

MACROSCOPIC ASPECTS AND SCANNING ELECTRON MICROSCOPY OF THE OVARIES OF THE SPINY LOBSTERS *Panulirus* (CRUSTACEA: DECAPODA)

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ABSTRACT

In spite of its importance as a major fishing resource in Northeastern Brazil, little is known regarding the reproductive biology of the spiny lobster. The present study describes the macroscopic morphology and scanning electron microscopic (SEM) structure of the ovaries of the species *Panulirus argus*, *P. laevis* and *P. echinatus*. The morphological study included the parameters developmental stage, color and surface texture. With samples fixed in Bouin and Karnovsky solution, SEM analyses identified four distinct stages (I. immature, II. prematuration, III. mature, IV. spawning/resorption) as well as changes in the organization of the somatic (fibrous tissue) and germinative (oocytes) components at each stage. At Stage I, ovaries consist of nodes or cysts and are lined with thick fibrous components which tend to distend toward Stage III. The oocytes are rounded and the nucleus/cytoplasm ratio decreases as the cells grow larger and move towards the periphery. As the first SEM-aided description of *Panulirus* ovaries, the present study represents a contribution to our understanding of the tridimensional organization of the ovarian components at different stages of maturation and sheds new light on the changes which occur during the maturation process of spiny lobsters.

Key words: Pleocyemata, morphology, gonads, reproduction, aquaculture

INTRODUCTION

Decapod ovaries are paired organs primarily located in the cephalothorax, dorsally or dorsolaterally to the digestive tube and in many cases surrounded by the hepatopancreatic lobes [1,19,23]. Each ovary is coupled to a thin, tubular oviduct connected to a gonopore opening onto the sternite of the appendage of the sixth thoracic segment, corresponding to the base of the third pair of pereopods.

Female gonads vary in shape, position, size and coloring as they mature [2,17]. As with crabs and other lobster species, the ovary of the spiny lobster is basically H-shaped with the posterior lobes extending onto the abdomen [1,19].

The morphological aspects of the gonads and germ cells are used by most workers to determine

the stage of sexual maturity, especially in the more economically important species.

The classification and designation of gonadal stages vary from author to author. In penaeid shrimp, three to eight stages have been recognized by Tan-Fermin and Pudadera [31]. Four and five stages are given, respectively, for the carid shrimp species *Macrobrachium acanthurus* [8] and *M. rosenbergii* [10]. Likewise, Galvão [13] identified five stages for the carid shrimp *Atya scabra*. Among the Reptantia, *Homarus americanus* ovaries mature in seven stages [2] while those of spiny lobsters require from four to six [3-5,12,16,22,24,25; Silva JRF, Master's dissertation, Federal University of Paraíba, Paraíba, Brazil*].

The first and last stages are generally termed "immature" and "spawn" (or "post-spawn"). The intermediary stages are variously termed "development", "inactive", "prematuration", "near-mature", "active", "mature" and "maturing". So far, apart from morphological descriptions of gonadal stages, the only available description of lobster ovaries using scanning electron microscopy (SEM)

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* Histomorfometria da reprodução da lagosta verde *Panulirus laevis* (Latreille, 1817) (Crustacea: Decapoda: Palinuridae) do litoral do Estado do Ceará, João Pessoa. 150 pp.

is a study by Talbot [29,30] involving the mature ovaries of *Homarus americanus*.

The objective of the present study was to make a SEM-based description of the ovarian morphology of the spiny lobster, using three species (*Panulirus argus*, *P. echinatus* and *P. laevicauda*) occurring in the tropical Western Atlantic.

MATERIAL AND METHODS

Fifty-four female spiny lobsters (*Panulirus argus*, *P. laevicauda* and *P. echinatus*) at different stages of sexual maturation were collected off Ceará, Brazil, and dissected for removal of the gonads. Aspects such as gonadal coloring and stage of development were registered. The macroscopic assessment included pressure-testing the ovary for firmness and examining the surface for texture indicative of the presence of developed oocytes. Moreover, females with spermatophores adhering to the sternite and/or eggs adhering to the pleopods were considered to be in the final stage of ovarian maturity when eggs are released or resorbed. The latter observation was helpful considering that dissected gonads at this stage resemble early-stage or immature gonads to the naked eye.

Subsequently fragments fixed in Karnovsky or Bouin solution mixed with filtered sea water were submitted to scanning electron microscopy (SEM). The material fixed in Karnovsky solution was immersed twice for 30 min in 0.2 M sodium cacodylate buffer and then dehydrated in a series of increasingly concentrated alcohol baths (70% - 100%), in alcohol mixed with acetone (1:1) and in pure acetone, each bath lasting 10 min. The dehydrated material was then submitted to critical point drying, fastened to a metal support and coated with gold. The material fixed in Bouin solution was prepared in the same manner, though not immersed in sodium cacodylate buffer.

All observations and images were produced with the Jeol JSM-P15 scanning electron microscope, operated at 60 kV.

RESULTS

The total length of the collected animals ranged from 11 to 30 cm. The weight ranged from 78 to 823 g.

Macroscopic description of ovaries

Spiny lobster (*Panulirus*) ovaries follow the morphological pattern observed in other Reptantia with regard to the parameters used in the determination of gonadal stage, such as location, shape and maturity-related color changes.

The morphological differences between the three species sampled for the present study were not

significant enough to warrant separate descriptions. Thus, in much of the following we will refer to all species simply as spiny lobster. Four stages of gonadal development were observed: *I. Immature* - slight development of ovaries with slender anterior and posterior lobes restricted to the body cavity. Ovaries are white and difficult to distinguish from surrounding muscles (Figs. 1A and 2A). *II. Prematuration* - Ovaries have grown in volume and extension and the color is pink or light yellow. The organ appears distended and firm to the touch, indicating the multiplication of germ cells and suggesting early-stage vitellogenesis (Fig. 2B). *III. Mature* - The gonad is now fully developed and will occupy all available space in the body cavity. It becomes more sinuous and may extend onto the second abdominal segment. The color is now orange or reddish and nodes or cysts give the surface a nubby look. When depressed, even if only lightly, the ovary releases a large number of mature cells (Figs. 1B and 2C). *IV. Spawning or resorption* - following ovulation oocytes and other cells may be resorbed by the ovaries. As observed on transparency, the gonad becomes flaccid with pigmented areas and empty spaces internally. At this point the ovary presents coloring and size much like in the immature or premature stage, although the gonad of a spawned lobster is never entirely restored to the condition of original immaturity (Fig. 2D).

The color of the gonads appears to vary very little between the species of spiny lobster examined, although no other macroscopic description of the gonadal stages of *P. echinatus* is available in the literature. However, the minimum total length of the animal at first spawning presents some interspecies variation: 16.3 cm for *P. echinatus*, 18.2 cm for *P. laevicauda* and 20.5 cm for *P. argus*.

Microscopic description of ovaries

Under SEM, the external lining of the ovary appears verrucose and entirely covered by branched and overlapping fibers. When the ovary is immature the surface is relatively smooth and homogenous, but as the organ grows the surface acquires an increasingly irregular and nubby appearance (Fig. 3).

Longitudinal and cross-sections of the ovary show the lining to be composed of several layers of filamentous or fibrous material disposed in lamellae (Fig 4A, B). Some of the inner layers invaginate toward the center of the ovary forming groups of

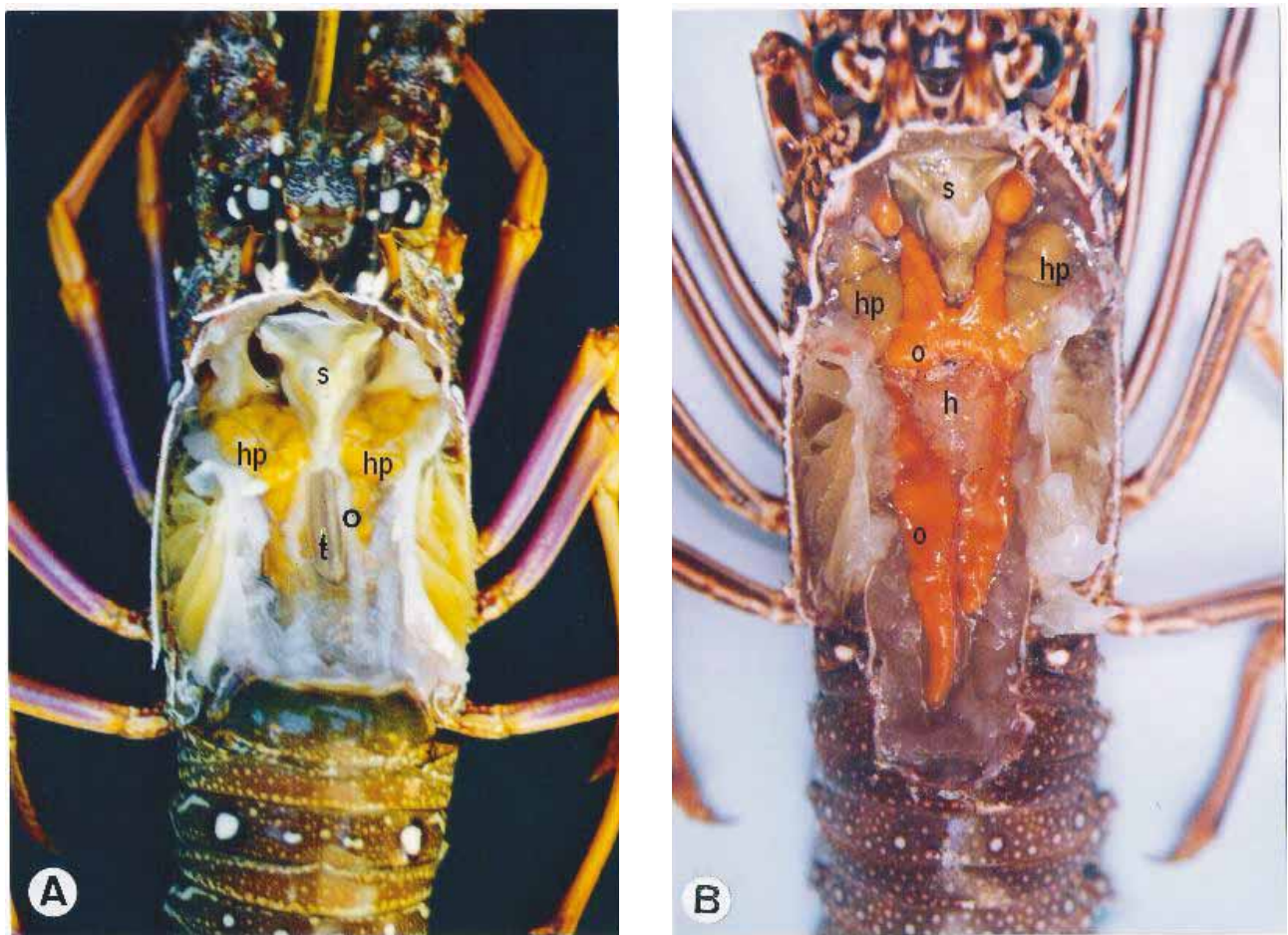


Figure 1. Location of ovaries in *Panulirus*. **A.** White-colored, immature ovary (o) in *P. argus*. **B.** Orange-colored, mature ovary in *P. echinatus* lobes developed. The rather asymmetrical posterior lobes of the gonad extend to the first abdominal segment. Hepatopancreas (hp); heart (h); stomach (s).

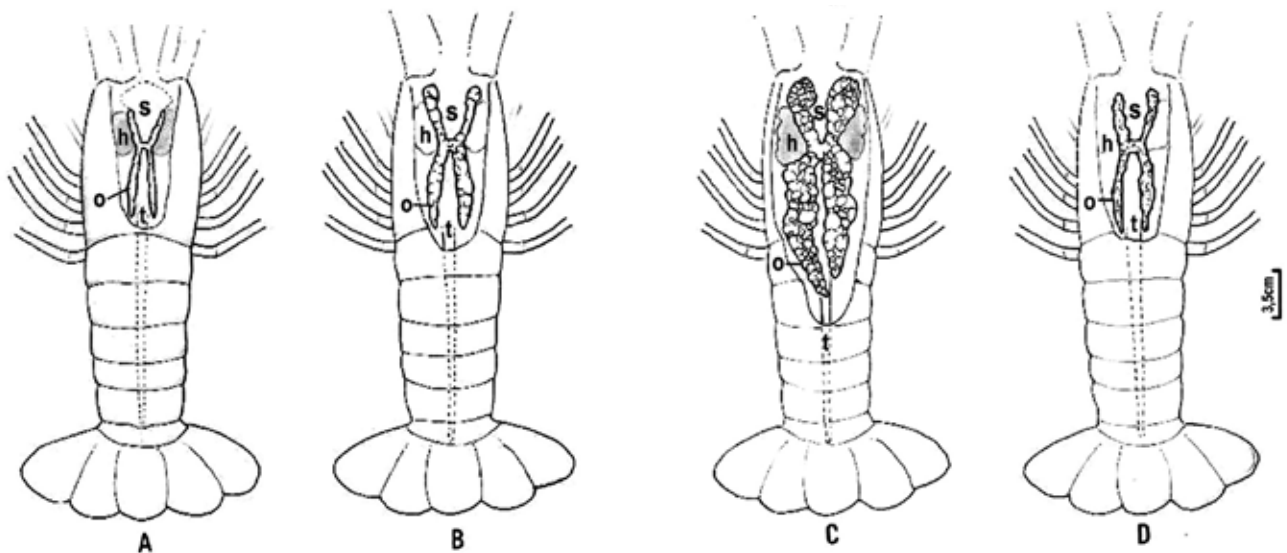


Figure 2. Diagram of the four stages of ovarian development in spiny lobsters (*Panulirus*). The exoskeleton has been partly removed disclosing the location and morphological aspects of the ovaries inside the cephalothorax. **A.** immature; **B.** prematuration; **C.** mature; **D.** spawning/resorption. Ovary (o); stomach (s); hepatopancreas (hp); posterior digestive tract (t). Bar = 3.5 cm

spherical bodies resembling cells of different sizes. The grainy material observed among the fibers possibly corresponds to the hemolymph circulating the ovary internally (Fig. 4C). A number of larger spherical bodies in between the lamellae were observed (Fig. 4C-E).

The bodies formed by the lamellae and their internal components, termed nodes or cysts, were identified during dissection of premature ovaries. The nodes tend to be organized internally with the smaller cells, which may be either follicles or early-stage germ cells, near the center (Fig. 5). However, because of their similar morphology and size, these two cell types could not be clearly distinguished.

The large previtellogenic oocytes and vitellogenic (mature) oocytes of the subsequent stages are easier to distinguish due to differences in diameter, cytoplasmic aspect and nucleus/cytoplasm ratio. Previtellogenic oocytes are smaller than mature oocytes but larger than oogonia near which they are located. The nucleus and nuclear envelope are visible and the cytoplasm appears slightly granular (Figs. 4D, 5B and 6A). In mature oocytes, the nucleus/cytoplasm ratio is reduced; the nucleus is at this point quite evident

and is filled with spherical vesicles containing yolk granules (Figs. 4E and 6B-D). These cells, which come closest to the external lining of the ovary, are separated by increasingly conspicuous envelopes. The external lining of intact cells appears membranous and branched (Fig. 6D), and may originate from extensions of the fibrous layer or follicular cells (the technique employed does not allow to make the distinction). The periphery of cross-sectioned mature oocytes displays a rather homogeneous and continuous band, possibly corresponding to the plasma membrane. In addition, extensions resembling microvilli may be seen projecting outward from the band (Figs. 6B, C) occupying an empty space corresponding to the chorion. Mature oocytes are covered by another protective envelope formed probably by surrounding follicular cells.

Premature and mature gonads are mostly filled with previtellogenic and vitellogenic oocytes and are bound by a slender external lining.

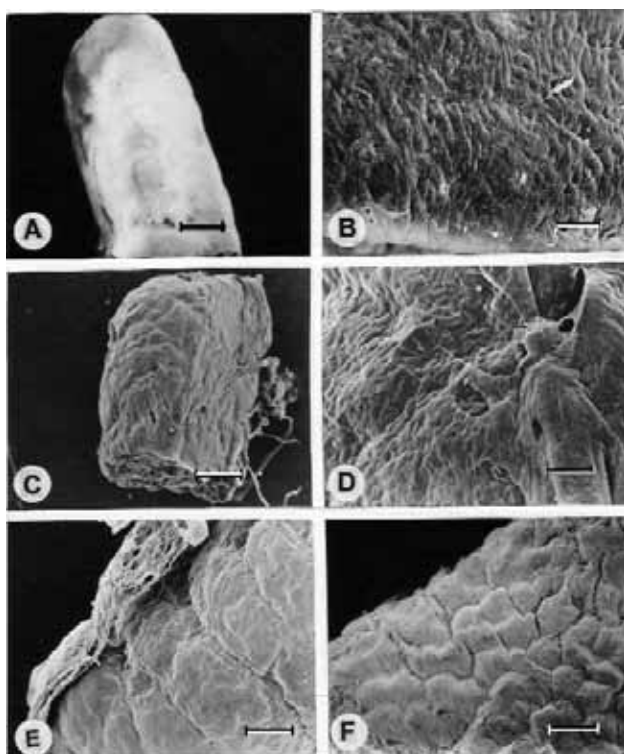


Figure 3. SEM-generated images showing external aspects of spiny lobster (*Panulirus*) ovaries at different stages of maturation. **A, B.** immature; **C, D.** prematuration; **E, F.** mature. Bars: A = 300 μ m; B = 20 μ m; C = 200 μ m; D = 30 μ m; E = 100 μ m; F = 200 μ m.

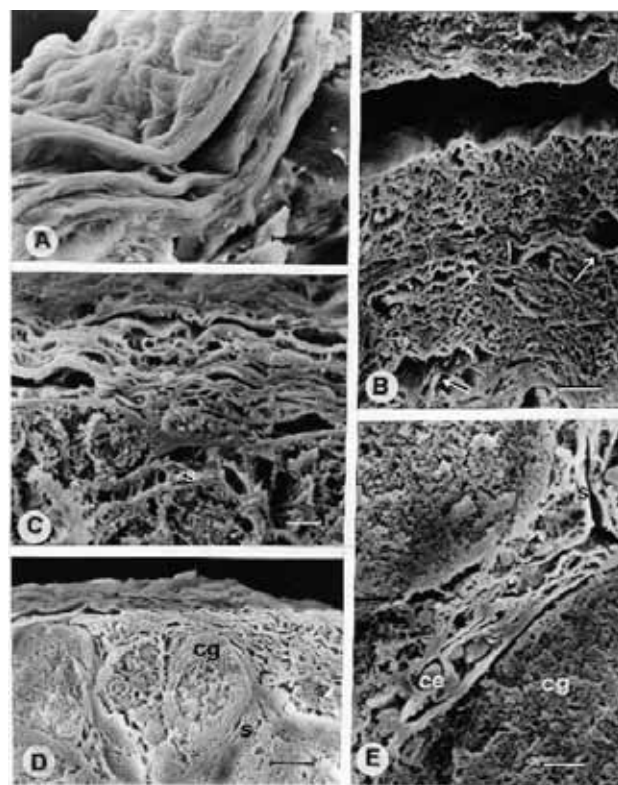


Figure 4. SEM ovarian coat of the spiny lobster (*Panulirus*). **A.** External layers. Bar = 5 μ m. **B.** Cross-section showing spaces corresponding to sinuses (arrows) containing hemolymph. Bar = 3 μ m. **C, D, E.** Fibers with visible formation of septae (s) surrounded by germ cells (cg) and spherical bodies (ce). Bars: C = 2.5 μ m; D = 10 μ m; E = 5 μ m.

Using scanning electron microscopy, the external and internal structure of the ovaries of the spiny lobster could be viewed and cysts and mainly the spatial relation between cell components could be identified, especially in late-stage germ cells.

DISCUSSION

Our morphological findings for the gonads of three species of spiny lobster (*Panulirus argus*, *P. echinatus* and *P. laevicauda*), collected off the coast of Ceará in the tropical Western Atlantic, match the general decapod pattern of position, coloring and volume changes.

As described for other decapods in reviews by Adiyodi and Subramoniam [1], McLaughlin [23] and Krol *et al.* [19], spiny lobster gonads are basically H-

shaped. Nevertheless, in a description of the gonadal structure of the slipper lobster (*Scyllarus arctus*), Cau *et al.* [9] mentioned two transversal commissures bridging the left and right lobes, one above and the other below the heart. However, as with other panulirids, the three species examined in the present study have a single commissure connecting the ovaries antero-ventrally to the heart. Since no other cases of double commissures have been reported for achelate lobsters (Scyllaridae and Palinuridae), comparisons and generalizations are not warranted.

In Reptantia, such as in Dendrobranchiata and Caridea, gonads tend to be located inside the cephalothorax, with the exception of Anomura in which these organs are basically located on the abdomen, as in the hermit crab species described by Carayon [7]. In the spiny lobsters examined by us, the volume and length of maturing gonads grow as far as to the second abdominal segment, with one of the posterior lobes being more developed. This lobe does not reach the telson in pleocyemates. In spite of the presence of lateral digitiform lobes in the cephalothorax, the posterior part of the gonads of the carid species *Macrobrachium acanthurus* does not extend beyond the first abdominal segment [8], while in *Atya scabra* it reaches as far as the third segment [13]. In the panulirids *Jasus lalandii* [12] and *Panulirus japonicus* [26] it extends to the first and fourth segment, respectively; in *Scyllarus arctus* to the first [9] and in *Homarus americanus* to the fifth [2]. In contrast, dendrobranchiates have lobes extending to the telson, as described for *Penaeus setiferus* [18], *P. paulensis* [27], *P. aztecus* and *P. setiferus* [6].

In the mature stage, the ovaries of *P. echinatus* and *P. laevicauda* take on an orange hue while those of *Panulirus argus* are consistently red. The same color range has been observed in other panulirids such as *Jasus lalandii* [12], *Panulirus interruptus* [21], *P. homarus* [4], *P. delagoae* [5], *P. ornatus* [22], *P. penicillatus* [16] and *P. japonicus* [26] as well as in *Scyllarus arctus* [9] and in the crab species *Gecarcinus lateralis* [32], *Portunus sanguinolentus* [28], *Libinia emarginata* [14], *Cancer pagurus* [11], *Carcinus maenas* [20] and *Callinectes sapidus* [15]. In contrast, the mature ovaries of astacuran lobsters have been described as being dark green (*Homarus*) or bluish (*Nephrops*) [2]. Both *Homarus americanus* and dendrobranchiate and carid shrimp of the genus

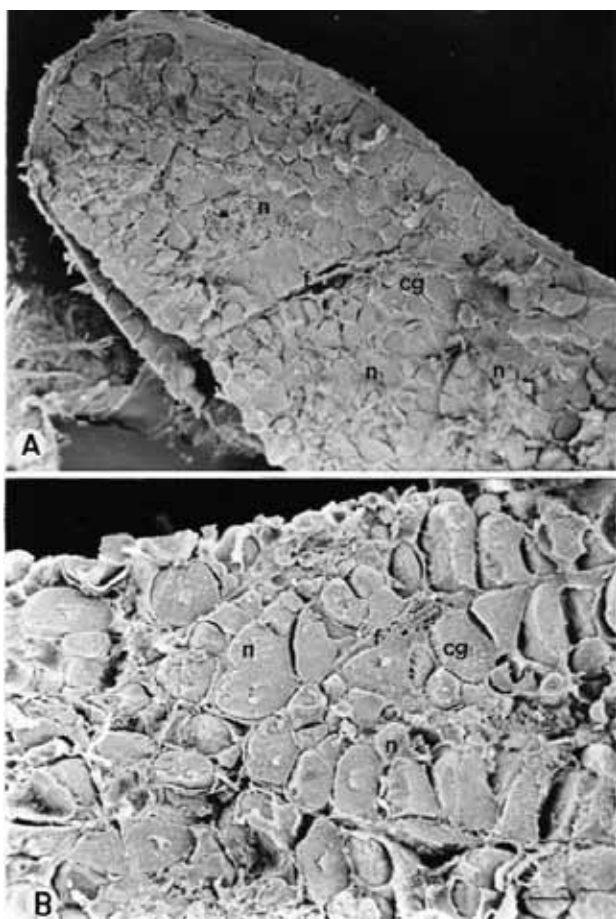


Figure 5. SEM of longitudinal sections of spiny lobster (*Panulirus*) ovaries. **A.** Internal organization of components involved in node formation. Nodes (**n**) are separated by fibers (**f**) and contain developing germ cells (**cg**). Bar = 140 μ m. **B.** Nodes (**n**) containing germ cells (**cg**) at different stages of development. The closer to the nodal envelope, the larger the germ cells. Bar = 5 μ m.

Macrobrachium have dark green or brownish ovaries, possibly as a result of the presence of the pigment ooverdin [29,31]. The carotenoid-induced reddish coloring of the ovaries of our species may serve as a shield to protect the embryo against solar radiation, as suggested by Adiody and Subramoniam [1].

The three panulirids included in our study differed with regard to the minimum total length at first spawning. The smallest species was *P. echinatus*, followed by *P. laevicauda* and *P. argus*. This may be

related to the fact that *P. argus* prefers greater depths where it would supposedly be exposed to a lesser degree of stress, while the two other species are generally found near the shore, as observed during the sampling for the present study.

The morphological criteria proposed by [Silva JRF, Master's dissertation, Federal University of Paraíba, Paraíba, Brazil] for classifying gonadal stages in *Panulirus laevicauda* yielded satisfactory results in our study and were easy to apply, especially with

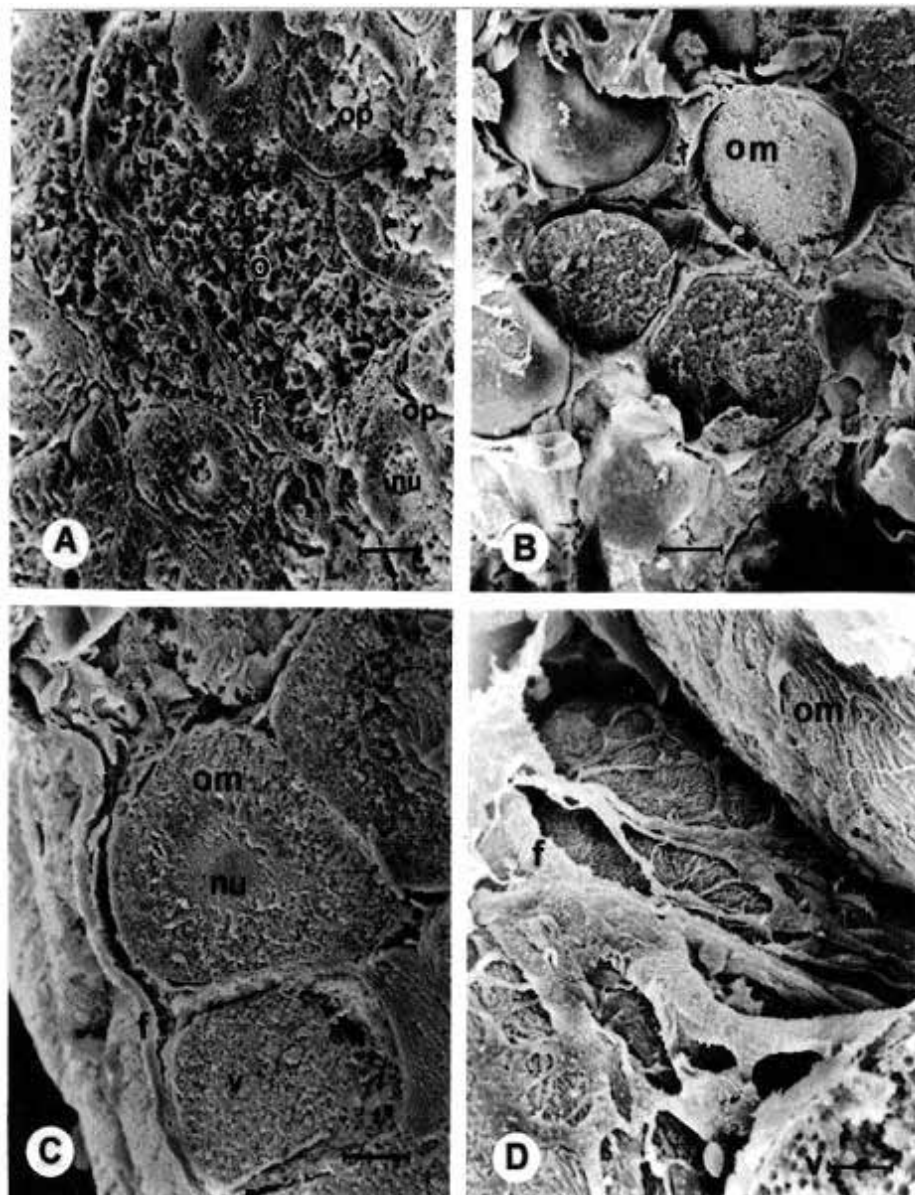


Figure 6. SEM of germ cells in the spiny lobster (*Panulirus*) at different stages of ovarian development. A. Oogonia (o) located centrally and separated by fibrous septae (f); previtellogenic oocytes (op) located off-center, with visible nuclei (nu). Bar = 20 μ m. B, C, D. Mature oocytes (om) and cytoplasm containing yolk granules (y) near the thinning ovarian fibrous (f) lining; nucleus (nu). Bars: B = 50 μ m; C = 30 μ m; D = 10 μ m.

regard to changes in gonad coloring and size. Thus, four gonadal stages were identified in our lobsters: immature, prematuration, mature and spawning/resorption. The mature stage may be recognized by the size of the ovaries and by the ease with which oocytes are released upon touch, indicating completed vitellogenesis and ovulation. Based on our findings, the four-stage pattern may now be associated with *P. argus* and *P. echinatus* as well. Despite variations in nomenclature, most authors classify decapod gonadal maturation in four stages. This is a practical approach with dendrobranchiates and carids whose carapace is transparent allowing examination of the gonads to be done without dissecting the animal, as described for penaeid shrimp [6].

The findings obtained by SEM in the present study make it possible to compare the ovarian architecture of our three lobster species with that of other decapods, as was done by Talbot [29,30] who studied mature follicles of *Homarus americanus*. Our description makes it possible to distinguish germ cells and their relationship with the fibrous components. The studies of Mota and Tomé [24] and of Mota-Alves and Tomé [25] do not mention any somatic components in ovaries of *Panulirus argus* and *P. laeviscauda*, respectively. The thinning of the ovarian wall from Stage I to Stage III indicates the fibrous components are being stretched to accommodate the growth of the germ cells. Although SEM provides information on these aspects, it was not possible to determine whether the fibers were conjunctive or muscular.

The node or cyst-like aspect of the ovaries in our species differs from findings for *H. americanus*. The apparent absence of such nodes in the latter species is possibly due to the fact that the large size of mature germ cells makes observation of the interstitial material impossible; however, the muscle fibers branch into the space containing the mature oocytes. The ovaries of our species presented similar ramifications although the type of fiber could not be determined.

Our analyses showed that fragments were better preserved in Bouin than in Karnovsky solution. In addition to being more commonly employed in both light and electron microscopy, Bouin solution is also the less expensive alternative.

The combination of morphological observations and ultrastructural analyses of the ovarian architecture of the spiny lobster suggests the existence of a

communication between germ cells and fibrous components, possibly contributing to the eventual incorporation of the yolk sac and the completion of vitellogenesis. The SEM and TEM-based findings of Talbot [29,30] on the relationship between mature oocytes and follicular cells in *Homarus americanus* were not observed here for *Panulirus*, but the fibrous components seen probably contained follicular cells. As the first SEM-aided description of *Panulirus* ovaries, the present study represents a contribution to our understanding of the tridimensional organization of the ovarian components at different stages of maturation and sheds new light on the changes which occur during the maturation process of spiny lobsters.

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