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### Monograph

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## New insights into *Diplogastrellus* Paramonov, 1952 (Nematoda: Rhabditida: Diplogastroidea) with three novel and three known species from India

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**Abstract.** Three new and three known species of the genus *Diplogastrellus* Paramonov, 1952 are described and illustrated from India. *Diplogastrellus didelphis* sp. nov. is characterised by a narrow stoma; a medium-sized dorsal tooth with ventrally directed apex; an oblong median bulb, a small pyriform basal bulb; an amphidelphic reproductive system; a large uterine pouch; ventrally arcuate spicules with a 90° curvature; a stout gubernaculum, proximally notched with a long dorsal arm, distally with a sleeve and nine pairs of genital papillae. *Diplogastrellus longipharyngis* sp. nov. is characterised by a short tubular stoma; a medium-sized ventrally directed dorsal tooth; an ovoid median bulb and a long isthmus; heavily cuticularized almost straight spicules; a keel-like triangular gubernaculum proximally notched, a caudal margin interrupted and nine pairs of genital papillae. *Diplogastrellus robustus* sp. nov. is characterised by a medium-sized dorsal tooth with a ventrally directed apex; an ovoid median bulb; a mono-prodelphic or amphidelphic reproductive system; robust spicules, with a blunt distal tip; a boat-shaped gubernaculum with a prominent distal sleeve and nine pairs of genital papillae. Three known species, viz., *D. gracilis*, *D. graciloides* and *D. monhysteroides* are redescribed/illustrated with emended diagnosis and SEM details. The comparative analysis of morphometrics and morphological traits as well as cladogram based on morphological characters are provided besides their biogeographic distribution.

**Keywords.** Description, *Diplogastrellus*, morphology, new species, SEM, taxonomy.

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## Introduction

The genus *Diplogastrellus* Paramonov, 1952 was placed in Diplogastridae Micoletzky, 1922 by Sudhaus & Fürst von Lieven (2003). Earlier, Kiontke & Sudhaus (1996) considered *Diplogastrellus* as a subgenus of *Diplogaster* Schultze in Carus, 1857, revised the species group, hypothesised on their phylogeny and provided keys to both sexes. They also added a new species *D. cerea* and synonymised *Metadiplogaster secundus* Dassonville & Heyns, 1984 and *Tawdenema indicus* Suryawanshi, 1971 with *Diplogastrellus*. In addition, some species from the genus *Acrostichus* Rahm, 1928 and *Diplogasteritus* Paramonov, 1952 were transferred to *Diplogastrellus*. Sudhaus & Fürst von Lieven (2003) considered *Diplogastrellus* as a separate genus with 12 species while Andrásy (2005) regarded *Diplogastrellus* as a junior synonym of *Acrostichus*. Ahmad *et al.* (2005) reported *D. thoubalicus* collected from farmyard manure in Manipur. Kanzaki *et al.* (2008) isolated a species of *Diplogastrellus*, viz., *D. metamasius*, phoretically associated with *Metamasius hemipterus* (Linnaeus, 1758). Khan *et al.* (2008) described a new species *D. sikorai*, collected from wet humus of a polluted drain at Quarsi, Aligarh. Mahamood *et al.* (2015) added one more species to the genus namely *D. latigubernacula* collected from farmyard manure in Jammu & Kashmir, thus making a total of 16 valid species under the genus *Diplogastrellus*. Later, Ledón-Rettig *et al.* (2018) showed that the nematode *Diplogastrellus monhysteroides* (Bütschli, 1874) was transgenerationally inherited and sexually transmitted by dung beetle *Onthophagus taurus* (Schreber, 1759) and enhances the growth of beetle offspring. Burdine *et al.* (2024) described the sexually transmitted mutualist nematode *Diplogastrellus monhysteroides* shape host growth across dung beetle species.

The present paper provides the description of three new species, *Diplogastrellus didelphis* sp. nov., *D. longipharyngis* sp. nov. and *D. robustus* sp. nov., as well as three known species, *D. gracilis* (Bütschli, 1876), *D. graciloides* (Skwarra, 1921) and *D. monhysteroides*, collected from different saprobic habitats of India. A morphology-based cladistic analysis of the genus *Diplogastrellus* is presented to resolve the exact status of the species and their relationships.

## Material and methods

### Collection and isolation of nematodes

Samples from rotten plant material, farmyard manure and soil were processed through Cobb's (1918) sieving, decantation, and modified Baerman's funnel techniques for extraction of nematodes.

### Preparation for light microscopy

For the light microscopic study, the extracted nematodes were fixed in 4% formalin for 24 hours, dehydrated (Seinhorst 1959) in glycerin-alcohol and mounted in anhydrous glycerin on slides using the wax ring technique (De Maeseneer & d'Herde 1963). Nematodes were measured using an ocular micrometer, and drawings were made using a drawing tube attached to an Olympus BX50 DIC microscope. Light microscopy images were taken with a Jenoptik ProgRes C5 digital camera (Jenoptik, Jena, Germany) attached to the DIC microscope.

### Preparation for scanning electron microscopy

The fixed specimens as well as those stored earlier in anhydrous glycerin, were processed for scanning electron microscopic study. The dehydrated specimens were hydrated by placing them in glycerin (100%)–ethanol (30%) graded series in descending proportion of glycerin (95%, 80%, 70%, 50%, 30% and 0%). The last step was repeated twice to ensure the complete replacement of glycerin with 30% ethanol following which the specimens were transferred to distilled water along with the fixed specimens. The specimens were then fixed in 2% glutaraldehyde for 24 hours, washed in 0.05 M sodium phosphate buffer (pH = 6.9) several times and dehydrated through a graded ethanol series. The dehydrated specimens were

subsequently critical-point dried using carbon dioxide, mounted on stubs, coated with 10 nm gold and observed under a Joel JSM 6510 microscope at 15 kV.

### **Cladistic analysis. Characters and character states**

Thirty morphological characters as listed in Appendix 1 were selected for the analysis. For cladogram construction, the species were compared based on plesiomorphic and apomorphic characters (Fig. 18). The characters present in most genera were considered plesiomorphic; in contrast, those possessed rarely were categorized as apomorphic characters. All the characters were important and informative with context to the differentiation of species of *Diplogastrellus*. The characters were ranked based on the commonality principle. For studying the relationship within the species of *Diplogastrellus*, character state '0' represented the most commonly occurring trait. A data matrix (Appendix 2) was prepared using character states for different species of the genus and owing to their similarity, *Acrostichus nudicapitatus* Steiner, 1914 was taken as an outgroup. The phylogenetic tree was constructed for nineteen species of *Diplogastrellus* containing 30 characters using PAUP ver. 4.0. (Swofford 2002).

### **Deposition of specimens**

One male and one female paratype each of *Diplogastrellus didelphis* sp. nov., *D. longipharyngis* sp. nov., *D. robustus* sp. nov. and specimens of *D. gracilis*, *D. graciloides*, *D. monhysteroides* were deposited in the Nematode Collection of the Zoological Survey of India (ZSI), Kolkata, whereas the remaining paratypes and holotypes of the above-mentioned species were deposited in the Nematode Collection, Department of Zoology, Aligarh Muslim University (AMU/ZD/NC), India.

### **List of abbreviations**

- a = Body length/greatest body diameter
- b = Body length/distance from anterior end to the pharyngo-intestinal junction
- c = Body length/tail length
- c' = Tail length/anal body diameter
- G1 = Length of anterior genital branch × 100/body length
- G2 = Length of posterior genital branch × 100/body length
- L = Total body length
- n = Number of specimens
- T = Total length of testes × 100/body length
- V = Distance of vulva from anterior end × 100/body length

## **Results**

### ***Systematics and taxonomy***

- Phylum Nematoda Cobb, 1932
- Class Chromadorea Inglis, 1983
- Order Rhabditida Chitwood, 1933
- Suborder Diplogastrina Micoletzky, 1922
- Superfamily Diplogastroidea Micoletzky, 1922
- Family Diplogastridae Micoletzky, 1922
- Genus *Diplogastrellus* Paramonov, 1952

### **Type species**

*Diplogastrellus gracilis* (Bütschli, 1876).

### Diagnosis

Body medium-sized. Cuticle usually appearing smooth. Stoma longer than wide. Cheilostom as long as gymnostom, with six flaps. Gymnostom anisotropic, dorsal gymnostomal wall being much shorter than subventrals. Stegostom anisotropic and anisomorphic; dorsal wall with dorsal flap or tooth. Subventral walls with a tooth or teeth or simply a ridge. Pharynx well-developed, muscular metacorpal bulb with valve plates, rounded to ovoid basal bulb. Female reproductive system mono-prodelphic or amphidelphic. Uterus divided into well-developed glandular and muscular parts. Genital sensilla nine pairs. Bursa absent. Tail long, filiform.

*Diplogastrellus didelphis* sp. nov.

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Figs 1–2, 17–19; Tables 1, 7–8

### Diagnosis

The new species *Diplogastrellus didelphis* sp. nov. is characterised by finely punctated cuticle (Fig. 2H); a narrow stoma longer than wide; a medium-sized dorsal tooth with ventrally directed apex; amphidial apertures small, elliptical, at the base of gymnostom; oblong median bulb with valve plates and a pyriform basal bulb; amphidelphic reproductive system, a large uterine pouch filled with sperm (Fig. 2I); spicules separate, strongly built, ventrally arcuate with a 90° curvature; proximally sickle-shaped or notched with a long anteriorly-directed dorsal arm and a short ventral arm, distal end thin and delicate with a sleeve and nine pairs of genital sensilla.

### Etymology

The name of the species is based on the didelphic-amphidelphic gonad.

### Material examined

#### Holotype

INDIA • ♀; Uttar Pradesh, District Muzaffarnagar; 29°31'27" N, 77°38'38" E; isolated from rotting banana rhizome; slide reference number AMU/ZD/NC/*Diplogastrellus didelphis*/1.

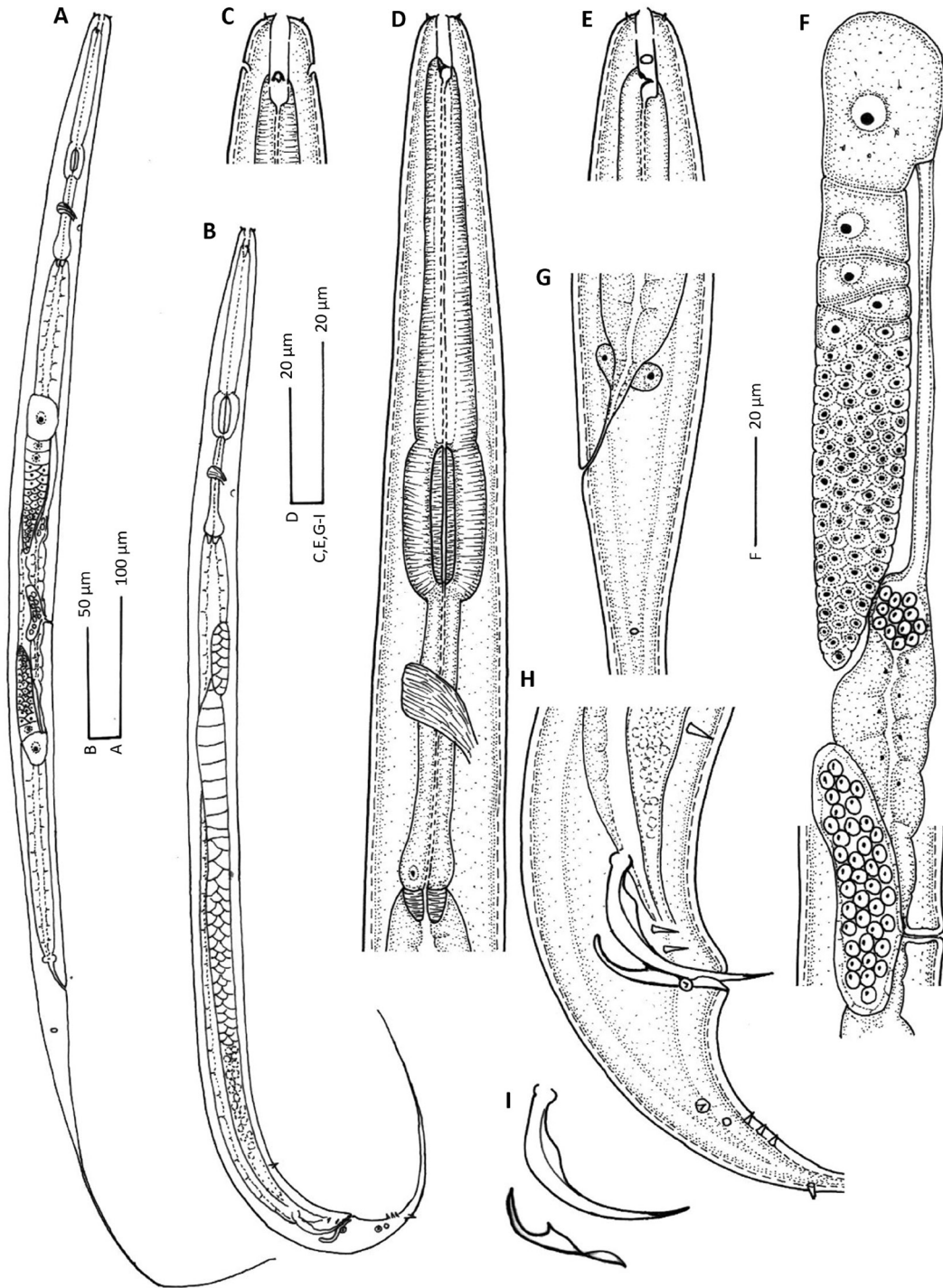
#### Paratypes

INDIA • 9 ♀♀, 10 ♂♂; same data as for holotype; slide reference number AMU/ZD/NC/*Diplogastrellus didelphis*/2–9.

### Description

#### Adult

Body slender, less than 1 mm long; almost straight after fixation, tapering only very slightly anteriorly, but more posteriorly forming a filiform tail. Cuticle with fine transverse and longitudinal striations. Cuticular punctations fine, transversely arranged (Fig. 2H). Lip region round, continuous with body contour. Lips six, amalgamated, each bearing a small papilliform labial sensilla. Amphidial apertures small, elliptical, 6–7 µm from anterior end of stoma, at the base of gymnostom. Stoma narrow, tubular, longer than wide, about 10–11 µm in depth. Cheilostom slightly longer than wide, cheilorhabdions arched inward anteriorly, cheilorhabdial flaps six, protruding above labial contour; gymnostom anisotropic, dorsal wall shorter than subventrals. Stegostom anisotropic and anisomorphic, dorsal metastegostomal wall with medium-sized tooth, with ventrally directed apex, subventral walls without armature. Pharynx with slender 65–78 µm long, muscular corpus of uniform diameter; median bulb set off from corpus, oblong, 23–29 µm long with strong valve plates. Isthmus 32–42 µm long, narrow, conspicuously differentiated from median bulb. Basal bulb small, pyriform, glandular, 12–15 × 9–10 µm, only slightly expanded from isthmus without any valve plate or grinder. Dorsal pharyngeal gland nucleus usually prominent, located



**Fig. 1.** *Diplogastrellus didelphis* sp. nov. **A, C–G.** Holotype, ♀ (AMU/ZD/NC/*Diplogastrellus didelphis* sp. nov./1). **B, H–I.** Paratype, ♂ (AMU/ZD/NC/*Diplogastrellus didelphis* sp. nov./2–9). **A.** Entire female. **B.** Entire male. **C.** Anterior region showing stoma (lateral view). **D.** Pharyngeal region. **E.** Anterior region showing stoma (dorsoventral view). **F.** Female reproductive system showing anterior branch. **G.** Female posterior region. **H.** Male posterior region. **I.** Spicule and gubernaculum.



**Fig. 2.** *Diplogastrellus didelphis* sp. nov. **A–F, H–I.** Holotype, ♀ (AMU/ZD/NC/*Diplogastrellus didelphis* sp. nov./1). **F–G, J.** Paratype, ♂ (AMU/ZD/NC/*Diplogastrellus didelphis* sp. nov./2–9). **A.** Pharyngeal region. **B.** Anterior region showing stoma (lateral view). **C.** Anterior region showing amphid. **D.** Female reproductive system showing anterior branch. **E.** Female posterior region. **F.** Male genital papillae. **G, J.** Male posterior region showing spicules and gubernaculum. **H.** Cuticular punctations. **I.** Uterine pouch. The arrowheads in C, F and I indicate amphidial aperture, genital sensilla and uterine pouch, respectively. Scale bars = 10 µm.

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

Characters	Holotype female	Paratype females (n = 9)	Paratype males (n = 10)
L	877	837.3 $\pm$ 61.1 (735–948)	668.9 $\pm$ 50.7 (592–770)
a	28.3	28.1 $\pm$ 1.6 (26.0–31.6)	28.2 $\pm$ 2.1 (23.9–31.3)
b	5.4	5.7 $\pm$ 0.3 (5.1–6.2)	5.1 $\pm$ 0.4 (4.5–5.8)
c	3.8	3.8 $\pm$ 0.2 (3.5–4.2)	4.6 $\pm$ 0.3 (4.1–5.1)
c'	16.4	15.6 $\pm$ 1.1 (13.5–17.0)	7.3 $\pm$ 0.4 (6.8–8.1)
V/T	47	45.7 $\pm$ 1.1 (44–47)	55.8 $\pm$ 5.3 (46–62)
G1	25.8	31.5 $\pm$ 4.2 (26–38)	–
G2	21.8	24.8 $\pm$ 3.8 (19–31)	–
Maximum body width	31	29.9 $\pm$ 2.6 (25–35)	23.8 $\pm$ 2.1 (22–29)
Lip width	7	6.9 $\pm$ 0.3 (6–7)	6.4 $\pm$ 0.5 (6–7)
Length of stoma	11	10.2 $\pm$ 0.4 (10–11)	9.2 $\pm$ 0.4 (9–10)
Corpus	78	71.4 $\pm$ 3.9 (65–78)	61.8 $\pm$ 2.7 (57–65)
Median bulb	28	26.9 $\pm$ 1.7 (23–29)	22.1 $\pm$ 1.5 (20–25)
Isthmus	42	36.1 $\pm$ 3.1 (32–42)	36.0 $\pm$ 3.3 (32–42)
Basal bulb	13	13.0 $\pm$ 0.9 (12–15)	11.1 $\pm$ 0.7 (10–12)
Pharynx	161	147.4 $\pm$ 6.3 (139–161)	131.0 $\pm$ 6.4 (123–141)
Cardia	5	5.0 $\pm$ 0.0 (5–5)	3.6 $\pm$ 0.5 (3–4)
Nerve ring from ant. end	121	111.6 $\pm$ 5.1 (105–121)	98.7 $\pm$ 4.2 (92–105)
Pharynx-gonad distance	119	78.6 $\pm$ 18.0 (57–119)	50.7 $\pm$ 18.9 (27–90)
Anterior gonad	227	265.1 $\pm$ 46.1 (188–343)	–
Posterior gonad	192	208.3 $\pm$ 36.1 (138–253)	–
Uterine pouch	67	61.8 $\pm$ 14.7 (35–80)	–
VBD	29	29.7 $\pm$ 2.6 (25–35)	–
Vulva-anus distance	237	232.9 $\pm$ 20.6 (203–273)	–
Vulva from anterior end	410	382.4 $\pm$ 26.7 (331–420)	–
Rectum	16	14.8 $\pm$ 1.0 (13–16)	19.3 $\pm$ 0.9 (18–21)
Tail	230	222.0 $\pm$ 19.0 (200–255)	146.1 $\pm$ 8.4 (132–165)
ABD	14	14.2 $\pm$ 0.6 (13–15)	20.0 $\pm$ 1.1 (18–22)
Phasmid from anus	23	21.3 $\pm$ 2.5 (18–25)	19.6 $\pm$ 1.3 (17–22)
Testis	–	–	374.1 $\pm$ 53.0 (282–477)
Spicule along axis	–	–	30.4 $\pm$ 0.5 (30–31)
Spicule along cord	–	–	22.3 $\pm$ 1.2 (20–24)
Gubernaculum	–	–	18.2 $\pm$ 0.4 (18–19)
No. of precloacal papillae	–	–	3
No. of postcloacal papillae	–	–	6

near base of basal bulb. Nerve ring encircling isthmus in anterior half, located at 73–77% of pharyngeal length. Hemizonid and excretory pore not visible. Cardia well-developed, 5 µm long, consisting of three flaps, one dorsal and two ventro-sublateral. Intestine composed of dark granulated cells with prominent nuclei, intestinal lumen uniformly wide, 5–6 µm without any bacterial pouch. Rectum 0.9–1.1 times anal body diameter long; rectal glands distinct.

### Female

Reproductive system didelphic, amphidelphic, anterior gonad on right side and posterior on left side of intestine. Posterior branch usually smaller than the anterior. Ovaries long reversed, with distal part of ovaries not reaching the level of vulva. Oocytes with large nuclei, arranged in multiple rows in germinal zone and single row in maturation zone. Oviduct long, narrow, tubular. Spermatheca not set off from uterus, with distinctly narrower walls, containing sperm. Uterus divisible into a distal smaller muscular part and proximally placed longer, glandular part made up of large cells and narrow lumen. A large uterine pouch, 35–80 µm long, opens into reproductive tract at uterus-vagina junction, filled with sperm. Vagina narrow, tubular, almost at right angle to longitudinal body axis, 6–7 µm long about one-fourth of corresponding body diameter. Vulval opening small, elliptical, flushing with body contour. Vulva-anus distance 7.2–9.1 times vulval body diameter. Phasmids located at a level 1.3–1.7 of anal body diameter posterior to anus. Tail long, filiform 0.8–1.1 times vulva-anus distance, divided into two parts, an anterior short conoid part and a posterior longer filamentous part.

### Male

Similar to females in general morphology but smaller in size. Reproductive system monorchic, testis reflexed laterally, on right side of intestine. Spermatocytes arranged in two rows distally followed by single row proximally. Vas deferens a long tube containing spermatocytes transforming into spermatozoa, tapering to an ejaculatory duct. Spicules paired, separate, strongly built, ventrally arcuate with a 90° curvature, 1.4–1.7 times cloacal body diameter long. Manubrium round connected to calamus/lamina complex expanding into ventral conoid process, posteriorly tapering to a pointed distal tip. Gubernaculum stout, 58–61% of spicule length, proximally sickle-shaped or notched with a long anteriorly-directed dorsal arm and a short ventral arm, distal end thin and delicate with a sleeve. Tail divisible into two parts, a short conoid part and a longer filamentous part. Genital sensilla nine pairs; three pairs precloacal and six pairs postcloacal. Genital sensilla formula:  $v1, v2d, v3/v4, ad, ph, (v5, v6, v7), pd$ . Precloacal pair  $v1$  located more than one cloacal diameter anterior to cloaca;  $v2$  and  $v3$  closely placed;  $v3$  just anterior and  $v4$  closely posterior to cloaca;  $ad$  less than one cloacal body diameter posterior to cloaca,  $v5-7$  grouped;  $pd$  far posterior to  $v7$ . Phasmids pore-like, 0.7–1.1 anal body diameter posterior to cloacal opening.

### Remarks

*Diplogastrellus didelphis* sp. nov. comes close to *D. gracilis* in most morphometric and morphological characteristics but differs in the shape of stoma (narrow, tubular vs comparatively broader), structure of dorsal tooth (anterio-ventral margin lacking serration vs serrated antero-ventral margin), position of amphids (at the base of gymnostom vs at the base of cheilostom), female reproductive system (amphidelphic vs mono-prodelphic), uterine pouch (present vs absent), size and shape of spicules (30–31 µm; strongly built, calamus/lamina complex expanded into ventral conoid process vs 42–58 µm; long, attenuated, calamus/lamina complex not expanded), shape of gubernaculum (proximally notched with a long dorsal arm vs keel-like proximal part) and in the arrangement of genital sensilla ( $v3$  anterior to cloaca vs  $v3$  adcloacal).

The new species differs from *D. monhysteroides* in the shape of dorsal tooth (ventrally directed apex vs anteriorly directed apex), shape of median bulb (oblong vs ovoid), female reproductive system (amphidelphic vs mono-prodelphic), uterine pouch (present vs absent), shape of spicules (strongly arcuate with a 90° curvature to a pointed distal end vs smoothly arcuate to a curved distal end), structure

of gubernaculum (proximally notched with a long dorsal arm, distal part thin with a sleeve vs proximally curved, distal part with a large rectangular sleeve) and arrangement of genital sensilla (*v*2 anterior to cloaca far away from *v*1 vs *v*2 more anterior near the spicule head, close to *v*1).

The new species resembles *D. metamasius* in most morphometric characters but differs in having more slender body ( $a = 26.0\text{--}31.6$  vs  $16.1\text{--}22.2$  in females), female reproductive system (amphidelphic vs mono-prodelphic), by uterine pouch (present vs absent), shape of spicules (strongly arcuate with a  $90^\circ$  curvature vs slightly curved), structure of gubernaculum (proximally notched with a long dorsal arm, distal sleeve present vs proximally notched, sleeve absent) and arrangement of genital sensilla (*v*2 anterior to cloaca within the spicular range vs *v*2 more anterior beyond the spicule head).

*Diplogastrellus didelphis* sp. nov. differs from *D. graciloides* in the position of amphids (at the base of gymnostom vs posterior to the base of stoma), female reproductive system (amphidelphic vs mono-prodelphic), uterine pouch (present vs absent), size of spicules ( $30\text{--}31\ \mu\text{m}$  vs  $24.5\ \mu\text{m}$ ), structure of gubernaculum (proximally notched with a long dorsal arm, distal sleeve present vs caudally projected keel-like without sleeve) and arrangement of genital sensilla (9 pairs; *v*1 beyond the spicular range vs 8 pairs; *v*1 located near the spicule head; *v*7 absent).

*Diplogastrellus didelphis* sp. nov. resembles *Acrostichus medius* Tahseen *et al.*, 2016 in having didelphic reproductive system in females amphidelphic, and by presence of uterine pouch and stomal morphology. *Acrostichus medius* shows features common to both *Diplogastrellus* and *Acrostichus*; however, *Diplogastrellus didelphis* markedly differs from it in the structure of spicules (strongly arcuate with about  $90^\circ$  curvature vs robust spicules with hood-like capitula, appearing deeply forked distally with fine extensions and a ventral attenuated arm) and gubernaculum (proximally notched with a long dorsal arm, distal sleeve vs curved and tapering proximal end and distal end with slight protuberances), bursa (absent vs with rudiments confined to posterior four genital pairs) in addition to other morphometric characters.

***Diplogastrellus longipharyngis* sp. nov.**

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Figs 3–5, 17–19; Tables 2, 7–8

**Diagnosis**

The new species *Diplogastrellus longipharyngis* sp. nov. is characterised by a narrow stoma longer than wide; elliptical amphidial aperture, at the level of dorsal tooth; a medium-sized dorsal tooth with ventrally directed apex; pharynx well-developed, muscular ovoid metacorporeal bulb with valve plates, a long isthmus and ovoid basal bulb; posterior pharynx 1.5–1.9 times anterior pharynx; mono-prodelphic reproductive system with a post-uterine sac filled with sperm; spicules separate, heavily cuticularized, slightly curved with small rounded manubrium; a proximally notched, keel-like triangular gubernaculum and nine pairs of genital sensilla.

**Etymology**

The name of the species is derived from the long isthmus part of pharynx.

**Material examined**

**Holotype**

INDIA • ♀; Uttarakhand, District Haldwani;  $29^\circ 12' 52''$  N,  $79^\circ 31' 40''$  E; isolated from rotting bark; slide reference number AMU/ZD/NC/*Diplogastrellus longipharyngis*/1.

### Paratypes

INDIA • 9 ♀♀, 10 ♂♂; same data as for holotype; slide reference number AMU/ZD/NC/*Diplogastrellus longipharyngis*/2–9.

### Description

#### Adult

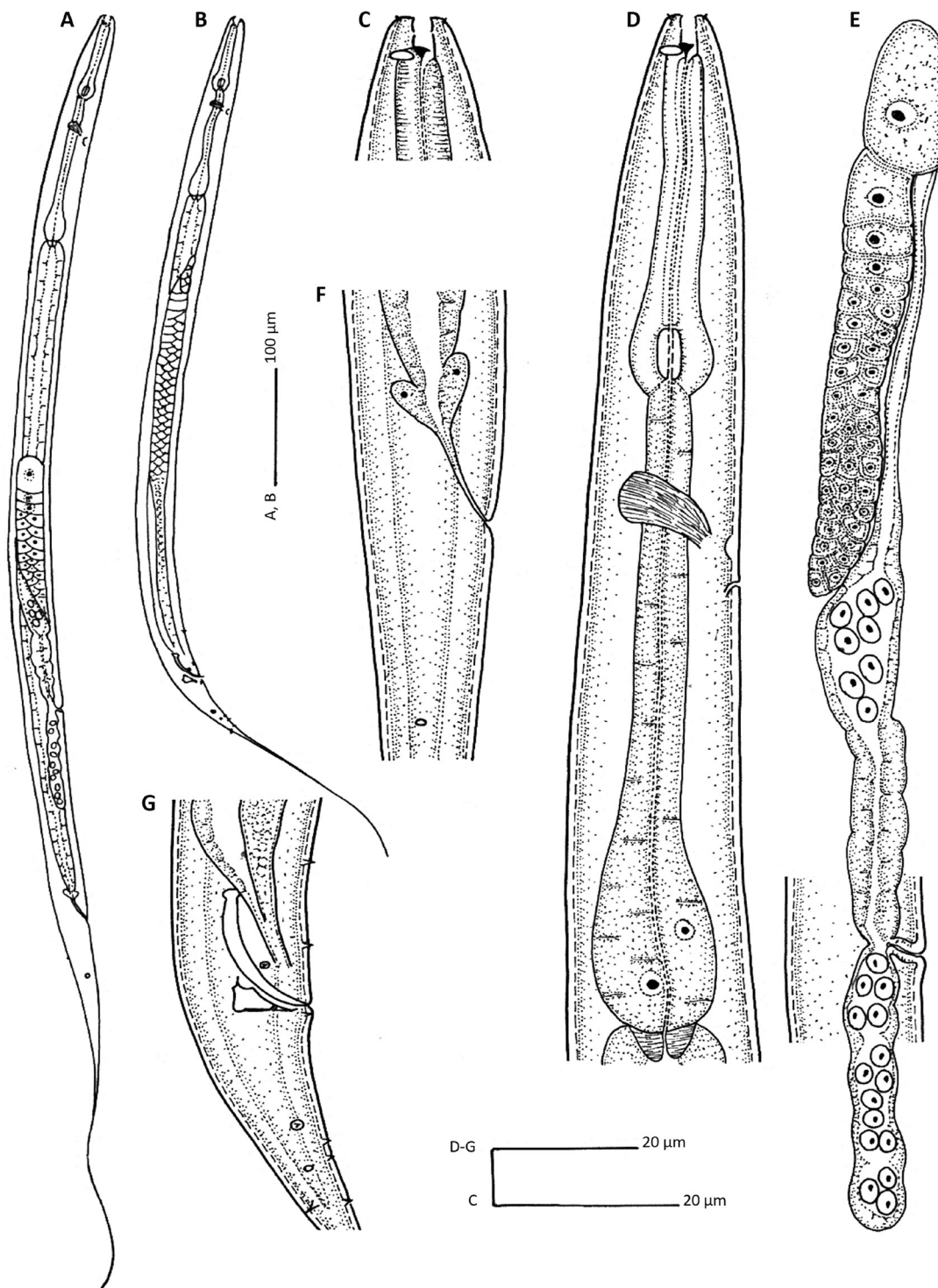
Body slender, about 1 mm long, almost straight after fixation, tapering towards both the extremes. Cuticle with transverse striations. Lip region continuous with body contour. Lips six, amalgamated, each bearing a small papilla. Amphidial apertures elliptical, 5–6 µm from anterior end of stoma, at the level of dorsal tooth. Stoma short, tubular, longer than wide, about 5–6 µm in depth. Cheilostom as long as wide, cheilorhabdions arched inward anteriorly. Gymnostom anisotropic, dorsal wall shorter than subventrals. Stegostom anisotropic and anisomorphic, dorsal metastegostomal wall with a medium-sized tooth, with ventrally directed apex, posterior-ventral aspect strongly cuticularised; subventral walls without armature. Pharynx with slender 42–51 µm long, muscular corpus of uniform diameter; median bulb set off from corpus, ovoid, 11–15 µm long with strong valve plates. Isthmus 65–85 µm long, narrow, conspicuously differentiated from median bulb. Basal bulb bigger than the median bulb, glandular, 25–33 × 13–17 µm, only slightly expanded from isthmus without any valve plate or grinder. Posterior pharynx 1.5–1.9 times anterior pharynx. Dorsal pharyngeal gland nucleus usually prominent, located near base of basal bulb. Nerve ring encircling isthmus in the anterior half, at 46–51% of pharyngeal length. Hemizonid conspicuous, just posterior to the nerve ring, at 51–56% of pharyngeal length. Excretory pore posterior to hemizonid, at 55–59% of pharyngeal length. Cardia well-developed, 5–6 µm long, consisting of three flaps, one dorsal and two ventro-sublateral. Intestine composed of dark granulated cells with prominent nuclei, intestinal lumen uniformly wide, 5–6 µm without any bacterial pouch. Rectum 1.2–1.5 times anal body diameter long; rectal glands distinct.

#### Female

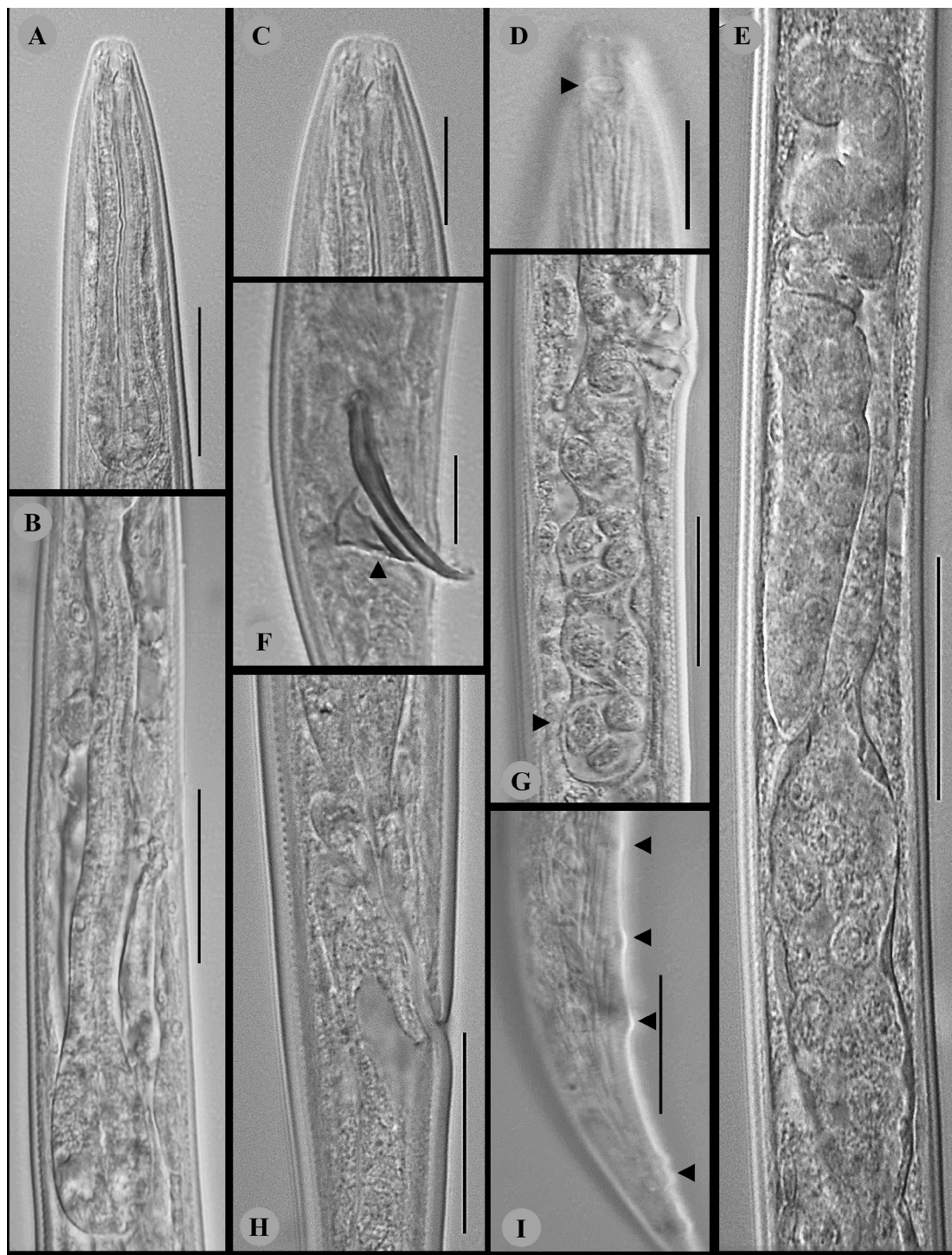
Reproductive system mono-prodelphic. Ovary reversed on right side of intestine. Oocytes with large nuclei, arranged in multiple rows in germinal zone and single row in maturation zone. Distal tip of ovary not reaching vulva. Oviduct long, narrow, tube-like. Spermatheca expanded, set off from uterus with distinctly narrower walls, containing sperm. Uterus divisible into a distal smaller muscular part and proximally placed longer, glandular part made up of large cells and narrow lumen. Post-uterine sac 35–49 µm long, 2.0–2.5 times vulval body diameter, filled with sperm. Vagina narrow, tubular, slightly inclined anteriorly, 11–16 µm long about one-third of corresponding body diameter long. Vulval opening small, pore-like. Vulva-anus distance 5.5–7.0 times vulval body diameter. Phasmids located at 1.8–2.4 anal body diameter posterior to anus. Tail long, filiform 1.5–1.9 times vulva-anus distance.

#### Male

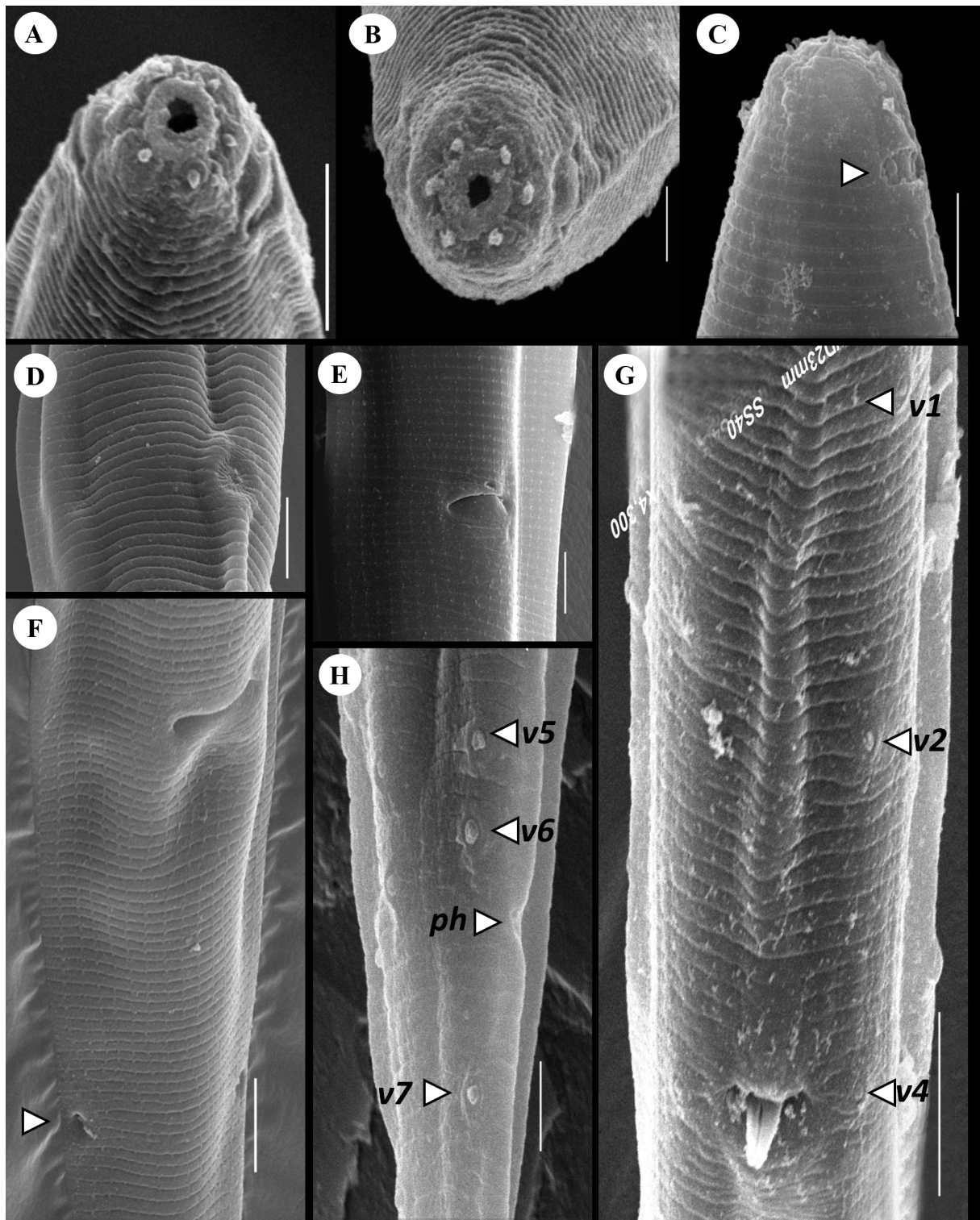
Similar to females in general morphology but smaller in size, curved in posterior region. Reproductive system monorchic, testis reflexed laterally, on right side of intestine. Spermatocytes arranged in two rows distally followed by single row proximally. Vas deferens a long tube containing spermatocytes transforming into spermatozoa, tapering to an ejaculatory duct. Spicules paired, separate, almost straight, heavily cuticularized, 1.4–1.6 times cloacal body diameter long. Manubrium small, rounded, lamina/calamus complex not expanded, smoothly tapering to a pointed distal tip. Gubernaculum 37–44% of spicule length, proximally notched, appearing triangular, keel-like with a pointed distal end. Tail divisible into two parts, a short conoid part and a longer filamentous part. Genital sensilla nine pairs; three pairs precloacal and six pairs postcloacal. Genital sensilla formula: *v1*, *v2*, *v3d/v4*, *ad*, (*v5*, *v6*), *ph*, *v7*, *pd*. *v1* located beyond the spicular range, more than one cloacal body diameter anterior to cloaca; *v2* less than one cloacal body diameter anterior to cloaca; *v3d* slightly posterior to *v2*; *v4* just posterior to cloaca; *ad* one cloacal body diameter posterior to cloaca; *v5–7* not grouped, *v5* and *v6* close to each other



**Fig. 3.** *Diplogastrellus longipharyngis* sp. nov. **A, C–F.** Holotype, ♀ (AMU/ZD/NC/*Diplogastrellus longipharyngis* sp. nov./1). **B, G.** Paratype, ♂ (AMU/ZD/NC/*Diplogastrellus longipharyngis* sp. nov./2–9). **A.** Entire female. **B.** Entire male. **C.** Anterior region showing stoma. **D.** Pharyngeal region. **E.** Female reproductive system. **F.** Female posterior region. **G.** Male posterior region showing spicules and gubernaculum.



**Fig. 4.** *Diplogastrellus longipharyngis* sp. nov. A–E, G–H. Holotype, ♀ (AMU/ZD/NC/*Diplogastrellus longipharyngis* sp. nov./1). F, I. Paratype, ♂ (AMU/ZD/NC/*Diplogastrellus longipharyngis* sp. nov./2–9). A. Anterior part of pharynx. B. Posterior part of pharynx. C. Anterior region showing stoma. D. Anterior region showing amphid. E. Female reproductive system showing anterior branch. F. Male posterior region showing spicules and gubernaculum. G. Post-uterine sac. H. Female posterior region. I. Male posterior region showing genital papillae. The arrowheads in D, F–G and I indicate amphidial aperture, distal part of keeled gubernaculum, sperm-filled post-uterine sac and genital papillae, respectively. Scale bars: A–B, E, G–I = 20 µm; C–D, F = 10 µm.



**Fig. 5.** *Diplogastrellus longipharyngis* sp. nov. **A–F.** Holotype, ♀ (AMU/ZD/NC/*Diplogastrellus longipharyngis* sp. nov./1). **G–H.** Paratype, ♂ (AMU/ZD/NC/*Diplogastrellus longipharyngis* sp. nov./2–9). **A–B.** Enface view. **C.** Anterior region showing amphid. **D.** Vulval opening. **E.** Anal opening. **F.** Female posterior region showing phasmid. **G–H.** Male posterior region showing genital papillae. The arrowheads in C, F and H indicate amphidial aperture, phasmid and genital papillae, respectively. Scale bars: A, C–G = 5 µm; B, H = 2 µm.

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

Characters	Holotype female	Paratype females (n = 9)	Paratype males (n = 10)
L	1009	954.5 $\pm$ 78 (865–1091)	692.6 $\pm$ 47.5 (597–752)
a	34.8	34.4 $\pm$ 2.5 (30–38)	32.4 $\pm$ 2.0 (28.4–35.9)
b	5.8	5.9 $\pm$ 0.5 (5.2–6.7)	4.9 $\pm$ 0.4 (4.3–5.6)
c	3.5	3.4 $\pm$ 0.2 (3.2–3.7)	3.9 $\pm$ 0.3 (3.4–4.4)
c'	15.2	15.0 $\pm$ 1.5 (12.3–17.5)	10.2 $\pm$ 1.1 (7.9–11.7)
V/T	54	54.0 $\pm$ 1.3 (51–56)	45.6 $\pm$ 5.4 (33–50)
G1	33.3	33.6 $\pm$ 8.9 (22–50)	–
Maximum body width	29	27.9 $\pm$ 2.8 (23–32)	21.4 $\pm$ 0.8 (20–23)
Lip width	7	6.6 $\pm$ 0.5 (6–7)	6
Length of stoma	6	5.3 $\pm$ 0.5 (5–6)	5.1 $\pm$ 0.3 (5–6)
Corpus	50	47.4 $\pm$ 2.8 (42–51)	41.7 $\pm$ 2.4 (38–45)
Median bulb	15	13.6 $\pm$ 1.4 (11–15)	12.8 $\pm$ 1.1 (11–15)
Isthmus	85	74.8 $\pm$ 6.6 (65–85)	64.7 $\pm$ 7.1 (55–77)
Basal bulb	25	26.8 $\pm$ 2.6 (25–33)	21.5 $\pm$ 2.1 (19–27)
Pharynx	175	162.6 $\pm$ 7.0 (152–175)	140.7 $\pm$ 10 (125–159)
Cardia	5	5.3 $\pm$ 0.5 (5–6)	4.6 $\pm$ 0.5 (4–5)
Nerve ring from ant. end	86	80.3 $\pm$ 4.1 (73–86)	70.2 $\pm$ 4.1 (65–78)
Excretory pore from ant. end	101	96.0 $\pm$ 4.3 (88–101)	83.5 $\pm$ 4.7 (76–92)
Hemizonid from ant. end	95	89.3 $\pm$ 4.0 (82–95)	77.6 $\pm$ 4.4 (70–85)
Pharynx-gonad distance	160	149.9 $\pm$ 24.5 (110–195)	73.5 $\pm$ 33.7 (32–150)
Anterior gonad	337	324.8 $\pm$ 104.0 (188–507)	–
Post-uterine sac	41	43.0 $\pm$ 4.4 (35–49)	–
VBD	27	26.7 $\pm$ 2.0 (23–29)	–
Vulva-anus distance	173	159.5 $\pm$ 15.4 (140–190)	–
Vulva from anterior end	547	515 $\pm$ 36 (469–564)	–
Rectum	27	24.8 $\pm$ 2.3 (22–29)	21.7 $\pm$ 1.2 (19–24)
Tail	289	280 $\pm$ 32 (245–346)	178.8 $\pm$ 22.2 (135–210)
ABD	19	18.7 $\pm$ 1.3 (17–20)	17.5 $\pm$ 0.5 (17–18)
Phasmid from anus	46	39.6 $\pm$ 4.4 (32–46)	26.6 $\pm$ 1.0 (25–28)
Testis	–	–	316.6 $\pm$ 48.8 (235–372)
Spicule along axis	–	–	25.9 $\pm$ 0.8 (25–27)
Spicule along cord	–	–	23.0 $\pm$ 1.2 (21–25)
Gubernaculum	–	–	10.7 $\pm$ 0.4 (10–11)
No. of precloacal papillae	–	–	3
No. of postcloacal papillae	–	–	6

while *v7* situated more posteriorly at the level of *pd*. Phasmids pore-like, just posterior to *v6*, 1.3–1.5 anal body diameter posterior to cloacal opening.

### Remarks

*Diplogastrellus longipharyngis* sp. nov. comes close to *D. heynsi* (Kiontke & Sudhaus, 1996) in most morphometric and morphological characteristics but differs in the size of stoma (5–6  $\mu\text{m}$  vs 11–13  $\mu\text{m}$ ), size of dorsal tooth (medium-sized, ventrally directed vs massive, ventrally directed), position of amphids (at the base of dorsal tooth vs posterior to the base of stoma), median bulb (ovoid vs oblong), isthmus (longer than procorpus vs shorter than procorpus), shape of spicules (strongly built, almost straight vs stout, strongly arcuate at the distal end), shape of gubernaculum (triangular, proximally notched vs short, keel-like, caudally projected loop) and in the arrangement of genital sensilla (*v4* just posterior to cloaca, *v5–7* not grouped vs *v4* more than one cloacal diameter posterior to cloaca, *v5–7* grouped).

The new species resembles *D. sikorai* and *D. stammeri* Weingärtner, 1955 in general morphometric values. However, it can be easily distinguished from both species by its short tubular stoma, long isthmus and in the structure of gubernaculum without sleeve. It further differs from *D. sikorai* in having larger body size ( $L = 865\text{--}1091 \mu\text{m}$  vs  $492\text{--}657 \mu\text{m}$  in females;  $597\text{--}752 \mu\text{m}$  vs  $395\text{--}470 \mu\text{m}$  in males), by structure of dorsal tooth (ventrally directed vs anteriorly directed), size of post-uterine sac (35–49  $\mu\text{m}$  vs 19–31  $\mu\text{m}$ ), length of spicules (25–27  $\mu\text{m}$  vs 17–19  $\mu\text{m}$ ) and arrangement of genital papillae (*v1* anterior to spicule head, *v7* more posterior at the level of *pd* vs *v1* within the spicule range, *v5–v7* grouped).

From *D. stammeri*, it can be further differentiated in the shape of dorsal tooth (ventrally directed vs anteriorly directed), position of amphids (at the base of dorsal tooth vs at the base of gymnostom), subventral armature (absent vs small tooth in both walls), size of post-uterine sac (more than twice vulval body diameter long vs less than one vulval body diameter) and structure of spicules (almost straight with rounded manubrium, anteriorly flattened, merging with shaft vs arcuate with ovoid manubrium separated from shaft by neck).

The new species differs from *D. thoubalicus* in having shorter stoma (5–6  $\mu\text{m}$  vs 12–15  $\mu\text{m}$ ), by position of amphids (at the base of dorsal tooth vs posterior to the base of stoma), median bulb (ovoid vs oblong), isthmus (longer than procorpus vs shorter than procorpus), shape of spicules (strongly built, almost straight vs slender, distally more curved), shape of gubernaculum (triangular, proximally notched vs posterior–dorsally directed loop with a small sleeve) and in the arrangement of genital sensilla (*v4* just posterior to cloaca, *v5–7* not grouped vs *v4* more than one cloacal diameter posterior to cloaca, *v5–7* grouped).

### *Diplogastrellus robustus* sp. nov.

[urn:lsid:zoobank.org:act:36D4BB43-EC22-481D-BE18-CDDF4784140D](https://doi.org/10.3897/zoobank.org/act:36D4BB43-EC22-481D-BE18-CDDF4784140D)

Figs 6–8, 17–19; Tables 3, 7–8

### Diagnosis

The new species *Diplogastrellus robustus* sp. nov. is characterised by a narrow stoma longer than wide; small, elliptical amphidial aperture, at the level of gymnostom; a medium-sized dorsal tooth with ventrally directed apex; pharynx well-developed, muscular ovoid metacorporeal bulb with valve plates, narrow isthmus and a pyriform basal bulb; amphidelphic or mono-prodelphic reproductive system with a post-uterine sac; spicules robust, heavily cuticularized, smoothly curved to a blunt distal end; boat-like gubernaculum with a sleeve and nine pairs of genital sensilla.

### Etymology

The name of the species is based on the robust spicule.

## Material examined

### Holotype

INDIA • ♀; Kerala, District Kottayam; 9°35'55" N, 76°30'39" E; isolated from soil near ginger plantation; slide reference number AMU/ZD/NC/*Diplogastrellus robustus*/1.

### Paratypes

INDIA • 10 ♀♀, 10 ♂♂; same data as for holotype; slide reference number AMU/ZD/NC/*Diplogastrellus robustus* /2–9.

## Description

### Adult

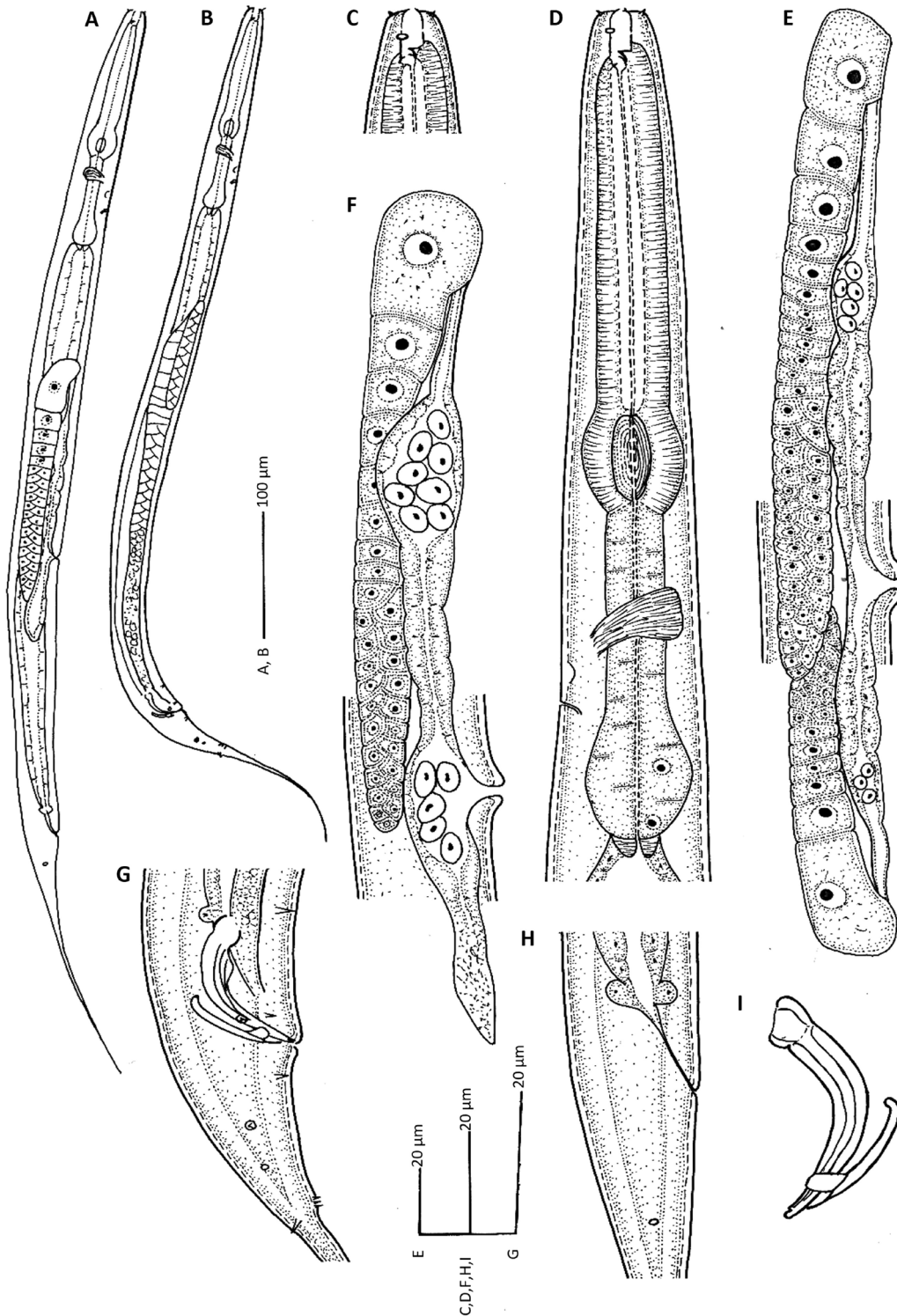
Body slender, medium-sized, less than 1 mm long; almost straight after fixation, tapering only very slightly anteriorly, but more posteriorly forming a filiform tail. Cuticle with fine transverse striations. Lip region round, continuous with body contour. Lips six, amalgamated, each bearing a small papilliform labial sensilla. Amphidial apertures small, elliptical, 5–6 µm from anterior end of stoma, at the level of gymnostom. Stoma longer than wide, about 9–11 µm in depth. Cheilostom longer than wide, cheilorhabdions arched inward anteriorly, cheilorhabdial flaps six, protruding above labial contour; gymnostom anisotropic, dorsal wall shorter than subventrals. Stegostom anisotropic and anisomorphic, dorsal metastegostomal wall with a ventrally directed medium-sized tooth. Postero-ventral aspect of dorsal tooth heavily cuticularized, supported posteriorly by cuticularised ridge. Subventral walls without armature. Pharynx with slender 78–88 µm long, muscular corpus of uniform diameter; median bulb set off from corpus, ovoid, 21–25 µm long with strong valve plates. Isthmus 33–57 µm long, narrow, conspicuously differentiated from median bulb. Basal bulb small, pyriform, glandular, 18–21 × 14–17 µm, only slightly expanded from isthmus without any valve plate or grinder. Nerve ring encircling isthmus in the middle or sometimes in the anterior half, located at 64–76% of pharyngeal length. Hemizonid posterior to nerve ring, 65–85% of pharyngeal length. Excretory pore posterior to hemizonid, 69–88% of pharyngeal length. Cardia well-developed, 3–4 µm long, consisting of three flaps, one dorsal and two ventro-sublateral. Intestine composed of dark granulated cells with prominent nuclei and intestinal lumen uniformly wide, 8–10 µm without any bacterial pouch. Rectum 1.0–1.2 times anal body diameter long; rectal glands distinct.

### Female

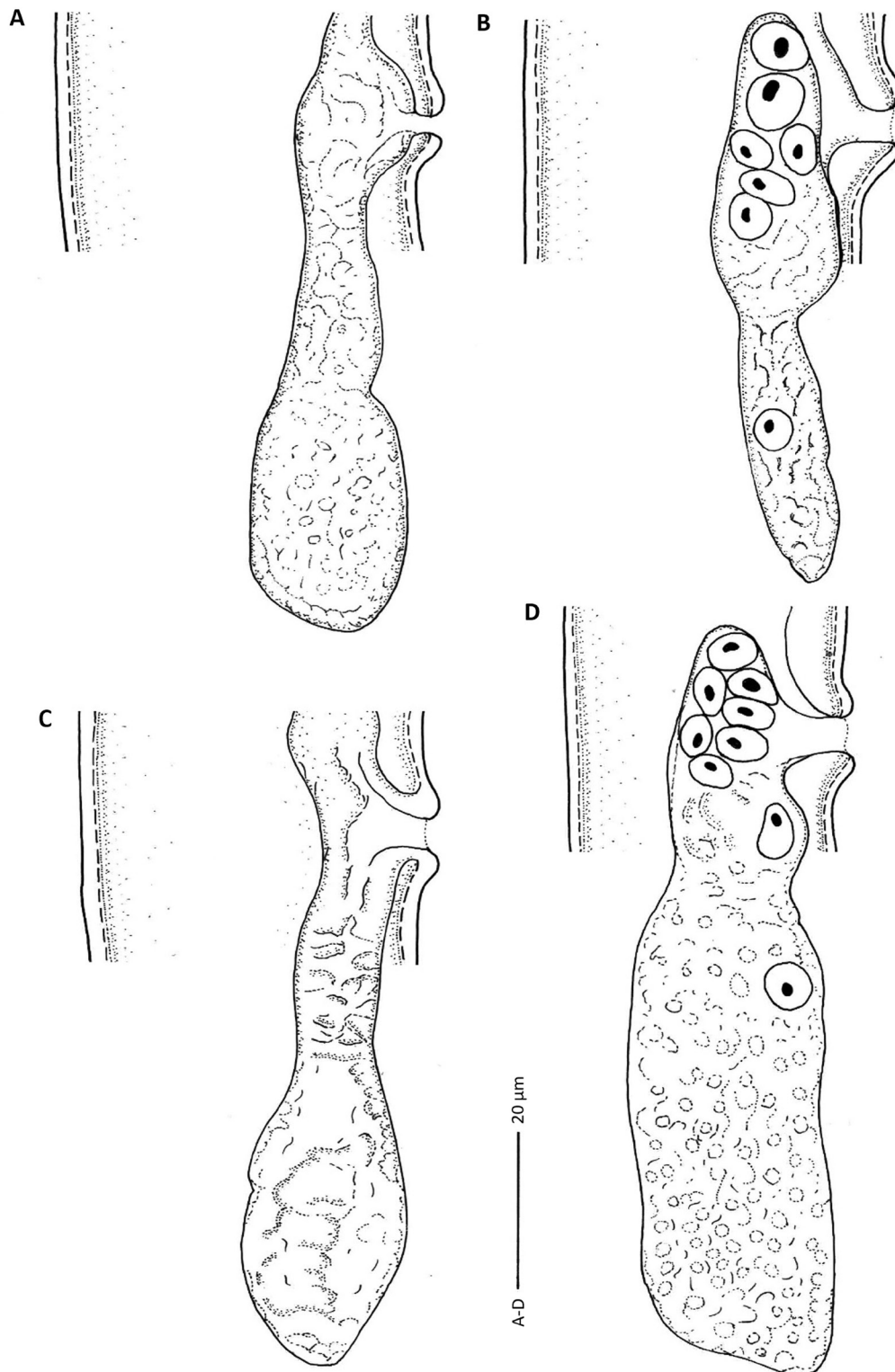
Reproductive system mono-prodelphic or amphidelphic. Anterior branch on the right and posterior on the left side of intestine. Ovary long reversed, extending beyond the level of vulva. Oocytes with large nuclei, arranged in multiple rows in germinal zone and single row in maturation zone. Oviduct narrow, tubular. Spermatheca expanded, not set off from uterus, with distinctly narrower walls, containing sperm. In most specimens, posterior genital branch appears to be reduced with sperm in the anterior part and vacuolated, degenerated cells in posterior part; sometimes posterior branch represented by a post-uterine sac. In two specimens, the posterior genital branch appeared well-developed and functional (amphidelphic). Uterus divisible into a distal smaller muscular part and proximally placed longer, glandular part made up of large cells and narrow lumen. Vagina short, at right angle to longitudinal body axis, 7–8 µm or about one-fourth of corresponding body diameter long. Vulval opening large, elliptical. Vulva-anus distance 5.6–7.9 times vulval body diameter. Phasmids located at a level 1.1–1.5 anal body diameter posterior to anus.

### Males

Similar to females in general morphology but smaller in size. Reproductive system monorchic, testis reflexed laterally, on right side of intestine. Spermatocytes arranged in two rows in anterior reflexed part, well-developed spermatocytes in anterior half of non-reflexed part. Vas deferens a long tube narrowing posteriorly to form an ejaculatory duct. Spicules robust, strongly ventrally arcuate 1.2–1.5 times cloacal



**Fig. 6.** *Diplogastrellus robustus* sp. nov. **A, C–F, H.** Holotype, ♀ (AMU/ZD/NC/*Diplogastrellus robustus* sp. nov./1). **B, G, I.** Paratype, ♂ (AMU/ZD/NC/*Diplogastrellus robustus* sp. nov./2–9). **A.** Entire female. **B.** Entire male. **C.** Anterior region showing stoma. **D.** Pharyngeal region. **E–F.** Female reproductive system. **G.** Male posterior region showing spicule and gubernaculum. **H.** Female posterior region. **I.** Spicule and gubernaculum.



**Fig. 7.** *Diplogastrellus robustus* sp. nov., paratype, ♀ (AMU/ZD/NC/*Diplogastrellus robustus* sp. nov./2–9). **A, C–D.** Posterior branch showing reduced gonad contains sperm in the anterior part and vacuolated degenerated cells in the posterior part. **B.** Post-uterine sac.



**Fig. 8.** *Diplogastrellus robustus* sp. nov. A–E, G, I. Holotype, ♀ (AMU/ZD/NC/*Diplogastrellus robustus* sp. nov./1). F, H, J. Paratype, ♂ (AMU/ZD/NC/*Diplogastrellus robustus* sp. nov./2–9). A. Pharyngeal region. B. Anterior region showing stoma. C. Female posterior region. D. Female reproductive system showing anterior branch. E, I. Posterior branch (female gonad). G. Vulval opening. F, H. Male posterior region showing spicules and gubernaculum. J. Male genital papillae. The arrowheads in E and H–J indicate posterior genital branch, sleeve and genital papillae, respectively. Scale bars =10 μm.

**Table 3.** Morphometric characteristics of *Diplogastrellus robustus* sp. nov. Measurements are in  $\mu\text{m}$  and in the form: mean $\pm$ standard deviation (range).

Characters	Holotype female	Female paratypes (n = 10)	Male paratypes (n = 10)
L'	648	653.7 $\pm$ 59.3 (523–730)	583.7 $\pm$ 31.6 (535–621)
a	20.3	19.8 $\pm$ 1.9 (17.8–25.1)	19.0 $\pm$ 2.1 (15.8–22.7)
b	4.1	3.9 $\pm$ 0.3 (3.3–4.2)	3.8 $\pm$ 0.2 (3.5–4.3)
c	14.4	15.9 $\pm$ 1.1 (14.4–17.5)	14.8 $\pm$ 0.5 (14.2–15.6)
c'	2.0	2.1 $\pm$ 0.2 (1.7–2.6)	1.6 $\pm$ 0.1 (1.5–1.8)
V/T	61	61.6 $\pm$ 1.2 (59–64)	66.7 $\pm$ 2.9 (62.0–71.7)
G1	45	43.5 $\pm$ 4.1 (35–50)	–
Maximum body width	32	33.2 $\pm$ 3.8 (27–41)	31.0 $\pm$ 4.2 (26–38)
Lip width	9	8.9 $\pm$ 0.7 (8–10)	8.3 $\pm$ 0.9 (7–9)
Length of stoma	11	10.5 $\pm$ 0.7 (10–12)	10.0 $\pm$ 0.8 (9–11)
Corpus	82	85.0 $\pm$ 3.6 (78–93)	77.5 $\pm$ 3.6 (71–82)
Median bulb	21	22.5 $\pm$ 1.4 (21–25)	20.5 $\pm$ 0.8 (20–22)
Isthmus	35	39.2 $\pm$ 6.2 (33–57)	37.3 $\pm$ 1.8 (34–40)
Basal bulb length	20	20.0 $\pm$ 1.0 (18–22)	18.7 $\pm$ 1.7 (15–21)
Pharynx length	158	166.7 $\pm$ 6.8 (158–180)	154 $\pm$ 5.3 (145–160)
Cardia	3	3.1 $\pm$ 0.3 (3–4)	3.2 $\pm$ 0.4 (3–4)
Excretory pore from ant. end	131	134.5 $\pm$ 10.3 (117–143)	123.0 $\pm$ 8.3 (112–132)
Hemizonid from ant. end	125	126.6 $\pm$ 8.8 (111–136)	116.4 $\pm$ 6.7 (104–126)
Nerve ring from ant. end	118	119.4 $\pm$ 5.4 (110–126)	112.3 $\pm$ 5.2 (100–119)
Pharynx-gonad distance	90	100.2 $\pm$ 10.3 (83–115)	76.0 $\pm$ 14.3 (50–99)
Anterior gonad	292	289.8 $\pm$ 46.2 (223–372)	–
Posterior gonad	–	245	–
Post-uterine sac	63	61.5 $\pm$ 7.6 (52–80)	–
VBD	33	32.9 $\pm$ 3.9 (27–41)	–
Vulva-anus distance	210	209.7 $\pm$ 20.5 (162–237)	–
Vulva from anterior end	393	402.8 $\pm$ 38.4 (325–450)	–
Rectum	21	21.1 $\pm$ 1.6 (18–23)	18.7 $\pm$ 1.2 (17–20)
Tail (broken)	45	46.1 $\pm$ 16.5 (36–97)	39.4 $\pm$ 1.9 (37–43)
ABD	22	19.9 $\pm$ 2.2 (17–25)	24.5 $\pm$ 2.1 (22–29)
Phasmid from anus	25	25.1 $\pm$ 1.5 (22–28)	27.4 $\pm$ 1.5 (26–30)
Testis	–	–	389.4 $\pm$ 28.9 (346–445)
Spicule along axis	–	–	33.5 $\pm$ 1.4 (31–36)
Spicule along cord	–	–	27.8 $\pm$ 1.2 (25–29)
Gubernaculum	–	–	17.9 $\pm$ 0.8 (17–20)
No. of precloacal papillae	–	–	3
No. of postcloacal papillae	–	–	6

body diameter long. Manubrium hood-like, lamina/calamus complex expanded posterior to manubrium, and then smoothly tapering to a blunt distal tip. Gubernaculum slender, boat-shaped, 51–56% of spicule length, proximally curved, provided with a sleeve followed by a pointed distal end. Tail divisible into two parts, a short conoid part and a longer filamentous part. Genital sensilla nine pairs; three pairs precloacal and six pairs postcloacal. Genital sensilla formula: *v1*, *v2*, *v3d*, *v4*, *ad*, *ph*, (*v5*, *v6*, *v7*), *pd*. Precloacal pair *v1* located just anterior to the spicule head; *v2* and *v3d* at the same level, anterior to cloaca and *v4* posterior to cloaca, *ad* less than one cloacal body diameter posterior to cloaca, *v5–7* grouped, located just anterior to base of filiform tail spike. Phasmids pore-like, posterior to *ad*, 1.0–1.3 anal body diameter posterior to cloacal opening; *pd* slightly posterior to *v7*.

### Remarks

*Diplogastrellus robustus* sp. nov. comes close to *D. graciloides* in most morphometric and morphological characteristics but differs in having more robust body (MBD = 27–41  $\mu\text{m}$  vs 20–26  $\mu\text{m}$  in females; 26–38  $\mu\text{m}$  vs 18–21  $\mu\text{m}$  in males), by position of amphids (at the level of gymnostom vs posterior to the base of stoma), shape of median bulb (ovoid vs oblong), length of the flexure of ovary (extending beyond the level of vulva vs not reaching the level of vulva), structure of spicules (robust, hood-like manubrium, blunt distal end vs slender, rounded manubrium, curved distal end), structure of gubernaculum (slender, boat-shaped, with distal sleeve vs globose, keel-like projection, without distal sleeve) and number of genital papillae (9 pairs; *v4* less than one cloacal diameter posterior to cloaca vs 8 pairs; *v4* more than one cloacal diameter posterior to cloaca, *v7* absent).

The new species differs from *D. metamasius* in the shape of median bulb (ovoid vs oblong), position of vulva (posteriorly located, 59–64% vs anterior, 48–56%), size of post-uterine sac (longer than vulval body diameter vs shorter than vulval body diameter), shape and size of spicules (31–36  $\mu\text{m}$ ; hood-like manubrium, with blunt distal end, vs 23.0–30.5  $\mu\text{m}$ ; small rounded manubrium, with pointed distal end), shape of gubernaculum (boat-shaped with distal sleeve vs keel-like without distal sleeve) and arrangement of genital sensilla (*v4* slightly less than one cloacal diameter posterior to cloaca vs *v4* just posterior to cloacal slit).

*Diplogastrellus robustus* sp. nov. differs from the new species *D. didelphis* sp. nov. in the position of amphids (at the anterior part of gymnostom vs at the base of gymnostom), shape of median bulb (ovoid vs oblong), length of the flexure of ovary (extending beyond the level of vulva vs not reaching the level of vulva), uterine pouch (absent vs present), structure of spicules (robust, hood-like manubrium, calamus/lamina complex not expanded with blunt distal end vs slender, rounded manubrium, calamus/lamina complex expanded into ventral conoid process with pointed distal end), structure of gubernaculum (boat-shaped, with distal sleeve vs proximally sickle-shaped, long curved dorsal arm and a short ventral arm with a distal sleeve) and arrangement of genital papillae (*pd* slightly posterior to *v7* vs *pd* far posterior to *v7*).

*Diplogastrellus robustus* sp. nov. also differs from the new species *D. longipharyngis* sp. nov. in having more robust body (MBD = 26–38  $\mu\text{m}$  vs 20–23  $\mu\text{m}$  in males), position of amphids (at the anterior part of gymnostom vs at the base of dorsal tooth), length of stoma (10–12  $\mu\text{m}$  vs 5–6  $\mu\text{m}$  in females; 9–11  $\mu\text{m}$  vs 6  $\mu\text{m}$  in males), isthmus (shorter than corpus vs longer than corpus), length of the flexure of ovary (extending beyond the level of vulva vs not reaching the level of vulva), size and shape of spicules (31–36  $\mu\text{m}$ ; robust, hood-like manubrium, with blunt distal end vs 25–27  $\mu\text{m}$ , slender, rounded manubrium with pointed distal end), structure of gubernaculum (boat-shaped, with distal sleeve vs triangular, proximally notched without distal sleeve) and arrangement of genital papillae (*v7* grouped and *pd* slightly posterior to *v7* vs *v7* separated from *v5*, *v6* and *pd* at the level of *v7*).

***Diplogastrellus gracilis* (Bütschli, 1876)**

Figs 9–11, 17–19; Tables 4, 7–8

**Emended diagnosis**

*Diplogastrellus gracilis* is characterised by a stoma longer than wide; cuticle with transverse and longitudinal striations; elliptical amphidial aperture, at the base of cheilostom; massive dorsal tooth with ventrally directed apex, antero-ventral margin serrated; small tooth on each subventral wall; pharynx well-developed, muscular oblong metacorpal bulb with valve plates, narrow isthmus and a pyriform basal bulb; mono-prodelphic reproductive system; spicules thin, long, attenuated, strongly arcuate with oval manubrium, smoothly arcuate to a pointed distal end; gubernaculum 33 – 40% of spicule length, with lateral sleeve at distal end surrounding terminal region of spicules and a prominent, keel-like proximal part. and nine pairs of genital sensilla – constituting two precloacal, two adcloacal and five postcloacal pairs.

**Material examined**

INDIA • 10 ♀♀, 10 ♂♂; Uttar Pradesh, District Aligarh; 27°54'24" N, 78°4'36" E; extracted from rotting banana rhizome; slide reference number AMU/ZD/NC/*Diplogastrellus gracilis*/1–10.

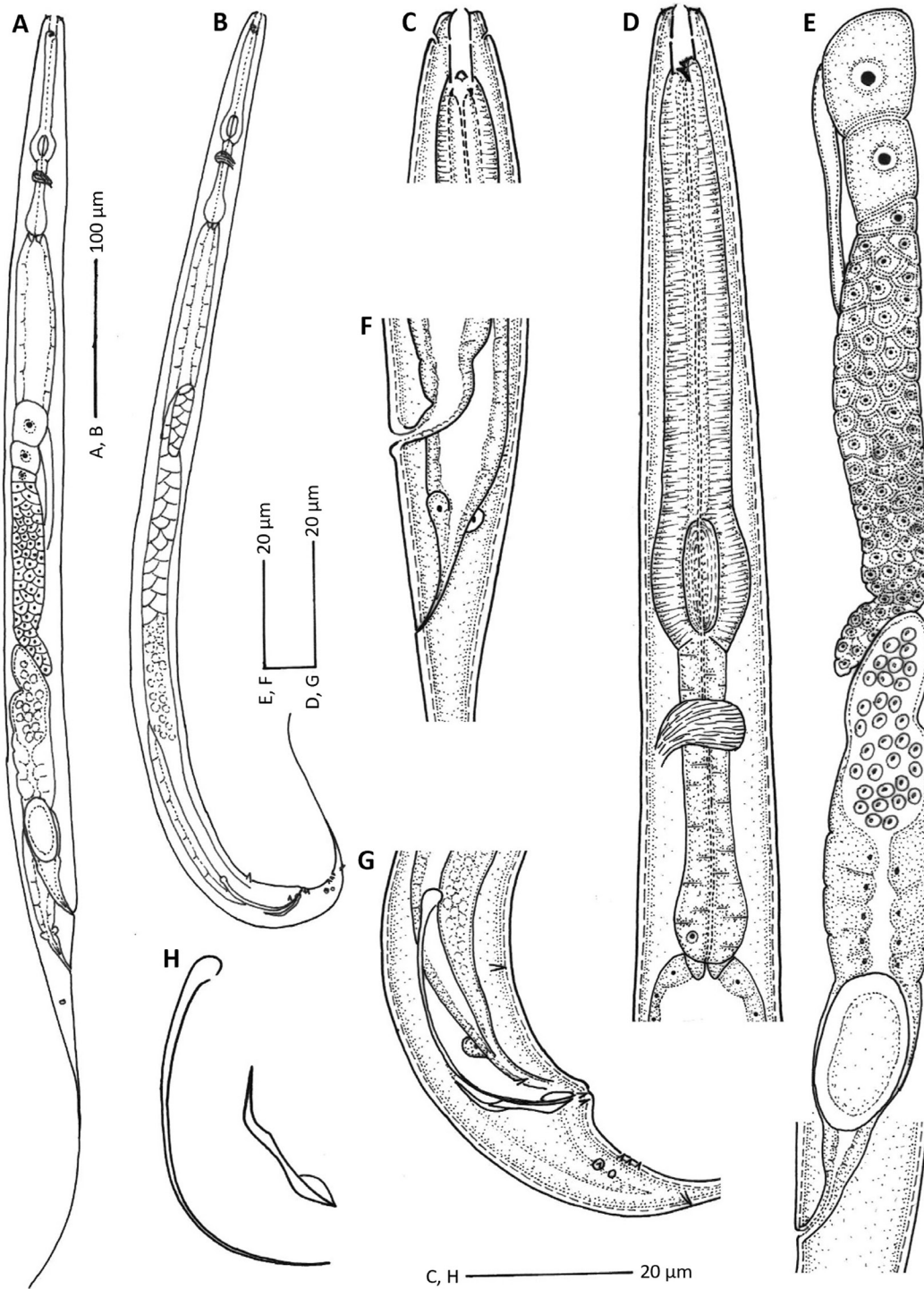
**Description**

**Adult**

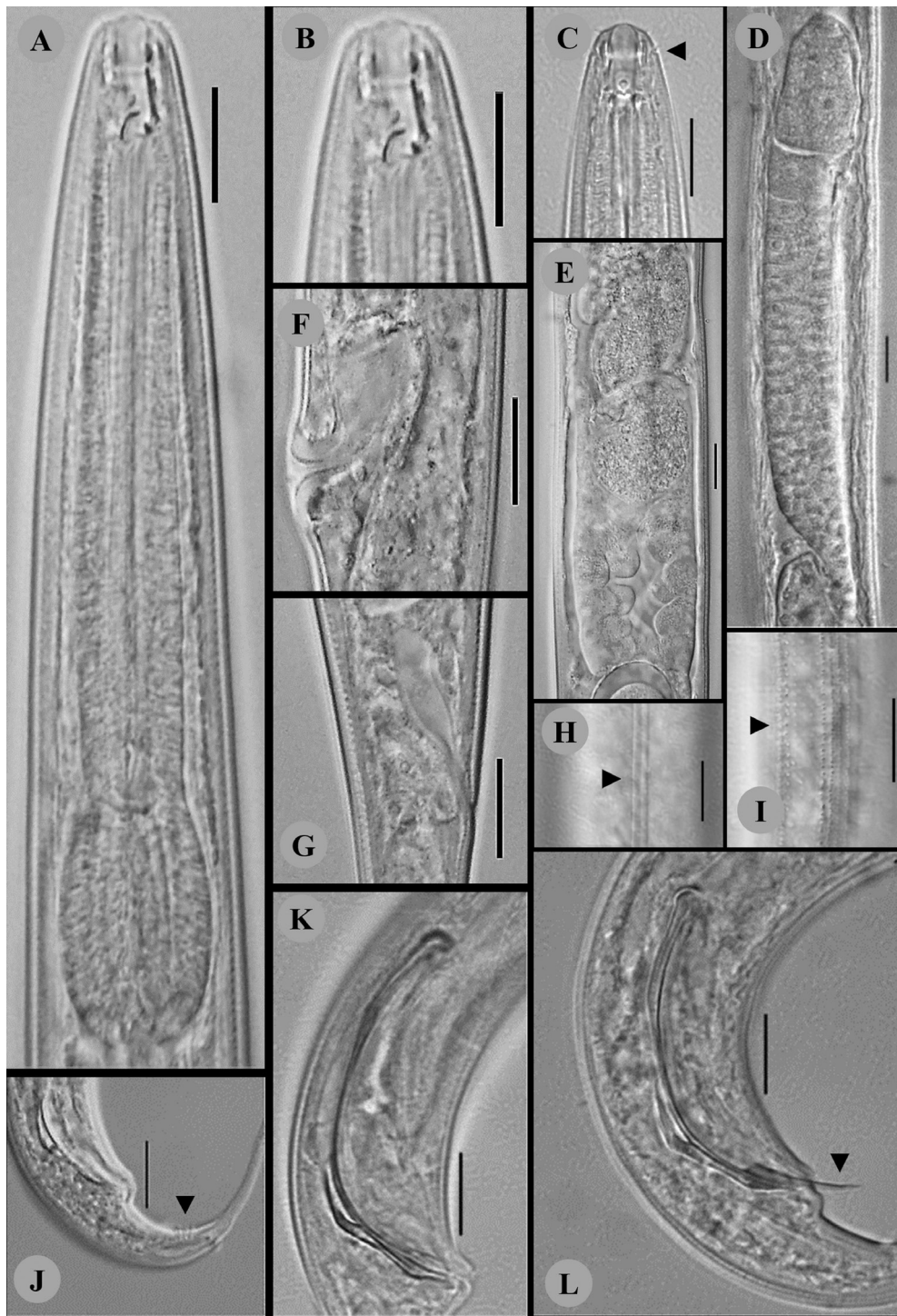
Body slender, medium-sized, less than 1 mm long; almost straight after fixation, tapering only very slightly anteriorly, but more posteriorly forming a filiform tail. Cuticle with fine transverse and longitudinal striations. Cuticular punctations fine, transversely arranged. Lateral fields with two conspicuous spaced ridges about 8.6–12.0% of maximum body width. Lip region continuous with body contour. Lips six, amalgamated, bearing a small papilla each. Amphidial apertures elliptical, 3 µm from anterior end of stoma, at the base of cheilostom. Stoma longer than wide, about 8–11 µm in depth. Cheilostom longer than wide, cheilorhabdions straight, cheilorhabdial flaps six, protruding above labial contour, gymnostom anisotropic, dorsal wall shorter than subventrals. Stegostom anisotropic and anisomorphic, dorsal metastegostomal wall with a large dorsal tooth, bulging into stoma, apex ventrally directed almost reaching the base of the ventral gymnostomal wall. Postero-ventral aspect of tooth strongly cuticularised, concave; antero-ventral aspect not cuticularised with an irregular surface. Subventral walls with small tooth each. Pharynx with slender 58–79 µm long, muscular corpus of uniform diameter; median bulb set off from corpus, oblong, 17–22 µm long with strong valve plates. Isthmus 23–40 µm long, narrow, conspicuously differentiated from median bulb. Basal bulb small, pyriform, glandular, 14–17 × 10–13 µm, only slightly expanded from isthmus without any valve plate or grinder. Nerve ring encircling isthmus in anterior half, located at 70–84% pharyngeal length. Excretory pore and hemizonid inconspicuous. Deirids at the level of basal bulb. Cardia small, 3–4 µm long, consisting of three flaps, one dorsal and two ventro-sublateral. Intestine composed of dark granulated cells with prominent nuclei intestinal lumen uniformly 11–13 µm wide, without any bacterial pouch. Rectum 1.0–1.6 times anal body diameter long; rectal glands distinct.

**Female**

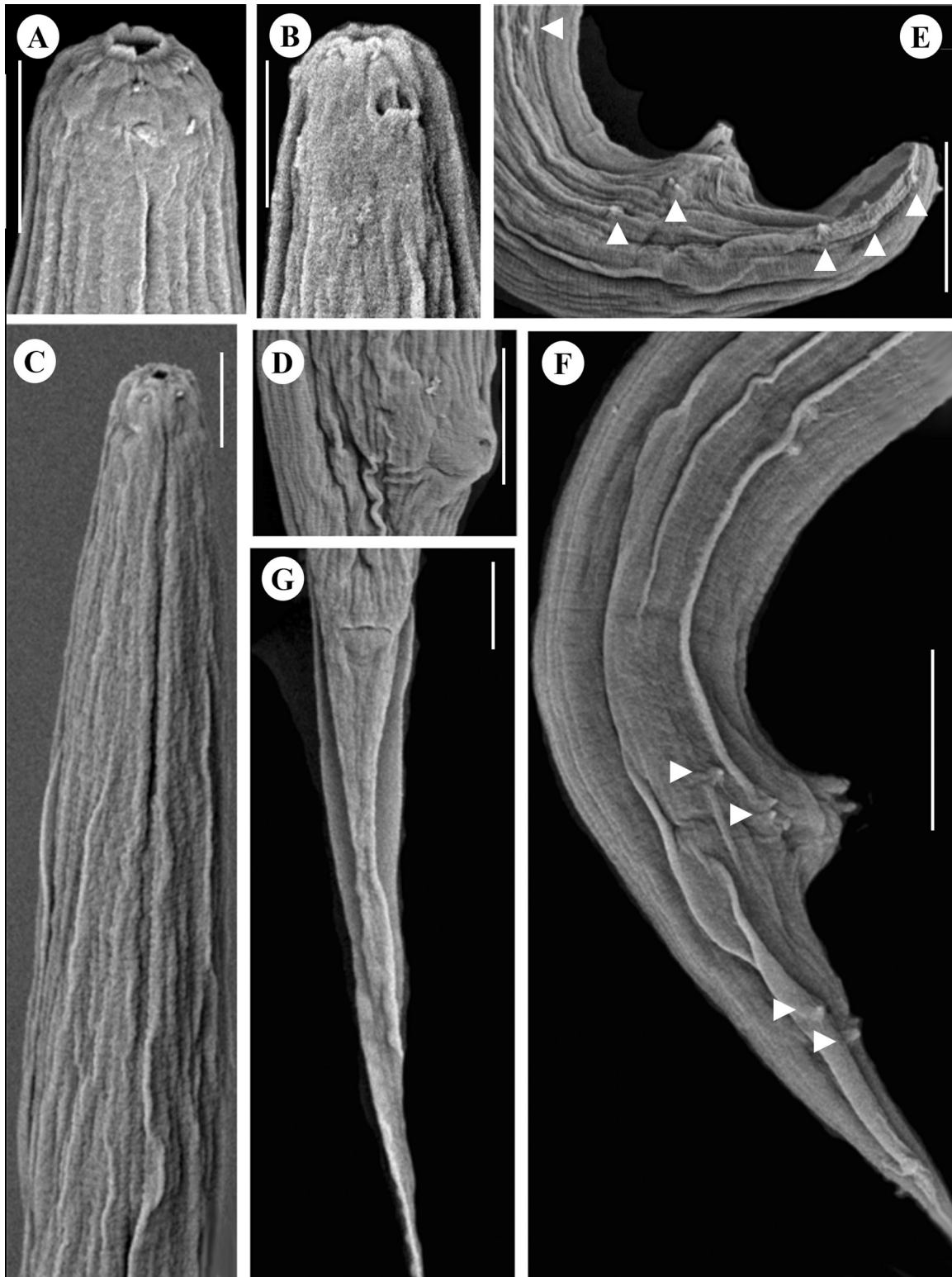
Reproductive system mono-prodelphic. Ovary reversed on right side of intestine, distal part of ovary not reaching the level of vulva. Oocytes with large nuclei, arranged in multiple rows in germinal zone and single row in maturation zone. Oviduct long, narrow, connecting spermatheca and ovary. Spermatheca expanded, generally filled with sperm, continuous with oviduct but separated by a constriction from uterus. Uterus divisible into a distal smaller muscular part and proximally placed longer glandular part made up of large cells and narrow lumen. Vagina tubular, inclined anteriorly, 11–13 µm long about half of the corresponding body diameter long. Vulval opening circular, vulval lips slightly protuberant. Post-uterine sac absent. Vulva-anus distance 1.0–1.7 times vulval body diameter. Phasmids located at about 1.3–2.1 anal body diameter posterior to anus. Tail long, filiform 4.8–7.8 times vulva-anus distance.



**Fig. 9.** *Diplogastrellus gracilis* (Bütschli, 1876). **A, C–F.** ♀ (AMU/ZD/NC/*Diplogastrellus gracilis*/1–10). **B, G–H.** ♂ (AMU/ZD/NC/*Diplogastrellus gracilis*/1–10). **A.** Entire female. **B.** Entire male. **C.** Anterior region showing stoma (dorsoventral view). **D.** Pharyngeal region. **E.** Female reproductive system. **F.** Female posterior region. **G.** Male posterior region. **H.** Spicule and gubernaculum.



**Fig. 10.** *Diplogastrellus gracilis* (Bütschli, 1876). **A–I.** ♀ (AMU/ZD/NC/*Diplogastrellus gracilis*/1–10). **J–L.** ♂ (AMU/ZD/NC/*Diplogastrellus gracilis*/1–10). **A.** Pharyngeal region showing procornu and median bulb. **B.** Anterior region showing stoma (lateral view). **C.** Anterior region showing amphids (dorsoventral view). **D.** Reflexed part of ovary. **E.** Uterus (glandular part). **F.** Vulval region. **G.** Female posterior region. **H.** Lateral lines. **I.** Cuticular punctations. **J.** Genital papillae (v5–7). **K–L.** Male posterior region showing spicules and gubernaculum. The arrowheads in C, H–J and L indicate amphidial aperture, lateral lines, fine punctations, grouped genital papillae and attenuated spicular distal end, respectively. Scale bars: A–B, E–G = 20 µm; C–D, H–L = 10 µm.



**Fig. 11.** *Diplogastrellus gracilis* (Bütschli, 1876). **A–D, G.** ♀ (AMU/ZD/NC/*Diplogastrellus gracilis*/1–10). **E–F.** ♂ (AMU/ZD/NC/*Diplogastrellus gracilis*/1–10). **A.** Anterior region showing labial papillae and cheilorhabdial plates. **B.** Anterior region showing amphid. **C.** Pharyngeal region showing cuticular striations. **D.** Vulval region. **E–F.** Male posterior region showing genital papillae. **G.** Female posterior region. The arrowheads in E–F indicate genital papillae. Scale bars = 10 µm.

**Table 4.** Morphometric characteristics of *Diplogastrellus gracilis* (Bütschli, 1876). Measurements are in  $\mu\text{m}$  and in the form: mean $\pm$ standard deviation (range).

Characters	Females (n = 10)	Males (n = 10)
L	777.4 $\pm$ 27.5 (735–817)	613.8 $\pm$ 66.3 (536–733)
a	27.5 $\pm$ 2.9 (22.7–30.3)	31.3 $\pm$ 2.7 (29.7–36.7)
b	5.8 $\pm$ 0.5 (4.9–6.4)	4.8 $\pm$ 0.3 (4.5–5.3)
c	3.9 $\pm$ 0.2 (3.6–4.2)	5.2 $\pm$ 0.3 (4.8–5.7)
c'	14.8 $\pm$ 1.6 (13.0–16.8)	6.5 $\pm$ 0.6 (5.7–7.4)
V/T	70.0 $\pm$ 1.7 (67–73)	55.0 $\pm$ 3.5 (49–59)
G1	52.9 $\pm$ 5.0 (48.4–62.0)	–
Maximum body width	28.6 $\pm$ 3.6 (25–35)	19.6 $\pm$ 1.0 (18–21)
Lip width	5.8 $\pm$ 0.7 (5–7)	6
Length of stoma	9.4 $\pm$ 1.0 (8–11)	8.8 $\pm$ 0.4 (8–9)
Corpus	68.2 $\pm$ 7.0 (58–79)	62.6 $\pm$ 5.1 (56–71)
Median bulb	20.4 $\pm$ 1.9 (17–22)	16.8 $\pm$ 1.2 (16–19)
Isthmus	30.2 $\pm$ 5.9 (23–40)	36.8 $\pm$ 1.6 (34–39)
Basal bulb	15.6 $\pm$ 1.0 (14–17)	11.8 $\pm$ 0.4 (11–12)
Pharynx	134.4 $\pm$ 13.4 (115–157)	128 $\pm$ 7.0 (120–139)
Cardia	3.2 $\pm$ 0.4 (3–4)	2.6 $\pm$ 0.5 (2–3)
Nerve ring from ant. end	102.6 $\pm$ 9.6 (85–112)	92.8 $\pm$ 7.9 (82–105)
Pharynx-gonad distance	141.0 $\pm$ 22.2 (105–173)	65 $\pm$ 13 (41–80)
Anterior gonad	411.8 $\pm$ 46 (356–492)	–
VBD	25.8 $\pm$ 2.6 (22–30)	–
Vulva-anus distance	33.8 $\pm$ 5.8 (26–42)	–
Vulva from anterior end	545.0 $\pm$ 27.6 (493–575)	–
Rectum	16.2 $\pm$ 1.9 (14–19)	20.0 $\pm$ 0.6 (19–21)
Tail	198.6 $\pm$ 8.5 (182–205)	117.4 $\pm$ 13.3 (105–140)
ABD	13.6 $\pm$ 1.4 (12–15)	18.0 $\pm$ 1.1 (16–19)
Phasmid from anus	22.7 $\pm$ 3.3 (18–25)	14.4 $\pm$ 1.9 (11–16)
Testis	–	339.2 $\pm$ 51.0 (295–432)
Spicule along axis	–	50.2 $\pm$ 3.2 (45–55)
Spicule along cord	–	41.4 $\pm$ 1.2 (40–43)
Gubernaculum	–	19.2 $\pm$ 2.1 (15–21)
No. of precloacal papillae	–	2
No. of adcloacal papillae	–	2
No. of postcloacal papillae	–	5

**Male**

Similar to females in general morphology but smaller in size. Reproductive system monorchic, testis reflexed laterally, on right side of intestine. Spermatocytes arranged in two rows in anterior reflexed part, well-developed spermatocytes in anterior half of non-reflexed part, as multiple rows in next half and containing small sperm in remaining gonad. Vas deferens a long tube tapering to an ejaculatory duct. Spicules long, thin, attenuated, strongly arcuate, 2.6–2.9 times cloacal body diameter long. Capitulum oval, calamus/lamina smoothly attenuated towards a pointed distal tip. Gubernaculum 33–40% of spicule length, with lateral sleeve and a prominent, proximal keel-like part. Tail divisible into two parts, a short conoid part and a longer filamentous part. Genital sensilla nine pairs; two pairs precloacal, two pairs adcloacal and five pairs postcloacal. Genital sensilla formula: *v1*, *v2d*, *v3/v4*, *ad*, *ph*, (*v5*, *v6*, *v7*), *pd*. Precloacal pair *v1* located more than one cloacal body diameter anterior to cloaca; *v2d* slightly anterior to cloaca; *v3* and *v4* adcloacal; *ad* less than one cloacal body diameter posterior to cloaca; *pd* far posterior to *v7*. Phasmids pore-like, at the level of *v5*, 0.7–0.9 anal body diameter posterior to cloacal opening.

**Remarks**

The original description of *D. gracilis* was given by Bütschli (1876) and further described or reported by Goodey (1929), Paramonov (1952), Weingärtner (1955), Timm (1961) and Kiontke & Sudhaus (1996). Later, Khan *et al.* (2008) provided the detailed description of the species collected from compost of mushroom, India. Our specimens' descriptions and morphometric measurements concur well with those of the original specimens of *D. gracilis* described by Bütschli (1876). However, differences were observed in total length of body in both sexes (L = 735–817 µm vs 1350–1390 µm in females and 536–733 µm vs 1000–1070 µm in males), longer pharynx (b = 4.9–6.4 vs 7.3) in females, longer tail (c = 4.8–5.7 vs 7.1) in males, and structure of dorsal tooth (massive tooth with serrated anterior-ventral margin vs smaller tooth lacking serration). The population reported by Khan *et al.* (2008) indicates a wide range of values (L = 666–750 µm, a = 26.8–32.6, b = 5.2–5.9, c = 3.5–3.8, c' = 11.1–12.0, V = 67.3–68.9% in females; spicule = 50–57 µm, gubernaculum = 20–25 µm) which nearly overlapped with the values of our population.

***Diplogastrellus graciloides* (Skwarra, 1921)**

Figs 12–13, 17–19; Tables 5, 7–8

*Diplogaster microstoma* Goodey, 1929: 27–62, figs 25–27.

*Diplogaster inaequidens* Paesler, 1946: 87–128.

**Emended diagnosis**

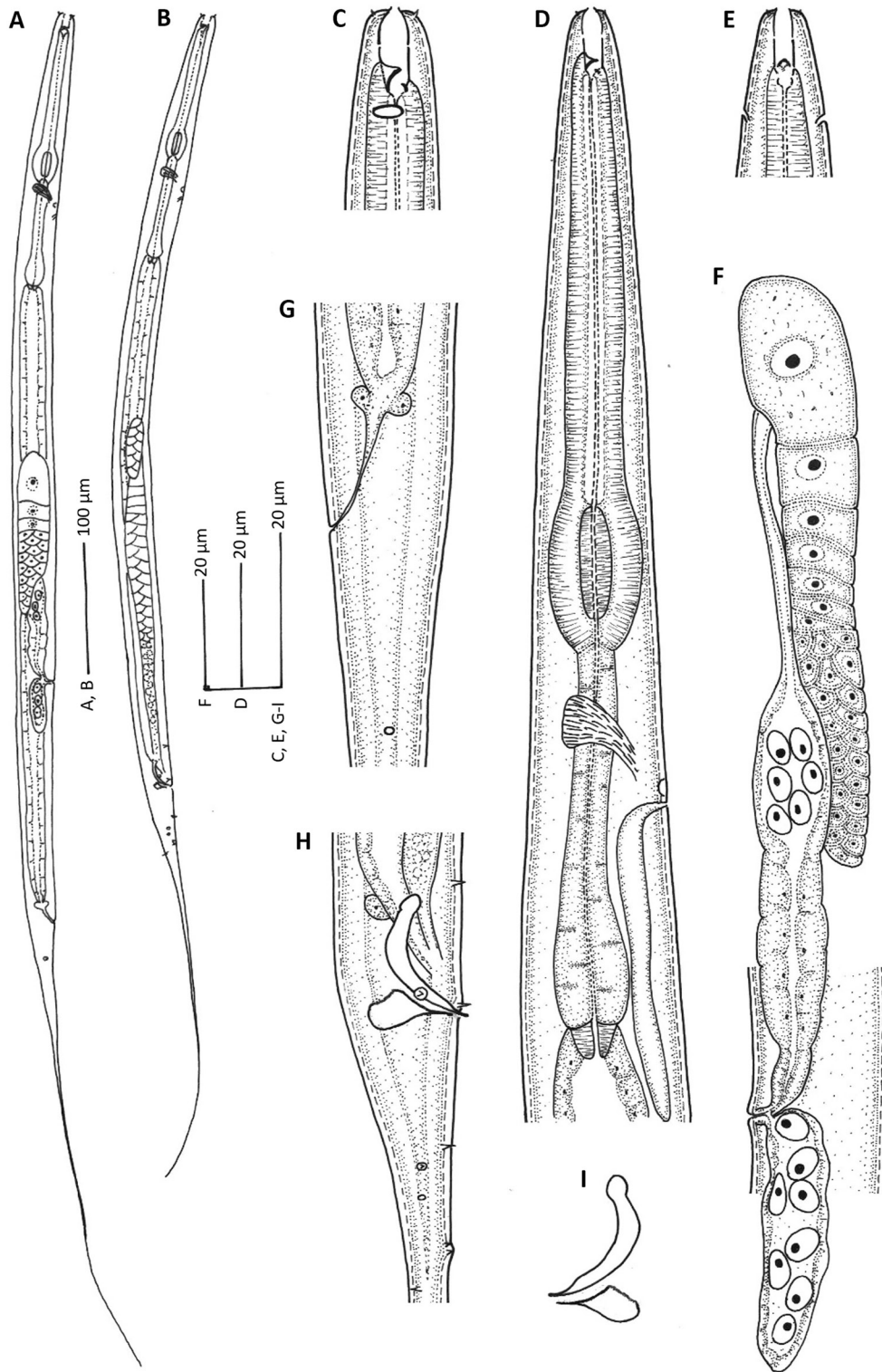
*Diplogastrellus graciloides* is characterised by a narrow stoma longer than wide; elliptical amphidial aperture, posterior to the base of stoma; massive dorsal tooth with ventrally directed apex, small tooth on each subventral wall; pharynx well-developed, muscular oblong metacorporeal bulb with valve plates, narrow isthmus and a pyriform basal bulb; mono-prodelphic reproductive system with a post-uterine sac; spicules slender with oval manubrium, smoothly arcuate to a curved distal end; globose gubernaculum with serrated proximal part and pointed distal tip and eight pairs of genital sensilla – with three precloacal and five postcloacal pairs and *v7* absent.

**Material examined**

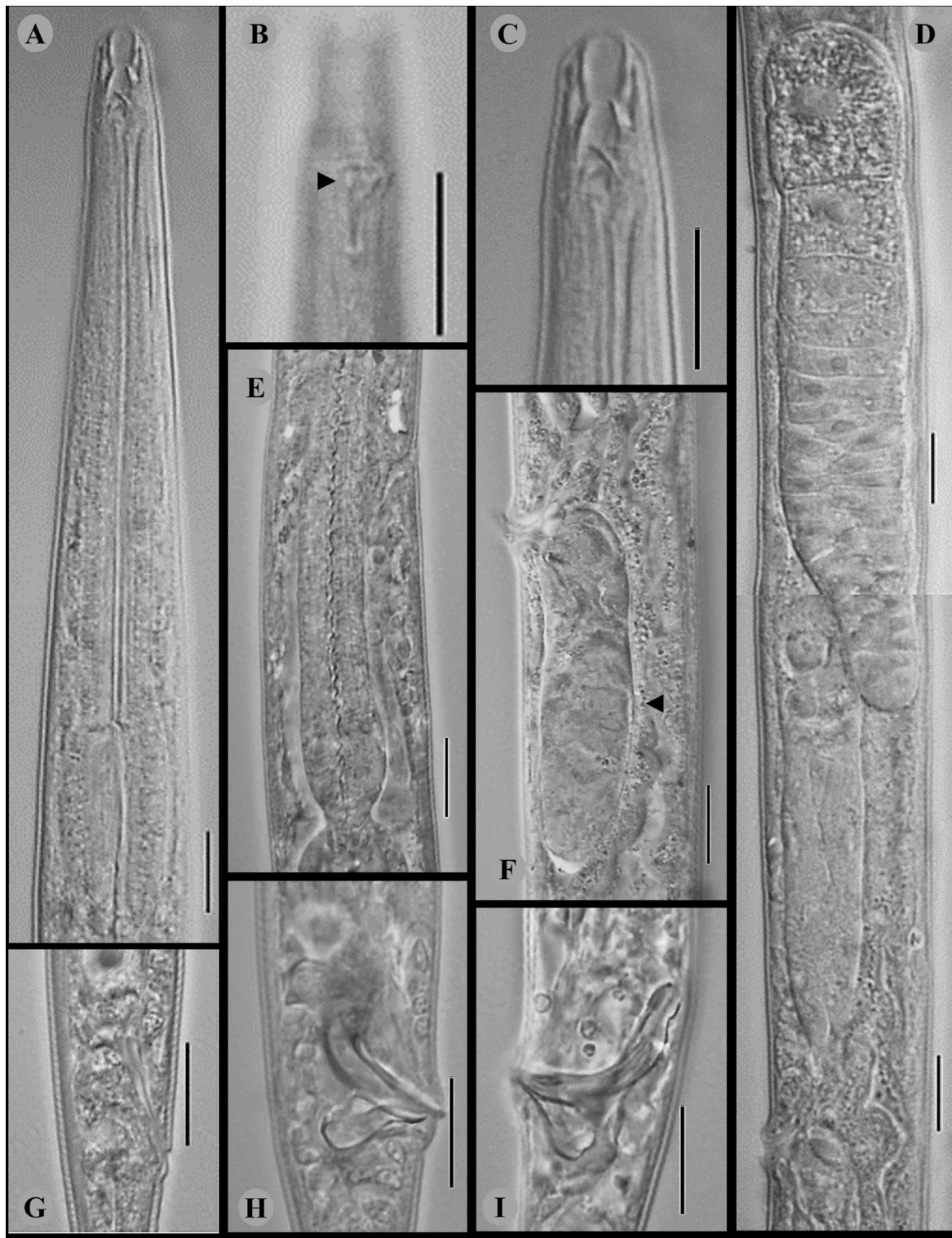
INDIA • 10 ♀♀, 10 ♂♂; Uttar Pradesh, District Muzaffarnagar; 29°31'27" N, 77°38'38" E; extracted from rotting banana rhizome; slide reference number AMU/ZD/NC/*Diplogastrellus graciloides*/1–10.

**Description****Adult**

Body slender, medium-sized, less than 1 mm long; almost straight after fixation, tapering only very slightly anteriorly, but more conspicuously posteriorly to a long filiform tail. Cuticle with fine transverse



**Fig. 12.** *Diplogastrellus graciloides* (Skwarra, 1921). **A, C–G.** ♀ (AMU/ZD/NC/*Diplogastrellus graciloides*/1–10). **B, H–I.** ♂ (AMU/ZD/NC/*Diplogastrellus graciloides*/1–10). **A.** Entire female. **B.** Entire male. **C.** Anterior region showing stoma (lateral view). **D.** Pharyngeal region. **E.** Anterior region showing stoma (dorsoventral view). **F.** Female reproductive system. **G.** Female posterior region. **H.** Male posterior region. **I.** Spicule and gubernaculum.



**Fig. 13.** *Diplogastrellus graciloides* (Skwarra, 1921). **A–G.** ♀ (AMU/ZD/NC/*Diplogastrellus graciloides*/1–10). **H–I.** ♂ (AMU/ZD/NC/*Diplogastrellus graciloides*/1–10). **A.** Anterior pharynx. **B.** Anterior region showing amphid. **C.** Anterior region showing stoma. **D.** Female reproductive system showing anterior branch. **E.** Posterior pharynx. **F.** Post-uterine sac. **G.** Female posterior region. **H–I.** Male posterior region showing spicules and gubernaculum. The arrowheads in B and F indicate amphidial aperture and post-uterine sac, respectively. Scale bars = 10  $\mu$ m.

striations, lateral fields inconspicuous. Lip region round, continuous with body contour. Lips six, amalgamated, each bearing a small papilla. Amphidial apertures elliptical, 13–14  $\mu\text{m}$  from anterior end, just posterior to the base of stegostom. Stoma narrow, longer than wide, about 9–11  $\mu\text{m}$  in depth. Cheilostom longer than wide, cheilorhabdions arched inward anteriorly, cheilorhabdial flaps six, protruding above labial contour; gymnostom anisotopic, dorsal wall shorter than subventrals. Stegostom anisotopic and anisomorphic, dorsal metastegostomal wall with a large tooth, having ventrally directed apex. Posterio-ventral aspect strongly cuticularized, smooth. Anterio-dorsal aspect not cuticularized. Each subventral wall provided with a small tooth. Pharynx with slender 74–89  $\mu\text{m}$  long, muscular corpus of uniform diameter; median bulb set off from corpus, elongate, 22–30  $\mu\text{m}$  long with strong valve plates. Isthmus 38–48  $\mu\text{m}$  long, narrow, conspicuously differentiated from median bulb. Basal bulb small, pyriform, glandular, 13–21  $\times$  9–12  $\mu\text{m}$ , only slightly expanded from isthmus without any valve plate or grinder. Nerve ring encircling isthmus in anterior half, located at 66–71% of pharyngeal length. Hemizonid posterior to nerve ring, 67–72% of pharyngeal length. Excretory pore just posterior to hemizonid, 70–74% of pharyngeal length. Cardia well-developed, 3  $\mu\text{m}$  long, consisting of three flaps, one dorsal and two ventro-sublateral. Intestine composed of dark granulated cells with prominent nuclei, intestinal lumen 6–8  $\mu\text{m}$  wide, without any bacterial pouch. Rectum 1.0–1.4 times anal body diameter long; rectal glands distinct.

#### Female

Reproductive system mono-prodelphic. Ovary reversed on right side of intestine, distal part of ovary not reaching the level of vulva. Oocytes with large nuclei, arranged in multiple rows in germinal zone and single row in maturation zone. Oviduct long, narrow, tubular, connecting spermatheca and ovary. Spermatheca expanded, not set off from uterus but with distinctly narrower walls, containing sperm. Uterus divisible into a distal smaller muscular part and proximally placed longer glandular part made up of large cells and narrow lumen. Vagina narrow, tubular, almost at right angle to longitudinal body axis, 5–6  $\mu\text{m}$  long, about one-fifth of corresponding body diameter. Vulval opening circular. Post-uterine sac 33–51  $\mu\text{m}$  long, more than one vulval diameter long, sometimes filled with sperm. Vulva-anus distance 6.4–7.0 times vulval body diameter. Phasmids located at about 1.7–2.2 anal body diameter posterior to anus. Tail long, filiform 1.7–2.2 times vulva-anus distance.

#### Male

Similar to females in general morphology but smaller in size. Reproductive system monorchic, testis reflexed laterally, on right side of intestine. Spermatocytes arranged in two rows distally followed by single row proximally. Vas deferens a long tube containing spermatocytes transforming into sperm, tapering to an ejaculatory duct. Spicules paired, ventrally strongly arcuate, 1.3–1.4 times cloacal body diameter long. Manubrium oval, calamus/lamina complex smoothly tapered to a curved distal end. Gubernaculum globose, 45–50% of spicule length, proximal part serrated, caudally projected keel-like with pointed distal tip. Tail divisible into two parts, a short conoid part and a longer filamentous part. Genital sensilla eight pairs; three precloacal and five postcloacal pairs. Genital sensilla in configuration *v1*, *v2d*, *v3/v4*, *ad*, *ph*, (*v5*, *v6*), *pd*. Precloacal pair *v1* located anterior to the spicule range, almost one cloacal body diameter anterior to cloaca; *v3* just anterior to cloaca; *v2d* slightly anterior to *v3*; *v4* more than one cloacal body diameter posterior to cloaca and slightly anterior to *ad*; *v5*, *v6* posterior to *ad*, *v7* absent. Phasmids pore-like, 1.8–2.3 anal body diameter posterior to cloacal opening.

#### Remarks

The original description of *D. graciloides* was given by Skwarra (1921) collected from farmyard manure, Kaliningrad, Russia. The morphometric measurements of *D. graciloides* of our sample concur well with that of Skwarra (1921). However, minor differences were observed in body size ( $L = 769\text{--}865\ \mu\text{m}$  vs  $1000\text{--}1670\ \mu\text{m}$  in females;  $699\text{--}857\ \mu\text{m}$  vs  $860\text{--}1220\ \mu\text{m}$  in males), position of amphids (just posterior to the base of stoma vs far posterior to the base of stoma) and shape of spicules (distally curved vs not curved).

**Table 5.** Morphometric characteristics of *Diplogastrellus graciloides* (Skwarra, 1921). Measurements are in  $\mu\text{m}$  and in the form: mean  $\pm$  standard deviation (range).

Characters	Females (n = 10)	Males (n = 10)
L	825.4 $\pm$ 34.9 (769–865)	744.8 $\pm$ 48.4 (699–857)
a	36.2 $\pm$ 2.1 (32.9–39.9)	38.4 $\pm$ 2.4 (34.0–42.3)
b	4.9 $\pm$ 0.1 (4.7–5.2)	4.9 $\pm$ 0.3 (4.5–5.4)
c	2.8 $\pm$ 0.2 (2.6–3.1)	2.8 $\pm$ 0.1 (2.7–3.1)
c'	23.4 $\pm$ 2.7 (18.3–26.5)	17.9 $\pm$ 1.5 (15.0–19.9)
V/T	45.7 $\pm$ 1.7 (43–49)	40.0 $\pm$ 2.9 (36–43)
G1	23.1 $\pm$ 3.1 (19–30)	
Maximum body width	22.9 $\pm$ 2.0 (20–26)	19.4 $\pm$ 1.1 (18–21)
Lip width	6.2 $\pm$ 0.6 (5–7)	5.3 $\pm$ 0.5 (5–6)
Length of stoma	10.0 $\pm$ 0.7 (9–11)	9.6 $\pm$ 0.5 (9–10)
Corpus	80.7 $\pm$ 3.8 (74–89)	72.2 $\pm$ 4.3 (65–81)
Median bulb	26.8 $\pm$ 2.6 (22–30)	23.6 $\pm$ 1.6 (22–27)
Isthmus	44.3 $\pm$ 3.3 (38–48)	42.4 $\pm$ 2.5 (38–45)
Basal bulb length	16.1 $\pm$ 2.2 (13–21)	14.2 $\pm$ 0.8 (13–15)
Pharynx	167.9 $\pm$ 9.3 (150–182)	152.4 $\pm$ 5.8 (141–158)
Cardia	3	3
Nerve ring from ant. end	113.6 $\pm$ 5.7 (102–122)	101.6 $\pm$ 4.2 (94–109)
Excretory pore from ant. end	126.7 $\pm$ 3.4 (122–130)	104.4 $\pm$ 2.9 (100–109)
Hemizonid from ant. end	118.5 $\pm$ 6.3 (108–128)	103.0 $\pm$ 1.9 (100–105)
Pharynx-gonad distance	100.2 $\pm$ 12.5 (80–115)	70.3 $\pm$ 24 (36–106)
Anterior gonad	190.6 $\pm$ 29.0 (146–257)	–
Post-uterine sac	39.2 $\pm$ 5.1 (33–51)	–
VBD	21.9 $\pm$ 1.6 (20–25)	–
Vulva-anus distance	148.2 $\pm$ 9.7 (134–159)	–
Vulva from anterior end	377.3 $\pm$ 27.0 (345–422)	–
Rectum	15.0 $\pm$ 1.4 (13–18)	15.1 $\pm$ 0.3 (15–16)
Tail	299.9 $\pm$ 13.0 (275–321)	262.7 $\pm$ 13.3 (240–280)
ABD	13.0 $\pm$ 1.4 (11–15)	14.8 $\pm$ 0.9 (14–16)
Phasmid from anus	25.0 $\pm$ 1.0 (23–26)	32.0 $\pm$ 2.2 (28–35)
Testis	–	298.0 $\pm$ 31.9 (259–368)
Spicule along axis	–	19.8 $\pm$ 0.4 (19–20)
Spicule along cord	–	18.0 $\pm$ 0.8 (17–19)
Gubernaculum	–	9.2 $\pm$ 0.4 (9–10)
No. of precloacal papillae	–	3
No. of postcloacal papillae	–	5

Our population of *Diplogastrellus graciloides* shows resemblance with its closely related species *D. gracilis*. However, it differs in having longer tail ( $c = 2.6\text{--}3.1$  vs  $3.5\text{--}3.8$  in females;  $2.7\text{--}3.1$  vs  $5.3\text{--}7.6$  in males), position of amphids (at the base of cheilostom vs posterior to the base of stoma), post-uterine sac (present vs absent), size and shape of spicules ( $19\text{--}20\ \mu\text{m}$ ; strongly arcuate, smoothly tapered to a curved distal end vs  $50\text{--}57\ \mu\text{m}$ ; long, thin, slender, attenuated to a pointed distal end), size and shape of gubernaculum ( $9\text{--}10\ \mu\text{m}$ ; globose with pointed distal tip vs  $20\text{--}25\ \mu\text{m}$ ; keel-like proximal end with a distal sleeve).

***Diplogastrellus monhysteroides* (Bütschli, 1874)**

Figs 14–19; Tables 6–8

*Diplogaster minor* Goodey, 1929: 27–62, figs 36–37.

*Diplogaster irregularis* Paesler, 1946: 87–128.

**Emended diagnosis**

*Diplogastrellus monhysteroides* is characterised by a broad stoma longer than wide; elliptical amphidial aperture, at the base of stoma; strong, dagger-like dorsal tooth, anteriorly-directed and small tooth on both subventral walls; pharynx well-developed, muscular ovoid metacorporeal bulb with valve plates, narrow isthmus and an oval basal bulb; mono-prodelphic reproductive system with a post-uterine sac; spicules slender with rounded manubrium, strongly arcuate to a curved and pointed distal end; shoe-like gubernaculum, distal end with a rectangular sleeve and nine pairs of genital sensilla.

**Material examined**

INDIA • 10 ♀♀, 10 ♂♂; Uttar Pradesh, District Bulandshahr;  $28^{\circ}16'22''$  N,  $78^{\circ}1'29''$  E; extracted from farmyard manure; slide reference number AMU/ZD/NC/*Diplogastrellus monhysteroides*/1–10.

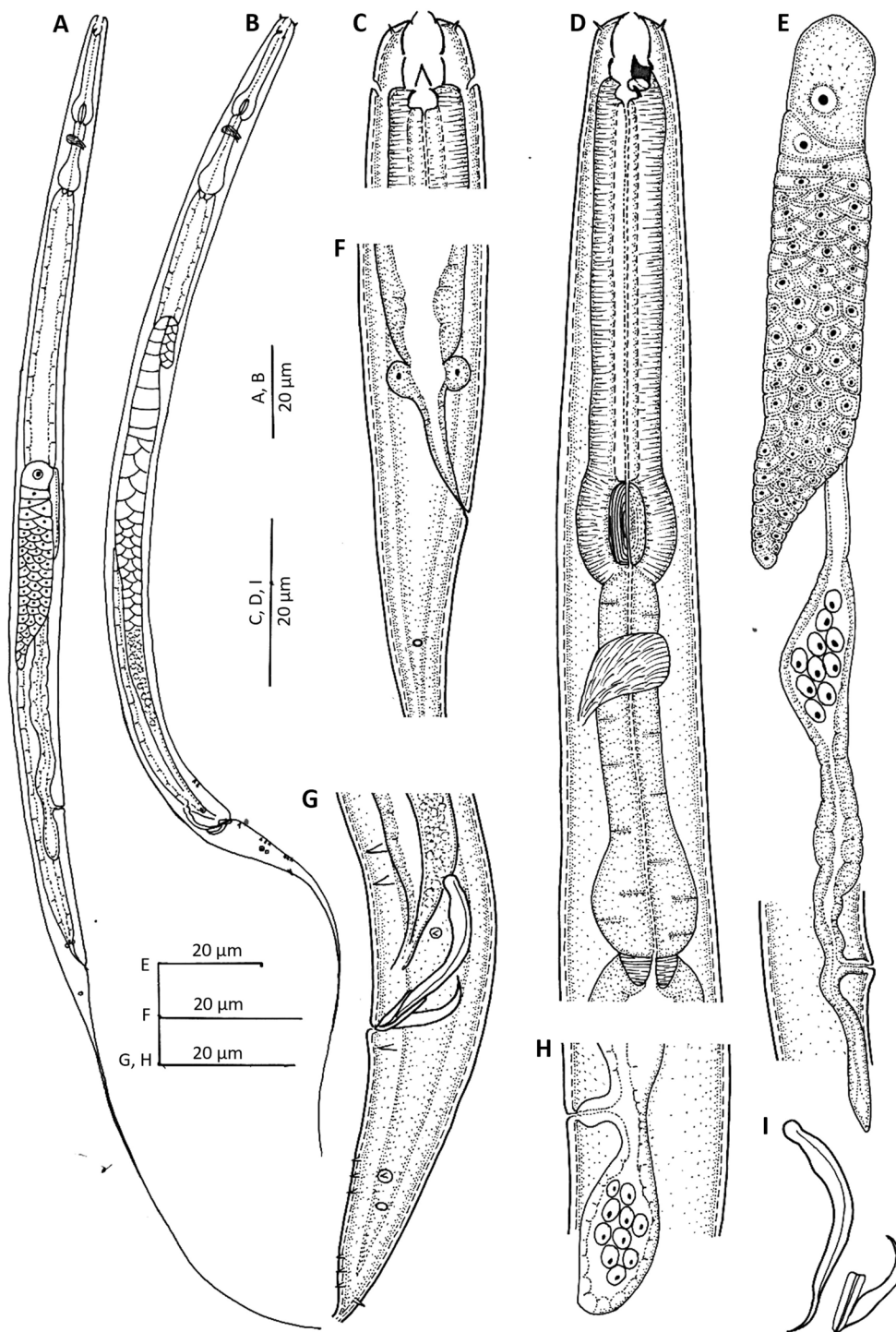
**Description**

**Adult**

Body slender, medium-sized, less than 1 mm long; almost straight after fixation, tapering at both extremities. Cuticle with fine transverse striations, longitudinal ridges faint. Lip region round, continuous with body contour. Lips six, amalgamated, each bearing a setose papilla. Amphidial apertures oval,  $7\text{--}9\ \mu\text{m}$  from anterior end of stoma, at the base of dorsal tooth. Stoma broad,  $9\text{--}11\ \mu\text{m}$  long. Cheilostom longer than wide, cheilorhabdions arched inward anteriorly, cheilorhabdial flaps six, protruding above labial contour; gymnostom anisotropic, dorsal wall shorter than subventrals. Stegostom anisotropic and anisomorphic, dorsal metastegostomal wall with a strong, dagger-like, anteriorly-directed tooth,  $3\text{--}4\ \mu\text{m}$  long; subventral walls provided with small tooth each. Pharynx with slender  $54\text{--}62\ \mu\text{m}$  long, muscular corpus of uniform diameter; median bulb set off from corpus, ovoid,  $15\text{--}16\ \mu\text{m}$  long with strong valve plates. Isthmus  $29\text{--}37\ \mu\text{m}$  long, narrow, conspicuously differentiated from median bulb. Basal bulb oval, glandular,  $13\text{--}16 \times 11\text{--}14\ \mu\text{m}$ , only slightly expanded from isthmus without any valve plate or grinder. Nerve ring encircling isthmus in anterior half, located at  $69\text{--}72\%$  pharyngeal length. Excretory pore and hemizonid not visible. Cardia well-developed,  $5\text{--}6\ \mu\text{m}$  long, consisting of three flaps, one dorsal and two ventro-sublateral. Intestine composed of dark granulated cells with prominent nuclei, intestinal lumen uniformly wide,  $5\text{--}7\ \mu\text{m}$  without any bacterial pouch. Rectum  $1.4\text{--}1.7$  times anal body diameter long, three rectal glands, one dorsal and two subventral, observed at intestine–rectum junction

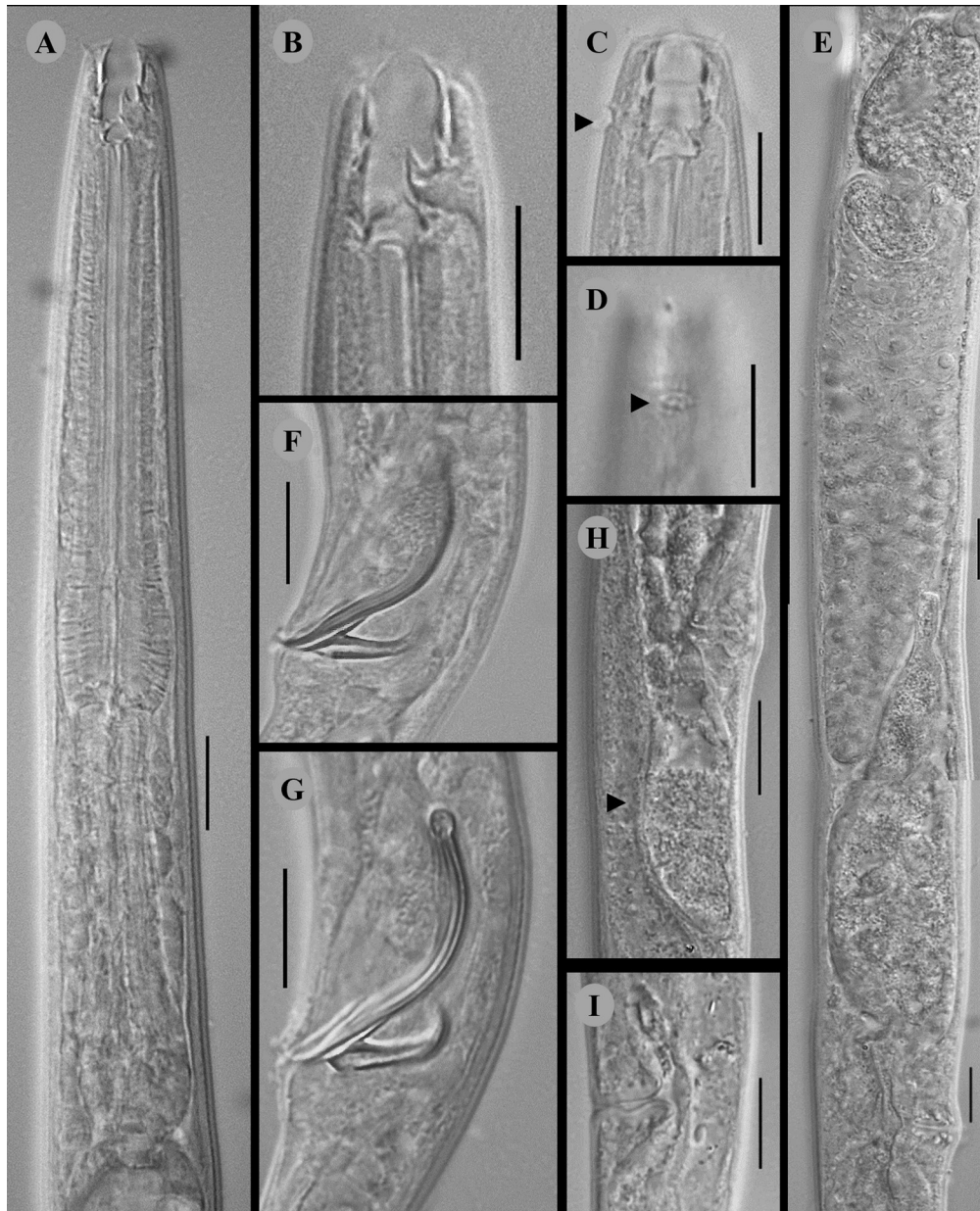
**Female**

Reproductive system mono-prodelphic. Ovary reversed on right side of intestine, distal part of ovary not reaching the level of vulva. Oocytes with large nuclei, arranged in multiple rows in germinal zone and single row in maturation zone. Oviduct long, narrow, tubular, connecting spermatheca and ovary. Spermatheca expanded, not set off from uterus, with distinctly narrower walls, containing sperm. Uterus

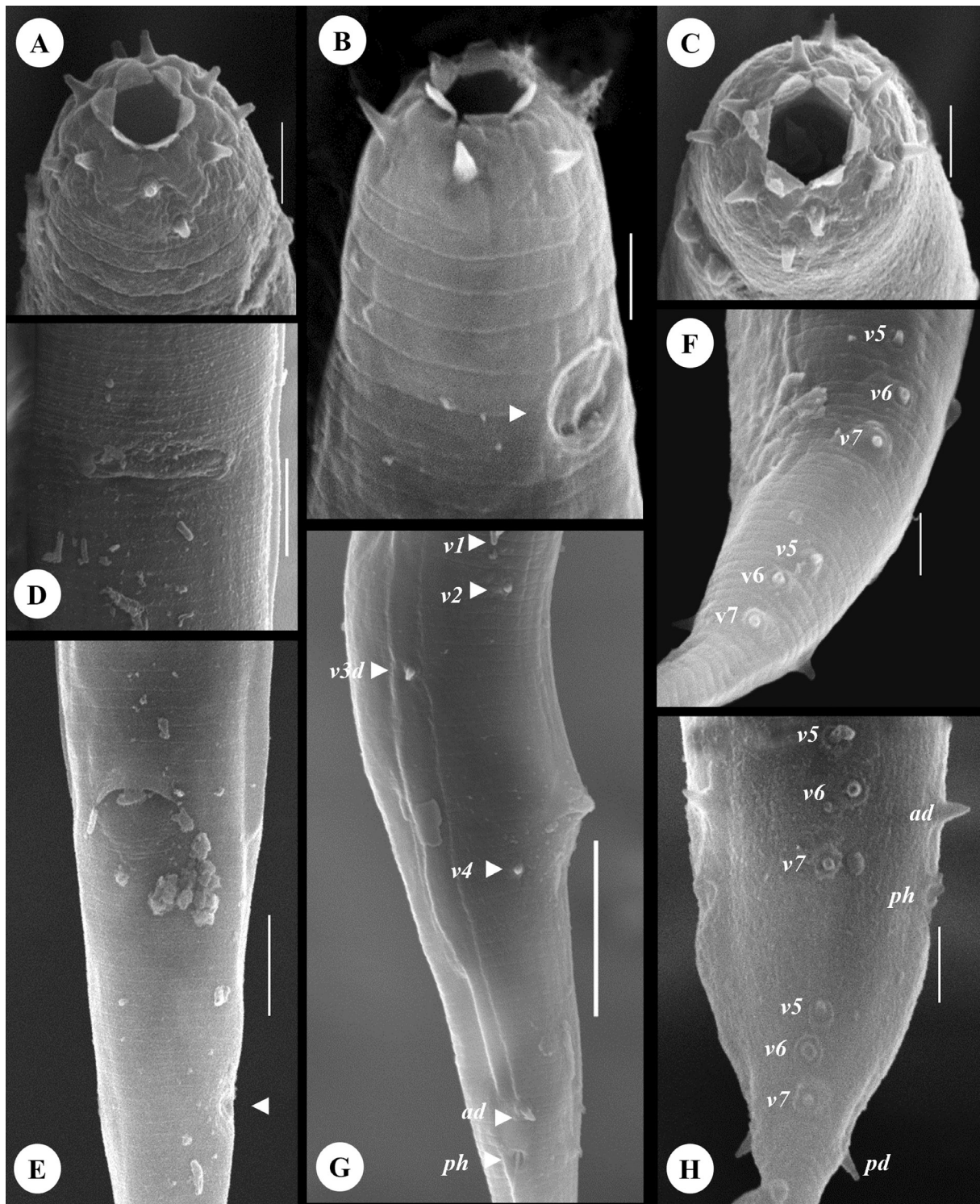


**Fig. 14.** *Diplogastrellus monhysteroides* (Bütschli, 1874). **A, C–F, H.** ♀ (AMU/ZD/NC/*Diplogastrellus monhysteroides*/1–10). **B, G, I.** ♂ (AMU/ZD/NC/*Diplogastrellus monhysteroides*/1–10). **A.** Entire female. **B.** Entire male. **C.** Anterior region showing stoma (dorsoventral view). **D.** Pharyngeal region. **E.** Female reproductive system. **F.** Female posterior region. **G.** Male posterior region. **H.** Post-uterine sac. **I.** Spicule and gubernaculum.

divisible into a distal small muscular and proximally placed longer, glandular part made up of large cells and narrow lumen. Vagina narrow, tubular, almost at right angle to longitudinal body axis, 11–14  $\mu\text{m}$  long about one-third of corresponding body diameter. Vulval opening circular. Post-uterine sac 27–40  $\mu\text{m}$  long, sometimes filled with sperm. Vulva-anus distance 4.1–5.2 times vulval body diameter. Phasmids located at 1.3–1.8 anal body diameter posterior to anus. Tail long, filiform 2.7–3.6 times vulva-anus distance.



**Fig. 15.** *Diplogastrellus monhysteroides* (Bütschli, 1874). **A–E, H–I.** ♀ (AMU/ZD/NC/*Diplogastrellus monhysteroides*/1–10). **F–G.** ♂ (AMU/ZD/NC/*Diplogastrellus monhysteroides*/1–10). **A.** Pharyngeal region. **B.** Anterior region showing stoma (lateral view). **C–D.** Anterior region showing amphid. **E.** Female reproductive system showing anterior branch. **F–G.** Male posterior region showing spicules and gubernaculum. **H.** Post-uterine sac. **I.** Vulval region. The arrowheads in C–D and H indicate amphidial aperture and post-uterine sac, respectively. Scale bars = 10  $\mu\text{m}$ .



**Fig. 16.** *Diplogastrellus monhysteroides* (Bütschli, 1874). A–E. ♀ (AMU/ZD/NC/*Diplogastrellus monhysteroides*/1–10). F–H. ♂ (AMU/ZD/NC/*Diplogastrellus monhysteroides*/1–10). A. Anterior region showing labial papillae and cheilorhabdial plates. B. Anterior region showing amphid. C. Enface view. D. Vulval opening. E. Female posterior region showing anal opening and phasmid. F–H. Male posterior region showing genital papillae. The arroheads in F–H indicate configuration of genital papillae. Scale bars: A–C, F, H = 2 µm; D–E = 5 µm; G = 10 µm.

**Table 6.** Morphometric characteristics of *Diplogastrellus monhysteroides* (Bütschli, 1874). Measurements are in  $\mu\text{m}$  and in the form: mean  $\pm$  standard deviation (range).

Characters	Females (n= 10)	Males (n = 10)
L	829.6 $\pm$ 69.9 (714–910)	629.0 $\pm$ 86.8 (535–769)
a	34.0 $\pm$ 3.9 (27.8–38.8)	34.0 $\pm$ 3.6 (29.7–40.5)
b	6.8 $\pm$ 0.2 (6.4–7.1)	6.0 $\pm$ 0.7 (5.4–7.2)
c	2.9 $\pm$ 0.2 (2.6–3.1)	3.0 $\pm$ 0.5 (2.2–3.7)
c'	22.6 $\pm$ 2.0 (20.4–25.5)	14.0 $\pm$ 3.6 (10.3–20.6)
V/T	53.9 $\pm$ 2.1 (50–56)	44.7 $\pm$ 2.9 (42–50)
G1	42.6 $\pm$ 3.4 (38–47)	–
Maximum body width	24.6 $\pm$ 3.0 (20–29)	18.4 $\pm$ 1.0 (17–20)
Lip width	8.2 $\pm$ 0.7 (7–9)	7.0 $\pm$ 0.6 (6–8)
Length of stoma	10.4 $\pm$ 0.8 (9–11)	9.2 $\pm$ 0.4 (9–10)
Corpus	57.4 $\pm$ 3.2 (54–62)	47.6 $\pm$ 3.2 (45–52)
Median bulb	15.4 $\pm$ 0.5 (15–16)	13.2 $\pm$ 1.5 (12–16)
Isthmus	34.0 $\pm$ 3.1 (29–37)	32.0 $\pm$ 1.9 (30–35)
Basal bulb	14.6 $\pm$ 1.0 (13–16)	11.8 $\pm$ 0.7 (11–13)
Pharynx	121.4 $\pm$ 6.3 (111–129)	104.6 $\pm$ 3.1 (100–109)
Cardia	5.2 $\pm$ 0.4 (5–6)	4.4 $\pm$ 0.5 (4–5)
Nerve ring from ant. end	86.0 $\pm$ 4.9 (80–93)	73.2 $\pm$ 1.3 (71–75)
Pharynx-gonad distance	115.0 $\pm$ 26.3 (89–160)	48.0 $\pm$ 17.9 (17–70)
Anterior gonad	354.2 $\pm$ 48 (268–411)	–
Post-uterine sac	32.8 $\pm$ 4.4 (27–40)	–
VBD	20.8 $\pm$ 1.5 (19–23)	–
Vulva-anus distance	97.8 $\pm$ 11.2 (77–110)	–
Vulva from anterior end	448.4 $\pm$ 52.9 (357–503)	–
Rectum	20.2 $\pm$ 1.0 (19–22)	18.6 $\pm$ 2.3 (16–23)
Tail	283.4 $\pm$ 11.6 (265–301)	219.8 $\pm$ 69.6 (155–350)
ABD	12.6 $\pm$ 1.0 (11–14)	15.4 $\pm$ 1.0 (14–17)
Phasmid from anus	19.7 $\pm$ 1.7 (18–22)	–
Testis	–	280.2 $\pm$ 34.3 (228–321)
Spicule along axis	–	26.8 $\pm$ 2.2 (25–31)
Spicule along cord	–	22.8 $\pm$ 1.8 (21–25)
Gubernaculum	–	13.2 $\pm$ 0.7 (12–14)
No. of precloacal papillae	–	3
No. of postcloacal papillae	–	6

### Male

Similar to females in general morphology but smaller in size. Anterior region with four cephalic papillae present posterior to circlet of six labial papillae. Reproductive system monorchic, testis reflexed ventrally, on right side of intestine. Spermatocytes arranged in two rows in anterior reflexed part, well-developed spermatocytes in anterior half of non-reflexed part, as multiple rows in next half and containing small sperm in remaining gonad. Vas deferens a long tube tapering to an ejaculatory duct. Spicules paired, slender, ventrally arcuate, 1.7–1.8 times cloacal body diameter long. Manubrium rounded, calamus/lamina complex slightly expanded and smoothly tapering to pointed and curved distal tips. Gubernaculum shoe-like, 45–52% of spicule length, proximally pointed and arcuate, distal end provided with a large rectangular sleeve. Tail divisible into two parts, a short conoid part and a longer filamentous part. Genital sensilla nine pairs; three pairs precloacal and six pairs postcloacal. Genital sensilla formula:  $v1, v2, v3d/v4, ad, (v5, v6, v7), phasmids, pd$ . Precloacal pair  $v1$  located anterior to the spicular range, more than one cloacal body diameter anterior to cloaca;  $v2$  at the level of the spicule head;  $v3d$  about one cloacal body diameter anterior to cloaca;  $v4$  just posterior to cloacal aperture;  $v5-7$  clusters greatly separated from each other with left subventral group at the level of  $ad$  and right subventral group just anterior to  $pd$ . Phasmids pore-like, slightly posterior to left subventral  $v5-v7$  group, 1.3–1.8 anal body diameter posterior to cloacal opening.

### Remarks

The original description of *D. monhysteroides* was given by Bütschli (1874). The values of morphometric characters of *D. monhysteroides* of our sample overlap with those of Bütschli (1874). Our population also resembles the type population in the shape of lip region, structure of stoma, pharynx, position of nerve ring and shape and size of gubernaculum. However, it differs in shape of spicules and number and arrangement of genital papillae. Spicules in our specimen bear curved distal end (Fig. 14I) while in the type population, they are smoothly arcuate with straight distal end. Bütschli (1874) mentions 12 pairs of genital papillae in which group  $v5-7$  was in two pairs. Both clusters of first pair are situated at the level of  $ad$  and both clusters of second pair just anterior to  $pd$  while in our population, only one pair of  $v5-7$  is evident. As clearly seen in the SEM images (Fig. 16F, H), the two clusters are separated from each other. The discrepancy in numbers (12 pairs vs 9 pairs) appears to have arisen because of the anterior-posterior segregation of the cluster of  $v5-7$  group which may have resulted in the unusual configuration of the genital sensilla in the type population.

Our population of *D. monhysteroides* resembles the closely related species *D. cerea*. However, it differs in position of amphids (at the base of dorsal tooth vs at the level of gymnostom), size of dorsal tooth (dagger-like vs medium-sized), subventral armature (small tooth on both walls vs small ridges on both walls), post-uterine sac (longer than vulval body diameter vs shorter than vulval body diameter), shape of spicules (curved distal end vs smoothly tapering to a distal end), shape of gubernaculum (shoe-like with rectangular sleeve vs keel-like, proximally notched without distal sleeve) and arrangement of genital sensilla ( $v1$  and  $v2$  closely placed; both cluster of  $v5-7$  at the same level vs  $v2$  far posterior to  $v1$ , situated near cloaca;  $v5-7$  separated, first pair at the level of  $ad$  and second pair just anterior to  $pd$ ).

### Bionomics

The nineteen species of *Diplogastrellus* are distributed worldwide. Seven species have been recorded from Asia, six from Europe, three from North America, two from Oceania, and one from Africa. Most species, e.g., *D. didelphis* sp. nov., *D. indicus*, *D. latigubernacula*, *D. longipharyngis* sp. nov., *D. robustus* sp. nov., *D. sikorai*, and *D. thoubalicus*, have been described from India, whereas *D. gracilis*, *D. monhysteroides*, and *D. stammeri* have been reported from Germany, and *D. cerea*, *D. metamasius*, and *D. prodelphis* from the USA. The rest of the species, viz., *D. graciloides*, *D. mikuschi*, *D. secundus*, *D. heynsi*, *D. parvus*, and *D. pulcher*, were described from Russia, The Netherlands, Denmark, South Africa, Fiji, and New Guinea, respectively. The species of *Diplogastrellus* have been predominantly reported from farmyard manure, rotting banana rhizome, decaying cactus, decay leaves of *Iris* L., rotting

**Table 7** (continued on next two pages). Comparative measurements of species of *Diplogastrellus* Paramonov, 1952 (all measurements are in  $\mu\text{m}$ ). Abbreviations: ABD = anal body diameter; L = length. Symbols: \* = calculated from drawing; – indicates unavailability of information in the type/original description.

Characters	<i>D. cerea</i>	<i>D. didelphis</i> sp. nov.	<i>D. gracilis</i>	<i>D. graclloides</i>	<i>D. heynsi</i>	<i>D. indicus</i>	<i>D. latigubernacula</i>
Country	USA	India	India	Russia	India, South Africa	India	India
Habitat	Decaying Saguaro cactus	Rotting banana rhizome	Compost of mushroom	Compost	Sewage drain	Soil	Farmyard manure
<b>Female</b>							
Body length	509–855	735–948	644–750	1000–1670	667–788	610–620	550–600
Stoma length	9.4–10.3	10–11	11–13	10*	11–13	12.5*	10–12
Pharynx length	107–131	139–161	115–143	220*	162–182	114–115	92–106
Body diameter	20–43	25–35	21–24	–	–	21.5–22.5	25*
ABD	20*	13–15	14–18	–	14–16	–	10–13
Tail	113–241	200–255	175–200	–	170–194	201–241	194–301
a	18.5–27.3	26.0–31.6	26.8–32.6	27–41	23.4–34.8	27–29	30.0–35.8
b	4.7–7.0	5.1–6.2	5.2–5.9	4.7–5.8	4.1–4.5	5.3–5.4	5.2–6.9
c	3.4–5.2	3.5–4.2	3.5–3.8	2.8–4.1	3.7–4.4	2.5–3.0	2.2–2.6
c'	8.9–15.0	13.5–17.0	11.1–12.0	–	11.1–13.3	–	19.2–28.3
V (%)	84–87	44–47	68–69	46–58	52.4–55.1	46–51	56–58
<b>Male</b>							
Body length	459–724	592–770	541–675	850–1200	515–615	460	530–600
Stoma length	9.4–11.3	9–10	11–12	–	10–11	10.6*	10–12
Pharynx length	103–120	123–141	113–122	–	143–155	102–109	81–88
Spicule length	24–28	30–31	50–57	24.5*	25–28	20–23	17.5–18.5
Gubernaculum I	12–14	18–19	20–25	20*	9–11	4–5	10–11
Tail	74–160	132–165	85–115	–	113–145	139	226–276
a	17.7–30.8	23.9–31.3	25.7–30.4	34–47	27.9–32.7	24	36.5–40.5
b	4.4–6.1	4.5–5.8	4.7–5.5	4.7–7.1	3.5–4.1	4.4	6.2–7.0
c	4.2–6.1	4.1–5.1	5.3–7.6	2.9–6.0	3.9–4.5	3.3	2.1–2.3
c'	4.6–9.1	6.8–8.1	4.6–6.3	–	6.1–8.3	–	15.6–18.8
References	Kiontke & Sudhaus 1996	sp. nov.	Büttschli 1876; Paramonov 1952; apud Khan <i>et al.</i> 2008	Skwarra 1921; Paramonov 1952	Kiontke & Sudhaus 1996; Sudhaus & Fürst von Lieven 2003; apud Ahmad <i>et al.</i> 2005	Suryawanshi 1971	Mahamood <i>et al.</i> 2015

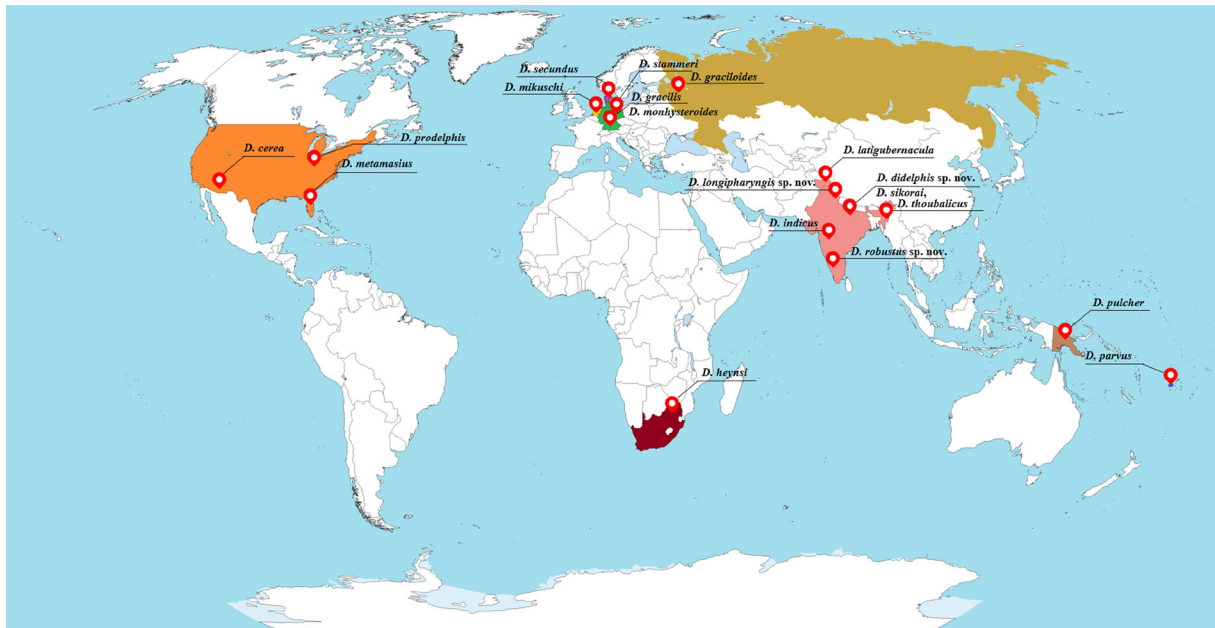
**Table 7** (continued). Comparative measurements of species of *Diplogastrellus* Paramonov, 1952 (all measurements are in  $\mu\text{m}$ ). Abbreviations: ABD = anal body diameter; L= length. Symbols: \* = calculated from drawing; – indicates unavailability of information in the type/original description.

Characters	<i>D. longipharyngis</i> sp. nov.	<i>D. metamasius</i>	<i>D. mikuschi</i>	<i>D. monhysteroides</i>	<i>D. parvus</i>	<i>D. prodelephis</i>
Country	India	USA	Holland	Germany	Fiji	USA
Habitat	Rotting bark	Insect (abdominal fold of <i>M. hemipterus</i> )	Frass of beetle	Cow and horse dung	Decaying sheaths of banana	Diseased leaves of <i>Iris</i>
<b>Female</b>						
Body length	865–1091	753–1155	600–700	630–1280	500–960	480–590
Stoma length	5–6	11–15	–	11.7*	–	–
Pharynx length	152–175	144–190	–	221.7*	–	–
Body diameter	23–32	40–53	–	–	–	–
ABD	17–20	23–30	–	18*	–	–
Tail	245–346	106–163	–	–	–	–
a	30–38	16.1–22.2	22–27	26.5–30.1	20–34	24–28
b	5.2–6.7	4.7–6.6	6–7	5.5–7.9	4.2–5.6	5–6
c	3.2–3.7	5.8–8.0	5.2–5.4	2.7–3.5	2.7–3.5	3.0–3.3
c'	12.3–17.5	3.8–6.3	–	–	–	–
V (%)	51–56	48–56	63–66	54–57	50–65	54–55
<b>Male</b>						
Body length	597–752	646–817	600	525–834	600–730	440
Stoma length	5–6	10–14	–	–	–	–
Pharynx length	125–159	136–174	–	–	–	–
Spicule length	25–27	23–30	–	20–37	29	–
Gubernaculum I	10–11	14–19	–	12–19	12	–
Tail	135–210	100–135	–	–	–	–
a	28.4–35.9	17.8–24.5	27–32	26.9–32.5	26–35	23
b	4.3–5.6	4.5–5.5	6	4.1–5.8	4.9–5.2	5
c	3.4–4.4	5.4–7.4	7	2.6–3.3	5.3–7.6	3.2
c'	7.9–11.7	3.9–5.1	–	–	3.5–4.6	–
References	sp. nov.	Kanzaki <i>et al.</i> 2008	Fuchs 1938; Paramonov 1952	Bütschli 1876; Paramonov 1952	Cobb 1893; Paramonov 1952	Steiner 1936; Sudhaus & Fürst von Ljeven 2003

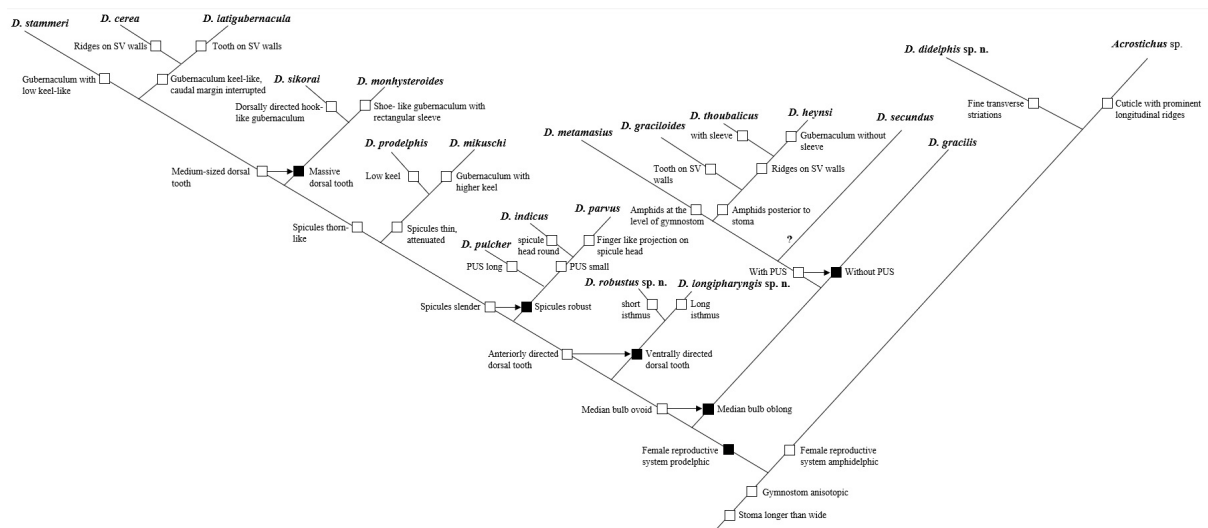
**Table 7** (continued). Comparative measurements of species of *Diplogastrellus* Paramonov, 1952 (all measurements are in µm). Abbreviations: ABD = anal body diameter; L= length. Symbols: \* = calculated from drawing; – indicates unavailability of information in the type/original description.

Characters	<i>D. pulcher</i>	<i>D. robustus</i> sp. nov.	<i>D. sikorai</i>	<i>D. stammeri</i>	<i>D. thouballicus</i>
Country/ Island	New Guinea	India	India	Germany	India
Habitat	Decayed leaves	Soil	Wet humus	Compost and manure piles	Farmyard manure
<b>Female</b>					
Body length	800–830	523–730 (L')	492–657	670–1000	750–1024
Stoma length	11–12	10–12	9–11	11.8*	12–15
Pharynx length	133–140	158–180	95–118	149.5*	171–228
Body diameter	–	27–41	17–25	–	–
ABD	–	17–25	10–16	–	17–20
Tail	330–355	–	195–270	–	230–335
a	30–34	17.8–25.1	24–30	25–32	23.8–34.8
b	5.8–6.1	3.3–4.2	4.9–6.3	5.2–7.0	4.3–4.9
c	2.2–2.4	14.4–17.5	2.4–2.8	2.6–3.0	3.0–3.5
c'	22–26	1.7–2.6	15.8–23.6	–	11.8–26.3
V (%)	44–46	59–64	45–48	50–57	49–59
<b>Male</b>					
Body length	660–680	535–621	395–470	680–970	713–818
Stoma length	–	9–11	7–9	–	10–14
Pharynx length	–	145–160	87–101	–	159–189
Spicule length	18–20	31–36	17–18.5	25*	25–28
Gubernaculum I	10	17–20	6.0–6.5	14.5	15–16
Tail	260–270	–	150–180	–	196–274
a	31–36	15.8–22.7	30.1–34.3	27–34	23.7–33.9
b	5.4–5.5	3.5–4.3	4.2–5.1	5.4–6.8	4.0–4.5
c	2.5–2.6	14.2–15.6	2.5–2.8	2.4–3.8	3.1–3.7
c'	20–22	1.5–1.8	12.5–15.0	–	8.8–11.3
References	Andrássy 1986; Sudhaus & Fürst von Lieven 2003	sp. nov.	Khan <i>et al.</i> 2008	Weingartner 1955; Meyl 1960	Ahmad <i>et al.</i> 2005

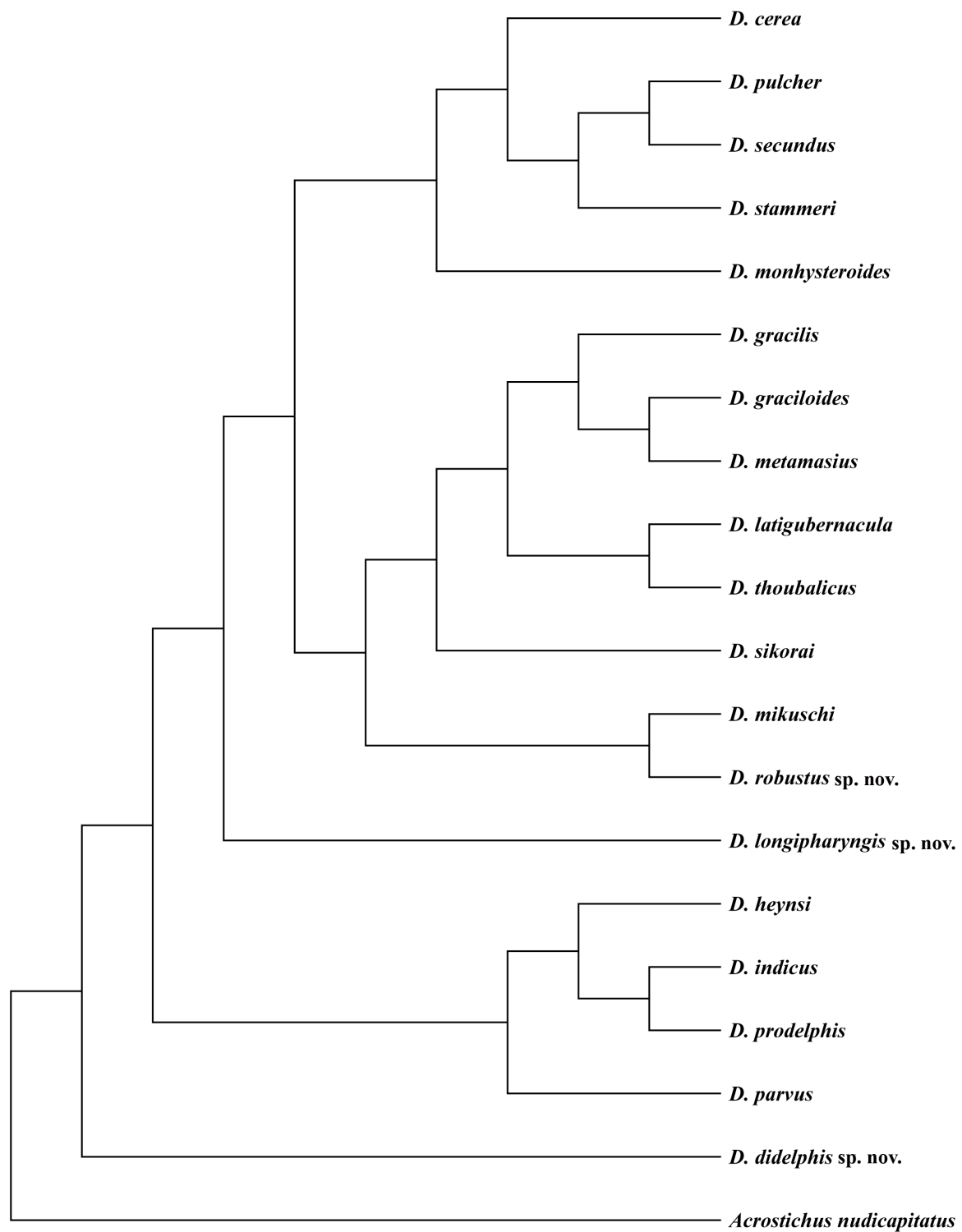
barks, wet humus, soil, compost of mushroom as well as from frass of beetle. However, two species (*D. metamasius* and *D. monhysteroides*) were reported to be associated with insects. *Diplogastrellus metamasius* was phoretically associated with sugarcane weevil (*Metamasius hemipterus*), recovered as dauer from the abdominal intersegmental folds and grown on xenic culture of bacteria. *Diplogastrellus monhysteroides* isolated from genitalia of male and female beetles (*Onthophagus taurus*).



**Fig. 17.** Map showing the worldwide geographical locations of the holotypes of species of *Diplogastrellus* Paramonov, 1952.



**Fig. 18.** Cladogram (constructed manually) showing the relationships of the species of *Diplogastrellus* Paramonov, 1952. Apomorphic morphological traits are designated by black squares and plesiomorphic traits by white squares.



**Fig. 19.** Cladistic analysis of the species of the genus *Diplogastrellus* Paramonov, 1952, indicating the position of the new species *D. didelphis* sp. nov., *D. longipharyngis* sp. nov., and *D. robustus* sp. nov. The phylogenetic tree was constructed for nineteen species using PAUP ver. 4.0. (Swofford 2002).

**Table 8** (continued on next two pages). Comparison of species of *Diplogastrellus* Paramonov, 1952 based on some important morphological characters. Abbreviations: adcl = adcloacal; LSV = left subventral; post. = posterior; postcl = postcloacal; precl = precloacal; PUS = post-uterine sac; RSV = right subventral; VBD = vulval body diam.; – indicates unavailability of information in the type/original description.

Characters	<i>D. cerea</i>	<i>D. didelphis</i> sp. nov.	<i>D. gracilis</i>	<i>D. graciloides</i>	<i>D. heynsi</i>	<i>D. indicus</i>
Stoma length vs diameter	~ 3 times longer	~ 4 times longer	~ 3 times longer	~ 3 times longer	~ 4 times longer	~ 2.5 times longer
Amphid position	At the level of gymnostom	At the base of gymnostom	At the base of cheilostom	Posterior to the base of stoma	Posterior to the base of stoma	At the level of cheilostom
Shape of dorsal tooth	Medium-sized, anteriorly directed	Medium-sized, ventrally directed	Massive, ventrally directed	Massive, ventrally directed	Massive, ventrally directed	Medium-sized, anteriorly directed
Subventral armature	Small ridges on both RSV and LSV	Absent	Small ridges on both RSV and LSV	Small tooth on both RSV and LSV	Small ridges on both RSV and LSV	Small tooth on both RSV and LSV
Shape of median bulb	Ovoid	Oblong	Oblong	Oblong	Oblong	Ovoid
Female reproductive system	Mono-prodelphic with PUS	Amphidelphic	Mono-prodelphic	Mono-prodelphic with PUS	Mono-prodelphic with PUS	Mono-prodelphic with PUS
Size of post-uterine sac	Shorter than VBD	Absent	Absent	Longer than VBD	Longer than VBD	Shorter than VBD
Male genital sensilla	9 pairs: 3 precl., 1 adcl., 5 postcl.	9 pairs: 3 precl., 6 postcl.	9 pairs: 2 precl., 2 adcl., 5 postcl.	8 pairs: 3 precl., 5 postcl.	9 pairs: 3 precl., 6 postcl.	5 pairs: 3 precl., 2 postcl.?
Position of <i>ad</i> sensilla	~ one cloacal diam. post. to cloaca	< one cloacal diam. post. to cloaca	< one cloacal diam. post. to cloaca	> one cloacal diam. post. to cloaca	> one cloacal diam. post. to cloaca	–
Position of <i>pd</i> sensilla	At the level of $v7$	Far post. to $v7$	Far post. to $v7$	Far post. to $v6$	At the level of $v7$	–
Relative size of trio $v5-v7$	$v7$ with two tips	$v5-v7$ equal	$v5-v7$ equal	$v7$ absent	$v5-v7$ equal	–
Shape of spicule	Strongly arcuate, thorn-like	Ventrally arcuate with 90° curvature	Long, slender, attenuated	Stout, strongly arcuate	Stout, strongly arcuate	Stout, strongly curved
Shape of gubernaculum	Keel-like, proximally notched	Proximally notched with a distal sleeve	Keel-like proximal end with a distal sleeve	globose, caudally projected keel-like	Short, caudally projected keel-like	Dorsally directed hook-like
Gubernaculum length vs spicule length	< 1/2 of spicule length	> 1/2 of spicule length	< 1/2 of spicule length	≤ 1/2 of spicule length	< 1/2 of spicule length	< 1/2 of spicule length

**Table 8** (continued). Comparison of species of *Diplogastrellus* Paramonov, 1952 based on some important morphological characters.

Characters	<i>D. latigubernacula</i>	<i>D. longipharyngis</i> sp. nov.	<i>D. metamasius</i>	<i>D. mikuschi</i>	<i>D. monhysteroides</i>	<i>D. parvus</i>
Stoma length vs diameter	~ 3 times longer	~ 3 times longer	~ 2.5 times longer	–	~ 2.5 times longer	–
Amphid position	At the base of gymnostom	At the base of dorsal tooth	At the level of gymnostom	–	At the base of dorsal tooth	–
Shape of dorsal tooth	Medium-sized, anteriorly directed	Medium-sized, ventrally directed	Flap-like tooth	–	Massive, anteriorly directed	Massive tooth
Subventral armature	Small tooth on both RSV and LSV	Absent	Small ridges on both RSV and LSV	–	Small tooth on both RSV and LSV	–
Shape of median bulb	Ovoid	Ovoid	Oblong	Ovoid	Ovoid	Ovoid
Female reproductive system	Mono-prodelphic with PUS	Mono-prodelphic with PUS	Mono-prodelphic with PUS	Mono-prodelphic	Mono-prodelphic with PUS	Mono-prodelphic with PUS
Size of post-uterine sac	Shorter than VBD	Longer than VBD	Shorter than VBD	–	Longer than VBD	Shorter than VBD
Male genital sensilla	9 pairs: 3 precl., 6 postcl.	9 pairs: 3 precl., 6 postcl.	9 pairs: 3 precl., 6 postcl.	–	9 pairs: 3 precl., 6 postcl.	8 pairs: 3 precl., 5 postcl.
Position of <i>ad</i> sensilla	> one cloacal diam. post. to cloaca	~ one cloacal diam. post. to cloaca	< one cloacal diam. post. to cloaca	–	> one cloacal diam. post. to cloaca	–
Position of <i>pd</i> sensilla	Slightly post. to $\nu 7$	At the level of $\nu 7$	At the level of $\nu 7$	–	Slightly post. to $\nu 7$	–
Relative size of trio $\nu 5$ – $\nu 7$	$\nu 5$ – $\nu 7$ equal	$\nu 7$ separated from $\nu 5$ , $\nu 6$	$\nu 5$ – $\nu 7$ equal	–	$\nu 5$ – $\nu 7$ equal, group separated from each other	–
Shape of spicule	Arcuate, curvature more at proximal end	Straight, slender with pined distal tips	Smoothly ventrally arcuate	Smoothly ventrally arcuate	Smoothly arcuate with curved distal end	Stout, strongly arcuate
Shape of gubernaculum	Keel-like, caudal margin interrupted	Triangular, with a notch at proximal end	Flat, boat-like, proximally notched	Boat-shaped, proximally notched	Shoe-like, distal end with a rectangular sleeve	Dorsally directed hook-like
Gubernaculum length vs spicule length	$\leq 1/2$ of spicule length	$\leq 1/2$ of spicule length	$> 1/2$ of spicule length	$< 1/2$ of spicule length	$\leq 1/2$ of spicule length	$< 1/2$ of spicule length

**Table 8** (continued). Comparison of species of *Diplogastrellus* Paramonov, 1952 based on some important morphological characters.

Characters	<i>D. prodelphis</i>	<i>D. pulcher</i>	<i>D. robustus</i> sp. nov.	<i>D. secundus</i>	<i>D. sikorai</i>	<i>D. stammeri</i>	<i>D. thoubalicus</i>
Stoma length vs diameter	~ 2.5 times longer	~ 3 times longer	~ 2.5 times longer	–	~ 2.5–3 times longer	~ 2.5 times longer	~ 2.5–3 times longer
Amphid position	At the base of gymnostom	At the level of cheilostom	At the level of gymnostom	–	At mid-level of dorsal tooth	At the base of gymnostom	Posterior to the base of stoma
Shape of dorsal tooth	Anteriorly directed tooth	Massive anteriorly directed	Medium-sized, ventrally directed	Small tooth	Dagger-like, anteriorly directed	Medium-sized, anteriorly directed	Massive, ventrally directed
Subventral armature	Small tooth on both RSV and LSV	Small tooth on both RSV and LSV	Absent	–	Small ridges on both RSV and LSV	Small tooth on both RSV and LSV	Small ridges on both RSV and LSV
Shape of median bulb	Ovoid	Ovoid	Ovoid	Oblong	Ovoid	Ovoid	Oblong
Female reproductive system	Mono-prodelphic	Mono-prodelphic with PUS	Mono-prodelphic or Amphidelphic	Mono-prodelphic with PUS	Mono-prodelphic with PUS	Mono-prodelphic with PUS	Mono-prodelphic with PUS
Size of post-uterine sac	Shorter than VBD	Longer than VBD	Longer than VBD	Same as VBD	Same or longer than VBD	Shorter than VBD	Longer than VBD
Male genital sensilla	–	9 pairs: 2 precl., 7 postcl.	9 pairs: 3 precl., 6 postcl.	–	9 pairs: 3 precl., 6 postcl.	8 pairs: 3 precl., 5 postcl.?	9 pairs: 3 precl., 6 postcl.
Position of <i>ad</i> sensilla	–	> one cloacal diam. post. to cloaca	< one cloacal diam. post. to cloaca	–	> one cloacal diam. post. to cloaca	–	> one cloacal diam. post. to cloaca
Position of <i>pd</i> sensilla	–	At the level of $v7$	Slightly post. to $v7$	–	Far posterior to $v7$	–	Slightly post. to $v7$
Relative size of trio $v5-v7$	–	$v7$ bigger in size	$v5-v7$ equal	–	$v5-v7$ equal	–	$v5-v7$ equal
Shape of spicule	Smoothly curved with pointed end	Stout, smoothly curved	Robust, strongly arcuate	Smoothly curved with pointed end	Smoothly curved with pointed end	Smoothly curved, thorn-like	Slender, distally more curved
Shape of gubernaculum	Boat-shaped, proximally notched	Keel-like without sleeve	Slender with a large sleeve	Flat and slender	Dorsally directed hook-like	Boat-shaped, proximally notched	Dorsally extending keel like, with a sleeve
Gubernaculum length vs spicule length	$\leq \frac{1}{2}$ of spicule length	$\leq \frac{1}{2}$ of spicule length	$> \frac{1}{2}$ of spicule length	$\leq \frac{1}{2}$ of spicule length	$< \frac{1}{2}$ of spicule length	$\sim \frac{1}{2}$ of spicule length	$> \frac{1}{2}$ of spicule length

## Discussion

The genus *Diplogastrellus* was proposed by Paramonov (1952) with *D. gracilis* as its type species. The genus contains many old and relatively poorly described species that obscure phylogenetic and systematic relationships (Andrássy 1984; Kiontke & Sudhaus 1996). *Diplogastrellus* was established for species of diplogastrids that were slender, cuticle with fine transverse striations, stoma longer than wide, dorsal stegostomal wall with anteriorly-directed or ventrally directed tooth, ovoid or oblong median bulb and with prodelphic or amphidelphic female reproductive system. All species of *Diplogastrellus* show a high level of intrageneric divergence in the structure of the dorsal tooth, shape of median bulb, position of vulva, length of post-uterine sac, morphology of spicules and gubernaculum, and in the arrangement of genital sensilla. Out of all nineteen nominal species, the majority viz., *D. cerea*, *D. indicus*, *D. latigubernacula*, *D. longipharyngis* sp. nov., *D. mikuschi*, *D. monhysteroides*, *D. parvus*, *D. prodelphic*, *D. pulcher*, *D. robustus* sp. nov., *D. sikorai* and *D. stammeri* have an ovoid median bulb, while *D. didelphis* sp. nov., *D. gracilis*, *D. graciloides*, *D. heynsi*, *D. metamasius*, *D. secundus*, and *D. thoubalicus* have an oblong median bulb. The second character, i.e., structure of dorsal tooth, also shows a variation within the species. Species having an oblong median bulb bear a ventrally-directed dorsal tooth; however, those having an ovoid median bulb bear an anteriorly-directed tooth except *D. longipharyngis* and *D. robustus*.

The third character that is shared by all species of *Diplogastrellus* is the presence of a post-uterine sac (PUS), except in *D. gracilis* and *D. didelphis* sp. nov., where the conditions demonstrating the loss of the feature are not the same. In *D. gracilis* the proximity of vulva and anus remove the possibility of PUS whereas in *D. didelphis* there exists a small posterior genital branch in place of PUS. Based on the size of the post-uterine sac, *D. cerea*, *D. indicus*, *D. latigubernacula*, *D. metamasius*, *D. parvus*, *D. prodelphis*, and *D. stammeri* with a sac shorter than the vulval body diameter, fall into one group, while *D. graciloides*, *D. heynsi*, *D. longipharyngis* sp. nov., *D. monhysteroides*, *D. pulcher*, *D. robustus* sp. nov., and *D. thoubalicus* with a sac longer than the vulval body diameter fall into another group. The rest of the species have a post-uterine sac the same size as the body diameter or slightly longer. The position of the vulva in all species is near to mid body, except in *D. cerea*, *D. gracilis*, *D. mikuschi*, *D. parvus* and *D. robustus* where it is situated posteriorly.

In the present study, *D. didelphis* sp. nov. is a unique species having an amphidelphic condition of gonad which is the first report within the genus *Diplogastrellus*. Typologically, we have chosen to affiliate this species with *Diplogastrellus* based on its stomatal morphology, similar length of cheilostom and gymnostom, medium-sized ventrally directed dorsal tooth, cuticle with fine transverse and longitudinal striations, and oblong median bulb. *Diplogastrellus didelphis* shares possible homologies with *Acrostichus*, the assumed sister taxon of *Diplogastrellus*, in the presence of a uterine pouch and the amphidelphic condition of the gonad. However, differences were observed in the cuticular longitudinal ridges which are prominent in *Acrostichus* but faint in *D. didelphis*, and the size of the gubernaculum apparently robust, covering more than half of the length of the spicules in *Acrostichus*.

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**Appendix 1.** Characters and character states for comparison of species of *Diplogastrellus* Paramonov 1952.

S. No.	Character	Character state
1	Female body length	more than 0.7 mm (0), up to 0.7 mm (1)
2	Longitudinal striations	inconspicuous (0), conspicuous (1)
3	Labial sensilla	papilliform (0), raised/setose (1)
4	Position of amphid	within the range of stoma (0), posterior to the base of stoma (1)
5	Size of amphidial aperture	prominent, circular to elliptical (0), small pore-like (1)
6	Shape of stoma	narrow tubular (0), wide tubular (1)
7	Stoma length: width	more than 2.5 times (0), 2.5 times (1)
8	Size of dorsal tooth	massive (0), medium-sized (1), flap-like (2)
9	Shape of dorsal tooth	anteriorly directed (0), ventrally directed (1)
10	Subventral armature	small tooth (0), small ridge (1), absent (2)
11	Shape of median bulb	ovoid (0), oblong (1)
12	Shape of basal bulb	pyriform (0), ovoid to elongate (1)
13	Length of posterior pharynx	shorter than anterior pharynx (0), longer than anterior pharynx (1)
14	Female reproductive system	mono-prodelphic (0), amphidelphic (1)
15	Uterine pouch	absent (0), present (1)
16	Size of post-uterine sac	shorter than VBD (0), same or longer than VBD (1), absent (2)
17	Position of vulva	more than 50% of the body (0), less than 50% of the body (1),
18	Shape of spicule	slender smoothly arcuate (0), thorn-like/stout strongly curved (1), almost straight (2)
19	Size of spicule	less than 30 $\mu$ m (0), more than 30 $\mu$ m (1)
20	Head of spicule	rounded (0), hood-shaped (1)
21	Distal end of spicule	smoothly pointed (0), pointed and curved (1), blunt/ rounded (2),
22	Shape of gubernaculum	boat-shaped (0), dorsally directed hook-like (1), caudally projected keel-like (2)
23	Proximal end of gubernaculum	proximally notched (0), without notch (1), keel-like (2)
24	Distal sleeve of gubernaculum	sleeve absent (0), sleeve present (1)
25	Gubernaculum vs spicule length	< 1/2 of spicule length (0), > 1/2 of spicule length (1), ~ 1/2 of spicule length (2)
26	Number of genital sensilla	9 pairs (0), 8 pairs (1)
27	Position of <i>vl</i> genital sensilla	beyond the spicular range (0), within the spicular range (1)
28	Position of <i>ad</i> genital sensilla	> one cloacal diam. post. to cloaca (0), < one cloacal diam. post. to cloaca (1), ~ one cloacal diam. post. to cloaca (2)
29	Post-cloacal grouped sensilla ( <i>v5-7</i> )	<i>v5-7</i> grouped (0), <i>v5-7</i> not grouped, <i>v7</i> far posterior (1)
30	Position of <i>pd</i> genital sensilla	at the level of <i>v7</i> (0), slightly posterior to <i>v7</i> (1), far posterior to <i>v7</i> (2)

Appendix 2. Data matrix for cluster analysis of the species in the genus *Diplogastrellus* Paramonov, 1952.

Species	Character																														
	01	02	03	04	05	06	07	08	09	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	
<i>A. nudicapitatus</i>	1	1	0	0	0	1	1	0	0	0	0	0	1	1	2	1	1	1	1	0	2	0	0	0	0	1	0	1	0	0	?
<i>D. cerea</i>	0	1	1	0	1	0	0	1	0	1	0	0	0	0	0	0	0	1	0	0	0	0	2	0	0	0	0	0	2	0	0
<i>D. didelphis</i> sp. nov.	0	0	0	0	0	0	0	1	1	2	1	0	0	1	2	1	1	1	1	0	0	1	0	1	0	1	0	0	1	0	2
<i>D. gracilis</i>	0	1	0	0	1	1	0	0	1	1	1	0	0	0	2	0	0	0	1	0	0	0	2	1	0	0	1	1	0	2	
<i>D. graciloides</i>	0	1	0	1	0	1	0	0	1	0	1	0	0	0	1	0	1	0	1	0	1	2	1	0	0	1	0	0	0	2	
<i>D. heynsi</i>	0	0	0	1	1	1	0	0	1	1	1	0	0	0	1	0	1	0	1	0	1	0	2	0	0	0	0	0	0	0	0
<i>D. indicus</i>	1	0	1	0	1	1	1	1	0	0	0	1	0	0	0	0	0	1	0	1	1	1	0	1	0	1	0	?	0	?	?
<i>D. latigubernacula</i>	1	0	1	0	0	1	0	1	0	0	0	1	0	0	0	0	0	0	0	0	2	2	0	0	0	0	0	1	0	0	1
<i>D. longipharyngis</i> sp. nov.	0	0	0	0	0	0	0	1	1	2	0	1	1	0	0	1	0	2	0	0	0	0	0	0	0	0	0	0	2	1	0
<i>D. metamasius</i>	0	0	0	0	0	0	1	2	0	1	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1	0	0	1	0	0
<i>D. mikuschi</i>	1	?	?	?	?	?	?	?	?	?	0	?	?	0	0	?	0	0	?	0	2	0	0	0	0	0	?	?	?	?	?
<i>D. monhysteroides</i>	0	0	1	0	0	1	1	0	0	0	0	1	0	0	1	0	0	1	0	1	0	1	1	2	1	0	0	0	0	0	1
<i>D. parvus</i>	0	?	?	?	?	?	?	?	?	?	0	?	?	0	0	0	0	1	0	1	0	1	0	0	0	0	1	?	?	?	?
<i>D. prodelpis</i>	1	0	0	0	0	1	0	0	0	0	0	1	0	0	0	0	0	1	?	0	0	0	0	1	0	0	?	1	?	?	?
<i>D. pulcher</i>	0	0	1	0	0	0	0	0	0	0	0	1	0	0	0	1	1	1	0	0	1	2	1	0	0	0	0	0	0	0	0
<i>D. robustus</i> sp. nov.	0	0	0	0	1	0	1	1	1	2	0	1	0	0	1	0	1	1	1	1	2	0	1	1	1	1	0	0	1	0	1
<i>D. secundus</i>	?	?	?	?	?	?	?	?	?	?	1	?	?	0	0	0	?	0	?	0	0	0	1	0	0	?	?	?	?	?	?
<i>D. sikorai</i>	1	1	1	0	0	0	0	0	0	1	0	0	0	0	0	1	1	0	0	0	0	1	0	1	0	0	1	0	0	0	2
<i>D. stammeri</i>	0	0	1	0	1	1	1	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1	2	1	1	?	?	?
<i>D. thoubalicus</i>	0	0	0	1	1	1	0	0	1	1	1	0	0	0	0	1	0	0	0	1	0	2	1	1	1	1	0	0	0	0	1