



Organogenesis and subsequent development of the genital organs in female and male Pacific white shrimp *Penaeus (Litopenaeus) vannamei*

Rodolfo Garza-Torres, Rafael Campos-Ramos*, Alejandro M. Maeda-Martínez

Centro de Investigaciones Biológicas del Noroeste (CIBNOR), Mar Bermejo 195, Col. Playa Palo de Santa Rita, La Paz, B.C.S. 23096, México

ARTICLE INFO

Article history:

Received 19 February 2009

Received in revised form 5 August 2009

Accepted 5 August 2009

Keywords:

Androgenic gland

Genital organ

Penaeus (Litopenaeus) vannamei

Organogenesis

Sexual differentiation

ABSTRACT

Timing of organogenesis and subsequent development of genital organs were studied in female and male Pacific white shrimp *Penaeus (Litopenaeus) vannamei* postlarvae. This was linked to the timing of differentiation of external structures that differentiate the genders. Anatomy of the gonad appears to be unique for penaeid species. The genital organ was fully recognized from postlarve day-16 (PL16) as a bilateral lobe located in the anterior region of the midgut gland (first anterior lobes) that connects to an anterior perpendicular collector tube that extends dorsally towards the posterior region of the midgut gland and forms an inverted U-shape collector. Eight bilateral lobes in females (second to ninth) and the bilateral oviduct between the seventh and eighth lateral lobes and seven bilateral lobes in the male (second to eighth) are connected along the inverted U-shape collector tube. These lobes extend over the surface of the midgut gland beneath the pericardium. Shortly after organogenesis of the female gonad, the tenth bilateral lobe emerges from the distal region of the collector tube and continues dorsally along the intestine, and the fourth bilateral lobe did not develop and regressed until apparently absorbed. In males, the posterior bilateral vas deferens emerges from the same region. Around PL50 (0.5–0.6 g; 45–50 mm), external gender differentiation was recognized in the form of the thelycum in females and the gonopores in males. Additionally, the male androgenic gland appears at the posterior-external wall of each anterior vas deferens, surrounded by connective tissue that attaches to the anterior vas deferens and the eighth testicular lobe. Gonad differentiation occurred from PL68 (1.8–2.2 g; 70–74 mm), where it was possible to differentiate the female ovary from the male testes. Timing of sex reversal studies in penaeids is discussed.

© 2009 Elsevier B.V. All rights reserved.

1. Introduction

Information concerning development of internal and external sexual characteristics in commercially reared decapods is useful because there is gender dimorphism, whereby one sex grows larger than the other does. In freshwater prawns, males grow larger than females, as in the Malaysian prawn *Macrobrachium rosenbergii* (De Mann) (Sagi and Aflalo, 2005; Aflalo et al., 2006). In penaeid shrimp, females grow larger than males, as in the Pacific white shrimp *Penaeus (Litopenaeus) vannamei* (Boone) (Chow and Sandifer, 1991), and other American, Asian, and Indian species reviewed by Campos-Ramos et al. (2006). The relevance of sexual dimorphism in *P. vannamei* is that it involves a significant differential growth at harvest size (Pérez-Rostro et al., 1999), and therefore, an all-female culture would increase profitability for farmers based on size and market weight (Zhang et al., 2007). Sex determination and differentiation is particularly interesting in crustacean malacostracans like isopods, amphipods, and decapods because the organ responsible for maleness is the androgenic gland (AG). This gland was discovered in the amphipod *Orchestia gammarellus* (Pallas)

(Charniaux-Cotton, 1953, 1954, 1960). In freshwater prawns, such as *M. rosenbergii* (Nagamine et al., 1980a,b; Sagi and Cohen, 1990; Sagi et al., 1990; Malecha et al., 1992), *Cherax destructor* (Clark) (Fowler and Leonard, 1999), *C. quadricarinatus* (von Martens) (Khalaila et al., 2001; Barki et al., 2003; Manor et al., 2004), and *Procambarus clarkii* (Girard) (Taketomi and Nishikawa, 1996), andrectomy and implantation of the AG leads to partial or total gonad sex reversal. However, the AG function in commercial penaeid species requires further investigation to understand its role in sex differentiation; the regulation of spermatogenesis during reproduction; sex reversal techniques; and in the production and secretion of hormones. Alfaro (1994) and Campos-Ramos et al. (2006) documented the structure of the AG in *P. vannamei*, which resembles those described in the literature for other malacostracans. The organogenesis of the genital organ, including the AG and the time of gender differentiation, has been studied in male *Penaeus japonicus* (Bate) by Chim (1983), Laubier et al. (1983), Charniaux-Cotton and Payen (1985), and Nakamura et al. (1992). These studies agree that organogenesis of the AG occurs before or at the onset of spermatogenesis by the end of the second month of the postlarval stage, long after gender differentiation takes place by the formation of the male vas deferens within the first two weeks of the postlarval stage. No other penaeid species male has been examined for this detail. Females

* Corresponding author. Tel.: +52 612 123 8451; fax: +52 612 125 3625.
E-mail address: rcampos@cibnor.mx (R. Campos-Ramos).

have been excluded from these analyses, having no record of the organogenesis of the oviduct and gonad differentiation into an ovary that would identify a female. The basic anatomy of the adult female genital apparatus in the literature is the original description by King (1948) for *P. setiferus* (L.), which describes the ovary as a partly fused, bilaterally paired structure with projecting lobes. The review of Dall et al. (1990) of female penaeids describes the ovary as two independent bilateral structures with projecting lobes, i.e., the anterior lobe, six to eight short lateral lobes, and the long posterior lobe, plus the oviduct at the sixth lateral lobe. In the case of males, the original description by King (1948) was complemented by Chim (1983) in *P. japonicus* and corroborated by Chow et al. (1991) in two species of shrimp (*P. setiferus* and *P. vannamei*) regarding the individuality of the testicular lobes connected to a horseshoe-shaped proximal vas deferens. Additionally, Chow et al. (1991) observed that it was a single, non-multiple, seminiferous tubule along each independent testicular lobe.

This study focused on the timing of organogenesis and subsequent development of the genital organs in female and male *P. vannamei* in concordance with the timing of the development of external structures that differentiate genders. We present a timeline of events of internal and external gender differentiation that is critical for developing techniques for sex reversal, with the objective of future production of female monosex populations for shrimp farming. We also provide new evidence on the morphology of the female genital organs that is different from descriptions in previous reports on related species. Further, we emphasized organogenesis and development of the AG, and how they interconnect the vas deferens and testis, apparently participating in spermatogenesis and testis maturation.

2. Materials and methods

Shrimp postlarvae were obtained from a shrimp laboratory in La Paz City, Mexico. The postlarvae, juvenile, preadult, and adult specimens were cultivated at the CIBNOR facilities. Shrimp were reared from postlarvae day-4 (PL4) in six 1000-L outdoor oval tanks maintained with filtered and aerated seawater (36–38 ppt salinity) at 27 ± 1 °C by submersible 300-W heaters at a density of 200 shrimp/tank. Exchange of seawater was ~50% three times weekly and temperature of the seawater was adjusted to that of tank water before the exchange. Shrimp up to PL20 were fed three times daily to apparent satiation with brine shrimp nauplii and commercial micro-particulates (250–500 µm); older shrimp (>PL20) were fed three times daily with live and frozen adult brine, brine shrimp flakes, and

crumbled, commercial pellets. Every four days (from PL4 to PL80), ten shrimp from the six tanks were randomly chosen and their weight (mg and g) and length (mm, rostrum tip to the end of the telson) were recorded. External sex structures were analyzed, as described by Pérez-Farfante (1988) and Campos-Ramos et al. (2006), and their internal organs, as described by King (1948), Kong and William (1988), and Dall et al. (1990). Shrimp were fixed in Davidson's solution (Howard and Smith, 1983; Bell and Lightner, 1988) for 24 h and then in 70% ethanol until processed with the hematoxylin and eosin histological technique (Bell and Lightner, 1988). External and internal examination was performed under a dissecting stereoscopic microscope (Olympus, Tokyo, Japan). Internal genital organs were stained with commercial aquarium methylene blue in 0.9% NaCl saline solution. Prepared slides of the tissues were examined with a compound light microscope (Olympus, Tokyo, Japan). Images were recorded with an attached digital camera and stored in the software Image Pro Plus 4.0 (Media Cybernetics).

3. Results

3.1. Timeline of events of internal and external differentiation of gender

A timeline of events of internal and external differentiation in females and males is shown in Fig. 1, which links each sex-development to a specific figure.

3.2. Organogenesis of the gonad (PL12 to PL16)

After histological preparations, no sign of gonad tissue was observed in PL4 and in PL8. The gonad was recognized in PL12 as a bilateral structure located dorsally at the anterior region of the midgut gland. The genital organ of each gender was fully recognized through dissection in PL16. During this time, the body weight of shrimp ranged from 80–130 mg and body length from 15–18 mm.

3.2.1. Morphology of the genital organ

The first bilateral anterior lobe is connected to a main anterior collector tube that is perpendicular to the body axes, extends dorsally towards the posterior region of the midgut gland, and forms an inverted U-shaped collector. Along and perpendicularly connected to the inverted U-shaped collector, eight bilateral lobes in the female, numbered from second to ninth lobe plus the bilateral oviduct (Fig. 2A), seven bilateral lobes in the male, numbered from second to

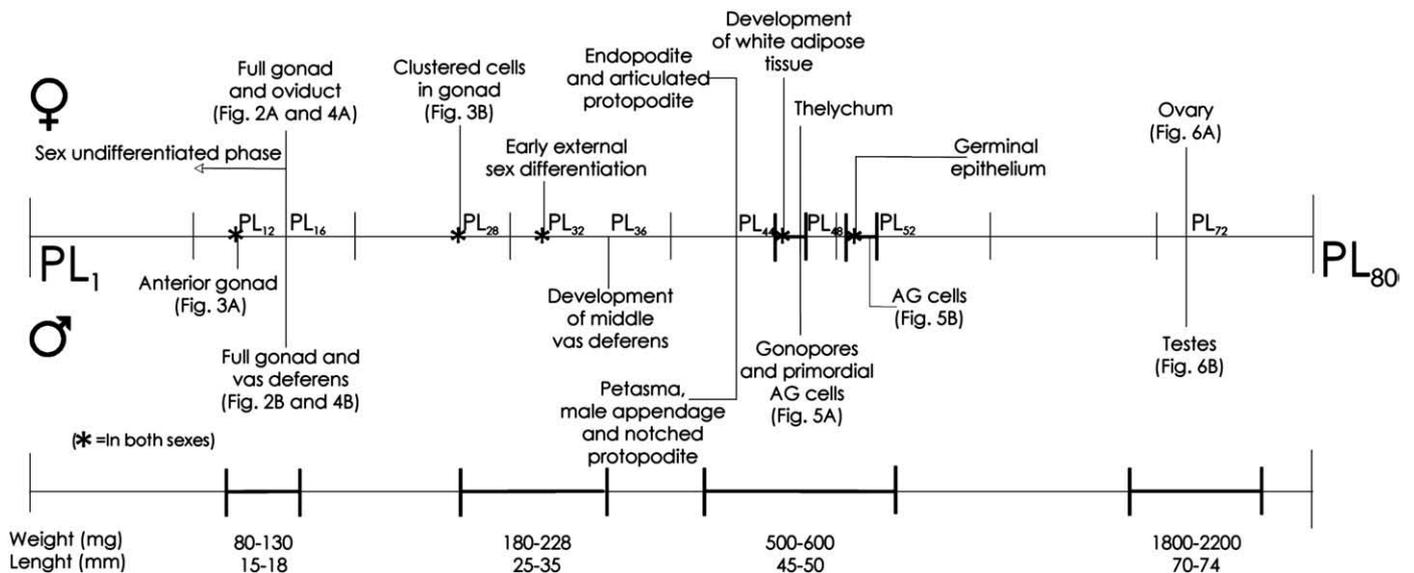


Fig. 1. Timeline of events of internal and external differentiation of female and male Pacific white shrimp *Penaeus vannamei*.

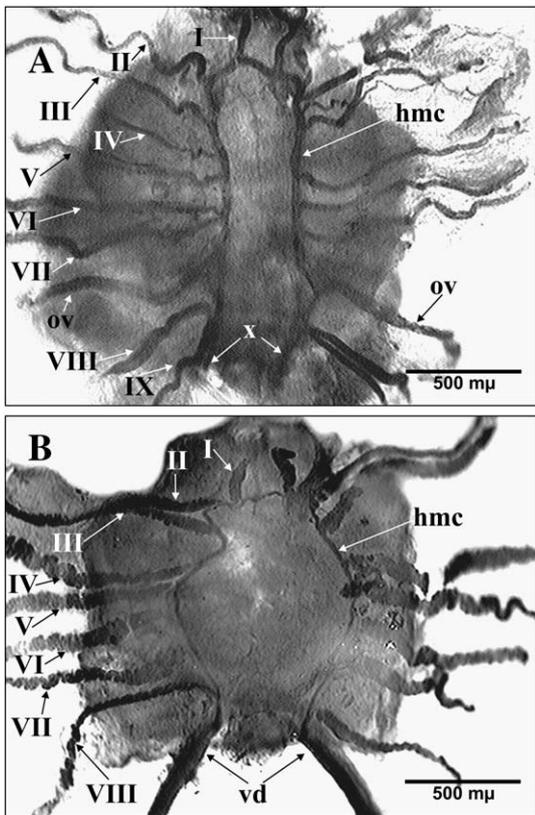


Fig. 2. *Penaeus vannamei*, PL24. Ventral view of genital organ in female (A) and male (B) located in the cephalothoracic region. Exo-skeleton and midgut gland were removed. Horseshoe-shape main collector tube (hmc), gonadic lobes (Roman numbers), female oviduct (ov), and male vas deferens (vd).

eighth lobe (Fig. 2B), extend over the surface of the midgut gland beneath the pericardium. They represent the basic anatomy of the gonad. In the female, the tenth bilateral lobe emerges from the distal region of the collector tube and develops dorsally along the intestine through the five abdominal segments during the following eight days. In addition, from PL28, the fourth lobe did not develop and regressed until apparently absorbed, leaving no rudimentary lobe-tissue and an evident space between the third and the fifth lobes (Fig. 3A). Therefore, the final morphology of the female gonad was composed of the first bilateral anterior lobe, seven bilateral lateral lobes, numbered from second to eight, plus the bilateral oviduct (Fig. 3B), and the ninth distal abdominal bilateral lobe.

3.2.2. Early development of the gonad (PL12 to PL28)

The initial morphology of the sex-undifferentiated bilateral anterior lobe was composed of unarranged cells of irregular shape (Fig. 4A), which by PL28 developed clusters of cells in all gonadic lobes (Fig. 4B).

3.2.3. Histology of female oviduct, and male vas deferens

The bilateral oviduct is a single, collapsed tube, with a half-moon-shaped cross-section that distinguishes it from the gonadic lobes (Fig. 4C). It is located between the sixth and seventh gonadic lobes, continuing laterally to the midgut gland, and then extends ventrally to the thoracic-abdominal muscle at the level of the third pereopod. In the male, the proximal bilateral vas deferens continues from the distal end of the U-inverted collecting tube behind the midgut gland. It extends upward and laterally, and then descends parallel to the aorta that runs down the left side of the body between the thoracic-abdominal muscle and the intestine, surrounded by a wide space of undifferentiated loose connective tissue (Fig. 4D). Each vas deferens extends towards the lateral junction of the thoracic-abdominal and the oblique flexor

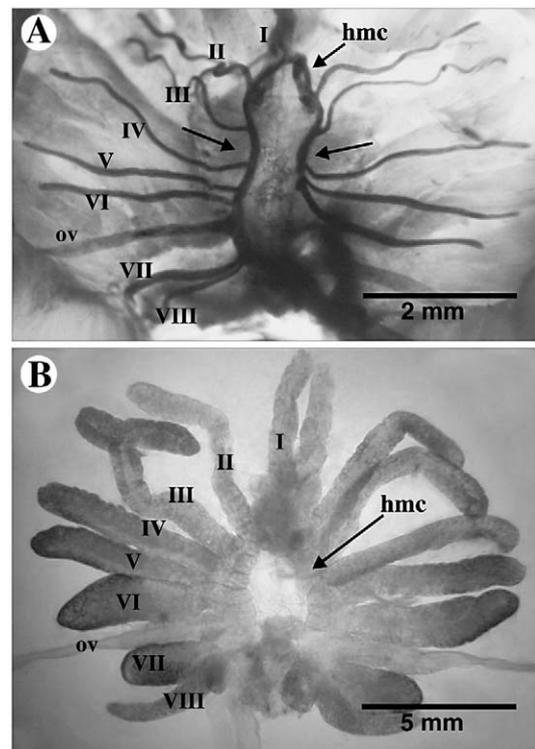


Fig. 3. *Penaeus vannamei*. Ventral view of genital organ in juvenile female PL80 (3 g) showing a bilateral physical space (arrows) that corresponded to the fourth lobe (A). Ventral view of genital organ in adult female (28 g) showing one anterior and seven lateral lobes plus the oviduct. Exo-skeleton and midgut gland were removed. Horseshoe-shape main collector tube (hmc), gonadic lobes (Roman numbers), and oviduct (ov).

abdominal muscles, descending laterally and straightforward as a distal vas deferens that reaches ventrally to the internal region of the base of the fifth pereopod as a primordial terminal ampoule. The vas deferens are filiform, with no distinguishable ejaculator medium bulb and composed of columnar epithelium cells with a lumen that forms a tubular structure and a longitudinal septum that divides the tube into a large chamber lined with epithelium and a smaller, incomplete cavity (Fig. 5). Earlier development of the male vas deferens was clear after PL36, when the anterior region became wider and differentiated into a middle vas deferens that will function as ejaculatory bulbs.

3.3. Early external sex differentiation (PL32 to PL44)

Early external sex differentiation began around PL32; body weight ranged from 180–280 mg and body length from 25–35 mm. Two external characteristics were evident: First, the endopodite emerged as a tiny protuberance with one or two apical setae. Over the following days, the endopodite of females became wider at the external proximal and middle regions and started developing three or four proximal setae along its ridge and maintaining the apical setae. The endopodite of males had one or two proximal and apical setae, which are lost in the following molts and remained as a tubular appendage forming the petasma. Second, the protopodite of the first pair of pleopods had a rectangular articulation in females and the same articulation was notched in the distal region in males. Detailed morphology of these external structures is provided in Campos-Ramos et al. (2006).

3.4. Further external sex differentiation (PL44 to PL48)

Further external sex differentiation began from PL44; body weight ranged from 0.5–0.6 g and body length from 45–50 mm. Females started developing a pair of oblique, sharp ridges in the thelycum that is

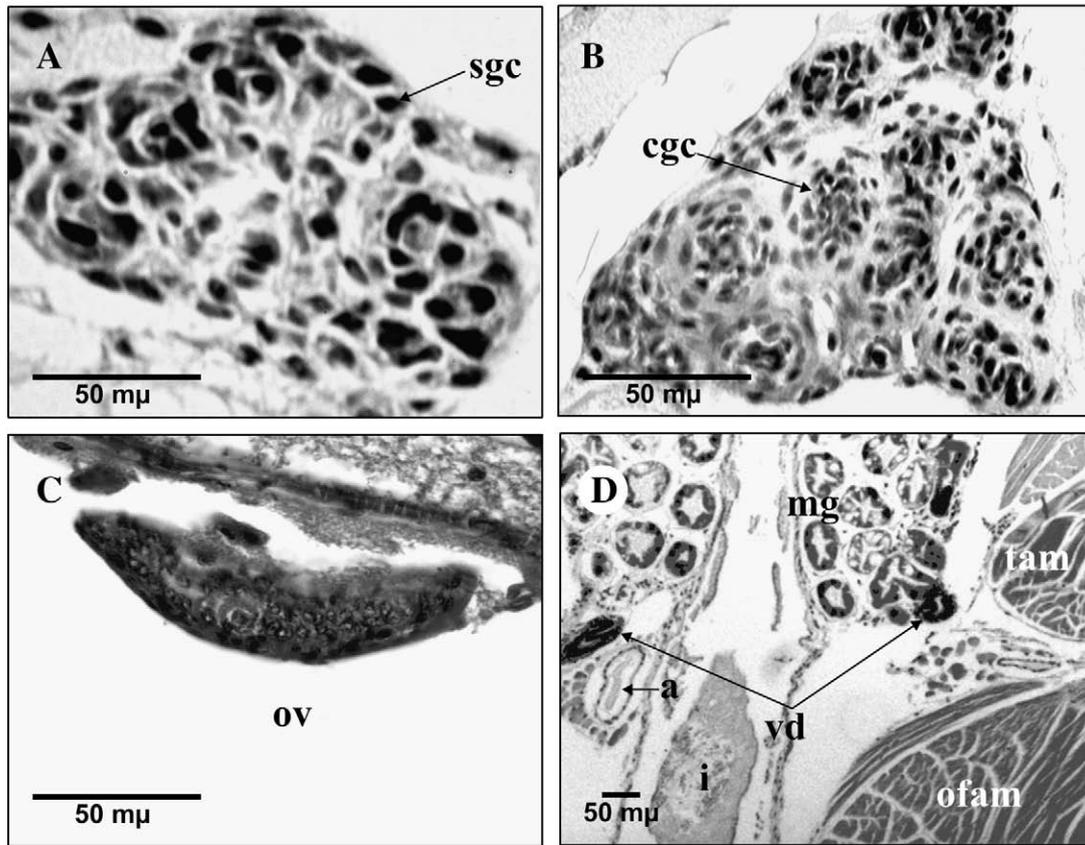


Fig. 4. *Penaeus vannamei*. Longitudinal sections of the gonadic anterior lobe in PL18 (A) and PL28 (B). Cross section of the oviduct located in the postero-lateral side of the midgut gland of a female in PL40 (C). Longitudinal internal section of the posterior cephalothoracic region and the anterior abdominal region of a male in PL32 (D). Aorta (a), semi-arranged gonadic cells (sgc), clustered gonadic cells (cgc), intestine (i), midgut gland (mg), oblique flexor abdominalis muscle (ofam), oviduct (ov), thoraco-abdominis muscle (tam), and vas deferens (vd).

characteristic of this species and males started developing gonopores at the coxa of the fifth pereiopods and the male appendage at the second pair of pleiopods. The morphological detail of these structures is provided in Campos-Ramos et al. (2006).

3.5. Organogenesis of the AG and white adipose tissue (PL48 to PL52)

In males, primordial AG cells appeared by PL48, gathering in line, and apparently held and orientated by a thin fibrous connective tissue at the posterior-external wall of each vas deferens, the side of the vas deferens that faces the oblique flexor abdominal muscle (Fig. 5A). Shortly afterward, by PL52, oval cells accumulated in the area forming the AG tissue (Fig. 5B).

During this time, females and males differentiated loose connective tissue into white adipose tissue filling the cavity in the posterior region of the midgut gland, the thoracic-abdominal muscle, the intestine, and the area around the descending aorta on the left side of the body. The nuclei of these cells are pushed to the periphery because of the fat content in the cytoplasm (Fig. 5).

3.6. Development of germinal epithelium and gonad sex differentiation (PL52 to PL72)

By PL52, the clusters of cells in the gonad differentiated into germinal epithelium. The germinal cells were larger than the original clustered cells and were rounded, forming an oval tubular structure where gonial cells were at the periphery, surrounding the lumen. However, the gonad was still undifferentiated. Secondary gonial cells transformed into primary oocytes that began previtellogenic primary growth by PL68–PL72, which distinguished the female gonad (Fig. 6A) from the male

gonad (Fig. 6B). The differentiation of the gonad occurred when both genders reached a body weight of 1.8–2.2 g and a body length of 70–74 mm. Female oocytes grew larger and formed a pear-like or triangular shape and began filling and expanding the lobes that characterize the ovary. Primary male spermatocytes, which were rounded and tightened, produced spermatids that were transferred into the lumen of the testicular lobe. By this time, the dissection of any independent testicular lobe showed a long and single seminiferous tubule.

3.7. Subsequent development of the vas deferens and the AG

The external appearance of the AG in juvenile shrimp was difficult to observe because it is small and transparent. However, development of an external connective tissue is evident at the wider middle region of the vas deferens, which indicates the location of the putative AG. The connective tissue of the vas deferens also attached along the eighth testicular lobe so that the vas deferens and testis are held to each other in parallel (Fig. 7A). As the male grows, the bilateral anterior vas deferens continues to develop and turns into a huge median and folded vas deferens that takes the form of an inverted U (named “double fixture” by Treece and Yates, 1988). Each of the median vas deferens includes the anterior and posterior ejaculator bulbs (Fig. 7B and C). In preadult and adult shrimp, the AG appears as cords immersed in the connective tissue, connecting the internal distal region of the posterior ejaculator bulb with the eighth testicular lobe (Fig. 8A and B). After dissecting the vas deferens, the AG appears as a slender and compact mass of oval cells with prominent nuclei under histological examination. Detailed morphology of the AG in preadult and adult penaeid shrimp is provided in Charniaux-Cotton and Payen (1985), Alfaro (1994), and Campos-Ramos et al. (2006).

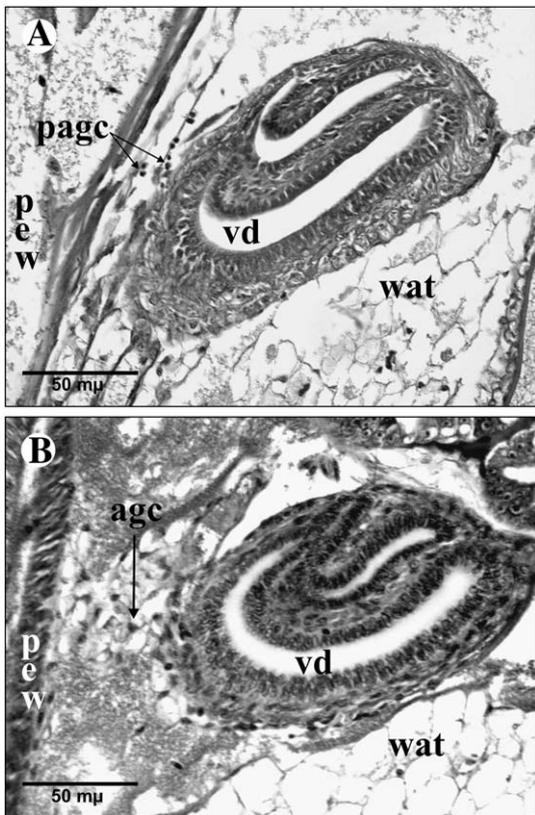


Fig. 5. *Penaeus vannamei*. Cross section of the vas deferens of male in PL44 (A) and in PL52 (B). Primordial androgenic cells (pagc), androgenic gland cells (agc), white adipose tissue (wat), posterior-external wall (pew), and vas deferens (vd).

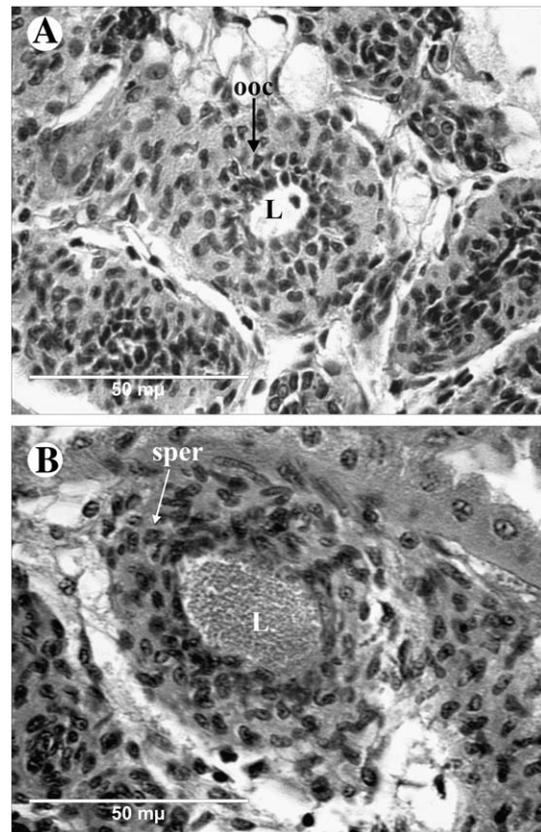


Fig. 6. *Penaeus vannamei*. Cross section of gonad in female (A) and male (B) in PL72. Arrow in (A) shows pear-like or triangular shape oocytes (ooc), and in (B) spermatogonia (sper), around a lumen (Lu).

4. Discussion

The anatomy of the genital organ in male *P. vannamei* matches the description of *P. japonicus* by Chim (1983) and in *P. vannamei* and *P. setiferus* by Chow et al. (1991). However, the female genital organ in *P. vannamei* and, in general, of penaeids described by King (1948) and Dall et al. (1990) does not match what we found. Both genders have a basic anatomy of a bilateral anterior lobe and independent lateral lobes arranged symmetrically on each side of a single collecting tube with an inverted U or horseshoe shape. This structure appears to be unique for penaeid species (Laubier et al., 1983; Chow et al., 1991; this study). However, many other species have not been studied with this detail.

Our description of genital organogenesis concurs, in general, with the few previous investigations of penaeids regarding internal and external morphology and sexual differentiation (Chim, 1983; Laubier et al., 1983; Charniaux-Cotton and Payen, 1985; Chow et al., 1991; Nakamura et al., 1992; Campos-Ramos et al., 2006). The evident separation between the third and fourth lobes in both genders (see Fig. 3A in female and Fig. 2B in male) may suggest that the fourth bilateral lobe forms during the early network of mesodermic cells that give structure of each gonadic lobe. The regression of this lobe is not clear, and the entire genital organ is difficult to analyze during these early stages. Once the gonad of each gender was developed, there was not a difference in gonad development among the ten shrimp sampled, at each PL-stage. From an anatomical point of view, the physical position of the fourth lobe would coincide with the wider and higher transversal axes of the anterior midgut gland. Therefore, if the fourth lobe would develop, it would be higher, and more external than the other lobes. Instead, this lobe regresses, possibly because of the morphology and development of the midgut gland.

It appears that in both *P. japonicus* and *P. vannamei*, the duration that postlarvae remain gender-undifferentiated is remarkably short after

larval metamorphosis. According to Laubier et al. (1983), the gonad first appears as two masses of germ cells associated with somatic cells by PL8. In *P. vannamei*, the bilateral anterior lobe was observed by histological examination in PL12 as the first recognizable gonadal structure. These authors recognized the entire male genital organ by PL11. In contrast, our earliest identification of the female oviduct and filliform male vas deferens was in PL16. Nevertheless, external gender differentiation was delayed until shrimp reached 0.5 g and 45 mm, which is about PL50, which agrees with the development of *P. japonicus* (Nakamura et al., 1992). This stage is represented by and, to some extent, synchronized by the external development of the female thelycum and male gonopores and internally by the appearance of the male AG. The organogenesis of the AG in *P. vannamei* begins at the posterior-lateral region of each vas deferens, as observed in *P. japonicus* (Nakamura et al., 1992). It appears that primordial AG cells differentiated independently from the loose connective tissue, which in turn, differentiated into white adipose tissue around the intestine and midgut gland in both genders. The AG cells may originate from mesoderm cells that formed the connective tissue that holds testicular lobes beneath the pericardium. The AG is immersed within a connective tissue that interconnects the vas deferens and the eighth testicular lobe, which was not previously observed in *P. japonicus* (Charniaux-Cotton and Payen, 1985) or *P. vannamei* (Alfaro, 1994; Campos-Ramos et al., 2006).

According to Charniaux-Cotton and Payen (1985), the clusters of cells gathering at the gonadal lobes are mesoderm cells, whereas oviducts and vas deferens grow from tissue from the genital organ that differentiates into columnar epithelium. By PL52 (0.5 g and 45 mm), these clusters of cells form oval tubular structures where gonial cells gather around a lumen, which indicate a germinal zone. From PL68 to PL72, (around 2 g and 72 mm) shrimp showed early developing oocytes, indicating the time of gonadal differentiation, which is eight days later than the time reported by Nakamura et al. (1992) in *P. japonicus*.

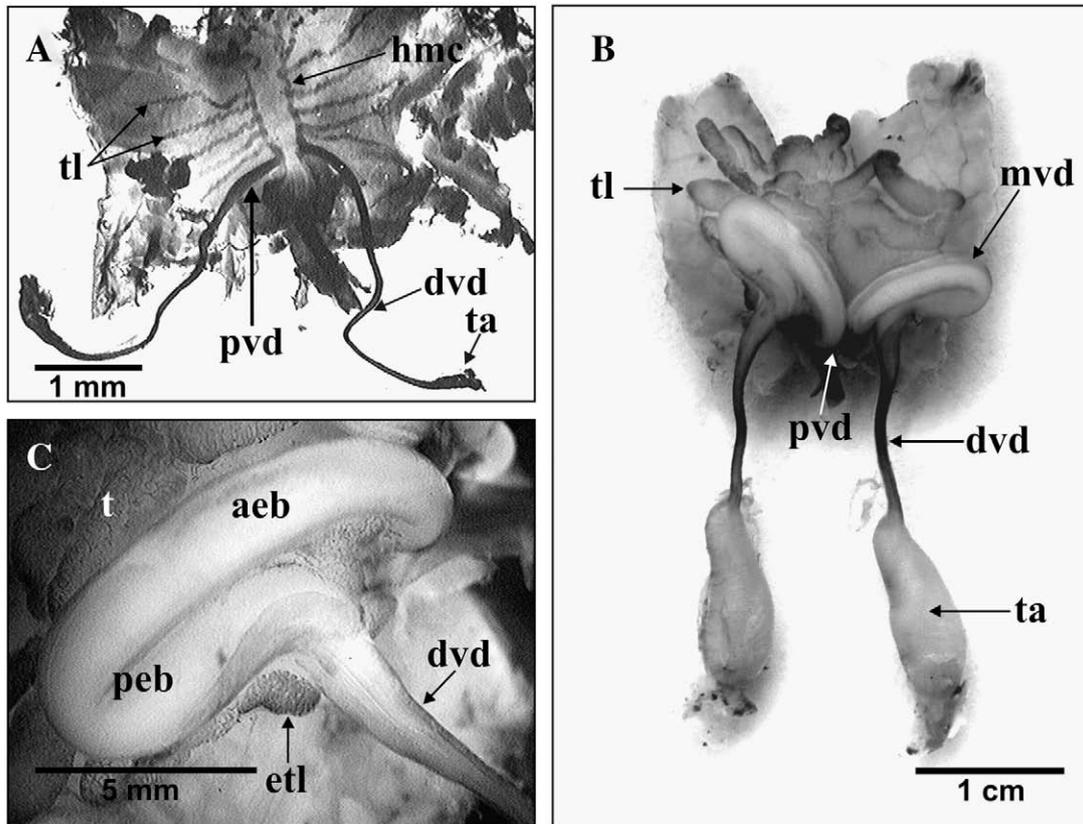


Fig. 7. *Penaeus vannamei*. Ventral view of male genital organ in PL48, 0.5 g (A), and adult (26 g) (B). Detail of vas deferens from B (C). Horseshoe-shape main collector tube (hmc), testicular lobes (tl), anterior ejaculatory bulb (aeb), posterior ejaculatory bulb (peb), eighth testicular lobe (etl), proximal vas deferens (pvd), medium vas deferens (mvd), distal vas deferens (dvd), and terminal ampoule (ta).

In males, spermatogonia gather at the periphery of the testicular lobe and the lumen is filled with secondary spermatocytes and spermatids, as described by King (1948). According to Chow et al. (1991), the gamete production cycle, independent in each seminiferous tubule, includes two distinguishable phases where sustentacular (supporting epithelial) cells play a role in expanding and contracting the lumen to begin a new cycle from primary spermatocytes produced by spermatogonia. Initially, the 'cord phase,' where spermatogenesis is supported by multi-functional sustentacular cells, generates late spermatids; secondarily, the lumen phase,' where sustentacular cells retreat and align to form a cavity where late spermatids are transferred to the posterior vas deferens, where spermiogenesis continues. According to Parnes et al. (2006), males also have reproductive molt cycles. The spermatid–spermatozoa mass is transported along the vas deferens, wrapped, and stored in the terminal ampoule, forming the spermatophore that will be expelled through the gonopores and renewed every molting cycle, if no reproductive behavior occurs.

According to Campos-Ramos et al. (2006), *P. vannamei* possesses a stable genetic sex determination system that is not influenced by environmental conditions. The size of shrimp was a good guide of gender development under the rearing conditions of this study. However, Campos-Ramos et al. (2006) found that external gender differentiation occurs during the same time of development under two temperature conditions at 27 and 32 °C, where shrimp size was variable, and can be delayed under low temperature at 18 °C and unfavorable biological and environmental conditions. Therefore, size must not be a factor to assume a gender development. It is necessary to identify external and internal gender structures, which are resumed in Fig. 1. Although the AG is unique among malacostracans, the evidence from the two species of penaeids that have been studied indicates that this gland is not involved in sex determination or sex differentiation because the gender differentiates earlier, soon after transformation of larvae.

Most likely, the interconnection between the vas deferens and testis seems to be related to spermatogenesis and testis maturation or some regulatory process of ovary inhibition, since gametogenesis begins after the AG is formed. In penaeids, it is still unknown if the AG is involved in the eyestalk–AG–testis endocrine axis, as reported for *C. quadricarinatus* (Khalaila et al., 2002).

In freshwater prawns, the AG induces masculinization and secondary sexual male characteristics and inhibition of vitellogenesis in implanted young females; andrectomy induces feminization (vitellogenesis) in young male prawns. The strategy to induce sex reversal and produce male monosex aquaculture in freshwater prawns, such as *M. rosenbergii*, is well documented in the literature (Nagamine et al., 1980a,b; Sagi et al., 1990; Aflalo et al., 2006). In contrast, Li and Xiang (1997) attempted reversing the gender of *Fenneropenaeus chinensis* (Kishinouye) without success. This unsuccessful experiment may be related to the lack of adequate knowledge of the process of sex differentiation in penaeid shrimp compared to freshwater prawns. It remains unknown whether implantation of the AG and andrectomy in penaeid shrimp will be successful in sex reversal. Certainly, previous studies and our study should be taken into account. We conclude that in both genders of *P. vannamei*, external gender differentiation develops around 15–20 days earlier than gonad differentiation. In theory, when external gender differentiation is observed, any surgical procedure on the vas deferens or an AG implantation in females would compromise the fate of gonad differentiation in *P. vannamei*, which is the same biotechnological sex reversal principle used in *M. rosenbergii*. Pro-insulin-like genes expressed exclusively in the AG of male *C. quadricarinatus* and male *M. rosenbergii* have been recently reported (Manor et al., 2007; Ventura et al., 2009). The discovery of these specific genes may show molecular processes of sex differentiation and may provide clues to innovative strategies for sex reversal techniques in commercially reared freshwater prawns. From a gene regulation perspective, this study may have

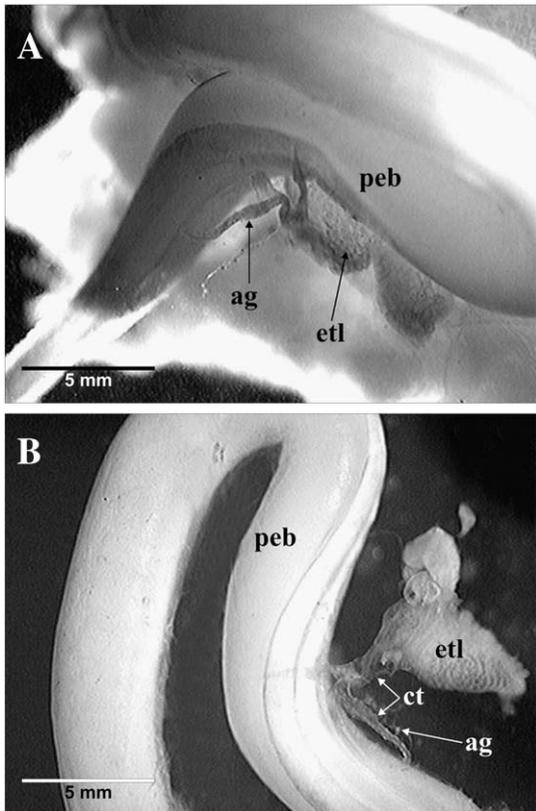


Fig. 8. *Penaeus vannamei*. External anatomy of the medium vas deferens in an adult male is showing the interconnection trough connective tissue (ct) among the distal region of the posterior ejaculatory bulb (peb), the androgenic gland (ag), and the eighth testicular lobe (etl). Lifted posterior ejaculatory bulb (A) dissected with the eighth testicular lobe (B).

implications in commercially reared penaeid shrimp, because it will allow establishing with detail the development, differentiation, and structural changes of the genital organs in further applied molecular aquaculture research.

Acknowledgements

We thank three anonymous reviewers for their comments, suggestions, and corrections to the manuscript. We also thank the shrimp lab "Acuicultura Mahr" for providing postlarvae for this study. Ira Fogel at CIBNOR provided editorial support. This investigation was funded by Centro de Investigaciones Biológicas del Noroeste. R.G.T. received a Consejo Nacional de Ciencia y Tecnología doctoral fellowship.

References

- Alfaro, J., 1994. Ultrastructure of the androgenic gland, spermatogenesis and oogenesis in marine shrimps (Decapoda: Penaeidae). *Rev. Biol. Trop.* 42 (Suppl. 2), 121–129.
- Aflalo, E.D., Hoang, T.T.T., Nguyen, V.H., Lam, Q., Nguyen, D.M., Trinh, Q.S., Raviv, S., Sagi, A., 2006. A novel two-step procedure for mass production of all-male populations of the giant freshwater prawn *Macrobrachium rosenbergii*. *Aquaculture* 256, 468–478.
- Barki, A., Karplus, I., Khalaila, I., Manor, R., Sagi, A., 2003. Male-like behavioral patterns and physiological alterations induced by androgenic gland implantation in female crayfish. *J. Exp. Biol.* 206, 1791–1797.
- Bell, T.A., Lightner, D.V., 1988. *A Handbook of Normal Penaeid Shrimp Histology*. Allen Press, Lawrence, Kansas, USA.
- Campos-Ramos, R., Garza-Torres, R., Guerrero-Tortolero, D.A., Maeda-Martínez, A.M., Obregón-Barboza, H., 2006. Environmental sex determination, external sex differentiation, and structure of the androgenic gland in the Pacific white shrimp *Litopenaeus vannamei* (Boone). *Aquac. Res.* 37, 1583–1593.
- Charniaux-Cotton, H., 1953. Étude du déterminisme des caractères sexuels secondaires par castration chirurgicale et implantation d'ovaire chez un Crustacé Amphipode (*Orchestia gammarella*). *Cr. Hebd. Acad. Sci.* 236, 141–143.
- Charniaux-Cotton, H., 1954. Découverte chez un Crustacé Amphipode (*Orchestia gammarella*) d'une glande endocrine responsable de la différenciation des caractères sexuels primaires et secondaires mâles. *Cr. Hebd. Acad. Sci.* 239, 780–782.

- Charniaux-Cotton, H., 1960. Sex determination. In: Talbot, H.W. (Ed.), *Metabolism and Growth. The Physiology of Crustacea*, vol. I. Academic Press, New York, pp. 441–447.
- Charniaux-Cotton, H., Payen, G., 1985. Sexual differentiation. In: Bliss, D.E., Mantel, L.H. (Eds.), *Integuments, Pigments, and Hormonal Processes. The Biology of Crustacea*, vol. 9. Academic Press, New York, pp. 217–299.
- Chim, L., 1983. La morphogénèse sexuelle, la gamétogénèse et leur contrôle neurohormonal chez la Crevette *Penaeus japonicus* Bate utilisation de l'aspartate transcarbamylase comme indice de croissance de l'appareil génital. Thèse de 3^e cycle, Université Pierre et Marie Curie, Paris VI, 107 pp.
- Chow, S., Sandifer, P.A., 1991. Differences in growth, morphometric traits and male sexual maturity among Pacific white shrimp, *Penaeus vannamei*, from different commercial hatcheries. *Aquaculture* 92, 165–178.
- Chow, S., Dougherty, W.J., Sandifer, P.A., 1991. Unusual testicular lobe system in the white shrimps, *Penaeus setiferus* (Linnaeus, 1761) and *P. vannamei* Boone, 1931 (Decapoda, Penaeidae); a new character for Dendobranchiata? *Crustaceana* 60, 304–318.
- Dall, W., Hill, B.J., Rothlisberg, P.C., Sharples, D.J., 1990. The Biology of the Penaeidae. In: *Advances in Marine Biology*, vol. 27. Academic Press, London.
- Fowler, R.J., Leonard, B.V., 1999. The structure and function of the androgenic gland in *Cherax destructor* (Decapoda: Parastacidae). *Aquaculture* 171, 135–148.
- Howard, D.W., Smith, C.S., 1983. *Histological techniques for marine bivalve mollusks*. NOAA Technical Memorandum NMFS-F/NEC-25, Woods Hole, Massachusetts.
- Khalaila, I., Katz, T., Abdu, U., Yehezkel, G., Sagi, A., 2001. Effects of implantation of hypertrophied androgenic glands on sexual characters and physiology of the reproductive system in the female red claw crayfish, *Cherax quadricarinatus*. *Gen. Comp. Endocrin.* 121, 242–249.
- Khalaila, I., Manor, R., Weil, S., Granot, Y., Keller, R., Sagi, A., 2002. The eyestalk-androgenic gland-testis endocrine axis in the crayfish *Cherax quadricarinatus*. *Gen. Comp. Endocrin.* 127, 147–156.
- King, J.E., 1948. A study of the reproductive organs of the common marine shrimp, *Penaeus setiferus* (Linnaeus). *Biol. Bull.* 94, 244–262.
- Kong, Ch.J., William, E.G., 1988. *Prawn Culture, Scientific and Practical Approach*. Chuson Printing Press, Calasiao, Pangasinan, Philippines. 32 + 323 pp.
- Laubier, A., Chim, L., Payen, G.G., 1983. Morphogénèse sexuelle et régulation hormonale de l'activité génitale chez la crevette *Penaeus japonicus* en élevage. *Actes de colloques-IFREMER* 1, 195–206.
- Li, F., Xiang, J., 1997. Preliminary studies on form, structure and function of androgenic gland in *Penaeus chinensis*. *Chinese Sci. Bull.* 42, 499–503.
- Malecha, S.R., Nevin, P.A., Ha, Ph., Barck, L.E., Lamadrid-Rose, Y., Masuno, S., Hedgecock, D., 1992. Sex-ratios and sex-determination in progeny from crosses of surgically sex-reversed freshwater prawns, *Macrobrachium rosenbergii*. *Aquaculture* 105, 201–218.
- Manor, R., Aflalo, E.D., Segall, C., Azulay, D., Ventura, T., Sagi, A., 2004. Androgenic gland implantation promotes growth and inhibits vitellogenesis in *Cherax quadricarinatus* females held in individual compartments. *Invertebr. Reprod. Dev.* 45, 151–159.
- Manor, R., Weil, S., Oren, S., Glazer, L., Aflalo, E.D., Ventura, T., Chalifa-Caspi, V., Lapidot, M., Sagi, A., 2007. Insulin and gender: an insulin-like gene expressed exclusively in the androgenic gland of the male crayfish. *Gen. Comp. Endocrin.* 150, 326–336.
- Nagamine, C., Knight, A.W., Maggenti, A., Paxman, G., 1980a. Effects of androgenic gland ablation on male primary and secondary sexual characteristics in the Malaysian prawn, *Macrobrachium rosenbergii* (de Man) (Decapoda, Palaemonidae), with first evidence of induced feminization in a nonhermaphroditic decapod. *Gen. Comp. Endocrin.* 41, 423–441.
- Nagamine, C., Knight, A.W., Maggenti, A., Paxman, G., 1980b. Masculinization of female *Macrobrachium rosenbergii* (de Man) (Decapoda, Palaemonidae), by androgenic gland implantation. *Gen. Comp. Endocrin.* 41, 442–457.
- Nakamura, K., Matsuzaki, N., Yonekura, K.I., 1992. Organogenesis of genital organs and androgenic gland in the kuruma prawn. *Nippon Suisan Gakkaishi* 58, 2261–2267.
- Parnes, S., Raviv, S., Scechter, A., Sagi, A., 2006. Males also have their time of the month! Cyclic disposal of old spermatophores, timed by the molt cycle, in a marine shrimp. *J. Exp. Biol.* 209, 4974–4983.
- Pérez-Farfante, I., 1988. Illustrated key to penaeoid shrimps of commerce in the Americas. NOAA Technical Report NMFS 64.
- Pérez-Rostro, C.I., Ramírez, J.L., Ibarra, A.M., 1999. Maternal and cage effects on genetic parameter estimation for Pacific white shrimp *Penaeus vannamei* Boone. *Aquac. Res.* 30, 681–693.
- Sagi, A., Aflalo, E.D., 2005. The androgenic gland and monosex culture of freshwater prawn *Macrobrachium rosenbergii* (De Man): a biotechnological perspective. *Aquac. Res.* 36, 231–237.
- Sagi, A., Cohen, D., 1990. Growth, maturation and progeny of sex-reversed *Macrobrachium rosenbergii* males. *World Aquac.* 21, 87–90.
- Sagi, A., Cohen, D., Milner, Y., 1990. Effect of androgenic gland ablation on morphotypic differentiation and sexual characteristics of male freshwater prawns, *Macrobrachium rosenbergii*. *Gen. Comp. Endocrin.* 77, 15–22.
- Taketomi, Y., Nishikawa, S., 1996. Implantation of androgenic glands into immature female crayfish, *Procambarus clarkii*, with masculinization of sexual characteristics. *J. Crustacean Biol.* 16, 232–239.
- Treece, G.D., Yates, M.E., 1988. *Laboratory Manual for the Culture of Penaeid Shrimp Larvae*. Marine Advisory Service, Sea Grant College Program, Texas A&M University. 75 pp.
- Ventura, T., Manor, R., Aflalo, E.D., Weil, S., Raviv, S., Glazer, L., Sagi, A., 2009. Temporal silencing of an androgenic-gland-specific insuline-like gene affecting phenotype gender differences and spermatogenesis. *Endocrinology* 150, 1278–1286.
- Zhang, L., Yang, Ch., Zhang, Y., Li, L., Zhang, X., Zhang, Q., Xiang, J., 2007. A genetic linkage map of the Pacific white shrimp (*Litopenaeus vannamei*): sex-linked microsatellite markers and high recombination rates. *Genetica* 131, 37–49.