The Development of the Female *Enterobius vermicularis* and the Morphogenesis of Its Sexual Organ

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**Abstract.** The development of the female *Enterobius vermicularis* L. is described from its larval stage with the first anlage of its sexual organ to the adult female, in which the function of its reproductive system terminates. The primary anlage of the reproductive system is cylindrical, attenuated at both ends. Its anterior portion differentiates into the vulva, which opens on the body surface. On the posterior portion originate two moderately anterlaid situated tubes, which continue in their growth. A major part of the original anlage behind the vulva differentiates into a sack-like formation. The posterior termination of the anlage changes into a common opening for both uteri, which originates from the gradual differentiation of the simple tubes into uteri, oviducts and ovaries. In the present paper a description of the macroscopic regressive changes of the reproductive system of the oldest female worms is given.

Although great interest has been taken in studies on the biology and pathogenicity of the human pinworm, information on its morphology is relatively scarce. Descriptions on its developmental stages, dating back only to the second half of the last century, are incomplete and disagreeing and nothing is known about its microscopic anatomy. In the histological diagnosis of these worms, obtained from pathological, biotopic and necrotic material, these data are of greatest importance. Therefore, the aim of our studies has been to give a description of the morphology and morphogenesis of the human pinworm in the course of its development, which data are also needed for the study of its pathogenicity. Our paper presents the development of the female *Enterobius vermicularis* from the larval stage to the first differentiation of its sexual organs.

**MATERIAL**

Our material, obtained from Šikl's Department of Pathology in Plzeň, was fixed in 10% formalin. The worms were extracted from biotopically examined human appendices and in post mortem from the contents of the large and small intestine in cases of a massive oxyurasis. Of the 1,010 worms acquired, 409 were males, 7 larvae with the anlage of the sexual organs, 3 younger larval stages with a smooth cuticle without cuticular alae and without an anlage of the sexual organs.
METHOD

The female worms were divided into five groups according to their outer morphological signs, mainly the size of the body, differentiation of the excretory organ, location of the excretory pore, distance of anal pore, differentiation of the sexual organ and distance of vulva from anterior portion. The characteristics of the head, i.e. the arrangement of the lips, the buccal capsule, the lip papillae, length and width of cervical alae, length and width of esophageal bulb are constant for the females of all groups. The worms were examined in native preparations and also by histological methods. The female sexual organs were extracted from the worms to obtain topographical data.

Histological procedure:

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A needle with a loop at its end was used for transferring the larvae into paraffin.

Methods used for the staining of the series: Mallory’s phosphowolfram hematoxyline, Weigert’s ferric hematoxyline, Azan, acid hematoxyline after Mayer, Bährer’s hematoxyline, trichrome after Masson, Sudan III, Millon, Comori.

RESULTS

Group I (10 larval stages)

The three youngest larvae observed were 0.8—0.9 mm long and 0.07 mm wide. Length of esophagus and bulb 0.15 mm. On the cephalic end three circular impressions resembling lips. Cuticular alae not developed. Body translucent, almost without granules, cuticle not striated. In the bulb a not very distinct incomplete partition, forming the anlage of the future valve. Intestine wide, packed with cells. Narrow lumen with irregular windings observed in the anterior portion only. Attenuated end of intestine empties into thin rectal tube with an indistinct opening to the outside. Anus indicated only as a slender groove terminating at about 0.1 mm from the end of the tail. Gonad anlage not formed. A large pear-shaped cell, considered by GÖBB (1890) to be the anlage of the excretory organ, is situated on the ventral side of the esophagus. The anterior end of this cell attenuates into a slender canal, which disappears in the cuticle (Fig. 1A).

Next, two larvae were observed with a developed sexual anlage in the middle of the body ventrally to the intestine. It is pear-shaped and composed of a greater
number of small cells. In the anterior part of this structure, a groove was observed extending to its middle, which is the vulva anlage. These larvae measured 1.21—1.26 mm in length and 0.08 mm in width. Length of esophagus and bulb 0.24 mm. Distance of the posterior end of the anlage of the reproductive system from the bulb is 0.16 mm. Body translucent, elongated. Cuticle smooth without transverse stria, cuticular wings not developed. Arrangement of intestine and bulb the same as in the preceding larvae. Mouth opening differentiated by three incompletely formed lips (Fig. 1B).

![Diagram of larval stages](image)

**Fig. 1.** Larval stages A — youngest larva without anlage of reproductive system and undifferentiated cephalic organs; B — older larva with oval anlage of reproductive system and three lips; C — older larva with differentiating reproductive system. (Explan. see p. 27.)

In 5 older and larger larvae, we observed extended cuticular alae on the cephalic end. Cuticle with fine striation; a dorsal and ventral cuticular ledge is visible. Length of body 1.40—1.43 mm, width 0.08 mm. Length of esophagus and bulb
0.30—0.35 mm. Vulva and short vagina developed from the transverse groove in the anlage of the reproductive system. Anlage of the reproductive system moderately extended behind the vagina, posteriorly passing into two very narrow tubes, which are translucent and shrivelled and turn anteriad without forming loops. In two larvae, the tubes reach the vulva. The intestine is wide, the border between the cells distinct. The lumen irregular, narrow, extended throughout the intestine. The esophagus contains distinct annuli of radial muscle fibers. The bulb, not completely separated from the esophagus, has a clearly developed trilobate valve. Excretory cell larger, containing a fine granular plasma. The opening of the excretory canal could not be observed on the sections (Fig. 1C). In all these larvae, the nerve ring was found on the anterior portion of the esophagus.

Group II (187 youngest females)

Length of body ranges from 2.6—2.9 mm, width from 0.11—0.14 mm. Mouth opening differentiated by the origin of a single dorsal and two ventrolateral lips. On histological sections, three pairs of lip papillae, the largest on the ventrolateral lip, were observed. The lip is composed of a thick cell layer, which becomes very attenuated medially, turns and forms the true circular valve, dividing the buccal cavity from the esophageal cavity.

An unclosed amphid, formed by a tubular sac, was observed on the ventral side of the ventrolateral lip. The length of the esophagus varies around 0.7 mm; a narrow corpus and a pear-shaped bulb with a trilobate valve are present. The lumen of the esophagus contains bundles of radial and marginal muscle fibers, which increase in numbers at the point of transition into the bulb. An unilobate esophageal gland is situated in the anterior portion of the esophagus. The subdorsal esophageal gland forms two lobes at the base of the corpus. On the ventral side of the esophagus, a sac-shaped excretory gland, covered with a thin membrane was observed. It empties into a thin tube, which opens through an excretory pore close to the nerve ring. The distance of the excretory pore from the cephalic end is 0.3—0.4 mm. The intestinal cells are distinctly separated from each other. Three large epithelial cells, widening the intestine dorsoventrally, are found in its anterior portion. The wide lumen is clearly visible throughout the intestine. The posterior portion of the intestine and the lumen is widened into the rectum, which is 0.14—0.15 mm long. The anal pore is situated at a distance of 0.8—0.9 mm from the end of the tail (Fig. 2A). By the development of the reproductive system these females can be distinguished from older larvae. The vulva opens at a distance of 1.12 mm from the cephalic end on the ventral side of the body, passing into a strong muscular vagina with a narrow lumen. The vagina widens into a sac-like structure, attenuated at both its ends and enters through a moderately constricted portion the slender translucent tubes. The posterior tube, forming several loops, is longer than the anterior one. The terminal region of the tubes, situated under the vulva, are moderately widened. The sac-like vaginal structure is translucent, covered on the surface with a thick membrane
of connective tissue. The muscle fibers and epithelial cells, situated under this membrane, fill in this formation and the spaces between them are filled with sperms (Fig. 2B).

In larger females of this group, the sac-like structure bifurcates at its posterior end into two thicker tubes, of which one runs forwards to the vulva, the other backwards to the anus. At their termination, the anterior tube turns backwards and the posterior tube forwards, emptying into a narrower tube, which forms 2—4 loops and passes into the attenuated, finely striated ovarian tubes. The terminal end of both ovaries is composed of a large cell with an indistinct wall and a bladder-like nucleus. Only at the junction with the sac-like formation, they attain the typical tube shape with a lumen packed with albuminous droplets. The wall is composed of a cubical epithelium. This part is the anlage of the future uterus and the oviduct (Fig. 2C).

Fig. 2. A — youngest female; B — with partly differentiated reproductive system (vulva, vagina, sac-like structure with sperms in the lumen, passing posteriorly into the common opening of both tubes, which turn in front of the vulva); C — reproductive system of larger females with the anlage of the uteri and their common opening. (Explan. see p. 27.)

Group III (103 older, sexually immature females)

According to body measurements and the development of the excretory and reproductive system, these females were divided into three subgroups: 1. youngest females 3.50—3.60 mm long, 0.17 mm wide; 2. older females 4.40—4.46 mm long, 0.19 mm wide; 3. oldest females 4.50—4.80 mm long, 0.20 to 0.21 mm wide. Body transluscent, containing few granules. Arrangement of head the same as in previous group. On the sections, two cephalic glands and fine secretory granules can be viewed. Esophagus fills one third of the body length of the youngest females, in the older females it is less long in relation to the length of the body. Position of dorsal and subventral esophagus gland unchanged, only in older and the oldest females the glands are branching into lobes at the base of the corpus and the bulb. Nerve ring situated at the level of termination of the cuticular alae. Behind the nerve ring are situated four ganglia, one each in the internal portion of both lateral chords.
laterally to the excretory canal. These are the lateral cephalic ganglia. The dorsal cephalic ganglion is placed in the dorsal chord and the ventral cephalic ganglion reaches the ventral chord.

![Diagram of excretory system in immature females](image)

**Fig. 3.** Older, sexually immature females. A — youngest female with excretory organ resembling a sac-like tube in shape. The digestive tract retained the same structure as in the foregoing stage. On a short portion, the posterior uterus is differentiated. B — older female with excretory organ of the „H“ type, esophagus relatively shortened, anterior and posterior uteri completely developed; C — oldest female of larger size; distended posterior uterus containing eggs. (Explan. see p. 27.)

The excretory gland of the youngest females, resembling a sac-like tube filled with granules, passes into a narrow duct, which opens through the excretory pore. In older females, the excretory system is of the type generally spoken of as the “II” system; it consists of two lateral, longitudinal excretory canals, which are tubes of an oval diameter, with a thin wall and a finely granulated plasma. The lumen is narrow and often almost indistinguishable. The anterior margin of the wall is close to the esophagus, the posterior border is formed by the lateral cell of the lateral
chord. The lateral ducts with the transversal duct unit in the so-called bridge, composed of the cell extension of the lateral chord. From the transverse duct arises the outletting duct, joint with the excretory pore. In the oldest females, the transverse duct is shifted to the level of the anteriorly distended part of the intestine. The lumen of the anterior portion of the intestine is distended dorsoventrally and heart-shaped. The cell borders in older and the oldest females are not distinct. In the middle portion of the intestine, the lumen is irregular and attenuated. The intestine of the youngest females has the same structure as that of the females in group II. Anterior to the emptying of the intestine into the rectum, the lumen widens and the intestinal cells form 2—3 layers. The rectum is 0.28—0.35 mm long. On the wall of the rectum a muscle ring and three rectal glands are situated. The subventral gland is placed vertically to the rectum and is sac-shaped. It was also observed on native preparations. The position of the anal pore changes with the length of the tail. In the youngest females it lies at a distance of 0.80 to 0.90 mm from the termination of the tail, in the older females at 0.89—0.95 mm and in the oldest females at 1.21 mm (Fig. 3).

The reproductive system consists of narrow tubes, which, in the oldest females, extend anteriorly to the bulb and posteriorly close to the anal pore. In all females of this group, the posterior portion of the reproductive system is more developed than the anterior one. The anterior uterus is connected with a translucent sac-like structure, attenuated in its anterior portion, which empties into the muscular vagina and differs by its folded wall. Its posterior portion attenuates into a short part, which is distinctly set off from the rest of the sac-like structure, from which the anterior and posterior uterine tubes arise distally. In the youngest females, the anterior oviduct, which bends and turns anteriad, arises directly from the attenuated portion of the sac-like structure. The oviduct and also the attenuated portion are translucent and the border between them is distinct. The attenuated portion of the sac-like structure empties opposite the anterior oviduct into a short, attenuated tube; this differentiates into the posterior uterus, which is still very narrow and short. In two places, this uterus becomes moderately distended and contains albuminous granules. Then follows the posterior oviduct and ovary, both longer than the anterior ones, forming loops round the intestine. The vulva is placed at a distance of 1.10—1.15 mm from the cephalic end (Fig. 4A).

In older females, the distal portion of the anterior uterus turns forward and distends immediately after leaving the attenuated portion of the sac-like structure, becoming constricted during its course in two or three places. No eggs were present in both uteri. The border between both uteri is marked by a deeper groove on the dorsal side, towards the attenuated part of the sac-like structure, which has now become distinctly differentiated into an opening, through which both uteri enter this formation. The oviducts form three S-bend loops and are divided into two portions, the wider with a transversely grooved wall, the narrower with a smooth wall. The borders between the uteri and oviducts are marked by folds in the wall. In the ovaries, a germinal and a growth zone were observed. The terminal regions
of the ovaries are formed by an epithelial cell of uncommon shape, attached like a conical coat to the terminal strip of pregenital cells (Fig. 4B).

The oldest females differ from the foregoing females in sizes, in an attenuated intestine and, in view of their body length, in a relatively shorter esophagus. The excretory canals and tubes of the reproductive system are elongated. Both uteri are distended and of dark colour. The posterior uterus contains immature eggs in addition to compact albuminous granules. Distance of vulva from cephalic end 1.56—1.59 mm. These females form the transition to the sexually maturing females (Fig. 4C).

Fig. 4. Reproductive system of older, sexually immature females. A — anterior oviduct arising from the attenuated termination of the sac-like structure. Close to its diversion lies the common opening of both uteri, connected with the narrow and short posterior uterus. B — border between posterior and anterior uterus marked by a deep groove on the dorsal side opposite the common opening. Both oviducts and ovaries fully differentiated into individual zones. C — immature eggs in the posterior uterus, which is wider and longer than the anterior uterus. (Explan. sec p. 27.)

Group IV (225 maturing females)

Body compact, the posterior portion opaque, filled with granules. The organs of the reproductive system at such stage of development that an orientation on their position and on the position of the organs, which they overlap, is impossible. These females have not yet reached the egg-laying phase, because the eggs in both uteri
are still cleaving. Length of body 7.60—7.90 mm, width 0.37 mm. The esophagus is relatively shortened, retaining the same absolute length of 0.6—0.7 mm and extended not quite to one third of the body length. The esophageal glands form lobate processes, multiplied especially at the base of the corpus and the bulb. Histological studies revealed a further extension of the lateral excretory canals and a shifting towards the bulb of the transverse bridge with the transverse connecting canal.

The lumen of the intestine is attenuated in its anterior and posterior portion and irregular. The borders between the cells are not distinct, the number of cells is not increased in the rectal region. The cells of this portion are narrower and appear as if fused together. The rectum is distended, 0.30—0.35 mm long. The anal pore opens at a distance of 1.49 mm from the termination of the tail. The arrangement of the rectal region is the same as in the females of the preceding group.

The tube of the reproductive system fills almost two thirds of the body. The anterior loops of the ovaries extend to the esophagus, the bend of the posterior uterus to the anus. The uteri are distended in several places and packed with cleaving eggs. Their common opening into the sac-like structure is becoming smaller (Fig. 5). Also in these females, distinct differences were found in the development of the anterior and posterior portion of the reproductive system. The posterior uterus and oviduct are longer and thicker than the anterior ones. Borders are formed between both uteri and oviducts. The cuboidal epithelium of the anterior uterus is shaped like a cushion, the cells of the posterior uterus are flattened, because the wall is dilated by the accumulated eggs. The oviducts are divided into a smooth portion and a portion with transverse grooves. The lumen is narrow and just large enough for a single egg to pass through. The structure of the ovaries is the same as in the females of the previous group. The sac-like structure of the vagina is narrow and reduced, but still containing sperms. The distance of the vulva from the anterior end is approximately the same as in the females of the preceding group.

Fig. 5. In the reproductive organ of maturing females the further reduced common opening of both uter in the sac-like structure; the eggs in both uteri at the cleaving stage. (Explan. see p. 27.)

**Group V (176 sexually mature females)**

This group comprises all females, will eggs containing developed, tadpole-shaped larvae. The group is not uniform and is composed of: 1. smaller females, length
8.6–8.8 mm, width 0.55–0.60 mm, characterized by narrower and shorter uteri and their still differentiated common opening into the sac-like structure, which is shortened and, through the presence of eggs, has changed into the ovijector emptying into the narrow vagina. The border between the ovijector and the common opening of both uteri is indistinct, their walls are merging. The vulva is placed at a distance of 2.4 to 3 mm from the head end. The function of the ovaries is retained and, histologically, they are in agreement with those of the younger females. The strip of pregenital cells is followed by the germinal growth zone. In the oviducts, a short portion of the wall is smooth and a long portion has transverse grooves.

2. Older and larger females, length 11.5–12 mm, width 0.69–0.70 mm. Their uteri are prolonged (Fig. 6 B) forming a continuous sac without a distinct border between them and filling a major part of the body. According to histological signs, the ovaries have stopped to function and are shorter, translucent and shrivelled. The lumen of the germinal zone is empty and the terminal regions of the ovaries are devoid of dividing pregenital cells. The eggs are situated in the bottom parts of the oviducts and uteri, where they mature; the majority of eggs contain a tadpole-like larva. The vagina is very distended, shortening the ovijector, which now represents only a communication with the sac of the fused uteri. Both the uteri and vagina are packed with departing eggs. Degenerative changes can be also observed in the cells of the intestine, in which cavities filled with fine granules can be found. The walls of the intestinal cells are fused, the lumen irregular. The rectum and the termination of the intestine are narrow. The excretory, cephalic and anal glands are the same as in the females of the preceding groups.

Fig. 6. Reproductive organ of mature females with a tadpole-shaped larva in the eggs. A — the common opening of the uteri into the sac-like structure, which has changed into an ovijector, is still present. Ovaries still in function and histologically in accord with younger females. B — uteri forming a continuous sac, ovaries showing degenerative changes. (Explan. see p. 27.)
DISCUSSION

In the papers by Leuckart (1865a), Grassi (1881), Callandrucio (1889) on the pinworm, a larval stage is mentioned with an incompletely developed esophagus and mid-gut. Similar larval stages were also described by Zenker (1868) and Heller (1903). Leuckart (1865b) found young developmental female stages in the anterior portion of the small intestine. Zenker (1868) considered the blind appendix to be the principal site of location. Vix (1860) found in the anterior portion of the small intestine sexually differentiated females and males and also moulting larvae, which had descended to the posterior portion of the small intestine. Galeb (1878), Trumpp (1913), Koch (1925), when trying to confirm the direct development of the pinworm from the egg in the mid-gut, found larvae 2 to 3-times larger than the eggs. Rodenwaldt and Röckemann (1921) obtained slightly bigger larvae than those described by Koch (1925). Heller (1903) observed larvae without lips, cuticular alae, dorsal and ventral cuticular ledges. Their esophagus was S-bend. The mid-gut packed with cells without lumen. In these larvae, the anlage of the reproductive system was found. Judging from the arrangement of the other parts of the body, these larvae belong to younger stages than those studied by us.

Leuckart (1876), during his studies on the development of Oxyuris ambigua and Enterobius vermicularis found a larva leaving the egg, which was 0.14 mm long and 0.01 mm wide. No valve was attached to the bulb of the pear-shaped esophagus, the lumen and the rectum in the mid-gut were clearly developed. However, in the youngest larvae of our observations (0.8—0.9 mm long) the anlage of the valve in the bulb was present, but the lumen was visible only in the anterior portion of the intestine. The rectum and anus resembled slender grooves. After comparing these larvae with larvae of other worms of the family Oxyuridae, Leuckart assumed that the older, 1.5 mm long larva was just at the point of moulting. Contrary to that, the cuticle of the larvae in our observations, which measured 0.8—0.9 mm in length, was still without stria, while the older larva (1.34 mm) had the same striated cuticle as the young female of group II. This indicates that the larvae start moulting when attaining a length of 0.8—1.3 mm, but so far we did not succeed to find such transitory stage. We observed the rounded head and impressed anlage of the lips, considered by Leuckart to be a larval sign, only in the youngest larvae. In older larvae (1.28 mm long) the lips were distinctly formed. In the oldest larvae (1.43 mm long), lips and cuticular alae were developed.

Contrary to Leuckart, we observed in older larvae on the ventral side of the intestine the anlage of the reproductive system, which became gradually differentiated. Leuckart described the differentiation of the gonad in females measuring 3—5 mm in length with a developed vulva, vagina, the sac-like structure and two ovaries. We observed these differentiated gonads in younger females measuring only 2.30—2.80 mm in length and the presence of sperms in the sac-like elongated vagina, which Leuckart found only in females of 6—7 mm in length. These are, with regard to their length, in accord with the maturing females of group IV.
The excretory system of Enterobius vermicularis has not yet been described and the terminology used is analogous with that of nematodes, in which the excretory system has similar arrangement as in Oxyuris equi (see Martini 1916, 1926), in Camallanidae (see Törnquist 1931), in Cephalobellus (see Chitwood 1931, 1933) and in Ascaris lumbricoides (see Musso 1930, Chitwood 1932). Cobb (1890, 1924) described the anlage of the excretory system in a young-stage larva of Enterobius vermicularis as a kidney-shaped cell with an outletting duct. We observed a similar anlage in the larval stages and in females of group II. In adult females, the excretory system is of the so-called “H-type” mentioned by Hyman (1951).

A brief description of the sexually mature female is given by Skryabin and Shikhobalova (1949) and also in the systematic paper by Skryabin, Shikhobalova and Lagodovska (1960). These authors divided the ovjector of the sexually mature female into two parts, the longer portion with the uteri, the shorter one with the vulva, describing it as an S-shaped muscular organ. We observed that the ovjector undergoes a certain development together with the whole gonad. It differed from that of the young and mature females not only in its shape, but also in its function. It was never S-shaped and very different from the ovjector described by Hyman (1951) from Strongylata. Also the position of the uteri in the adult females, observed by Skryabin and Shikhobalova (1949) is not in complete accord with our observations, because the anterior and posterior uterus never formed a backward loop, but extended straight forward and backward from the common opening into the sac-like structure.

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Received 20 February 1967.

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EXPLANATIONS TO LETTERING IN THE FIGURES:

a = lips, b = esophagus, c = buidge, d = bulbus, e = bulbus valve, f = excretory organ, g = mid-gut, h = mid-gut lumen, i = rectum, j = anal pore, k = vulva, l = vagina, m = sac-like structure, n = ovaries, o = oviduct, p = anterior uterus, r = posterior uterus, x = place, where the ovaries had been cut off to make the drawing clearer, s = common opening of ovaries, t = eggs, u = cuticular alae, v = nerve ring, y = smooth portion of oviducts.