

ANATOMY AND HISTOLOGY OF THE FEMALE REPRODUCTIVE SYSTEM OF *Boophilus microplus* (ACARI: IXODIDAE)

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ABSTRACT: The knowledge of the anatomy and histology of the female genital system in ticks is very important as a basic tool in all species in relation to morphological sciences and evolution, physiological events like ovulation, sexual behavior, maturation and mating, and also to research the interrelation tick-hemoparasite. The anatomy and histology of the female genital system in *Boophilus microplus* are scarcely studied. In the present paper, the female genital system of this tick has been considered as a whole and morphological and functional interrelations between different structures and also some other related items has been presented. Unfed and partially engorged females were employed in the study. The quality and resolution of images are remarkable because of the improvement by authors of a histological technique specially for arthropods.

(Key words: ticks; Ixodidae; *Boophilus microplus*; anatomy and histology; female genital system)

ANATOMIA E HISTOLOGIA DEL SISTEMA REPRODUCTOR FEMENINO DE *Boophilus microplus* (ACARI: IXODIDAE)

RESUMEN: El estudio de la anatomía y la histología del sistema genital femenino en las garrapatas es de gran importancia como una herramienta básica para el conocimiento de las diferentes especies en relación con las ciencias morfológicas y la evolución, eventos fisiológicos como la ovulación, conducta sexual, maduración y apareamiento, y además para investigar la interrelación garrapata-hemoparásito. La anatomía e histología del sistema genital femenino de *Boophilus microplus* están escasamente estudiadas. En el presente artículo se describe de forma integral el sistema genital femenino en esta especie y se presentan interrelaciones morfológicas y funcionales entre las diferentes estructuras, así como otros aspectos relacionados. Se emplearon hembras no alimentadas y parcialmente ingurgitadas. La calidad y resolución de las imágenes son notables como resultado del perfeccionamiento de una técnica histológica específica para artrópodos, por parte de los autores.

(Palabras clave: garrapatas; Ixodidae; *Boophilus microplus*; anatomía e histología; sistema genital femenino)

INTRODUCTION

The anatomy and histology of the female genital system in ticks have been studied only in some species by different authors (1,2,3,4,5,6). The knowledge of these subjects is very important as a basic tool in all species in relation to morphological sciences and evolution, physiological events like ovulation, sexual

behavior, maturation and mating, and also to research the interrelation tick-hemoparasite. The midgut, salivary glands and ovary in ticks, are organs of the great interest for their role in tick physiology and/or pathogen transmission (7).

The structure of female genital system in Ixodidae consists of a single U-shaped tubular ovary in the

posterior region of the body, paired and folded oviducts are in both extremes of the ovary joint into a common oviduct or uterus. The uterus opens into the vagina, which is divided in a cervical vagina and a vestibular vagina. A seminal receptacle, absent in argasids (3,4) as well as the uterus, open also in the cervical vagina. A pair of tubular accessory glands pours out their secretions at the level of the union of both cervical and vestibular vagina. Surrounding the vestibular vagina there is a glandular epithelium, the lobular accessory gland, found only in ixodid ticks (4).

The anatomy and histology of the female genital system in *Boophilus microplus* are scarcely studied; only it is possible to refer to articles about oviducts and fertilization (8,9) and studies of the ovary by Saito *et al.* (10). In the present paper, the general objective is to consider the female genital system of this tick as a whole and to analyze the morphological and functional interrelations between different structures. Also, the mating process in the species is approached and the semen transport inside the female's tract is shown. The quality and resolution of images are remarkable because of the improvement by authors of a histological technique specially for arthropods and for the employ of an original procedure for feminine tract dissection that makes it easier and saves time avoiding technical complications.

MATERIALS AND METHODS

Procedures: Histological and dissection Techniques:

The histological technique of Schneider and Rudinsky (11) modified by de la Vega and Fernández (12) was used, to accomplish good results the initial technique needs from 8 to 9 days, whereas that present demands only from 8 to 9 hours and images retain the same quality. Coloration was done with hematoxylin-eosine. A vertical microtome was used; tissue thickness of 3-5 μ was achieved. Some serial slicing were performed transversely and other longitudinally in horizontal or in sagittal sense, this procedure permits a better comprehension of three-dimensional disposition of organs inside the arthropod. Dissection of tick specimens was performed with an original method (13) allowing a faster and secure procedure that could be done in 10 to 15 minutes per tick with a great percentage of success and without loss of genital structure parts.

Ticks: Unfed and partially engorged females of *B. microplus*, before or after mating, were employed in the study.

RESULTS AND DISCUSSION

GENERAL SCHEME OF FEMALE GENITAL STRUCTURE

Figure 1 shows a general scheme of the principal organs that form the female genital system of *B. microplus*, in a lateral view, based on the mentioned papers and present results. As all schemes, it is a generalization from a number of individuals; reader ought to keep in mind that variations

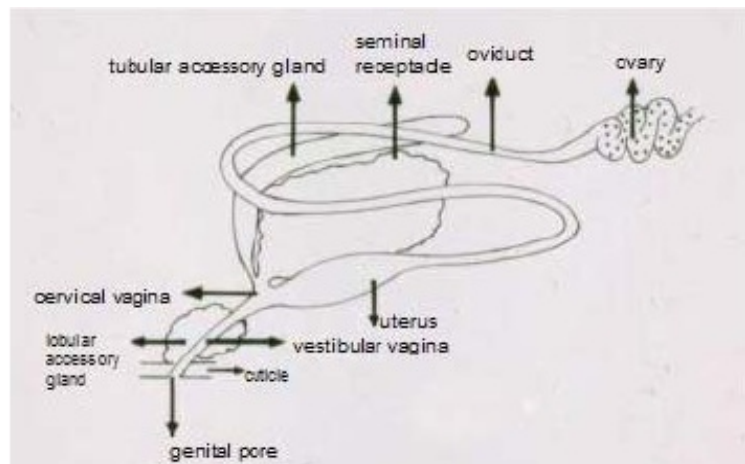


FIGURE 1. *B. microplus* female genital system scheme: lateral view./ *Esquema del sistema genital femenino: vista lateral.*

in each item from different specimens are actually a very frequent fact. Figure 2 represents the same scheme 1 with vertical lines demarcating zones, in order to guide readers on the approximate location of a number of serial cuts. So, next photographs will represent an approach of the anatomical location of these shown structures in relation to these conventional lines.

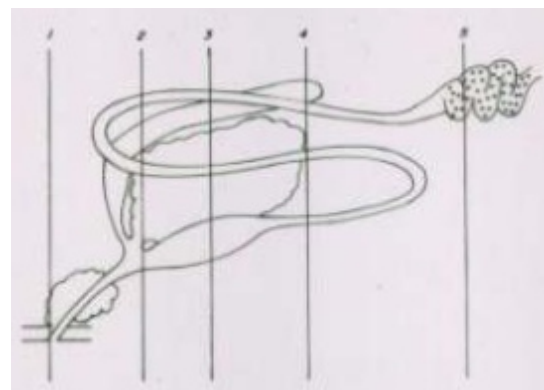


FIGURE 2. General scheme with vertical lines guiding reader about locations of cuts./ *Esquema general con líneas verticales como guía para la localización de los cortes.*

Ovary, oviducts, seminal receptacle and tubular accessory glands obtained by dissection of non-fed and fed, unmated and mated females are shown in Figures 3-5.



FIGURE 3. Ovary, oviducts, seminal receptacle and tubular accessory glands, obtained by dissection of a non-fed female./ *Ovario, oviductos, receptáculo seminal, glándulas accesorias tubulares, obtenidos por disección de una hembra no alimentada. 16X*

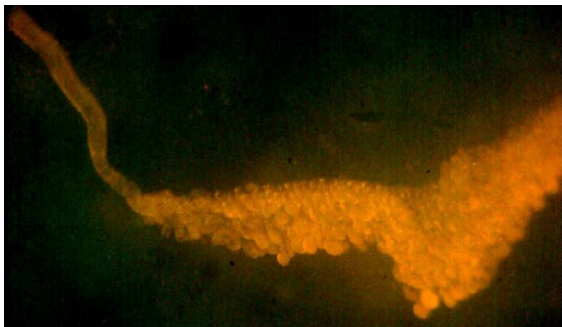


FIGURE 4. Extreme of an ovary and oviduct beginning, by dissection, in a non-fed female./ *Extremo del ovario e inicio del oviducto, por disección, en una hembra no alimentada. 25X*

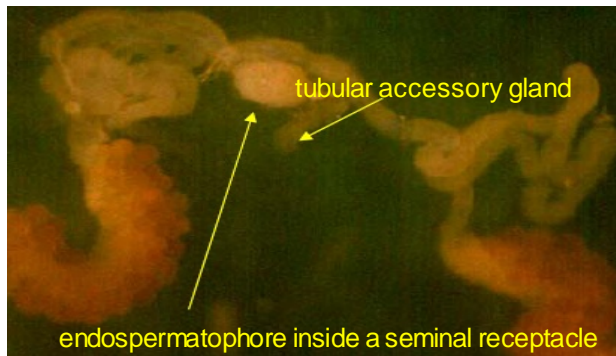


FIGURE 5. A close up of ovary extremes, oviducts, a full seminal receptacle and a tubular accessory gland, by dissection, in a fed and mated female./ *Un acercamiento a los extremos del ovario, oviductos, receptáculo seminal lleno y una glándula tubular accesorias, por disección, en una hembra que se alimentó y copuló. 25X*

Ovary: The histological structure of ovary with oocytes and pedicel cells is shown in figures 6 and 7. The ovary is composed by a layer of small epithelial cells with rounded nuclei surrounding the lumen, and oocytes in different developmental stages: I, II, III, IV and V (10), attached through the pedicel cells to the ovarian wall. Also, the degenerative stage VI has been described (5,10). Cells very similar to the epithelial ones form the pedicels. Pedicel cells holding oocytes I and II stages are cylindrical with large elliptic nuclei occupying most of the cytoplasm, there are numerous round mitochondria as well as rough endoplasmic reticulum, instead, pedicel cells attaching oocytes IV and V show signs of degeneration (14). In *Rhipicephalus sanguineus*, de Oliveira *et al.* (15) considered that there are three probable sources of elements for the oocytes' growth: an endogenous one, through synthesis processes inside the oocyte, and two exogenous ones: the haemolymph and the pedicel cells. Results of studies in *Amblyomma triste* by de Oliveira *et al.* (16) suggest that besides the exogenous production of vitellogenic elements, endogenous

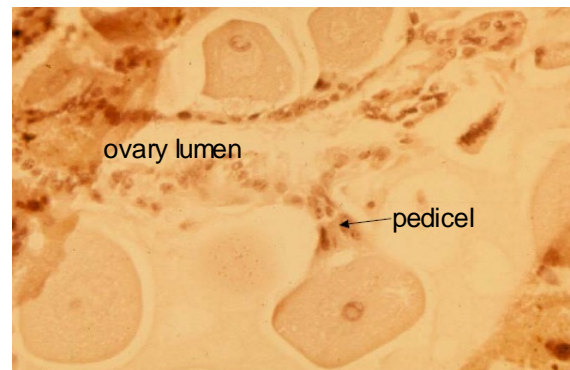


FIGURE 6. Transversal section of the ovary. Relationship between pedicel cells and oocytes is clearly exposed./ *Sección transversal del ovario. Se aprecia la relación entre las células pedicelares y los oocitos. 560X*

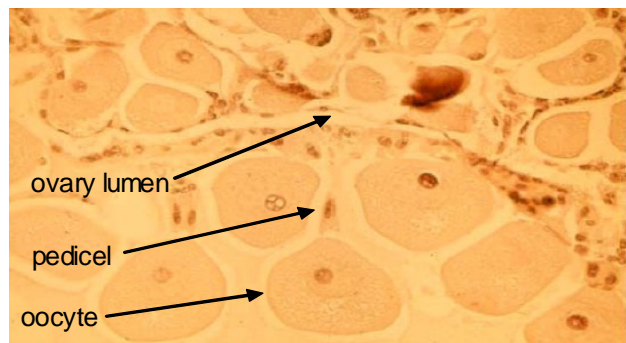


FIGURE 7. Another view of ovary, pedicels and oocytes./ *Otra vista del ovario, pedicelos y oocitos. 560X*

production can take place simultaneously contributing to the development and growth of the oocytes.

Central structures of female genital system

Oviducts: in both endings of the ovary there are two thin folded tubes, in *B. microplus* the diameter of oviducts oscillated between 75 and 140 μ in females feed up to 48 hours; they fuse under the seminal receptacle to form the common oviduct or uterus.

Uterus: It is in communication with the cervical vagina by means of an epithelial folded muscular tube: the **connecting tube** (Fig. 8). In the **cervical vagina** (Fig. 9), besides the uterus, the **seminal receptacle**, **tubular accessory glands (TAG)** and the **vestibular vagina** converge. There are some anatomical particularities in *B. microplus* that are evident when sections obtained in this tick (Fig. 9) are compared with schemes of other species like *Rhipicephalus appendiculatus* and *Hyalomma asiaticum* (1,3). In *R. appendiculatus* and *H. asiaticum*, the cervical vagina is very developed, the debouchment of the seminal receptacle and the uterus form an angle of 180° and are relatively distant one from the other. On the contrary, in *B. microplus*, the cervical vagina is very little, the debouchment of the seminal receptacle and the uterus in the mentioned vagina form an angle less than 90° and the seminal receptacle is completely above the uterus. This fact could facilitate the way for spermiphores to found the course toward the oviducts and ovary.

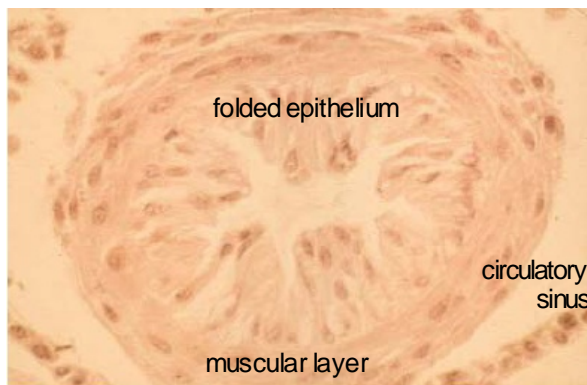


FIGURE 8. A close-up of connecting tube (between cervical vagina and uterus) near line 2. / *Un acercamiento al tubo de conexión (entre la vagina cervical y el útero) cerca de la línea 2. 400X*

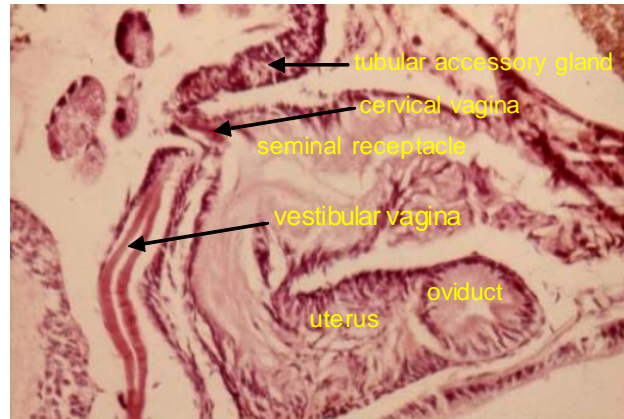


FIGURE 9. Sagittal cut involving vestibular vagina, cervical vagina, tubular accessory gland, empty seminal receptacle, empty uterus and an oviduct debouching in the uterus. / *Corte sagital que interesa la vagina vestibular, vagina cervical, glándula tubular accesoria, receptáculo seminal vacío, útero vacío y un oviducto desembocando en el útero. 200X*

An anatomical view of one of TAG is shown in Fig. 5, and longitudinal and transversal histological views of one of these glands are presented in Fig. 10 and 11. In the female fixed for six days to the host and with 24 hours with a male near it, these glands have a length of 540 μ and 90 μ of wide. **The vestibular vagina:** in a sagittal histological cut (Fig. 12), it is seen that it extends from the genital pore to the cervical vagina. Also, the sagittal sections, and in close up of the genital pore and the final part of the vestibular vagina can be seen in Fig. 13, 14, 15. The **lobular accessory gland** is also shown in Fig 12; it is an enlargement of the vestibular vaginal epithelium which produces a secretion for the initial waterproofing layer for the eggs and also it is the most likely source of the genital sex pheromone (4, 17). Transversal sections of vestibular vagina appear in Fig. 16-18, they show that this structure is irregular in shape along it. Next transversal cuts (Fig. 19-22) show images of the central part of the genital organs. In these images, all structures seem to be surrounded by circulatory sinus (in fact they were never shown in images of *B. microplus*). It is conspicuous the likeness of the images in all photographs and this fact discards the possibility of being a histological technic artifact.

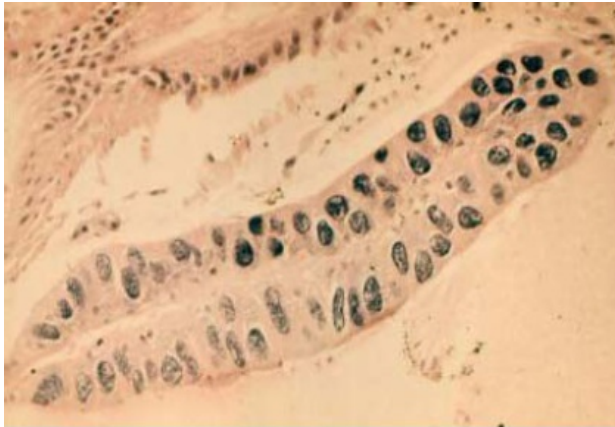


FIGURE 10. Sagittal cut of a tubular accessory gland./ *Corte sagital de una glándula tubular accesoria.* 240X

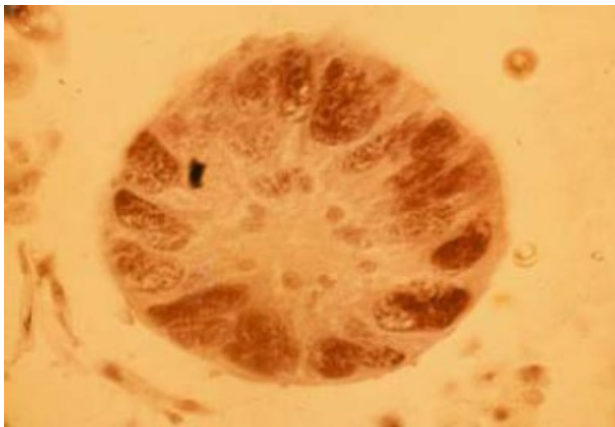


FIGURE 11. Transversal cut of a tubular accessory gland./ *Corte transversal de una glándula tubular accesoria.* 350X

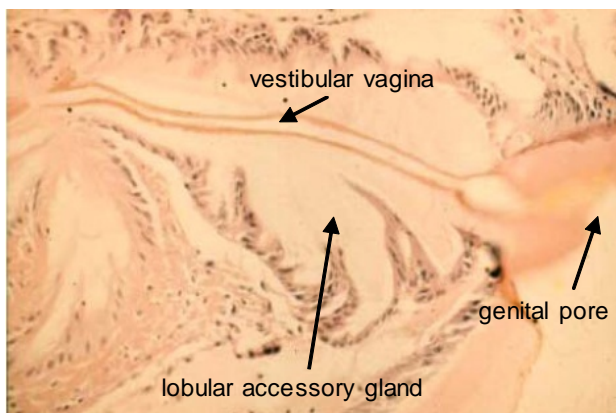


FIGURE 12. Sagittal cut of the vestibular vagina and its lobular accessory gland, view of the genital pore./ *Corte sagital de la vagina vestibular y su glándula lobular accesoria, vista del poro genital.* 230X

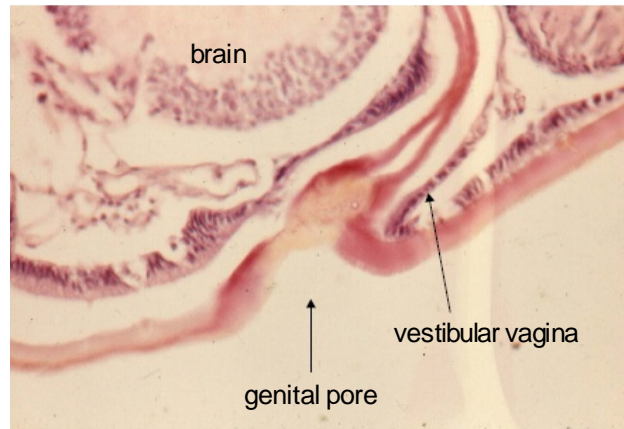


FIGURE 13. Sagittal section, in close up with the distal part of the vestibular vagina and the genital pore./ *Sección sagital, en un acercamiento de la porción distal de la vagina vestibular y el poro genital.* 250X

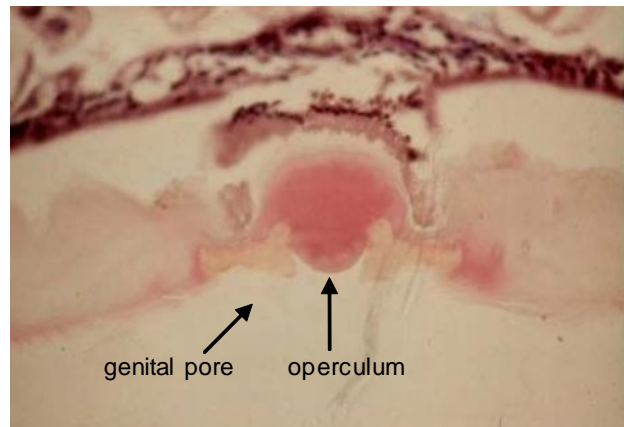


FIGURE 14. Transversal cut (a close up) made through the genital pore line 1./ *Sección transversal, con acercamiento a nivel del poro genital línea 1.* 250X

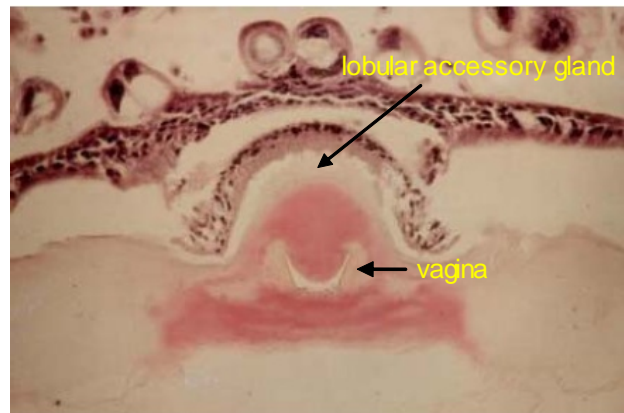


FIGURE 15. Section made near line 1, it begins to be apparent the lobular accessory gland./ *Corte realizado cerca de la línea 1, comienza a apreciarse la glándula lobular accesoria.* 275X

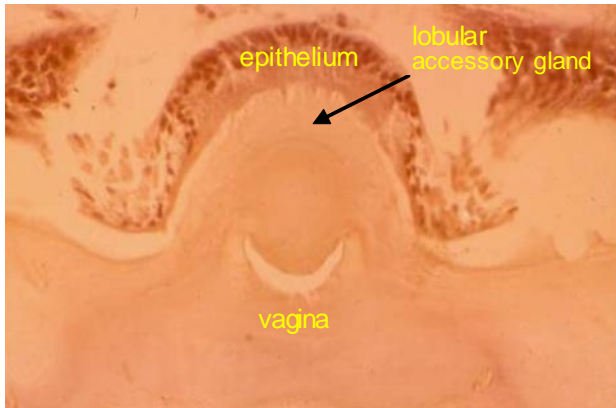


FIGURE 16. Transversal cut made between lines 1 and 2./ *Corte transversal realizado entre las líneas 1 y 2. 200 X*



FIGURE 17. Transversal cut made between lines 1 and 2./ *Corte transversal realizado entre las líneas 1 y 2. 200 X*



FIGURE 18. Transversal cut between lines 1 and 2./ *Corte transversal entre las líneas 1 y 2. 200 X*

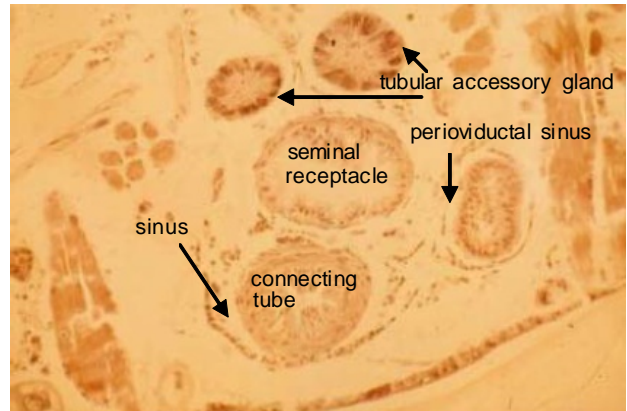


FIGURE 19. Transversal cut between lines 2 and 3, different sinus are seen surrounding seminal receptacle, oviducts, connecting tube and tubular glands./ *Corte transversal entre las líneas 2 y 3, se aprecian diferentes senos rodeando el receptáculo seminal, oviductos, tubo de conexión y glándulas tubulares. 200X*

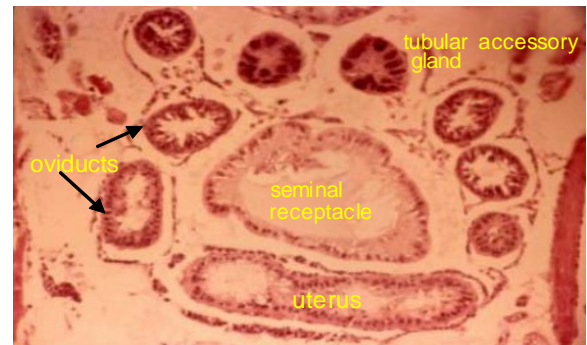


FIGURE 20. Transversal cut of oviducts, seminal receptacle, uterus and tubular accessory glands. It seems that all these structures are surrounded by vascular sinus, this image is situated between lines 2 and 3./ *Corte transversal de oviductos, receptáculo seminal, útero y glándulas tubulares accesorias. Al parecer todas estas estructuras están rodeadas por senos vasculares, esta imagen está situada entre las líneas 2 y 3. 125X*

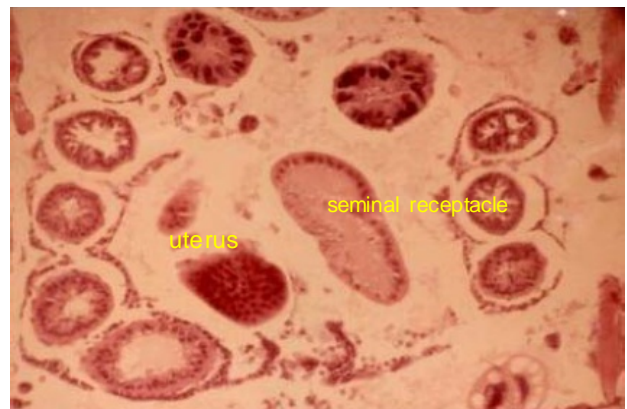


FIGURE 21. This histological cut passed near line 4./ *Corte histológico que pasó cerca de la línea 4. 125 X*

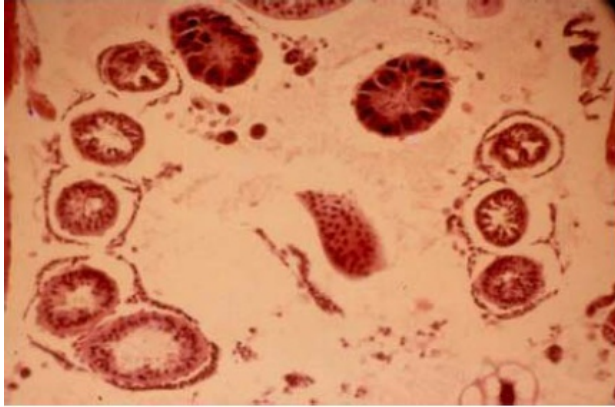


FIGURE 22. This is scraping the end of both, uterus and seminal receptacle cut made near or between lines 4 and 5./ *Corte rozando el extremo distal del útero y el receptáculo seminal cercano a la línea 4 o entre las líneas 4 y 5. 125 X*

Gené's organ: It is an organ only observed in ticks, it is located in the interior of the body cavity near the anterior end of the scutum (2,4). It is a paired structure formed by globular or finger like sacs sometimes called horns and glandular tissue. Different authors (1,2,3,4) have made a full description of this organ. When not in use, the organ is retracted within the anterior region of the body near the capitulum. In Fig. 23, a transversal cut can be appreciated at the cheliceral level, a horn and glandular tissue of the Gené's organ can be seen.

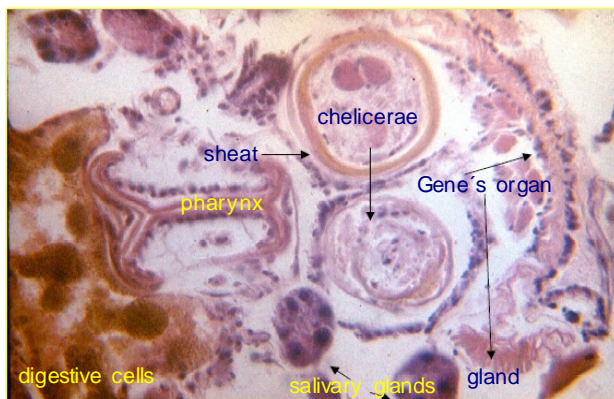


FIGURE 23. Transversal cut through the capitulum, chelicerae with their sheaths, the double Y form of pharynx is also seen and some salivary glands and elements of Gené's organ: two horns and one of the glandular structures./ *Corte transversal a través del capitulum, queléceros con sus vainas, se aprecia la doble Y característica de la faringe y algunas glándulas salivares y elementos del órgano de Gené: dos cuernos y una de las estructuras glandulares. 220X*

When oviposition begins, the swollen structure is projected forward by hydrostatic pressure, the finger-like processes are everted from the body (4). In Fig. 24, it can be appreciated an everted Gené's organ through the camerostomal fold just before oviposition begins. Gené's organ deposits wax on each egg as it is delivered from the vagina. The wax coat waterproofs the eggs and causes them to stick together (17) forming huge masses that also contribute to retard desiccation.

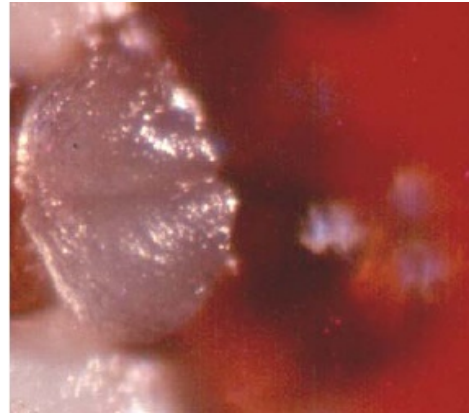


FIGURE 24. Two horns of Gené's organ have protruded through the camerostomal fold just before oviposition./ *Dos cuernos del órgano de Gené proyectados a través del pliegue camerostomal justamente antes del inicio de la oviposición. 15X*

Copula and semen transport

Male climbs over the back of the female and then slide down beneath the couple looking for the genital pore (18,19) and put itself venter to venter with the female, grasping it with its legs (Fig. 25). The male



FIGURE 25. A couple of *B. microplus* in mating / *Pareja de B. microplus en posición de cópula. 12X*

bends its capitulum toward the female genital aperture and then introduces its chelicerae which protrude out of its shafts into the female genital pore (18,19, 20); both hypostome and palps briefly touch the surface of the female, receptors on the palps mediate pheromone perception, these sensilla are concentrated on the fourth segment of the palp on hard ticks (20). A detail study of mating and sexual tick behavior could be read in Kiszewski *et al.* (20).

Male introduces its chelicerae inside the female genital pore and then ejaculates into a sac, named ectospermatophore; inside it, there is another sac the endospermatophore full of immature seminal cells named spermatids. The male takes the ectospermatophore with its chelicerae by the neck of the sac and introduces it into the female genital pore (18). In Fig. 26, a female with the ectospermatophore hanging from the genital pore can be seen, it is a weak union, and for this reason, it is not frequent to see it. The ectospermatophore is pear-like in shape and the maximum diameter oscillates between 400-500 μ in this tick. The endospermatophore has a rounded shape (350-400 μ) with two appendices, one greater than the other one. These two appendices have been also described in other ticks and are characteristic of Ixodidae, whereas in Argasidae, there is only one (18).

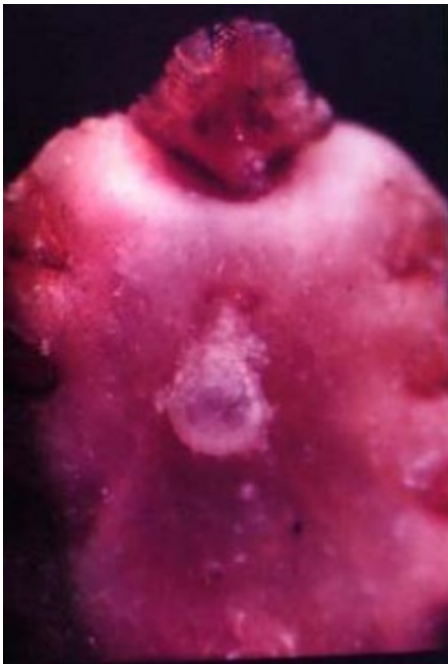


FIGURE 26. Ectospermatophore attached to the female genital pore shortly after copulation./ *Ectoespermatóforo unido al poro genital femenino poco después de la cópula.* 20 X

Spermatids suffer a capacitation inside the seminal receptacle in order to complete maturation; already mature cells are called spermiofores (Fig. 27, 28, 29, 30). The minimum time required after the copula is performed is seven days. When sexual cells are already mature, the endospermatophore's wall breaks down and spermiofores form a compact packet passing into the uterus (Fig. 31). Finally in Fig. 32, it can be seen how spermiofores are released inside the feminine tract. It has been suggested (9) that fertilization takes place in the internal cylinder which extends from the uterus to the ovary itself.

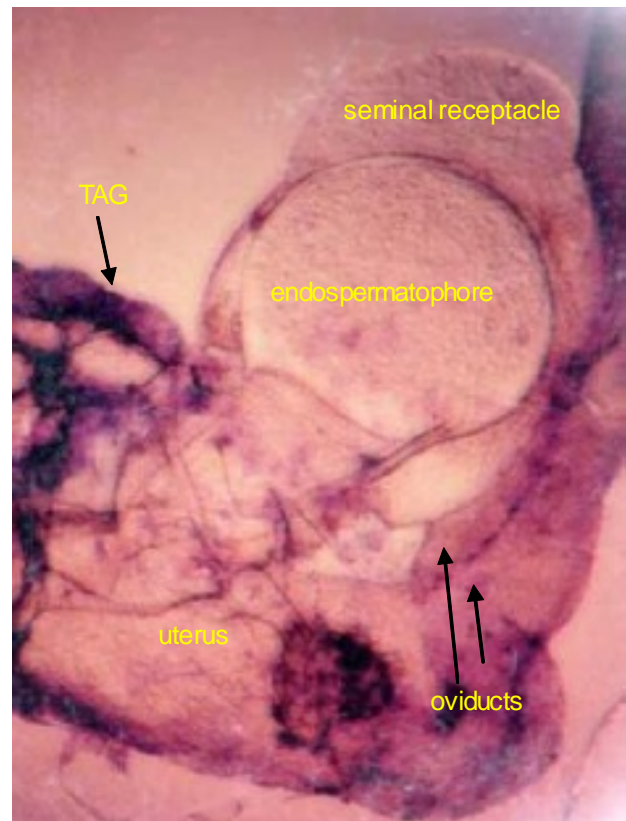


FIGURE 27. A detailed view, by dissection, of a seminal receptacle containing an endospermatophore, two hours after the meeting of the female with a male. Also there are images of the uterus, oviducts and a tubular accessory gland (TAG). Organs are slightly crushed between glass sheets./ *Vista detallada, por disección, del receptáculo seminal conteniendo un endoespermatóforo dos horas después del encuentro de una hembra con el macho. Además, imágenes del útero, oviductos y una glándula tubular accesorria (TAG). Los órganos se encuentran ligeramente aplastados entre dos láminas de vidrio.* 125X

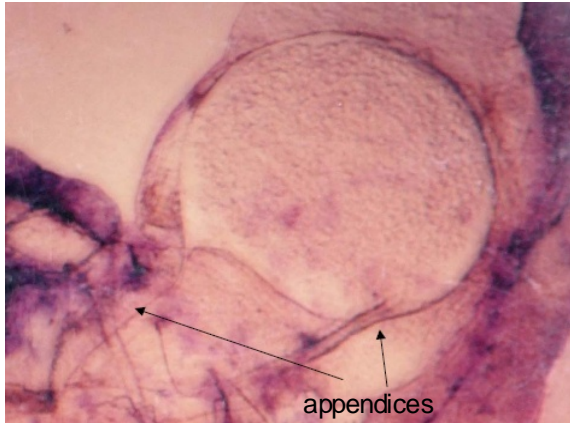


FIGURA 28. A close-up of the endospermatophore showing in detail two prominent appendices./ *Acercamiento del endoespermatóforo mostrando en detalle dos apéndices prominentes.* 150 X

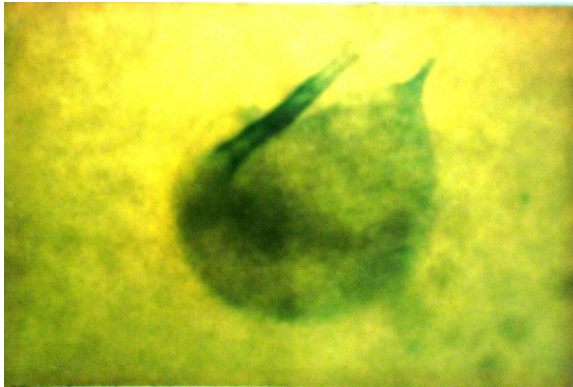


FIGURE 29. Endospermatophore obtained by dissection of the seminal receptacle, 2% toluidine blue was used to tint it./ *Endoespermatóforo obtenido por disección del receptáculo seminal, se utilizó azul de toluidina al 2% para la tinción.* 150X

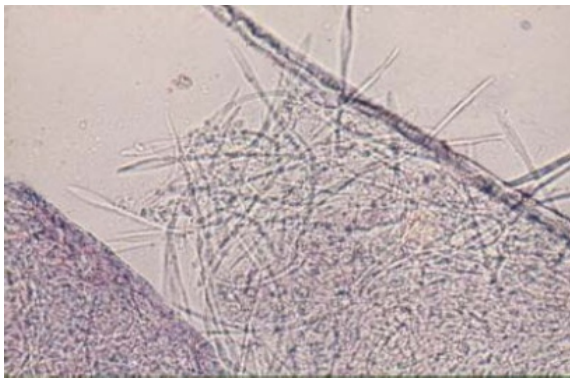


FIGURE 30. Spermiofores obtained by splashing a dissected endospermatophore./ *Espermióforos obtenidos por aplastamiento de un endoespermatóforo diseccionado.* 325X

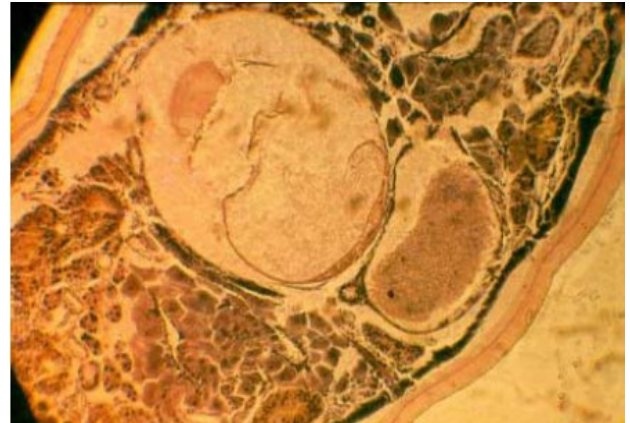


FIGURA 31. Rupture of an endospermatophore into the seminal receptacle and pass of spermiofores into the uterus./ *Ruptura de un endoespermatóforo dentro del receptáculo seminal y paso de los espermióforos hacia el útero.* 200X

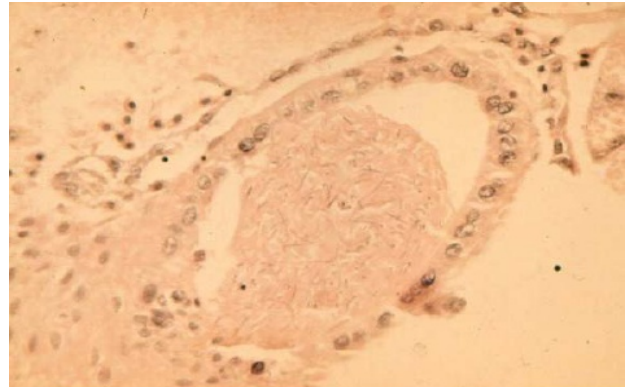


FIGURE 32. A mass of spermiofores inside an oviduct moving toward the ovary./ *Masa de espermióforos en el interior de un oviducto que se dirige hacia el ovario.* 400X

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