Zoology. — *The Nemas Anchylostoma and Necator.* By J. H. SCHUURMANS STEKHOVEN Jr. (Communicated by Prof. J. F. VAN BEMMELEN.)

(Communicated at the meeting of December 18, 1926).

The Hookwormproblem will reach its final solution, when we will be able to distinguish the different earth-living larval stages of all *Anchylostoma-* and *Necator*species.

Until now our knowledge of the morphology of the hookwormlarvas still is superficial, notwithstanding LOOSS has done very prominent work on this subject. Therefore the finest technique, combined with the keenest observation are wanted to conquer all difficulties.

The present research considers the third, i.e. infective larval stage only. It will however be of great use to apply the same technique to the first and second stage larvae as well as to the hookworms, just after their arrival in the intestine or to the sexually mature individuals, as their histology has not been studied sufficiently.

Now the third stage larva, generally enveloped by a sheath, — the skin of the second stage larva, — is not only the most important stage, by its relation with mankind, but may be discriminated also from other freeliving nemas, according to its structure and movements. Chemical substances which destroy other earth-living larvae easily, do not attack the hookwormlarvae. Its snakelike movements are typical enough to identify the third stage larvae of the hookworm, although it is very difficult to give an adequate description. So I am quite convinced that BAERMANN did not mistake other larvae for hookwormlarvae, although he never gave an exact description of the incriminated larvae.

Moreover, it will be rather easy, I suppose, to gather sufficient knowledge about the social conditions in the examined regions, as for instance about the presence or absence of dogs to exclude in many cases a soilinfection with canine hookworm larvae. A decision, however, can be given by exact morphological research only.

Last years these larvae were studied by the SCHUURMANS STEKHOVEN's along biometrical lines, by VAN THIEL, who found some differences between the Genera *Anchylostoma* and *Necator*, although he is not always very clear in the interpretation of his observations, by SVENSSON, who paid special attention to the esophageal sphincter, by COBB,

who gave a minute description and beautiful figures of the head of *Necator americanus*.

During a Travelling Fellowship, granted to me by the ROCKEFELLER Education Board I got the opportunity to make a thorough study of some freeliving marine nemas in the office of Dr. STEINER, Nematologist at the Bureau of Plant Industry, Department of Agriculture, Washington D.C. U. S. A. and to gather more complete knowledge on the infective larvae of *Necator americanus* and *Anchylostoma caninum* during the last fortnight of my stay at Washington. Dr. STEINER was so kind to give me the necessary technical attendance, whereas Dr. SCHILLINGER furnished me with pure cultures of *Necator americanus* larvae, with human and canine excrementa and some living females of *Anchylostoma caninum*.

This research was made with the same technique as the nematologists of the Bureau of Plant Industry generally use. Confer COBB's and STEINER's paper. To isolate larvae from a soilculture I used wet, hairy tomato-seeds on which the larvae crept. So the attached larvae could be transported easily to a drop of water on a slide.

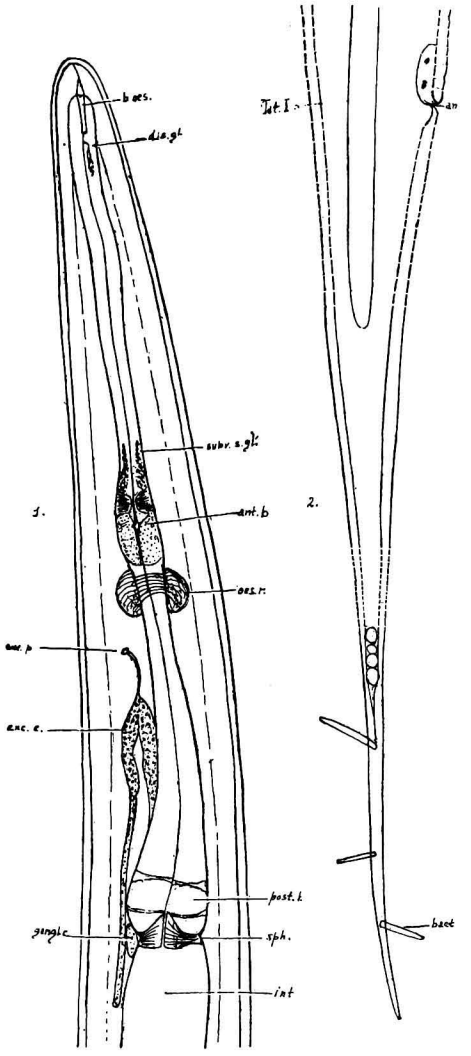
The third stage larva of Necator americanus.
Textfigure A., Plate I.

VAN THIEL emphasized the fact that the cuticular striations are much more pronounced and also more widely separated in the larvae of *Necator* than in those of *Anchylostoma*. This is true for the 3^{d} stage larva as well as for the second. Moreover the tail of the sheath is long and tapers to a very fine point, thus resembling a tube, pointed in a flame. To the empty skin of the larva (confer Textfigure A 2) some bacteria are attached. The head of the larva, when seen in lateral view (Pl. I, Fig. 2—4) shows no lips; this is still more evident when the head is seen on top (Pl. I, Fig. 1). Now the triangular mouth opens widely and marks the centre of the rectangular oral field. The oral aperture is encircled by the four papillae (pap.) and the lateral organs or amphids (amph.), both lying on the same level.

The latter near the oral opening very much, which is particularly clear, when the head is seen in lateral view (Pl. I, Fig. 2, 3). The amphids are composed of a short tubelike pouch (amph. p.) opening distally into a widening, where nervous fibrillae distinctly are to be seen, whereas its proximal end bears a median slit and resembles therefore an opening bud. The amphids are small in size.

The cylindrical, short oral tube passes into the beginning of the esophagus (b. oes.). In Literature some controverse exists about the beginning of the esophagus. According to COBB the cylindrical tube, lined with chitinised bars (oes. b.), to which the oral cavity passes, belongs to the esophagus. VAN THIEL describes the same as oral cavity. I think

we are allowed to assume, that the esophagus begins at the point where the esophageal cells are attached to the intestinal tract at its proximal end (Pl. I, Fig. 3). In this case the mentioned part undoubtedly belongs to the esophagus and COBB is right, unless one supposes that the oral cavity, together with its chitinisations is pushed into the esophagus. In this I agree with COBB. The cuticularisations of the beginning of the esophagus are very characteristic. Here the esophagus is lined by two, equally thick parallel bars, each ending proximally into a curved clasp. These clasps are more or less bean-shaped, faintly curved outwards at their apices. The supposition seems logical that this clasp articulates with the long rod, although I could not prove this. In a former paper my wife and I compared this rod, together with its clasp with the stamen of a grass, bearing one pollensack only. If one turns the larva round its longitudinal axis, this whole apparatus gets a quite different appearance. If I compare a bar with its clasp with a spoon, both spoons, which sometimes touch each other almost with their apical ends (Pl. I, Fig. 2) — will be seen as individual objects in optical section (Pl. I, Fig. 4) in the case mentioned above; if however the animal is rolled over, the backside of one of the spoons will come up. Apparently COBB has figured this case in his paper, where he describes this apparatus as an onchium and compares it with the spear of the Mermithids. Confer also Pl. I, Fig. 3.

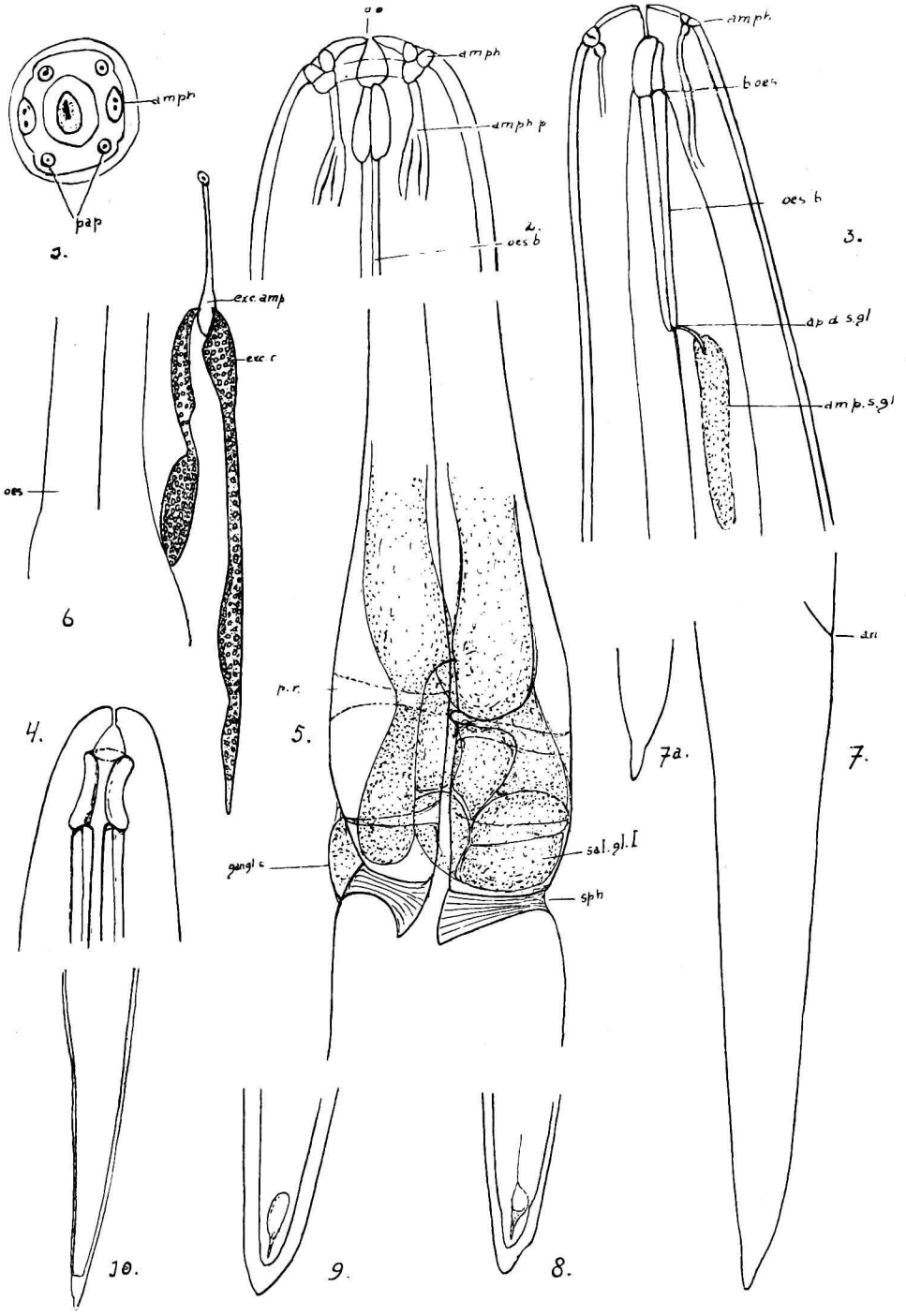


Textfig. A.

Fig. 1. *Necator americanus*, third stage larva. Enlarg. Öl Imm. 2 mM., Oc. 12, Tub. 140 mM. × 1/2.

Fig. 2. The sheath (skin second stage larva) Enlarg. Öl Imm. 2 mM., Oc. 6, Tubus 140 mm. × 1/2.

ant. b. = anterior bulbus; *an.* = anus; *b. oes.* beginning of oesophagus; *bact.* = bacteria attached to the skin; *d. s. gl.* = dorsal salivary gland; *exc. c.* = excretory cell; *exc. p.* = excretory porus *gangl. c.* = ganglion cell; *int.* = intestine; *lat. l.* lateral line, *oes. n.* = esophageal nervering; *post. b.* = posterior bulbus; *sph.* = sphincter; *subv. s. gl.* = subventral salivary gland.



Necator americanus, third stage larva.

Fig. 1. Apex of head, enlarg. Öl Imm. 2 mm., Oc. 12, Tubus 140 mm. × 2/3.

Fig. 2. The head in profile, enlarg. as 1. × 2/3.

Fig. 3 and 4, idem × 2/3.

Fig. 5. Esophagus, posterior bulbus.

Fig. 6. Intestine and excretory apparatus; enlarg. Öl. Imm. 2 mm., Oc. 4. Tub. 140 mm. × 2/3.

Fig. 7—9. Tailend.

Fig. 10. Tail. enlarg. Öl Imm. 2 mm., Oc. 4. Tubus 140 mm. × 2/3.

amph. = amphid; *amph. p.* = amphidial pouch; *amp. s. gl.* = ampulla of the salivary gland; *ap. d. s. gl.* = aperture dorsal salivary gland; *an.* = anus; *b. oes.* = beginning of esophagus; *exc. amp.* = excretory ampulla; *exc. c.* = excretory cell; *gangl. c.* = ganglioncell; *o. o.* = oral opening; *oes.* = esophagus; *oes. b.* = esophageal rods; *pap.* = papilla; *sal. gl. l.* = lobus of salivary gland, *sph.* = sphincter.

With good reason VAN THIEL protests against this view. Former papers of the SCHUURMANS STEKHOVEN's pointed into the same direction.

When rolling the larva over, it may seem sometimes if one of the rods is thicker than the other; in reality this is not the case and I think this gives the solution of the controversy between COBB and VAN THIEL. But apart from the given morphological arguments my physiological observations speak against COBB's idea of the function of these structures.

Once I found the clasps protruding from the oral opening in a dead animal. This proves however nothing in favour of the real function of these parts, as changes in osmotic pressure of the bodyfluid may have occurred during the death of the animal as the cause of this.

Moreover, when a quiet ensheathed larva is observed for longer periods, one may have the chance to catch a larva in the act of shedding its sheath. The head curves backward, during which the skin of the third stage larva shrivels and folds; the mouth of the larva sucks at a definite spot the enveloping sheath into the oral aperture; the larva tugs at it violently, till it suddenly looses its hold and straightens again. Often the same manipulation is repeated, during which the rods do not change their place. It is not possible to conclude anything about the presence of chemical substances, helping to perforate the sheath at its apical end. I am however inclined to think at the possibility that the products of the salivary glands participate in the exsheating of the larva. At any rate the larva suddenly lifts the lid of the sheath, which often remains connected with the tailend of the sheath at one side. The larva will attain its aim easiest, if it gets the opportunity to anchor itself in the sand, or if the distance between slide and coverslip becomes smaller and smaller, thus urging the larva to remain in the same position, or if the sheath sticks to a rough portion of the underlayer. So this phenomenon will be mainly mechanical.

If once the lid has been lifted, it is curious to observe the larva while turning round its longitudinal axis, and by doing this, freeing itself from its envelope. Sometimes the head glides backwards along the body as if to strip off the sheath. Each moment the larva emerges further; the empty skin falls down after each turn. Therefore the empty sheath presents a number of oblique folds just as in a towel turned round its axis in the same way. Textfigure C 6 gives an idea of such a sheath for a larva of *A. canium* in which things happen in the same manner.

In a waterculture of larvae a considerable number of animals had stripped off their sheath and I suppose that the same thing will happen in the soil. When a stain is added to the water of the culture, as for instance methylenblue or methylgreen the percentage of larvae, which sheds their sheath becomes larger.

The esophagus of the third stage larva presents 2 bulbi (Textfigure A 1) a rudimentary anterior bulb (ant. b.) which precedes the compar-

atively narrow nerve ring (oes. r), thus dividing the esophagus into 2 equal portions. This bulb was described already by VAN THIEL, which however did not identify it as such. The posterior bulb (post. b.) is very pronounced and marks the end of the esophagus.

Clear transverse (p. r.) rings divide the posterior bulb (Textfigure A 1 and Plate I, Fig. 5) into 3 strongly granulated portions. Here 3 esophageal glands (1 dorsal gland and 2 ventral glands), the salivary glands of other authors, originate. Both ventral glands open into the esophageal lumen just in front of the nervous ring (Textfigure 1, subv. s. gl.), whereas the dorsal gland forms an ampulla, (amp. d. s. gl.) just behind the mentioned cuticularisations of the anterior end of the esophagus, and opens into the latter by means of a short tube (Textfigure 1 and Plate I, Fig. 3 ap. d. s. gl.). The part of the salivary glands, lying in the esophageal bulb is lobed (Pl. I, Fig. 5 sal. gl. l.). Next to the sphincter, see below, one finds a ganglionic. cell. The posterior esophageal bulbus does not show any thickenings or valvae at its lumen.

SVENSSON and CORT emphasize the existence of a clear space at the junction of the esophageal bulb and the intestine. SVENSSON is undoubtedly right to call this structure a sphincter (sph.). The muscular fibres are clearly visible. The sphincter is low at its junction to the intestinal wall and becomes higher at the lumen (Textfig. 1 and Pl. I, Fig. 5).

Its proximal side is perpendicular to the body-axis, therefore its distal portion protrudes into the intestine as a plug and resembles very much a cone, which is separated from the intestinal wall by a circular groove. As I will describe below for *Anchylostoma* this sphincter is the only part which really acts as such, providing the ingested food a passage to the intestine, whereas the bulbus functions as a sucking apparatus. VAN THIEL is not very clear in this point. The lumen of the intestine (int.) is wide, the cells forming its wall are scanty granular.

The excretory apparatus, H-shaped in the typical representants of this group, has lost its anterior horns (Textfig. 1 and Pl. I, Fig. 6). The excretory ampulla (exc. amp.) is easily to be seen in front of the excretory cells (exc. c.) which open into the caudal end of the ampulla. These excretory cells lay astride over the intestine. Each excretory cell is rather narrow, its foremost portion resembles a peanut as it is narrowed in the middle, the caudal part of the excretory cells is narrow and paralel-sided; it reaches till behind the esophageal sphincter. Apparently a sphincter is found at the end of the curved tube, which leads from the excretory ampulla to the exterior. Another sphincter connects the ampulla and this tube. When the larva is alive it is very easy to observe the functioning of the excretory apparatus. Just after the ampulla has fallen down, having ejaculated its contents, it appears again in the same manner as a pulsatile vacuole of protozoa. I think it would be of great importance to compare the histology and the physiology of the pulsatile vacuole of protozoa with the excretory ampulla of nemas. I figured some phases of the ex-

cretory ampulla (Pl. III, Fig. 5 and 6) of the larve of *A. caninum*, which behaves in the same manner.

Finally I will point to the fact that the tail of the third stage larva, opposite to the tail of the second stage larva (sheath) is rather short and ends bluntly (Pl. I, Fig. 7—10).

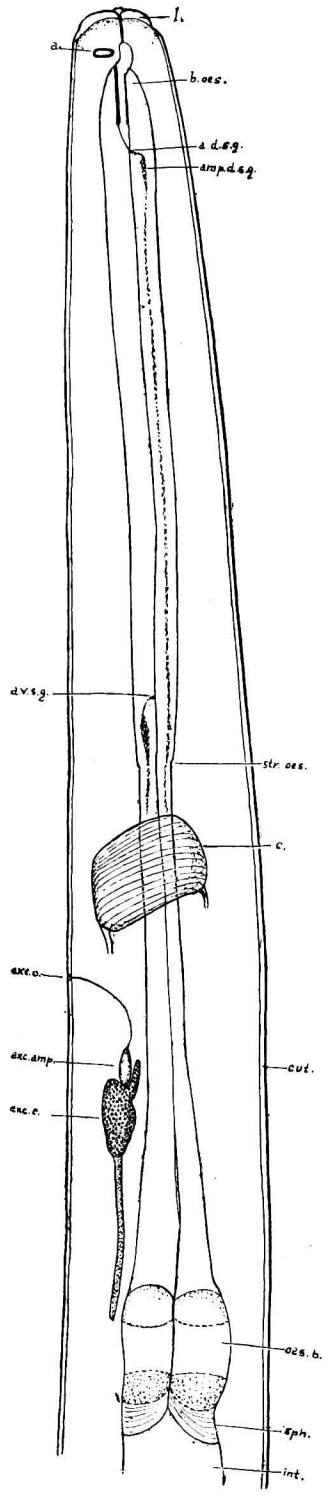
The third stage larva of Anchylostoma caninum.
Textfigures B, C, Plate II en III.

Seen on top the larvae of *A. caninum* are easily to distinguish from those of *Necator americanus* as the first ones possess three lips, which reduce the oral aperture to a small triangular space. If once observed, these lips (*l*) are also very obvious, when seen from aside (Pl. 2, Fig. 2—5, Pl. 3, Fig. 1).

It is very interesting to note that PERRONCITO first found these lips in *A. duodenale*, whereas LOOSS denied their existence. The papillae (Pl. 2, Fig. 4 pap.) are situated on the lips; each dorsal lip bears one papilla only (Pl. 2, Fig. 1 d. p.), whereas the subventral lip is in the possession of 2 papillae (Pl. 2, Fig. 1, subv. p.). The amphids (amph.) are larger than in *Necator* (Pl. 2, Fig. 4—6), dont show a lateral slit, which character is very obvious, when the larva is observed from aside. Moreover they are shifted further caudal. Therefore when the papillae are focussed, the amphids are not to be seen (Pl. 2, Fig. 1). The amphidial pouch (amph. p.) is much wider in this species than in *Necator*, its wall is however thinner, which character is not figured in my drawings (Confer Textfig. C, Fig. 5).

The oral cavity is short and narrow (Pl. 2, Fig. 2—5). The apical end of the esophageal wall is provided with three rodlike thickenings, which VAN THIEL correctly described to be of unequal thickness. The ventral thickening is the thickest and at their apical end these rods are connected two by two. A widening of the oral wall forms the junction between the short oral cavity and the esophageal lumen. In this cavity the spoon-shaped parts, which articulate with the longitudinal rods, project. Thus the oral cavity gets the shape of an urn. Compare also VAN THIEL's description. The latter author did not understand the mutual relations of the described parts.

The esophagus differs in its shape and structure in many points from that of *Necator*. For instance a distinct anterior bulb is absent, although the oesophagus is slightly constricted just in front of the nerve ring (Textfig. B, C, Pl. 2, Fig. 8, Pl. 3, Fig. 4, oes. r.), which is broader than in *Necator*. The structure of the esophagus has not been changed however interiorly at this spot. Slightly more apical (Textfig. B, a. v. s. g.) the ventral salivary glands open into the esophageal lumen (Confer Pl. 2, Fig. 8) which LOOSS figured minutely in his well known monograph. Sometimes, viz. when the larvae are sufficiently transparent,

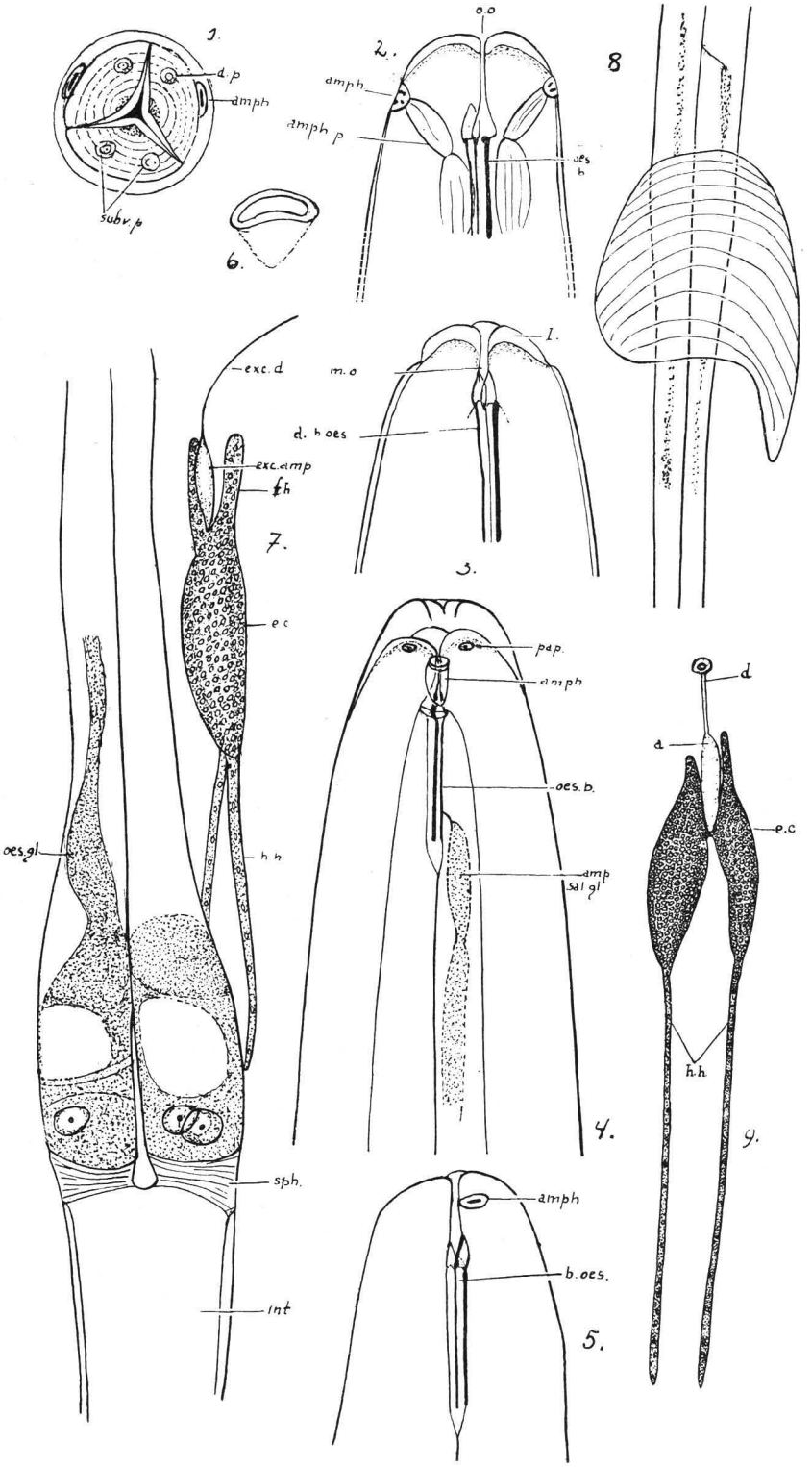


Textfigure B.

one is able to observe nerves (Pl. 3, Fig. 4 *n*) originating from the nervering and running in caudal and apical direction. The posterior bulbus (Textfig. B, oes. b.) misses the valvulae, LOOSS ascribes to it. Its lumen has somewhat thickened walls only. Compare Pl. 2, Fig. 7 and Pl. 3, Fig. 2 and 3. Moreover the bulbus is not sharply separated from the rest of the esophagus, but gradually passes into it. In structure this bulbus also differs from that of *Necator*. Its granulation is scanty. The clear rings (Pl. 2, Fig. 7), which were very obvious in *Necator* are rather inconspicuous here. The nuclei of the salivary glands however are more pronounced (Pl. 3, Fig. 2, n. oes. c.). The middle portion of the bulbus is vacuolised. I mentioned already the ventral salivary glands, the dorsal salivary gland stretches further apical, its ampulla (amp. d. sal. gl.) is narrower than that of *Necator*, whereas the efferent tube opens into the esophagus just in front of the proximal end of the esophageal cylinder (Textfig. B., a. d. s. g.). In exceptional cases only it is possible to follow the course of the esophageal glands (oes. gl. Pl. 2, Fig. 7) in the bulbus of the living animal. The larva of *Anchylostoma* possesses a similar sphincter (Textfig. B, Pl. 3, Fig. 7, Pl. 3, Fig. 2, 3, sph.) at the junction of the esophagus (l. oes.) and intestine (int.). Here the intestinal wall forms a continuous curved line with the sphincter, therefore this structure resembles the mediastinum of mammals. Once I had the opportunity to observe the sphincter,

Textfigure B.

Anchylostoma caninum, third stage larva.
Enlarg. Öl Imm. 1,5 mm, Oc. 8, Tubus 140 mm. × 1/2.
a. = amphid; *a. d. s. g.* = aperture dorsal salivary gland; *a. v. s. g.* = aperture ventral salivary gland; *amp. d. s. g.* = ampulla dorsal salivary gland; *c* = oesophageal nervering; *b. oes.* = beginning of esophagus; *exc. c.* = excretory cell; *exc. amp.* = excretory ampulla; *exc. o.* = orifice excretory apparatus; *int.* = intestine; *l.* = lip; *oes. b.* = esophageal bulbus; *sph.* = sphincter; *str. oes.* = constriction of esophagus.



Anchylostoma caninum, third stage larva

Fig. 1. The head, frontview, enl. Öl Imm. 2 mm., Oc. 12, Tubus 140 mm. × 2/3.
Fig. 2. Head in profile, Enlarg, Öl Imm. 1.5 mm., Oc. 12, Tubus 140 mm. × 2/3.
Fig. 3. enlarg. as in 2.
Fig. 4. enlarg. as in 2.
Fig. 5. enlarg. as in 1.
Fig. 6. Amph., enlarg. as in 1.
Fig. 7. Oesophagus with the excretory apparatus, enlarg. as in 1.
Fig. 8. Oesophagus and nervering, enlarg. as in 1.
Fig. 9. Excretory apparatus enlarg. Öl Imm. 1.5 mm., Oc. 8, Tubus 140 mm. × 2/3.

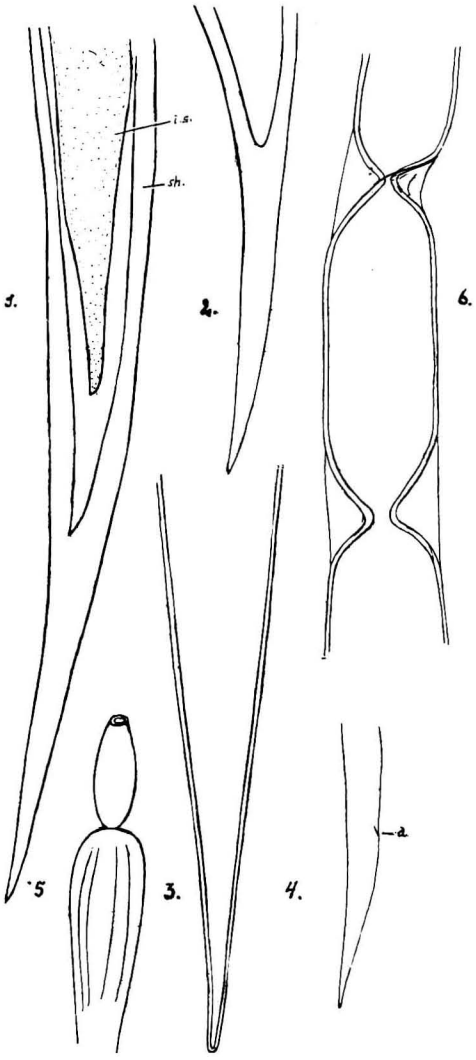
a = ampulla excretory apparatus; *amph.* = amphid; *amph. p.* = amphidial pouch; *amp. sal. gl.* = ampulla of salivary gland; *b. oes.* = beginning of esophagus; *d* = excretory porus; *d p.* = dorsal papilla; *e. c.* = excretory cell; *exc. amp.* = excretory ampulla; *exc. d.* = excretory duct; *h.h.* = posterior horns of excretory apparatus; *f.h.* = anterior horns of the same; int. = intestine; *l* = lip; *m.o.* = oral aperture; *m.o.* = mouthcavity; *oes. b.* = esophageal rods; *oes. gl.* = esophageal gland; *pap.* = papilla; *d. b. oes.* = dorsal oesophageal rod; *subv. p.* = subventral papilla.

on which the bulbus rests as a recipient in the ring of a water-bath ("au bain marie"), while functioning. The esophagus was filled with bacteria (Pl. 3, Fig. 3.). Now and then the sphincter (sph.) opened and moved the esophageal contents to the intestine (int.). The latter has a rather narrow lumen in *Anchylostoma*, whereas its wall is thickly granulated. So SVENSSON's supposition has been prooved. LOOSS described the junction of esophagus and intestine in *Anchylostoma duodenale* as follows:

"Die vordersten Zellen des Darmrohres heben sich deutlich von den volgenden ab, dadurch dass sie kürzer bleiben und in ihrem Innern keine Körnchen abschieden, auch sich so dicht an den Bulbus anlegen, dass sie eher zu diesem als zu dem Darme zu gehören scheinen. Sie representieren die Anlage des späteren zelligen Verschlusapparatus am Ende des Oesophagus".

Now apparently LOOSS has not catched the true meaning of the sphincter, as the sphincter really belongs to the esophagus and contains muscular fibres. The intestine commences caudal from the sphincter. VAN THIEL speaks in this connection about some cells serving for the purpose of closing the entrance of the intestine ("Schliesszellen").

The excretory apparatus is H-shaped (Pl. 2, Fig. 7, 9, Textfig. B), the anterior horns (f. h.) are very short and embrace the excretory ampulla (exc. amp.), the posterior horns (p. h.) lay astride over the intestine. The apical portion of this apparatus is wide, shows no constriction and reaches till about



Textfigure C.

Anchylostoma caninum,

Fig. 1. Sheath enveloping the third stage larva Enlarg.: Obj. 12 mm., Oc. 8. Tub. 140 mm. × 1/2.

Fig. 2. Empty sheath, enlarg. as in 1.

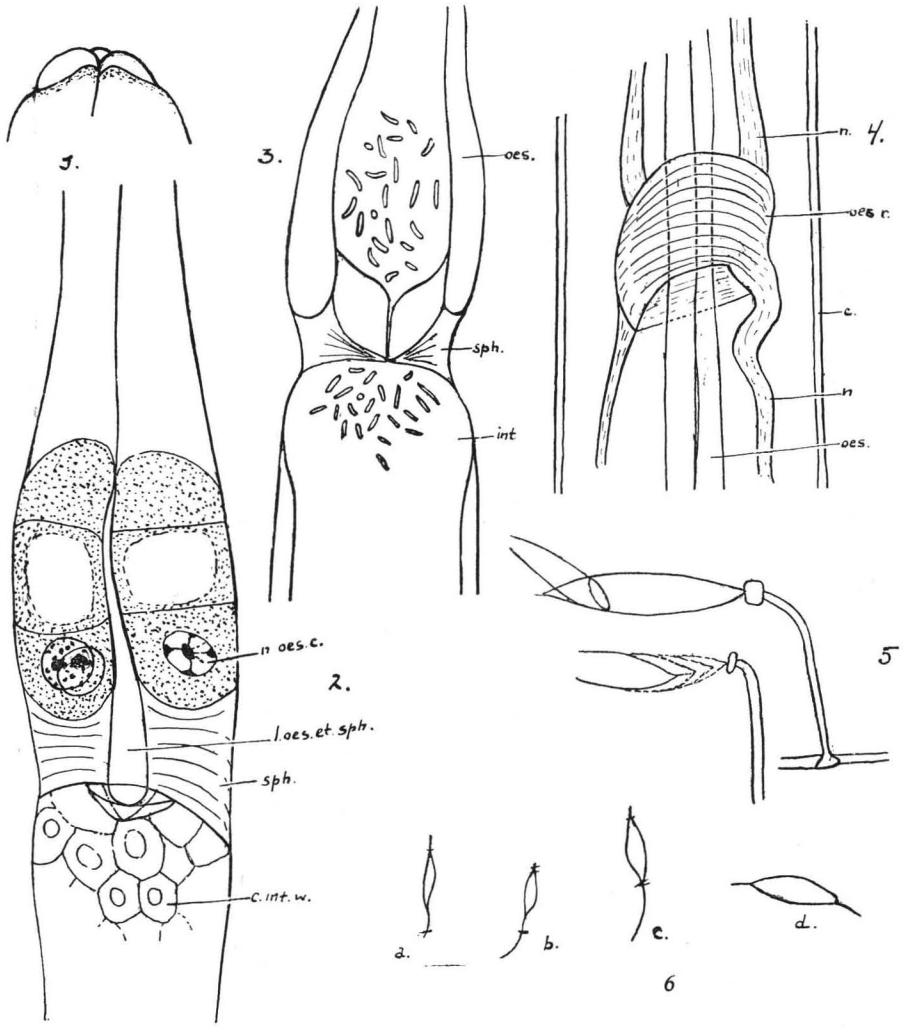
Fig. 3. Tail of third stage larva; enlarg. as in 1.

Fig. 4. Tail of third stage larva; enlarg. Obj. 16 mm., Oc. 4, Tub. 140 mm. × 1/2.

Fig. 5. Amphid: enlarg. Öl Imm. 4 mm., Oc. 12 Tub. 140 mm. × 1/2.

Fig. 6. Shedded skin of second stage larva; enlarg. Öl Imm. 2 mm. Oc. 4, Tub. 140 mm. × 1/2: *a* = anal opening; *i. s.* = third stage larva; *sh.* = sheath.

PLATE III.



Anchylostoma caninum, third stage larva.

Fig. 1. Head with papillae, Enlarg. Öl Imm. 2 mm., Oc. 12, Tubus 140 mm. × 2/3.

Fig. 2. Esophagus and esophageal bulbus Enlarg. Öl Imm. 1.5 mm., Oc. 12, Tubus 140 mm. × 2/3.

Fig. 3. Sphincter between intestine and oesophagus; Enlarg. Öl. Imm. 1,5 mm. Oc. 8, Tubus 140m m. × 2/3.

Fig. 4. Esophageal nervering and nerves, enlarg. as in 3.

Fig. 5 & 6. Excretory apparatus while functioning, enlarg as in 2, the stripes indicate the points of maximum expansion.

c. = cuticula; *c. int. w.* = cells intestinal wall; *int.* = intestine; *l. oes. et sph.* = lumen of esophagus and sphincter; *oes.* = oesophagus; *oes. r.* = esophageal nervering; *n.* = nerve; *n. oes. c.* = nucleus of esophageal cell, *sph.* = sphincter.

the middle portion of the posterior esophageal bulbus. In Plate 3, Fig. 5 and 6 I have figured how the ampulla gradually enlarges till the utmost tension has been reached and an ejaculation follows: compare p. 118.

The tail of the sheath (Textfig. C, Fig. 1 and 2) is shorter than the same part in *Necator*. VAN THIEL compares its point with a sharpened pencil-point. The tail of the third stage larva (Textfig. C, Fig. 1, 3, 4), is considerably longer than the tail of the same stage larva of *Necator*, whereas its point is also finer. The transverse striations visible on the sheath as well as on the skin of the third stage larva are rather inconspicuous; striae much closer than in *Necator*.

One is inclined to ask what bearing thesef acts have on the relationship of these parasites with free-living nematodes. They differ widely from *Rhabditis* in the structure of the mouth cavity, number of papillae and structure of esophagus.

Concerning other free-living forms as *Rhabdolaimus* (especially *Rh. terrestris*) to which these show an unmistakable resemblance, we know too little to enable us to arrive at trustworthy conclusions. Moreover, the adult forms of *Necator*, as well as of *Anchylostoma*, up to the present have not been studied along these lines, so that further speculation must be postponed until at least a part of this program has been fulfilled.

SUMMARY.

An accurate morphological research on the larvae of two species of the Genera *Anchylostoma* and *Necator* gave a number of differences between the concerned nemas. The main differences were found to consist in the structure of the head and its organs, lips, papillae, amphids, in the covering of the apical end of the esophagus, the presence or absence of the anterior esophageal bulbus, in the structure of the posterior esophageal bulbus, the structure of the esophageal (salivary) glands, in the sphincter forming the junction between esophagus and intestine, in the structure and shape of the excretory apparatus, in the shape of tail and sheath of the third stage larva and in the distribution of the transverse striations on the skin of second and third stage larvae.

Now it is possible to distinguish between the larvae of *Necator* and those of *Anchylostoma*. In large parts of America, where *Necator americanus* is the only human hookworm it is rather easy to conclude at the presence or absence of human or canine hookworm larvae in infested soil.

As far as the Dutch East Indies are concerned we are not allowed to go so far in our conclusions, but as for instance *Anchylostoma duodenale* is a rather rare parasite in several provinces of Java the above mentioned facts may be sufficient for those regions to arrive at trustworthy conclusions. Further I expect that a new research taking into

consideration the larvae of *A. duodenale* and *A. ceylanicum* will provide us with new data, which will enable us to identify all concerned larvae by means of easy characteristics.

The present research emphasizes also that a study of free living nemas is indispensable for Parasitologists and that a uniformity in methods of research is quite necessary for both groups of Nematologists.

POSTSCRIPTUM.

When controlling my proofs I saw a paper of SVENSSON and KESSEL (Journal of Parasitology Vol. 13, Dec. 1926) which brings more extensive data about the larvae of *Necator americanus*, *Anchylostoma duodenale* and *A. caninum*. The differences which they emphasize especially are the so called protusile onchium of COBB, which they think acts as a spear in the sense of COBB and the esophageal sphincter. Some illustrations are added. With respect to the morphology this paper is only a somewhat more minute description of the facts contained in the previous paper of SVENSSON of 1925.

LITERATURE.

COBB, N. A. The Pharynx and alimentary Canal of the Hookworm larva, Necator americanus, Jl. of Agric. Research Vol. **25**, p. 359, 1923.

CORT, Journal of Parasitology Vol. **11**, p. 228, 1925.

LOOSS, A. The Anatomy and Life History of Anchylostoma duodenale Dub. I, Rec. Egypt. Government School of Medic. 1905.

PERRONCITO, E. La maladie des mineurs du St. Gothard au Simplon. Archives Italiennes de Biologie T. **3**, p. 315, 1882, p. 7, 1883.

SCHUURMANS STEKHOVEN, J. H. Jr. en SCHUURMANS STEKHOVEN—MEIJER, A. W. Anchylostomiasisbekämpfung und Bodeninfektion, Verh. des 4ten Kongr. der F. E. A. T. M.

SCHUURMANS STEKHOVEN, J. H. Jr. en SCHUURMANS STEKHOVEN—MEIJER, A. W. Voorbereidende onderzoekingen met betrekking tot de differentiatie van de larven van de mijnwormen van mensch en hond, Tijdschr. v. Vergel. Geneesk. Dl. **10**, p. 1, 1923.

SVENSSON, R. A morphological distinction between infective larvae of Anchylostoma and Necator. Proc. Soc. f. Experim. Biology and Medicine **22**, 1925.

VAN THIEL, P. H. De differentieeldiagnose van de strongyloïde larven van Necator americanus en Anchylostoma caninum, Tijdschr. v. Vergel. Geneesk. **10**, 1923.

VAN THIEL, P. H. Verdere onderzoekingen omtrent de differentieeldiagnose van de strongyloïde larven van Necator americanus en Anchylostoma caninum. Geneesk. Tijdschr, v. Nederl. Indië, Deel **66**, p. 14, 1926.

VAN THIEL, P. H. Diagnostie différentiel des larves strongyloïdes du Necator americanus et de l'Anchylostoma caninum. Annales de parasitologie humaine et comparée T. **4**, p. 228, 1926.

Utrecht, 21 November 1926.