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Life cycle of *Verutus mesoangustus* Minagawa (Nematoda : Heteroderidae) on *Vetiveria zizanioides* (L.) Nash. (Gramineae)

Harish K. BAJAJ and Mange R. DALAL

Department of Nematology, CCS Haryana Agricultural University, Hisar-125 004, India.

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Summary – Second stage juveniles are the infective stage of *Verutus mesoangustus*. They hatch out of the eggs 10 days after initiating embryonic development. Sex can be determined during the second moult by genital primordium. Third stage juveniles retain the cuticle of second stage juveniles in the posterior region of body. Males develop without metamorphosis. Fourth stage male juveniles come out of the cuticle of the third stage juveniles and enter the soil where they moult to become adults. Female development occurs *in situ* on the roots. Fourth stage and adult females are posteriorly enclosed within the exuvia of preceding stages. Eggs are initially laid singly in the soil but are retained and embryonate inside the bodies of older females. The life cycle is completed in 34 days at mean soil temperature of 35.6 ± 1.8 (32.1-39.0) °C on *Vetiveria zizanioides* roots.

Résumé – ***Cycle de* Verutus mesoangustus *Minagawa (Nematoda : Heteroderidae) sur* Vetiveria zizanioides *(L.) Nash. (Gramineae)*** – Les juvéniles de deuxième stade représentent le stade infestant de *Verutus mesoangustus*. Ils sortent des œufs 10 jours après le début du développement embryonnaire. Le sexe peut être déterminé au moment de la 2e mue grâce au primordium génital. Les juvéniles de 3e stade conservent la cuticule du 2e stade sur la partie postérieure de leur corps. Les mâles se développent sans métamorphose. Les juvéniles mâles de 4e stade sortent de la cuticule du 3e stade, pénètrent dans le sol où ils muent pour devenir adultes. Le développement des femelles s'accomplit *in situ*, dans les racines. La partie postérieure du corps des juvéniles de 4e stade et des femelles demeure enfermée dans l'exuvie des stades prédécents. Les œufs sont au début pondus isolément dans le sol, mais sont ensuite retenus dans le corps des femelles agées où ils se transforment en embryons. Sur *Vetiveria zizanioides*, le cycle s'accomplit en 34 jours, à une température moyenne de 35,6 ± 1,8 (32,1-39,0)°C.

Key-words : Biology, development, Heteroderidae, *Verutus mesoangustus*, vetiver grass, nematode.

Verutus mesoangustus Minagawa, 1986 was described from around the roots of *Miscanthus sinensis* L. from Japan. Bajaj and Walia (1996) reported it parasitizing the roots of vetiver grass, *Vetiveria zizanioides* (L.) Nash., in the Haryana State of India. Nothing is known about the life cycle and host parasite relationships of this species although there are some reports on such aspects for *V. volvingentis* Esser, 1981.

Materials and methods

Soil infested with *V. mesoangustus* was planted with slips of vetiver grass in 30 cm earthen pots in a greenhouse. Second stage juveniles were collected from soil on a 300-mesh sieve using Cobb's sieving and decanting technique. Roots were examined after staining in 0.1 % acid fuchsin lactophenol for 24 h in an oven at 55 °C.

For the study of embryonic development, eggs were either obtained from the vicinity of females or they were dissected from young gravid females. The hanging drop technique (Seshadri, 1965) was used to observe development. For studying postembryogenesis, 3 week-old vetiver grass was transplanted singly in 15 cm earthen pots containing steam sterilized soil. Each pot was inoculated with *ca* 500 juveniles obtained from stock cultures. The plants from three pots were uprooted daily and developing nematodes were examined from roots and soil. The soil for this purpose was processed through a 100-mesh sieve placed over a 300-mesh sieve. Contents of the 100-mesh sieve were examined directly under a stereozoom binocular microscope to record the sedentary stages. Residue on the 300-mesh sieve was further processed by modified Baermann's funnel technique and the motile stages were collected after 24 h. Contents of tissue paper, after this period, were then processed by sugar centrifugal flotation technique (Jenkins, 1964) for collecting immobile juvenile stages/females. Detailed observations on the developmental stages were made after killing and fixing the nematodes in hot 4 % formalin and processing them to anhydrous glycerine by slow method.

In another set of experiment, juveniles collected from the culture pots were kept in sterilized distilled water in cavity blocks and examined daily to record the development, if any.

Air temperature during these studies varied from 18.6-28.1 to 37.4-45,1 (mean = 23.2-41.4) °C and soil temperature measured 26.9-32.5 (morning) and 35.9-45.4 (evening) with an average of 35.6 ± 1.8 (32.1-39.0) °C.

Results

Embryogenesis

The pattern of embryonic development was similar to other heteroderids (Raski, 1950; Siddiqui & Taylor, 1970; Bajaj & Bhatti, 1983). An egg took about 1, 3, and 4 days to reach blastula, gastrula, and tadepole stage, respectively. The first stage juveniles (J1) was formed on the 8th day after the first cell cleavage. The first moult was observed on the 9th day inside the egg and the second stage juvenile out of first stage exuvium was recorded on the 10th day of development.

Post-embryogenesis (Figs 1-3)

Penetration

Second stage juveniles penetrated all along the roots within 24 h of inoculation and preferred the young, thin roots. Only the neck region of J2 penetrated the cortical region of the roots as reported by Cohn *et al.* (1984) for *V. volvingentis*. There was a slight increase in the body diameter and in the size (14-24 × 10 µm) and number of cells (14-18) of the genital primordium after the juveniles penetrated the roots.

The second moult occurred *in situ* and was first noticed on the 10th day after inoculation (DAI). During this moult there was further increase in the size of the genital primordium and the sexes became distinguishable. In case of juveniles destined to become males, the germinal cells lied just above the cap cells in the posterior region of the genital primordium. This primordium first increased in length posteriorly due to the proliferation of epithelial cells, then became inverted and " U " shaped at the 48 (approx.) cell stage, and finally straightened up prior to the completion of moult. Sexes could also be distinguished by the presence of spicular primordial cells in the males. In case of juveniles destined to become females, the genital primordium increased in length both anteriorly and posteriorly and measured 32-40 µm when consisting of 24 cells.

Third stage juvenile (male)

Measurements (n = 10) : L = 271.6 ± 26.1 (251-309) µm; a = 14.6 ± 1.5 (13-16); b′ = 3.79 ± 0.30 (3.5-4.2); c = 42.45 ± 11.0 (31-53); stylet = 14 µm; median bulb at 33.3 ± 2.3 (32-37) µm from anterior end.

Body slightly swollen in the middle. Stylet with well developed knobs. Oesophageal bulb long, thin and overlapping the intestine ventrally. Genital primordium 61.3 ± 13.6 (48-112) µm long, consisting of a cap cell, two germinal cells and with the rest of oocytes arranged in two rows anteriorly and in a single row posteriorly. Spicular primordium prominent. Tail short with rounded tip.

Male as well as female J3 retain the cuticles of J2 at the posterior end; therefore, they appear to have spikelike tails. They may be enclosed within subcrystalline layers as well.

Fourth stage juvenile (male)

Measurements (n = 7) : L = 384.6 ± 10.3 (371-410) µm; a = 18-19; b′ = 3.71 ± 0.19 (3.5-4.1); c = 3.57 ± 0.11 (3.4-3.7); stylet = 14 µm; median bulb at 45.2 ± 1.7 (43-48) µm from anterior end; tail = 11-13 µm.

Body vermiform. Stylet and oesophagus well developed. Oesophageal lobe 30-35 µm long, overlapping the intestine ventrally. Genital primordium 131-168 µm long, with or without sperm and emptying into rectum posteriorly. Spicular primordia well developed.

The fourth stage male juveniles undergo a direct development (without metamorphosis); they left the roots and were collected from the soil on the 14th DAI. The fourth moult of male juveniles was noticed on 18 DAI in the soil. During this moult, testis increased in length and became reflexed in a few cases. Spicules and gubernaculum were also formed. Newly moulted adult males were collected from soil 19 DAI onwards. During the third moult the genital primordium of female juveniles did not undergo major changes but the didelphic nature of the genital tract became apparent.

Fourth stage juvenile (female)

Measurements (n = 20) : L = 349.8 ± 24.9 (304-378) µm; W = 40-53 µm; a = 7.24 ± 0.74 (6.3-8.6); b′ = 3.74 ± 0.21 (3.4-4.1); stylet = 14 µm; DGO = 6-7 µm; median bulb at 47.6 ± 2.6 (43-51) µm from anterior end; G = 59.4 ± 1.2 (57-61); tail = 8 µm.

The fourth stage female juveniles remained loosely attached to the roots and were enclosed in sticky materials. Body swollen, elongated oval, covered posteriorly with the cuticles of J2 and J3, thus appearing to have a spiked tail. Stylet and oesophagus well developed. Basal oesophageal bulb short, elongated-oval lobe, 16-32 × 11-16 µm in size. Genital primordium distinctly made up of two branches with many oocytes in each branch. Length of each sexual branch variable (116.0 ± 39.0 (45-160) µm depending upon the age of juvenile. It is undifferentiated and made up of few cells in the newly moulted juveniles but is well developed and differentiated into various parts and coiled in the older ones. Vaginal primordium distinct.

During the final moult, which was first noticed at 20 DAI, the differentiation of the genital tract was completed and the vaginal primordium gave rise to vagina. Young females (A♀1) were recorded at 21 DAI. Initially, their bodies were enveloped in the posterior region with the exuvia of J2, J3, and J4 (Fig. 1D). A subcrystalline layer also surrounded the body. Later in the development, vaginal lips became more prominent and the cuticles of the preceding stages were cast off. Such fe-

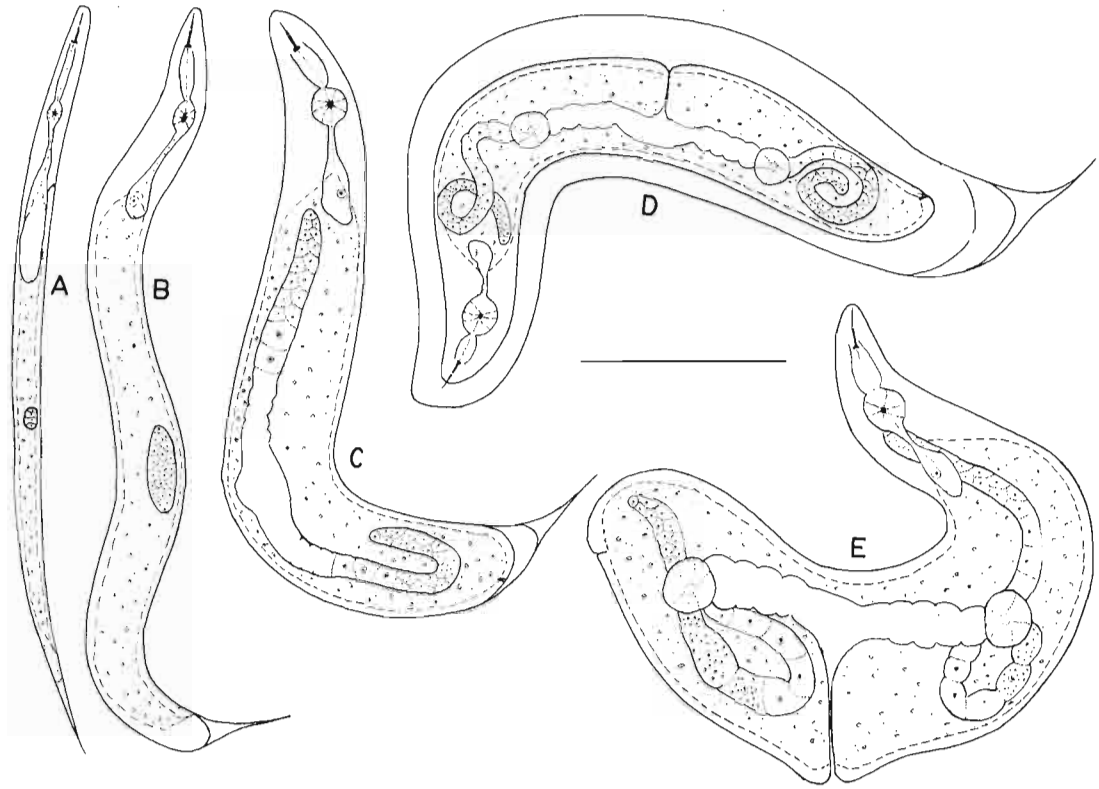

Fig. 1. *Female development in* Verutus mesoangustus *Minagawa. A : Second stage juvenile; B : Third stage juvenile; C : Fourth stage juvenile; D : Adult female posteriorly enclosed within exuvia of preceeding stage; E : Young female out of exuvia (Scale bar = 86 µm).*

males (A♀2) were also loosely attached to the roots from where they easily got detached while processing the soil for nematode recovery. Colonization of roots as reported for *V. volvingentis* by Cohn *et al.* (1984) was, however, not observed.

Young females laid the eggs singly in the soil; the eggs were glued with sticky substance presumably secreted by the female and deposited near its body. One to twelve eggs were seen in the lumps near female bodies. As their age increased, it became increasingly difficult for the females to oviposit in the soil (may be due to atrophy of the dorso-lateral dilator vulval muscles). Embryonic development in such cases occurred inside the female bodies themselves (A♀3). The number of embryonated eggs gradually increased inside the old female bodies. Subsequently, all the body contents except for the eggs disintegrated. Such females (A♀4) resembled bags (made up of female body walls) filled with embryonated eggs (n = 7-23). Similar sacs containing embryonated eggs were also found associated with the roots during the senescence period of the grass and formed the inocula for the new roots of the next growth season.

Second stage juveniles kept in water failed to develop further.

Discussion

Second stage juveniles, adult males, and females of Indian population of *V. mesoangustus* resemble those described by Minagawa (1986) from Japan. Other developmental stages of this species have been described for the first time in this paper. Detailed descriptions of these stages are not available either for other species of this genus, *viz, V. volvingentis* and *V. californicus* Baldwin *et al.,* 1989. Development of *V. mesoangustus* is apparently similar to *V. volvingentis* as the retention of cuticles by juvenile stages and adult females observed in *V. mesoangustus* has also been reported for *V. volvingentis* (Cohn *et al.,* 1984). The J4 male juveniles of the latter species also are migratory since Carta and Baldwin (1990) could collect them using a hot mist. However, the development of the reproductive system of *V. mesoangustus* differs from that of *V. volvingentis* as described by Esser (1983). The proliferation of cells of genital primordium starts during the J2 stage soon after the nematode penetrates the root in case of former species, but is delayed till the initiation of moulting of *V. volvingentis*. The male genital primordium reaches the rectum before or just after the second moult in the latter species

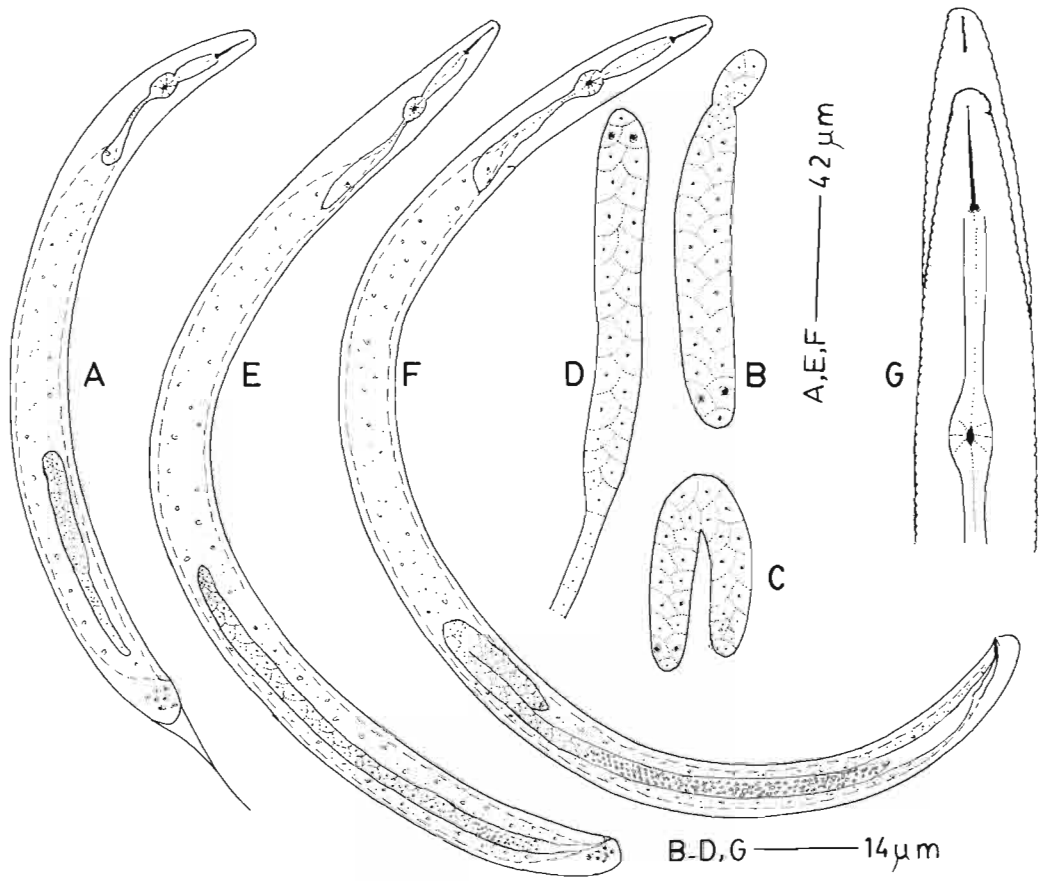

Fig. 2. *Male development in* Verutus mesoangustus *Minagawa. A : Third stage juvenile; B-D : Changes in the genital primordium during second moult; E : Fourth stage juvenile; F : Adult male; G : Fourth moult, anterior region.*

whereas this process occurs after the third moult in *V. mesoangustus*. Reversal of male genital primordium as recorded here for *V. mesoangustus* is also known to occur in other tylenchid genera like *Ditylenchus, Pratylenchus, Radopholus, Subanguina,* etc. (van Weerdt, 1960; Hirschmann, 1962, 1977; Roman & Hirschmann, 1969) but had not been previously reported for *V. volvingentis* (Esser, 1983).

The life cycles of a number of Heteroderidae genera are not fully known which may help in the better understanding of phylogenetic relationships among them. The development of *V. mesoangustus* which is regarded as a primitive genus among heteroderids (Wouts, 1985; Luc *et al.,* 1988) or even an out group (Baldwin & Schouest, 1990) is unique and that may be traced to its distinct habitat (sedentary ectoparasite of cortical cells). It differs from that of the related genus *Meloidodera* since males do not develop when J2 are incubated in water as has been reported in pine cyst nematode by Hirschmann and Triantaphyllou (1973); also J3 and J4 males are not enclosed anteriorly in the cuticles of preceding stages; J3 and J4 females retain the cuticle of J2 in the posterior region of body in *V. mesoangustus*. In retention of cuticles of preceding stages (though only in the posterior region) by J3 and J4, the development of *V. mesoangustus* is analogue to *Meloidogyne* species (Siddiqui & Taylor, 1970) but, in the former species, the duration of J2 is not very long, J3 and J4 have well developed stylet and oesophagus, and males develop in soil after J4 stage without metamorphosis. J3 and J4 females of cyst nematodes also possess well developed stylet and oesophagus but they develop inside the roots and are not enclosed posteriorly in the exuvia of J2; males in these genera also develop through metamorphosis.

At the end of the crop growth season, female bodies of *V. mesoangustus* are transformed into sacs that contain embryonated eggs. Among heteroderids, there is a tendency of retention of embryonated eggs/J2 inside the female bodies, *e.g., Rhizonema sequoiae* Cid del Prado *et al., Thecavermiculatus gracililancea* Robbins, *Heterodera, Globodera, Punctodera* spp., etc. The cuticle toughens in

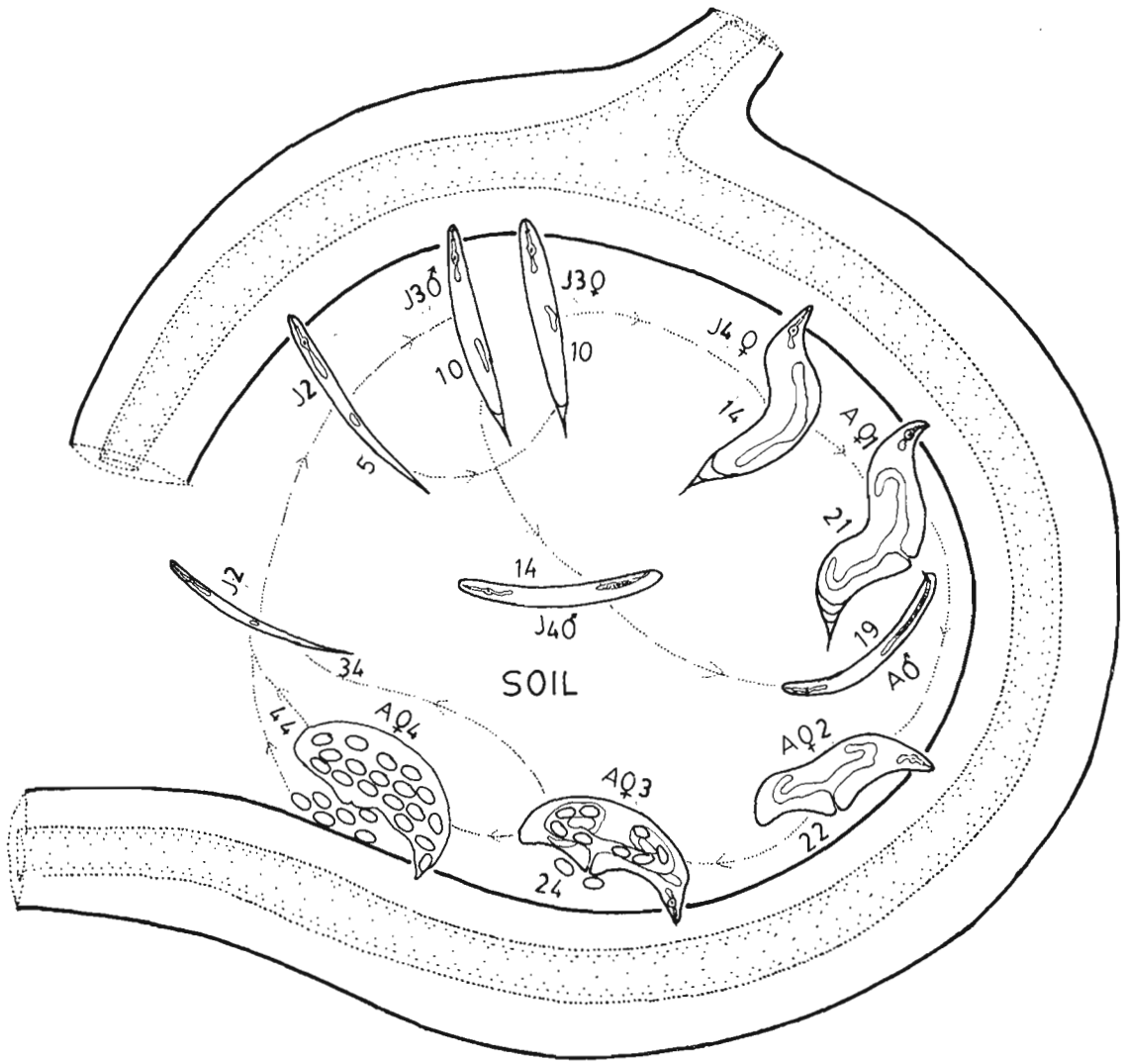

Fig. 3. *Schematic representation of development of* Verutus mesoangustus *Minagawa on vetiver roots. A♀1 : Female enclosed within exuvia of preceding stages; A♀2 : Young female out of exuvia; A♀3 : Gravid female ovipositing in soil; A♀4 : Female body transformed into an egg sac (Numbers indicate days after inoculation).*

the last three genera to form cysts. In *V. mesoangustus* however, the cuticle remains thin and subsequently disintegrates. This tendency of retention of eggs inside the female bodies in a primitive genus like *Verutus* might have led to the evolution of cysts in more advanced groups of heteroderids.

References

BAJAJ, H. K. & BHATTI, D. S. (1983). Life history of *Heterodera mothi* Khan & Husain, 1965 on *Cyperus rotundus* L. *Indian J. Nematol.,* 13 : 48-63.

BAJAJ, H. K. & WALIA, R. K. (1996). *Verutus mesoangustus* Minagawa, 1986 (Nematoda : Heteroderidae), a new record from India. *Indian J. Nematol.,* 25 : 48-51.

BALDWIN, J. G., BERNARD, E. C. & MUNDO-OCAMPO, M. (1989). Four new species of Heteroderidae including *Ekphymatodera* n. gen. from California. *J. Nematol.,* 21 : 48-68.

BALDWIN, J. G. & SCHOUEST Jr, L. P. (1990). Comparative detailed morphology of the Heteroderinae Filipjev & Schuurmans Stekhoven, 1941 *sensu* Luc *et al.,* 1988. Phylogenetic systematics and revised classification. *Syst. Parasit.,* 15 : 81-106.

CARTA, L. K. & BALDWIN, J. G. (I990). Phylogenetic implications of phasmid absence in males in three genera of Heteroderinae. *J. Nematol.,* 22 : 386-394.

COHN, E., KAPLAN, D. T. & ESSER, R. P. (1984). Observations on mode of parasitism and histopathology of *Meloidodera floridensis* and *Verutus volvingentis* (Heteroderidae). *J. Nematol.,* 16 : 256-264.

ESSER, R. P. (1981). *Verutus volvingentis* n. gen., n. sp. (Heteroderidae : Tylenchida) in Verutinae n. subf. a phytoparasitic nematode infesting buttonweed in Florida. *Proc. helminth. Soc. Wash.,* 48 : 230-240.

ESSER, R. P. (1983). Reproductive development of *Verutus volvingentis* (Tylenchida : Heteroderidae). *J. Nematol.,* 15 : 576-581.

HIRSCHMANN, H. (1962). The life cycle of *Ditylenchus triformis* (Nematoda : Tylenchidae) with emphasis on postembryonic development. *Proc. helminth. Soc. Wash.,* 29 : 30-43.

HIRSCHMANN, H. (1977). *Anguina plantaginis* n. sp. parasitic on *Plantago aristata* with a description of its developmental stages. *J. Nematol.,* 9 : 229-243.

HIRSCHMANN, H. & TRIANTAPHYLLOU, A. C. (1973). Post embryogenesis of *Meloidodera floridensis* with emphasis on development of males. *J. Nematol.,* 5 : 185-195.

JENKINS, W. H. (1964). A rapid centrifugal flotation technique for separating nematodes from soil. *Pl. Dis. Reptr,* 48 : 692.

LUC, M., MAGGENTI, A. R. & FORTUNER, R. (1988). A reappraisal of Tylenchina (Nemata). 9. The family Heteroderidae Filip'ev & Schuurmans Stekhoven, 1941. *Revue Nématol.,* 11 : 159-176.

MINAGAWA, N. (1986). Description of *Verutus mesoangustus* n. sp. (Tylenchida : Heteroderidae) from Japan. *Ann. Ent. Zool.,* 21 : 277-282.

RASKI, D. J. (1950). The life history and morphology of sugar beet nematode, *Heterodera schachtii* Schmidt. *Phytopathology,* 40 : 135-152.

ROMAN, J. & HIRSCHMANN, H. (1969). Embryogenesis and postembryogenesis in species of *Pratylenchus* (Nematoda : Tylenchidae). *Proc. helminth. Soc. Wash.,* 36 : 164-174.

SESHADRI, A. R. (1965). Investigations on the biology and life cycle of *Criconemoides xenoplax* Raski, 1952 (Nematoda : Criconematidae). *Nematologica,* 10 : 540-562.

SIDDIQUI, I. A. & TAYLOR, D. P. (1970). The biology of *Meloidogyne naasi. Nematologica,* 16 : 133-143.

VAN WEERDT, L. G. (1960). Studies on the biology of *Radopholus similis* (Cobb, 1893) Thorne, 1949. Part III. Embryology and postembryonic development. *Nematologica,* 5 : 43-52.

WOUTS, W. M. (1985). Phylogenetic classification of the family Heteroderidae (Nematoda : Tylenchida). *Syst. Parasit.,* 7 : 295-328.