

THE FEMALE GENITALIA MORPHOLOGY AND THE ARRANGEMENT OF  
SPERM CELLS OF THE BROWN SPIDER *Loxosceles intermedia* MELLO-LEITÃO  
(1934) (ARANEAE: SICARIIDAE)

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## ABSTRACT

*Loxosceles intermedia* transfer spermatozoa to the female body in the synspermia form. These are produced inside the male testes, pass through the vasa deferentia channels where a secretion is deposited over them, as well as a proteinaceous sheath that surrounds each synspermium. Reaching the exterior the synspermia are captured by the male palps, and stay inside the simple bulb spermophor until copulation. At this time, the male palp is inserted into the female genital opening and its embolus reaches the female spermathecae where the synspermia are deposited. Two spatially and cul-de-sac type spermathecae are present. Each one have a single duct, through which connects the spermatheca to the *uterus externus*, for the transit of the seminal fluid, typical condition for haplogynae spiders. Four situations were examined at light microscopy level: virgin females, females just after copulation, females collected when oviposition was occurring, and females which had laid eggs two or three months before. The adult spermathecae glandular tissue is composed by gland unities, each one composed by several secretory cells. These cells discharge their secretions in a single space continuous to a ductile that pass through the sclerotized internal wall of the spermathecae and reach its lumen. This secretion has a neutral to basic glycoproteic composition and appears more concentrate in the young and virgin females. During copulation, when a mixing of female and male secretions occurs, some alterations in the proteinaceous components of these secretions are observed since they reacted negatively to the ninhidryn-Schiff treatment. Females at the moment of oviposition had their spermathecal stalk portion containing free sperm cells surrounded by a *uterus*-like secretion. Free sperm cells were also founded inside the *uterus externus*, *uterus*, oviduct and ovary lumens, but no evidence was found regarding the fertilization site. Females which had laid eggs some months ago had also free sperm cells inside their spermatheca, indicating that females? or sperm cells? may stay prepared to another oviposition.

KEY-WORDS: *Loxosceles intermedia*, synspermia, spermathecae morphology, spider reproduction

## 1. INTRODUCTION

The brown spider *Loxosceles intermedia* Mello-Leitão 1934 (Araneae: Sicariidae) (Platnick, 2005), is a venous haplogyne species that is widely distributed in southern Brazil. Aspects of its reproductive behavior were described by Fischer (1996), the oogenesis and oviposition were described at light microscopy level by Morishita and coworkers (2003), and the genitalia gross anatomy is described in Gertsch (1967). Details on the morphology of the female reproductive tract or on the condition of sperm cells storage inside the female spermathecae have not been described for this species of spider yet. Those aspects are the focus of debate nowadays, especially on view of a better understanding of the mechanisms involved on female choice and sperm selection, and on the precise dynamics of the fertilization process.

During copulation sperm cells are transferred to the female body where they are kept until the moment of fertilization and egg deposition. As in insects, insemination and fertilization are temporally separated events (Fritz and Turner, 2002), and many spiders store spermatozoa inside the spermathecae for weeks or months (Uhl, 1994b, 1996). Before copulation male spiders fill their palpal organs with a sperm mass delivered from the epigastric furrow (Michalik et al. 2004) and sperm cells are transferred to the female body in an inactivated state (Uhl, 1994; Michalik et al., 2003). An interesting aspect of the reproductive biology of spiders is the survival of viable sperm cells for long periods of time and the possibility of multiple copulations and thus the development of mechanisms of sperm selection. Spermathecal secretions seem to contribute to retain sufficient spermatozoa for successive egg-laying events, minimizing loss through the genital opening (Uhl, 1996, for *Pholcus phalangioides*; Araneae).

Haplogyne spiders are characterized by presenting a genital apparatus with a cul-de-sac type spermathecae, each one with a single duct leading to the genital cavity. This feature represents a morphological evidence for last male sperm priority (Uhl, 2000). Despite the current knowledge, this general hypothesis has been discussed as

there are variations on the morphological pattern of haplogyne spermathecae, and there are works showing that the mode of insemination predicts that the male embolus reaches the lumen of the spermatheca allowing mixing of sperm. The site of fertilization, an important point for the comprehension of sexual selection, is not well defined in spiders. Evidences point to the distal oviduct (*uterus*) as the site of fertilization, despite the fact that Suzuki (1995) describes fertilization occurring inside the ovary for the spider *Achaearanea tepidariorum*. For a general review concerning these subjects, report to Michalik and coworkers (2005).

The objective of this study is to describe the female genital system and the conditions of transference and maintenance of sperm cells, in view of a better comprehension of the function in keeping the sperm cell integrity and also the influence of the secretions in preparing sperm cells for the fertilization process, in the brown spider *L. inermidia*. The data will contribute for the discussion of the survival of the spider spermatozoa in the female spermatheca environment, and the possible mechanisms of selection inside the female reproductive tract prior to fertilization.

## **2. MATERIAL AND METHODS**

### **2.1. Animals**

Adult brown spider (*Loxosceles inermidia*) specimens were donated by inhabitants of the city of Curitiba, Paraná, Brazil (25°25'40'' S/ 49°16'23'' W). The animals were maintained in the laboratory inside small plastic vials, receiving *Tenebrium sp* larvae and water (in a saturated small cotton ball) twice a month. Juvenile spiders, which birth was accompanied in our laboratory, were kept separately and utilized in this work only after the 6<sup>th</sup> molt, when it is possible to distinguish between males (selected by observation of pedipalp bulb differentiation, Fig. 1) and females.

## 2.2. Copulations

Copulations were programmed between adult females and males. Females were selected by observation of an open quitinous epigastric furrow (Fig. 1). Couples were formed and placed inside transparent plastic vials to permit observation of the moment of palp insertion. All females were selected just after the last molt to make sure they didn't have old stored sperm cells in their spermathecae. Four different situations were established for analysis: virgin females, females just after copulation, females collected when oviposition was occurring, and females which had laid eggs two or three months before.

## 2.3. Light Microscopy

Specimens were etherized and dissected for isolation of the male pedipalps and female genital apparatus, which were fixed in 4% paraformaldehyde in 0.1M phosphate buffer pH 7.4, for 3 hours at 4<sup>0</sup>C. The pieces were dehydrated using graded ethanol series and embedded in JB4 historesin. Sections of 2 to 5µm were stained with hematoxylin-eosin. Female apparatus tissues were also submitted to PAS and ninydrin-Schiff reactions, for neutral carbohydrates and basic proteins detection respectively. Photomicrographs were taken with a Leitz Photomicroscope.

## 2.4. Electron Microscopy

Specimens were dissected and spermathecae and uterus (for females), pedipalps, testes, and vasa deferentia (for males) were prefixed in 2.5% glutaraldehyde, 2% paraformaldehyde in 0.2 M cacodylate buffer pH 7.4 for 3 hours at 4<sup>0</sup>C.

#### 2.4.1. Transmission Electron Microscopy

After pre-fixation and several washes in the same buffer, samples were post fixed in 1% OsO<sub>4</sub> (1 hour, room temperature) and treated with 2% uranyl acetate. Tissues were dehydrated using graded ethanol series and embedded in Spurr's resin. Semithin sections were placed on glass slides and stained with toluidin blue. Ultrathin sections (70nm) were stained with 2% uranyl acetate and lead citrate. Semithin and ultrathin sectioning was done on a Leica Ultracut ultramicrotome. Electronmicrographs were obtained by using a JEOL-JE1200 EXII transmission electronic microscope (operating at 80 KV) and GATAN-MULTISCAN 600W software.

#### 2.4.2. Scanning Electron Microscopy

After fixation male pedipalps were dehydrated in a graded ethanol series and critical point dried. Dried testes and vasa deferentia were cut in small pieces and placed on a metallic holder, sputter-coated with gold and analyzed in a JEOL JSM-6360LV Scanning Electron Microscope

### 3. RESULTS

#### 3.1. The synspermia trajectory

After formation inside the testicles, synspermia are released inside the vasa deferentia, a sequence of channels where a glycoprotein secretion surrounds groups of spermatozoa. At the final vasa deferentia portion an additional proteic capsula is deposited over each synspermium (Figs. 2A, 2B, 2C).

The synspermia and the vasa secretion form a gelatinous mass (Fig. 3B), which is conducted to a common ejaculatory channel (Fig1D), and then released through the genital opening to the exterior of the male abdomen. This gelatinous mass is collected

by the palp bulb (Fig. 3A) and the sperm cells are stored inside the spermophor until copulation (Figs. 3C, 3D). *L. intermedia* present a simple type bulb, a quitinous structure that is formed at the tarsal segment of the modified palp (pedipalp). The quitinous bulb is composed by a quitinous external capsule and a spiraled and quitinous internal channel, the spermophore. It is a sclerotized, spiraled and cul-de-sac type channel, where synspermia are stored in its more dilated internal and terminal portions (Fig. 1B).

### 3.2. *L. intermedia* genital apparatus

The *L. intermedia* female genital apparatus is composed of an ectodermal *uterus externus* (Figs. 4B, 4C) and two spatially separated cul-de-sac spermathecae. Each spermatheca has a single and long duct that connects the spermatheca to the *uterus externus* and provides the transit of seminal fluid (Figs. 4B, 4C). The *uterus externus* is separated from the internal structures of the genital apparatus (ovaries, oviducts, and the secretory *uterus*) by projections forming a valve, which opening is probably controlled by the muscles that extend from an adjacent depression of the *uterus externus* wall (Figs. 4B, 4C). The *uterus externus* makes the connection between the internal structures of the genital apparatus and the spermathecae, which opens at its roof, and to the exterior via the genital opening (Figs. 1A, 4A).

### 3.3. Spermatheca morphology

*Loxosceles intermedia* spermathecae are seen, by transparency, at the ventral surface of the abdomen, in the sclerotized genital opening region (Figs. 1A, 4A). During copulation the male introduces its pedipalp's bulbs inside the female *uterus externus* positioning the embolus through the long, thin and sclerotized spermatheca stalk. This one finishes in a dilated portion, the bulb, in which the synspermia are deposited and stay until fertilization and egg laying (Fig. 4C).



The spermatheca wall is formed by a glandular tissue and a sclerotized pore plate that delimitates a lumen (Fig. 4D). This one is larger at the cul-de-sac portion of the spermatheca, and smaller at the stalk portion. The glandular tissue has an arrangement similar to that of a pseudostratified tissue, and is formed by glandular unities separated by intercalary cells which appear as columnar cells, with a basal and elliptical nucleus. The glandular unities are composed by large secretory cells, which have a broad basal region where the nucleus and a great number of secretory granules are seen. Their apical surface faces a common space where the secretion product of many of them is discharged. From this reservoir the secretion is carried to a pore on the internal and sclerotized surface of the spermathecal lumen by a thin duct. (Fig. 4D).

#### 3.4. The virgin female spermathecae

Virgin females presenting slightly sclerotized and open genital opening have their spermathecae with the glandular tissue displaying the same features of those of adult individuals. The spermathecae are filled with a PAS and ninyhydrin-Schiff positive secretion, indicating that it has a neutral to basic glycoproteic composition (Figs. 5A, 5B). No spermatozoa are seen inside the spermathecae, confirming that these females never copulated.

#### 3.5. The spermatozoa transfer

Spermathecae of recently copulated females have a great number of synspermia embedded in a secretion, which must be a mixture of female and male secretions, showing positive reaction to PAS treatment, but none to ninyhydrin-Schiff treatment (Figs. 6A, 6B). A small amount of synspermia is seen inside the *uterus externus*, but not at the *uterus* region, demonstrating that male bulbs are inserted directly into the *uterus externus* and that the embolus reaches the spermathecal ducts (data not shown). The few synspermia present at the *uterus externus* might represent leakage at the

moment of retraction of the bulb. The connection between the *uterus* and the external portions of the genital apparatus probably remains closed during male contact.

### 3.6. Adult females at oviposition

Females collected at the during oviposition have intact synspermia in the spermathecal bulb and in the distal stalk portions, but the spermathecae ducts are full of free sperm cells (Figs. 7A, 7B). These free sperm cells are surrounded only by the *uterus* secretion (Fig. 7B). Free sperm cells were also found at the *uterus externus*, *uterus*, oviducts, and ovaries lumens (Figs. 7C, 7D, 7E, 7F) always surrounded by a local secretion.

### 3.7. Females which had laid eggs two or three months before

These ones have the spermathecae with free sperm cells inside the stalk portion duct, surrounded by the local PAS and nynthidrin-Schiff positive secretion. This secretion does not have a direct contact with sperm cells, which are immersed in a light medium (Figs. 8A, 8B).

## 4. DISCUSSION

*L. intermedia* synspermia are produced inside the testes and pass through the vasa deferentia where a secretion is deposited over them before being released to the exterior. At the final vasa portion, each synspermium is involved by a proteic capsule, as described by Michalik and coworkers (2004) for Dysderidae spiders, and the synspermia plus secretion are liberated to the exterior as a droplet, passing by the genital opening. The droplets are captured by the simple palpal bulb, where the sperm cells stay until the transference to the female body during copulation. No alterations

seem to occur in the sperm cells inside the pedipalp spermophor and there is no published description of such alterations.

The *L. intermedia* female external genital apparatus has the typical composition described for haplogyne spiders (Uhl, 1994, 2000): an ectodermal genital cavity (*uterus externus*, ectodermal *uterus*, *bursa copulatrix*, vulva or vagina) and two spatially separated spermathecae (also called *receptacula seminis*), cul-de-sac type which have a single duct that connects each spermatheca to the *uterus externus* and that conducts the transit of seminal fluid,. The *uterus externus* makes the connection between the spermathecae and the internal organs (*uterus*, *uterus internus*, mesodermal *uterus*, oviducts, and ovaries).

A general rule for haplogyne spiders is that during copulation, the male inserts the palp bulb into the female genital opening with sperm deposition in the seminal receptacles (Foelix, 1996, Uhl, 2002, Michalik et al., 2005). For members of some spider genera like *Grammostola* and *Acanthoscurria* (De Carlo, 1973), and for *Oligoxystre argentinensis* (Costa and Pérez-Miles, 2000), it was demonstrated that sperm cells are directly deposited by the male pedipalp embolus deep into the spermathecal receptacles. For *L. intermedia* the male bulb is likely to be inserted inside the genital opening, reaching the *uterus externus* and sequentially in the spermathecal stalk and bulb. There is a valve that isolates this anterior region from the rest of the internal organs of the female reproductive tract so sperm cells do not spread throughout the whole system, being deposited directly in the spermathecal bulb, as observed in all the females examined in this work. Another spider species, *Pholcus phalangioides*, is reported to possess a valve separating the internal organs of the external *bursa copulatrix* (Uhl, 1994). In this case, the valve is formed by grooves and is in the dorsal and ventral sides of the *uterus externus*, which fit exactly one in the other, each side functioning as a counterpart of the other. For *L. intermedia* we observed a correspondence between the ventral and dorsal sides of the *uterus externus* in the valvar region, not as conspicuous as the one observed in *P. phalangioides*, but certainly functioning as the valve observed by Uhl (1994).

The cul-de-sac type spermathecae of most of the haplogyne spiders represent a morphological evidence for last male sperm priority (Uhl, 2000). Besides the fact that there are variations on the morphological pattern of haplogyne spermathecae, our observations in *L. intermedia*, in accordance with other works show that the mode of insemination allows the male embolus to reach the lumen of the spermatheca probably contributing for the mixture of different batches of sperm, from successive inseminations. Our experimental procedures using females which copulated just once restrict our analysis to the description of conditions of the cells at storage and release, not allowing inferences on multiple male copulation and choice of sperm.

The spermathecal gland in *L. intermedia* is composed by different kinds of cells including the class 3 cells type, according to the classification of Noirot and Quenedey (1974, 1991) for insect gland cells. These ones are cells that discharge the secretion in a space named *reservoir*, and this secretion pass through a conducting canal built by other cells. In *Loxosceles intermedia* more than one gland cells are visible at light microscopy level, and the *reservoir* space is always filled with glandular secretion. Canal cells, like those described for *P. phalangioides* (Uhl, 1994) and *Dysdera erythrina* (Uhl, 2000), were not observed in our light microscopy preparations, apart from an occasional nucleus in the region occupied by the ductules. Intercalary cells similar to those described by Uhl (1994, 2000) are also seen in our preparations, in which elyptical and dark stained nuclei appears at the basal region of the epithelia, between the glandular unities. A better investigation at electron microscopy level must be accomplished to have a better understanding of the fine morphology of the *L. intermedia* spermathecal secretory tissue.

The *L. intermedia* spermathecal secretion has neutral to basic glycoproteic composition and a viscous aspect. Uhl (1996) investigated the genital cavity secretion in *P. phalangioides* concluding that it is composed by glycoproteins (which give the secretion its viscous aspect), and lipoproteins, and the same author reviews other works in which the investigations lead to a similar result. This viscosity of the spermathecal secretion may serve to retain sufficient spermatozoa at the storage site for successive egg-laying events, and the lipoprotein component may serve to prevent

desiccation of the sperm mass (Uhl, 1996). The male secretion in *L. intermedia* also seems to have a viscous aspect and at scanning electron microscopy it appears like droplets of secretion surrounding the synspermia. The sperm maintenance, a function for long time attributed to the spermathecal secretion in the spider literature, is another aspect that has been discussed in many works (Michalik et al., 2005). Works on sperm cytochemistry and spermathecal secretion biochemistry demonstrate that the spermatozoa maintain different amounts of nutrients within its cytoplasm and inside its capsule. Therefore, there would be no need for nutrients from the female body. The main function attributed to the female secretion is the assurance of the retention of the sperm cells inside the spermathecal duct and the creation of an environment favorable for their survival (Uhl, 1996, Michalik et al., 2005).

During copulation the male and female secretions mix and some alterations in these secretions take place. An example is the decapsulation of sperms in the female genital system of the spider *Leucauge mariana*, soon after insemination, observed by Eberhard and Huber (1998). In *L. intermedia* the spermathecal secretion reacted negatively to the ninyhydrin-Schiff treatment in females analyzed soon after copulation. This result suggests that some alterations in basic protein content occur in the local secretions, when male and female secretions are mixed, despite the fact that the synspermia remain intact.

Analysis of the genital apparatus of *L. intermedia* females during oviposition revealed that the sperm cells were still encapsulated at the more distal regions of the spermathecae, but at the proximal stalk region free sperm cells were observed, surrounded by a *uterus*-like secretion. In females that were sacrificed 2 or 3 months after oviposition, free sperm cells were still present at the stalk of the spermathecae, but no *uterus*-like secretion was observed in this region. In this case the free sperm cells were surrounded by a light secretion, which was involved by the local glycoproteic secretion. The free sperm cells might represent a pool that was retained since copulation and was just about to be used in a second oviposition. This is the more plausible scenario, as usually spermatozoa are stored in the spermatheca in an inactive state that probably is maintained by the synspermial arrangement.

The fertilization site is an important variable in sperm selection (Michalik et al., 2005). That is not well understood for spiders yet. The more accepted hypothesis is that fertilization takes place inside the *uterus externus* (Morishita, 2003, Michalik et al., 2005). Observations of Suzuki (1995) contrast with the general idea, once this author describes the incorporated sperm nuclei in eggs, inside the ovarian cavity, and postulate that in many other species of spiders fertilization may occur inside the ovary. In our work, we found sperm nuclei inside the *uterus externus*, *uterus*, oviduct, and ovary lumen of *L. intermedia*, but no oocytes were inside these compartments at the same time. Presently, the fertilization site still needs to be elucidated in spiders. In the present work, the female genital apparatus morphology was described for *L. intermedia* at light microscopy level. The results demonstrated that *L. intermedia* genital apparatus has a typical morphology for haplogynae spiders, but the spermathecal morphology has to be investigated at ultrastructural level to obtain a better understanding of its cellular composition. The female secretions are of glycoproteic composition and sperm mixing seems to occur, once there were observed no stratifications of sperm masses inside the spermathecae. Our experimental protocol ensured that just one copulation occurred for the examined females, so we can not discuss the results of multiple male inseminations. The influence of the male secretions on the female genital tract physiology, the influence of female secretions on the sperm cells state, and the site of fertilization are also questions to be answered in view of the comprehension of the aspects involved in the *L. intermedia* reproduction, and for spiders in general.

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**FIGURES**

Fig. 1. A. Ventral view of a female *L. intermedia* abdomen, showing the epigastric furrow (arrow head) and the right spermatheca (arrow), which appears by transparency of the sclerotized body wall. B. Simple type palp of male *L. intermedia*, in which the spiraled and sclerotized spermophor (SP) can be seen, by transparency, through the bulb (B); Ti – tibia, Ta – tarsus.

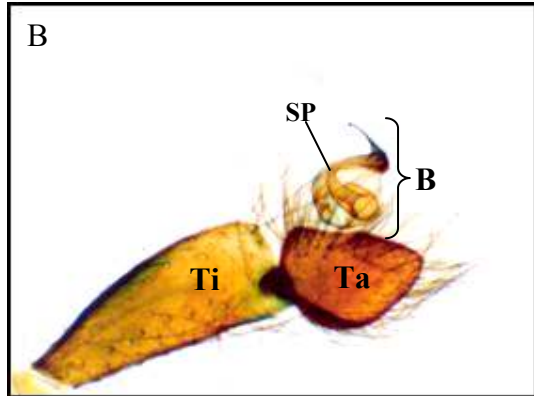
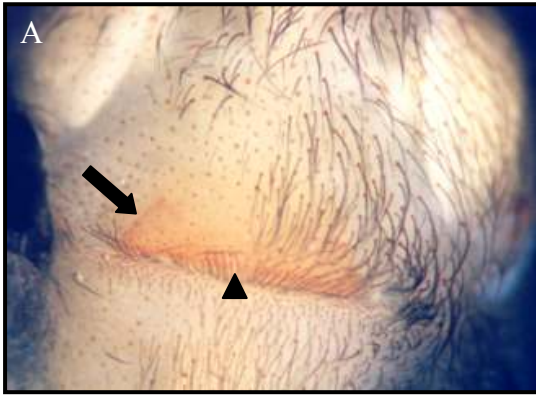


Fig. 2. Transmission electron microscopy, showing the synspermium/synspermia inside the testicle (A), and at the proximal (B) and final vasa (C); and light microscopy of the synspermia reaching the common ejaculatory channel (D, scale bar – 42  $\mu\text{m}$ ); \* - vasa secretion (notice the difference between vasa proximal and vasa distal secretions, this one presenting droplets of a clear secretion, which has a similar appearance to the synspermium proteic capsula); C – common ejaculatory channel, E – epigastric furrow, S – synspermium, V – vas deferens tissue (notice the microvilli of the epithelial and secretory cells, in Fig.2C), white arrow – proteic capsula, Z - sperm cell.

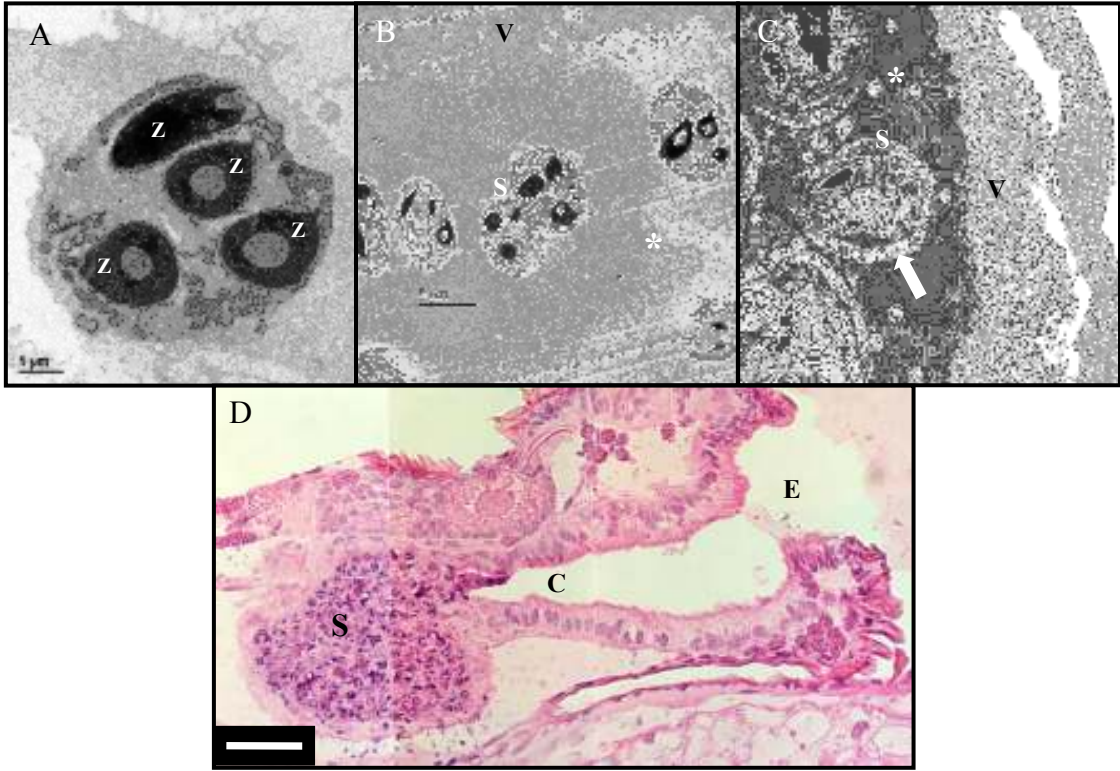


Fig. 3. *L. intermedia* simple bulb (in A) and the synspermia and secretion (in B) showed by scanning electron microscopy. Longitudinal (in C, scale bar – 71  $\mu\text{m}$ ) and cross (in D, scale bar – 64  $\mu\text{m}$ ) sections of the bulb, light microscopy, HE staining; B – palp bulb, S – synspermium or synspermia, SP – sclerotized spermophor, white arrow – male secretion.

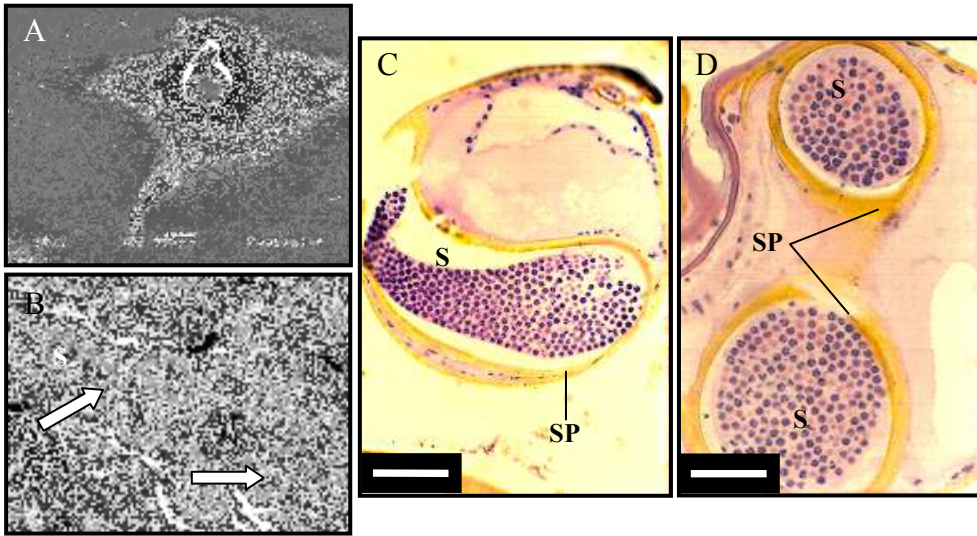




Fig. 4. A. Representation of the ventral surface of a female *L. intermedia* abdomen, in which the pointed line indicates the direction of section showed in Figs. 4B and 4C. B. Longitudinal section through the genital opening region of a female abdomen, HE staining, scale bar – 53  $\mu\text{m}$ . The spermatheca wall is composed by a secretory tissue (ST), and a sclerotized pore plate that delineates the lumen. C. Schematic representation of the longitudinal section of the female abdomen showed in Fig. 4B. D. The spermathecal stalk of an adult female in cross section, HE staining, scale bar – 31  $\mu\text{m}$ . Notice the secretory tissue (ST) composed of gland cells unities. Arrow – sclerotized internal pore plate, arrow head – epigastric furrow, \* - secretion, black arrow – spermatheca, D – duct, L – lungs (showed as a point of reference), M – abdominal muscle, N – nuclei of spermathecal cells, S – synspermia inside the spermatheca lumen, ST – spermathecal tissue, thin arrow – spermatheca pore plate, U – uterus, UE – uterus externus, white arrow – spermathecal duct.

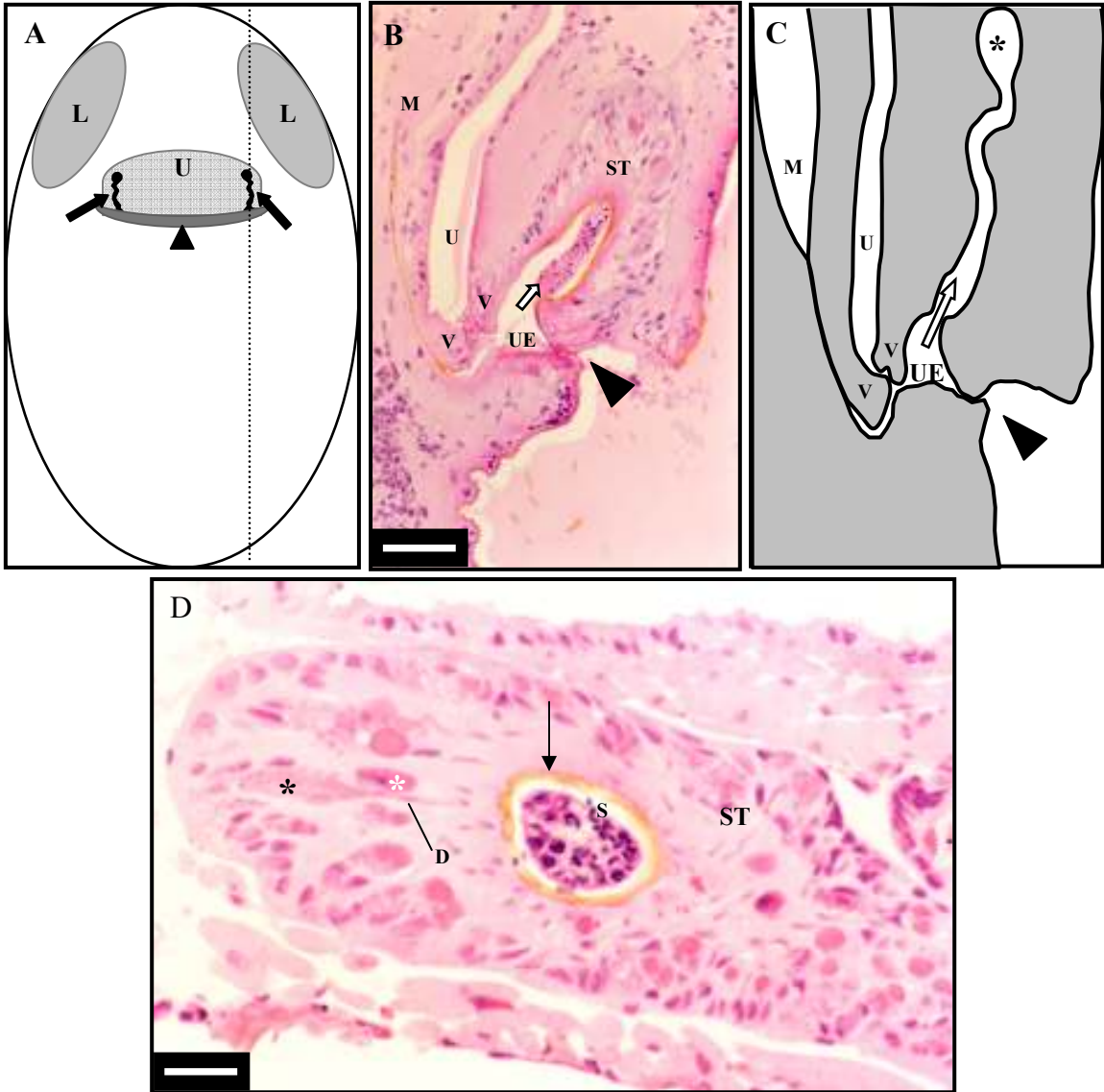


Fig. 5. Secretion inside the spermatheca of the virgin spider, which reacted positively to PAS (in A, scale bar – 18  $\mu\text{m}$ ) and nynhidrin-Schiff (in B, scale bar - 18  $\mu\text{m}$ ) treatments; S - secretion, ST – secretory tissue.

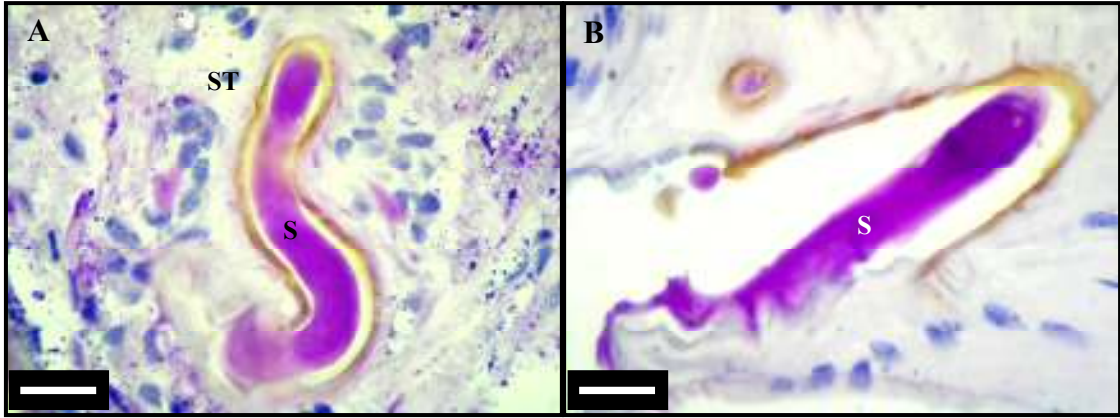


Fig. 6. Spermatheca of a recent coupled female, cross sections. The spermatheca secretion is positive for PAS (in A, scale bar – 18  $\mu\text{m}$ ) and negatively for nynthidin-Schiff (in B, scale bar – 18  $\mu\text{m}$ ) reactions; arrow – spermatheca sclerotized stalk wall, S - synspermia.

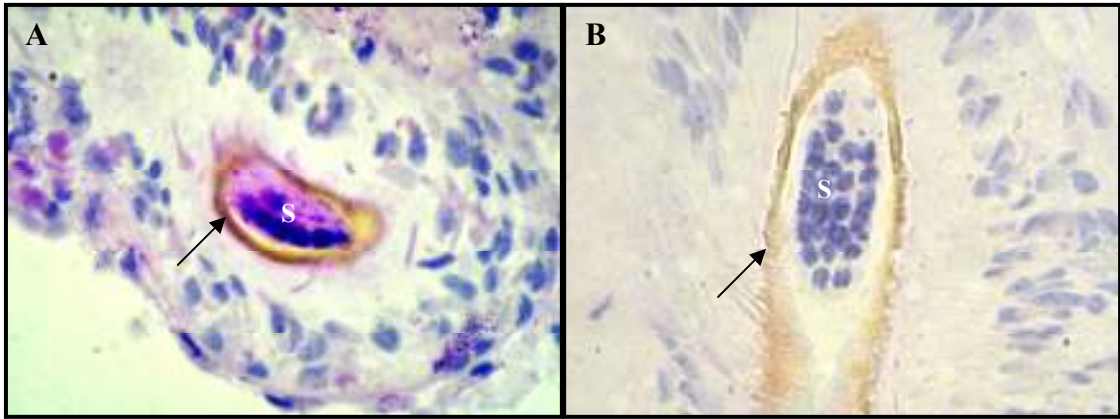


Fig. 7. Situations in females collected when during oviposition, HE staining; A. cross section of the distal stalk portion of the spermatheca containing still encapsulated sperm cells (synspermia), scale bar – 18  $\mu\text{m}$ ; B. longitudinal section of the proximal portion of the spermatheca, containing free sperm cells surrounded by the uterus secretion, scale bar – 20  $\mu\text{m}$ ; C – F. different situations in which the free sperm cells were founded: C – inside the *uterus externus*, scale bar – 20  $\mu\text{m}$ ; D – inside the *uterus*, between the cell apical projections of the epithelium, scale bar – 20  $\mu\text{m}$ ; E – inside the oviduct, scale bar – 20  $\mu\text{m}$ ; and F – inside the ovary, scale bar – 20  $\mu\text{m}$ ; arrow – the sclerotized spermatheca sclerotized pore plate, S – synspermia, ST – secretory tissue, \* - *uterus* secretion.

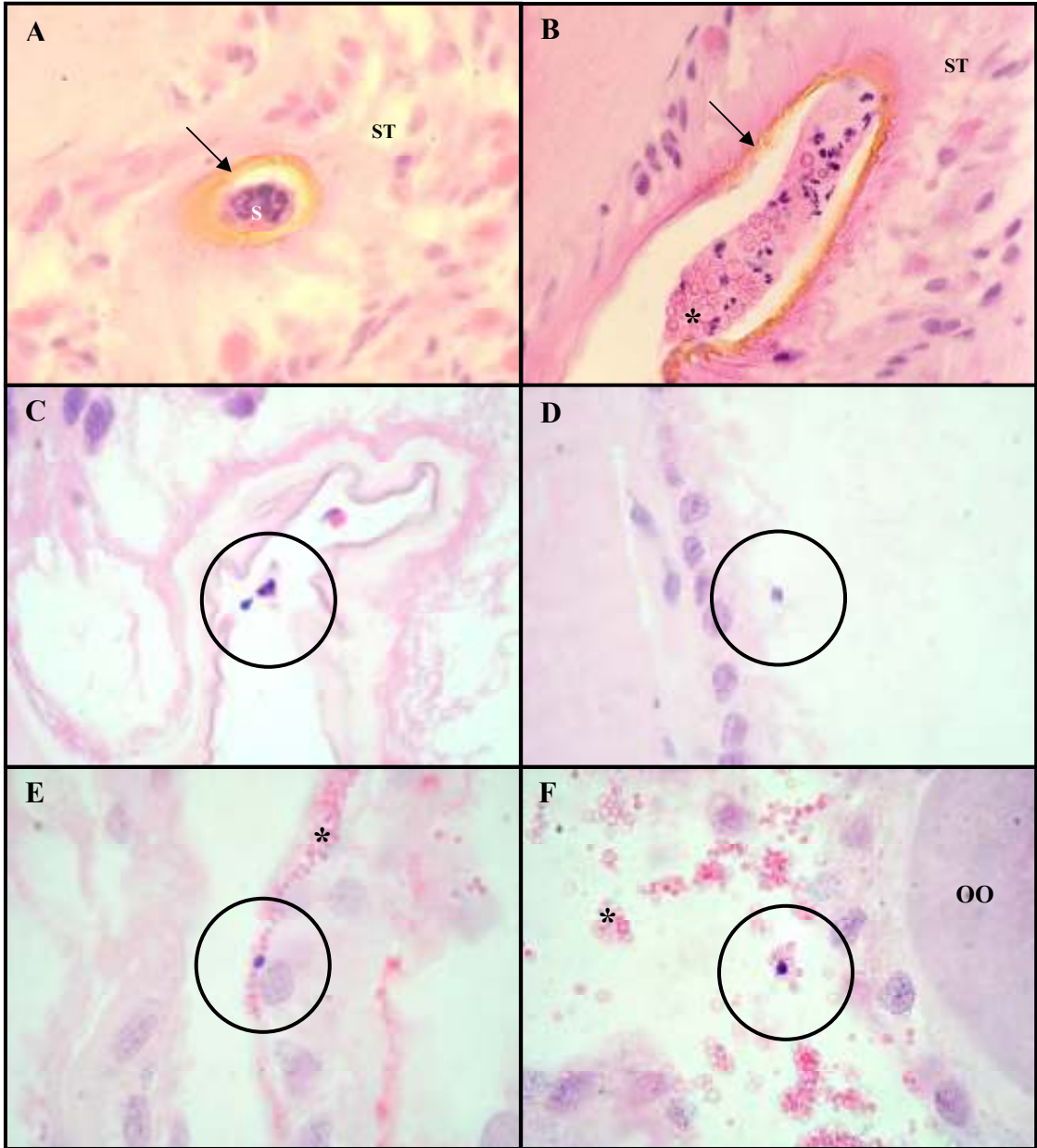
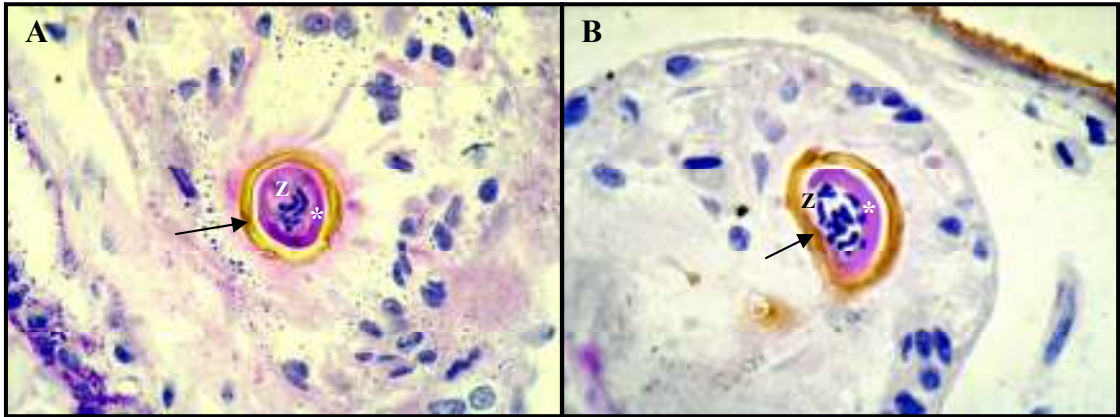




Fig. 8. Spermatheca of the female that had laid eggs two or three months before, PAS (in A, scale bar – 18  $\mu\text{m}$ ) and nynthidin-Schiff (in B, scale bar - 18  $\mu\text{m}$ ) treatments. Notice the free sperm cells inside the spermathecal lumen (Z); arrow – spermathecal sclerotized pore plate, \* - secretion.



## CONCLUSÕES

O presente trabalho teve como objetivo geral o estudo da espermatogênese e da morfologia dos espermatozoides da aranha marrom *Loxosceles intermedia*, antes e após a transferência para o corpo da fêmea.

Através dos experimentos realizados as seguintes conclusões podem ser delineadas:

- 1) A osmolalidade da hemolinfa da aranha marrom é, em média, 478 mOsm/Kg H<sub>2</sub>O. Duas das fórmulas fixativas testadas deram resultados positivos na fixação dos tecidos dos testículos da *Loxosceles intermedia*: a. glutaraldeído a 2,5%, paraformaldeído a 2%, em tampão cacodilato de sódio a 200 mM, cuja osmolalidade média do tampão foi de 454 mOsm/Kg H<sub>2</sub>O; e b. glutaraldeído a 2,5%, paraformaldeído a 2%, em tampão fosfato 100 mM e sacarose a 200 mM, cuja osmolalidade do tampão foi de 439 mOsm/Kg H<sub>2</sub>O. Ambos os tampões foram aproximadamente isosmóticos à hemolinfa da aranha marrom. O tampão cacodilato mostrou-se mais eficiente em preservar os tecidos, porém o tampão fosfato, adicionado de sacarose, que é uma alternativa que expõe o usuário a uma menor toxicidade, mostrou-se eficiente na preservação das membranas. A combinação de glutaraldeído e paraformaldeído nas concentrações indicadas acima resultou em uma melhor preservação das estruturas celulares.
- 2) A estrutura geral do aparelho reprodutor masculino e feminino da aranha marrom segue o padrão normal descrito para aranhas haplóginas. No entanto, algumas características, como a presença de um ducto e uma ampola ligando o testículo ao vas deferens no sistema genital masculino, e a presença de uma valva, entre o *uterus* e o *uterus externus*, que separa os órgãos internos e externos do aparelho genital feminino, fogem à regra geral.

- 3) O espermatozóide da aranha marrom apresenta um capuz acrosomal alongado e uma elongação pós-centriolar curta, o que são características consideradas plesiomórficas para as aranhas.
- 4) A forma de transferência de espermatozóides em *L. intermedia* é o *synspermium*, condição considerada altamente derivada em Araneae, em que 4 espermatozóides encontram-se encapsulados, ainda na forma sincicial, compartilhando o citoplasma remanescente comum, e envoltos por uma cápsula protéica, que é depositada sobre a membrana plasmática que envolve o conjunto.
- 5) O sêmen da aranha marrom é constituído pela secreção glicoprotéica secretada na vasa deferentia, e pelos *synspermia*.
- 6) As espermatecas da aranha marrom são do tipo fundo-de-saco, com um ducto simples que leva e traz o sêmen, desembocando no teto do *uterus externus*. Seu epitélio é do tipo pseudoestratificado e secretório, constituído por unidades glandulares compostas por mais de uma célula secretora.
- 7) A secreção produzida pela espermateca varia de acordo com a condição reprodutiva da fêmea. Em fêmeas recém copuladas, uma alteração na marcação para proteínas básicas foi observada.
- 8) Fêmeas que foram coletadas no momento da oviposição apresentavam espermatozóides livres, envolvidos por uma secreção similar àquela produzida pelo *uterus*, no interior do ducto da espermateca. Espermatozóides livres foram ainda encontrados nas regiões do *uterus externus*, oviduto, e ovário de uma dessas fêmeas.
- 9) Fêmeas que haviam colocado os ovos há 2 ou 3 meses continham ainda espermatozóides livres no interior do ducto da espermateca, porém a secreção do *uterus* não estava presente. Os espermatozóides se encontravam em meio a uma secreção clara, e o conjunto estava envolto pela secreção da espermateca.
- 10) A fêmea da aranha marrom, além de não utilizar todos os espermatozóides livres, contidos no ducto da espermateca, para a fecundação, ainda guarda,

no bulbo do mesmo órgão, *synspermia* intactos para serem utilizados em uma nova oviposição.

- 11) A aranha marrom mostra ser um rico modelo para estudos futuros em Biologia Celular e do Desenvolvimento, pois se trata de um animal de fácil acesso e manutenção em laboratório, além de apresentar aspectos teciduais e celulares próprios das aranhas e dos invertebrados.
- 12) O presente trabalho constitui uma contribuição para o debate das questões ainda controversas relacionadas com as variações morfológicas encontradas no aparelho reprodutor de aranhas e que devem ser definidas para esclarecimento de aspectos evolutivos e filogenéticos do grupo.

## PERSPECTIVAS

- 1) É grande a eficiência da fixação das gônadas das aranhas utilizando-se das fórmulas fixativas determinadas no presente trabalho. No entanto, para elementos ricos em gordura, como os ovócitos mais desenvolvidos, os ovos e embriões da aranha marrom, alguns ajustes no protocolo deverão ser efetuados de modo a obter uma melhor preservação.
- 2) Existe a necessidade de se realizar uma análise ultraestrutural dos *synspermia* e espermatozóides contidos no interior das espermatecas da fêmea com vistas a um melhor entendimento das transformações ocorridas nessas células, em diferentes situações: quando ainda encapsuladas, no momento da cópula, no decorrer do seu armazenamento no interior das espermatecas, e no momento que antecede a fertilização.
- 3) A caracterização das proteínas presentes no sêmen, nas secreções da espermateca e do *uterus*, da mistura das secreções do macho e da fêmea resultante da cópula, e da secreção contida na espermateca após a oviposição é de suma importância para o entendimento das alterações ocorridas no corpo da fêmea e nos espermatozóides da aranha marrom.
- 4) A avaliação dos hormônios circulantes no corpo da fêmea, em cada situação acima descrita, nos dará informações sobre a influência do sêmen depositado nas espermatecas sobre a ovogênese na aranha marrom, pois existem suspeitas de que a vitelogênese só ocorre nas aranhas, após a cópula.
- 5) Deve-se investigar a situação do sêmen nas espermatecas das fêmeas que copularam mais de uma vez, visando obter informações a respeito da ocorrência de uma possível estratificação desses semens, o que nos dará base para a discussão sobre a possibilidade de a fêmea escolher ou não o lote de sêmen que irá utilizar em cada oviposição. Este é um assunto muito discutido atualmente na literatura sobre reprodução em aranhas e, como em nosso trabalho analisamos o conteúdo das espermatecas de fêmeas que

copularam uma só vez, não foi possível discutir esse aspecto para a aranha marrom ainda.