outer and inner labial sensilla not visible; cephalic sensilla setose, about  $3\mu\text{m}$  long. Amphids inconspicuous. Adam's apple-like collar present in neck region. Stoma prismatic, rhabditoid type, about  $1/4^{\text{th}}$  of pharyngeal length or 3-4 labial diameter long. Cheilostom thick; cuticularised, rod-like; gymnostom moderately

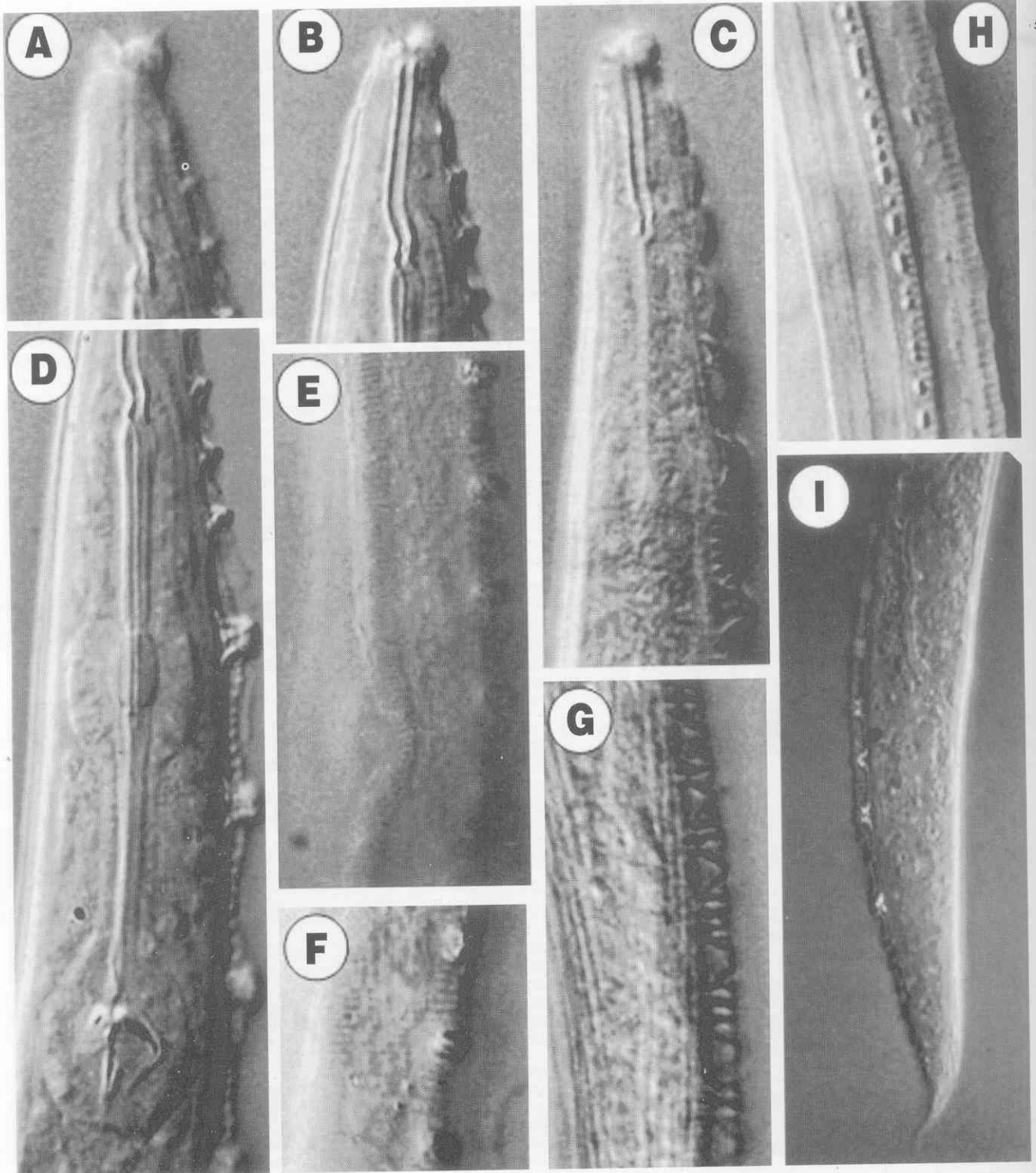


Fig. 4. *Bunonema flexistoma* sp. n. A, B. Anterior end; C. Anterior pharyngeal region (fourth stage juvenile) D. Pharyngeal region; E: Vulval region (ventral); F. Tubercles (upper view) with inconspicuous network; G. Tubercles (fourth stage juvenile); H. Smooth and striated longitudinal ridges; I. Female posterior end.

**Table 2.** Morphometric characteristics of *Bunonema flexistoma* sp. n. Measurements are in  $\mu\text{m}$  and in the form: mean  $\pm$  standard deviation (range).

Character	Holotype ♀	Paratype ♀♀ (n=3)
Body length	236	198 $\pm$ 14.4 (172-206)
Body diameter	18	18 $\pm$ 0.8 (17-19)
a	13.1	12.4 $\pm$ 1.2 (10-14.5)
b	3.5	3.1 $\pm$ 0.4 (2.9-3.6)
c	13.8	13.6 $\pm$ 0.5 (13.2-14.7)
c	3.4	3.8 $\pm$ 0.6 (2.1-3.5)
V/T	56.5	56.4 $\pm$ 1.2 (56-58)
G <sub>1</sub>	35.5	32.4 $\pm$ 2.8 (26.7-37.5)
G <sub>2</sub>	21.9	20.4 $\pm$ 1.9 (15.7-24.4)
Lip diameter	5.5	5.4 $\pm$ 0.5 (5-6)
Lip height	3	2.8 $\pm$ 0.2 (2.5-3)
Cephalic setae length	3	3 $\pm$ 0 (3-3)
Stoma length	18	16.7 $\pm$ 1.7 (14-19)
Pharyngeal length	68	62 $\pm$ 3.6 (58-69)
Nerve ring	53	50.5 $\pm$ 3.2 (47-55)
Tail length	17	17.5 $\pm$ 2.1 (13-19)
ABD	5	5.5 $\pm$ 4.2 (5-6)

cuticularised, little longer than stegostom. Stegostom half of stomal length with small prostegostom, dorsally arcuate anisomorphic mesostegostom and thick, strongly cuticularised metastegostom with a fine denticle. Telostegostom indistinct. Pharynx differentiated into strongly swollen corpus with thickened lumen, narrow isthmus and ovoid, valvate basal bulb of 13 $\times$ 11  $\mu\text{m}$

dimension. Corpus 60-63% of pharyngeal length. Body at pharyngeal end 3-3.5 times labial diameter. Corpus 1.5-1.6 times longer than isthmus and basal bulb together. Nerve ring encircling isthmus at 74-79% of pharyngeal length. Excretory pore faint, rarely visible in isthmus region. Hemizonid not seen. Cardia small, conoid, 3.5-4  $\mu\text{m}$  long. Intestine with large nucleated cells and distinct lumen. Rectum 2.6-4.1 anal body diameters long.

Reproductive system didelphic, amphidelphic. Ovaries dorsally reflexed, with two rows of oocytes in germinal zone. Anterior genital branch 1.0-1.6 times longer than posterior one. Eggs not observed in uteri. Vulval opening small ovoid slit, slightly post-equatorial. Vulva-anus distance 5-5.5 times tail length. Tail conoid, uniformly tapering to a fine terminus.

**Male:** Not found.

**Fourth stage juvenile:** Juveniles resemble adults in general morphology except for number and structure of cuticular tubercles. Tubercles 41 pairs, simple, without internal cuticularised rods, 4-5  $\mu\text{m}$  apart with 4-6 ribs in between.

**Type habitat and locality:** Organic manure collected from Narora, Uttar Pradesh, India.

**Type specimens:** Holotype female on slide *Bunonema flexistoma* sp. n NON/1, paratype female and one fourth stage juvenile on slides *Bunonema flexistoma* sp. n NON/2,3 deposited in 'Nematode Collection' of Department of Zoology, Aligarh Muslim University, Aligarh. One paratype female on slides *Bunonema flexistoma* sp. n NON/4 deposited at the Laboratory of Nematology, Wageningen University and Research Center (WUR), 6700 ES, Wageningen, The Netherlands.

**Differential diagnosis:** *Bunonema flexistoma* sp. n is characterized by small body; 28-30 paired tubercles on right side with faint or obscure network between tubercles pairs; presence of thick, strongly cuticularized cheilostom and metastegostom, dorsally arcuate mesostegotom and a sharply pointed tail of 2-3.5 anal body diameters.

*Bunonema flexistoma* sp. n. most closely resembles *B. reticulatum* Richters, 1905 in morphometric characteristics but differs in having paired tubercles over entire body length; smaller labial sensilla; obscure network between paired warts; presence of strongly cuticularised cheilostom and metastegostom; dorsally arcuate mesostegostom and absence of uterine eggs (2-4 unpaired tubercles present in caudal region; labial sensilla long spike-like; network between warts pairs conspicuous; cheilostom and metastegostom not cuticularised; mesostegostomal walls straight and uterine eggs reported in *B. reticulatum* Richters, 1905).

*Bunonema flexistoma* sp. n. also resembles *B. multipapillatum* Stefański, 1914 but differs in having smaller body length; smaller 'V' value; lesser number of tubercles on body with lesser supporting cuticularised rods; presence of

strongly cuticularised cheilostom and metastegostom and posteriorly dorsally arcuate mesostegostom ( $L=0.30-0.38$  mm;  $V=59-66\%$ ; tubercles 35-50 pairs with 5-6 internal cuticularised rods; cheilostom and metastegostom not cuticularised; mesostegostomal walls straight in *B. multipapillatum* Stefański, 1914).

*Bunonema flexistoma* sp. n. differs from *B. richtersi* Jägerskiöld, 1905 in having smaller 'V' value; greater number of tubercles over body as well as in pharyngeal region with lesser cuticularised rods; inconspicuous network between wart pairs; presence of strongly cuticularised cheilostom and metastegostom and dorsally arcuate mesostegostom ( $V=58-61\%$ ; total tubercle pairs = 18-24 with 6 pairs in pharyngeal region; warts with 4 cuticularized rods; network between wart pairs conspicuous or massive; cheilostom and metastegostom not cuticularised and mesostegostomal walls straight in *B. richtersi* Jägerskiöld, 1905).

The new species further differs from *B. franzi* Andrassy, 1971 in having greater number of tubercles over body as well as in pharyngeal region; smaller labial sensilla; obscure network between wart pairs; longer stoma; presence of strongly cuticularised cheilostom and metastegostom; dorsally arcuate mesostegostom and smaller rectum in relation to anal body diameter (total tubercle pairs =17-18 with 4 pairs in pharyngeal region; labial sensilla long, sharply pointed; network between wart pairs distinct, larger near warts; stoma 12  $\mu\text{m}$  long; cheilostom and metastegostom not cuticularised; mesostegostomal walls straight and rectum 10 anal body diameters long in *B. franzi* Andrassy, 1971).

**Etymology:** The species name is based on its posteriorly arcuate stoma.

**Remarks:** Most tubercles of third and fourth stage juveniles showed a superficial resemblance with tubercles of *B. irregularis* Huseni, Ahmad and Firdausi, 1997 where internal cuticularised rods were absent and 4-6 ribs existed between two tubercles. However, the juveniles lacked a conspicuous cuticular network between tubercle pairs unlike *B. irregularis* Huseni, Ahmad and Firdausi, 1997. Some large tubercles of *P. saphophila* sp. n. juveniles appeared thick with faint internal cuticularised rods thus indicating the simple, rodless tubercles to be an early stage in the development of a full fledged tubercle.

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