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Cell Structure and Seasonal Changes of the Androgenic Gland of the Mud Crab *Scylla paramamosain* (Decapoda: Portunidae)

Hong Liu[1,2], Kwok-Chu Cheung[1], and Ka-Hou Chu[1,*]

[1]*Department of Biology, Chinese University of Hong Kong, Shatin, Hong Kong, China*

[2]*Key Laboratory of Aquatic Genetic Resources and Aquacultural Ecology, Shanghai Ocean University, Shanghai, 200090, China*

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Hong Liu, Kwok-Chu Cheung, and Ka-Hou Chu (2008) Cell structure and seasonal changes of the androgenic gland of the mud crab *Scylla paramamosain* (Decapoda: Portunidae). *Zoological Studies* **47**(6): 720-732. In the mud crab, *Scylla paramamosain*, the androgenic gland (AG) is an elongated structure, situated along the posterior vas deferens. There are 3 types of cells (types I, II, and III) in different seasons and in different parts of the AG of *S. paramamosain*. They could be easily distinguished based on the cell size, relative proportion of the nucleus in cells, and the abundance of vacuoles. Type I cells are packed together in a high density and have a small cell size, a relatively large nucleus, and a small amount of cytoplasm. These are newly formed gland cells. Type II cells represent the majority of AG cells with the highest activity during the secretory cycle. They have much more cytoplasm with bigger cell sizes than type I cells. Type III cells are characterized by abundant vacuoles and the disappearance of cell boundaries in some cases, which represent the degeneration stage of the secretory cycle. In different seasons, there are significant differences in terms of gland size, cell boundary, the presence of multinucleated cells, and the occurrence of immature gland cells. The size of the AG increased from January and reached a maximum in the major mating season (July-Sept.). The AG began to degenerate in Oct. In Nov., cell boundaries were difficult to locate. The number of hemocytes greatly increased in Dec., indicating regeneration of the AG. In Mar. to Apr. is a minor mating season, and some spent testes were observed. The AG further developed to the next major mating season. Therefore, the activity of the AG is synchronized with the reproductive cycle. http://zoolstud.sinica.edu.tw/Journals/47.6/720.pdf

Key words: Histology, Ultrastructure, Reproduction, *Scylla paramamosain*, Brachyura.

Charniaux-Cotton (1954) discovered the androgenic gland (AG) in amphipods and believed it to be related to sexual differentiation. This gland was later found in isopods and decapods (Charniaux-Cotton et al. 1966). The AG is responsible for the development of all male sexual characters (Payen 1973, Charniaux-Cotton and Payen 1985, Sagi et al. 1990 1997, Taketomi and Nishikawa 1996, Okumura and Hara 2004). Most studies on the AG in decapods have been focused in prawn, shrimp, and crayfish. In crabs, the structure of the AG was described in *Ocypoda platytarsis* (Thampy and John 1970), *Callinectes sapidus* (Payen et al. 1971), *Rhithropanopeus harrisii* (Payen et al. 1971), *Ranina ranina* (Minagawa et al. 1994), and *Pachygrapsus crassipes* (King 1964). Except the early study conducted by Payen et al. (1971), who described the histological structure and some ultrastructure of the hypertrophied AG of juvenile animals, the other researchers studied either the histological structure or ultrastructure but did not simultaneously compare the structural relationship using histology, scanning electron microscopy (SEM), and transmission electron microscopy (TEM) in a single species. So a comprehensive understanding of the structure of the AG in crabs is limited. Mud crabs, *Scylla* spp., are commercially important species

*To whom correspondence and reprint requests should be addressed. Tel: 852-26096772. Fax: 852-26035391.
E-mail:kahouchu@cuhk.edu.hk

in fisheries throughout the Asian-Pacific region and are cultured in Japan, China, Taiwan, and Southeast Asian countries (Robertson and Kruger 1994, Keenan 1999, Liu and Zhang 2001). In the present study on the mature male mud crab *S. paramamosain*, we conducted both TEM and SEM, as well as histological, observations of the AG. Histological and ultrastructural differences between immature and mature male *S. paramamosain* were investigated. Structural changes in the AG in an annual cycle were also elucidated by examining mature crabs collected monthly. The results provide background information for further studies of the physiology of the AG in this species.

MATERIALS AND METHODS

Crabs

Male mud crabs *S. paramamosain* were acquired from local fish markets in Hong Kong and used for dissecting microscopic, histological, TEM, and SEM studies. Wu (2002) found that male *S. paramamosain* (called *S. serrata* in that study, see Keenan et al. 1998) was capable of mating when the carapace width reached 8.2 cm. To follow the annual changes in the histological structure of the AG among mature male *S. paramamosain*, 3-5 crabs with similar carapace widths (11.9 ± 0.4 cm) were collected monthly from June 2001 to May 2002. Crabs with a carapace width of < 8.0 cm were regarded as immature male crabs. The carapace widths of collected immature crabs were 6.1-7.8 cm, and those of mature crabs were 8.0-13.6 cm.

Crabs were dissected immediately after collection. The posterior vas deferens (PVD) with the AG attached was isolated and fixed for different studies.

Dissecting microscopy

Isolated samples were kept in crab saline (Grau and Cooke 1992, Toullec et al. 1996), observed under a dissecting microscope, and photographed with a Nikon (Tokyo, Japan) digital camera.

Histological observations

Samples were fixed in a 10% phosphate buffered formalin solution (0.045 M Na_2HPO_4, 0.028 M NaH_2PO_4, and 10% formalin) for 3-4 d, embedded in paraffin wax, sectioned at 5 μm, and stained with hematoxylin and eosin. Sections were observed under a light microscope and photographed using a Nikon digital camera and ACT-1 software (Nikon). The widths of the PVD and AG were measured using the scale bar on the pictures of the longitudinal sections. During observation, the number of cells belonging to each cell type (see "Results") was counted in 3-5 visual fields in order to determine the major cell type in the AG of crabs collected in different months. The biggest proportion represented the major cell type.

Scanning electron microscopy (SEM)

Samples were fixed in 2.5% glutaraldehyde buffered with 0.1 M phosphate buffer for 4-6 h at 4°C, washed twice with phosphate buffer for 15 min, and then post-fixed in 1% phosphate-buffered osmium tetroxide for 2 h. After that, samples were washed, dehydrated, then critical point-dried with liquid CO_2, and coated with gold/palladium. Specimens were observed under a scanning electron microscope (JEOL JSM-5300;Tokyo, Japan).

Transmission electron microscopy (TEM)

Samples were fixed as for SEM, dehydrated, infiltrated, and then embedded in Spurr resin and sectioned at 70-75 nm. Ultrathin sections were stained with 1% (w/v) methanolic uranyl acetate and Reynold's lead citrate, and observed under a transmission electron microscope (JEOL JEM-1200EX).

RESULTS

Overall structure of the AG and AG cells of *S. paramamosain*

In *S. paramamosain*, the AG is an elongated structure, situated along the PVD between the levator and depressor muscles of the 5th periopod (Fig. 1). The gland extends from a point close to the entrance of the PVD through the large foramen mediodorsally to a point near the beginning of the ejaculatory duct, running alongside the nerve to the 5th periopod, almost occupying the entire length between the end of the median vas deferens (MVD) and the penial structure. The gland is bound by a very thin connective tissue membrane and is attached to one side of the PVD (Figs. 2A, B).

The AG in *S. paramamosain* is thread-like and roughly circular in shape in transverse section, and light yellow to greenish-yellow (Fig. 2A). The length of the gland is almost the same as the PVD, and about 1/4 to 1/3 of the carapace width of the crab. The width of the gland was not uniform, but varied with the size of the crab, ranging from a very fine thread-like structure (1/10 of the diameter of the PVD, 50 μm) (Fig. 7B) and a cord-like structure (150-400 μm in diameter) (Figs. 5A, B), to a massive rope-like structure (500-600 μm in diameter) (Fig. 7A) thicker than the PVD.

Cells of the AG are packed together in an acinar shape, and have a diameter of 8-25 μm (Figs. 2C, D). They are transparent with an oval or granular shape (Figs. 3A-C). The large nucleus is round to oval, with a diameter of up to 7 μm (Fig. 3D). There is often an oblong and eccentrically situated nucleolus. The large nuclei are rich in chromatin granules. The nucleus-to-cytoplasm ratio is 0.2-0.5. The cytoplasmic matrix shows a high electron density (Fig. 3B).

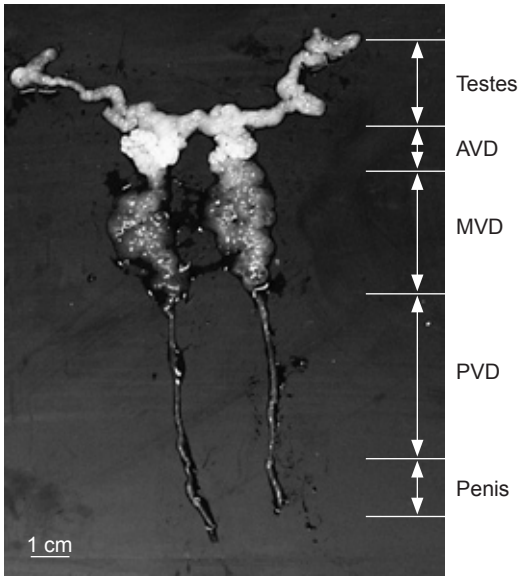

Fig. 1. Male reproductive system of *Scylla paramamosain* (carapace width 12.0 cm). AVD, anterior vas deferens; MVD, median vas deferens; PVD, posterior vas deferens.

Three cell types in AG of *S. paramamosain*

Three types of cells, named types I, II, and III, could be distinguished in different parts of the AG of *S. paramamosain* (Fig. 4A). Type I cells (Figs. 3A, 4B) were found mainly at the 2 ends of the gland. They are located near the epithelium and on the periphery of each strand of the AG behind the growing tip, packed together in a high density, and containing a relatively large nucleus with a small amount of cytoplasm (Figs. 3A, 6A). The cell size was about 8-2 μm. The nucleus-to-cytoplasm ratio exceeded 0.5 (Figs. 3A, 4B).

Type II cells were the majority of AG cells (Figs. 3B, 4C). There was much more cytoplasm in type II cells with a bigger cell size (14-25 μm) (Figs. 2C, D) than type I cells. The nucleus-to-cytoplasm ratio was 0.2-0.5. The cell boundaries of type I and II cells were always very sharp.

Type III cells were characterized by abundant vacuoles and the disappearance of cell boundaries in some cases (Figs. 3C, 4D). The cell size was about 20-25 μm. The 3 types of AG cells could easily be distinguished based on cell size, the relative proportion of the nucleus in the cell, and the abundance of vacuoles (Table 1). In type I cells, more than 1/2 of the cellular space was occupied by the nucleus. In type III cells, vacuoles occupied most of the cellular space. Growth in type III cells had ceased. The sizes of the cell and nucleus in type II cells were intermediate between those of type I and III cells. The cytoplasm was more or less homogeneous with the occurrence of vacuoles in type II cells.

Structural differences of the AG between immature and mature male *S. paramamosain*

In immature crabs, AG cells were packed together very tightly (Fig. 6A). The nuclei had

Table 1. Comparison of the 3 cell types in androgenic glands of *Scylla paramamosain*

Cell type	Cell diameter (μm)	$V_{nucleus} : V_{cell}$	Vacuoles
I	8-12	> 1/2	Absent
II	14-25	1/2-1/5	Present in some cells
III	20-25	< 1/5 (~1/12)	Abundant, occupying most of the cytoplasm

condensed chromatin, and there was only small amount of cytoplasm with few rough endoplasmic reticula, some smooth endoplasmic reticula, and rare Golgi bodies. The characteristics of AG cells in immature crabs were the same as those of type I AG cells. In mature crabs, AG cells were packed together much more loosely (Fig. 6B). Their ultrastructure was mostly similar to that of type II AG cells.

Seasonal changes of the AG of *S. paramamosain*

Seasonal changes in morphology and histology of the AG were found in *S. paramamosain*. Differences including gland size, cell boundary, the presence of binucleated cells, and the occurrence of immature gland cells were observed in crabs sampled in different months. The proportions of the 3 cell types in the AG also changed with the season.

There were significant differences in the size of the AG in different months. In July-Sept. (summer), the gland reached its maximum size. In July, the width of the AG was 1.2 times that of the PVD (Fig. 7A). In Jan. (winter), the size of the gland reached a minimum, with a width only 1/10 that of the PVD (Fig. 7B). From Jan. to Mar., the size of the AG increased.

Cell boundaries in the AG also varied in different months (Figs. 8A-D). From June to Sept., cells were loosely arranged, and most cells were characterized as type II. During this period, cell boundaries were very sharp (Fig. 8A). From Oct., the proportion of type III cells increased, and the cell boundaries began to disappear. In Nov.,

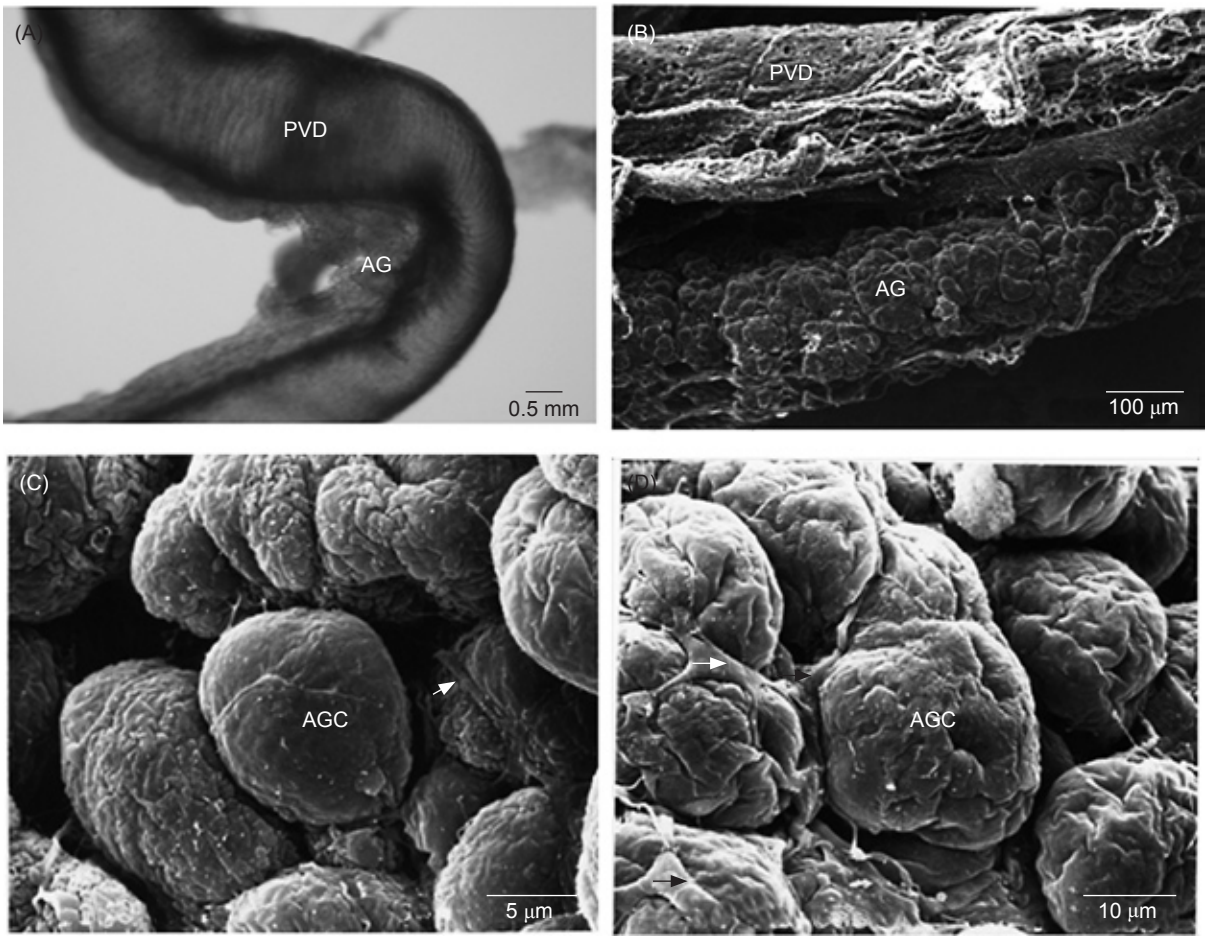

Fig. 2. Posterior vas deferens (PVD) and androgenic gland (AG) under dissecting microscopy (A, carapace width 10.7 cm) and scanning electron microscopy (SEM) (B, carapace width 11.1 cm) and groups of AG cells (AGCs) connected by connective tissue (arrow) under SEM (C, D). The AG is tightly connected to the PVD in (A). (B) The loose connection between the AG and PVD. Tight and loose connections were observed in different AGs of different crabs and even in the same AG but in different regions. The diameters of the AGCs are 14 and 25 μm in (C, carapace width 12.3 cm) and (D, carapace width 11.3 cm), respectively.

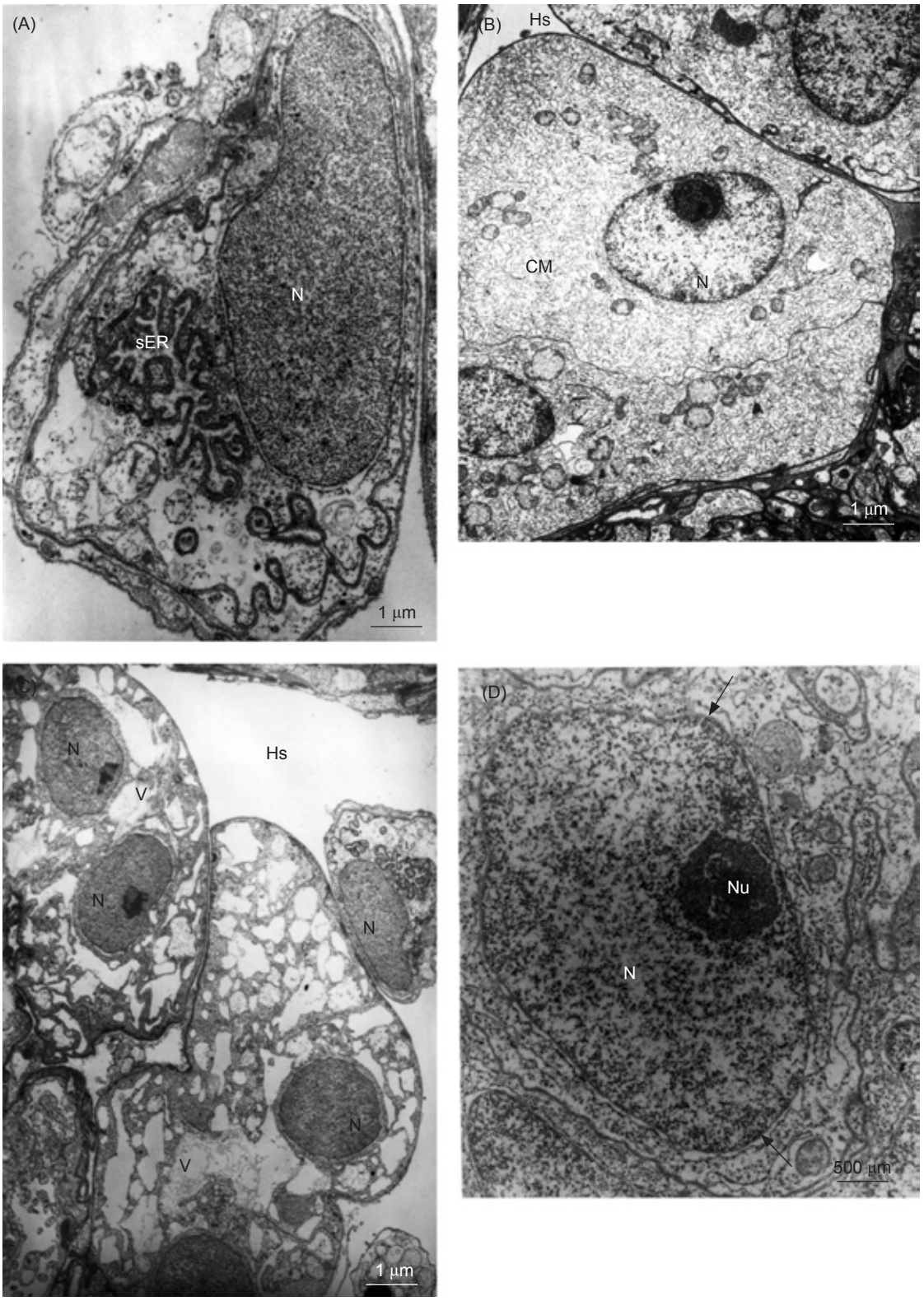

Fig. 3. Differences in the cell size and organelles in type I (A, carapace width of 6.4 cm), II (B, carapace width of 9.3 cm), and III (C, carapace width of 9.9 cm) androgenic gland cells (AGCs) and a nucleus (D, carapace width 9.3 cm) of an AGC of *Scylla paramamosain*. Cell boundaries of type III cells had disappeared (C). CM, cytoplasmic matrix; N, nucleus; Nu, nucleolus; sER, smooth endoplasmic reticulum; V, vacuole; Hs, hemocoel space or intercellular space. Arrow, space between 2 nuclear membranes or perinuclear space.

type III cells were dominant, and most of the cell boundary had disappeared (Fig. 8B). In Dec., the number of hemocytes in the AG had greatly increased. In Jan., very clear cell boundaries appeared again along with a majority of type I gland cells (Fig. 8C). Type II cells were present, but type III cells were seldom found. From Jan. to Mar., many immature (type I) AG cells were observed, and the number of type II cells also increased. In Apr., the cell boundary in some AG cells was once again not so clear (Fig. 8D).

In months other than Nov. and Dec., binucleated or multinucleated cells were observed in a large number of small AG cells (Fig. 8E). Two or more nuclei might be located very closely together. Binucleated or multinucleated gland cells were also observed by TEM (Fig. 8F). These small cells represent type I AG cells.

Significant differences in AG cells including the appearance and ultrastructure were found between summer (June-Sept.) and winter (Nov.-Jan.) months. According to Wu (1995), June-

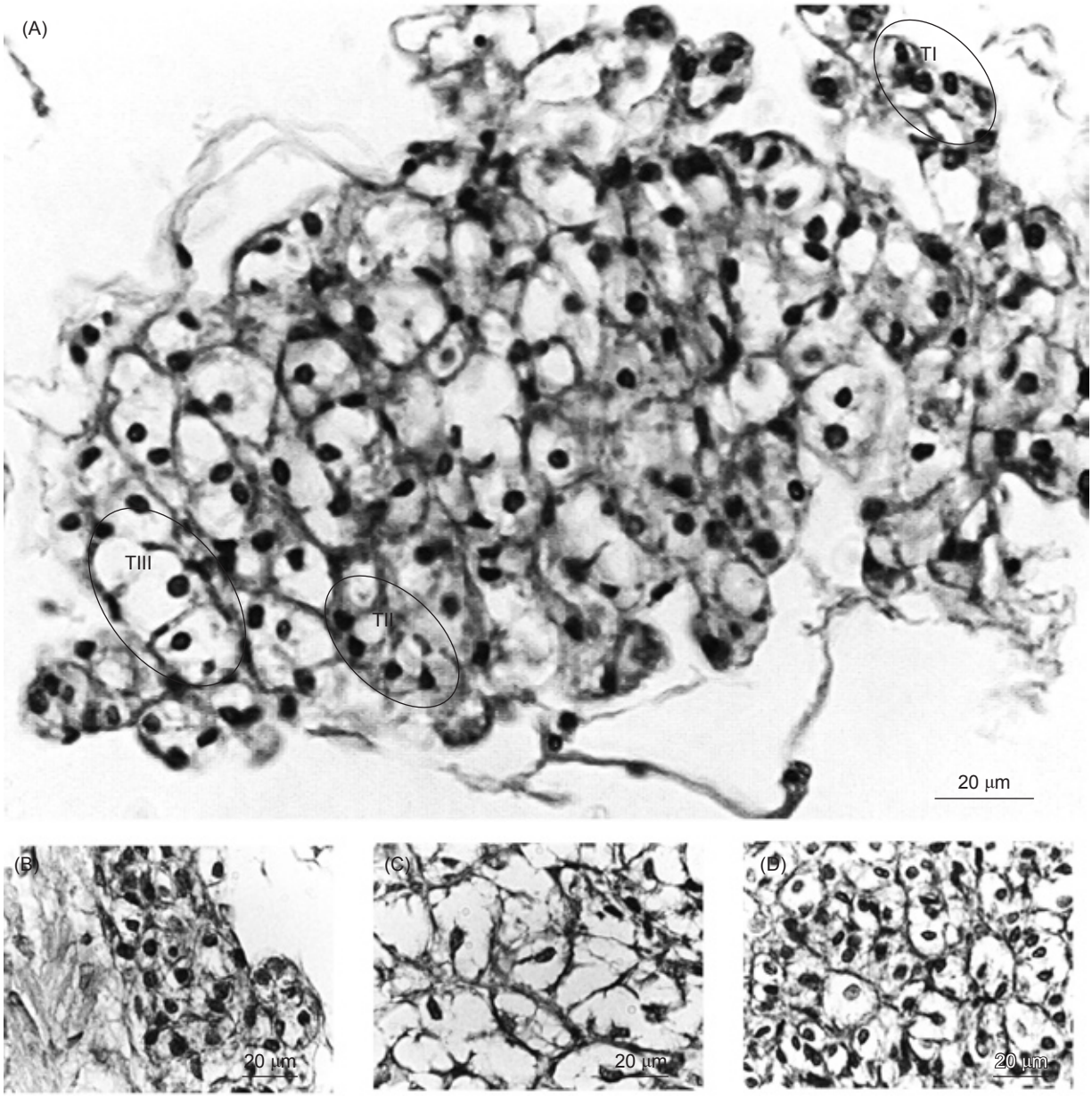

Fig. 4. Three types of androgenic gland cells of *Scylla paramamosain* (A, carapace width 11.4 cm). TI, type I cell (B); TII, type II cell (C); TIII, type III cell (D). Transverse section.

Sept. is the mating season of the mud crab *S. paramamosain* (called *S. serrata* in that study, see Keenan et al. 1998) on the coast of Guangdong Province, China. Mating and spawning seldom occurs in winter. During the mating season, the AG in *S. paramamosain* reached its maximum size and appeared to be highly active. The majority of cells were type II with extensive occurrence of organelles such as rough endoplasmic reticula, Golgi bodies, and mitochondria.

Seasonal variation in the proportions of the 3 types of AG cells was also observed. From Jan. to Mar., cells were mainly type I. The AG was still inactive and in the process of regeneration. From July to Sept., most AG cells were type II with very few type III cells. The AG was most active during this period. From Oct. to Nov., type II cells predominated, but the number of type III cells also increased indicating that the AG was still active, but beginning to degenerate. Cellular degeneration might represent termination of the secretory cycle. In Dec., the AG began to regenerate as the number of hemocytes began to greatly increase. In Jan., type I cells predominated, and type II and III cells with sharp cell boundaries were seldom found. These results indicate that the AG in Jan. was in an inactive and regenerating stage. Annual changes in morphology and histology of the AG of *S. paramamosain* are summarized in table 2.

DISCUSSION

The width of the AG in mature *S. paramamosain* ranges 50-600 μm. The width of the PVD is about 500 μm. The length of the AG is 1/3 to 1/4 of the carapace width of crabs, as in *Ocypoda platytarsis* (Thampy and John 1970). In immature

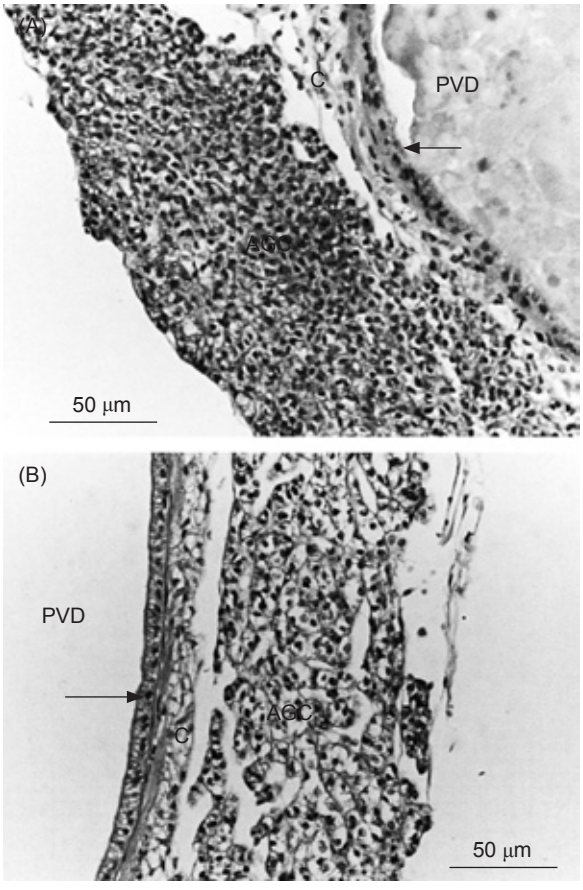

Fig. 5. Type I (A) and II (B) androgenic gland (AG) cells (AGCs) in different parts of the same AG of *Scylla paramamosain* (carapace width 11.8 cm). The AG loses its connection with the connective tissue (C) of the posterior vas deferens (PVD). Diameters of the AG in (A) and (B) are both 113.5 μm. Arrow, epithelium of the PVD. Longitudinal section.

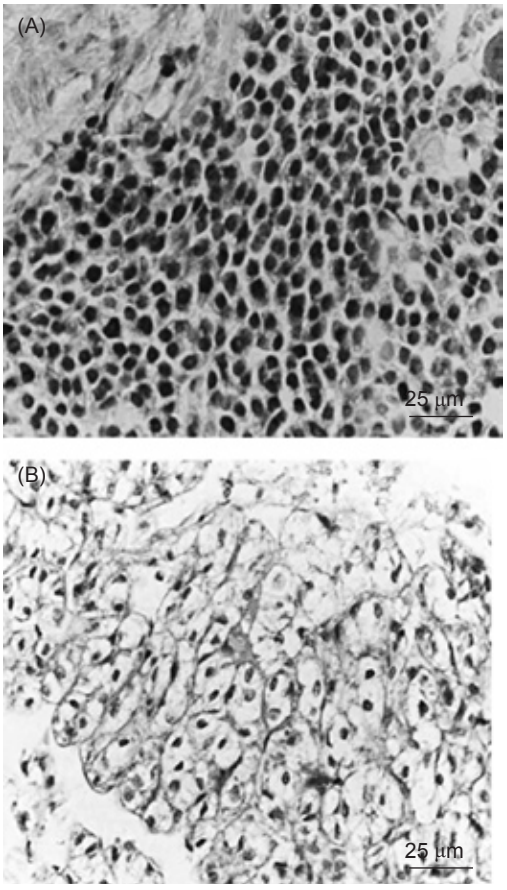

Fig. 6. Androgenic gland (AG) cells (AGCs) in immature (A, carapace width of 7.5 cm, showing type I AGCs) and mature (B, carapace width of 8.9 cm, showing type II AGCs) *Scylla paramamosain*. The AGCs are tightly (A) or loosely (B) packed. Transverse section.

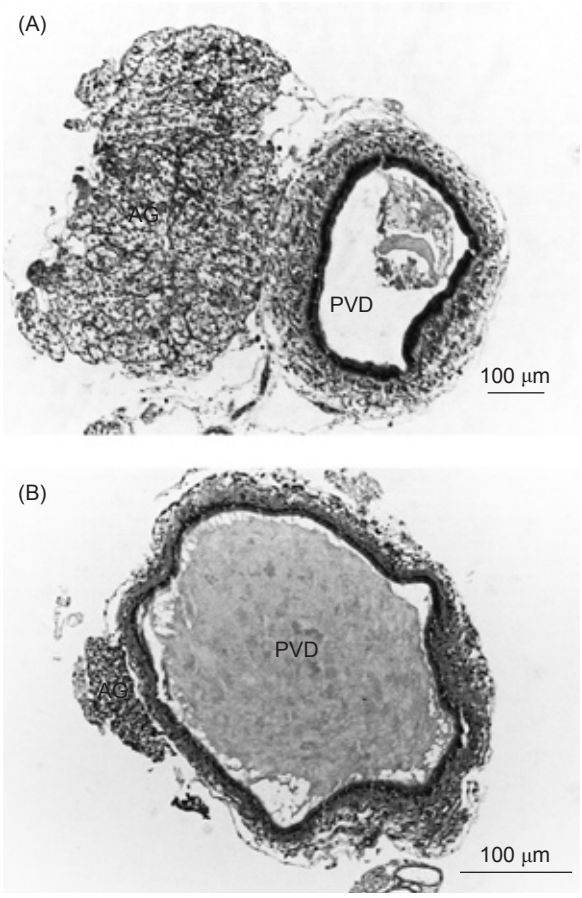

Fig. 7. Androgenic gland (AG) in July (A, carapace width of 11.7 cm) and Jan. (B, carapace width of 13.8 cm). Widths of the AG and posterior vas deferens (PVD) are 600 and 500 mm, respectively in (A), while those in (B) are 50 and 500 μm, respectively. Transverse section.

males, the width of the AG was 2 times smaller than that of the PVD. In the prawn *Macrobrachium rosenbergii*, the diameter of the AG is 6.08-14.86 μm (Awari and Dube 1999). The width range of the blue crab AG is 200-300 mm (Tcholakian and Reichard 1964). In the crayfish *Cherax destructor*, the AG is a gel-like cord with a length of 5 mm and width of 100-300 μm (Fowler and Leonard 1999). In other crustaceans such as *Carcinus maenas* (Demeusy 1960) and *Ocypoda platytarsis* (Thampy and John 1970), the growth of the male genital apparatus, as represented by the diameter of the ejaculatory duct, closely parallels that of the AG. This differs from the situation in *S. paramamosain*. In some mature male crabs, the width of the AG was much smaller than that of the PVD. These were small, narrow, thread-like AGs. In some crabs, the width of the AG was equal to or even greater than that of the PVD. In the ghost crab *Ocypoda platytarsis*, it was observed that the gland actively grows at its 2 ends, where the narrow and small thread-like structure attaches the growing tips to the ejaculatory duct (Thampy and John 1970). Therefore, the very small AG in mature *S. paramamosain* indicates that the AG is actively growing similar to that in *Ocypoda platytarsis* (Thampy and John 1970).

In the present study, different types of the AG cells were observed at different points along the length of the gland. The major cells near the 2 ends were type I cells. During the early

Table 2. Annual changes in androgenic glands (AGs) of *Scylla paramamosain*

Month	Size of the AG	Major cell types	Remarks
June 2001	Medium	I, II	No spermatogonia in testes; binucleated
July	Large	I, II	Multinucleated
Aug.	Large	II, III	Cytoplasm not homogeneous; some spent testes present
Sept.	Large	II	No spermatophores in PVD
Oct.	Large	II	Small nucleus with small cells; indistinct cell boundaries
Nov.	Medium	III	
Dec.	Medium	I, III	Hemocytes increased
Jan., 2002	Small	I	
Feb.	Medium	II	
Mar.	Medium	I, II	Some spent testes present
Apr.	Medium	I	Vacuoles increased in number
May	Medium	I	Some vacuoles; binucleated; indistinct cell boundaries in some cells

PVD, posterior vas deferens.

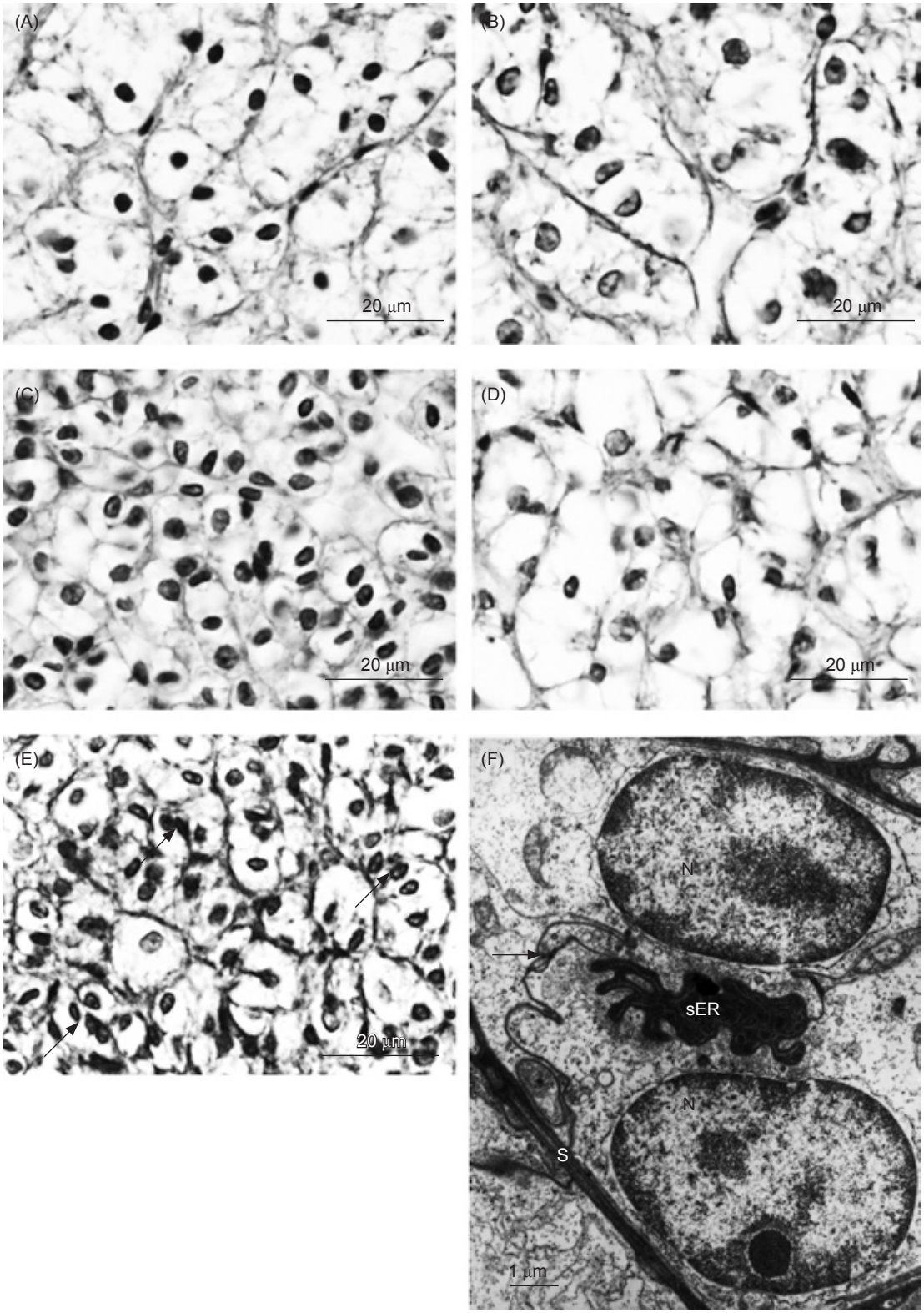

Fig. 8. Androgenic gland cells (AGCs) with sharp boundaries in July (A, carapace width of 12.1 cm) and Jan. (C, carapace width of 14.5 cm), and indistinct boundaries in Nov. (B, carapace width of 11.4 cm) and Apr. (D, carapace width of 11.8 cm). Transverse section. Binucleated AGCs under light microscopy (E, indicated by arrows, carapace width of 13.8 cm) and transmission electron microscopy (TEM) (F, carapace width of 13.4 cm). N, nucleus; sER, smooth endoplasmic reticulum; S, sheath of AG; arrow in (F), cell membrane.

developmental stage of growth of the AG in *S. paramamosain*, most of the AG cells were type I cells. They were relatively small (8-12 μm in diameter), and bi- or multinucleated cells were observed. Similarly, a large number of binucleated small cells were observed in the AG of *Ocypoda platytarsis* (Thampy and John 1970). This binucleate condition is thought to be the result of amitotic division (Thampy and John 1970), in which small cells give rise to new cells by amitosis. The binucleated cells and daughter cells seem to be type I cells. The mother cells in *O. platytarsis* are similar to type II cells in *S. paramamosain*, and highly vacuolated cells (also called the oldest cells in *O. platytarsis*) are similar to type III cells of *S. paramamosain* and other decapods.

Originating from the differentiating epithelium of the PVD as reported in *Pandalus platycero* (Hoffman 1969) and *M. rosenbergii* (Veith and Malecha 1983), the AG increased in length and width until its width equaled or exceeded that of the PVD. During growth, the AG was tightly connected to the PVD by connective tissue (Fig. 2A). This connection became looser after the growth of the AG, suggesting that the activity of the AG hormone was the highest, and degeneration may have begun (Figs. 2B, 5A, B). Growth was more active at the 2 ends of the gland since type I cells were abundant there.

The structure of the AG in the mud crab *S. serrata* was first reported by Rangneker et al. (1971), although a very clear and complete description was not given in that paper. In the present study on *S. paramamosain*, AG cells were tightly packed together in an acinar shape mounted on the PVD and extending along the entire length of the PVD under dissecting microscopy and SEM (Figs. 2A, B). The structure of AG cells in *S. paramamosain* was similar to that reported in decapods such as the crabs *Pachygrapsus crassipes* (King 1964), *Rhithropanopeus harrisii*, and *Callinectes sapidus* (Payen et al. 1971), the crayfish *Procambarus clarkii* (Taketomi 1986, Taketomi et al. 1990) and *Cherax destructor* (Fowler and Leonard 1999), and marine shrimp (Alfaro 1994). Similar ultrastructures were also reported in the isopod *Armadillidium vulgare* (Negishi et al. 2001). The size of AG cells in *S. paramamosain* was about 20 (range, 8-25) μm, and the nucleus was about 7 μm. In *C. sapidus*, the sizes of the AG cell and nucleus are 15-20 and 5-7 μm, respectively (Tcholakian and Reichard 1964). In *M. rosenbergii*, the AG cell is 15-25 μm in diameter (Sun et al. 2000). The nucleus in *S. serrata* is 3-5 μm (Rangneker et al. 1971). Therefore, the sizes of the AG cell and its nucleus appear to be similar among these decapods. Based on the ultrastructural study of AG cells of *S. paramamosain*, it can be concluded that AG cells resemble vertebrate protein-producing cells rather than steroid-producing cells.

Three types of AG cells were observed in the present study based on differences in cell size, the nucleus-to-cytoplasm ratio, and the abundance of vacuoles. More significant differences were observed among these 3 types of cells using TEM. Type I cells were mainly found at the 2 ends of the gland, near the epithelium of the PVD (Fig. 5A). They appeared to be new cells produced by the germinal epithelium. Type II cells were actively secreting hormones, and type III cells seemed to be in the degeneration phase. Different cell types of the AG were also reported in other decapods. In *Carcinus maenas*, 3 different regions of the AG can be distinguished (Paoli unpublished data, cited in Charniaux-Cotton et al. 1966). In the freshwater prawn *M. rosenbergii* (Awari and Dube 1999, Veith and Malecha 1983) and *M. kistnensis* (Mirajkar et al. 1984), 3 types of AG cells were identified as type I, II and III cells. According to their description, type I is the smallest cell with dense cytoplasm, type II is a slightly larger cell with vacuoles, and type III is the largest cell consisting almost entirely of vacuoles. These characteristics are the same as those of AG cells of *S. paramamosain* in the present study. In addition, in the AG of the ghost crab *Ocypoda platytarsis*, there are 3 histologically different cells according to their cell size (11-13, 13-15, and 15 μm), dense or homogeneous cytoplasm, and the presence or absence of vacuoles (Thampy and John 1970). In *Penaeus chinensis*, 2 kinds of AG cells were identified (Li and Xiang 1996). The smaller cell is 6-10 μm in diameter with clear cell boundaries. These cell types may be in different phases of secretory activity.

There are 5 developmental stages in the male crayfish *Procambarus clarkii* based on its external and internal male sexual characteristics (Taketomi et al. 1996). The activity of the AG differs in each developmental stage, with stage E representing the stage of complete sexual maturation. AG cells at this stage are highly active and characterized by a large nucleus with diameter of 7 μm and a large amount of cytoplasm. In our study, the highly active AG mainly contained type II cells with a large amount of cytoplasm and a condensed nucleus (7 μm

in diameter). Type II cells in *S. paramamosain* appeared to be equivalent to AG cells in stage E in *P. clarkii*. The proportions of the 3 types of AG cells in the AG seem to represent different phases of secretory activity as suggested by Thampy and John (1970).

In the present study, it was found that vacuoles existed in type II and III cells, and occupied almost all of the cytoplasm in type III AG cells (Figs. 3B, C). Vacuoles were described as indicating the degeneration phase of the AG (Carpenter and DeRoos 1970). In the crayfish *P. clarkii*, the presence of inclusion bodies in AG cells is independent of season (Miyawaki and Taketomi 1978). Inclusion bodies represent a unique process of hormone secretion. The structural similarity of the inclusion bodies in *P. clarkii* and vacuoles in *S. paramamosain* indicates that they might represent the same kind of organelle involved in hormone secretion.

There were significant differences in the AG of *S. paramamosain* between summer (mating season) and winter (non-mating season). In winter, the AG was small, and the major cell type was type I. In summer, the AG reached its maximum size, and the major cell was type II. In Guangdong Province and Hong Kong, China, there are 2 reproductive seasons consisting of a major (June-Sept.) and minor peak (Mar.-Apr.) in the mud crab *S. paramamosain* (Wu 1995, Liu and Zhang 2001). In the present study, the size and activity of AG cells seemed to correlate with these reproductive cycles. Spent testes were observed in Mar. and Aug. indicating the occurrence of mating, which was synchronized with the 2 reproductive seasons of *S. paramamosain* in Guangdong Province. In Jan., the AG was at its minimum size with type I cells occupying almost the entire gland, indicating that the AG was inactive and regenerating. From Jan. to Mar., the size of the AG and the number of type II cells increased. These changes might be related to the minor peak of the reproductive season. From June to Aug., AG cells were mostly type II with very few type III. Therefore, the AG was the most active from June to Aug., which is synchronized with the major reproductive season. From Oct. to Nov., the number of type III cells increased, indicating that the AG had begun to degenerate. When the activity of the AG decreased, the cell boundaries became indistinct. Cellular degeneration may represent termination of the secretory cycle. In Dec., the AG began to regenerate as the number of hemocytes greatly increased, indicating the start of a new reproductive cycle.

Seasonal changes in the AG have been reported in the crayfish *Orconectes nais* (Carpenter and DeRoos 1970) and *Procambarus clarkii* (Taketomi 1986, Taketomi et al. 1996), the freshwater prawn *Macrobrachium kistnensis* (Mirajkar et al. 1984), the marine shrimp *Penaeus chinensis* (Li and Li 1993), and the isopod *Armadillidium vulgare* (Katakura 1984). In *O. nais*, the AG was also found to regress during winter (Carpenter and DeRoos 1970). In *P. clarkii*, the number of highly active cells increases during summer, in contrast to the low number in winter (Taketomi et al. 1996). Water temperature is not the main environmental factor influencing structural changes and function of the AG in this crayfish. In *M. kistnensis*, the AG attains a maximum size during the breeding season and becomes reduced during non-breeding months (Mirajkar et al. 1984). In *M. rosenbergii*, the androgenic gland is the largest in blue-claw males, which is the final morphotypic stage having high mating activity (Okumura and Hara 2004). During the inactive phase of the gland, cell boundaries become indistinct, and only a syncytium can be seen. There are a major peak, a minor peak, and a lean period in between these 2 peaks in the annual reproductive cycle. During the lean period, the AG is composed of only a small number of cells closely associated with the vas deferens (Mirajkar et al. 1984). In *S. paramamosain*, the increase in the size of AG or increase in the number of hypertrophied cells indicates an increase in AG activity, and there is a direct correlation between the size and activity of the AG as well as testicular activity. In Mar. and Aug., some spent testes were observed, indicating that Mar. and Aug. are mating seasons. In June, spermatogenesis frequently occurs, as in *M. kistnensis* (Mirajkar et al. 1984). In *P. chinensis*, the AG appears after sexual differentiation, and the number of AG cells varies with season. Secretion by the AG is the most active when mating occurs in the middle of Oct. (Li and Li 1993). Further studies found that cell morphological differences were also correlated to differential gene expression. In *S. paramamosain*, the β-actin analog gene was highly expressed in the AG in Jan., but was not expressed in Apr. or July/Aug., nor in the hemolymph control (Liu and Chu 2007). In Jan., the AG in *S. paramamosain* is inactive and characterized by the smallest gland size. Differential accumulation of β-actin messenger RNA often occurs during cleavage (Taylor and Piko 1990, Sarmiento et al.

2000). This also proves that in Jan., the AG in *S. paramamosain* is in the regeneration stage.

The present study defined 3 types of cells in the AG of the mud crab *S. paramamosain*, which can be distinguished based on cell size, the relative size of the nucleus, and the abundance of vacuoles. Cells corresponding to AG cells have been observed in many other decapods (Charniaux-Cotton et al. 1966, Thampy and John 1970, Veith and Malecha 1983, Mirajkar et al. 1984, Awari and Dube 1999) and represent different stages of the secretory cycle of the gland but not different functional cell types. This was also evident from the seasonal changes in the size of the gland and the relative abundances of the cell types showing that the activity of the gland is synchronized with the reproductive cycle of the mud crab.

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