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Bilobodera flexa gen. n., sp. n. (Nematoda: Heteroderidae) from Andhra Pradesh, India

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Abstract. *Bilobodera flexa* gen.n., sp.n. from soil and roots of *Allmania nodiflora* (L.) in Andhra Pradesh, India, is described and illustrated. *Bilobdera* gen.n. belongs to the Verutinae of Heteroderidae and is characterised by bilobed females with a sharp dorsal depression opposite the median vulva, a subterminal anus, a spheroidal postanal region, and eggs that are generally retained in body. There is no immature slender female stage. The male body is small, under 0.6 mm long, C-shaped when fixed; but unlike other Heteroderidae, never twisted in the posterior region; bursa absent. Second-stage juveniles are small, under 0.4 mm long and have lateral fields with four incisures and punctiform phasmids located slightly behind the anus but anterior to the middle of the tail.

Keywords: *Allmania nodiflora*, *Bilobodera flexa* gen.n., sp.n., India, systematics, Verutinae.

INTRODUCTION

During surveys for plant-parasitic nematodes associated with different crops on Alfisols (red soil) at the research farm of the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, Andhra Pradesh, India, some horse-shoe shaped, swollen female nematodes, full of eggs, were observed in the soil residue collected on 150 and 180 um-pore sieves. Detailed examination of the females, eggs, and second-stage juveniles revealed that this nematode population is distinct from those of the existing genera of Heteroderidae and represent a new genus and species, for which the name *Bilobodera flexa* gen.n., sp.n. is proposed.

MATERIALS AND METHODS

Second-stage juveniles and females containing many eggs inside their bodies were collected from the soil around the roots of *Allmania nodiflora* in the Manmool area of the ICRISAT farm, India. Juveniles, females and eggs were studied under a high resolution light microscope in water mounts, and also as glycerine-mounted specimens. The perineal region of the female was gently cut from the body, cleared in clove oil and mounted in DPX(R) so the end-on view could be seen. For scanning electron microscopy, using JSM 35 CF scanning electron microscope at 10 and 15 KV, specimens were fixed in 3% glutaraldehyde, 0.2 M sodium phosphate buffer pH 7.5 and postfixed in 2% osmium tetraoxide for four hours. The fixed specimens were washed with double distilled water and dehydrated in a graded series of acetone before processing through critical point drying. They were then mounted on stubs and coated with 20 nm thick gold layer.

SYSTEMATICS

Bilobodera gen. n.

Diagnosis: Verutinae, Heteroderidae. Mature female: Body bilobed with a sharp dorsal depression opposite the median vulva. Cuticle striated, transparent. Two ovaries coiled within the two lobes of the body. Anus subterminal; post-anal region rounded. Eggs generally retained in the body. Cyst stage absent. Immature slender female stage absent. Male: Body small, under 0.6 mm long, C-shaped when fixed, but unlike other Heteroderidae, never twisted in the posterior region. Stylet small, under 20 µm. Spicules with a truncate, or indented tip. Gubernaculum linear, lacking titillae, fixed. Tail almost absent, a hemispherical post-anal region present. Bursa absent. Second-stage juvenile: Small, under 0.4 mm long, straight to arcuate when relaxed. Cuticle annulated. Lateral fields with four incisures. Stylet small, 16 µm or less long. Oesophageal glands extending ventrally over intestine. Tail elongate-conoid with an attenuated and conspicuous hyaline terminal region. Phasmids punctiform, a little below anus but anterior to middle of tail.

Type species: *Bilobodera mesoangustus* (Minagawa, 1986) comb. n. Syn. *Verutus mesoangustus* Minagawa, 1986.

Other species: *Bilobodera flexa* gen. n., sp. n.

Relationship: *Bilobodera* gen.n. differs from *Venutus* Esser, 1981 in having a smaller body and stylet, the mature female body being bilobed, cephalic framework lacking conspicuous sclerotization, face views of male and second-stage juveniles with an unbroken labial plate formed by the fusion of all six pseudolips and bearing large amphidial openings and indistinct oral disc (Minagawa, 1986). Labial plates are marked by indentations separating lateral pseudolips and bearing distinct oral disc in *Venutus* (Othman & Baldwin, 1985).

Bilobodera has some affinity with *Meloidodera* Chitwood, Hannon & Esser, 1956 of Meloidoderinae from which it differs in having a bilobed female body (spheroidal in the latter), smaller body and stylet, and a continuous cephalic region lacking conspicuous sclerotization. *Rotylenchulus* Linford & Oliveira, 1940 and *Senegalonema* Germani, Luc & Baldwin, 1984 of the Rotylenchulinae have kidney-shaped mature females, a slender immature female stage, curved juveniles, different face views, and males with a conoid tail carrying a bursa.

Bilobodera flexa gen. n., sp. n.

(Figs 1, 2, 3)

Mature females

30 paratypes: L = 0.32 - 0.69 (0.46 ± 0.05) mm; width at vulva = 60 - 125 (97 ± 24) µm; L/W ratio = 3 - 5 (3.5 ± 0.13).

Holotype (mature female): L = 0.62 mm; width of anterior lobe = 200 µm; width of posterior lobe = 150 µm; width at vulva = 75 µm; vulva length = 40 µm; eggs in body = 67 - 81 (72.4 ± 3.75) µm X 33 - 40 (35.2 ± 2.48) µm.

Body swollen, bilobed (hence the generic name) with a characteristic dorsal depression opposite vulva (Figs 1J-M, 2B, 3A,B) making it dorsally flexed (hence the specific epithet). Cuticle transparent and thick, with distinct transverse striae. Many eggs retained in both lobes of the body, easily seen through transparent cuticle. Dead females retain eggs inside; cyst stage absent. Small cluster of eggs seen attached to the vulval region in some females (Fig. 3B). Body may break in the middle in gravid females. Stylet 13 um long. Vulva almost median, transverse, 20 - 45 µm long slit, with slightly raised lips. Underbridge seen in some females (Fig. 3C).

Males

Not found; females were not inseminated.

Second-stage juveniles

30 paratypes: L = 0.29 - 0.38 (0.32 ± 0.022) mm; a = 19 - 26 (23 ± 1.7); b = 3.9 - 5.2 (4.6 ± 0.41); b' = 2.4 - 3.0 (2.9 ± 0.19); c = 7.6 - 9.3 (8.3 ± 0.46); c' = 4.0 - 4.8 (4.5 ± 0.2); stylet = 11 - 13 (12 ± 0.6) µm; m = 37 - 45 (41 ± 2.4); O = 50 - 62 (54 ± 5.2).

Body straight or generally arcuate ventrally when relaxed (Fig. 1A). Cuticle distinctly annulated; annules averaging 1.6 µm wide at neck and 1.4 - 1.6 µm wide at midbody region. Lateral field beginning as a narrow streak at middle of stylet shaft, gradually expanding posteriorly to hemizonid level where it expands rather suddenly to become 22-25 percent of body width over most of body, narrowing on tail and disappearing behind its middle; with four equidistant incisures, outer ones smooth to slightly crenate; not areolated.

Cephalic region continuous, hemispherical, rather low, with 3 to 4 annules and indistinct oral disc; sclerotization weak, its outer margins extending posteriorly 1-2 body annules. Cephalids distinct in some specimens; anterior ones just behind outer margins of cephalic framework; posterior ones 3-4 annules behind the anterior ones. Stylet of medium strength; conus shorter than shaft, 4.5 - 5.5 (5 ± 0.3) µm long; shaft cylindrical, stout; knobs closely packed together, rounded with anterior surfaces convex to posteriorly sloping, 1.6 - 2.0 µm across. Orifice of dorsal oesophageal gland 6.0 - 7.6 (7 ± 0.5) µm behind stylet base Fig. 1B-D).

Median oesophageal bulb oval, 10 - 11 X 6.5 - 7.5 µm, with a distinct, oval, valvular apparatus 2.5 X 2.0 µm, located at 40 - 46 (43 ± 0.21) µm from anterior end. Excretory pore opposite or anterior to oesophago-intestinal junction, 65 - 73 (69 ± 1.6) µm from anterior end. Hemizonid distinct, 1-2 annules long, just anterior to excretory pore. Oesophageal glands lobe-like, mostly ventral but also ventro-lateral to intestine; nucleus of dorsal gland anterior to those of subventrals (Fig. 1E).

Genital primordium 2-celled, oval, 8 - 9 X 5 - 6 µm (Fig. 1A), at 45 - 53 (49) percent of body length from anterior end, usually on right, but occasionally on left side of intestine. Rectum about one anal body width long. Tail elongate-conoid, tapering to a narrow, attenuated terminal region with a finely rounded to sharply pointed tip; 35 - 46 (39 ± 4) µm long; hyaline terminal portion 13 - 24 (20) µm or 40 - 59 percent of tail length. Phasmids distinct, punctiform, at 12-15 µm or 34 - 40 percent of tail length from anus; 21 - 26 µm from tail tip.

Eggs

30 intrauterine eggs: Oval, usually with slight concavity to one side; shell smooth, about 2 µm thick; length almost double the width, 68-83 (75 ± 4.57) X 32 - 40 (35.5 ± 2.03) µm. Juveniles within eggs with 5 (Fig. 1I), rarely 4, folds.

Type habitat and locality: Soil and roots of *Allmania nodiflora* (L.) (Amaranthaceae) growing in Manmool area, ICRISAT farm, located at 18°N 78°E near Patancheru village, Andhra Pradesh, India.

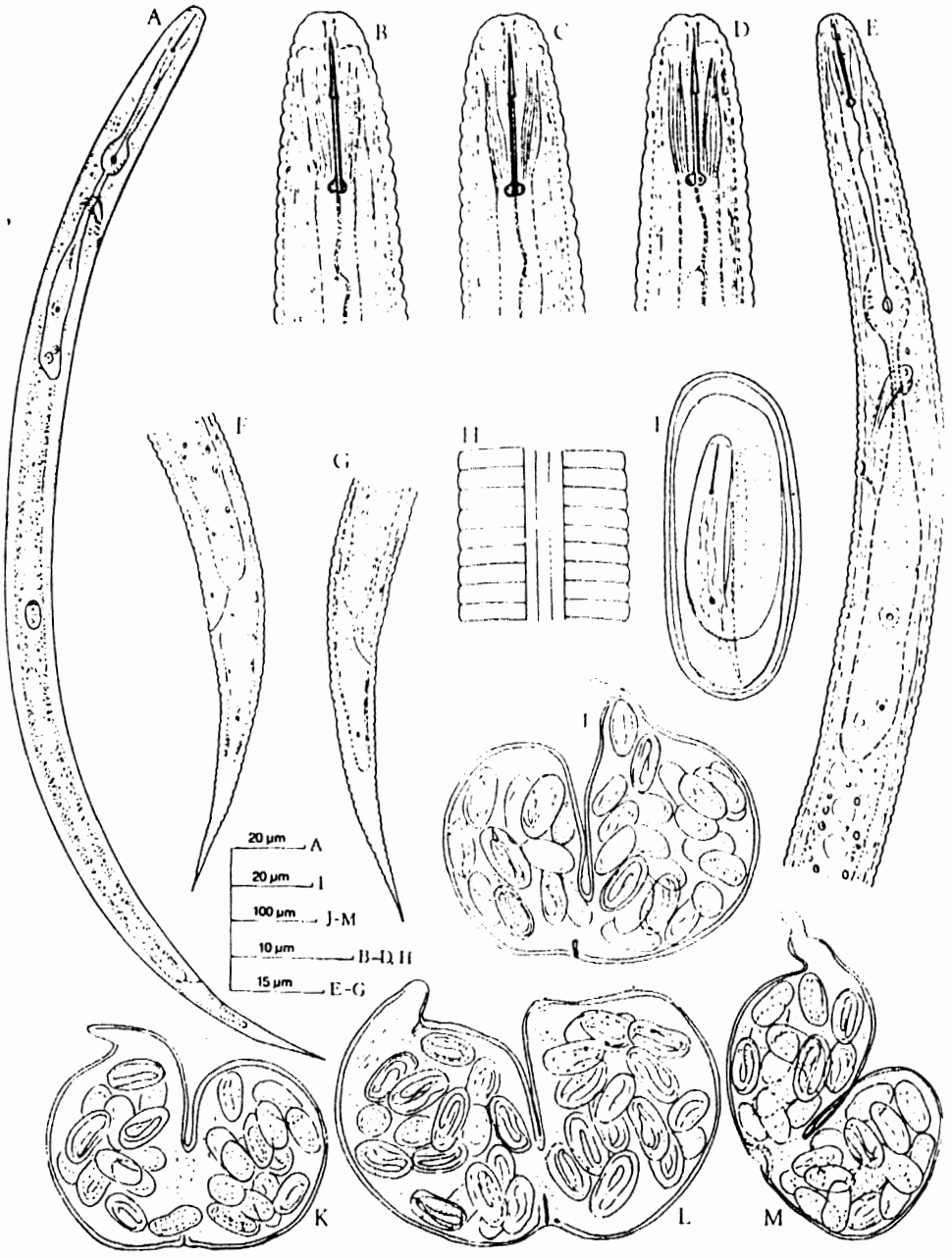

Fig. 1. *Bilobodera flexa* gen. n., sp. n. A–I. Juveniles (J2). A. Entire body. B–D. Head ends. E. Oesophageal region. F, G. Tail ends. H. Lateral field at midbody. I. Juvenile in 5-folds within egg. J–M. Mature females filled with eggs.

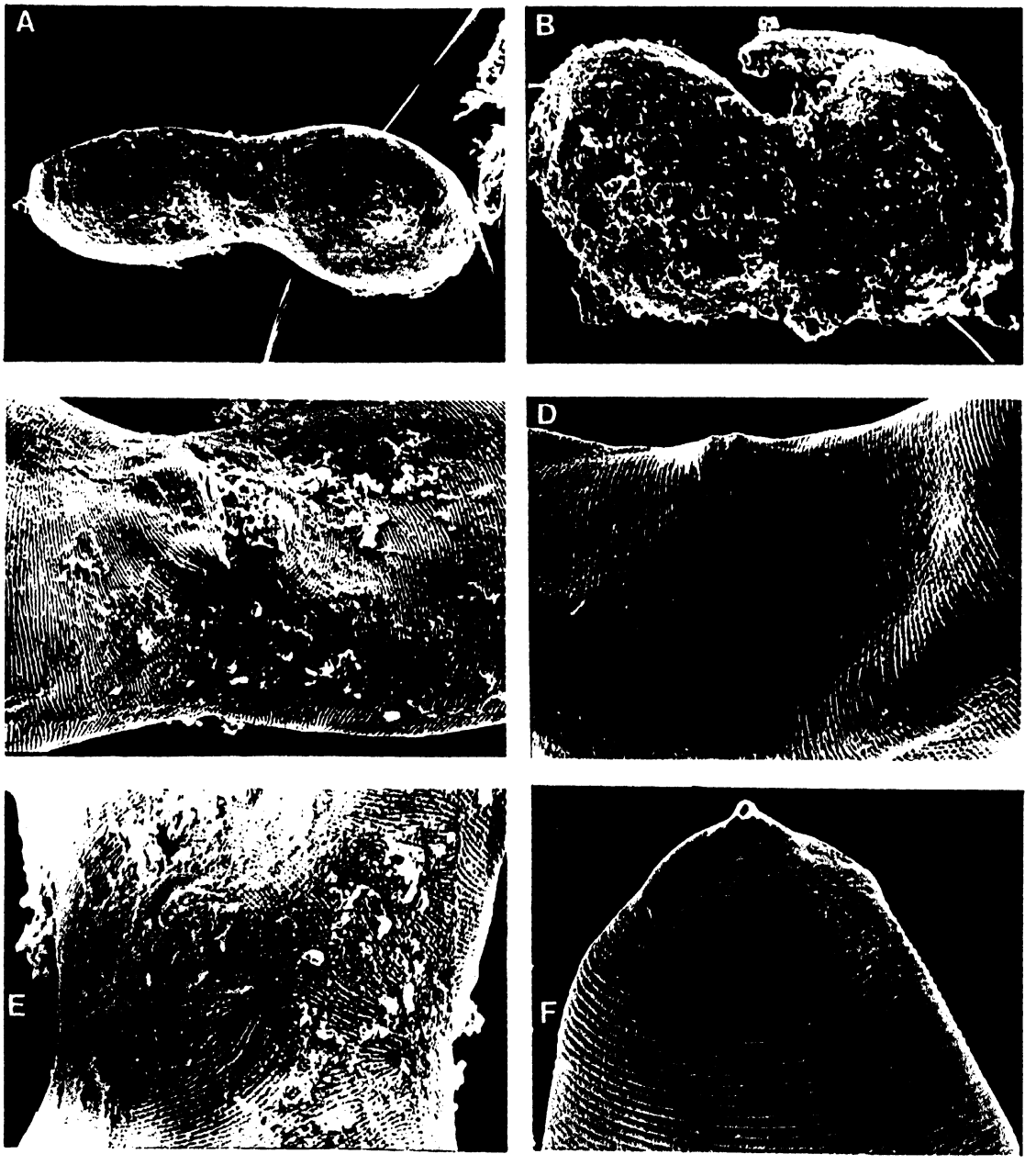

Fig. 2. *Bilobodera flexa* gen. n., sp. n. SEM photomicrographs of females. A, B. Entire body. C, D, F. Vulva regions. E. Tail region.

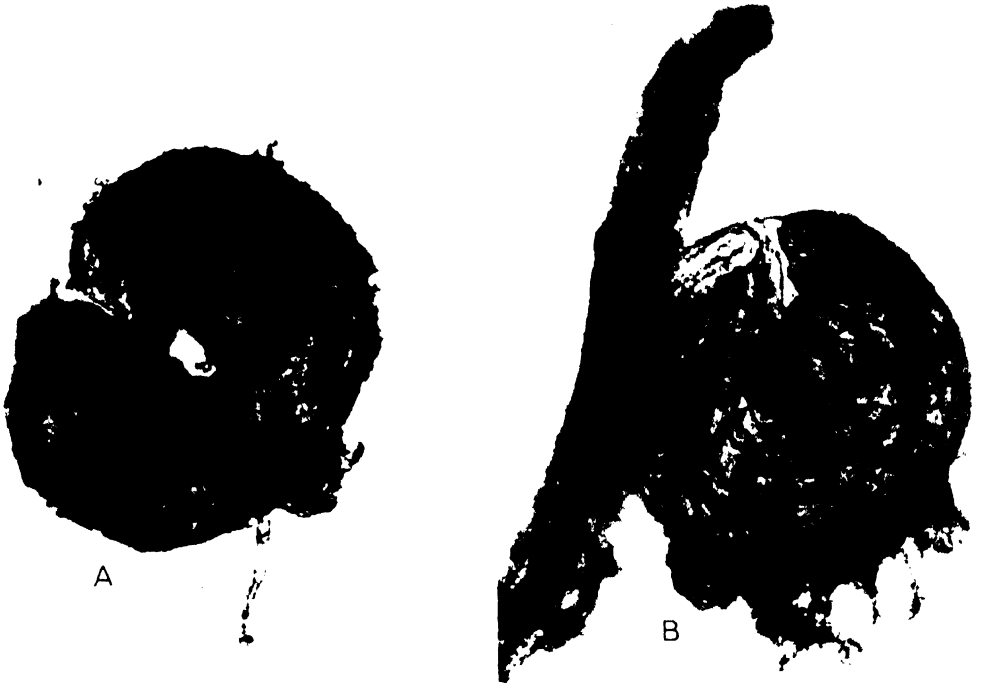

Fig. 3. *Bilobodera flexa* gen. n., sp. n. Light photomicrophraphs. A. Female with eggs inside body. B. Female with cluster of eggs outside vulva region.

Type specimens: Holotype female, 25 paratype females and 30 paratype juveniles on permanent slides at International Institute of Parasitology, St. Albans, England. Ten females in 4% formalin in glass vials at each of these centres: Nematology Unit, ICRISAT, Patancheru, Andhra Pradesh 502 324, India; Division of Nematology, Indian Agricultural Research Institute, New Delhi 110 012, India.

Relationship: *Bilobodera flexa* gen. n., sp. n. differs from *B. mesoangustus* Minagawa, 1986 in being unisexual and having a shorter stylet (female stylet 14.5–19.0 (17.7) µm, juvenile stylet 13.7–16.7 (14.7) µm long in the latter) and the orifice of its dorsal oesophageal gland located at half or more of the stylet length behind the stylet base.

Remark: *Bilobodera flexa* did not reproduce on pigeonpea (*Cajanus cajan*), castor (*Ricinus communis*), pearl millet (*Pennisetum glaucum*) and sorghum (*Sorghum bicolor*). However, females full of eggs were found in the root zones of *Celosia argentea* (Amaranthaceae) and *Cynodon dactylon* (Poaceae).

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