579.5:595.11:595.2

MOUNTING OF HELMINTHS AND SMALL ARTHROPODS

A new, simple and efficient method

BY

P. DE TOLEDO ARTIGAS

In a recent paper, C. Pereira and Z. Vaz (1) report the difficulties helminthologists encounter in preparing permanent mounts of nematodes between slide and cover-slip. In addition to a review of the already known methods employed in nemathelminthic toto-mouting, these authors complete their work describing a new technic which permits them to obtain very fine nematode preparations, mounted in Canada balsam.

Like these researchers I have been interested for a long time in the solution of such an important problem of parasitological technic as the manner of obtaining permanent nematode preparations. Upon that solution depends the preservation of helminthological material, porticularly the new species types and the organization of collections for teaching purposes.

At the time when Pereira and Vaz published their paper (December 1934) I had made some rather advanced observations concerning a new toto-mounting method. The researches upon this subject having been completed, it was thought advisable to publish the new technic, the characteristics of which are its simplicity and its possibility of yielding uniformily good results.

Technic

In routine technic of investigating helminthological material the method used by Travassos (2 and 3) was followed. This particularly useful method may be thus described:

The helminths fixed in saline-formalin or saline acetic-formalin, are treated with acetic acid and then with phenol. These two substances clarify the mate-

rial to such an extent that the microscopical examination of the nematode is easily carried out.

After some trials it was resolved to use beeck tar creosote instead of phenol. With this variation of the technic very good results have been obtained. In addition to its facility, the creosote is less corrosive and does not cause the burning of one's fingers. This happens very frequently in working with phenol, while manipulating the material to place it in a good position on the microscope for examination. The employement of the acetic acid followed by plenol or creosote brings about a perfect clearness of the nematode without any structural deformation.

This has induced me to seek for a resinous substance, which, being soluble in phenol or creosote, would allow a quick inclusion of the material, previously treated by acetic acid. The steps of a hard and slow dehydration would thus be avoided.

In this regard experiments were carried out with *Pistacia lentiscus* resin (mastic resin), a substance which is easily dissolved in phenol or creosote. The first mounting attempts were made with mastic-phenol, a fluid obtained by the dissolution of resin in phenol (10 gm. mastic to 20 cc. phenol). The results were not entirely satisfactory as the wrinkling of the nematodes was frequently noticed.

Better results were obtained, however, in experiments made with mastic-creosote prepared in the same manner as the mastic-phenol. Good preparations were made using material fixed in saline-formalin or saline-acetic-formalin. The following procedure was used:

1. Treatment of the nematode with acetic acid.
2. Treatment with acetic acid and creosote (equal parts).
3. Treatment with pure creosote.
4. Treatment with creosote-mastic (3 parts creosote, 1 part mastic).
5. Mounting in mastic-creosote (2 parts mastic, 1 part creosote).

At that time two great difficulties were present, the slow hardening of the resin and the presence of many colloidal particles which made rather unsatisfactory the appearance of the preparation. It seemed possible to avoid this by heating the preparation on the Malassez platine. In this way the resin could be solidified in a short time along the sides of the cover-slip and the colloidal particles vanished. This disappearance of the colloidal particles was unstable, however, for the colloidal state often reappeared on returning to the laboratory temperature.

These defects were avoided by making a mounting medium as follows:

30 cc. of 95% alcohol are added to 10 gm. of pulverized mastic. The mixture is left in an incubator at 55°C., for 24 hours. Then, the alcoholic

mastic is taken off and, after cooling, centrifuged. The sediment is poured off by decantation and, if necessary, the fluid is centrifuged once more. 30 cc. of pure creosote are added to the purified alcoholic mastic and the whole mixture is placed in the incubator at 55°C., until the complete evaporation of the alcohol.

The resulting fluid is clear and sirupy; after solidification it takes a yellowish coloration.

Continuing the research, the conclusion was reached that steps 2 and 4 of the above method, although not being harmful, could be omitted. It was also found that the nematode, after successive treatment with acetic acid and creosote, became remarkably resistent to deformation. Even after heating the mastic-creosote to the boiling point, the nematode thoroughly retains its morphological particularities. It may be further added that even very fragile species as *Enterobius vermicularis* (L., 1758), *Habronema megastoma* (RUD., 1819), etc., can be treated successively and with no inconvenience with acetic acid, creosote and mastic-creosote, as they do not show any somatic alteration.

Consequently the following very simple technic has since been adopted:

1. Treatment with acid. The length of time required varies, according to the thickness and size of the nematode and it is completed with its clearing.
2. Treatment with creosote. In this substance the nematode remains until fully impregnated. There is no harm to the specimen in prolonging this step for some hours.
3. Mounting between slide and cover-slip in mastic-creosote fluid.
4. Hardening of mounting medium in the incubator at 50°-55°C.

Small Petri dishes or porcelain "godets" containing acetic acid and creosote are used. The nematodes to be mounted are successively dropped into these receptacles. Finally, mounting is done directly on the slide, after pouring sufficient drops of mastic-creosote on its surface. If the nematode shows any somatic retraction, it is possible to prevent this defect by carefully heating the slide on the Bunsen flame. The preparation is allowed to solidify in an incubator at 50°-55°C. As a result thin preparations at the end of 2-3 days solidify sufficiently at the sides of the cover slip to allow the manipulation necessary for microscopical examination. In thick preparations, however, as soon as the resin becomes firm, the volume of resin decreases. This makes necessary the addition of some more drops of mastic-creosote, without any harmful results.

In order to obtain colored preparations hydrochloric carmine or Semichon's acetic carmine have been used, prepared according to Langeron's indications (4). With both, good results have been obtained. The hydrochloric carmine has, however, a greater power of penetration into the helminths and consequently the nematodes are more rapidly stained. The staining precedes the other

steps of the mounting technic. Its duration varies according to the size, thickness and permeability of the nematode. In case of over-staining, differentiation can be obtained by means of hydrochloric water or hydrochloric alcohol (1% HCl in 70% alcohol). Usually the specimens to be stained are first submitted to the action of acetic acid, which seems somehow to make the nematode cuticle permeable, allowing the easy staining of the material, as well as the penetration of the creosote into it. The acetic acid acts as a mordant. The stained specimens do not lose their coloration by the action of acetic acid and after clearing with creosote present a very fine and clear appearance in the mount.

Using the above described technic, preparations of the following species have been made: *Enterobius vermicularis* (Linn., 1758); *Oesophagastomum (Proteracrum) columbianun* (Curtice, 1890); *Strongylus (Delafondia) vulgaris* (Looss, 1900); *Aspidotera raillieti* (Trav., 1913); *Habronema megastoma* (Rud., 1819); *Heterakis gallinae* (Gmelin, 1890).

In species of small size, one can, generally, notice the arrangements of the different internal organs. In larger species with a more complex organization, the particular analysis of which is difficult, the details of external genital organs (copulatrix bursa, rays of the bursa, spicules, gubernaculum), as well as the structures of the head and eosophagus regions (buccal cavity, teeth, dentigerous ridges, pharynx, eosophagus, eosophageal valves) are always made evident. Cuticular striation and other cuticular formations are usually entirely retained.

The mounting of plathelminths (*Cestoda* and *Trematoda*) have also been accomplished with good results. Small arthopods mounting is also successfully performed by the method.

According to the results thus far obtained, it seems that mounting in mastic-creosote, although not representing an ideal method, permits in a solidifiable medium the preservation of nematodes with the greater part of their morphological characters as usually employed in classification. The simplicity and security of the method may be added to the above reported quality.

SUMMARY

In this paper a new method is described for toto-mounting nematodes. This new method can also be used with plathelminths and small arthropods.

The mounting medium is obtained by dissolving mastic resin (from *Pistacia lenticus*) in creosote (Creosote from beeck tar, F. Merck).

This mastic-creosote fluid is prepared as follows:

30 cc. of 95% alcohol are added to 10 gm. of pulverized mastic. The mixture is left in an incubator at 55°C., for 24 hours. Then, the alcoholic mastic is taken off and, after cooling, centrifuged. The sediment is then poured off by decantation and, if necessary, the fluid is centrifuged once more.

30 cc. of pure creosote are added to the alcoholic mastic and the whole mixture is placed in the incubator at 55° until complete evaporation of the alcohol. The resulting fluid, which is clear and sirupy, after solidification takes a yellowish coloration.

A very simple mounting technic has thus been adopted:

1. Treatment with acetic acid. The lenght of time required varies according to the thickness and size of the nematode and is completed with its clearing.
2. Treatment with creosote. In this substance remains the nematode until fully impregnated. There is no harm to the specimen in prolonging this step for some hours.
3. Mounting between slide and cover-slip in mastic-creosote fluid.
4. Hardening of the mounting medium in the incubator at 50°-55°C.

In order to obtain colored preparations, the material must be treated with hydrochloric carmine or Semichon's acetic carmine (prepared according to Langeron's indications). The staining precedes the other steps of the mounting technic. Over-staining differentiation is done by means of hydrochloric water or hydrochloric alcohol.

Mem. Inst. Butantan
Vol. X — 1935-1936.

Fig. 1. *Enterobius vermicularis* (Linn., 1758).
Femea gravida mostrando os apparelhos digestivo e reproductor.
Pregnant female showing the digestion and reproduction apparatuses.

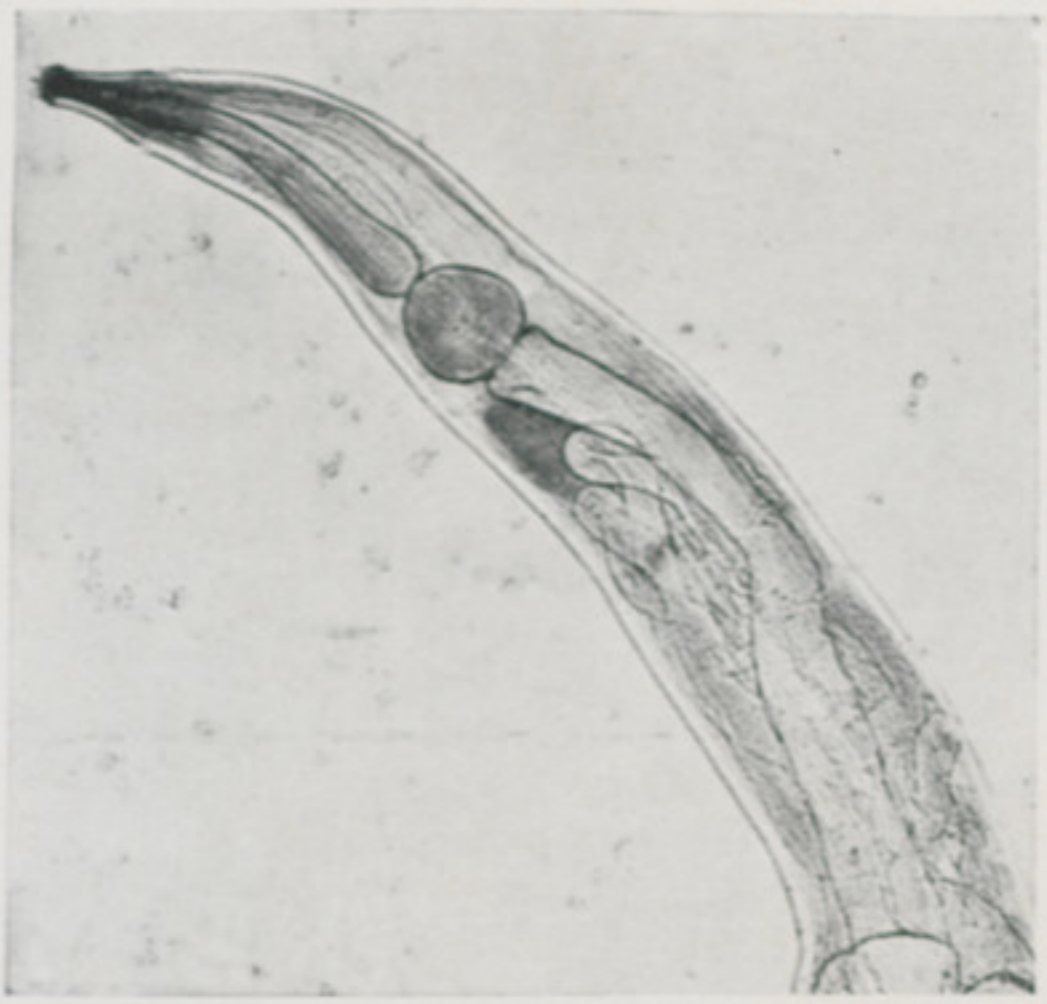

Fig. 2. *Enterobius vermicularis* (Linn., 1758).
Terço anterior de femea visto com maior augmento.
Anterior third of female as seen under greater magnification.

Fig. 3. *Enterobius vermicularis* (Linn., 1758).
Extremidade cephalica de femea vista com grande augmento.
Cephalic end of female as seen under great magnification.

Fig. 4. *Habronema megastoma* (Rud., 1819).
Extremidade buccal observada com grande augmento. (Preparado do dr. F. Fonseca)
Buccal end as seen under great magnification.

Mem. Inst. Butantan
Vo. X — 1935-1936.

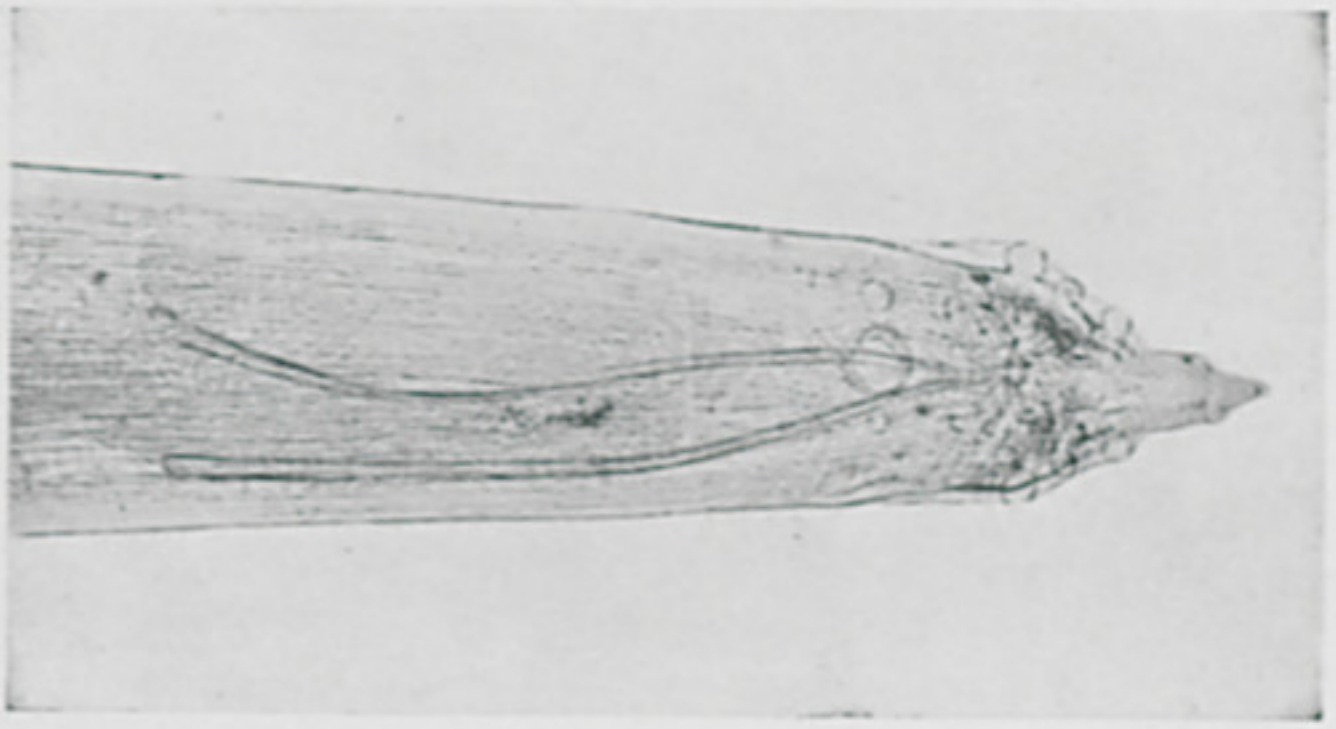

Fig. 5. *Ascaridia galli* (Schrank, 1788).
Extremidade caudal de macho. (Preparado do dr. F. Fonseca).
Tail end of male.

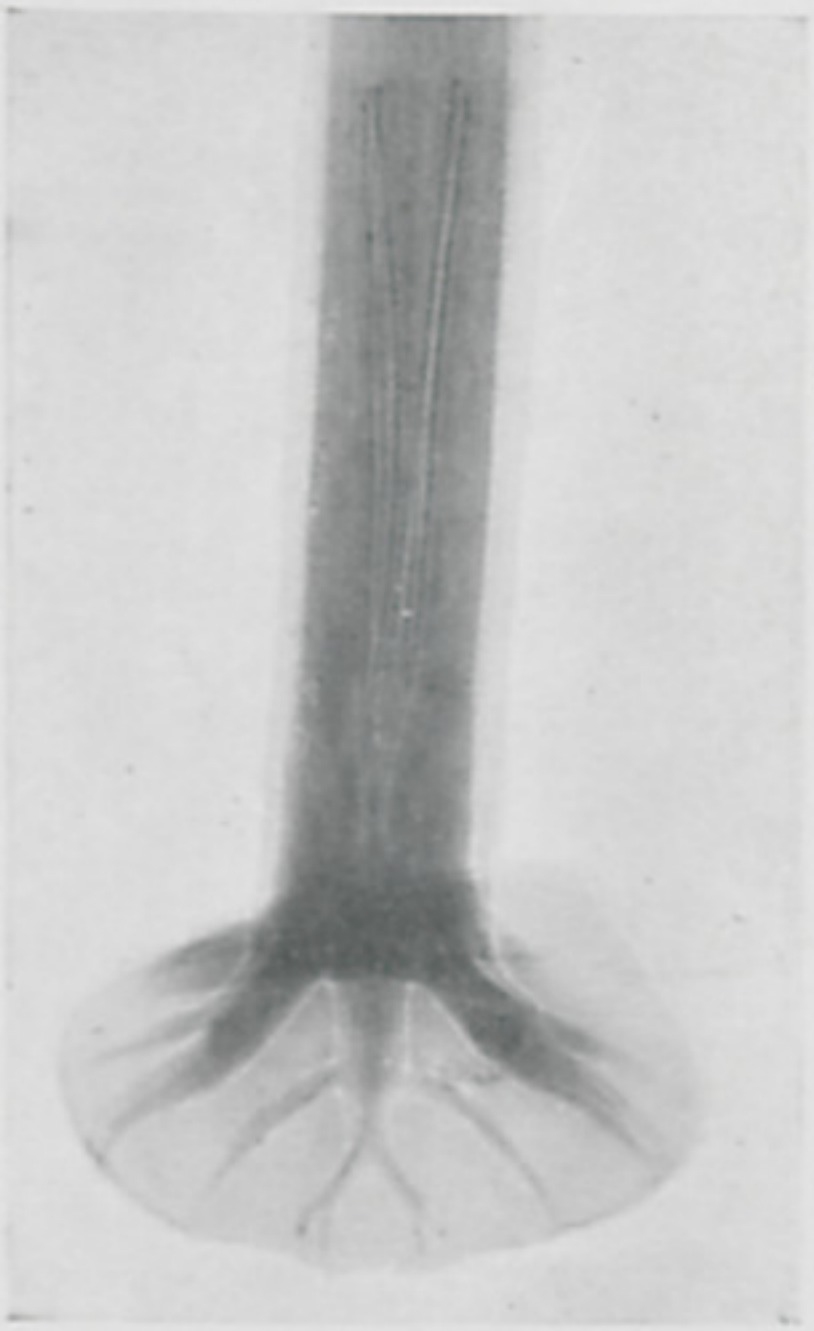

Fig. 6. *Oesophagostomum* (Prot.) *columbianum* Curtice, 1890.
Bolsa copuladora em posição.
Copulatrix bursa.

Fig. 7. *Strongylus* (Del.) *vulgaris* (Looss, 1900).
Capsula bucral de macho.
Buccal capsule of male.

Mem. Inst. Butantan
Vol. X — 1935-1936.

Fig. 8. *Strongylus* (Del.) *vulgaris* (Looss, 1900).
Bolsa copuladora vista de lado.
Copulatrix bursa as seen laterally.

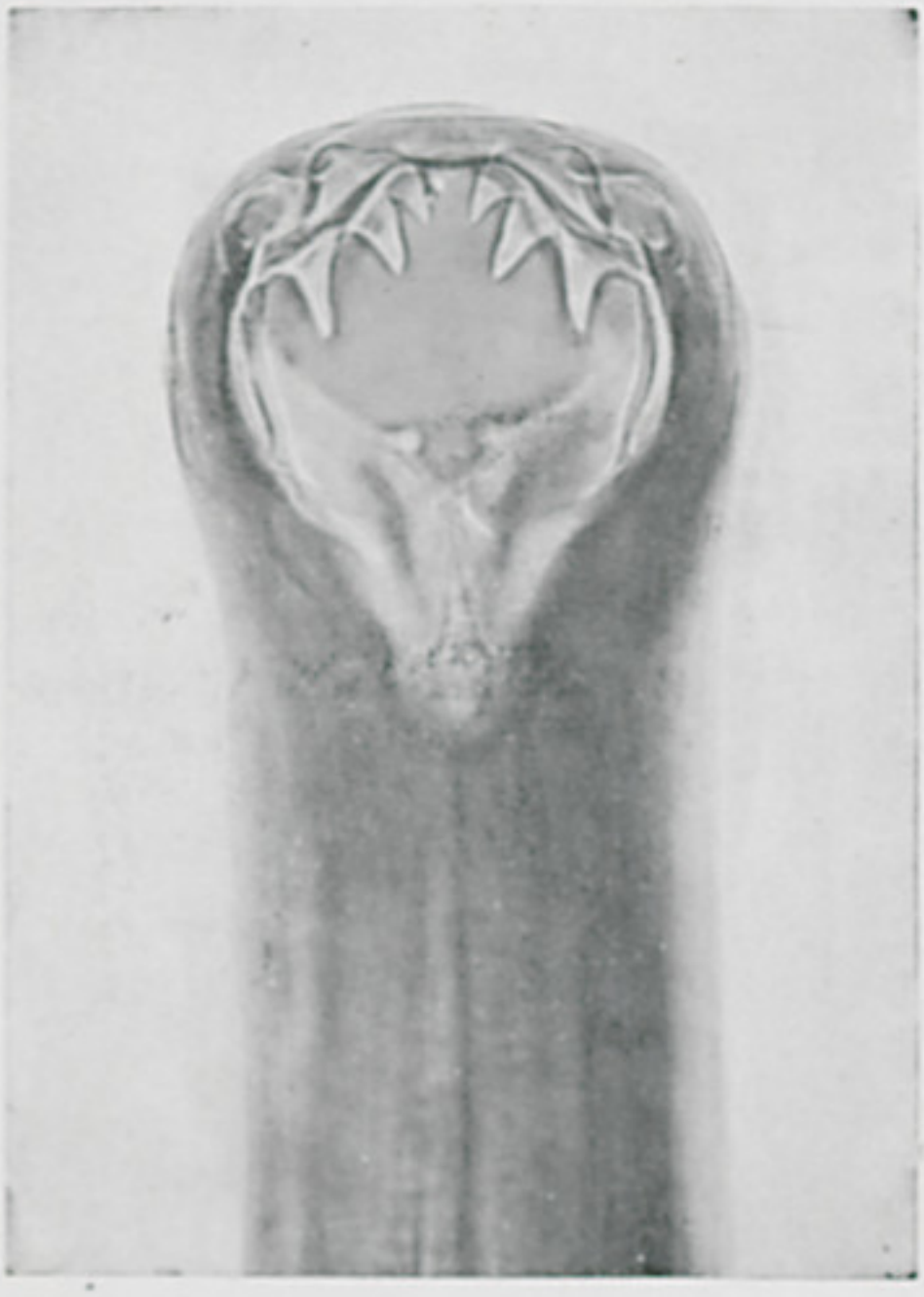

Fig. 9. *Ancylostoma caninum* (Ercolani, 1859).
Capsula buccal de femea vista de frente.
Buccal capsule of female (front view).

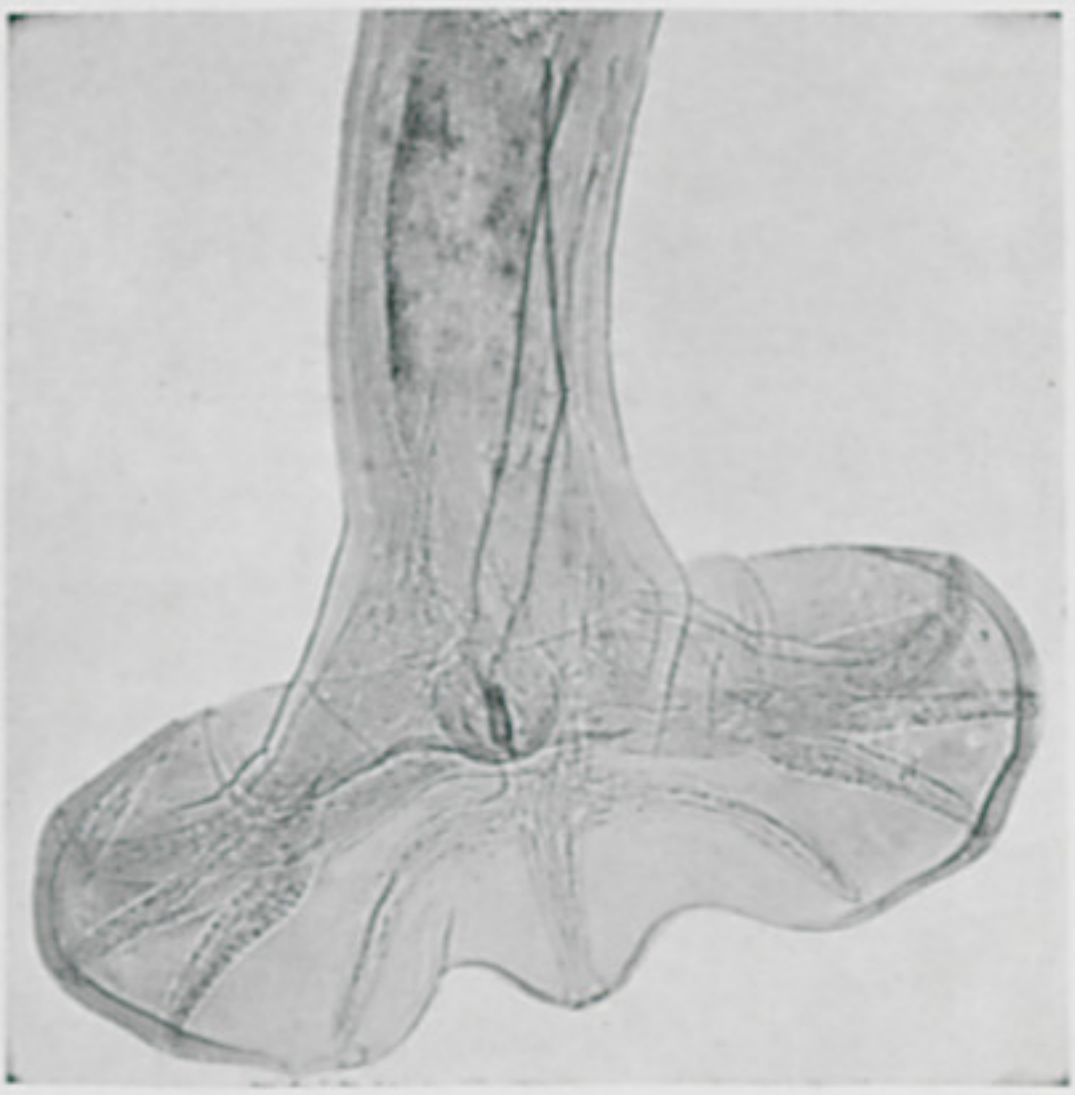

Fig. 10. *Ancylostomum caninum* (Ercolani, 1859).
Bolsa copuladora em posição.
Copulatrix bursa.