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Three New Species of *Brevitobrilus* Tsalolikhin, 1981 (Nematoda) with a Discussion on Relationships within the Genus

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Abstract: Three new species of the genus *Brevitobrius* Tsalolikhin, 1981 are described. *Brevitobrilus glandulatus* n. sp. is characterized by conspicuous sphincter between pars dilatata and uterus; two pairs of vaginal glands; spicules having elliptical capitula with small proximal stiffening piece; proximally-arcuate gubernaculum; S3 and S4 smaller than other supplements; S6 out of spicular range and 57-60 micropapillae. *Brevitobrilus dimorphicus* n. sp. is diagnosed by sexual dimorphism in labial sensilla and amphids; thick-walled rectum with a diverticulum protruding into intestinal lumen and males with boat-shaped spicules and S6 occasionally slightly smaller than other supplements. *Brevitobrilus allahabadensis* n. sp. possesses large amphids of 28-33% of corresponding labial diameter in both sexes; vagina and uterus with muscular, plicate walls; well developed sphincter between vas deferens and ejaculatory duct; capitulate spicules with sloping ventral and angular dorsal walls; S3, S4 and S6 smaller than other supplements, S6 close to cloaca and 28-37 micropapillae. The relationships of the species of genus *Brevitobrilus* have been assessed using morphological characters subjected to parsimony and a non cladistic key to identification of species is given.

Key words: Brevitobrilus glandulatus n. sp., *B. dimorphicus* n. sp., *B. allahabadensis* n. sp., description, morphology, nematode.

Members of Tobrilidae De Coninck, 1965 are predominantly aquatic with the ability to resist oxygen stress (Schiemer and Duncan, 1974; Arthington et al., 1996). *Tobrilus longus* has been one of the oldest known aquatic species described by Leidy (1851) as *Anguillula longa* which was named as a new genus *Trilobus* by Bastian (1865) and later renamed as *Tobrilus* by Andrássy (1959). As the genus accommodated a large number of species, its taxonomy has been studied and revised by several workers (Andrássy, 1964; Schiemer, 1971; Loof and Riemann, 1976; Joubert and Heyns, 1979 etc.). Tsalolikhin (1981) splitted *Tobrilus* into thirteen different genera on the basis of similarities primarily in body size, stoma, lip sensilla, supplements and spicules. This categorization removed much heterogeneity from the earlier known genus *Tobrilus*. De Ley et al. (2006) in their new scheme of classification placed Tobrilina as a suborder of Triplonchida. Within Tobrilidae, they considered nine genera under subfamily Tobrilinae and four under Neotobrilinae with *Brevitobrilus* a member of the latter taxon. Zullini (2006) considered eight species under *Brevitobrilus* viz., *B. stefanskii* (Micoletzky, 1925) Tsalolikhin, 1981; *B. fesehai* Eyualem and Coomans, 1997; *B. findeneggi* (Schiemer, 1971) Tsalolikhin, 1981; *B. graciloides* (Daday, 1908) Tsalolikhin, 1981; *B. kenyensis* Tsalolikhin, 1992; *B. sardus* (Vinciguerra and Zullini) Tsalolikhin 1981; *B. montanus* Ocana, Hernandez and Martin, 1996 and *B. tsalolikhini* Eyualem-Abebe and Coomans, 1997. He regarded *B. confusus* (Khera, 1975), *B. consimiloides* (Altherr, 1965), *B. fontinalis* (Altherr, 1976) and *B. sexsetiferus* (Khera, 1975) as species inquirenda.

During a screening of soil samples from India and China, three new species of *Brevitobrilus viz., Brevitobrilus glandulatus*[a] n. sp., *B. dimorphicus*[b] n. sp., *B. allahabadensis*[c] n. sp. were found that are reported hereunder along with a key to identification of species of the genus.

Materials and Methods

Collected soil samples were processed by Cobb's (1918) sieving and decantation and modified Baerman's funnel techniques. The nematodes extracted were heat-killed and fixed in Formalin-glycerol fixative, dehydrated by slow evaporation method (Seinhorst, 1959) and mounted in anhydrous glycerine. The measurements were taken by ocular micrometer and drawings made using drawing tube attached to Olympus BX-51 DIC Microscope. LM photographs were taken by Olympus digital camera DP-11. For Scanning Electron Microscopy (SEM), the specimens were fixed in 2% glutaraldehyde, post-fixed in 2% osmium tetroxide, dehydrated in alcohol series, critical point dried using CO_2, mounted on stubs, coated with 10 nm gold before viewing under Electron microscope.

Morphological characters and analysis: Twenty four morphological characters that were important and informative with context to differentiation of species of *Brevitobrilus*, were used. The characters were ranked on the basis of commonality principle. Character state '0" represented the most commonly occurring trait whereas a gradual increase in value represented more

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[a] The species name indicates presence of conspicuous vaginal glands
[b] The species name is based on the sexual dimorphism shown in amphids and lip sensilla
[c] The species name is based on its type locality, Allahabad.

deviation. A data matrix was prepared using character states for different species of *Brevitobrilus*. The data matrix was analysed using online PHYLIP 3.5c software. The rooted/ unrooted trees were constructed using Phylip: pars-Discrete character parsimony method (Felsenstein) online program (http://cmgm.stanford.edu/phylip/). TREEVIEW (win 32) (Roderic, 1996) was also used to cross-examine the out tree files. All morphological characters were given equal weight and consensus tree was constructed using maximum parsimony.

Description

Brevitobrilus glandulatus n. sp.
(Figs 1, 2)

Measurments of holotype, allotype and paratypes are given in Table 1.

Female (n = 12): Body slender, slightly curved ventrally and tapering towards both extremities. Outer and inner cuticles finely transversely striated and 2-3 μm thick at different body regions. Fine longitudinal lines are present (Fig. 2J, K) on the inner cuticle. Somatic setae 32-35 in number scattered all over body with 7-9 confined to pharyngeal region. Crystalloids appear fine and densely packed (Fig. 2F) in pseudocoelom. Lip region flattened, low and continuous with the adjoining body. Inner labial sensilla papilliform whereas outer labial sensilla setose (4-5 μm long) and plump. Cephalic sensilla 4-5 μm long, slightly thinner than outer labials and located at their bases. Amphids stirrup-shaped with transverse-oval, 5-7 μm wide fovea at level of anterior pocket of stoma or 14-18 μm from anterior end. Fovea occupies 25-28% of corresponding body diameter (Fig 1C, 2D). Cheilostom arched, weakly sclerotized. Rest of stoma divided into three parts. Anterior funnel-shaped buccal cavity of dimension 11.5-14.0 x 9.0-13.0 μm with thick sclerotised walls followed by 7.5-9.0 x 5.0-7.0 μm anterior pocket and 7.0-9.0 x 4.0-6.0 μm posterior pocket. Each pocket armed with a well sclerotised tooth. First tooth situated at 14-20 μm from anterior end while second tooth about 8-9 μm posterior to the first one. Pharynx cylindroid and muscular. Nerve ring located at 38-45% of pharyngeal length. Body width at pharyngeal end 37-51 μm. Cardia with

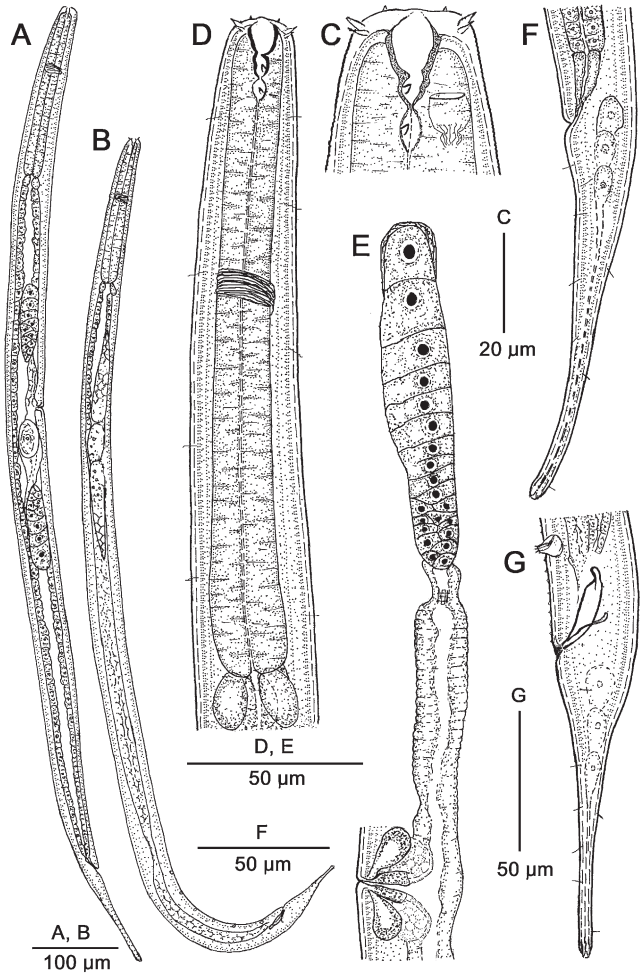

Fig. 1. *Brevitobrilus glandulatus* n. sp. A) Entire female. B) Entire male. C) Pharyngeal region. D) Anterior region. E) Female genital branch: anterior. F) Female tail. G) Male tail.

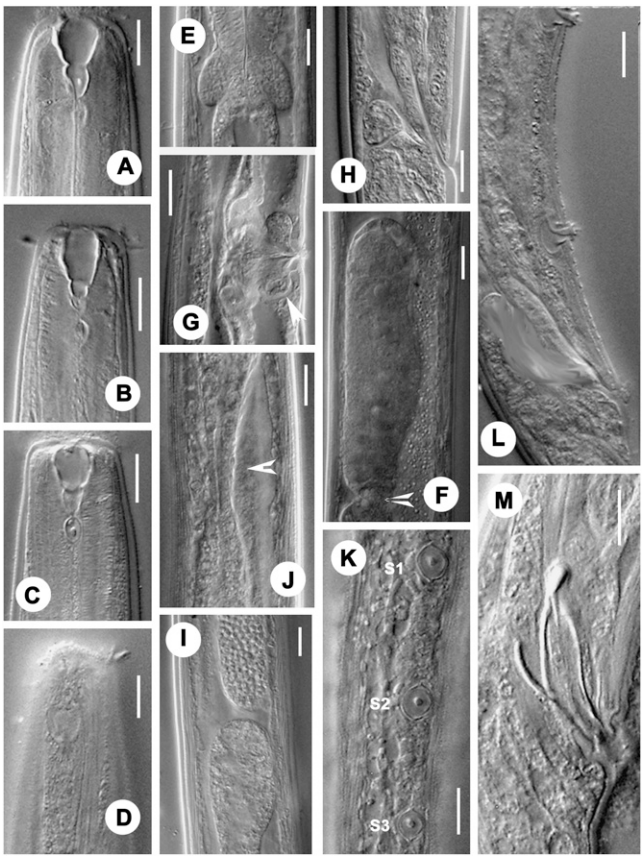

Fig. 2. *Brevitobrilus glandulatus* n. sp. A-D) Anterior end female. E) Pharyngeo-intestinal junction. F) Reflexed ovary and pars dilatata with sphincter. G) Vulval region: arrow indicates vaginal gland. H) Rectal region female. I) Male genital tract showing reflexed testis and seminal vesicle. J) Sphincter between vas deferens and ejaculatory duct. K) Posterior region showing supplements: ventral. L) Posterior region showing supplements: lateral. M) Cloacal region. (Scale bar = 10 μm).

TABLE 1. Measurements (μm) of *Brevitobrilus glandulatus* n. sp. with mean ± standard deviation (range).

Characters	Holotype female	Allotype male	Paratype female (n=11)	Paratype male (n=8)
Body length	1434	1413	1393.6±110.8 (1254-1583)	1348.6±168.0 (1070-1463)
Body diameter	54	43	50.4±4.8 (42-55)	43.8±4.7 (35-48)
a	26.5	32.8	27.7±1.9 (24.7-29.8)	30.7±1.9 (28.6-33.1)
b	5.9	6.3	5.7±0.2 (5.4-6.1)	6.2±0.4 (5.4-6.7)
c	9.3	13.7	9.3±0.5 (8.6-10.3)	13.3±1.7 (10.4-15.9)
c'	5.4	4.1	5.6±0.3 (5.1-6.0)	4.1±0.1 (4.0-4.2)
V/T	40.4	83.1	43.6±1.7 (40.4-45.2)	80.9±5.2 (74.5-89.1)
G_1	20.4	-	20.2±3.6 (14.2-24.8)	-
G_2	25.3	-	20.5±5.0 (14.4-25.3)	-
Lip height	2	2	2±0 (2-2)	2±0 (2-2)
Lip diameter	16	17	18.6±1.5 (16-20)	17.4±0.4 (17-18)
Stoma length	27	24	27.4±0.48 (26-30)	23.4±0.8 (23-25)
Stoma diameter	9	8	9.4±0.4 (8-11)	7.6±0.4 (7-8)
Pharyngeal length	242	216	240.4±11.2 (228-260)	213.8±12.5 (198-236)
Nerve ring	95	85	101.6±3.7 (95-105)	88.0±4.6 (85-97)
Anal Body Diameter	28	25	26.4±1.8 (24-28)	27.1±0.6 (24-30)
Rectum length	33	31	26.6±3.9 (24-33)	31.6±1.0 (30-33)
Vulva-anus distance: Tail	4.5	-	3.8±0.6 (3.5-4.9)	-
Tail length	153	103	149.6±14.5 (129-174)	101.4±1.2 (100-103)
Spicule length	-	30	-	27.6±1.5 (26-30)
Gubernaculum length	-	22	-	18.6±1.7 (17-22)

ovoid pericardial cells, 20-25 x 13-16 μm long (Fig. 1D, 2E). Excretory pore can not be observed. Intestine thin-walled, granular with wide lumen, compressed in the region of gonad. Rectum thick-walled, 24-33 μm long or 0.8-1.1 times anal body diameter. Three conspicuous caudal glands arranged in tandem, open through common duct at the tail tip. Anus a crescent-shaped slit. Reproductive system didelphic, amphidelphic with conspicuous lining of epithelial cells. Ovaries reflexed laterally, oocytes arranged in double rows in germinal zone and in single file beyond that. A prominent sphincter present between pars dilatata and uterus (Fig. 2F). Uteri with striated, muscular but not plicate walls. 1-2 intrauterine eggs of 47-52 x 22-27 μm dimension observed in few individuals. A moderately-developed ovijector present (Fig 2G). Vagina 1/3-1/4 of corresponding body diameter long, strongly muscular, provided with two pairs of globular gland cells opening distally (Fg. 1E, 2G). Vulval opening small slit. Tail slightly ventrally curved, uniformly tapering. Spinneret sclereotized 1-2 μm long. Caudal setae 6-7. Subterminal seta located 2 times corresponding tail diameters anterior to tail tip.

Male (n=9): Similar to female in characteristics except smaller body size and strongly ventrally curved posterior region. Male reproductive system represents diorchic testes with posterior one reflexed and on ventral side of intestine. The flexure is mostly on the right side of intestine. Ejaculatory glands absent. Seminal vesicle distinct. Sphincter demarcating vas deferens from ejaculatory duct well developed situated 12-30 μm anterior to the level of first supplement (Fig. 2J). Ventromedian supplements six, bulb-shaped; S-3, S-4 slightly smaller than rest (Fig 2L). Each supplement having an internal canal arising from anterior wall of ampulla connected to its distal end. The distal ends of supplements show an elliptical outline surrounding a central protrusion (Fig 2L). Distance between supplements variable- shortest distance (25-32 μm) observed between S2 and S3 while longest distance (40-50 μm) between S5 and S6. The latter is outside spicular range but closely anterior to spicules or about 33-41 μm anterior to cloaca. Around 56-60 conspicuous micropapillae are present in the region of supplements: 10-11 anterior to S1; 8 between S1 and S2; 6 between S2 and S3 and further between S3 and S4; 7 between S4 and S5; 10-11 between S5 and S6 while 9-10 papillae between S-6 and cloacal opening. Spicules slightly curved with elliptical heads, 1.0-1.2 anal body diameters long; a short median stiffening piece is confined to proximal end of spicules (Fig. 2M). Gubernaculum with arcuate proximal end, 0.6-0.7 times spicule length. Tail similar to female; caudal glands less conspicuous; caudal setae 7-8 in number; spinneret weakly sclereotized.

Diagnosis: Brevitobrilus glandulatus n. sp. is characterized by longitudinally and transversely striated body cuticle; slenderer cephalic sensilla; amphidial fovea 25-28% of corresponding body diameter; conspicuous sphincter between oviduct and uterus; two pairs of vaginal glands; spicules having elliptical capitula with small proximal stiffening piece; proximally-arcuate gubernaculum; smaller S3 and S4 supplements; S6 located outside spicular range and 56-60 micropapillae.

Relationship: Brevitobrilus glandulatus n. sp. differs from *B. montanus* Ocana, Hernandez and Martin, 1996 in having shorter cephalic setae (4-5 μm *vs* 5.5-7.5 μm), presence of two pairs of conspicuous vaginal glands and a sphincter between pars dilatata and uterus (*vs* vaginal glands and sphincter not reported), smaller supplements

(S3, S4 *vs* S5, S6), relatively smaller (26-30 µm *vs* 28-38 µm) spicules with elliptical (*vs* indistinguishable) capitula, greater number of micropapillae (57-60 *vs* 35-39) and subterminal seta located (2 *vs* >3 corresponding tail diameters anterior to tail tip); from *B. granatensis* Ocana and Zullini, 1988 in having smaller females (L=1.2-1.5 mm *vs* 1.4-2.4 mm) with cephalic and outer labials of (equal *vs* unequal) length, presence of two pairs of conspicuous vaginal glands (*vs* vaginal glands not reported), smaller (26-30 µm *vs* 34 µm) spicules with elliptical (*vs* elongate) proximal ends, supplement S5, S6 equal (*vs* smaller than other anterior supplements), the greatest distance existing between S5-S6 (*vs* S3-S4) supplements, S6 located outside (*vs* within) spicular range, greater (*vs* lesser) number of micropapillae and posterior most caudal seta located (anterior to *vs* at the tail terminus); from *B. stefanski* (Micoletzky, 1925) Tsalolikhin, 1981 in having greater 'c' value (5.1-6.0 *vs* 3.4-3.6), presence of two pairs of conspicuous vaginal glands (*vs* vaginal glands not reported), smaller (26-30 µm *vs* 35 µm), straight (*vs* arcuate) spicules, supplements with rounded (*vs* compressed) ampullae and S6 equal (*vs* distinctly smaller); from *B. graciloides* (Daday, 1908) Tsalolikhin, 1981 in having smaller females (L=1.2-1.5 mm *vs* 1.9 mm), larger amphids (25-28% *vs* 14.1-20.0% of corresponding body diameter), shorter outer labials (4-5 µm *vs* 7-12 µm), presence of two pairs of vaginal glands (*vs* vaginal glands not reported), smaller (26-30 µm *vs* 49-51 µm), unequal (*vs* equal) supplements and lesser micropapillae (57-60 *vs* over 80 in *B. graciloides apud* Eyualem and Coomans, 1997).

Type habitat and locality: Wet humus collected from a polluted drain at Narora, Uttar Pradesh, India.

Type designation: Holotype female, allotype male, ten paratype females and seven paratype males on slides *Brevitobrilus glandulatus* n. sp. No. NON/1-9 deposited at Nematode Collection Department of Zoology, Aligarh Muslim University, Aligarh. One paratype female and one paratype male on slide *Brevitobrilus glandulatus* n. sp. No. NON/10, deposited at the Laboratory of Nematology, Wageningen University and Research Center (WUR), 6700 ES Wageningen, The Netherlands.

Brevitobrilus dimorphicus n. sp.
(Figs 3, 4)

Measurments of holotype, allotype and paratypes are given in Table 2.

Female (n = 8): Body is slender, slightly ventrally curved upon fixation, tapering towards ends. Cuticle is 1-2 µm thick in lip region, 2-3 µm in vulval region while 0.5-1.0 µm in tail region. Outer cuticle is finely striated, inner cuticle smooth. Body setae number 22-26 including 6-8 cervical setae. Crystalloids are fine and sparse. Lip region is truncate and continuous with adjoining body. Inner labial sensilla are papilloid; outer labials plump

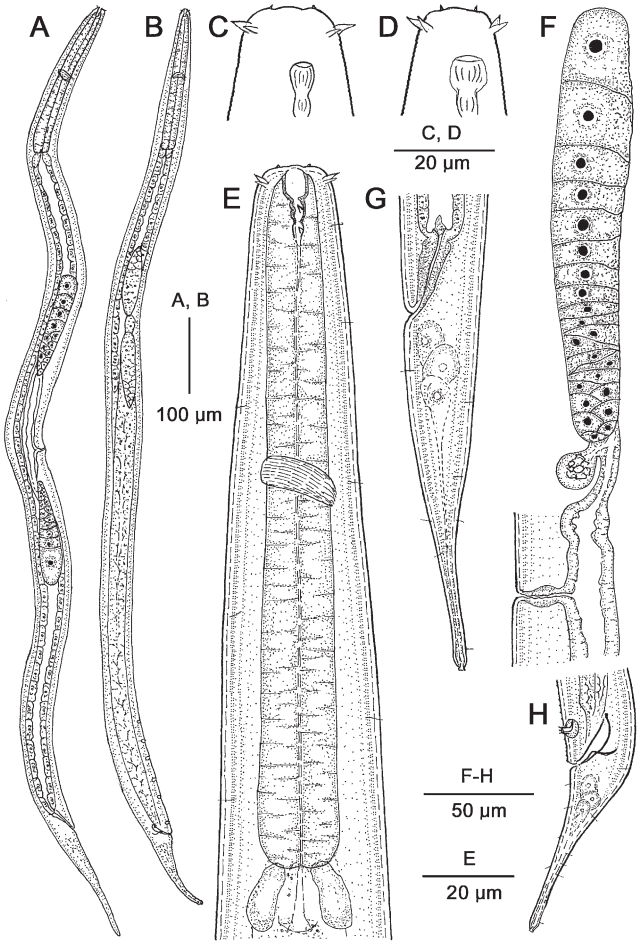

FIG. 3. *Brevitobrilus dimorphicus* n. sp. A) *Entire female.* B) Entire male. C) Anterior region female. D) Anterior region male. E) Pharyngeal region. F) Female genital branch: anterior. G) Female tail. H) Male tail.

and 4-5 µm long. Four slender cephalic sensilla of 3-4 µm length, are located at base of outer labials. Amphids are dimorphic with fovea smaller in females i.e., 4-5 µm or 20-25% corresponding body diameter and located at 12-18 µm from anterior end (Figs 3C, 4D). Cheilostom is arched and slightly sclerotized. Rest of stoma is strongly sclerotized, divisible into three parts: buccal cavity cup-shaped 7-10 x 6-8 µm followed by 5-6 x 3.0-4.5 µm anterior pocket and 4.0-5.5 x 2.5-3.5 µm posterior pocket long. Each pocket is armed with a well sclerotized tooth. First tooth is situated at 9-12 µm from anterior end whereas second tooth 4-6 µm posterior to first one. Nerve ring at 44-48% of pharyngeal length. Body width at pharyngeal end is 43-50 µm. Cardiac glands are oblong and expanded with 20-24 x 8-12 µm dimension. Excretory pore indiscernible. Intestine is thin-walled with wide lumen and is compressed in the region of gonad. Rectum is thick-walled, 0.8-1.0 times anal body diameter and proximally protrudes into intestine to form a valve like structure (Figs. 3G, 4I). Caudal glands are faintly visible with an arrangement showing two anterior gland cells and a closely placed posterior gland cell. Reproductive system is didelphic, amphidelphic.

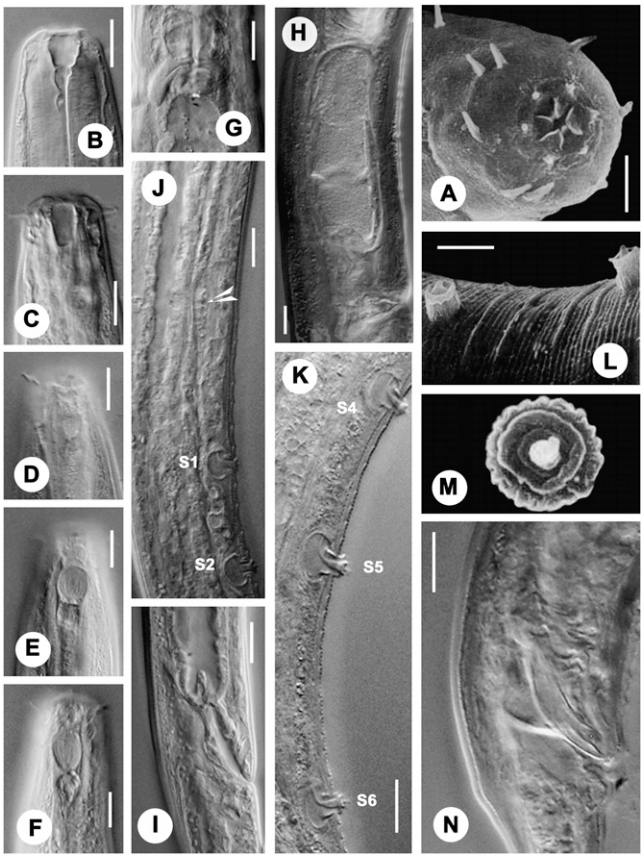

FIG. 4. *Brevitobrilus dimorphicus* n. sp. A) SEM micrograph of En face view male. B-D) Anterior end female. E, F) Anterior region male. G) Pharyngeo-intestinal junction. H) Anterior genital branch showing uterus. I) Anal region female. J) Sphincter between vas deferens and ejaculatory duct. K) Posterior region showing supplements: lateral. L) SEM micrograph showing supplements and micropapillae. M) Posterior region showing supplements: ventral N) Cloacal region.

Genital tract is devoid of distinct epithelial lining. Ovaries are robust and reflexed laterally, each with a large growing oocyte at distal end. Spermatheca is small and adaxial (Fig. 3F). Uteri are less muscular, without striated walls and accommodate occasionally 1-3 ovoid eggs of 30-40 x 19-25 μm dimension (Fig. 4H). Vagina is moderately muscular without plicate walls and 30-35% of corresponding body diameter. Vulva is a transverse slit. Tail is slightly ventrally curved and uniformly tapering. Spinneret is 1-2 μm long and sclereotized. Caudal setae number 5-6 with subterminal seta located 1.5-2.0 times corresponding tail width anterior to tail tip.

Male (n=10): Appears similar to female in most morphological characters except size of amphids and labial sensilla. Outer labial and cephalic sensilla are found to be equal in size and thickness (Fig. 4A). The amphids appear exceptionally large i.e., 7-8 μm or 38-43% of corresponding body diameter with conspicuously large fovea, fusus and ductus amphidialis (Figs. 3D, 4E, F). Male gonad is diorchic with posterior reflexed testis on ventral side of intestine. Ejaculatory glands are absent. Sphincter between vas deferens and ejaculatory duct is weakly developed, situated 35-45 μm anterior to the level of the first supplement (Fig. 4J). Supplements six, bulb-shaped, sizes not consistently same in ten males with S6 slightly smaller occasionally (Fig. 4K). Some specimens showed a slightly larger S2 compared to others (Fig. 4J). In SEM (Fig. 4L, M) the distal end of each supplement comprises of an outer thick frilled ring surrounding an inner smooth ring. The centre shows a protruding sensory plug. In SEM, micropapillae between supplements appear as cuticular folds running ventrally at right angle to longitudinal body axis and range from 34-38 in the configuration: 8-10 micropapillae anterior to S1; 4 between two consecutive supplements from S1-S5, 4-6 micropapillae between S5 and S6 while 6 between S6 and cloacal opening. Distances between consecutive supplements from S1 to S6 are found to be 19-30 μm, 20-30 μm, 19-32 μm, 21-34 μm, 25-46 μm respectively with greatest distance usually between S5 and S6. S6 is located nearly at level of head of spicules, about 18-24 μm anterior to cloaca. Spicules are weakly cephalated, 0.9-1.3 anal body diameter long, boat-shaped with straight/ slightly curved ventral wall and convex dorsal wall (Figs. 3H, 4N). Gubernaculum is angular with lateral sleeves, 0.7-0.8 times the spicule length. Caudal setae are 10-14 in number.

Diagnosis: Brevitobrilus dimorphicus n. sp. is characterized by sexual dimorphism in labial sensilla and amphids; weakly-developed uterus; conspicuous vagina; thick-walled rectum protruding into intestinal lumen; arcuate, boat-shaped spicules; angular gubernaculum; S6 occasionally slightly smaller than other supplements, located at level of spicular head; 34-38 micropapillae in supplement region and subterminal seta located 1.5-2.0 times corresponding tail width anterior to tail tip.

Relationship: Brevitobrilus dimorphicus n. sp. differs from *B. fesehai* Eyualem and Coomans, 1997 in having sexual dimorphism present in lip sensilla (males with equal-sized *vs* longer outer labials), relatively wider amphids (38-43% *vs* 27.3-31.8% corresponding body width) in males, relatively shorter (*vs* longer) stoma; smaller (*vs* greater) distance between two stomal teeth, indiscernible (*vs* conspicuous) excretory pore, intrauterine eggs (ovoid *vs* rounded), vagina 30-35% (*vs* 10%) of corresponding body diameter, supplements variable (*vs* equal) in size, lesser number of micropapillae (34-38 *vs* 140) in supplement region and terminal seta present (*vs* absent); from *B. graciloides* (Daday, 1908) Tsalolikhin, 1991 in having relatively smaller body (1.0-1.5 *vs* 1.9), sexual dimorphism present (*vs* absent) in lip sensilla and amphids, smaller outer labial sensilla (4-5 μm *vs* 7-12 μm), smaller distance (4-6 μm *vs* 8.5-11.5 μm) between stomal teeth, shorter (20-25 μm *vs* 49-51 μm) spicules, smaller distance (18-24 μm *vs* 35-48 μm) between S6 and cloaca and lesser number of micropapillae (34-38 *vs* over 80 in *B. graciloides apud* Eyualem and Coomans, 1997); from *B. granatensis* Ocana and Zullini, 1988 in having smaller

TABLE 2. Measurements (μm) of *Brevitobrilus dimorphicus* n. sp. with mean ± standard deviation (range).

Characters	Holotype female	Allotype male	Paratype female(n=7)	Paratype male(n=9)
Body length	1305	1170	1263.4±74.4 (1134-1327)	1140.4±70.9 (1017-1221)
Body diameter	49	42	51.8±1.9 (49-54)	41.2±2.4 (38-45)
a	26.6	27.8	24.4±1.6 (21.3-26.6)	27.9±2.9 (24.7-32.1)
b	7.2	7.8	6.8±0.4 (6.3-7.6)	6.9±0.4 (6.6-7.8)
c	8.1	13.7	8.6±0.8 (7.4-9.9)	14.9±1.2 (13.7-15.2)
c'	5.0	3.4	5.2±0.5 (4.3-5.8)	3.3±0.4 (2.7-3.9)
V/T	41.3	67.6	42.2±1.6 (40.6-45.3)	76.64±5 (67.6-82.7)
G_1	29.6	-	26.4±3.3 (21.1-29.6)	-
G_2	28.1	-	25.1±1.7 (23.1-28.4)	-
Lip height	3	3	2.4±0.489 (2-3)	2.3±0.4 (2-3)
Lip diameter	17	15	17.2±0.4 (17-20)	14.8±0.4 (14-20)
Stoma length	15	18	17±1.8 (17-21)	17.2±1.3 (16-19)
Stoma diameter	6	6	6.4±0.8 (5-7)	6±0 (6-6)
Pharyngeal length	170	150	184.6±11.9 (170-195)	164.8±15.6 (145-188)
Nerve ring	78	55	85.0±6.4 (78-95)	68.1±8.2 (55-80)
Anal Body Diameter	32	25	29.2±2.1 (26-32)	23.4±1.7 (22-26)
Rectum length	28	28	25.0±2.9 (20-28)	28.2±1.1 (27-30)
Vulva-Anus distance: Tail	3.7	-	3.8±0.8 (3.0-4.8)	-
Tail length	162	85	147.6±12.8 (134-164)	77.2±9.7 (60-87)
Spicule length	-	20	-	22.6±2.2 (20-25)
Gubernaculum length	-	16	-	16.8±1.2 (15-20)

body (1.0-1.5 mm *vs* 1.5-2.3 mm), presence (*vs* absence) of sexual dimorphism in length of lip sensilla and amphid size, smaller (20-25 μm *vs* 32-46 μm) spicules, supplement S5 (equal *vs* smaller), greatest distance between S5-S6 (*vs* S3-S4) supplements and sub terminal seta (anterior to *vs* at the tail terminus); from *B. tsalolikhini* Eyualem and Coomans, 1997 in having sexual dimorphism present (*vs* absent) in lip sensilla, larger amphidial fovea (38-43% *vs* 25% of corresponding body diameter) in males, smaller outer labial sensilla (4-5 μm *vs* 10-11 μm) in females, angular (*vs* straight) gubernaculum, greater distance between S5 and S6 (*vs* S3 and S4) supplements, internal canals of supplements merging with their anterior (*vs* dorsal) walls and lesser number of micropapillae (34-38 *vs* 46 in *B. tsalolikhini*).

Type habitat and locality: Slurry from a drain at Meerut, Uttar Pradesh, India.

Type designation: Holotype female, allotype male, six paratype females and eight paratype males on slides *Brevitobrilus dimorphicus* n. sp. No. Meerut/1-7 deposited at Nematode Collection Department of Zoology, Aligarh Muslim University, Aligarh. One paratype female and one paratype male on slide *Brevitobrilus dimorphicus* n. sp. No. Meerut/8, deposited at the Laboratory of Nematology, Wageningen University and Research Center (WUR), 6700 ES Wageningen, The Netherlands.

***Brevitobrilus allhabadensis* n. sp.**
(Figs 5-7)

Measurments of holotype, allotype and paratypes along with other populations are given in Table 3.

Female (n = 7): Body is slender, slightly ventrally curved and markedly tapering posteriad. Cuticle is 1-2 μm to 3-4 μm thick in different body regions with outer cuticle smooth to very finely striated. Somatic setae are sparse. Crystalloids are fine round to ovoid in shape, present in pseudocoelom. Lip region is rounded, narrow, completely merging with adjoining body. Inner labial sensilla are papilliform while outer labials are 4-5 μm long, stout and plump. Cephalic sensilla are almost of same length but thinner than outer labials. Amphids cup-shaped, located 15-19 μm from anterior end or 28-33% of corresponding body diameter (Figs. 5D, 6D). Cheilostom not sclerotized; rest of stoma can be divided into three distinctly sclerotized sections. An anterior rectangular to barrel-shaped buccal cavity of 9-11 x 7-9 μm dimension is followed by two adjacent pockets. The anterior pocket (5-7 x 4.0-5.5 μm) is separated occasionally by a distinct isthmus from the posterior spindle-shaped pocket (4-5 x 3.0-4.5 μm). Each pocket is provided with a tooth. First tooth located at 11-13 μm from anterior end while second tooth is 5-8 μm posterior to it. Nerve ring encircles pharynx at 38-45% of its length. Pericardial cells spherical to ovoid, (15-21 x 10-14μm) in dimension. In some individuals observed to be of unequal dimensions (Fig. 6F). Intestine thin-walled with wide lumen, granulated and heavily compressed in region of gonad. Rectum is muscular with wide lumen and 0.9-1.3 anal body diameters long. Caudal glands conspicuous, three arranged in a group with one ahead of the other two (Figs. 5G, 6K). Anus a crescent-shaped transverse slit. Reproductive system amphidelphic with ovaries reflexed laterally. The spermathecae are long, offset and adaxial. Genital tract well developed and covered by epithelium cells with conical (protruded) appearance. Uterine lining is thick and plicate in most individuals (Fig. 6I). Vagina, 1/3 of corresponding body diameter provided with muscles that appear as

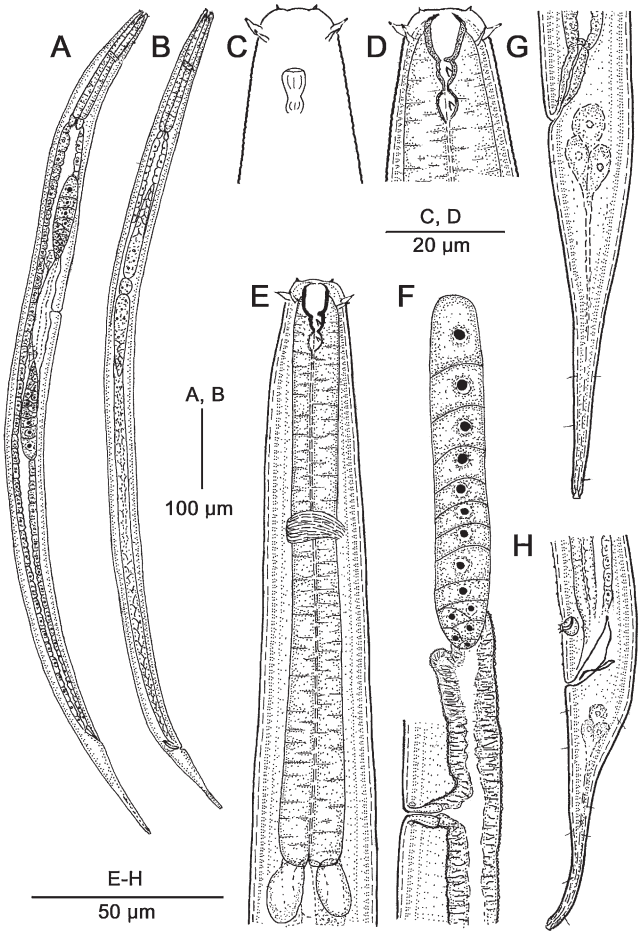

FIG. 5. *Brevitobrilus allahabadensis* n. sp. A) *Entire female.* B) Entire male. C) Anterior end male. D) Anterior region female. E) Pharyngeal region. F) Female genital branch: anterior. G) Female tail. H) Male tail.

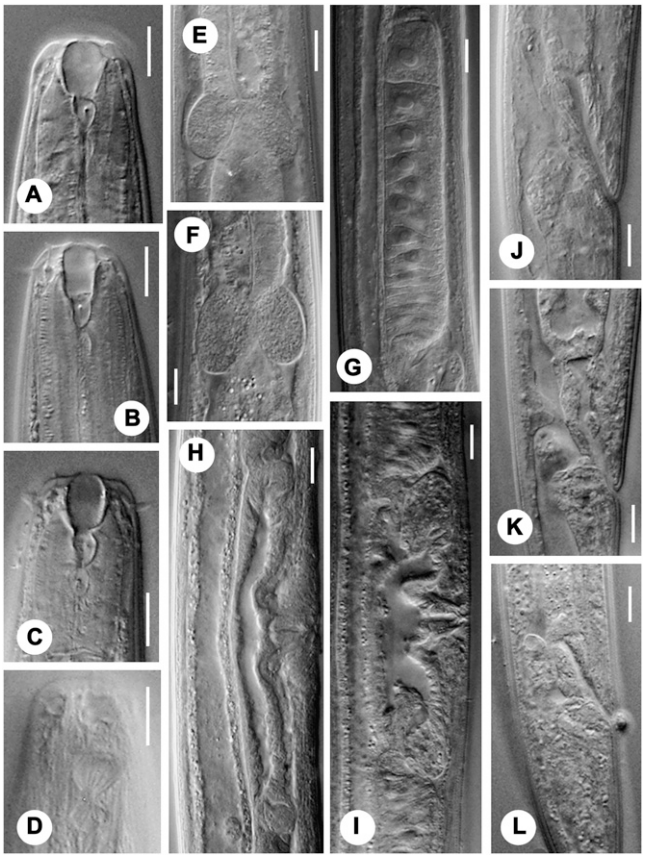

FIG. 6. *Brevitobrilus allahabadensis* n. sp. female. A-D) Anterior region. E, F) Pharyngeo-intestinal junction. G) Reflexed anterior ovary with small spermatheca. H, I) Reproductive system showing uteri with striated and plicate walls. J-L) Anal region. (Scale bar = 10 µm).

distal ovoid pieces in cross section. In few specimens vagina connected proximally with small chamber lined with thick striated muscles (Fig. 6I). Tail slightly ventrally curved, uniformly tapering. Caudal setae 4-6. Spinneret sclerotized, terminal seta at 2.0-2.5 tip width anterior to spinneret.

Male (n = 11): Male shows differences from female in body size and posterior body curvature. Male reproductive system is diorchic with posterior testis reflexed and placed on ventral of intestine. Sphincter between vas deferens and ejaculatory duct well developed (Fig. 7C). Of the six ventromedian supplements, S1, S2 and S5 are larger than S3, S4 and S6 (Fig. 7D, E). Each supplement is having an internal canal arising from anterior wall of ampulla connected to its distal end. Number of micropapillae between supplements is variable: 6-10 micropapillae anterior to S1, 4 micropapillae between consecutive supplements from S1 to S5; 4-8 micropapillae between S5 and S6 while 2-3 between S6 and cloacal opening. Distances between consecutive supplement from S1 to S6: 24-30µm, 22-30µm, 19-27 µm, 21-27 µm, 33-45 µm respectively with greatest distance between S5 and S6. Distance of S6 from cloaca variable ranging from 12-24 µm. Spicules are 0.8-1.1 anal body diameter long, cephalated with narrow calomus, without median piece, with sloping ventral wall and angular dorsal wall bent nearly at right angle (Figs. 5H, 7E,G). Gubernaculum angular, 0.6-0.7 times spicule length (Fig. 7F). Tail similar to female. Caudal setae 7-10. Terminal seta longer than that of female though present at same location.

Diagnosis: Brevitobrilus allahabadensis n. sp. is characterized by presence of large-sized amphids, uterus and vagina with muscular plicate walls; and males having well developed sphincter between vas deferens and ejaculatory duct; capitulate spicules with sloping ventral and angular dorsal walls; S3, S4 and S6 smaller than other supplements, S6 located within spicular range, 12-24 µm anterior to cloaca and 28-37 micropapillae in supplement region.

Relationship: Brevitobrilus allahabadensis n. sp. differs from *B. montanus Ocana, et al., 1996* in having relatively smaller females (L=0.9-1.3 mm *vs* 1.3-1.8 mm) with shorter cephalic setae (4-5 µm *vs* 5.5-7.5 µm), smaller (20-25 µm *vs* 28-38 µm) spicules, smaller supplement/s S3, S4, S6 (*vs* S5, S6) and terminal seta 1.5-2.5 (*vs* 4 times corresponding tail width anterior to tail terminus); from *B. sardus* Vinciguerra and Zullini, 1991 in outer labials and cephalic setae of same (*vs* different)

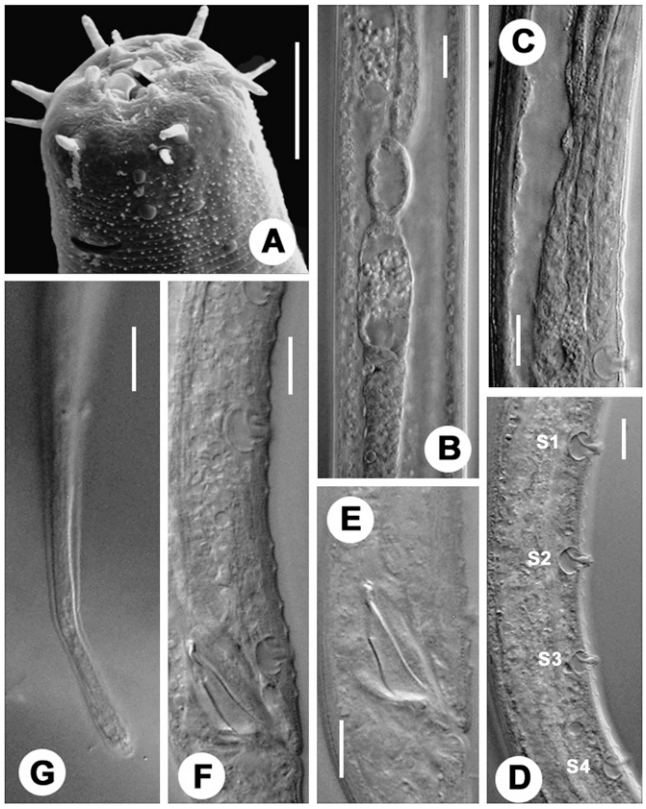

FIG. 7. *Brevitobrilus allahabadensis* n. sp. male. A) SEM micrograph showing anterior region. B) Genital tract with reflexed testis and seminal vesicle. C) Sphincter between vas deferens and ejaculatory duct. D, F) Posterior region showing supplements and micropapillae. E) Cloacal region: lateral. G) Tail end. (Scale bar = 10 μm).

sizes, larger (*vs* smaller) amphids, relatively smaller (20-25 μm *vs* 23-31 μm) and well developed (*vs* slender and deformable) spicules, smaller supplement/s S3, S4, S6 (*vs* S6), greatest distance between S5 and S6 (*vs* S3 and S4) supplements and relatively greater distance (12-24 μm *vs* 9-15 μm) between S6 and cloaca; from *B. tsalolikhini* Eyualem and Coomans, 1997 in having absence (*vs* presence) of sexual dimorphism in amphids, smaller outer labial sensilla (4-5 μm *vs* 10-11 μm), relatively fewer (2 *vs* 4) intra-uterine eggs, angular (*vs* straight) gubernaculum, greater distance between S5 and S6 (*vs* S3 and S4) supplements, internal canals of supplements merging with their anterior (*vs* dorsal) walls and lesser number of micropapillae (28-37 *vs* 46); from *B. graciloides* (Daday, 1908) Tsalolikhin, 1991 in having relatively smaller body (1.0-1.5 mm *vs* 1.9 mm), smaller outer labial sensilla (4-5 μm *vs* 7-12 μm), smaller distance (5-6 μm *vs* 8.5-11.5 μm) between stomal teeth, shorter (20-25 μm *vs* 49-51 μm) spicules, unequal (*vs* equal) supplements, smaller distance (12-24 μm *vs* 35-48 μm) between S6 and cloaca and lesser number of micropapillae (28-37 *vs* over 80 in *B. graciloides apud* Eyualem and Coomans, 1997); from *B. glandulatus* in having larger amphids (28-33% *vs* 25-28% of corresponding body width), absence of vaginal glands and the sphincter between pars dilatata and uterus, smaller spicules (20-25 μm *vs* 26-30 μm), smaller supplements S3, S4 and S6 (*vs* S3, S4), S6 within (*vs* outside) spicular range and lesser micropapillae (28-37 *vs* 56-60); from *B. dimorphicus* in lacking sexual dimorphism in amphids and lip sensilla, in having plicate (*vs* weak) uterine wall, a normal rectum (*vs* rectum protruding into intestinal lumen), smaller supplement/s occasionally S6 (*vs* S3, S4 and S6) and different structure of spicules (non capitulate *vs* capitulate with dorsal wall angular (*vs* curved) in *B. dimorphicus*.

Type habitat and locality: Slurry from drains at Allahabad, Uttar Pradesh, India.

Other populations were collected from ditches at Muzaffarnagar (Uttar Pradesh) and Bharatpur (Rajasthan), India; and from a drying pond at Yuan Ming Yuan Park, Beijing, China.

Type designation: Holotype female, allotype male, five females and nine males on slides *Brevitobrilus allhabadensis* n. sp. No.B.S/1-10 deposited at Nematode Collection Department of Zoology, Aligarh Muslim University, Aligarh. One paratype female and one paratype male on slide *Brevitobrilus allhabadensis* n. sp. No.B.S/1-11 deposited at the Laboratory of Nematology, Wageningen University and Research Center (WUR), 6700 ES Wageningen, The Netherlands.

Voucher specimens: Nine females and eight males of Bharatpur population, six females and six males of Muzaffarnagar population and five females and ten males of Beijing population deposited in Nematode Collection, Department of Zoology, AMU, Aligarh, India.

Remarks: In field samples the sex ratio was 1:1 in all populations of *B. allahabadensis* n. sp. indicating amphimixis to be the reproductive mode. Ovoviviparity or segmentation was not observed in the intra-uterine eggs that did not exceed two in a female. Eggs were ovoid and smooth-shelled. The three populations conform well to the type population in morphological characters, however, due to continuum some minor differences were considered as inter population variations. The minor differences noticed in the Beijing population include the rounded to globular pericardial cells compared to elongate ovoid cells in other populations. Out of ten males of Beijing population, S3, S4 and S6 were distinctly smaller in eight specimens while all the supplements appeared more or less equal in one male whereas S3 and S4 were smaller in another.

DISCUSSION

Genus *Brevitobrilus* Tsalolikhin, 1981 represents smaller species of about 1.5 mm (0.9-2.4 mm). Other features of the genus include smooth or finely striated cuticle with sparse somatic setae; short labial sensilla usually up to 1/3 lip diameter; stoma with relatively large buccal cavity, distinctly separated from two adjacent pockets, each bearing a tooth and separated occasionally by a narrow isthmus; amphidial fovea usually

TABLE 3. Measurements (μm) of populations of *Brevitobrilus allahabadensis* sp. n. with mean ± standard deviation (range).

Characters	Holotype female	Allotype male	Allahabad, India Paratypes 6 ♀♀ 10 ♂♂	Bharatpur, India 9 ♀♀ 8 ♂♂	Muzaffarnagar, India 6 ♀♀ 6 ♂♂	Beijing, China 5 ♀♀ 10 ♂♂
Body length	1148		1063±84.3 (947-1172)	1132.6±66.6 (1014-1216)	1301.2±43.8 (1164-1363)	1188.8±90.7 (1080-1340)
Body width	48		43.8±4.4 (36-48)	45.4±4.3 (38-51)	46.8±4.8 (38-52)	46.2±7.0 (39-59)
a	23.9		24.4±2.1 (22.5-28.4)	25.1±3.1 (21.5-30.7)	27.9±1.5 (26.1-30.6)	25.9±1.6 (22.7-27.5)
b	5.8		5.9±0.1 (5.7-6.0)	6.0±0.2 (5.6-6.3)	6.5±0.3 (6.2-7.0)	6.3±0.3 (5.9-6.8)
c	9.3		8.8±0.4 (8.1-9.6)	9.7±0.3 (9.2-10.2)	10.4±0.7 (9.7-12.0)	10.5±0.3 (10.1-11.2)
ć	5.2		5.3±0.3 (4.7-5.6)	5.0±0.3 (4.7 -5.6)	5.3±0.4 (4.4-5.7)	4.9±0.3 (4.3-5.4)
V	40.2		42.8±1.9 (40.2-45.2)	43.2±1.5 (41.6-46.3)	43.7±0.6 (42.7-44.8)	44.3±2.7 (41.1-47.8)
G1	26.8		22.8±3.1 (18.5-26.8)	23.4±4.1 (17.3-29.2)	22.6±4.0 (16.0-26.8)	20.1±4.1 (15.1-26.4)
G2	23.6		25.0±0.8 (23.1-26.1)	22.9±2.1 (20.5-25.5)	25.1±2.5 (22.8-29.2)	18.2±3.3 (12.4-20.8)
Stoma length	18		19.4±1.5 (18-21)	20.4±0.4 (20-21)	17.1±2.2 (16-21)	18.4±0.4 (17-21)
Stoma width	7.5		7.9±0.9 (7.0-9.5)	8.4±0.4 (7-9)	8±0 (8-8)	7.7±0.4 (7-9)
Lip width	16		14.8±2.1 (11-17)	15.8±1.2 (14-17)	16.2±0.6 (15-17)	15.4±0.4 (15-16)
Lip height	3		2.3±0.6 (1.5-3.0)	2.2±0.4 (2-3)	2.2±0.4 (2-3)	2.5±0 (2.5-2.5)
Pharynx	195		179.8±14.1 (158-195)	186.2±6.9 (178-195)	199.8±14.1 (191-219)	179.4±11.1 (165-197)
Nerve ring	78		76±2.8 (73-80)	77.2±2.5 (75-82)	84.8±4.2 (78-90)	75.8±2.9 (70-78)
Rectum length	29		25.8±2.9 (21-30)	23.6±2.7 (20-27)	27.8±1.3 (26-30)	24.2±2.2 (21-27)
V-A distance	4.5		489.7±18.9 (460-531)	540.4±32.3 (529-612)	606.6±26.4 (561-633)	523.0±68.9 (445-645)
ABD	23.5		22.3±1.2 (20.0-26.5)	23.0±1.2 (22-25)	23.2±0.7 (22-24)	23.0±2.4 (20-27)
Tail	123		120±9.4 (104-128)	116.5±6.1 (105-119)	125.2±14.5 (97-138)	112.4±10.5 (100-130)
Body length		1007	1016±29.6 (995-1056)	1102.2±44.4 (1060-1184)	1135.6±45.2 (1079-1194)	1027.1±94.6 (869-1118)
Body width		36	28±7.5 (32-36)	37.0±3.5 (32-42)	29.2±2.3 (26-30)	36.2±5.0 (30-44)
a		27.9	29.9±1.4 (27.9-32.1)	29.9±2.4 (26.0-33.1)	39.0±2.3 (35.6-42.1)	28.7±2.4 (25.4-33.0)
b		6.8	6.4±0.4 (5.9-7.2)	6.4±0.4 (5.8-7.2)	6.2±0.3 (5.8-6.7)	6.3±0.1 (6.1-6.5)
c		12.9	13.0±0.4 (12.5-13.6)	13.7±0.6 (12.8-14.5)	14.8±1.4 (13.3-17.2)	14.4±1.3 (12.5-16.5)
ć		3.9	4.1±0.5 (3.4-5.2)	3.9±0.2 (3.8-4.3)	3.8±0.5 (3.1-4.8)	3.6±0.4 (3.3-4.5)
T		85.1	87.3±2.2 (85.1-92.6)	77.3±5.5 (67.9-83.9)	78.6±2.7 (75.9-83.4)	78.3±3.5 (74.5-84.3)
Stoma length		17	18.0±1.8 (16-19)	16.8±1.9 (14-19)	17.6±1.3 (16-18)	16.2±2.1 (15-19)
Stoma width		8	8.2±0.4 (8-9)	7.5±0.4 (7-8)	7±0 (7-7)	7.2±0.7 (7-9)
Lip width		14	13.1±0.7 (12-14)	14.6±0.8 (13-15)	13.4±0.8 (13-15)	13.2±1.4 (11-15)
Pharynx		148	158.6±13.6 (139-165)	171.8±9.5 (163-189)	180.8±4.7 (174-185)	160.2±11.3 (141-173)
Nerve ring		63	69.1±5.1 (63-78)	60.0±16.7 (70-80)	78.6±3.6 (72-83)	68.1±4.6 (62-73)
Rectum length		25	26.4±1.3 (25-29)	27.4±1.6 (25-29)	25.2±1.8 (23-27)	23.6±2.6 (20-27)
Tail length		78	77.8±3.2 (72-82)	80.6±5.4 (75-89)	77.1±6.1 (66-82)	71.4±7.9 (60-80)
ABD		20	19.6±1.0 (18-21)	20.6±1.6 (18-23)	20.2±1.7 (17-22)	20.3±3.5 (14-24)
Spicules		23	23.2±1.7 (21-25)	21.4±1.0 (20-23)	20.8±1.2 (20-23)	20.6±0.4 (20-24)
Gubernaculum		17	18.8±3.1 (16-24)	15.8±1.3 (14-17)	15.2±1.3 (13-17)	15.3±2.2 (12-18)

conspicuous, at level of anterior pocket; female reproductive system with reflexed ovaries, usually with conspicuous spermathecae; uterine and vaginal musculature moderately developed; male supplements six, bulb-shaped with slightly protruding neck; occasionally one or two supplements (generally the posterior most) smaller than rest; distance between supplements variable; fine papillae of variable number present between the supplements as well as flank them. Sexual dimorphism as demonstrated by some species (*B. fesehai* and *B. tsalolikhini*) in anterior body region needs to be carefully addressed while studying the species of genus.

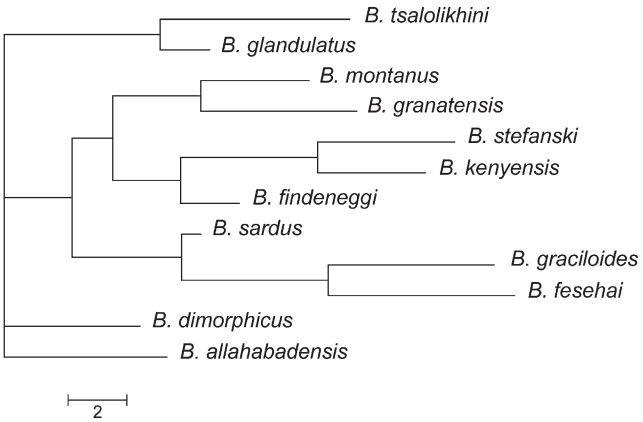

FIG. 8. Rooted phylogram showing relationship between the species of *Brevitobrilus* based on morphological data and representing the consensus tree of maximum parsimony.

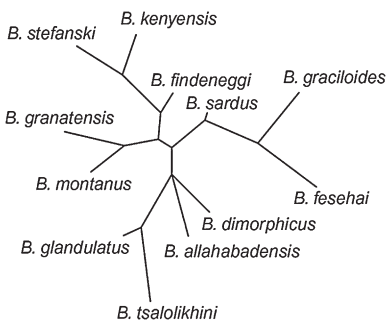

FIG. 9. Unrooted phylogram showing relationship between the species of *Brevitobrilus* based on morphological data and representing the consensus tree of maximum parsimony.

Some of the characters considered taxonomically relevant have been found to be diffuse and variable in populations of the present study viz., continuous/ slightly offset state of lip region, the isthmus between stomal pockets, the shape of pericardial cells and the length of genital tract. In view of present observations, the size of stoma, distance between two teeth and the position of amphids donot seem to be very reliable features for differentiaiton as these appear to be more or less same for species having similar body lengths. Some other features that also show intra-population variations but can be considered cumulatively include the size of supplements, the distances between them and the number of micropapillae. The length and structure of spicules and gubernaculum still hold good for the differentiation of species. Some other reliable characters include the distance of S6 from cloaca, the farthest consecutive supplements, shape of sperms as well as the orientation of the duct within ampulla of supplement.

The analysis of rooted and unrooted consensus trees (Fig. 8, 9) using morphological characters indicates distinct identities of the species of *Brevitobrilus*. The three newly described species stand apart in the cladogram. The twelve species of *Brevitobrilus* seem to arise from three main branches. *B. dimorphicus* and *B. allahabadensis* represent separate branches. *B. glandulatus* while sharing some features viz., more or less equal spicules, relatively straight gubernaculum and about 50-60 micropapillae, groups with *B. tsalolikhini*. *B. montanus* shows a close link with *B. granatensis* and despite some differences resembles the latter in having almost equal lip sensilla, spicules of overlapping sizes, more or less same number of micropapillae and similar ć value. *B. fesehai* and B. *graciloides* forming a closer group reflect some affinities in having all supplements of same size, S6 located far from cloaca, longest distance between S5 and S6 and presence of more than 80 micropapillae. The shared features of closely grouped species *B. stefanski* and *B. kenyensis* are the more or less similar ć value and lip sensilla, longest distance between S5 and S6 and presence of 40-50 micropapillae. The species of *Brevitobrilus* can be identified non cladistically by considering some key characters as given below:

1. All supplements equal and alike 2
- Supplements unequal 6
2. Outer labials more than 7 µm long 3
- Outer labials less than 7 µm long 4
3. Supplement duct merging with dorsal wall of ampulla . . . . . *tsalolikhini*
- Supplement duct merging with anterior wall of ampulla . . . . . *graciloides*
4. Amphids in males conspicuously larger than females 5
- Amphids similar in both sexes 6
5. Micropapillae fewer, 34-38 . . . . . *dimorphicus*
- Micropapillae numerous, up to 140 . . . . . *fesehai*
6. One supplement (S6) smaller than others 7
- Two supplements smaller than others. 8
7. Supplements compressed, spicules well built, straight . . . . . *stefanski*
- Supplements globose, spicules deformable. . . . . *sardus*
8. S5, S6 smaller than others, greatest distance between S3-S4. . . . . *granatensis*
- S5, S6 smaller than others, greatest distance between S5-S6 . . . . . *montanus*
9. Females with conspicuous vaginal glands . . . . . *glandulatus*
- Females without vaginal glands. 10
10. Supplement duct merging with dorsal wall of ampulla . . . . . *findeneggi*
- Supplement duct merging with anterior wall of ampulla 11
11. Subterminal seta 4-5 corresponding tail width anterior to terminus, a= 51.4. . . . . *kenyensis*
- Subterminal seta 1.5-2.5 corresponding tail width anterior to terminus, a = 24.4-42.0 . . . . . *allahabadensis*

Acknowledgments. The financial assistance provided by the TWAS, Italy, CAS, China and DST, New Delhi is gratefully acknowledged.

Appendix 1. Characters and character states for species of *Brevitobrilus*

1. Body up to 1.5 mm (0), greater (1) in size
2. Outer cuticle smooth (0), finely striated (1) under LM
3. Inner (sub)cuticle smooth (0), striated (1) under LM
4. Crystalloids fine granular (0), large (1)
5. Amphids similar in both sexes (0), showing sexual dimorphism (1)
6. Amphids in male 20-30% (0) or greater (1) to corresponding body width
7. Outer labials equal (0), larger (1), smaller (2) than cephalic sensilla
8. Maximum length of sensilla up to 4-5 µm (0), greater (1)
9. Stomal teeth less up to 8 µm (0) or greater distance (1)apart
10. Excretory pore discernible (0), indiscernible (1)
11. Spermatheca indistinct (0), axial (1), adaxial (2)
12. Uteri with moderate musculature (0), well developed striated musculature (1)
13. Vaginal glands absent (0), present (1)
14. Intra-uternie egg usually one (0), more (1)
15. Intra-uterine eggs more or less spherical (0), elongate (1)
16. Sphincter between vas deferens and ejaculatory duct weak (0), moderate (1), well developed (2)
17. Spicules upto 25 µm (0), 30 µm (1)or greater (2)
18. Capitulum absent (0), elliptical (1), attenuated (2)
19. Spicule with (0), without (1) median piece
20. Gubernaculum straight (0), curved (1), angular (2)
21. Greater distance between S5-S6 (0), S3-S4 (1), S1-S2 (2)

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
B. allahabadensis	0	1	0	0	1	0	0	0	0	1	2	1	0	0	1	2	0	1	1	2	0	3	0	1
B. dimorphicus	0	1	1	0	1	1	1	0	0	2	1	0	0	1	1	0	0	1	0	2	0	1	1	1
B. glandulatus	0	1	1	0	0	0	0	0	0	1	2	1	1	0	1	1	1	1	0	1	0	0	1	1
B. fesehai	0	0	0	0	1	1	1	0	0	0	0	1	0	1	0	0	2	1	1	1	0	0	1	0
B. findeneggi	0	0	0	0	1	0	1	0	0	1	0	1	0	1	0	0	0	2	1	1	1	0	0	0
B. graciloides	1	0	0	0	0	0	1	1	1	1	1	1	0	1	1	0	2	0	0	1	0	0	2	1
B. granatensis	1	1	1	0	0	0	1	1	1	0	0	1	0	1	1	0	2	2	1	0	1	2	0	0
B. kenyensis	1	0	0	0	0	0	1	0	1	1	1	1	0	0	0	0	2	2	0	1	0	1	0	0
B. montanus	1	0	1	0	0	0	1	1	1	1	0	0	0	0	1	1	2	1	1	1	0	1	1	2
B. sardus	1	0	0	0	0	0	1	1	1	1	0	1	0	1	0	1	1	2	1	0	1	1	0	1
B. stefanski	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	2	2	0	0	1	0	1	2	1
B. tsalolikhini	0	0	0	0	1	1	1	1	1	1	0	1	0	1	0	1	1	1	1	0	1	1	0	1

22. All supplements equal (0), S6 smaller (1), S5,S6 smaller (2), S3, S4, S6 smaller (3), S1,S6 smaller (4)
23. Distance between S6 and cloaca 5-20 μm (0), 20-35 μm (1), 35-50μm (2)
24. Terminal seta at tail tip (0), 1.5-3 times tail diameter (1) from terminus, greater (2) than that

Literature Cited

Altherr, E. 1965. Le faune des sables submerges des rivers du Rhin pres de Krefeld. Gewässer and Abwässer 39/40:80–101.

Altherr, E. 1976. Nématodes des eaux stygorhitrales des Alpes autrichiennes. Revue Suisse de Zoologie 83:779–847.

Andrássy, I. 1959. Neubenennungen einiger homonymer Nematoden-Gattungen. Nematologica 4:223–226.

Andrássy, I. 1964. Süsswasser-Nematoden aus Grossen Gebirgsgegenden Ostafricas. Acta Zoologica 9:1–59.

Arthington, A. H., Yeates, G. W., and Conrick, D. L. 1996. Nematodes include a new record of *Tobrilus diversipapillatus* in Australia, as potential indicator of sewage effluent pollution. Australian Journal of Marine and Freshwater Research 37:159–166.

Bastian, H. C. 1865. Monograph on the Anguillulidae, or free Nematoids, marine, land and freshwater; with descriptions of 100 new species. Transactions of the Linnean Society of London 25:73–184.

Cobb, N. A. 1918. Estimating the nema population of the soil. U.S. Department of Agriculture. Agricultural Technical Circular of US Department of Agriculture 1:48p.

De Coninck, L.A.P. 1965. Classe des Nématodes- Systématique des Nématodes et sous-classe des Adenophorea 217p *in* P. Grasse, ed. Traité de Zoologié. Tome IV, Fasc. II. Paris: Masson et Cie.

De Ley, P., Decaemer, W., and Eyualem, A. 2006. Introduction: Summary of present knowledge and research addressing the ecology and taxonomy of fresh water nematodes. Pp. 3–30. *in* A. Eyualem, I. Andrássy, and W. Traunspurger, eds. Fresh water nematodes: ecology and taxonomy. CABI Publishing.

Eyualem, A., and Coomans, A. 1997. Aquatic nematodes from Ethiopia VIII. Enoplids, with descriptions of *Brevitobrilus fesehai* n. sp. and *B. tsalolikhini* n. sp. (Enoplida: Nematoda). Hydrobiologia 345:149–184.

Felsenstein, J. 1993. PHYLIP (Phylogeny Interference Package) Version 3.5 c. Distribution by the author. Department of Genetics, University of Washington, Seattle.

Joubert, A. P., and Heyns, J. 1979. Fresh water nematodes from South Africa 3. *Tobrilus* Andrássy, 1959. Journal of the Limnological Society of Southern Africa 5:17–26.

Khera, S. 1975. On some nematodes belonging to the Orders Chromodorida and Enoplida from India. Records of Zoological Survey of India 68:273–286.

Leidy, J. 1851. Contributions to Helminthology. Proceedings of the Academy of Natural Sciences of Philadelphia 5:205–209.

Loof, P.A.A., and Riemann, F. 1976. Taxonomy of the *Tobrilus longus* group (Enoplida: Tripylidae). Nematologica 22:5–48.

Micoletzky, H. 1925. Die freilebenden Süßwasser- und Moornematoden Dänemarks. Nebst Anhang: Über Amobosporidien und andere Parasiten bei freilebenden Nematoden. Det Kongelige Danske Videnskabernes Selskabs Skrifter Naturvidenskabelig og Mathematisk Afdeling 8:57–310.

Ocana, A., and Zullini, A. 1988. A new species of *Tobrilus* (Nematoda) from spring water. Nematologica 34:1–5.

Ocana, A., Harnandez, J. A., and Martin, I. 1996. A new species and new combinations (Nematoda-Tobrilidae) from Spain. Journal of Nematology 28:190–195.

Roderic, D. M. 1996. Treeview: An application to display phylogenetic trees on personal computers. Computer applications in Biological Sciences 12:357–358.

Schiemer, V. F. 1971. Diagnose von *Tobrilus findeneggi* n. sp. mit Bemerkungen zur Gattung Tobrilus (Nematoda). Carinthia 31:147–157.

Schiemer, V. F., and Duncan, A. 1974. The oxygen consumption of a fresh water benthic nematode *Tobrilus gracilis* (Bastian). Oecologia 15:121–126.

Seinhorst, W. 1959. A rapid method for the transfer of nematodes from fixative to anhydrous glycerin. Nematologica 4:67–69.

Tsalolikhin, S. J. 1981. Revision of the genus *Tobrilus* (Nematoda-Tobrilidae). Zoologicheskii Zhurnal 60:1302–1313.

Tsalolikhin, S. J. 1992. Taxonomic notes on African species of the genus *Brevitobrilus* (Nematoda: Enoplida: Tobrilidae). Zoosystematica Rossica 1:1–5.

Vinciguerra, M. T., and Zullini, A. 1991. Two new species of *Tobrilus* (Nematoda) from Italy. Nematologica Mediterranea 19:269–274.

Zullini, A. 2006. Order Triplonchida. Pp. 203–325. *in* Eyualem-Abebe, I. Andrássy and W. Traunspurger, eds. Fresh water nematodes: ecology and taxonomy. CABI Publishing, 203–325.