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Copulatory system of the spider crab *Libinia spinosa* (Crustacea: Brachyura: Majoidea)

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Functional morphology of the male gonopods, female holding systems and vulvae of Libinia spinosa *are described using scanning electron microscopy and histological sections, establishing differences between immature and mature individuals. The first gonopod of mature males, larger than 35.1 mm of carapace width (CW), is long, slender, and ending in two wing-like processes; abdominal, characterized by the presence of a sub-terminal seminal canal, and sternal, closed and where the cuticle holds setae. In immature males, smaller than 29.9 mm CW, both processes are shorter, and the tip of the abdominal process closed. Six different types of setae are described for both gonopods. Internally, the gonopods have rosette glands in immature and mature individuals. The presence of secretions, which may be involved in the molting process, was observed in immature males towards the external cuticle corresponding to the rosette glands. The rosette glands may be related to reproduction in mature males. Females presented a press-button holding system and a biconcave vulva. In immature females, smaller than 36.1 mm CW, the vulva aperture was closed by integument bulging to the outside. In mature females, larger than 42.6 mm CW, the locking system was almost fused to the cuticle, and the vulvae showed an open biconcave form with a more inner flexible membrane. Morphological characteristics shown by the gonopods of mature males develop synchronously with the acquisition of physiological maturity, not with morphometrical maturity. The mature holding systems and vulvae of females are developed synchronously with physiological and morphometrical maturity.*

Keywords: gonopods, rosette glands, vulva, holding systems, spider crab, maturity, molt, insemination

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INTRODUCTION

Male spider crabs (Majoidea *sensu* Ng *et al.*, 2008) exhibit complex reproductive strategies related to the condition of the female (Jones & Hartnoll, 1997) and to their morphotype within the population (Sagi *et al.*, 1993; Laufer *et al.*, 1994). They reach sexual maturity at the terminal moult, exhibiting a disproportionally large cheliped that plays an important role in mating displays (Donaldson & Adams, 1989; Sainte-Marie *et al.*, 2008). Another male secondary sexual character, as in other Brachyura, is the modification as gonopods of the first and second pairs of pleopods (Beninger *et al.*, 1991). The gonopods are used in the transfer of spermatophoric masses to the female seminal receptacles during mating events (Bauer, 1986). These organs are rather stable and little affected by environmental variables (Chambers *et al.*, 1980), a condition that underlies hypotheses on decapod phylogeny as they are significant taxonomic characters (Bauer, 1986; Martin & Abele, 1986).

Early descriptions of Brachyura male gonopods were limited to the gross morphology under dissecting microscopes, although some studies have used scanning electron microscopy (SEM) for detailed observation of microstructures (Beninger *et al.*, 1991; Neumann, 1996; Beninger & Larocque, 1998; Brandis *et al.*, 1999; Chen *et al.*, 2007; Rorandelli *et al.*, 2008). The first descriptions dealt only with the first pair of gonopods, although they are part of a complex spermatophore transfer mechanism together with the second pair (Beninger *et al.*, 1991; Brandis *et al.*, 1999). Knowledge concerning the internal anatomy and histology of brachyuran gonopods are mainly focused on the rosette glands located in the first pair (Beninger *et al.*, 1991), for which possible functions have been suggested: hardening of the cuticle (Johnson & Talbot, 1987); or a role in reproduction as accessory sex glands (Diesel, 1989; Beninger *et al.*, 1991, 1995; Beninger & Larocque, 1998).

Morphological studies of majoid gonopods are scarce compared to the attention given to the reproductive biology of females (Hartnoll, 1965; Diesel, 1989; Beninger *et al.*, 1993; Sainte-Marie *et al.*, 2002; Corgos & Freire, 2006; Barón *et al.*, 2009). Brachyuran gonopores, or vulvae, may take one of three forms of closure: (1) a membrane opened by simple muscular action; (2) a soft membrane; or (3) a calcified operculum (Hartnoll, 1968, 2006; Thompson & McLay, 2005). Majoid females present vulvae of the concave type (Hartnoll, 1968). In mature individuals the opening of the vulva remains permanently soft (Diesel, 1991).

Juvenile females, as well as males, are capable of locking their abdomen using a variety of structures, while in mature or ovigerous females those structures may remain functional, become non-functional scars, or disappear completely (Ng *et al.*, 2008). Guinot & Bouchard (1998) argue that the acquisition of a locking mechanism may be regarded as a

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synapomorphy for the Brachyura. They showed that in primitive podotremes the locking structures are associated to the coxae of thoracopods, while in eubrachyurans they have moved onto the sternal plate, consisting of a prominence on the fifth thoracic sternite and a socket on the sixth abdominal segment. The Majoidea shows the typical press-button system (Guinot & Bouchard, 1998).

Several majoid species occur in the Argentinean Biogeographic Province (south-western Atlantic; Boschi, 2000), among which *Libinia spinosa* (Milne Edwards, 1834) is the most abundant along the coast of Buenos Aires Province (Boschi *et al.*, 1992; Scelzo *et al.*, 2002). Boschi (1964) published line drawings of the first pair of gonopods of *Libinia spinosa*. The genitalia and gonadal stages of development were described in detail for both sexes through morphological and histological studies (Sal Moyano, 2007; Sal Moyano *et al.*, 2010a, b). Physiological maturity, as defined by the presence of spermatophores in the male vas deferens, occurs before morphological maturity. The latter is determined by the enlargement of the chelae at the terminal moult (Sal Moyano, 2007; Sal Moyano *et al.*, 2010b). In contrast, the development of mature ovaries in females (physiological maturity) is synchronized with the enlargement of the abdomen at the terminal moult (morphological maturity) (Sal Moyano, 2007; Sal Moyano *et al.*, 2010b). Reported here are results of investigations on the functional morphology of the copulatory system of *L. spinosa* based on: (1) the detailed micromorphology of the male gonopods using SEM and histological sections; (2) the female vulva and the abdomen holding system; and (3) differences between mature and immature individuals.

MATERIALS AND METHODS

Crabs were collected along the coast of Mar del Plata (38°S 57°33′W), Argentina, using trawl nets towed by commercial boats, between September 2006 and October 2007. Carapace width (CW) was measured with a digital caliper and used as the reference variable. Comparison of mature and immature individuals was conducted following Sal Moyano *et al.* (2010b); accordingly, males larger than 33.6 mm CW and females larger than 40.3 mm CW were considered physiologically mature.

The gonopods of 15 individuals ranging from 17.6 to 82 mm CW were removed and selected for SEM studies, while those of 28 males ranging from 34.3 to 71.4 mm CW were used for histological sections. The area surrounding the vulvae and holding systems of twenty females (8 to 52.3 mm CW) were dissected and used for SEM. Samples were fixed in 2.5% glutaraldehyde in 0.1 M sodium cacodylate buffer, at pH 7.2–7.4. Samples were dehydrated in a graded alcohol series, dried in hexamethyldisilazane replacing the critical-point, coated with gold-palladium and viewed with a JEOL 6460 microscope. For histological studies, samples were fixed in Bouin's solution for 48 hours. The fixed tissues were dehydrated in a series of graded alcohol solutions, cleared in xylene agent and embedded in paraffin. 5–7 μm serial sections were cut on a rotary microtome. Sections were mounted on glass slides and stained with standard haematoxylin–eosin and Masson's Trichrome for observation under light microscope.

The terminology used mostly follows that of Nishimura (1967), Hartnoll (1975), Beninger *et al.* (1991), Neumann (1996), Guinot & Bouchard (1998) and Garm (2004).

RESULTS

External morphology of the gonopods

The first gonopod (G1) of mature males larger than 35.1 mm CW is composed of two articles: the basal protopodite and the long, slender distal endopodite that narrows towards its terminal end. The cuticle is ventro-laterally infolded forming a tube that functions as a seminal canal (Figure 1A). The apical tip exhibits two wing-like processes, sternal and abdominal, of different shape. The abdominal process is characterized by the presence of a sub-terminal seminal canal, expanded as a funnel and surrounded by a cuticle girdle of simple short setae, denominated as setae of type 1, characterized by a conical shape and fused-cuticle insertion (Figure 1B, C, D). The sternal process is closed, with a spatula-like ending; the folded cuticle bulges on its surface, having simple, grouped setae of type 1 (Figure 1B, D). Pits are arranged on the external surface of the gonopod, close to the tip of both processes and continuing to the median region. Short, conical setae with terminal pores and infracuticular insertion protrude from the pits; these are referred to as setae of type 2 (Figure 1C, E). The mid-region has two other different types of setae (types 3 and 4) distributed along the gonopod (Figure 1F). Type 3 setae are characterized by a conical shape, and have secondary setules with infracuticular insertions on the median part (Figure 1G). Setae of type 4 are pinnate, with pinnules growing randomly along its axis and with infracuticular insertion (Figure 1H). The latter extend to the basal region, forming dense groups (Figure 1H). Types and position of setae are summarized in Table 1.

The G1 of immature males smaller than 29.9 mm CW present incomplete development of the apical processes mainly the abdominal one, which corresponds to the seminal canal. The whole process is shorter, is not funnel-shaped and the tip is closed (Figure 1I). Secretions extruding through pores are observed over the entire surface of the gonopod (Figure 1J, K). The type and position of setae were similar in immature and mature individuals. Bacterial colonies were observed inside the seminal opening of the G1 abdominal process of some mature individuals (Figure 1L).

The second gonopod (G2) of mature males is approximately five times smaller than the G1, with the endopodite of a tubular shape (Figure 2A). The distal region consists of an apical girdle, an appendix masculina, and a central protuberance (Figure 2B). The apical girdle has isolated type 5 setae, characterized by conical form and infracuticular insertion. In the central protuberance, type 5 setae are found together with other types of setae, referred to as type 6 setae (Figure 2C, D). These are characterized by the presence of two projections at their base and fused-cuticle insertion. No setae were observed in the median region. The basal region is surrounded by setae of type 4. Types and position of setae are summarized in Table 1.

No differences between mature and immature individuals were observed in the general morphology of the G2. Type and position of setae were similar in both mature and immature individuals. Cuticle secretions arising from cuticle pores
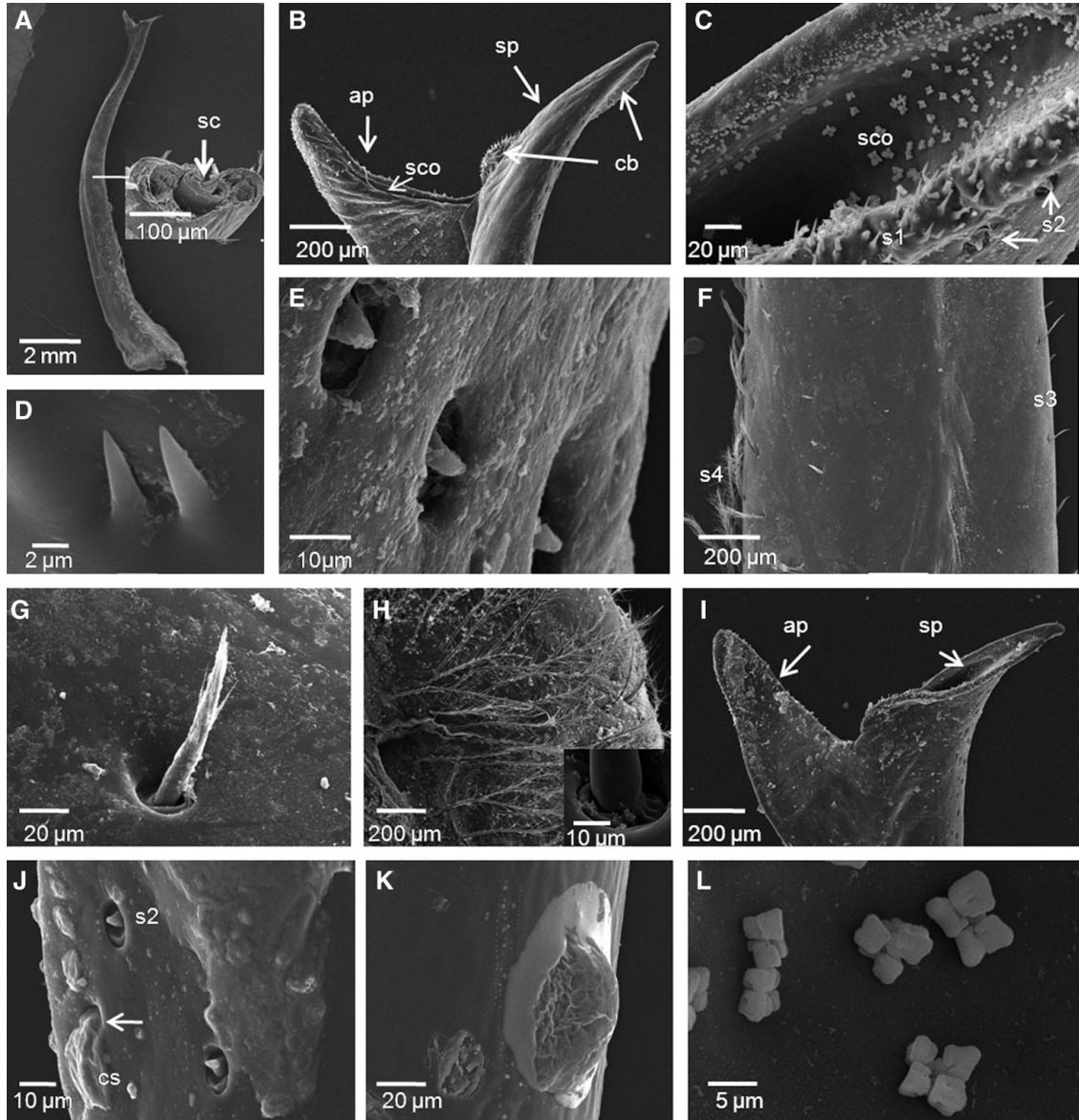
Fig. 1. *Libinia spinosa*, scanning electron micrographs of the first gonopod, G1. Mature males: (A) general structure of G1. Inset: transversal section of the median portion showing the cuticle infolded, forming the seminal canal (sc); (B) apical tip with wing-like processes, the sternal process (sp) showing cuticular bulges (cb) holding setae type 1, and the abdominal process (ap) with the seminal canal opening (sco); (C) detailed structure of the seminal canal opening (sco) of the abdominal process, showing setae of type 1 (s1) on its cuticle girdle and setae of type 2 (s2) on its external surfaces; (D) detailed structure of setae of type 1; (E) detailed structure of setae of type 2; (F) mid- region showing setae of type 3 (s3) together with setae of type 4 (s4); (G) detailed structure of setae of type 3, note the setules growing from its median region; (H) general view of grouped pinnate setae type 4. Inset: infracuticular insertion of setae of type 4. Immature males: (I) apical tip showing the shorter sternal (sp) and abdominal (ap) processes; the abdominal one is not funnel-shaped and its tip is closed; (J) surface of the gonopod showing cuticle secretions (cs) arising from the cuticle pores (arrow), and the setae of type 2 (s2); (K) detailed structure of cuticle secretions; (L) colonies of bacteria inside the seminal opening of mature male G1.

were observed over the entire length of the G2 of immature males (Figure 2E, F), as in the G1.

Internal anatomy of the gonopods

Cross-sections of the G1 showed the arrangement of the infolded cuticle which demarcates the seminal canal (Figure 3). Internally, in its apical region, rosette glands encircle the seminal canal (Figure 3A). Glands are composed of secretory cells with a basal nucleus, radially arranged around a central pore cell, and a canal cell that connects to the secretory surface (Figure 3B, C). Glandular ducts of the rosette glands are observed through the cuticle of the

seminal canal (Figure 3D). The median region presents a thick cuticle and a subjacent hypodermis, characterized by a cubic epithelium and loose connective tissue (Figure 3E). The basal region shows the presence of striated muscle, surrounded by loose connective tissue (Figure 3F). Immature and mature individuals show differences in the arrangement and quantity of rosette glands. In immature males they are more abundant and arranged along the entire length of the G1 (Figure 3G, H), while they are restricted to the apical region, surrounding the seminal canal (Figure 3A) in mature individuals.

The internal anatomy of both immature and mature G2 showed, in their apical regions, the central protuberance

Table 1. Position and characterization of the different types of setae encountered in the first (G1) and second (G2) gonopods of *Libinia spinosa* males.

Type setae	Gonopod	Position	Characteristics
1	1	Apical	Simple conical shape, fused-cuticle insertion
2	1	Apical/median	Simple conical shape, short length, terminal pore, infracuticular insertion, in pits
3	1	Median	Conical shape, secondary setules, infracuticular insertion
4	1/2	Basal	Pinnate, infracuticular insertion
5	2	Apical	Simple conical shape, infracuticular insertion
6	2	Apical	Conical shape, with two projections, fused-cuticle insertion

bordered by loose connective tissue and the hypodermis (Figure 3I). Only loose connective tissue was observed in the median portion, and striated musculature similar to G1 was recognized in the basal region. Longitudinal sections show the presence of rosette glands distributed along the entire length of the G2 (Figure 3J).

Morphology of the holding system and the vulvae

In immature females smaller than 36.1 mm CW the locking system is a typical eubrachyuran press-button composed by an acute cuticle protuberance of the fifth cephalothoracic sternite and a deep socket on the sixth abdominal segment (Figure 4A, B). The vulvae are located near the cuticle protuberance and are of biconcave shape (Figure 4C). The aperture is closed by integument on the ventral side, which bulges resembling an 'operculum' (Figure 4D). The holding system and vulvae are surrounded by long pinnate setae, similar to the setae of type 4 present in male gonopods.

In mature females, larger than 42.6 mm CW, the locking system is reduced; the protuberance and the abdominal socket are almost completely fused with the cuticle (Figure 4E, F). The vulvae are open, biconcave in shape, with a more inner flexible membrane hardly seen from the outer surface but easily displaced (Figure 4G, H). Long pinnate setae surround both structures.
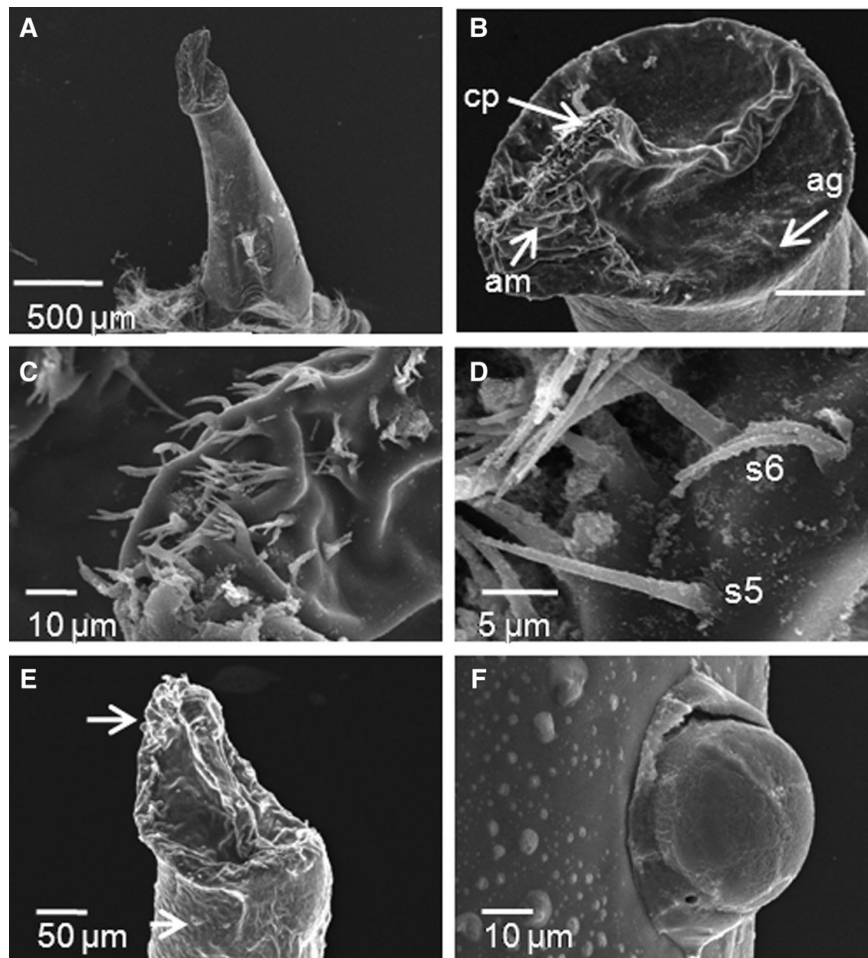
Fig. 2. *Libinia spinosa*, scanning electron micrographs of the second gonopod, G2. Mature males: (A) general view of G2; (B) apical region showing the central protuberance (cp), the apical girdle (ag) and the appendix masculina (am); (C) detailed structure of the central protuberance holding setae of types 5 and 6; (D) detailed structure of setae of types 5 (s5) and 6 (s6); notice the two projections of setae of type 6. Immature males: (E) general view of G2 with cuticle secretions (arrows); (F) detailed structure of cuticle secretions.
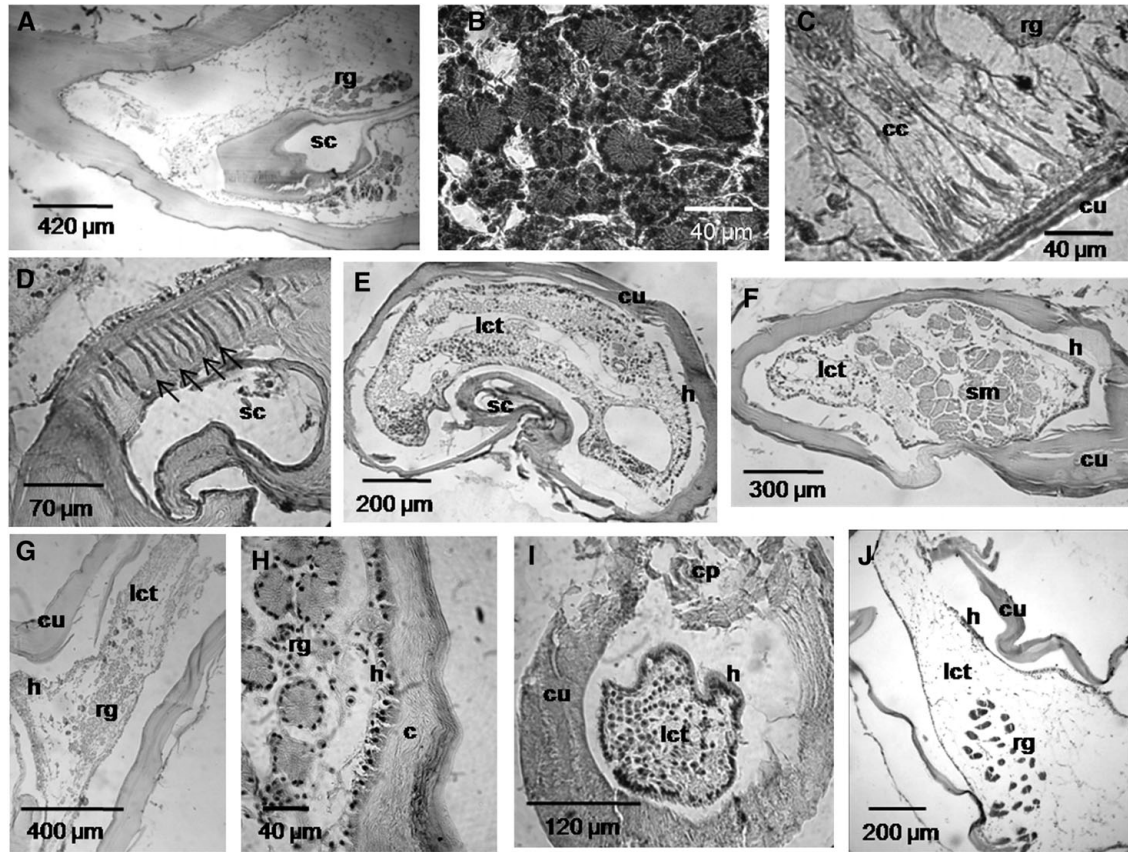
Fig. 3. *Libinia spinosa*, histological sections of mature and immature male's gonopods. First gonopod, G1, of mature males: (A) transversal section of the apical tip showing the rosette glands (rg) surrounding the seminal canal (sc); (B) detailed structure of rosette glands; (C) detailed structure of the canal cells (cc) of rosette glands (rg) opening through the cuticle (cu); (D) pores (arrows) corresponding to the rosette glands traversing the cuticle of the seminal canal (sc); (E) transversal section of the median region showing the seminal canal (sc), cuticle (cu), hypodermis (h), and loose connective tissue (lct); (F) transversal section of the basal region showing the striated muscle (sm), cuticle (cu), hypodermis (h) and loose connective tissue (lct). G1 of immature males: (G) longitudinal section showing the presence of rosette glands (rg) distributed along the gonopod, cuticle (cu), hypodermis (h) and loose connective tissue (lct); (H) detailed structure of the rosette glands distributed along the gonopod, cuticle (cu) and hypodermis (h). Second gonopod, G2, of mature and immature males: (I) transversal section of the apical tip showing the central protuberance (cp), cuticle (cu), hypodermis (h) and loose connective tissue (lct); (J) longitudinal section showing the presence of rosette glands (rg) distributed along the gonopod, cuticle (cu), hypodermis (h) and loose connective tissue (lct).

DISCUSSION

Copulatory system: mechanism of insertion

Male first gonopods of higher brachyurans are characterized by the complete folding of the cuticle, forming a tube-shaped structure (Hartnoll, 1975; Beninger *et al.*, 1991; Neumann, 1996; Brandis *et al.*, 1999) that is inserted in the female vulvae during copulation (Guinot & Quenette, 2005). The G1 transfers ejaculates into the female seminal receptacle. Coupled with the G2 it acts as a hydraulic pump to push the seminal fluid through the seminal canal (Beninger *et al.*, 1991). The apical tip of the G1 shows specific variation, and it is an important taxonomic character. While in the genera *Chionoecetes* (Beninger *et al.*, 1991), *Maja* (Neumann, 1996) and *Inachus* (Rorandelli *et al.*, 2008) the tip has a single ending, in *Libinia spinosa* the two wing-like processes, described in detail in this study, have a feature that has been also mentioned for *Leurocyclus tuberculosus* (Milne Edwards & Lucas, 1842) (Boschi, 1964). In *L. spinosa* each process has a particular shape and micro-structures, prompting the hypothesis that they have different roles during copulation. The form of the apical tip matches the biconcave shape of

the female vulva, suggesting that both G1 processes are inserted during mating. Due to its funnel-like morphology, it is unmistakable that the abdominal process is responsible for the transfer of spermatophores through its seminal canal, clearly visible on the gonopod tip. The sternal process has a complex shape, consisting of a cuticular expansion on its closed terminal portion and a bulging site with simple setae of conical form, close to the proximal branching point of the gonopod's tip. The whole tip of the abdominal process could be inserted into the female vulva and anchored by the structures of the gonopod sternal process described earlier. This fit would keep the abdominal process, which holds the seminal canal, fixed in its position while the ejaculate is being transferred during the copula.

Contrasting hypotheses have been proposed to explain the specific role of morphological structures considered to be involved in the process of gonopod insertion. Jennings *et al.* (2000) described the presence of a hook in the G1 process of the ocypodoid *Macrophthalmus hirtipes* (Heller, 1862), and suggested that it might help the male to be positioned during copulation. The presence and function of that hook resembles the sternal process described here for *L. spinosa*. Mature majoid females are characterized by the lack of
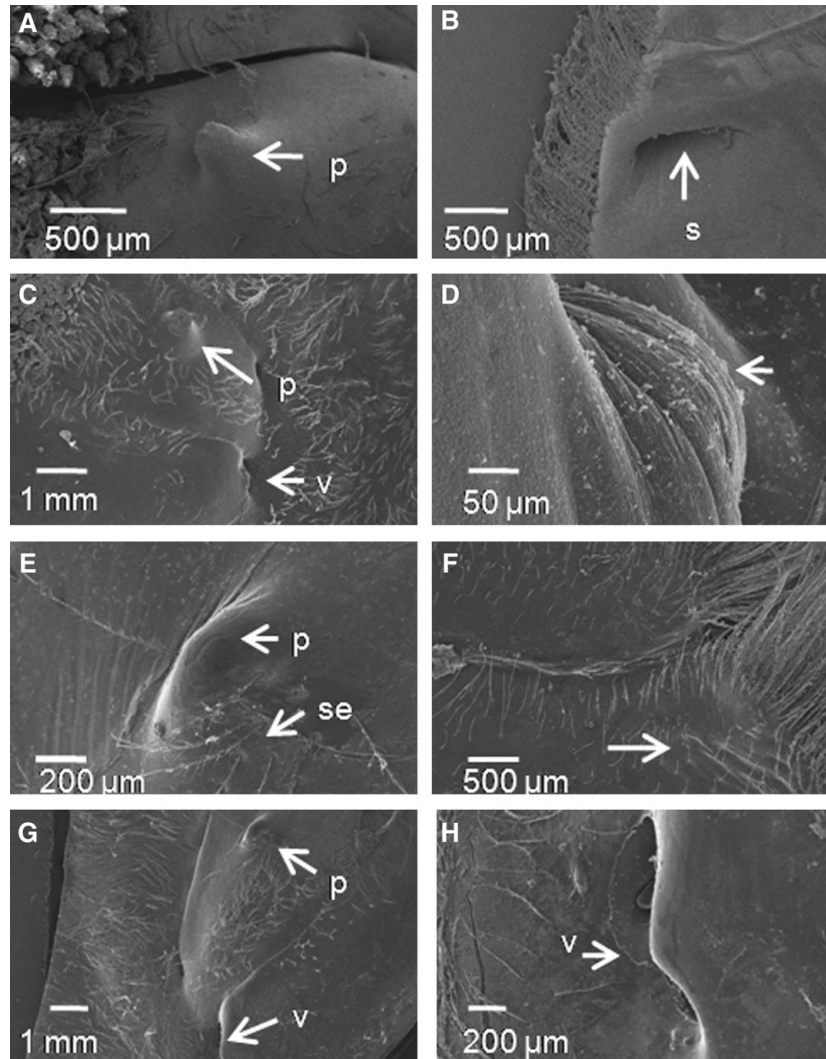
Fig. 4. *Libinia spinosa*, scanning electron micrographs of holding systems and vulvae of females. Immature females: (A) well developed sternal protuberance (p) of the holding system; (B) abdominal socket (s) of the holding system; (C) fifth cephalotoracic sternite showing the sternal protuberance (p) and the biconcave vulva (v); (D) detailed structure of the vulva with its aperture closed by integument that bulges (arrow). Mature females: (E) sternal protuberance (p) of the holding system fused with the cuticle and pinnate setae (se); (F) abdominal socket of the holding system fused with the cuticle (arrow); (G) fifth cephalotoracic sternite showing the sternal fused cuticle protuberance (p) and the biconcave vulva (v); (H) detailed of the open biconcave shape of the vulva (v).

opercula (Hartnoll, 1968), as shown also for *L. spinosa* in this study. It has been suggested that in *Chionoecetes opilio* (Fabricius, 1788) the insertion of the G1 process is involved in the removal of seminal receptacle contents corresponding to previous mating (Beninger *et al.*, 1991). In *L. spinosa* that function seems improbable because the histological study of female seminal receptacle revealed the presence of distinct sperm packets, indicative of multiple mating events without removal of previous ejaculate (Sal Moyano *et al.*, 2010a).

External anatomy of the gonopods: function of the setae

Distribution of the setae on the gonopods was addressed in a number of studies dealing with brachyuran crabs. Beninger *et al.* (1991) attributed functions to the setae based on their position, structure and insertion in the cuticle in *C. opilio*. Setae of type 1 were found here in the apical tip of the G1

and were similar to the 'pyramid-like' setae described in the G2 of three species of the genus *Maja* (Neumann, 1996), the 'cuspidate' setae observed in the maxillae and maxillipeds of *Cherax quadricarinatus* (Martens, 1868) (Garm, 2004) and the 'tooth-like' setae described for the G1's apical tip of *Inachus phalangium* (Fabricius, 1775) (Rorandelli *et al.*, 2008). Rorandelli *et al.* (2008) suggested that the function of these structures surrounding the seminal canal is to break down spermatophores during the transfer to the female seminal receptacle. Such a function seems implausible in *L. spinosa* due to the fact that setae of this type are located in the abdominal process, surrounding the seminal canal, but also in the sternal process. We suggest a mechanical function: they would allow keeping the gonopod positioned during copulation while inserted in the female vulva. The type 2 setae described here are similar to setae present in the G1 of species of *Maja* (Neumann, 1996) and in the antennae of the lobster *Homarus americanus* (Milne Edwards, 1837) (Derby, 1982).

379 Derby (1982) proposed that these setae, which emerge from a
380 cuticle depression, have a sensory function. In *L. spinosa* the
381 distribution of pits with setae of type 2, present only in the
382 mid and apical portions of G1, would indicate that males
383 are capable of sensing female condition during gonopod
384 insertion, and that the setae would be protected from
385 harmful events. Setae of type 3 have been also observed in
386 *C. opilio*, for which Beninger *et al.* (1991) suggested a poss-
387 ible mechanoreceptor role related to their infracuticular
388 insertion, helping to position the gonopod and facilitating
389 the copula. The completely pinnate setae (type 4) observed
390 at the basal region of the G1 of *L. spinosa* are morphologi-
391 cally similar to those described by Phillips & Macmillan
392 (1987), Beninger *et al.* (1991) and Garm (2004) for other
393 crustaceans. These setae could rub against the abdomen
394 during copulation, while those of the G2 could rub against
395 the protopodite of the first gonopod (Beninger *et al.*, 1991).

396 The presence of bacterial colonies in the apical opening of
397 the male gonopod seminal canal, reported here for *L. spinosa*,
398 has been reported before in other species (Benhalima &
399 Moriyasu, 2001), as well as inside the female seminal recepta-
400 cle and on the vaginal wall (Beninger *et al.*, 1993; Elner &
401 Beninger, 1995; Benhalima & Moriyasu, 2001). Their presence
402 in *C. opilio* male gonopods indicates that they infect the
403 female's seminal receptacles during mating (Benhalima &
404 Moriyasu, 2001), a phenomenon that is likely to occur also
405 in *L. spinosa*. In the case of female seminal receptacles the pro-
406 duction of melanin by the epithelium has been involved in
407 maintaining the bacterial population, being responsible for
408 the exclusion of opportunistic microbes, or to provide sub-
409Q1 strates for the sperm to metabolize (Elner & Beninger, 1992;
410 Beninger *et al.*, 1993; Jensen *et al.*, 1996).

411 The G2 of *L. spinosa* is short and highly modified, as
412 reported also for *Chionocetes* and *Maja*, consisting of an
413 apical girdle, an appendix masculina, and a central protuber-
414 ance which could represent a vestigial appendix interna.
415 Hartnoll (1975) and Beninger *et al.* (1991) suggested that
416 the G2 would be involved in the direct transport of seminal
417 fluid through the seminal canal due to its short size. The
418 apical girdle could act as a seal in the seminal canal, allowing
419 the seminal fluid to be hydraulically pushed down the canal
420 (Beninger *et al.*, 1991). Neumann (1996) compared the G2
421 morphology of three species of *Maja* with *C. opilio*, conclud-
422 ing that the functional interpretation proposed by Beninger
423 *et al.* (1991) could apply to all Majoidea. A special case
424 occurs in freshwater crabs of the genus *Potamon*, where the
425 G2 is unusually long; ending in a long sclerotized tube,
426 which receives the ejaculate from the lumen of the first
427 gonopod, and transfers it directly to female genital ducts
428 (Brandis *et al.*, 1999).

429 The type 5 setae, described here for the apical tip of G2, are
430 similar to the long cuspidate setae with cup insertion
431 described by Beninger *et al.* (1991) for the first gonopod of
432 *C. opilio* and by Garm (2004) for the maxilla of *C. quadricar-
433 inatus*. These setae, of infracuticular insertion, could have a
434 role in mechanoreception, probably detecting the movements
435 of G1 during copula and allowing the insertion of G2 inside
436 the seminal canal. Setae of type 6 resemble the complexly
437 branched setae described by Neumann (1996) as 'star-shaped'.
438 Because of the fused-cuticle insertion it is difficult to hypoth-
439 esize on their function. Beninger *et al.* (1991) suggested that
440 the non-articulating setae of the G2 probably assist in the
441 pumping of seminal fluids during ejaculation.

Internal anatomy of the gonopods: rosette glands

The internal anatomy of both gonopods showed significant development of the musculature in the basal region, and in the presence of rosette glands. The striated musculature suggests that positioning of the gonopods is adjusted through basal movements. Rosette glands are present in the pleopods of both immature and mature females and in adult males of *L. spinosa*, as is also the case for the lobster *H. americanus* (Johnson & Talbot, 1987) and the G1 of several species of Portunoidea, Cancroidea, Grapsoidea (Beninger & Larocque, 1998) and Majoidea (Diesel, 1989; Beninger *et al.*, 1991). Secretions of these glands could play multiple functions. In females they would serve to maintain the eggs attached to the pleopods (Johnson & Talbot, 1987), while in males they could participate in the formation of seminal fluid and sperm plugs (Beninger *et al.*, 1991, 1993); they may participate in the moulting process, in both sexes (Gorvett, 1946; Talbot & Zao, 1991). In this study, external glandular secretions were restricted to both gonopods of immature individuals, while internal rosette glands were found in mature and immature crabs. Diverse roles could be assigned to rosette glands in the G1. In immature males they are likely to play a role in the moulting process (Gorvett, 1946; Stevenson, 1961; Arsenault *et al.*, 1979) and in the hardening of the new cuticle after the moult (Johnson & Talbot, 1987). Majoid crabs are characterized by the presence of a terminal moult, usually coinciding with the puberty moult, after which mature individuals cease moulting. For that reason the most likely function of the rosette glands in mature *L. spinosa* males may be their participation in the formation of seminal fluid, as they are arranged surrounding the seminal canal. The abundant glands, localized along the entire length of the gonopods of immature crabs, would be restricted after the puberty moult to the seminal canal, their role changing in the transition from the puberty to the adult stage.

In G2, as in G1, the presence of cuticle secretory products in immature individuals is an indication of their role in the moulting process, while in mature ones secretions would act as a lubricant fluid, facilitating the displacement of G2 inside G1 during ejaculation.

Female holding system and vulva

In the immature females of *L. spinosa* the abdominal locking system has a pointed button and a deep socket, but both structures change in the course of successive moults. The button remains smooth and the socket disappears in mature females, and so the abdomen cannot be fastened to the sternum, implying that females are morphologically ready to mate. According to metabolic advantage explanations, the presence of a locking system in immature females enhances their mobility (Ng *et al.*, 2008). Most brachyuran mature females lack the locking system, and their abdomen enlarges to form a brood chamber carrying the developing eggs. An exception, for instance, is the Parthenopidae, in which females have a well developed locking system. This is intriguing because parthenopid females hold large egg masses (Ng *et al.*, 2008).

Following the puberty moult, the vulvae reach the size required to permit insertion of the male gonopods during

mating in the Majoidea (Hartnoll, 1965). In the case of *L. spinosa* not only the size of the gonopods but also their morphology would be essential for the intrusion of the two processes in the bifid gonopod tip. Immature females showed the presence of a bulged portion covering the posterior part of the vulva which, together with the locking device, would restrict the access of males and prevent mating.

Copulatory system of immature and mature individuals: relationship to morphological and physiological maturity

Males of *Libinia spinosa* mature physiologically at a small size, approximately at 33.6 mm CW (Sal Moyano, 2007; Sal Moyano *et al.*, 2010b). Morphometric maturity is reached on average at 58.9 mm CW, following terminal moult (Sal Moyano, 2007; Sal Moyano *et al.*, 2010b). It was observed that the morphological characters shown by the 'mature gonopods' appear coincidentally with the development of spermatophores inside the vas deferens. This character thus develops synchronously with the acquisition of physiological maturity, not with morphological maturity. In females, in contrast, physiological and morphometric maturity are reached simultaneously at an average size of 40.3 mm CW (Sal Moyano, 2007; Sal Moyano *et al.*, 2010b), which coincides with the acquisition of the mature holding systems and vulvae described here. An experimental study of mating behaviour currently under way will shed new light on inferences made on the basis of comparative morphology.

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