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ONTOGENY OF FEMALE PRIMARY SEXUAL CHARACTERS IN THE MAJID CRABS *CHIONOECETES OPILIO* AND *HYAS COARCTATUS*

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ABSTRACT

To investigate the development of primary sexual characters in the family Majidae, the spermathecae, ovaries, and oviducts of juvenile female *Chionoecetes opilio* and *Hyas coarctatus* were examined, using histology, histochemistry, and electron microscopy, and compared to those of mature females. Females were divided into 3 developmental categories, with corresponding size ranges, based on anatomical criteria: immature, premature, and mature. Immature crabs were still temporally remote from the terminal molt, had white ovaries, and the spermatheca presented a thin tissule separation between the dorsal and ventral regions. No stratified glandular epithelium was present; only a columnar epithelium lined the lumen. This epithelium tested slightly positive for amine-containing substances. Premature females were temporally close to terminal molt, had orange ovaries, and retained the tissule separation between the dorsal and ventral regions of the spermatheca. In addition, they presented a stratified glandular epithelium which developed beneath the columnar epithelium lining the lumen. The latter degenerated and sloughed into the lumen as the former developed. Mature females had undergone the terminal molt, and were examined both prior to and following egg extrusion. No trace remained of the columnar epithelium which was present in the preceding two categories. In mature females which had extruded eggs, the tissule separation between dorsal and ventral regions of the spermatheca was also absent. Neutral mucopolysaccharides dominated in the secretions of the dorsal glandular epithelium of both types of mature female. These results establish the primary sexual characters in female *C. opilio* and *H. coarctatus,* and also document the sequence of aquisition of these characters.

The family Majidae comprises 385 species worldwide (Griffin and Tranter, 1986). Crabs of the genus *Chionoecetes* constitute an important fishery resource in the Northern hemisphere (Anonymous, 1989); in the past 20 years the snow crab *Chionoecetes opilio* (Fabricius) has become a major invertebrate fisheries species in Atlantic Canada, with landings of 38,048 metric tons and a value of $61,575,000 in 1992 (Department of Fisheries and Oceans, 1994). Considerable variation in landings both within the same fishing zone in successive years, and between fishing zones within the same years, has emphasized the importance of improved knowledge of the biology of this species, and of its reproduction in particular. A persistent research effort has been directed at this goal over the past decade for both *C. opilio* and its Pacific congener *C. bairdi* Rathbun (see Paul, 1982, 1984; Davidson *et al.,* 1985; Taylor *et al.,* 1985; Conan and Comeau, 1986; Hooper, 1986; Ennis *et al.,* 1988, 1990; Beninger *et al.,* 1988, 1991, 1993; Paul and Paul, 1990, 1992; Comeau and Conan, 1992; Cormier *et al.,* 1992; Govind *et al.,* 1992; Sainte-Marie and Hazel, 1992; Sainte-Marie, 1993; Stevens *et al.,* 1993, 1994; Claxton *et al.,* 1994; Sainte-Marie and Lovrich, 1994). Such extensive investigations have yielded a plethora of information, and a deeper appreciation of the complexities of reproduction in this genus; consequently, *C. opilio* has been proposed as a model for crustacean reproductive strategies (Elner and Beninger, 1995).

Recent investigations have underscored the central role of the spermatheca in snow crab reproduction and reproductive strategies (Beninger *et al.,* 1993; see also Elner and Beninger, 1995). There is an unfortunate dearth of detailed knowledge concerning the structure, ultrastructure, and function of the spermatheca in most other species. Some histological information exists for the majid *Inachus phalangium* (Fabricius) (see Diesel, 1989), as well as for the portunids *Portunus sanguinolentus* (Herbst) and *Callinectes sapidus* Rathbun (see George, 1963; Johnson, 1980), the potamonid *Paratelphusa hydrodromus* (Herbst)

Table 1. Maturity criteria and carapace widths for female *Chionoecetes opilio* and *Hyas coarctatus* in the present study.

Female type	Carapace widths (mm)		Maturity criteria
	C. opilio	*H. coarctatus*	
Immature	19–60	15–30	No setae visible on pleopods White ovaries Immature oocytes (nonvitellogenic)
Premature	24–68	31–38	Pleopod setae visible beneath exoskeleton Ovary color increasingly orange Vitellogenic oocytes
Mature, noncopulated, nonovulated	52–82	45–50	Setae on pleopods Abdomen rounded Ovaries brilliant orange Mature oocytes

(see Anilkumar and Adiyodi, 1977), and the grapsid *Eriocheir sinensis* H. Milne Edwards (see Lee and Yamazaki, 1990). Since with the notable exception of *Callinectes sapidus* (see Johnson, 1980), only mature females were examined, developmental data are particularly lacking.

Basic understanding of the reproductive biology of any species must include information on the nature of primary sexual characters, as well as information on the ontogenetic acquisition of such characters. While some attention has been paid to this aspect for male snow crabs (Conan and Comeau, 1986; Beninger *et al.*, 1988; Comeau and Conan, 1992), no data are available for females. To this end, histological, histochemical, and ultrastructural techniques have been used in the present study to elucidate the development of the female reproductive system of *Chionoecetes opilio*. The noncommercial majid *Hyas coarctatus* Leach was also investigated, in order to ascertain whether the results are applicable to other members of the Majidae.

Table 2. Numbers of female *Chionoecetes opilio* (C) and *Hyas coarctatus* (H) used for histology and histochemistry.

Female type	Histochemistry				Histology	
	Lipids		Other tests			
	C	H	C	H	C	H
Immature	2	1	13	6	35	6
Premature	2	—	24	3	31	3
Mature, noncopulated, nonovulated	1	—	5	—	5	—
Mature, noncopulated, ovulated	1	—	12	2	15	3

Materials and Methods

Sampling and Maturity Criteria.—Female crabs were collected by a modified *Nephrops* trawl (Conan *et al.*, 1994) in the Baie des Chaleurs from 1992–1994. All crabs were maintained in an open circuit aquarium at the Aquarium and Marine Centre, Shippagan, New Brunswick, for less than 6 months prior to dissection. Temperature and salinity conditions varied from 0–6°C and 26–31‰, respectively, and the crabs were fed shrimp and smelt once a week. Females were assigned to three groups (immature, premature, and mature) based on the maturity criteria presented in Table 1. Mature, noncopulated females were obtained from isolated premature females that molted to maturity in captivity.

Female *C. opilio* were dissected throughout the year, while female *Hyas coarctatus* were dissected from September to November and from January to May. The numbers, developmental categories, and size ranges of female *C. opilio* and *H. coarctatus* used for histology and histochemistry are shown in Table 2.

Histology.—In crabs larger than 30-mm carapace width (CW), the entire reproductive system was dissected out and fixed. Females less than 30-mm CW were cut along the median plane, and one-half was fixed after removing the carapace and pereiopods. Tissues were fixed in aqueous Bouin's solution and processed using standard paraffin embedding methods. Longitudinal serial sections (7 μm) were stained with the Goldner variation of the Masson trichrome technique (Martoja and Martoja, 1967) or with a modified Mallory's aniline blue (after Johnson, 1980) for cuticle differentiation. Sections were observed and photographed with an Olympus BHS microscope.

Histochemistry.—The spermatheca, hepatopancreas, and ovary of immature, premature, and noncopulated mature female *C. opilio* and *H. coarctatus* were fixed and tested according to each histochemical protocol (Tables 2, 3). Tissues destined for lipid test were fixed in calcium-formol; for the remaining tests, tissues were fixed in Bouin's, Helly's, or Böhm-Sprenger solution.

Table 3. Histochemical tests performed on the reproductive system of female *Chionoecetes opilio* and *Hyas coarctatus*.

Target substance	Test	Positive control	Negative control	Reference
Lipid	Sudan black	Hepatopancreas	Lipid extraction	High, 1984
Amines	Orange G	Muscle	—	James and Tas, 1984
Neutral mucopolysaccharide	PAS	Hepatopancreas	Amylase digestion	Vacca, 1985
Acid mucopolysaccharide	Alcian blue	Hepatopancreas	Amylase digestion	Vacca, 1985

Total lipids were tested using the bromine-Sudan black B technique (High, 1984); negative controls were extracted using lipid solvent (chloroform, methanol, water, hydrochloric acid 66:33:4:1) for 1 h at room temperature. Orange G was used to test for amines (James and Tas, 1984). Muscle associated with the vagina and spermatheca was used as positive control for amines. Periodic acid-Schiff (PAS) and alcian blue (pH 2.5) were used to test for neutral and acid mucopolysaccharides, respectively; they were differentiated from glycogen by salivary amylase digestion of control slides (Vacca, 1985). Hepatopancreas sections were used as positive controls for all histochemical tests other than proteins.

Electron Microscopy.—The spermatheca of a postmolt, premature female *Chionoecetes opilio* (65-mm CW) was dissected for transmission electron microscopy in March 1993. Small pieces were initially fixed for 2 h in Karnovsky fixative and postfixed for 1 h in osmium tetroxide. After rinsing in sodium cacodylate buffer, tissues were dehydrated in an ascending ethanol series and embedded in Spurr resin. Semithin sections of 1 μm were stained with toluidine blue for preliminary observation under light microscopy, while thin sections were contrasted with lead citrate and uranyl acetate for transmission electron microscopy. The sections and observations were performed at the Centre de Microscopie Electronique Appliquée à la Biologie et à la Géologie (UCB/Lyon I), using a Hitachi HU12 TEM operating at 75 kV.

RESULTS

Immature

General Morphology.—The relative positions of the spermatheca and ovary in immature, premature, and mature *Chionoecetes opilio* and *Hyas coarctatus* are shown in Fig. 1. The dorsal region of the spermatheca resembled a blind, collapsed, narrow tube, and was joined to the ovary via the thin, transparent oviduct. Observation of the ventral region was difficult due to the small size of the structure. The histological sections, however, showed the presence of small pouches on the anterior and posterior aspects of the spermatheca, with a continuation of the vaginal cuticular lining between the two pouches. The vagina was cresent-shaped in cross section, as was the case for all categories observed (Fig. 3.4), and entered the spermatheca between the two pouches at an angle; muscles ran between the concave face of the vagina and the carapace. The cuticular lining was folded in the ventral region of the spermatheca, in contrast to the nonfolded condition in the vagina. The ventral region of the spermatheca retained the crescent-shaped cross section of the vagina, as did the gonopore. The oviduct inserted between the two pouches of the ventral region of the spermatheca just above the cuticular lining. The oviduct and the muscles of the vagina were situated on opposite sides of the spermatheca.

Ovaries were observed as thin strands of white tissue even in the smallest immature crabs observed (*C. opilio,* 18.9-mm CW; *H. coarctatus,* 12.4-mm CW). The histology and histochemistry of these structures is described below.

Histology and Histochemistry.—In immature females, as in premature and mature females, oogonia were located in the center of the ovary, while the oocytes were peripherally located; the gametes were bounded by a vitelline envelope and accessory cells (also termed follicular cells; Adiyodi and Subramoniam, 1983). The gonad itself was surrounded by a fibrous capsule (Fig. 2.5). The oocytes contained a prominent nucleus, with one or two dense nucleoli (Figs. 2.1–2.5). Throughout the cytoplasm, clear inclusions as well as inclusions which stained red with the Masson trichrome technique were observed (Fig. 2.2). The incidence of these inclusions could not be correlated with oocyte maturation. The ovaries were negative for acid mucopolysaccharides, positive for lipids (strongly positive in *Hyas coarctatus*), and strongly positive

for amines and neutral mucopolysaccharides (Table 4).

In both species, the dorsal and ventral regions of the spermatheca were separated by an obliquely oriented transverse layer of tissue, herein termed the transverse membrane (Fig. 3.2). Since the cuticle of the ventral region extended farther dorsally between the pouches on the medial face compared to the lateral face, the transverse membrane thus presented an oblique orientation (Fig. 3.2). The dorsal region consisted of an outer layer of connective tissue and an inner columnar epithelium resting on a thick basal lamella (Fig. 4.1). The nuclei of the columnar cells were elongated and located next to the basal lamella. The columnar epithelium tested negative for lipids, acid and neutral mucopolysaccharides, and positive for proteins; a gradient of protein-positive substances was observed toward the apex of the columnar cells (Table 4). Apart from some isolated debris, the lumen of the spermatheca was empty.

The histology of the ventral part of the spermatheca was similar in all developmental categories. The cuticular portion was composed of two thin layers, corresponding to an epicuticle and procuticle. Beneath the cuticle lay a columnar epithelium, surrounded by muscle and musculo-connective tissue. The transverse membrane separating the dorsal and ventral regions was continuous with this columnar epithelium. The pouches of the ventral region presented the same histological features, but the cuticular layer was absent. The columnar epithelium and lumen of the ventral region yielded negative results for all substances tested, with the exception of acid mucopolysaccharides in the lumen of some females.

Premature

General Morphology.—In both species, the general organization of the spermatheca conformed to that of the immature female; however, the dorsal region and the pouches of the ventral region were progressively more well developed with increasing crab maturity (Fig. 1.2).

Histology and Histochemistry.—The histology of the ovaries was virtually identical in *Chionoecetes opilio* and *Hyas coarctatus*; observed stages of oocyte development are presented in Fig. 2. The ovaries enlarged and became orange around July when the oocytes began vitellogenesis; vitelline granules first appeared at the periphery of the cells (Fig. 2.3). Around December following the molt to prematurity, vitellus constituted the majority of the cytoplasm in the oocytes. The metaphase of the first meiotic division was observed at the cortex of the primary oocyte between January and April (as for mature females, Fig. 2.6). Based on nine measurements in premature *C. opilio,* the mean length of the meiotic spindle was 13.1 $\pm$ 1.5 μm, while that of the equatorial plate was 17.0 $\pm$ 3.0 μm. The ovaries tested negative for acid mucopolysaccharides, but strongly positive for lipids, amines, and neutral mucopolysaccharides (Table 5).

The columnar epithelium lining the lumen of the spermatheca dorsal region was continuous with that of the pouches of the ventral region. As was observed in the immature females, the cells of this layer presented a positive reaction for amines, with an increasing gradient toward the lumen; in addition, a strongly positive reaction for neutral mucopolysaccharides with a similar gradient was observed (Table 5). In newly molted females, the subjacent thin layer of small round cells began to proliferate, forming a germinal zone (Fig. 4.2). Intense mitosis continued with increasing age of the premature females, resulting in a well-developed stratified epithelium (Fig. 4.3). This epithelium tested negative for lipids, amines, and acid mucopolysaccharides, and positive for neutral mucopolysaccharides. Mucopolysaccharide-positive substances appeared from November onward, and both these and amines presented an increasing gradient toward the lumen of the spermatheca (Table 5). Mitotic activity was ob-

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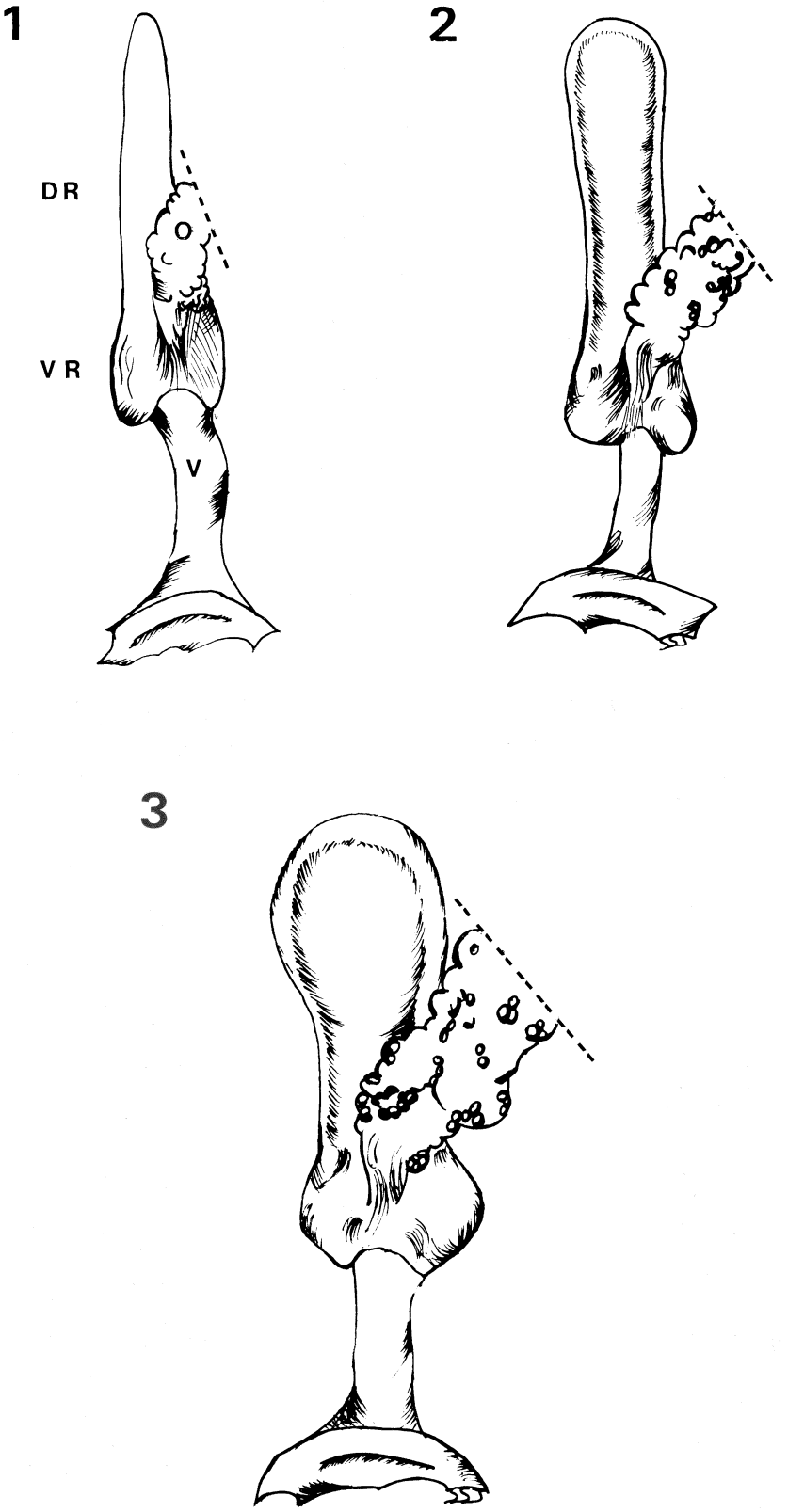
Fig. 1. *Chionoecetes opilio* and *Hyas coarctatus.* Schematic drawings of the morphological aspect of the spermatheca, vagina, and ovary in the three developmental categories. 1.1, immature female. DR = dorsal region, O = ovary, V = vagina, VR = ventral region; 1.2, premature female; 1.3, mature female.



served during the summer after the maturity molt, as early as 43 days after the molt, before any macroscopic or microscopic changes in the ovaries. Around August, mitotic figures were observed scattered throughout the stratified epithelium. In late autumn, the mitotic figures were mostly found in the stratified epithelium adjacent to the outer connective tissue. Beginning in October, connective fibers from the outer covering ramified into the stratified layer, assuming a reticulate appearance. The columnar epithelium and the basal lamella began to degenerate around the month of November prior to the maturity molt (Figs. 4.3, 4.4). The sloughed material accumulated in the lumen, and tested strongly positive for amines and neutral mucopolysaccharides (Table 5).

A single-layered transverse membrane separated the dorsal and ventral regions of the spermatheca, as was observed for the immature females (Fig. 3.1, 3.5).

The histology and histochemistry of the nonchitinous parts of the ventral region (pouches) was identical to that of the dorsal region. The histology and histochemistry of the chitinous parts were identical to that of the corresponding region of the immature spermatheca: a columnar epithelium underlying cuticular folds, some isolated cells testing positive for acid mucopolysaccharides; these cells were somewhat more common in *H. coarctatus* (Table 5, Fig. 3.5). The alcianophilic secretions of these cells accumulated beneath the cuticle throughout the year, eventually displacing the old cuticle and escaping into the ventral region lumen. Some of these secretions appeared to remain in the lumen of the ventral region after the molt.

Mature, Noncopulated, Preovulation

General Morphology.—The general morphology of the reproductive system of mature, noncopulated, preovulation females was similar to that of the premature females, except for a more well-developed dorsal region and ventral region pouches (Fig. 1.3). After the terminal molt to maturity, ovulation was delayed, usually approximately 41 days before the release of nonfertilized oocytes. The oocytes were shed from the abdomen approximately 110 days after oviposition; this was associated with abnormal funiculus development and egg necrosis. The ovaries then reached maturity within approximately one year, and the mature, noncopulated, postovulation females were able to copulate in a hard carapace condition the following spring and extruded viable eggs.

Histology and Histochemistry.—The dorsal region of the mature spermatheca consisted of the outer connective tissue, the subjacent reticulate fiber layer, the germinative layer, and the stratified epithelium. The rate of cellular division (evidenced by the number of mitotic figures) in the germinative layer appeared constant throughout the gametogenic cycle in mature, noncopulated females. The stratified epithelium tested negative for lipids and acid mucopolysaccharides, and positive for amines and neutral mucopolysaccharides, with an increasing gradient toward the lumen. The lumen of the dorsal region tested strongly positive for both amines and neutral mucopolysaccharides (Table 6). No trace of the dorsal columnar epithelium remained in mature females. The transverse membrane was present in these females prior to egg extrusion (Fig. 3.4).

The nonchitinous parts of the ventral region (pouches) presented a histological and histochemical profile identical to that of the dorsal region. The chitinous parts presented the same histological and histochemical

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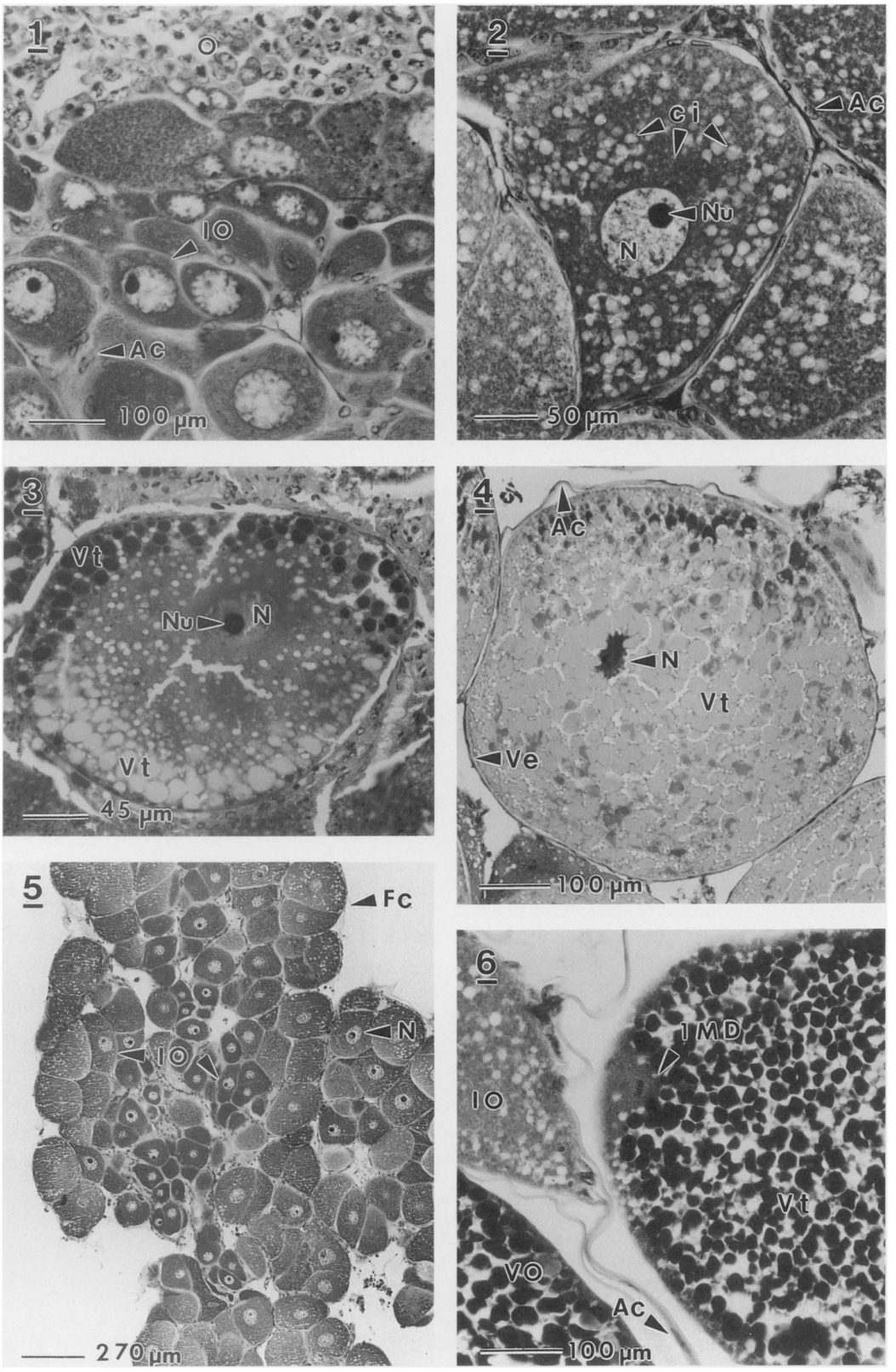
Fig. 2. *Chionoecetes opilio.* Stages of gonadal development. All sections stained with Masson trichrome. 2.*1,* Immature female (18.9-mm CW). Ac = accessory (=follicular) cells, IO = immature oocyte, O = oogonia; 2.*2,* Immature female (40.0-mm CW). Ac = accessory cells, ci = cytoplasmic inclusion, N = nucleus, Nu = nucleolus; 2.*3,* Early vitellogenic oocyte in premature female (August; 55-mm CW). N = nucleus, Nu = nucleolus, Vt = vitellus; 2.*4,* Vitellogenic oocyte in premature female (February; 31.7-mm CW). Ac = accessory cell; N = nucleus; Ve = vitelline envelope; Vt = vitellus. 2.*5,* General view of immature oocytes in immature female (39.9-mm CW). Fc = fibrous capsule, IO = immature oocyte, N = nucleus; 2.*6,* Oocyte in first meiotic division in mature, noncopulated, ovulated female (73.1-mm CW). Ac = Accessory cells, IO = immature oocyte, 1MD = first meiotic division, VO = vitellogenic oocyte, Vt = vitellus.



features as the corresponding regions of the immature and premature females (Table 6).

Mature, Noncopulated, Postovulation

The morphological, histological, and histochemical profile of the mature, noncopulated females following ovulation was identical in all respects to that of the mature, noncopulated female prior to ovulation, with the notable exception of the complete absence of a transverse membrane. Rare alcianophilic cells were present beneath the cuticle in the ventral region of the spermatheca, as was the case for premature females (Fig. 3.3).

Discussion

The location of the gonial cells in the center of the ovary in both species is consistent with the observations of Hinsch (1970) for the majid *Libinia emarginata* Leach. The features of oogenesis visible under light microscopy conform to those described for various other decapods by Charniaux-Cotton (1973), Payen (1974), Armstrong (1988), and Minagawa *et al.* (1993), and thus appear to be relatively uniform in the Decapoda.

The ovary is the only site where lipids are secreted; they are incorporated into the developing oocytes. The acid mucopolysaccharide secretions which originate in the immature oviduct epithelium become more abundant in premature and mature females. The bacteriostatic nature of acid mucopolysaccharides has been discussed by Sasikala and Subramoniam (1987). The results of the present study suggest that these secretions may coat the oocytes as they are extruded, affording some degree of protection from microbial attack after oviposition.

It should be noted that the oviduct inserts into the spermatheca between the two pouches of the ventral region. In the first such study on *C. opilio,* its insertion was depicted as between but slightly dorsal to these pouches, at that time termed an "intermediate sac-like structure" (Beninger *et al.* 1988). Recently, Sainte-Marie and Lovrich (1994) designated the ventral region a "fertilization chamber." This appears to be derived from the term "insemination chamber" (Diesel, 1989). While the ventral region is almost certainly the site where eggs and sperm are mixed together for the first time, the exact site of fertilization is as yet unknown—indeed eggs could be fertilized both within the spermatheca and on the abdomen. The term "fertilization" is also ambiguous, since it refers to a series of events which take place as the egg travels from the ovary to the abdomen. The use of this term is, therefore, discouraged.

The spermathecal characters corresponding to each female developmental type are summarized in Table 7. The major morphological primary sexual characters of the spermatheca in *Chionoecetes opilio* and *Hyas coarctatus* are the development of the dorsal region and the ventral region pouches. The major histological primary sexual characters are the mechanical loss of the transverse membrane, the degeneration and loss of the columnar epithelium in the glandular regions, and the proliferation of the secretory epithelium in the glandular regions.

The presence of a transverse membrane in the spermatheca has been reported in several crab species. The location and nature of this membrane, however, are not consistent. For example, a "valve-like tissue" was observed at the junction of the oviduct and spermatheca in the fresh-water

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Fig. 3. *Chionoecetes opilio.* General organization of the spermatheca and transverse membrane in relation to maturity. Masson's trichrome stain. 3.*1,* Premature female (September; 32.4-mm CW). cf = cuticular folds, od = oviduct, ov = ovary, mct = musculo-connective tissue, M = muscle, TM = transverse membrane, V = vagina; 3.*2,* Immature female (20-mm CW). cf = cuticular folds, H = hepatopancreas, ov = ovary, TM = transverse membrane; 3.*3,* Ventral region of mature, noncopulated, recently ovulated female (68.6-mm CW), showing alcianophilic secretions. AS = alcianophilic secretion, ce = columnar epithelium, cf = cuticular folds, ct = connective tissue. Alcian blue-PAS stain; 3.*4,* Mature, noncopulated, nonovulated female (56.2-mm CW). Longitudinal section of spermatheca, oblique section of dorsal part of vagina. cf = cuticular folds, cu = cuticle, DR = dorsal region, s = secretions, M = muscle, ov = ovary, TM = transverse membrane, VR = ventral region; 3.*5,* Ventral region of premature female (August; 55-mm CW), showing the transverse membrane. ce = columnar epithelium, cf = cuticular folds, dce = degenerating columnar epithelium, se = stratified epithelium, TM = transverse membrane.

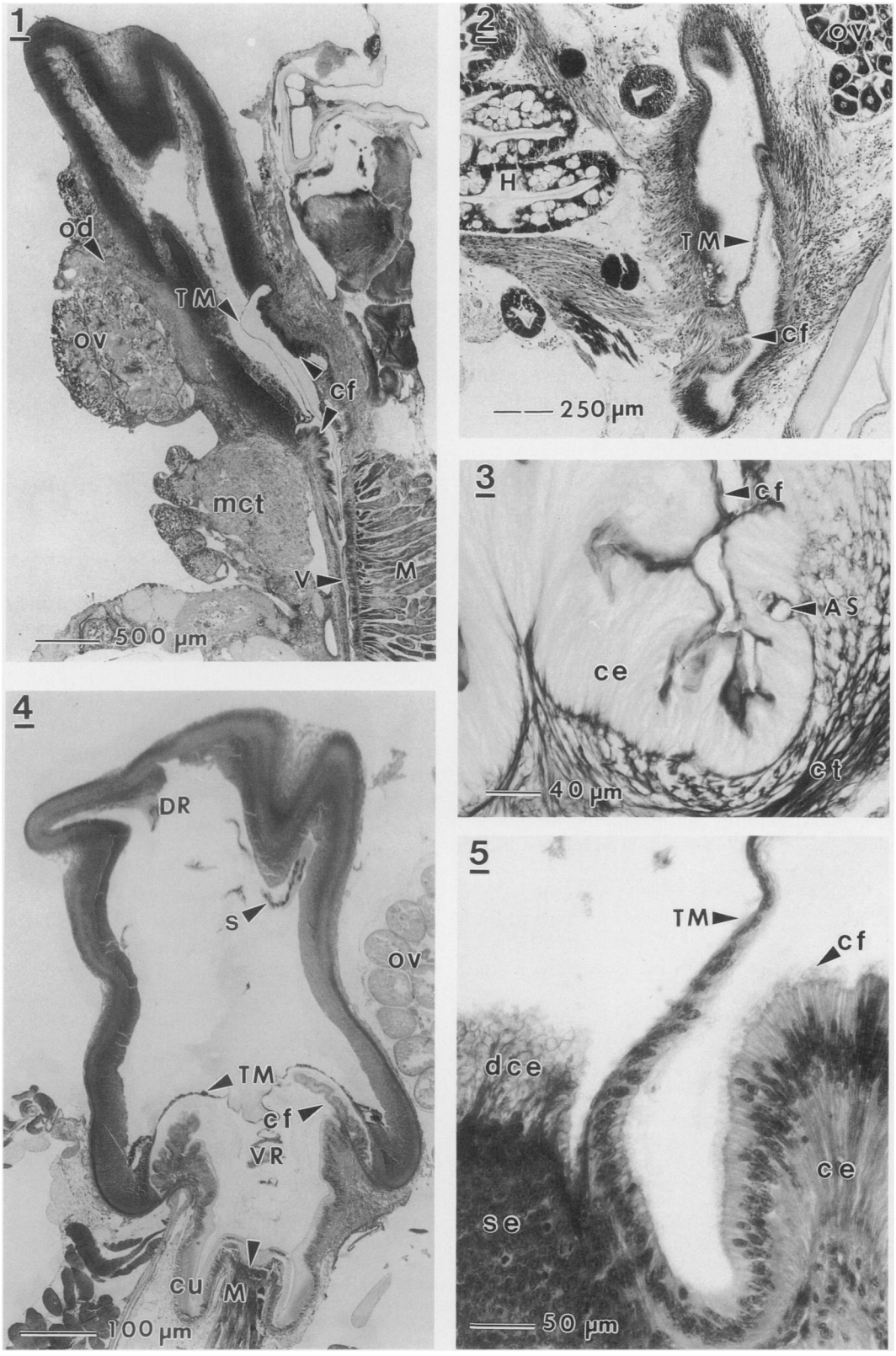

Table 4. Histochemical results for immature female crab reproductive system (*Chionoecetes opilio* and *Hyas coarctatus*). MPS = mucopolysaccharides.

	Dorsal region				
Target substance	Columnar epithelium	Lumen	Ventral lumen	Oviduct	Ovary
Lipids	—	—	—	—	+
Amines	+(1)	—	—	—	++
Neutral MPS	—	—	—	—	++
Acid MPS	—	—	+	+	—

— negative.
+ positive.
++ strongly positive.
(1) increasing intensity toward lumen.

grapsid crab *Eriocheir sinensis* (see Lee and Yamazaki, 1990). However, this tissue was said to reform after each ovulation, contrary to the transverse membrane of the two majids in the present study. Similarly, a diaphragmlike "velum" was reported to separate the dorsal and ventral regions of the spermatheca in the majid *Inachus phalangium* (see Diesel, 1989). This structure was present in mature females, in contrast to the two majids of the present study. It is possible that the "velum" of *I. phalangium* corresponds to the cuticular part of the ventral region in *Chionoecetes opilio,* which presents numerous folds (Beninger *et al.,* 1993; present study). However, we are unaware of any studies which demonstrate a diaphragmlike action of this feature, and the low-magnification micrographs of Diesel (1989) do not allow such conclusions to be drawn.

The transverse membrane reported herein is similar to that described for the portunid *Callinectes sapidus* (see Johnson, 1980). In *C. sapidus* the membrane is present in immature and premature crabs, but is absent in mature crabs (presumably due to mechanical rupture during copulation and ovulation), as was observed for both crabs of the present study. However, Johnson (1980) stated that this membrane is a continuation of the dorsal region epithelium in *C. sapidus,* whereas in *Chionoecetes opilio* and *Hyas coarctatus* it is a continuation of the ventral region epithelium. Such variation in the position, structure, regenerative ability, and derivation of the transverse membrane underscores the need for a more detailed, systematic study of this feature in the Brachyura. One advantage that such a membrane would confer would be the opportunity for a female to complete the ovogenesis of at least her first batch of eggs without any possibility of microbial contamination from the external environment via the gonopore.

The second major histological maturity character of the spermatheca is the degeneration of the columnar epithelium in the nonchitinous regions. This degeneration is accompanied by a proliferation of the underlying stratified epithelium, which assumes a holocrine secretory role in mature females (Beninger *et al.,* 1993). While a columnar epithelium is obviously not suited for a sustained holocrine secretory function, it does appear to contribute some neutral mucopolysaccharides to the lumen of the premature spermatheca. The accumulation of neutral mucopolysaccharides begins in the immature state, when the columnar epithelium is slightly positive for amines. Due to the ephemeral nature of this structure, it is unclear what function, if any, it might have in immature crabs. A similar situation was reported in *Callinectes sapidus* (see Johnson, 1980). In another portunid, *Portunus sanguinolentus,* Ryan (1967) also observed a proliferation of the stratified epithelium in premature and mature females, but did not observe a degenerating columnar epithelium in the premature females. Again, more research is required in order to elucidate the prevalence and eventual function of this structure in other Brachyura.

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Fig. 4. *Chionoecetes opilio* and *Hyas coarctatus.* 4.*1*–4.*3:* Longitudinal section of the columnar epithelium in the dorsal region. 4.*1, H. coarctatus,* immature female (27.0-mm CW). bl = basal lamella, ce = columnar epithelium, ct = connective tissue, L = lumen. 4.*2,* Premature female (May; 61-mm CW). bl = basal lamella, ce = columnar epithelium, ct = connective tissue, gz = germinal zone, L = lumen. 4.*3,* Premature female (November; 56.6-mm CW). bl = basal lamella, ct = connective tissue, dce = degenerate columnar epithelium, gz = germinal zone, L = lumen, s = secretions, se = stratified epithelium. 4.*4,* Transmission electron micrograph of recently molted premature female *C. opilio* (April; 65-mm CW). bl = basal lamella, cd = cell debris, dm = degenerating membranes, N = nucleus, se = stratified epithelium.

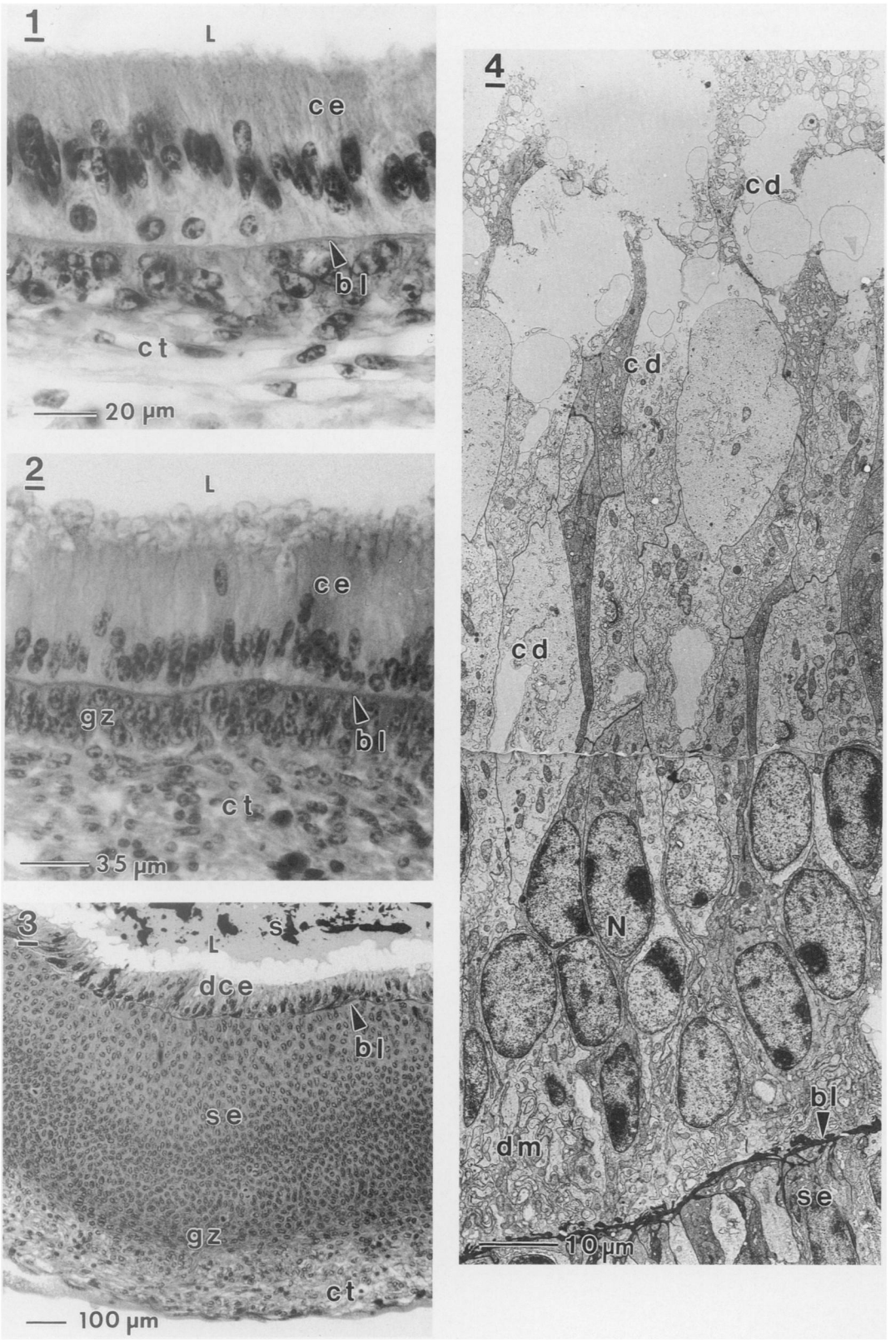

Table 5. Histochemical results for premature female crab reproductive system (*Chionoecetes opilio* and *Hyas coarctatus*). MPS = mucopolysaccharides.

Target substance	Dorsal region: Columnar epithelium	Dorsal region: Stratified epithelium	Dorsal region: Lumen	Ventral lumen	Oviduct	Ovary
Lipids	—	—	—	—	—	++
Amines	+(1)	—	++	—	—	++
Neutral MPS	++(1)	+(1,2)	++	—	+/—	++
Acid MPS	+(3)	—	—	++	++	—

— negative.
+ positive.
++ strongly positive.
(1) increasing intensity toward lumen.
(2) from November.
(3) some isolated cells only.

The major secretory product of the spermathecal stratified epithelium is neutral mucopolysaccharide, which appears as soon as this epithelium begins to proliferate in premature crabs. This confirms previous histochemical results for mature, copulated females; it has been postulated that these secretions maintain a homogeneous bacterial culture in the lumen, which acts to exclude opportunistic bacteria from the spermatheca (Beninger *et al.*, 1993).

As mentioned above, the acid mucopolysaccharides found in the ventral lumen of the spermatheca of immature, premature, and mature females probably originates in the oviduct, as well as from the isolated secretory cells in the cutinized portion of the ventral region of the spermatheca. It is unclear whether this is the same source for the abundant acid mucopolysaccharides found in mature, copulated females (Beninger *et al.*, 1993), since the male seminal products would push these secretions dorsally. Furthermore, the tegumental glands in the male first gonopod constitute another potential source of acid mucopolysaccharides at copulation (Beninger *et al.*, 1995).

The present study has described the female primary sexual characters and their acquisition in two representatives of the

Table 6. Histochemical results for noncopulated mature (before and after ovulation) crab reproductive system (*Chionoecetes opilio* and *Hyas coarctatus*). MPS = mucopolysaccharides.

Target substance	Dorsal region: Stratified epithelium	Dorsal region: Lumen	Ventral lumen	Oviduct	Ovary
Lipids	—	—	—	—	++
Amines	+(1)	++	—	—	++
Neutral MPS	+(1)	++	—	—	+
Acid MPS	—	—	+	++	—

— negative.
+ positive.
++ strongly positive.
(1) increasing intensity toward lumen.
(2) from November.

Table 7. Summary of spermathecal characters for female types in *Chionoecetes opilio* and *Hyas coarctatus*. DR = dorsal region. VR = ventral region.

Female type	Spermathecal characters
Immature	Transverse membrane Columnar epithelium+1 layer germinal tissue in DR No secretions in lumen DR blind, straight tube VR pouches poorly developed
Premature	Transverse membrane Degenerating columnar epithelium Active germinal zone → stratified epithelium Secretions from columnar epithelium cells in lumen Enlarged DR VR pouches well developed
Mature, noncopulated, nonovulated	Transverse membrane DR columnar epithelium totally absent Stratified epithelium well developed Considerable secretion in lumen Pronounced DR enlargement VR pouches very well developed
Mature, noncopulated, ovulated (also copulated; Beninger *et al.*, 1993)	Transverse membrane absent Otherwise same as preceding

family Majidae. The close similarity in the findings for *Chionoecetes opilio* and *Hyas coarctatus* suggest that the observed ontogeny of female primary sexual characters is typical of this brachyuran family.

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