

# Reproductive Development of *Verutus volvingentis* (Tylenchida: Heteroderidae)

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*Abstract:* *Verutus volvingentis* Esser, 1981 deposits eggs in the rhizosphere without a gelatinous matrix. Ecdysis was not observed to occur in the egg. Spicular primordia in the rectal area of a second-stage larva were well defined. One larva increased in width from 28.2  $\mu\text{m}$  to a maximum of 51.7  $\mu\text{m}$  after 176.5 hours of feeding, prior to the second ecdysis. It then decreased steadily in width to 33.3  $\mu\text{m}$ , at which time it had molted to a fully developed male. Males leave the third-stage larval integument embedded in the root following final ecdysis. The unique feature of female development was the occurrence of large vaginal primordial cells. Male and female development took from 6 to 15 days and 17 days, respectively. *Key words:* *Diodia virginiana*, life cycle.

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*Verutus volvingentis* Esser, 1981 (16) is a semi-endoparasitic nematode which feeds in roots of *Diodia virginiana* L. (buttonweed), a plant of no known economic importance occurring in wetland habitats in Florida. Buttonweed is usually found near but not in bodies of water located in ditches, canals, lakes, or ponds. The objective of this investigation was to study the life cycle of *Verutus volvingentis* with emphasis on the postembryonic development.

## MATERIALS AND METHODS

A 4-mm layer of sterile 1% water agar was poured into a plastic petri dish. A 5-mm glass rod heated over a gas flame until slightly red was used to burn a hole into the side of both the top and bottom of a 9.2-cm-d plastic petri dish. The edges of the hole were sanded so the dish was easy to take apart. A stem cutting of buttonweed bearing one or two small leaves was inserted through the hole into the agar with the leaves outside the dish. When primary roots emerged and elongated into the agar, a 5-mm well was cut into the agar 1 cm lateral

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to a primary root. Twenty-five first-stage larvae and five mature males were placed in the agar well in a small drop of sterile water. Similar methods have been previously used in postembryonic studies (2,3). A small drop of water was placed on a coverslip which was inverted and placed over the root site where larvae were attached. Observations and measurements were made under an oil immersion lens. Nematodes were fixed in lactophenol (15) to study stages and progress of development.

## RESULTS

Mature, usually uncleaved ova were deposited externally in the rhizosphere and accumulated in the ventral arc formed by the female body. A gelatinous matrix was not produced by the female.

Four hundred eggs, in lots of 50, were examined under the oil immersion lens to determine if a molt occurred in the egg as described for *Heterodera rostochiensis* Wollenweber (18) and *Meloidogyne* sp. (9). In no case was evidence of ecdysis observed. After examination, the larvae were expelled from the eggs by exerting gentle pressure with a fine needle tip on the cover slip. None of the larvae expelled from the

400 eggs showed evidence of ecdysis. It was concluded, based on these data, that a molt did not occur in the egg (16).

First-stage larvae possess binucleate genital primordia with posterior and anterior cap cells (Fig. 1A). Shortly after the first molt, sex identification can be made by examination of the rectal area. If spicular primordia cells are present (Fig. 1D-1), a male will develop, whereas absence of these cells indicates a female.

*Male development:* Shortly after the first molt but before the first-stage integument is cast off, the genital primordium cells begin to divide and proliferate posteriorly (Fig. 1B,C). The body widens and the genital primordium joins the spicular primordium shortly before, or shortly after, the first molted integument is lost (Fig. 1D). Spermatocytes are large and angular at this stage. Following the second molt, little development is evident in the testis and spicular primordia. The esophagus is not clearly differentiated at this stage of development. The gubernaculum is the anlage of sclerotization as found in *Ditylenchus destructor* Thorne (2), followed by the lamina of the spicules. The calomus and capitulum are the last to become sclerotized. The tails of second-stage larvae are hemi-

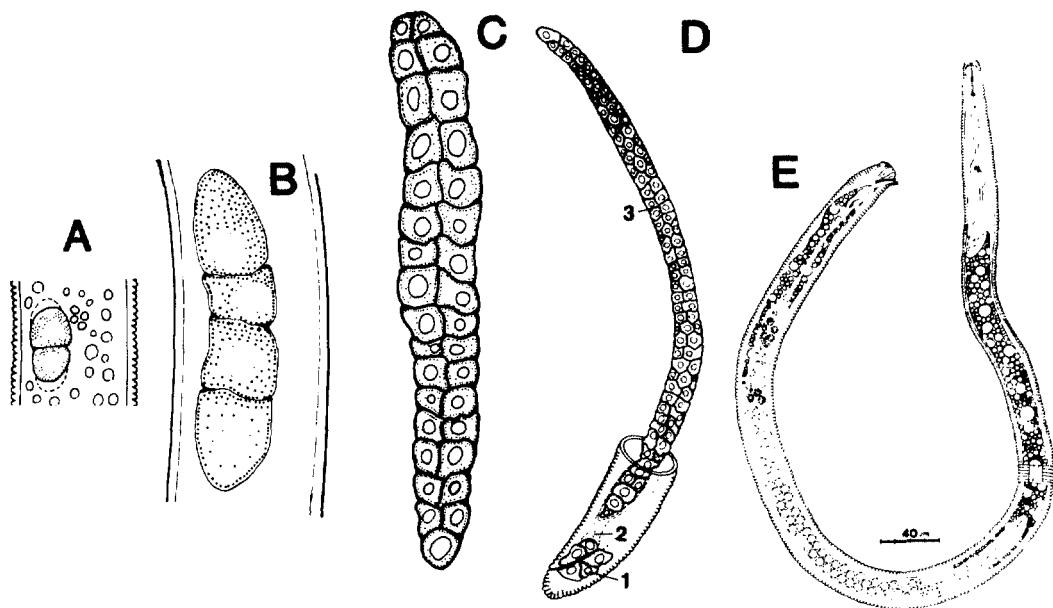


Fig. 1. Gonad development of male *Verutus volvingentis*. A) Genital primordium. B) Genital primordium in four-cell stage. C) Proliferated genital primordium. D) Gonad in third-stage male, nearly complete. 1 = spicular primordia, 2 = vas deferens, 3 = testes. E) Adult male.

spherical. Development proceeds to completion after the second exuvium is cast.

The reproductive system in a mature male (Fig. 1E) consists of an elongate testis originating anterior to mid-body, usually filled with spermatozoa. Germinal and growth zones are rarely well defined. Since the growth and germinal zone are not well defined, the elongate seminal vesicle (5) is not clearly set off from the testis. A narrow vas deferens extends about 90  $\mu\text{m}$  from the seminal vesicle to the ventral face of the spicule (16). The cloaca terminates exteriorly in a tubus. In two cases observed, the male left the third-stage exuviae embedded in the root. Empty exuviae of *V. volvingentis* are not uncommon in infected roots. Three first-stage larvae that entered a root about the same time molted to the second stage in 48 hours and molted to the third stage 24 hours later. Three days later the final molt occurred for all three males within 9.5 hours. Total average time required for male development was 10 days and 20 hours. The three males completed their life cycles in 15, 10, and 6 days.

The width of a feeding larva increased with time until 176.5 hours when a maximum width of 51.7  $\mu\text{m}$  was attained (Table 1). After the second molt, the width decreased until the male was fully developed with a width of 33.3  $\mu\text{m}$ . Feeding had ceased at this time, and it is postulated that the decrease in width is due to energy expended during ecdysis and migration.

Table 1. Measurements of the body width of a *Verutus volvingentis* male during postparasitic development and the nematode activity associated with stage of development.

Time (hours)	Activity	Width ( $\mu\text{m}$ )
0	Penetration	28.2
24, 32.5	Feeding	32.9
48	"	42.3
72, 81, 96	Molt (first)	37.6
105	Feeding	42.3
122	"	49.3
145	Shedding integument	49.3
168	Feeding	47.0
176.5	"	51.7
192	Molt (second)	42.3
195	Third-stage larva emerged from exuviae which remained in root	42.3
197	Migration along root (length 700 $\mu\text{m}$ ), stylet 24 $\mu\text{m}$	37.6
202, 212	Migration to a new root, no physical change	38.6
236	Molt (third and fourth)	38.6
244	Development complete	33.3
TOTAL TIME = 10 days, 4 hours		

*Female development:* Shortly after the first molt the body swells and the genital primordium proliferates anteriorly and posteriorly (Fig. 1A,B). The cells in the center of the genital primordium bulge toward the body wall forming the mesodermal vaginal primordium, after which the anterior and posterior branches elongate and develop (Fig. 2A). The vagina first appears as a large opening with a very large vaginal primordia cells on either side (Fig. 2B). After the second molt, the body swells and the gonad completes its development (Fig. 2C). The reproductive system is complete when the third-stage exuvium is cast (Fig. 3).

The reproductive system of a mature female consists of two reflexed ovaries with poorly differentiated oocytes. A definitive cap cell was not observed. A germinal zone followed by a growth zone was seen in a few specimens. The oviduct consists of three to four large cells attached closely to the large, thick-walled, roughly spheroid spermatheca. Spermatozoa were seen in one spermatheca only. The uteri are large oval sacs (70  $\times$  35  $\mu\text{m}$ ) attached directly to the spermatheca (Fig. 4). They are comprised of 4–5 rows of epithelial cells with a medial furrow. In young females the vagina uterina is quite narrow (Fig. 4) and appears as two separate tubes extending from the vagina to the uteri. In older females the vagina uterina is enlarged, convoluted, and appears as a single amalgamated structure. The vagina

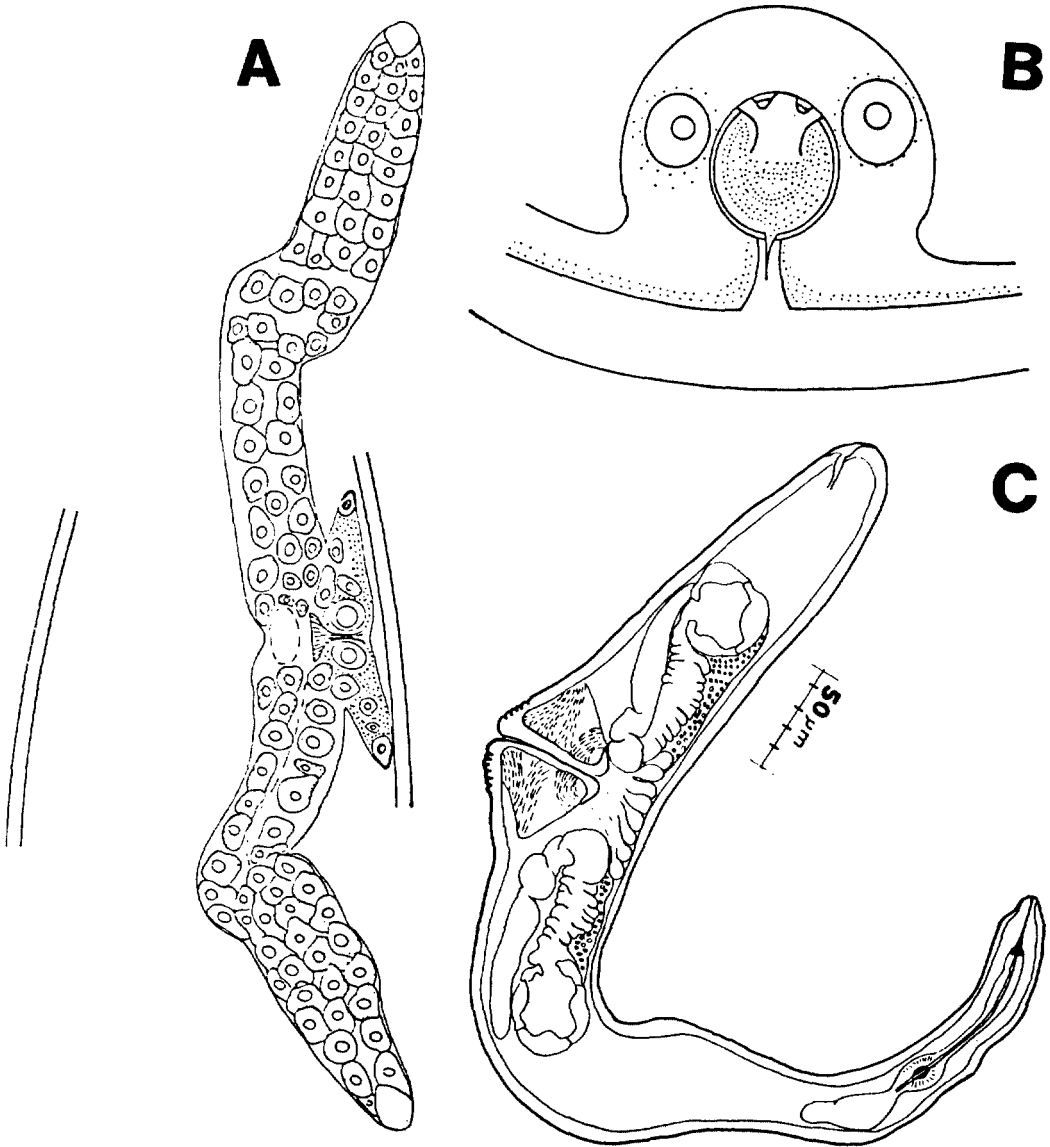


Fig. 2. Gonad development of female *Verutus volvingentis*. A) Early third-stage female gonad. B) Vaginal development of a late third-stage female showing vaginal primordia cells. C) Virgin female.

is pronounced and quite long (42–70.5  $\mu\text{m}$ ). The transverse prominent vulva is about 62  $\mu\text{m}$  long. In several females a severely prolapsed vagina was noted.

*Life cycle:* Only one female developed to maturity in life cycle tests. The onset of ecdysis was not observed. Vulva development was seen 4 days after root penetration by the first-stage larva. The ovaries were defined 7 days after penetration. Ventral chord nuclei were not observed. A fully developed female was evident 17 days after penetration. Seventeen eggs were deposited

the first day development was considered complete. Males were not observed near the female prior to oviposition.

#### DISCUSSION

Spicular primordia are discrete shortly after the first molt, and have been observed in a number of other studies (1,2,6,7,11,20, 23). In all cases reviewed, many cells were present, not the few large block-like cells detected in *V. volvingentis*. Several studies have measured larval growth during devel-



Fig. 3. Final female ecdysis of *Verutus volvingentis*.

opment (6,10,14,29). Growth increases have been uniform in such studies. In this study, a decrease in body width occurred following each molt. A peak body width of 51.7  $\mu\text{m}$  occurred after 168 hours feeding, which decreased to 33.3  $\mu\text{m}$  in the mature male (244 h).

Female development proceeds to the point of vagina formation quite similar to that of other members of the Tylenchina. Ventral chord nuclei have been described in *Amidostomum raillieti* Skrjabin, 1915 (23), *Chiloplacus symmetricus* (Thorne, 1925) Thorne, 1929 (1), *Cylindrocorpus longistoma* (Stefanski, 1922) Goodey, 1939 (6), *Diploscapter coronata* (Cobb, 1893) Cobb, 1913 (19), *Ditylenchus destructor* Thorne, 1945 (2), *D. triformis* Hirschmann and Sasser, 1955 (20), *D. myceliophagus* Goodey, 1958 (5), *Helicotylenchus dihystra* (Cobb,

1893) Sher, 1961 (21), *Pratylenchus scribneri* Steiner, 1943 (26), and *Radopholus similis* (Cobb, 1893) Thorne, 1949 (28). Post-embryonic studies in which ventral chord nuclei have not been noted include *Heterodera rostochiensis* Wollenweber, 1923 (8), *H. schachtii* (Schmidt, 1871) Orley, 1880 (25), *H. trifolii* (Goffart, 1932) Oostenbrink, 1949 (24), *Nacobbus serendipiticus* Franklin, 1959 (12), *Rotylenchulus macrodoratus* Dasgupta, Raski, and Sher, 1968 (22), and *Tylenchulus semipenetrans* Cobb, 1913 (27). In the aforementioned species in which ventral chord nuclei were not observed, all mature females possess a swollen body. In the previously mentioned species characterized by the presence of ventral chord nuclei, mature females are vermiform.

In the present study, several hundred larvae in various stages of development were critically examined to detect the presence of ventral chord nuclei with negative results.

Vaginal formation begins when the cells in the center of the genital primordia bulge toward the ventral body wall. Two very large vaginal primordia cells may be unique to this species (16). Single vaginal primordia cells have been observed in *Ditylenchus destructor* (2), more than two in *D. myceliophagus* (5), *Helicotylenchus dihystra* (21), *Amidostomum skrjabini* Boulenger, 1926 (23), and *Radopholus similis* (28). Female development from root penetration to oviposition occupied 17 days. Although a

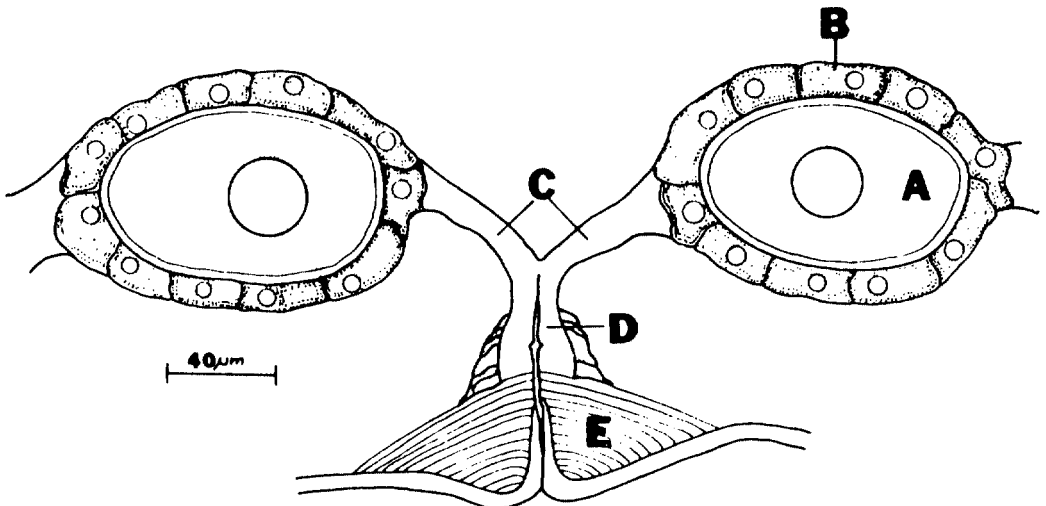


Fig. 4. Anatomy of the female reproductive system of *Verutus volvingentis* from vulva to uteri. A) Egg in uterus. B) Uterus cells. C) Narrow vagina uterina. D) Vagina E) Vaginal muscles.

molt was not observed inside the egg, the developmental pattern of the larvae after hatching proceeded similarly to that of other heteroderids.

Embryonic development of members of the Tylenchida is quite similar (6), and it is doubtful if such development can be utilized in establishing relationships. Despite a paucity of data, a review of post-embryonic development suggests an area where the biogenetic law (4,13,17) might have application in the interpretation of evolutionary trends. It is postulated here that the heterochronism (17) exhibited in vagina and spicule development might provide a key to nematode relationships as more postembryonic developmental studies are completed.

### LITERATURE CITED

1. Ahmad, I., and M. S. Jairajpura. 1977. Developmental biology of *Chiloplacus symmetricus*. Indian J. Nematol. 7:123-139.
2. Anderson, R. V., and H. M. Darling. 1964. Embryology and reproduction of *Ditylenchus destructor* Thorne, with emphasis on gonad development. Proc. Helminthol. Soc. Wash. 31:240-256.
3. Anderson, R. V., and H. M. Darling. 1964. Spear development in *Ditylenchus destructor* Thorne. Nematologica 10:131-135.
4. Andrassy, I. 1976. Evolution as a basis for the systematization of nematodes. London: Pitman Publishing Co.
5. Cayrol, J. C. 1964. Études préliminaires sur le cycle évolutif de *Ditylenchus myceliophagus* Goodey. Nematologica 10:361-368.
6. Chin, D. A. 1977. Embryonic and post-embryonic development of *Cylindrocorpus longistoma* (Nematoda: Cylindrocorpidae). Nematologica 23: 62-70.
7. Chitwood, B. G., and M. B. Chitwood. 1937. An introduction to Nematology, Section 1, Anatomy. Rev. Ed., 1950. Baltimore: Monumental Printing Co.
8. Chitwood, B. G., and E. M. Buhner. 1946. The life history of the golden nematode of potatoes, *Heterodera rostochiensis* Wollenweber, under Long Island, New York, conditions. Phytopathology 36: 180-189.
9. Christie, J. R., and G. S. Cobb. 1941. Notes on the life history of the root-knot nematode *Heterodera marioni*. Proc. Helminthol. Soc. Wash. 8: 23-26.
10. Chuang, S. H. 1962. The embryonic and post-embryonic development of *Rhabditis teres* (A. Schneider). Nematologica 7:317-330.
11. Clapham, P. A. 1933. On the life-history of *Heterakis gallinae*. J. Helminthol. 11:67-86.
12. Clark, S. A. 1967. The development and life history of the false root-knot nematode *Nacobbus serendipiticus*. Nematologica 13:91-101.
13. Darlington, P. J., Jr. 1980. Evolution for naturalists. New York: John Wiley and Sons.
14. Das Gupta, D. R., and D. S. Raski. 1968. The biology of *Rotylenchulus parvis*. Nematologica 14: 429-440.
15. Esser, R. P. 1973. A four-minute lactophenol fixation method for nematodes. Plant Dis. Rept. 57: 1015-1046.
16. Esser, R. P. 1981. *Verutus volvingentis* n. gen., n. sp. (Heteroderidae: Tylenchida) in *Verutinae* n. subf., a phytoparasitic nematode infesting button-weed in Florida. Proc. Helminthol. Soc. Wash. 48: 220-240.
17. Gould, S. J. 1977. Ontogeny and phylogeny. London: Belnap Press.
18. Hagemeyer, J. W. 1951. A new stage in the life cycle of the golden nematode *Heterodera rostochiensis* Wollenweber. Proc. Helminthol. Soc. Wash. 18:112-114.
19. Hechler, H. C. 1968. Postembryonic development and reproduction in *Diploscapter coronata* (Nematoda: Rhabditidae). Proc. Helminthol. Soc. Wash. 35:24-30.
20. Hirschmann, H. 1962. The life cycle of *Ditylenchus trififormis* (Nematoda: Tylenchida) with emphasis on post-embryonic development. Proc. Helminthol. Soc. Wash. 29:30-43.
21. Hirschmann, H., and A. C. Triantaphyllou. 1967. Mode of reproduction and development of the reproductive system of *Helicotylenchus dihystra*. Nematologica 13:558-574.
22. Inserra, R. N., and N. Volas. 1980. The biology of *Rotylenchulus macrodoratus*. J. Nematol. 12:97-102.
23. Leiby, P. D., and O. W. Olson. 1965. Life history studies on nematodes of the genera *Amidostomum* (Strongyloidea) and *Epomidiostomum* (Trichostrongyloidea) occurring in the gizzards of waterfowl. Proc. Helminthol. Soc. Wash. 32:32-49.
24. Mulvey, R. H. 1959. Investigations on the clover cyst nematode *Heterodera trifolii* (Nematoda: Heteroderidae). Nematologica 4:147-156.
25. Raski, D. J. 1950. The life history and morphology of the sugar-beet nematode, *Heterodera schachtii* Schmidt. Phytopathology 40:135-152.
26. Roman, J., and H. Hirschmann. 1969. Embryogenesis and postembryogenesis in species of *Pratylenchus* (Nematoda: Tylenchidae). Proc. Helminthol. Soc. Wash. 36:164-174.
27. Van Gundy, S. D. 1958. The life history of the citrus nematode *Tylenchulus semipenetrans* Cobb. Nematologica 3:283-294.
28. Van Weerd, L. G. 1960. Studies on the biology of *Radopholus similis* (Cobb, 1893) Thorne, 1949. Nematologica 5:43-51.
29. Zuckerman, B. M., and D. Strich-Harri. 1963. The life stages of *Helicotylenchus multicinctus* (Cobb) in banana roots. Nematologica 9:347-353.