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Functional anatomy of the female reproductive systems of two spider crabs (Decapoda, Majoidea)

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Abstract. To better understand the mating systems of majoid crabs, we studied the functional anatomy of the female reproductive systems of the spider crabs *Leurocyclus tuberculosus* and *Libinia spinosa,* comparing them with those of other Majoidea. Adult females were measured and dissected, and their reproductive systems described macroscopically and histologically. In females of both species, the seminal receptacles are paired globular structures of ecto-mesodermal origin. The mesoderm-derived region is lined by a stratified epithelium. The anchoring, proliferative, and secretory strata are clearly recognizable**.** The ectoderm-derived region is lined by a simple cylindrical epithelium underlying a cuticle that increases in thickness toward the vagina. The transition between the ectoderm and mesoderm-derived regions is abrupt, with differences between the studied species: *Li. spinosa* has a "velum," whereas *Le. tuberculosus* presents prominent "folds." In both species, the position in which the oviduct is connected to the seminal receptacles is intermediate between the dorsal and ventral types previously described in other eubrachyurans. The seminal receptacles of the studied species show four different conditions, which can be distinguished macroscopically based on their shape and amount of sperm stored. We compare our data with those from other Majoidea in an attempt to determine whether the morphology of the seminal receptacles is related to different mating strategies or behaviors.

Additional key words: reproductive anatomy, histology, crustacean, decapod

Studies of reproductive morphology in a phylogenetic framework can answer questions about the coevolution of anatomy, mating behaviors, and fertilization mechanisms (Wortham-Neal 2002). In many species, including some crustaceans, there is a correlation between the morphology of the sperm-storage chambers and the pattern of sperm precedence (last/first mate) at fertilization, the arrangement of stored ejaculates within the seminal receptacles (SR), and the molt status of females at mating (Parker 1970; Diesel 1991; Sainte-Marie 2007).

The female genital ducts of eubrachyuran crabs extend from the ovary to the vulva (Hartnoll 1968). The ovary releases the oocytes into the oviducts, which lead them to the SR. Each SR connects ventrally with a vagina, which opens to the exterior through the sternal vulva (Hartnoll 1968; Diesel 1991; Guinot & Quenette 2005). The SR are classified into two types according to the position in which the oviducts are connected to them: the dorsal type (dorsal-type SR) and the ventral type (ventral-type SR) (Diesel 1991). In the dorsal-type SR, the opening of each oviduct is located more or less dorsal to the seminal receptacle, and the vagina lies ventrally (e.g., Portunidae and Xanthidae: Diesel 1991). In the ventral type, both oviduct and vagina open into the ventral region of the SR, close to each other (e.g., Calappidae, Geryonidae, Leucosiidae, Parthenopidae, Parathelphusidae, Corystidae, Ocypodidae, and some species of Majoidea: Diesel 1991). The topographical arrangement of the connection between the oviduct and the SR has implications for how spermatozoa from different males are used for fertilization (Diesel 1991; Urbani et al. 1998; Sainte-Marie 2007). The dorsal-type SR is

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frequently observed in "soft-shelled mating" species, whereas the ventral-type SR is common in "hard-shelled mating" species, in which last-male sperm precedence is likely to occur (Diesel 1991). Many authors have used the term "spermathecae" when referring to structures storing spermatophores or spermatozoa (McLay & López-Greco 2011). In the present article, these structures are designated as "seminal receptacles" based on their mixed, ecto-mesodermal origin, and in accordance with the term used by Tavares & Secretan (1993) and Guinot & Quenette (2005) for Eubrachyura.

Seminal receptacles are extensions of the genital tract used for the reception, storage, and maintenance of sperm. They typically show high secretory activity (Hartnoll 1968; Diesel 1989; Anilkumar et al. 1996; Jensen et al. 1996; González-Gurriarán et al. 1998; Sainte-Marie & Sainte-Marie 1998; López Greco et al. 1999, 2009). It is suspected that secretions of the SR epithelium participate in the conditioning of spermatophores in preparation for dehiscence, or may even be the agents that cause dehiscence (Diesel 1989; Beninger et al. 1993), as was suggested for the snow crab by the close association between the secretory activity of the SR wall and neighboring concentrations of spermatophores in pre-oviposition females (Sainte-Marie & Sainte-Marie 1998; Sainte-Marie et al. 2000). Sperm can be stored for long periods, thus allowing the fertilization of consecutive broods without intervening matings (Diesel 1988, 1989, 1991; Hinsch 1991; Jensen et al. 1996; González-Gurriarán et al. 1998; Sampedro et al. 1999; Sainte-Marie 2007).

Sperm competition is defined as the contest between the sperm from at least two males for oocyte fertilization (Parker 1970, 1984; Parker et al. 1990). There is a great potential for sperm competition in species in which females store viable sperm for extended periods in specialized organs and mate with more than one male before oocytes are fertilized (Parker 1970; Diesel 1991; Wortham-Neal 2002). The function of these storage organs is not only of biological interest, as storage patterns (mixed or layered) of rival ejaculates can determine expression of paternity (multiple or single) in offspring (Koga et al. 1993; Sévigny & Sainte-Marie 1996), but has also been the subject of fisheries-oriented research for at least two reasons (Paul & Paul 1992; Gardner et al. 1998). On one hand, examination of SR provides a means of determining the occurrence of mating, which may be reduced when only males are harvested. On the other hand, they confer on females the ability to use stored sperm to fertilize separate broods, possibly allowing them to "buffer" the impact of male scarcity in fisheries (Paul & Paul 1992).

Eubrachyurans have simple or concave vaginae (Hartnoll 1968). A simple vagina is a straight tubular structure of circular cross section with a cuticular wall of uniform thickness surrounding the lumen. The term concave designates a vagina with a basal portion having a wall in part thick and rigid and in part thin and flexible, and crescent-shaped in cross section (Hartnoll 1968). According to Hartnoll (1969), females with simple vaginae mate shortly after the female has molted, while the exoskeleton is still uncalcified (soft-shelled mating), and those with concave vaginae mate during the inter-molt (hard-shelled mating). In addition, some species have opercula occluding the vulvae (Hartnoll 1968).

In Majoidea, a eubrachyuran group commonly known as "spider crabs," the morphology of the reproductive system has been studied in several species, including *Chionoecetes bairdi* RATHBUN 1924 (Paul & Adams 1984), *Chionoecetes opilio* FABRICIUS 1788 (Beninger et al. 1988; Sainte-Marie & Sainte-Marie 1998; Urbani et al. 1998; Sainte-Marie & Sainte-Marie 1999a, b; Benhalima & Moriyasu 2000), *Inachus phalangium* FABRICIUS 1775 (Diesel 1989), *Libinia emarginata* LEACH 1815 (Hinsch & Walker 1974; Hinsch 1986), *Maja brachydactyla* BALSS 1922 (García-Flores & Fernández-Rueda 2000; Rotllant et al. 2007), and *Maja squinado* HERBST 1788 (González-Gurriarán et al. 1998). Females of these species share the following characters: (1) ventral-type SR; (2) lumen of the SR partitioned by a "velum" or "folds"; (3) vagina of the concave-type. Members of the Majoidea usually show "hard-shelled mating," although in some species the first mating after the puberty molt can occur in the post-molt stage (Hartnoll 1969; Sainte-Marie et al. 1997; Jadamec et al. 1999).

Leurocyclus tuberculosus MILNE EDWARDS & LUCAS 1843 (Inachoididae) and *Libinia spinosa* MILNE EDWARDS 1834 (Epialtidae) are amphi-oceanic majoids distributed from Chile to Río de Janeiro, Brazil (Boschi et al. 1992). The largest members of this group are found in southern South America, where they co-exist on sandy-muddy substrates from the intertidal zone to depths of at least 45 m (González-Pisani 2011). These species are not currently of importance for fisheries, but are important members of the benthic fauna. Knowledge of their biology is limited. The few studies concerning these species have focused on the description of their external morphology, geographic distribution (Boschi et al. 1992), and larval development (Boschi & Scelzo 1968; Santana & Marques 2005). Moreover, size at morphological maturity was determined for *Le. tuberculosus* (Barón et al. 2009). The aim of the present work was to characterize the functional anatomy of the female reproductive system of members of both species, and to compare it with that of other Majoidea.

Methods

A total of 860 females of *Leurocyclus tuberculosus* (22–68.79 mm carapace width (CW)) and 136 of *Libinia spinosa* (44.2–63 mm CW) were sampled between 2007 and 2009 in waters of Nuevo Gulf, Argentina (42°–43°S, 64°–65°W) at depths between 5–45 m. Specimens were collected with baited traps or hand-picked by SCUBA divers. Once in the laboratory the CW of all females was measured; only individuals larger than 42 mm CW were characterized as morphometrically mature, in both species (González-Pisani 2011). All were cold-anesthetized, dissected, and their reproductive system organs (external: vulvae; internal: ovaries, oviducts, SR, and vaginae) were macroscopically characterized according to their relative position, color, consistency, and relative size. Detailed illustrations were made with a camera lucida based on 10 individuals of each species, and photographs were taken using a digital camera (Nikon Coolpix). Vaginae were stained for 5 min with methylene blue (10% in seawater) to improve contrast. Some SR together with the vagina and vulva were observed using a JEOL LSM-6460 LV scanning electron microscope. For this purpose, the complete reproductive system was removed, cleaned with distilled water, and mounted with a double-sided adhesive tape on a labeled slide. Dry SEM samples without gold coating were viewed using the back-scattered electron technique at low vacuum (15 –20 Pa).

For histological analysis, the entire reproductive systems of another 20 individuals of each species were fixed in Bouin's solution for 4 h and processed for routine histological analysis according to López Greco et al. (2007). Serial sections of 5–6 μm thickness were cut using a Carl Zeiss microtome, stained with hematoxylin-eosin, periodic acid-Schiff (PAS), and Masson-Trichrome, and examined and photographed with a Carl Zeiss Axioimager A1 light microscope.

Results

Anatomy of the female reproductive system

The ovaries of *Leurocyclus tuberculosus* and *Libinia spinosa* consist of two lateral lobes connected by a transverse bridge in the region between the pyloric stomach and the heart, and longitudinally attached to each other at their posterior ends, along the sagittal plane. The anterior part of both lobes extends medially from the transverse bridge to the orbital region, passes between the posterior mandible tendons, and then curves laterally at the anterior margin of the carapace (Fig. 1A). The oviducts jut ventro-laterally from the lateral lobes of the ovary, posteriorly to the bridge at the level of the sixth sternite, and extend to reach the mesoderm-derived region of the SR (Fig. 1B,C). In females of both species, the insertion of the oviduct is located in the intermediate zone of the mesoderm-derived region of the SR (Fig. 1B–D).

In females of both species, the SRs are paired, rounded, opalescent structures; they possess a ventral, asymmetrical cup-shaped cuticular portion of ectodermal origin, and a dorsal, sac-shaped soft-tissue portion of mesodermal origin (Fig. 1B–D). The following four SR conditions can be recognized macroscopically according to their shape and amount of sperm stored: stage I, SR of virgin females unfilled; stage II, SR partially full; stage III, SR full; and stage IV, SR of postspawning females emptied (Table 1).

The vaginae of *Le. tuberculosus* and *Li. spinosa* are short, cuticle-lined ducts showing a concave invagination, with their concavities facing the lateral side of the animal (Fig. 2A–D). The inner lateral sides of their walls are thick, rigid, and calcified, whereas the outer lateral sides are thin and flexible. In females of both species, the vaginae are fastened to the sterna by muscle fibers (Figs. 1C, 2B), which are inserted along the length of the flexible portion of the vagina wall and into part of the ectoderm-derived region of the SR (Figs. 1C, 2B–D). The vulvae of both species consist of elliptical openings in the wall of the sixth sternite. The openings are internally blocked by a "vulvar valve" formed by an evagination of the concave flexible portion of the vagina wall (Fig. 2E,F). No sperm plug was found in females of either species.

Histology of the female reproductive system

The oviducts of *Le. tuberculosus* and *Li. spinosa* are lined by a simple epithelium of cylindrical cells, which is continuous with the mesoderm-derived epithelium of the SR (Fig. 3A,B). The SR has a dorsal region formed of mesoderm-derived epithelium, and a ventral region formed of ectoderm-derived epithelium (Fig. 3C,D). These structures are entirely surrounded by an outer thin layer of connective tissue with embedded muscle fibers (Fig. 3A). The wall of

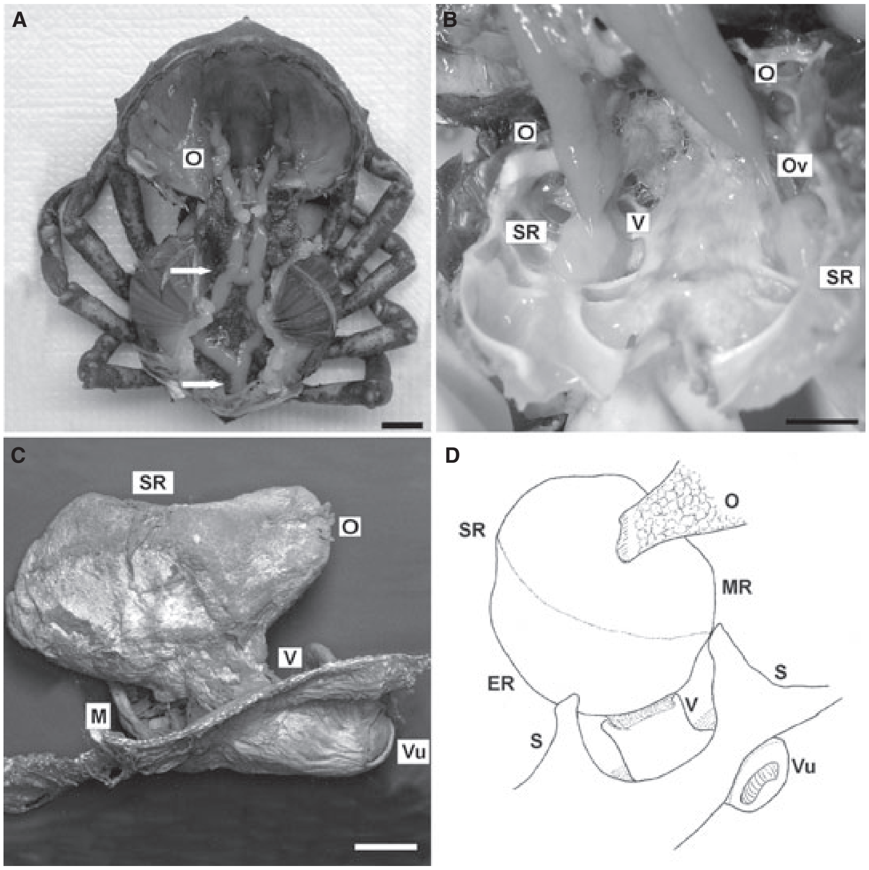

Fig. 1. **A.** Dorsal view of a mature ovary of *Libinia spinosa*. Arrows indicate the bridge between the lobules and the longitudinal junction between the posterior lobules. Scale bar=1 cm. **B.** Dorsal-lateral view of *Leurocyclus tuberculosus,* showing the site of connection of the oviduct to a stage II seminal receptacle and the relative position of the vagina. Scale bar=0.5 cm. **C.** SEM micrograph of the seminal receptacle, vagina, and vulva of *Leurocyclus tuberculosus* in lateral view. Scale bar=1 mm. **D:** Schematic drawing of the oviduct, seminal receptacle, vagina, and vulva in medial-lateral view. The dotted line indicates the middle region of the seminal receptacle. ER, ectoderm-derived region; M, muscles; MR, mesoderm-derived region; O, ovary; Ov, oviduct; S, septa; SR, seminal receptacle; V, vagina; Vu, vulva.

the mesoderm-derived region of the SR is lined by a stratified epithelium with strongly acidophilic cuboidal and cylindrical cells (Fig. 4A). Three well-defined strata can be recognized: an outer anchoring stratum, a middle proliferative stratum, and an inner secretory stratum (Fig. 4B**)**. In stage II SR, the anchoring stratum is composed of 10–12 cell layers (in *L. tuberculosus*) or 12–14 cell layers (in *Li. spinosa*). This stratum is continuous with the epithelium of the oviduct. The middle stratum, which is the site of cell proliferation, is approximately 10 cell layers thick in *Le. tuberculosus* and 15 cell layers thick in *Li. spinosa*. In both species, the secretory stratum consists of many layers of holocrine gland cells with large nuclei. Cells of this stratum show an increasing degeneration toward the lumen, where they release their secretions. In both species, the secretory stratum varies considerably in thickness according to the SR stage, becoming thicker at stages II (partially full) and IV (emptied)**.** Oocytes and free spermatozoa are found in the mesoderm-derived region (Fig. 4C,D).

In females of both species, the wall of the ectoderm-derived region is formed by an epithelium composed of a single outer layer of cylindrical cells with large nuclei, and inner layers of cuticle **(**Figs. 4C, 5A). In the dorsal, ectoderm-derived portion, the cuticle is thin and consists of a single layer. It increases in thickness as it approaches the ventral portion, and becomes thickest and composed of clearly distinguishable layers at the level of the vagina. Spermatophores and free spermatozoa are found in the ectoderm-derived region (Fig. 5B–D).

In females of *Li. spinosa*, the transition between the meso- and ectoderm-derived regions of the SR is abrupt, and includes a structure formed by columnar cells from the ectoderm-derived epithelium and many muscle fibers, referred to as a “velum” by

Table 1. Anatomical and histological description of different seminal receptacle stages (SR) of *Leurocyclus tuberculosus* and *Libinia spinosa*. TR, transition zone between the ectoderm and mesoderm-derived regions.

	Common features	Species-specific features	
	Leurocyclus tuberculosus and *Libinia spinosa*	*L. tuberculosus*	*L. spinosa*
Stage I	SR unfilled. Lumen reduced, without seminal content. Typical of virginal females	Many relaxed, long folds in TR	Velum relaxed and presence of folds in TR
Stage II	SR round. Lumen partially filled with seminal content, with spermatophores and free spermatozoa. Occurring in both pre- and post-spawn females	Absence of folds in TR	Velum short and absence of folds in TR
Stage III	SR oval and relatively large. Lumen completely filled with seminal content, with free spermatozoa. Occurring in both pre and post-spawn females		
Stage IV	SR emptied, with flaccid walls. Lumen conspicuous, with reduced seminal content; with spermatozoa, without spermatophores. High secretory activity in the mesoderm-derived inner walls. Typical of spawned females	Folds in TR are erect and extended	Velum long and presence of folds in TR

Diesel (1991) (Figs. 3C, 6). This structure is observed in different forms depending on SR condition (Table 1). At stage I, the velum is relaxed and its distal margin faces a series of folds from the opposite inner surface of the SR (Fig. 7A). At SR stages II and III, the velum is shorter and folds in the opposite wall are absent, so that the seminal contents occupy the whole lumen of the SR (Fig. 7B–D). At stage IV, the velum is longer and completely separates the mesoderm-derived region from the ectoderm-derived region of the SR. As a result, the seminal content is confined to the lumen of the mesoderm-derived region (Fig. 7B).

In females of *Le. tuberculosus*, no velum is present at the transition between the ecto- and mesoderm-derived regions. Instead, the SR of this species presents many radial folds projecting into the lumen from the ectoderm-derived epithelium (Figs. 3D, 6). These folds are observed in different conditions in accordance with different SR stages (Table 1). At SR stage I, the margin of the transition region shows numerous long and relaxed folds. At SR stages II and III, folds are absent and the wall of the SR is stretched by the seminal content. At stage IV, the folds are erect and shorter than at stage I (Table 1). Two types of amorphous material are observed within the SR, one being part of the seminal contents, and the other produced by the mesoderm-derived secretory stratum (Fig. 4C). Spermatozoa are transferred to females packed in elliptic spermatophores embedded in a strongly acidophilic, PAS-positive and Masson-Trichrome-negative seminal plasma (Fig. 5C). No discrete ejaculates or sperm packets *sensu* Diesel (1991) were observed within the SR.

The vaginae of both *Le. tuberculosus* and *Li. spinosa* are lined by a simple cylindrical epithelium underlying thick cuticular layers, continuous with that found in the ectoderm-derived region of the SR (Fig. 8A,B). Packets of muscle fibers attach to the flexible concave part of the vagina wall (Fig. 8B) and vulvar valve (Fig. 8C).

Discussion

The female reproductive tracts of the spider crabs *Leurocyclus tuberculosus* and *Libinia spinosa* display the same general anatomy observed in

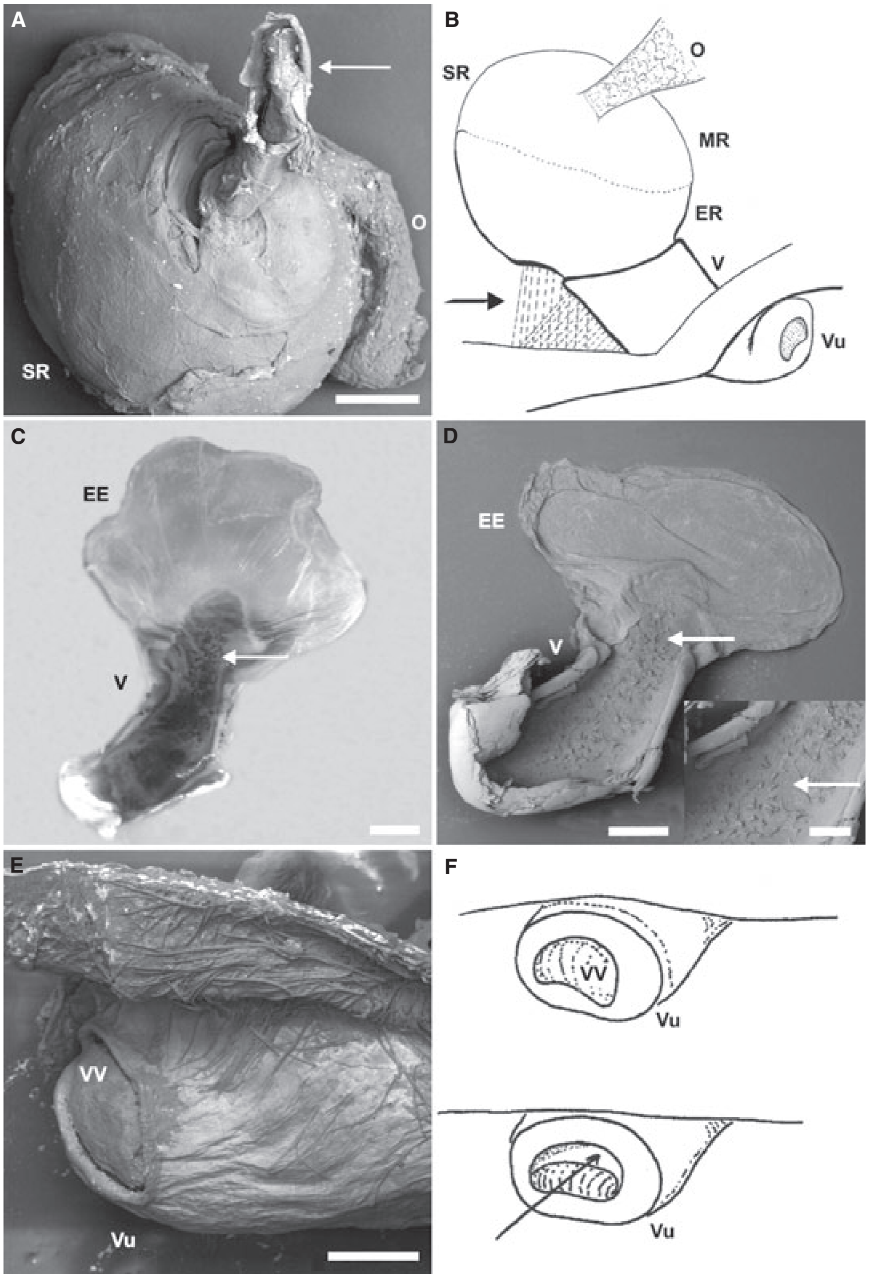

Fig. 2. A. SEM micrograph of the stage III seminal receptacle and ovary in ventral view of *Leurocyclus tuberculosus*. The arrow indicates the concave face of the vagina. Scale bar: 1 mm. **B.** Schematic drawing of the seminal receptacle and vagina indicating the position of the muscle fibers (white arrow). **C.** Concave face of the vagina of *Leurocyclus tuberculosus*. The arrow indicates muscle insertion. Scale bar: 1 mm. **D.** SEM micrograph of the concave face of the vagina of *Leurocyclus tuberculosus*. Scale bar: 1 mm. In the detail, the white arrow indicates the attachment of the muscle fibers. Scale bar: 0.5 mm. **E.** SEM micrograph of the vulva with vulvar valve of *Leurocyclus tuberculosus*. Scale bar: 1 mm. **F.** Schematic drawing of the vulvar valve. The arrow indicates the opening of the vulvar valve. EE, ectoderm-derived epithelium; ER, ectoderm-derived region; MR, mesoderm-derived region; O, ovary; SR, seminal receptacle; V, vagina; Vu, vulva; VV, vulvar valve.

other eubrachyurans (Ryan 1967; Hartnoll 1968; Johnson 1980; Diesel 1989; Krol et al. 1992). However, the ovaries of both species do not show the typical "H-shape" (Krol et al. 1992). Instead, the medial walls of the lateral lobes are fused together at their posterior ends. This morphological pattern

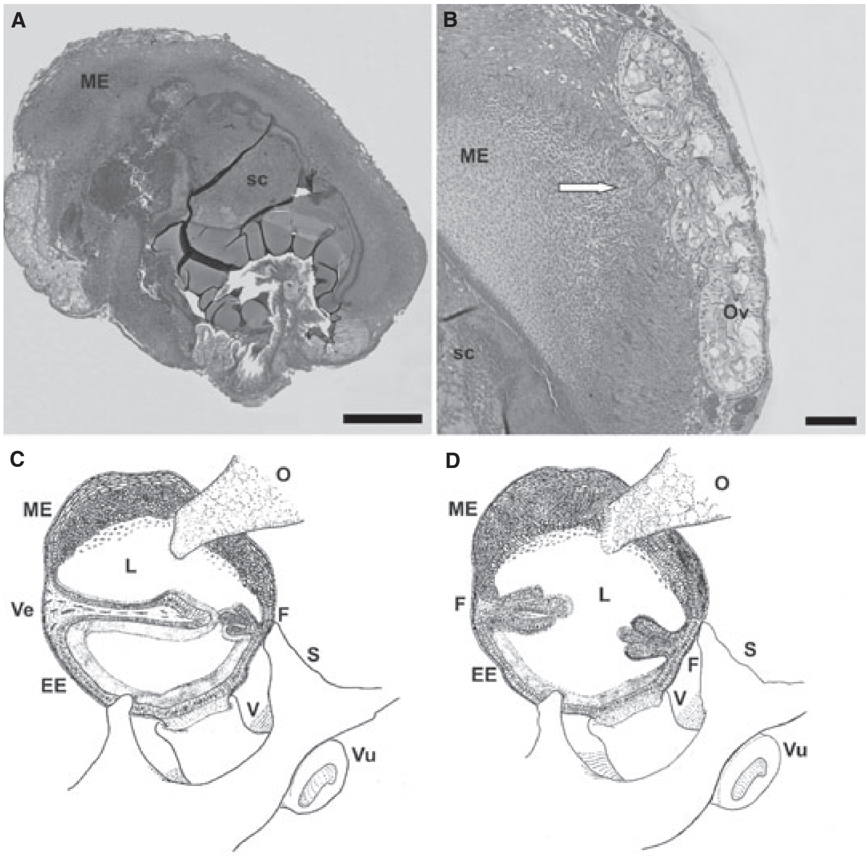

Fig. 3. **A.** Histology of the mesoderm-derived region of the stage II seminal receptacle at the level of oviduct insertion of *Leurocyclus tuberculosus*. Scale bar: 1 mm. **B.** Detail of the site of connection of the oviduct to the stage II seminal receptacle (arrow) of *Leurocyclus tuberculosus*. Scale bar: 0.5 mm. **C.** Schematic drawing of a section of the stage IV seminal receptacle of *Libinia spinosa*. **D.** Schematic drawing of a section of the stage IV seminal receptacle of *Leurocyclus tuberculosus*. EE, ectoderm-derived epithelium; F, folds; L, lumen of the seminal receptacle; ME, mesoderm-derived epithelium; O, ovary; Ov, oviduct; S, septum; SC, seminal content; V, vagina; Ve, velum; Vu, vulva.

is similar to that of *Maja brachydactyla* (Rotllant et al. 2007).

In members of *Le. tuberculosus* and *Li. spinosa*, the oviducts connect with the SR at the midlength of the mesoderm-derived region. Therefore, we propose naming this type of connection the “intermediate type”, between the ventral and dorsal-type connections described by Diesel (1991). Among Majoidea, this intermediate type has previously been observed in *Microphrys bicornutus* (LATREILLE 1825) and *Macrocoeloma trispinosum* (LATREILLE 1825) (McLay & López-Greco 2011). In contrast, the rest of the majoid species studied so far show the ventral-type connection (Diesel 1991; Sainte-Marie & Sainte-Marie 1998; McLay & López-Greco 2011). In species with this ventral type of connection, the last mate of a polyandrous, pre-ovulating female may have a higher chance to fertilize the oocytes. On this basis, it is proposed that in eubrachyurans with an “intermediate-type” connection, such as *Le. tuberculosus* and *Li. spinosa*, neither last-male nor first-male sperm precedence is favored. Instead, the spermatophores/spermatozoa from many males would undergo intense sperm competition in the lumen of the SR, or there would be no sperm competition at all in the event females are monandrous.

The condition of the SR could be used as an indicator of the reproductive condition of mature females (those greater than 45 mm CW for *Li. spinosa*, and 42 mm CW for *Le. tuberculosus*: González-Pisani 2011). Females with SR at stage I—without spermatophores/spermatozoa—could be considered virginal females (morphologically mature females that have not copulated yet). In addition, the amount of sperm stored at the other stages can be used to infer the seasonal pattern of filling (stages II and III) and emptying (stage IV) of the SR.

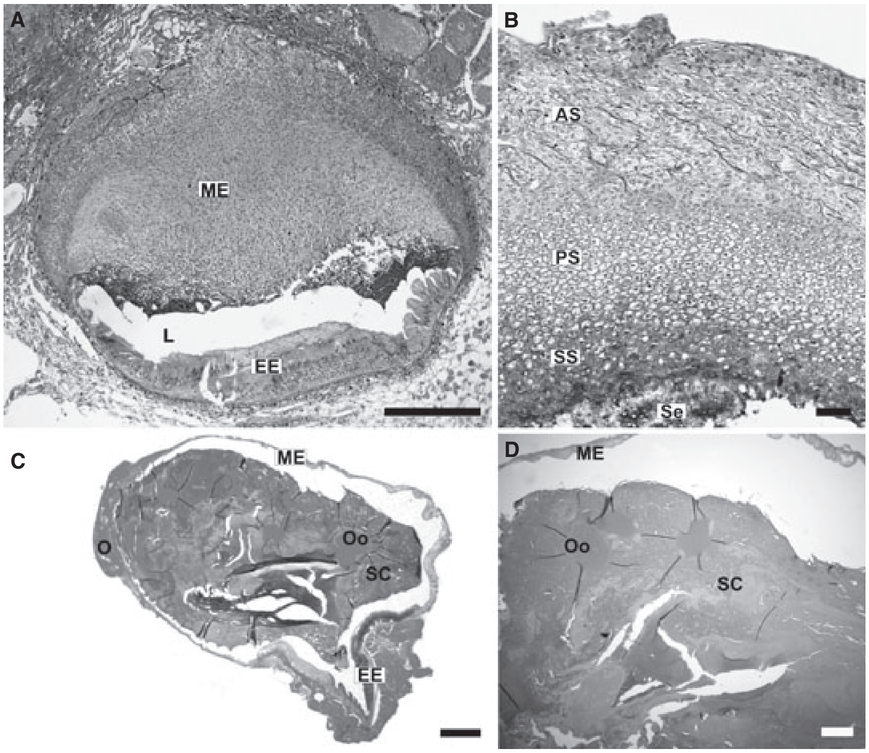

Fig. 4. **A.** Cross section of the stage II seminal receptacle of *Leurocyclus tuberculosus*. Scale bar: 100 µm. **B.** Detail of the mesoderm-derived region of the stage II seminal receptacle with the three strata of *Leurocyclus tuberculosus*. Scale bar: 10 µm. **C.** Histological longitudinal section of the stage III seminal receptacle with female's (pink) and male's (blue) secretions of *Libinia spinosa*. Scale bar: 500 µm. **D.** Detail of oocytes surrounded by spermatozoa in the stage III seminal receptacle of *Libinia spinosa*. Scale bar: 100 µm. AS, anchoring stratum; EE, ectoderm-derived epithelium; L, lumen of the seminal receptacle; ME, mesoderm-derived epithelium; O, ovary; Oo, oocytes; PS, proliferative stratum; SC, seminal content; Se, secretion; SS, secretory stratum.

The absence of discrete ejaculates or sperm packets is a difference between the studied species and other majoids such as *Inachus phalangium*, *I. communissimus* Rizza 1839*, Pisa tetraodon* (Pennant 1777)*, Macropodia rostrata* (Linnaeus 1761)*, Maja verrucosa* H. Milne Edwards 1834 (Diesel 1989), and *Maja squinado* (Diesel 1991). This could be related to different mating strategies, quick dehiscence of spermatophores, or it could reflect the monogamous condition of the females (they could have one mate within mating season and expend most or all stored sperm at each spawning).

In females of both studied species, the SR are clearly smaller than those of females of *Chionoecetes opilio* (Sainte-Marie & Sainte-Marie 1998). Furthermore, because the SR at stages I and IV have thick walls, the lumen is quite small. The SR at stage II and III have thinner walls, so the macroscopic measures are more similar to the size of the lumen. The differences in the size of the lumen (available space to load with spermatophores) could be related to the extent of reproductive period, which is prolonged in both studied species. It is possible that the small SR of the two studied species implies that they do not need to store much sperm, since males are available for mating over extended periods.

The seminal contents of other Majoidea studied so far are stored in the mesoderm-derived region of the SR, so in some species the fertilization occurs in the ectoderm-derived region (Diesel 1989; Sainte-Marie & Sainte-Marie 1998). In contrast, histological sections of the SR of *Le. tuberculosus* and *Li. spinosa* show that both sperm and oocytes are found in the mesoderm-derived region of the SR. This evidence points to the latter region as the most likely fertilization site.

The SR of *Le. tuberculosus* and *Li. spinosa* have a histological structure similar to that of other Majoidea. These have a mesoderm-derived region with three cell strata and an ectoderm-derived region with chitinized walls of ectodermal origin. These regions have received different names from different authors. The dorsal (mesoderm-derived) region was

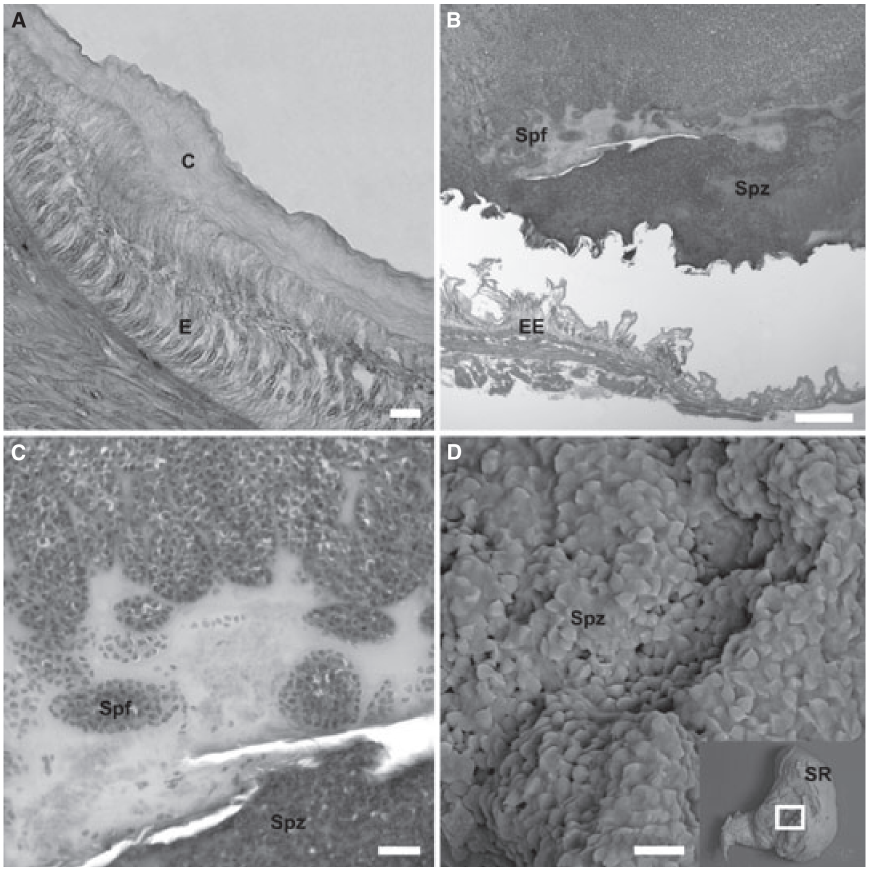

Fig. 5. **A.** Histological detail of the ectoderm-derived region of the stage II seminal receptacle of *Libinia spinosa*. Scale bar: 10 µm. **B.** Histological cross section of the ectoderm-derived region of the stage II seminal receptacle with spermatophores and free spermatozoa of *Leurocyclus tuberculosus*. Scale bar: 300 µm. **C.** Detail of the spermatophores and free spermatozoa in the stage II seminal receptacle of *Leurocyclus tuberculosus*. Scale bar: 50 µm. **D.** SEM micrograph of the spermatozoa in the stage III seminal receptacle of *Libinia spinosa*. Scale bar: 10 µm. C, cuticle; E, epithelium; EE, ectoderm-derived epithelium; Spf, spermatophores; Spz, spermatozoa; SR, seminal receptacle.

referred to as the dorsal chamber (or dorsal sperm storage chamber) in *I. phalangium* (Diesel 1989), and as the "proper spermatheca" (or storage chamber) in *C. opilio* (Beninger et al. 1988; Sainte-Marie & Sainte-Marie 1998). The ectoderm-derived region was referred to as the ventral chamber (or ventral insemination chamber) in *I. phalangium* (Diesel 1989) and as the intermediate chamber (or cuticle-lined portion) in *C. opilio* (Sainte-Marie & Sainte-Marie 1998).

The secretory cells in the mesoderm-derived region of *Le. tuberculosus* and *Li. spinosa* are of the holocrine type. This feature is shared by *I. phalangium* (Diesel 1989). However, in *C. opilio*, merocrine secretory cells are reported in this region (Sainte-Marie & Sainte-Marie 1998). Nonetheless, the cells of all these species contain acidophilic secretion.

Females of *Li. spinosa* have a "velum" in the transition zone between the mesoderm-derived and ectoderm-derived regions, which is also observed in females of *I. phalangium*, *I. communissimmus*, *P. tetraodom*, and *Maja verrucosa* (Majidae) (Diesel 1989). This structure is anatomically similar to that described by Diesel (1989) for *I. phalangium*, but functionally it is different. In members of *I. phalangium*, the velum contracts before and during ovulation, forming a ventral fertilization chamber (Diesel 1989). In females of *Li. spinosa*, depending on the degree of SR loading, this structure displays different forms. In virginal females, with SR at stage I, the velum is relaxed and short. In mated females with SR at stages II and III, the velum is short, the lumens of both regions are not separated, and both oocytes and spermatozoa are found together in the mesoderm-derived region. In recently spawned females with SR at stage IV, the velum is erected, separating the mesoderm- and ectoderm-derived regions; as a

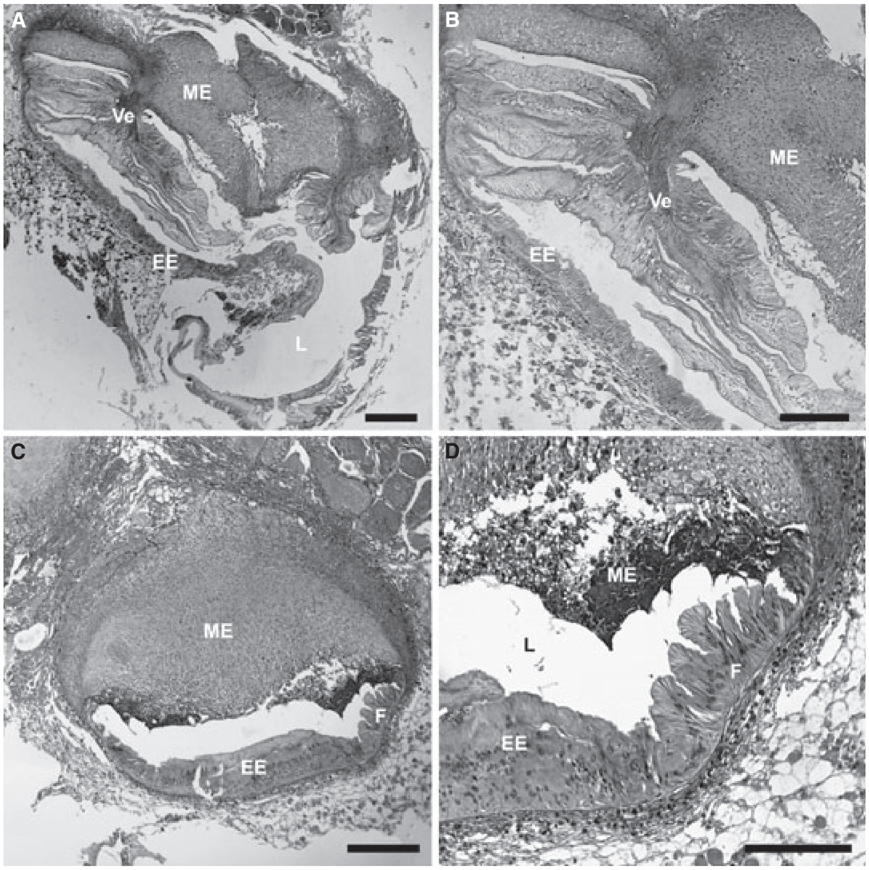

Fig. 6. **A.** Histological section of the stage IV seminal receptacle of *Libinia spinosa*. The velum and folds in the transition zone between the mesoderm- and ectoderm-derived regions are seen. Scale bar: 25 μm. **B.** Detail of the velum in stage IV seminal receptacle of *Libinia spinosa*. Scale bar: 15 μm. **C.** Histological section of the stage II seminal receptacle of *Leurocyclus tuberculosus*. The radial folds in the transition zone between the mesoderm- and ectoderm-derived regions are seen. Scale bar: 100 μm. **D.** Detail of the folds in stage II seminal receptacle of *Leurocyclus tuberculosus*. Scale bar: 50 μm. EE, ectoderm-derived epithelium; F, folds; L, lumen of the seminal receptacle; ME, mesoderm-derived epithelium; Ve, velum.

result, the seminal contents are restricted to the former region. This may indicate that at stage IV the velum acts like a valve delimiting the mesoderm-derived region, preventing the loss of residual seminal contents when the SRs are emptied after fertilization. Females of *Le. tuberculosus* show folds surrounding and projecting into the lumen in the transition zone between the mesoderm- and ectoderm-derived regions, acting as a diaphragm. Similar structures were reported by Beninger et al. (1993), Lanteigne et al. (1996), and Sainte-Marie & Sainte-Marie (1998). Only Sainte-Marie & Sainte-Marie (1998) proposed a function for this structure, suggesting that the cuticular folds in *C. opilio* (Oregoniidae) could serve to displace ejaculates and also to mix gametes and direct eggs toward the exterior. In *Le. tuberculosus*, these folds are absent in SR at stages II and III, suggesting that they would not be involved in these functions; rather, they probably restrict the seminal fluid to the mesoderm-derived region at stage IV, as does the velum of *Li. spinosa*.

Leurocyclus tuberculosus and *Li. spinosa* have concave-type vaginae as observed in other majoids (Hartnoll 1968; Sainte-Marie & Sainte-Marie 1998). In majoid females with this morphology, strong muscles attach to the concave, flexible portion of the vagina's wall, so that they can open or close the lumen by muscular contraction or relaxation (Hartnoll 1968). This would suggest that these females are not passive during copulation and spawning. In the species studied, muscle fibers attach not only to the flexible concave portion of the vagina wall, but also to a thickening of the ventral wall in the ectoderm-derived region of the SR. This probably allows the female to assist the release

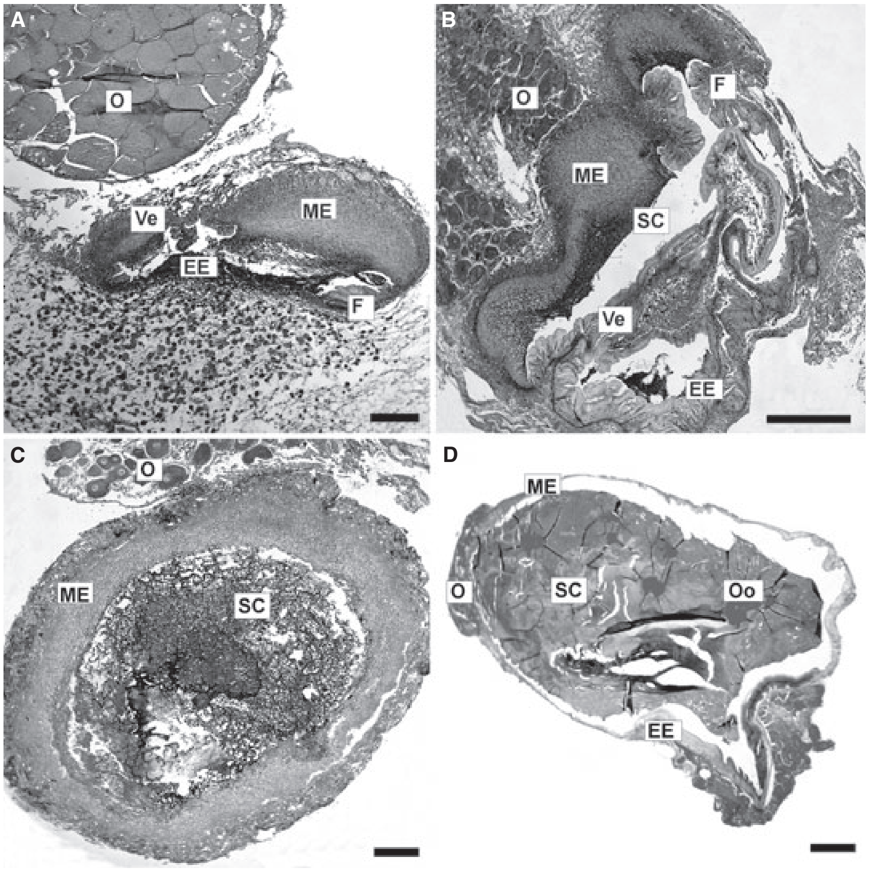

Fig. 7. Cross section of the seminal receptacles of *Libinia spinosa* at different stages. **A.** **S**tage I with SR unfilled. Scale bar: 500 µm. **B.** Stage IV with SR emptied. Scale bar: 500 µm. **C.** Stage II with SR partially full. Scale bar: 500 µm. **D.** Stage III with SR full. Scale bar: 500 µm. EE, ectoderm-derived epithelium; F, folds; ME, mesoderm-derived epithelium; O, ovary; Oo, oocytes; SC, seminal content; Ve, velum.

of fertilized ova into the vagina during spawning. The vulvae of *Le. tuberculosus* and *Li. spinosa* are internally blocked by a flexible membrane continuous with the inner wall of the vagina "vulvar valve," as in *Hyas araneus* (LINNAEUS 1758) (Hartnoll 1968).

The present study describes the morphology of the female reproductive system of *Le. tuberculosus* and *Li. spinosa* and provides a comparison with other Majoidea. In a complex taxon like the Majoidea, differences related to the SR (e.g., type of SR, morphology and function of the velum and internal folds) may be associated with different mating strategies and sperm competition mechanisms. Indeed, the "intermediate type" of insertion of the oviduct in the SR seems to indicate that last/first mate precedence is not an issue in the species studied here. Moreover, the absence of discrete ejaculates or sperm packets could be related to the short lifespan of spermatophores, whose walls are broken soon after mating. These two observations suggest that females of both species are normally monandrous (have one mate) within mating seasons, and expend most or all stored sperm at each spawning.

The small size of the SR in comparison with the SR of other Majoidea could indicate that these species do not need to store a great deal of sperm. This could imply a high frequency of mating in *Le. tuberculosus* and *Li. spinosa*, in comparison to other studied majoids.

These hypotheses can be further tested via several types of studies, including analyses of mating behavior to permit description of male-male competition before mating, and studies of postmating behavior and reproductive tract morphology to explore the timing of spermatophore dehiscence.

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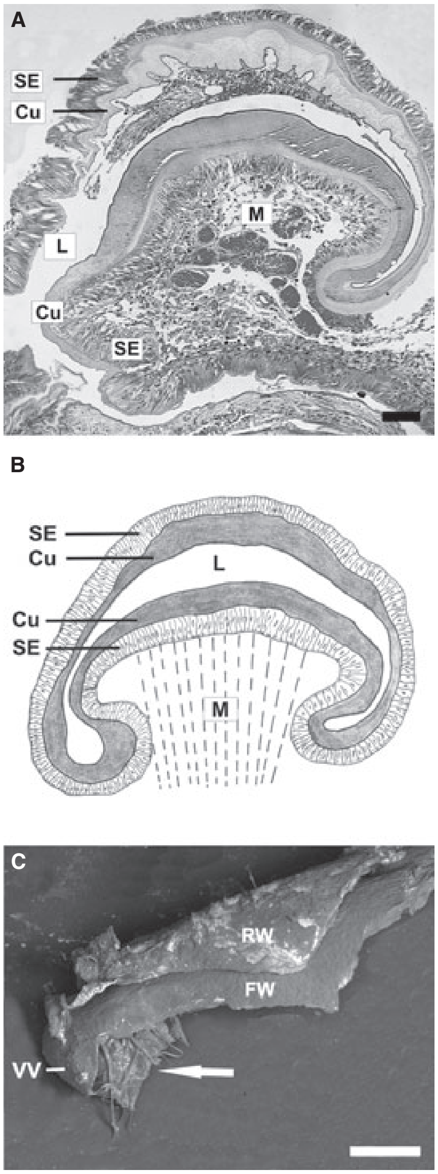

Fig. 8. A. Cross section of the vagina in *Libinia spinosa*. Scale bar: 100 μm. **B.** Schematic drawing of the vagina in the transverse plane indicating the insertion of the muscle fibers. **C.** SEM image of the longitudinal medial section of the vagina in *Leurocyclus tuberculosus*. The arrow indicates the muscle fibers. Scale bar: 500 μm. Cu, cuticle; FW, flexible wall; L, lumen of the vagina; M, muscle; RW, rigid wall; SE, simple epithelium; VV, vulvar valve.

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