Description and developmental biology of Teratorhabditis andrassyi n. sp. (Nematoda : Rhabditida)

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SUMMARY

Teratorhabditis andrassyi n. sp. has 58-77 μm long spicules fused at tips and bursal papillae arranged as 2 + 4 + 1 + 3. It is closely related to T. mariannae Farkas, 1973 and T. rovinjensis (Sudhaus, 1974) Andrassy, 1983 but differs from both in the length of spicules, the extent of their fusion and in the arrangement of bursal papillae. T. andrassyi n. sp. is amphimictic with a high reproductive potential. Fertilization is internal with external embryonic development. The time taken from egg laying to hatching varies from 12-14 h at 29-30°. Juvenile stages show a high degree of variability in body lengths. Ratios a, b, and c are not significant for their differentiation but the number of genital primordial nuclei along with some allometric ratios (body length/anus position from anterior end; tail length/position of primordium from anterior end and tail length/anus position) are important. Primordial nuclei multiply even during active stages besides the moulting. The flexure in gonad is formed at the time of the fourth or final moulting in females but at the third moulting in males. Total duration of life cycle is only 3-4 days at 29-30°.

RESUME

Description et étude du développement de Teratorhabditis andrassyi n. sp. (Nematoda : Rhabditida)

Teratorhabditis andrassyi n. sp. possède des spicules longs de 58-77 μm, soudés à leur extrémité, et des papilles caudales correspondant à la formule 2 + 4 + 1 + 3. Cette espèce est proche de T. mariannae Farkas, 1973 et T. rovinjensis (Sudhaus, 1974) Andrassy, 1983, mais en diffère par la longueur des spicules, l’étendue de leur soudure et la disposition des papilles caudales. T. andrassyi n. sp., amphimictique, a un potentiel de reproduction élevé. La fécondation est interne tandis que le développement embryonnaire est externe. Le temps compris entre ponte et éclosion est de 12 à 14 heures, à 29°-30°. Les stades juvéniles présentent un degré élevé de variabilité quant à la longueur du corps. Les rapports a, b et c ne sont pas significatifs pour la différenciation des stades, mais une certaine importance peut être accordée au nombre de noyaux du primordium génital et à certains rapports allométriques : longueur du corps/distance de l’avant à l’anus, longueur de la queue/distance de l’avant au primordium génital, longueur de la queue/distance de l’avant à l’anus. Les noyaux du primordium génital se multiplient même pendant les stades d’activité, entre les mues. La flexion de la gonade apparaît avec la quatrième et dernière mue, chez la femelle, mais dès la troisième chez le mâle. La durée totale du cycle est de trois à quatre jours à 29-30°.

Nematodes of the order Rhabditida have extensively been used in many biochemical and nutritional studies (Dougherty, et al., 1959; Nicholas, 1959; Vanfleteren, 1973) due to their short life cycles and a high reproductive potential. However, the developmental biology of only a few species has been studied so far. Chuang (1962) and Thomas (1965) gave accounts of the life cycles of Pelodera teres Schneider, 1866 and Acrobeles complexus Thorne, 1925 respectively. Chin and Taylor (1969) and Chin (1977) made detailed observations on the biology of Cylindrocorpus sp., while Hechler (1970) studied the reproduction, chromosome numbers and post-embryonic development of Panagrellus redivivus (Linnaeus, 1767) Goody, 1945. The life cycle of Caenorhabditis elegans (Maupas, 1900; Dougherty, 1953 was studied by Byerly, Cassada and Russell (1976) and the reproductive behaviour of Acrobeleoides sp. by Jairajpuri and Azmi (1977). Ahmad and Jairajpuri (1979) have provided a detailed account of the developmental biology of Chiloplacus symmetricus (Thorne, 1925) Thorne, 1937. Andrassy (1983) has given a detailed account on the systematics of rhabditid nematodes.

In the present study the description of a new species, Teratorhabditis andrassyi n. sp., is provided with observations on its embryonic and post-embryonic developments.

Materials and methods

Soil samples, having Teratorhabditis andrassyi n. sp., collected from Burdwan (West Bengal) were processed by Baermar’s funnel technique and the nematodes so obtained were cultured in 1 % water agar (1 g of agar + 100 ml of water) in 5 cm diam. Petri-dishes. 5 mg of milk powder (Lactogen) was spread over the surface of agar to enhance growth of bacteria serving as diet of the nematodes. The specimens used in embryological stud-
ies were the progeny of a single female. Observation chambers as designed by Ahmed and Jairajpuri (1979) were used to study the embryonic and post-embryonic developments.

**Teratorhabditis andrassyi** n. sp.

(Figs 1, 2)

**DIMENSIONS**

**Female** (paratypes; n = 25): L = 0.8-1.2 mm; a = 17-22; b = 3.5-4.6; c = 29-43; c' = 1-2; V = 92-96; stoma = 25-29 μm; oesophagus = 227-290 μm; ABD = 15-22 μm; tail = 25-33 μm.

**Male** (paratypes; n = 24): L = 0.7-1.0 mm; a = 18-23; b = 3-4.2; c = 22-29; c' = 1-2; T = 52-66; stoma = 25-27 μm; oesophagus = 203-230 μm; spicules = 58-77 μm; gubernaculum = 28-31 μm; ABD = 17-23 μm; tail = 23-32 μm.

**Holotype** (female): L = 1.05 mm; a = 19; b = 4.0; c = 29; c' = 1.6; V = 93; stoma = 29 μm; oesophagus = 260 μm; ABD = 22 μm; tail = 33 μm.

**Allotype** (male): L = 0.9 mm; a = 23; b = 4.2; c = 27; c' = 1.3; T = 65; stoma = 25 μm; oesophagus = 216 μm; spicules = 64 μm; gubernaculum = 31 μm; ABD = 23 μm; tail = 32 μm.

**DESCRIPTION**

**Female**: Body large and robust, cuticle marked with transverse rows of punctations, anterior 20-25 rows more prominent and twice in size of those on rest of body. Hypodermis with large flattened lateral chords, somatic musculature polymyarian with coelomyarian muscle cells. Lip region setoff, lips separate, equal and arranged as 2 precloacal, 4 + 1 post-cloacal and 3 caudal.

**TYPE HABITAT AND LOCALITY**

Soil near a ditch rich in humus and dung from Burdwan (West Bengal), India.

**DIFFERENTIAL DIAGNOSIS**

*T. andrassyi* n. sp. is characterized by the presence of prominent transverse rows of punctations on the entire body; spicules long, fused only at tip, and bursal papillae arranged as 2 + 4 + 1 + 3.

**Developmental biology**

After copulation, the impregnation of ovum by sperm occurs within the uterus but the actual fusion of the two nuclei normally takes place outside the body, except in older females where cleavage up to the four-cell stage takes place inside the body but eggs become non-viable, if retained further. The fusion of pronuclei involves movement of sperm pronucleus towards that of ovum and their simultaneous migration towards the centre of oocytes; oviduct, crustaform era and uterus well defined. No post-vulval uterine sac present. Vagina sclerotized with strong muscle bands attached to dorsal and ventro-lateral body walls; vulva transverse, situated a little below intestine-rectal junction, with cuticularized lips and well developed vulval muscles.

**Male**: Smaller than female. Testis single, reflexed; spicules long, slender with rounded heads, fused at tips. Gubernaculum 28-31 μm long or about half of spicular length. Tail short, conoid 1.0-1.5 anal body widths long. Bursa open, peloderan with 10 pairs of papillae arranged in four groups: 2 pre-cloacal, 4 + 1 post-cloacal and 3 caudal.

*Species is named in honour of Professor I. Andrassy (Budapest) in recognition of his work on rhabditid nematodes.*
Teratorhabditis andrassyi n. sp., description and development

Fig. 1. Teratorhabditis andrassyi n. sp. A: Entire female; B: Anterior end; C: Anterior region showing punctuation; D: Esophageal region; E: Lateral field; F: Female reproductive system; G: Posterior region (lateral); H: Posterior region (ventral).

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Fig. 2. *Terorhabditis andrassyi* n. sp. A: Entire male; B: Male reproductive system; C: C.S. through testis and vas deferens; D: C.S. through blade of spicules; E: C.S. through distal part of spicules; F: Male posterior region; G: Male posterior end (lateral); H: Male posterior end (ventral); I: En face view; J: C.S. at the base of lips; K: C.S. through metastom.

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followed by instantaneous fusion. The eggs laid are normally elongate-oval measuring \(63 \times 28 \mu m\)
\((60-66 \times 26-30 \mu m)\) but those laid by older females are larger, \(73 \times 32 \mu m\) \((71-75 \times 29-35 \mu m)\). The eggshell has longitudinal markings in the form of ridges (Fig. 3, A). Upon fertilization the shape of the egg cytoplasm changes giving the impression of false cleavage but soon transforms into a distinct single-cell stage with a prominent nucleus (Fig. 3, B). The first cleavage furrow is horizontal i.e., at right angle to the longitudinal axis of the egg (Po) giving rise to an anterior larger (S1) and a posterior smaller (P1) blastomere (Fig. 3, C). S1 further divides obliquely into blastomeres A & B thus forming a three-cell stage (Fig. 3, D). P1 divides into S2 and P2 (Fig. 3, E) and B and A into B1, B2 (Fig. 3, F) and A1, A2 respectively thereby advancing the egg into the six-cell stage (Fig. 3, G). The time taken for the completion of any of the above cell stages ranges from 5-15 min. Eight, ten and sixteen-cell stages are formed subsequently after every 10-15 min intervals. The divisions after sixteen-cell stage are difficult to observe because of heavy granulation and rapid multiplication of blastomeres. The “morula” stage, reaches within 20-30 min after the sixteen-cell stage, is followed by “blastula” 40-45 min later and leads to the “gastrula” within 1 1/4-1 1/2 h. In the later stage the developing embryo differentiates into anterior broader hyaline and posterior narrower granular portions (Fig. 3, K). At this point, invagination starts as a depression in the granular region slightly away from the centre of embryo. “Lima bean” stage (Ehrenstein & Schierenberg, 1980) reaches within 20 min of the initiation of invagination (Fig. 3, L) and 10-15 min later is followed by “comma” stage showing twitching movements and formation of a depression at the broader end. The “tadpole” stage is formed 25 min afterwards and the frequency of movements increases considerably (Fig. 3, M). The latter changes into “plum” stage within 20-25 mn and is characterized by nearly two-fold elongation of embryo (Fig. 3, N). The “loop” stage reaches in another 20-25 mn and involves continuous movement of embryo in antero-posterior direction with simultaneous sideways turning of body. The stomatal cavity appears on the broader (anterior) end while the other end forms the conoid tail. Oesophageo-intestinal junction and adjacent parts of basal bulb and intestine make appearance after 30 min and finally the embryo changes into early “pretzel” stage 60-75 min later (Fig. 3, O). The continuously moving embryo at this point possesses an oesophagus, faint stomatal rhabdions, lateral lines and punctuations. All body organs, except gonads, appear completely formed after 1 1/2 h (Fig. 3, P). The oesophageal pumping commences first irregularly but becomes steady later. Hatching takes place 12-14 h after egg-laying at 29-30°C. The first stage juvenile emerges through a slit in the egg shell but retreats back inside the shell and remains there for a brief period. The feeding commences immediately upon hatching and the body enlarges (length as well as width) considerably within a short period. The genital primordium becomes visible at this point.

The de Manian ratios a, b and c are not reliable in differentiating the various juvenile stages. Other ratios (body length/position of anus; tail length/position of anus; tail length/position of primordium from anterior end) besides the number of primordial nuclei are suitable for distinguishing different juvenile stages (Tab. 1).

The genital primordium in the first stage juvenile is 8-12 \(\mu m\) long consisting of two germinal and two somatic nuclei and is situated at 50-54% from anterior end (Fig. 4, A). The ventral chord nuclei numbering 54-58 located between the oesophageal bulb and anus are arranged in single row up to 46-48 nuclei and then in 2-3 rows onwards. Moulting occurs 6-8 h after hatching and is indicated by loosening of cuticle at both ends of body (Jantunen, 1964). The time taken for completion of the first moult varies from 4-6 h at 29-30°C. The body length of second-stage juvenile, thought larger than the first stage, shows considerable variations. Genital primordium 12-16 \(\mu m\) in length, possessing 4-6 germinal and 6-8 somatic nuclei (Fig. 4, B) is situated at 50-53% from anterior end. The somatic nuclei are present at both ends of primordium thus making the sex determination difficult at this stage of development. The time taken from the completion of first moult to the initiation of the second moult is 8-10 h. In the third stage juveniles is it possible to differentiate the sexes. The male juveniles have genital primordium 22-64 \(\mu m\) long and situated at 47-56% from anterior end. The proliferation of somatic nuclei results in an anterior elongation of primordium with 10-16 somatic nuclei, but the germinal nuclei number only 8-10 situated in the posterior part of primordium (Fig. 4, C). The moulting starts 10-14 h after second moult. At the time of moulting a small flexure develops in the anterior somatic tube, formed by somatic nuclei. The spicular primordium is represented by an aggregation of nuclei on the dorsal side of rectum (Fig. 4, H). In female juveniles the genital primordium is 22-56 \(\mu m\) long situated at 48-56% without any flexure but with a posterior elongation brought about by the multiplication of somatic nuclei. The number of germinal nuclei is 8-10 while somatic nuclei are 12-16 (Fig. 5, A). A mass of light and dark staining nuclei makes its appearance ventrally near the junction of intestine and rectum with specialized nuclei and represents the location of future vulva. In the fourth stage juveniles the differentiation of various parts of gonad in both sexes takes place. In male juveniles the genital primordium is 63-263 \(\mu m\) long with a flexure measuring 24-60 \(\mu m\) and is situated at 46-55% from anterior end. The germinal nuclei range from 15-50 (Fig. 4, D & E). The flexure elongates further representing the gonoduct having 40-80 somatic nuclei (Fig.
The spicular primordium becomes more compact with refractory lines of spicules. The moultng starts 14-16 h after the completion of third moultng and takes 4-6 h. After completion of moultng all parts of the gonad are fully differentiated. The primordium in female is 64-252 μm long (measured from tip to tip including flexure) situated at 48-56 % from anterior end. The degree of variation in length of primordia is considerably high. The long somatic tube with paired somatic nuclei shows considerable increase and a flexure.

Fig. 3. *Teratorhabditis andrassyi* n. sp. Embryonic development. A : Egg shell; B : Single-celled stage; C : Two-celled stage; D : Three-celled stage; E : Four-celled stage; F : Five-celled stage; G : Six-celled stage; H : Eight-celled stage; I : Sixteen-celled stage; J : “Blastula” stage; K : “Gastrula” stage; L : Lima bean stage; M : Tadpole stage; N : “Plum” stage; O : Early “pretzel” stage; P : Late “pretzel” stage.
Table 1
Differentiating ratios of various juvenile stages of Teratorhabditis andrassyi n. sp.

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Characters</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>First stage juvenile</td>
</tr>
<tr>
<td></td>
<td>n = 15</td>
</tr>
<tr>
<td>1.</td>
<td>Length: position of anus from anterior end</td>
</tr>
<tr>
<td>2.</td>
<td>Tail length: position of anus from anterior end</td>
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<tr>
<td>3.</td>
<td>Tail length: position of primordium from anterior end</td>
</tr>
<tr>
<td>4.</td>
<td>No. of germinal nuclei in genital primordium</td>
</tr>
<tr>
<td>5.</td>
<td>No. of somatic nuclei in genital primordium</td>
</tr>
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7-48 μm long with oogonial cells is formed which elongates further in later stage. The number of germinal nuclei ranges from 12-60 while that of somatic from 50-110 (Fig. 5 B & C). Specialized nuclei numbering 6-8 form vagina which opens to the exterior through a transverse vulval slit (Fig. 5, G & H). At the completion of moulting the somatic tube first becomes continuous with the vagina and then heavily cuticularized. The gonad then differentiates into ovary, oviduct, crustaformeria, uterus, vagina and vulva and the juvenile becomes an adult female.

Discussion

The pattern of fusion of pronuclei in Teratorhabditis andrassyi is similar to that of Caenorhabditis elegans (Ehrenstein & Schierenberg, 1980). No intra-uterine development took place and the eggs did not remain viable in the body if retained beyond the four-cell stage, as in Chiloplacus symmetricus (Ahmad & Jairajpuri, 1979) perhaps due to the senility of uterine muscles in ageing females. The cleavage patterns of T. andrassyi resembled C. elegans with large anterior blastomeres in contrast to Ditylenchus tricornis (Hirschmann, 1962), Helicotylenchus vulgaris (Yuen, 1965), H. dihytiera (Hirschmann & Triantaphyllou, 1967) and Cylindrocorpus longistoma (Chin, 1977) which possess smaller anterior blastomeres. The oblique division in the second cleavage was similar to that of Pelodera teres (Chuang, 1962). The increase in the rate of oesophageal pumping as the hatching time approached resembled Acrobeles complexus (Thomas, 1965) and Acrobeleoides sp. (Jairajpuri & Azmi, 1977). The oil globules that were observed within the shell of Teratorhabditis andrassyi were most probably the product of emulsification of the lipid layer of the shell (Bird, 1968). The embryonic development of T. andrassyi lasting for 12-14 h is quite similar to that of C. elegans in which it is less than 14 h (von Ehrenstein & Schierenberg, 1980). Byrly, Cassada and Russell (1976) observed that the life cycle of C. elegans was completed in 3 3/4 days at 20°. In T. andrassyi the entire life cycle took 3-4 days at 29-30°. The initial hesitation and retraction of the juvenile to come out of the shell at the time of hatching could be due to the sudden change in its environment as was also observed by Ahmad and
Fig. 4. A-E : Development of gonad. A : First stage juvenile; B : Second stage juvenile; C : Third stage juvenile (male); D : Early fourth stage juvenile (male); E : Moulting fourth stage juvenile (male). F-I : Posterior region; F : First stage juvenile; G : Second stage juvenile; H : Third stage juvenile (male); I : Fourth stage juvenile (male).
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Fig. 5. A-C: Development of female gonad. A: Third stage juvenile; B: Early fourth stage juvenile; C: Late fourth stage juvenile. D-H: Female posterior region - D: Third stage juvenile (lateral); E: Third stage juvenile (ventral); F: Early fourth stage juvenile (lateral); G: Early fourth stage juvenile (ventral); H: Late fourth stage juvenile (ventral).
Jairajpuri (1979). Yuksel (1960), Hirschmann (1962) and Ahmad and Jairajpuri (1979) found one germinal nucleus in the newly formed germinal primordium in the monodelphic-prodelphic species D. dipsaci, D. triformis and C. symmetricus respectively, while von Ehrenstein and Schierenberg (1980) and Chin (1977) reported two germinal nuclei in C. elegans and C. longistoma respectively. The monodelphic prodelphic T. andrassyi, however, resembles the amphidelphic species in having two germinal nuclei. It was further reported in D. dipsaci (Yuksel, 1960), D. triformis (Hirschmann, 1962), H. vulgaris (Yuen, 1965) H. dihystera (Hirschmann & Triantaphyllou, 1967), C. longistoma (Chin, 1977) and Chiloplacus symmetricus (Ahmad & Jairajpuri, 1979) that the number of germinal nuclei remain unchanged in the second stage in contrast to T. andrassyi which showed a multiplication of germinal nuclei during its first moulting. The continuous multiplication of primordial nuclei during moulting as well as active stages in T. andrassyi was similar to D. triformis (Hirschmann, 1962), Pratylenchus sp. (Roman & Hirschmann, 1969) and C. longistoma (Chin, 1977). There was an accumulation of dark and light staining nuclei around the developing vagina as was also observed by Ahmad and Jairajpuri (1979). For the differentiation of different juvenile stages a, b and c values were not of much help. Other morphometric and allometric ratios e.g., the number of primordial nuclei, ratio between total body length and position of anus, length of tail and position of primordium and length of tail and position of anus may be taken into consideration.

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