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Histology of the male reproductive system of the prawn *Macrobrachium lanchesteri* (Crustacea: Decapoda)

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Abstract

The testis of the freshwater prawn *Macrobrachium lanchesteri* consists of numerous branching lobules, bound by thin limiting membranes. There is very little inter-lobular space with indistinct blood sinuses and the lobules are filled with germ cells in different stages of development. The different regions of the vas deferens of the prawn *viz.*, anterior vas deferens (AVD), mid vas deferens (MVD) and posterior vas deferens (PVD), indicate structural variations reflecting the specific role of each region, in sperm transport, spermatophore formation and ejaculation. The nature of the glandular epithelium and presence of secretory material in the lumen suggest that packaging of sperms into a spermatophore is initiated in the AVD itself. The epithelial cells of the inner margin of the AVD and MVD are highly columnar, forming a 'typhlosole'-like region. The histology of the MVD differs from that of the AVD in the presence of prominent circular and longitudinal muscle layers. Presence of copious secretory material in the MVD indicates further packaging of the sperms. Extensive muscle layers and reduced secretory activity are characteristic of the PVD. The male hormone-producing tissue, the androgenic gland, is situated mesially in the curvature of the ampulla, the distal end of the PVD. The histology of the gland confirms its secretory role.

Key words: Reproduction, histology, testis, vas deferens, spermatophore, androgenic gland, male *Macrobrachium*, Crustacea.

1. Introduction

Macrobrachium lanchesteri is a commercially important freshwater prawn that inhabits lentic habitats of Asian countries. Despite the worldwide attention on the culture of prawns including that of *M. lanchesteri*, organised prawn culture (extensive or intensive), is still in its infancy. While importance of controlled breeding, involving manipulation of the reproductive process is stressed, structural details of the reproductive system of prawns is not well understood[1,2]. This basic information is imperative in any attempt to control the reproductive process of prawns. To fulfill this lacuna, morphology of the reproductive systems and sexual dimorphic features of *M. lanchesteri* were described earlier[3]. The present paper details the histology of the male reproductive system of *M. lanchesteri*.

2. Material and methods

Males of *Macrobrachium lanchesteri* (de Man) used during the present studies, were

collected from the freshwater habitat, the Hulimau tank (Bangalore, South India). Histological analyses of the reproductive system of the prawn were made on individuals measuring 30 mm total length and above. The specimens were dissected and their testes, vasa deferentia were excised and quickly fixed in Bouin's fluid. They were embedded in paraffin (58–60°C), sectioned (at 4 to 6 μ) and stained following standard light microscopic procedures[4,5] and examined under a Leitz microscope.

For electron microscopic studies, small pieces of the vas deferens were fixed in ice-cold (4°C) 3% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.4) for 3–4 h. Tissues were repeatedly washed in buffer and post-fixed in 1% cold osmium tetroxide for 1–2 h, dehydrated in concentrations of ethanol and propylene oxide and embedded in Agar 100 (Agar Aids, U.K.)[6]. Ultra thin sections were cut on an LKB-300 microtome and after staining with uranyl acetate and lead citrate, were examined using a transmission electron microscope (Model: Phillips 300).

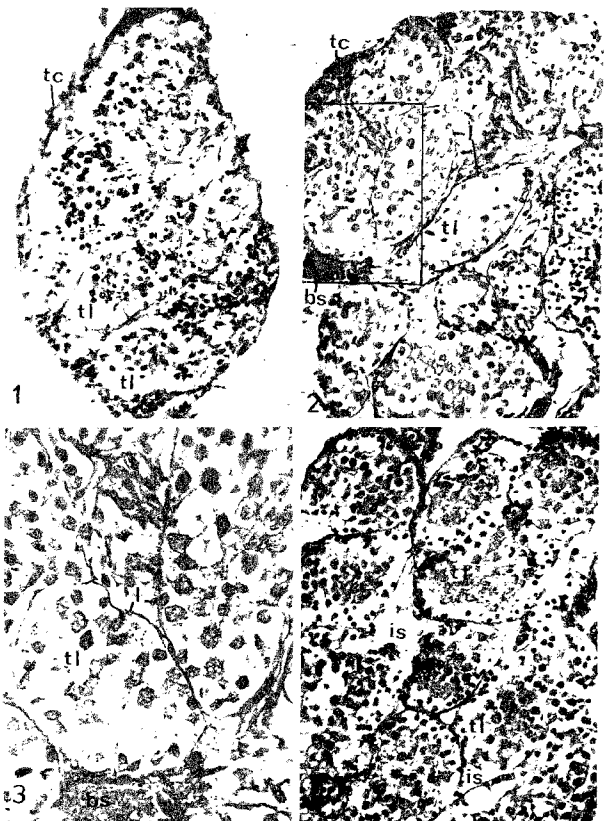
3. Results and discussion

3.1. *Testis*

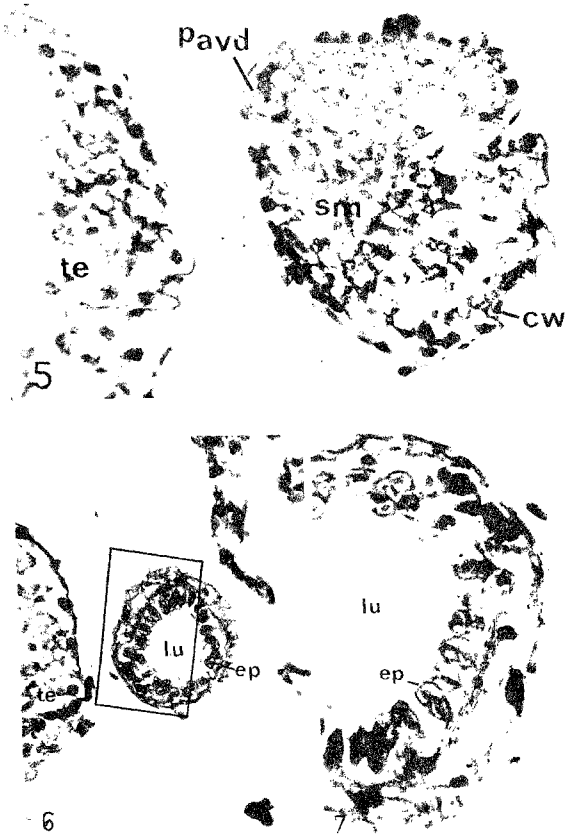
The testis of *Macrobrachium lanchesteri* is enclosed in a connective tissue capsule and is made up of numerous branching lobules, varying in their diameter (average 136.13 ± 18.48 μ: figs. 1–4). The testicular lobules are closely packed, with very little inter-lobular space. The lobules are separated from one another by thin limiting membranes. At some places, the lobules are ensheathed in fairly thick connective tissue. Here and there, in the inter-lobular spaces, rather indistinct blood sinuses can also be seen (fig. 3). Sometimes adjacent lobules are interconnected. This, rather complex and irregular lobular arrangement of the testis of *M. lanchesteri* differs from that of the simple tubular type as reported in other natantians (*Caridina laevis*[2]; *Crangon septemspinosa*[7]) as well as from the spermatogenic cysts/tubules of reptantians (*Orconectes limosus*[8]; *Potamon koolooense*[9]). However, the lobular arrangement, as observed presently, bears a closer resemblance to that reported for another Palaemonid, *M. rosenbergii*[10]. Each testicular lobule of *M. lanchesteri* was observed to be filled with germ cells in different stages of development, without conforming to any particular pattern. Structurally, there was not much variation in the testis in relation to the size of the prawn. Males of a size of even 30 mm TL exhibited marked spermatogenic activity comparable to that of higher size classes (figs. 1–4). This has also been confirmed by the gonadosomatic indices[11]. In comparison to this, females of the species mature only around 37 mm TL[12].

3.2. *Vas deferens*

The different regions of the vas deferens of *M. lanchesteri* indicate variations in their histology, perhaps reflecting the specific role of each region in sperm transport and spermatophore formation.



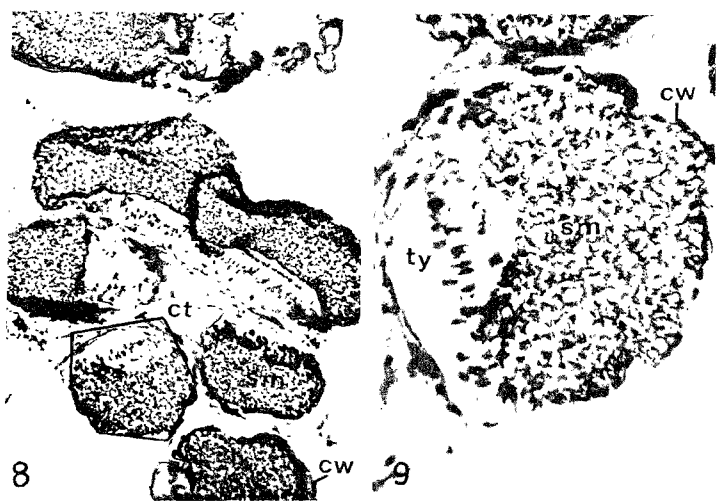
FIGS 1–4. (1) Histology of the testis (in size range) of: *Macrobrachium lanchesteri*, 30–35 mm TL(T.S.) ×250; (2) the prawn : 35–40 mm TL(T.S.)×250; (3) magnifed view of the inset marked in fig. 2×675; (4) the prawn: ≥ 40 mm TL(T.S.)×250. Note the presence of more than one type of cells in each testicular lobule. bs: blood sinus; l: limiting membrane of the testicular lobule; is: interlobular space; tc: testicular capsule, and tl: testicular lobule.



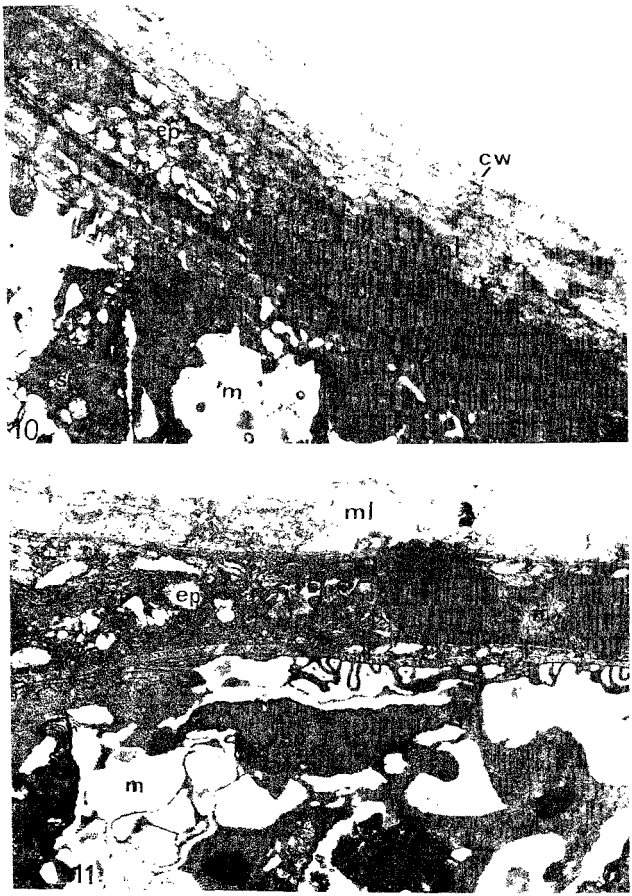
FIGS 5–7. (5) *Macrobrachium lanchesteri*: Transverse section of the proximal portion of the anterior vas deferens (PAVD) close to the testis. Note the sperm mass occupying the entire lumen × 425; (6) same as above but devoid of sperm mass; before the coils of the anterior vas deferens. × 600; (7) magnified portion of the inset marked in fig. 6 giving the histological details. × 1600. cw: connective tissue wall; ep: epithelium; lu: lumen and sm: sperm mass.

3.2.1. *Anterior vas deferens (AVD)*: Figures 5 to 7 represent the histological details of the simple, tubular proximal part of the AVD. Histologically, the portion of the duct nearer the testis, does not differ much from that of the testis (fig. 5). Beyond this region, it differentiates into a simple glandular duct (fig. 6). In transverse sections, an outer thick connective tissue wall (approx. 13 μ in thickness) surrounding a 2–3 layered epithelium is evident. The epithelium is made up of more or less spherical cells (each measuring approx. 13.75 μ in height) with prominent oval nucleii (measuring 7.5 μ at its longer axis; fig. 7). The secretory function of this epithelium is evident.

The histology of the coiled portion of the AVD (fig. 8) differs from that of the above. The outermost layer of this region of AVD becomes more prominent with the addition of a thin muscle layer, below the connective tissue (figs. 9, 10). The inner glandular epithelial layer becomes more prominent. The cells of this layer, towards the inner margin of the coils are formed into a highly columnar 'typhlosole'-like region. The cells of this region are taller than that of the rest of the epithelium (57.25 μ) with prominent oval nuclei (11.88 μ), projecting towards the interior of the lumen (fig. 9). The presence of such a 'typhlosole'-like ingrowth in the vas deferens has also been reported in an atyid prawn[2]. The lumen of the AVD is filled with sperms and secretory material (fig. 11). The



FIGS. 8–9. (8). Histology of the coiled portion of the anterior vas deferens of *Macrobrachium lanchesteri*. × 150; (9) magnified portion of the inset marked in fig. 8. × 560. Note the sperm mass occupying the entire lumen. ct: connective tissue binding the different coils of the anterior vas deferens; cw: connective tissue wall; sm: sperm mass and ty: 'typhlosole'-like ingrowth.



FIGS 10–11. Electron micrograph indicating the structure of a portion of anterior vas deferens of *M. lanchesteri* (10). ×5600; (11). ×6000. cw: connective tissue wall; ep: epithelium; m: matrix of secretions binding the sperms; ml: muscle layer; n: nucleus and sp: sperm.

nuclei of the epithelial cells have electron dense material scattered in the homogeneous chromatin. The cells have numerous mitochondria and abundant endoplasmic reticulum. Large vacuoles are seen distributed all over the cell (fig. 12). The secretions of these cells appear to be in the form of compact network, with fine granulations. These observations on the secretory nature of the duct with sperms suggest that packaging of sperms into a spermatophore is initiated in the AVD itself. While there are no comparable reports on other natantian decapods, elaboration of secretory material in the proximal segment of the vas deferens has been shown in some copepods[13-15].

3.2.2. *Mid vas deferens* (*MVD*): Figure 13 represents the transverse section of the MVD.

The histology differs from that of the AVD in the presence of (a) prominent outer circular and inner longitudinal muscle layers (fig. 14), (b) a columnar epithelium made up of closely packed glandular cells and (c) presence of copious secretory material. The 'typhlosole'-like ingrowth continues the extent of MVD and is comparable to that of the AVD (fig. 15). The inner longitudinal muscle layer surrounding the glandular epithelium

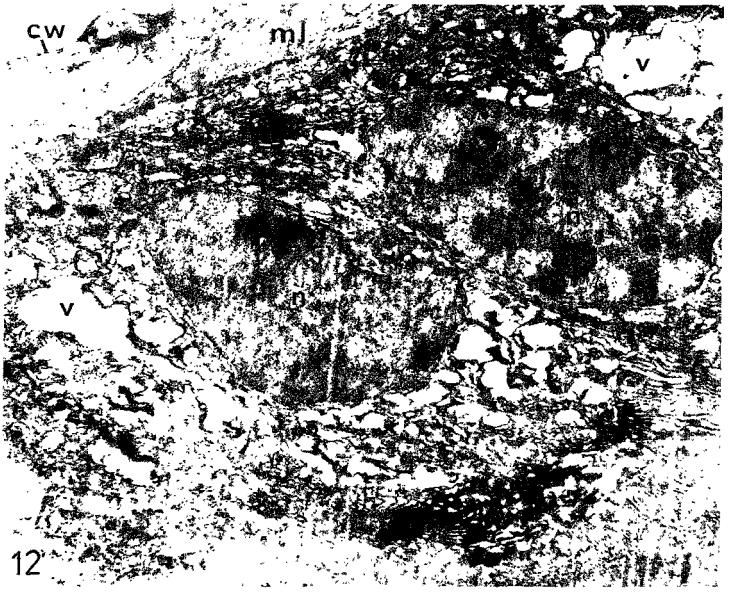
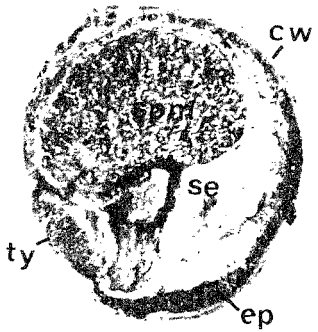


FIG. 12. Ultrastructure of the glandular epithelial cells of anterior vas deferens of *M. lanchesteri*. ×3600. cw: connective tissue wall; ml: muscle layer; n: nucleus and v: vacuole.



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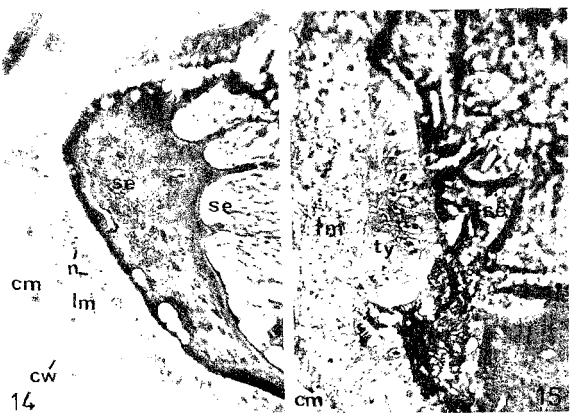
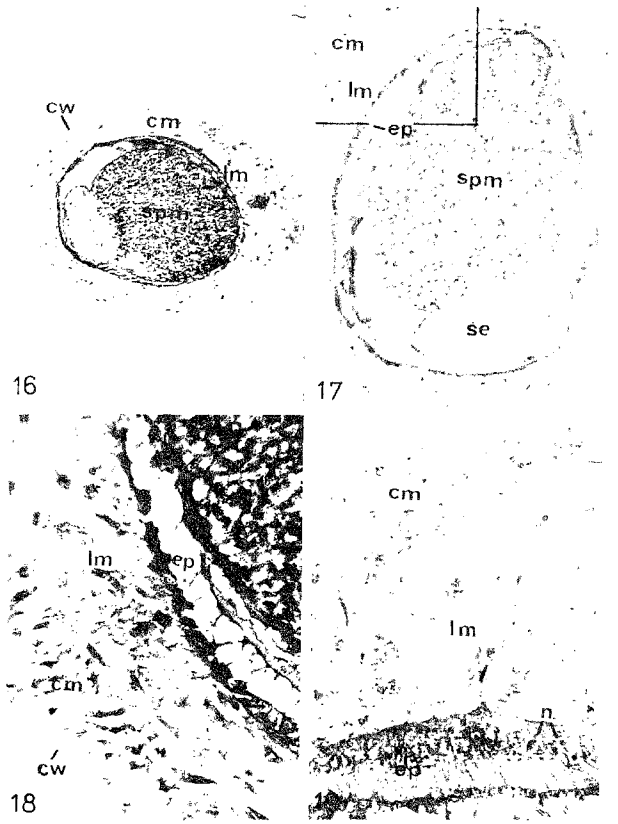
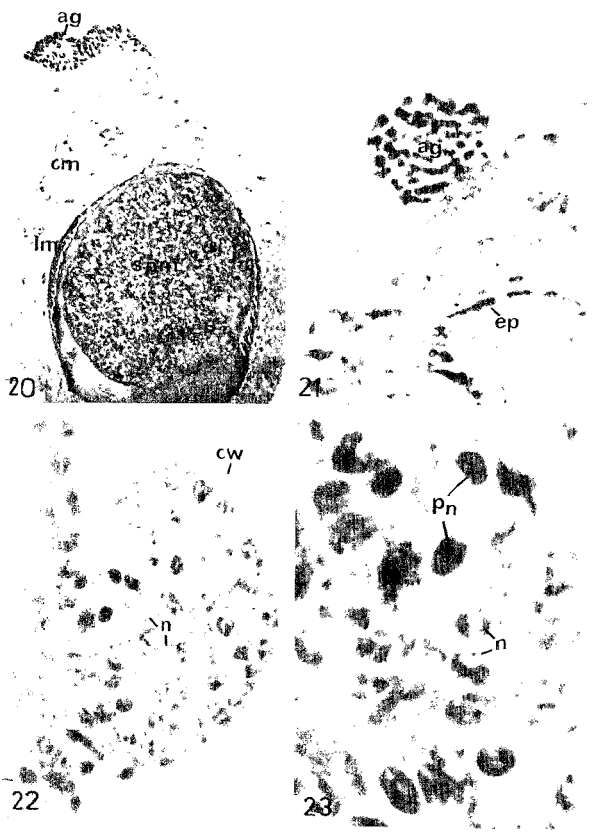


FIGS 13–15. (13) Transverse section of the mid vas deferens of *M. lanchesteri* ×200; (14) in another region. Note the presence of copious secretions filling up the lumen. ×550; and (15) a magnified portion of the mid vas deferens indicating the histological details. ×660. cm: circular muscles; cw: connective tissue wall; lm: longitudinal muscles; n: nucleus; se: secretions; spm: spermatophore and ty: 'typhlosole'-like ingrowth.



FIGS 16–19. (16) Transverse section of the posterior vas deferens of *M. lanchesteri*. Note the absence of 'typhlosole'-like ingrowth. ×175; (17) transverse section of the distal portion of the ampulla of the posterior vas deferens. ×200; (18) magnified view of the inset marked in fig. 17. Note the increased thickness of the muscle layers and presence of smaller nuclei situated at the basal lamina of the epithelium. ×700; and (19) transverse section of the posterior vas deferens showing the histology of a portion of the wall. ×560. cm: circular muscles; cw: connective tissue wall; ep: epithelium; lm: longitudinal muscles; n: nucleus; se: secretions; spm: spermatophore and v: vacuoles.



FIGS 20–23. (20) Transverse section of the ampulla of the posterior vas deferens with the androgenic gland of *M. lanchesteri*. ×190; (21) transverse section of the posterior vas deferens with the androgenic gland — a portion magnified. ×425; (22) histology of the androgenic gland (T.S.). ×825; and (23) a magnified portion of the androgenic gland showing the cellular details. ×2000. ag: androgenic gland; cm: circular muscles; cw: connective tissue wall; ep: epithelium; lm: longitudinal muscles; n: nucleus; pn: pycnotic nuclei and spm: spermatophore.

is thicker (10 μ) than the outer circular muscle layer (7.5 μ). The cells of the epithelium, at the 'typhlosole'-like ingrowths, are larger (50.0 μ) than elsewhere. These cells have large oval nuclei (8.13 μ) and the free ends have amoeboid appearance. A few vacuoles are also observed inside the cells, indicating the ability of these cells to secrete the sperm binding substance.

Two types of secretions appear to be produced by the glandular epithelial cells of the vas deferens (also referred to as 'spermiductal glands'[16]), which surround and package the sperms. As a result of intense secretory activity, the sperm mass gets pushed to one side of the mid vas deferens, rest of the lumen being filled by the secretions. At this stage, in a transverse section, the sperm mass roughly has an oval shape, a situation reported in another natantian *Caridina laevis*[2]. The secretions in the lumen of the MVD and those immediately surrounding the sperm mass are in the form of fibrillar network, as observed in the AVD (fig. 11). As a result, a further packaging of sperms into a compact spermatophore is evident in the MVD. Another type of secretion surrounding the sperm mass, adjoining the epithelial layer, is homogeneously granulated, staining bright pink with eosin. This secretion appears not only to surround the sperm mass but also to infiltrate into the mass and bind it (fig. 14). While the presence of two types of secretions is evident, a discussion on the nature of these secretions, can be undertaken only after further histochemical studies.

3.2.3. *Posterior vas deferens (PVD)*: The structure of the PVD is more or less comparable to that of the MVD. However, it differs in the presence of a highly muscular wall and nature of the epithelium. The outer circular muscle layer of the PVD measures approximately 25.0 μ in thickness, and the inner longitudinal muscle layer measures 30.0 μ (figs. 16–19). The epithelial cell layer is approximately 11.25 μ in thickness, cells bearing large basal nuclei with secretory granules and vacuoles (fig. 19). The 'typhlosole'-like region which was prominent in AVD and MVD, becomes less and less conspicuous in the PVD. As observed in the MVD, the two types of secretions are clearly distinguishable in the PVD. The outer homogenous granular secretions of the PVD surround the inner tightly packed sperm mass, forming more or less rigid coat of the sperm mass (fig. 17). The epithelial cells at the distal end of the PVD seem to have less secretory activity, which is evident by the presence of smaller nuclei situated almost at the basal lamina of the epithelium and devoid of any prominent cell inclusions (fig. 18).

3.3. *Androgenic gland*

The androgenic gland of *M. lanchesteri* is connected with the PVD by connective tissue (figs. 20, 21). The gland is compact and is enclosed in a thin connective tissue capsule. The gland consists of cells with indistinct cell boundaries. The arrangement of the cells of the androgenic gland of *M. lanchesteri* bears a close resemblance with those of *Orchestia* and *Lysmata* species[17] and also of *M. rosenbergii*[18] and differs from the simple cellular strands of *Cancer* and *Carcinus* species and from the tubular arrangement in *Maja* and

Gecarcinus species[17]. As in most of the malacostracans, the nuclei of the androgenic cells of *M. lanchesteri* are ovoid, measuring 5–10 μ in diameter and are endowed with dense chromatin (figs. 22, 23). The nuclei stain purple with haematoxylin. In certain regions of the gland, cells have darkly stained irregular pycnotic nuclei and small quantities of cytoplasm, resembling that of the 'degenerated areas' reported in the androgenic glands of certain malacostracans[17]. These observations suggest that the mode of secretion in the androgenic gland may be of a holocrine nature as claimed by Charniaux-Cotton *et al*[17]. While a conclusive endocrine role has been ascribed to the androgenic gland of *M. rosenbergii* and other malacostracans[10,17–20], the nature of tissue in *M. lanchesteri* suggests that it has an important role in the physiology of reproduction of the male.

References

1. PATWARDHAN, S. S. — *Palaemon (The Indian river prawn)*. In *The Indian zoological memoirs on Indian animal types* (Bahl, K. N., Ed.), Lucknow Publishing House, Lucknow, 1937, p. 100.
2. PILLAI, R. S. — Studies on the shrimp *Caridina laevis* (Heller). II. The reproductive system, *J. Mar. Biol. Ass. India*, 1960, **2**, 226–236.
3. RAO, CH. N., KATRE SHAKUNTALA AND RAVICHANDRA REDDY, S. — Morphology of the reproductive system of the freshwater prawn *Macrobrachium lanchesteri* (de Man), *Biol. Bull. India*, 1985, **7**, 31–37.
4. HUMASON, G. L. — *Animal tissue techniques*, W. H. Freeman & Co., San Francisco, 1972, p. 641.
5. PEARSE, A. G. E. — *Histochemistry: Theoretical and applied*, Vol. I, Churchill Livingstone, Edinburgh, 1977, p. 759.
6. WEAKLEY, B. S. — *A beginners handbook in biological electron microscopy*, Northumberland Press Ltd, Gateshead, 1972, p. 228.
7. ARSENAULT, A. L., CLATTENBURG, R. E. AND OPENSE, P. H. — Spermiogenesis in the shrimp, *Crangon septemspinosa*, Say, *Can. J. Zool.*, 1979, **57**, 486–498.
8. WEILGUS-SERAFINSKA, E. — Morphological and histological changes in the male gonad of the American crayfish *Orconectes limosus* (Rafinesque) in the annual cycle, *Acta Biologica Cracoviensia, Ser: Zoologia*, 1976, **19**, 89–105.
9. JOSHI, P. C. AND KHANNA, S. S. — Structure and seasonal changes in the testes of a freshwater crab, *Potamon koolooense* (Rathbun), *Proc. Indian Acad. Sci. (Anim. Sci.)*, 1982, **91**, 439–450.
10. NAGAMINE, C., KNIGHT, A. W., MAGGENTI, A. AND PAXMAN, G. — Effects of androgenic gland ablation on male primary and secondary sexual characteristics in the Malaysian prawn, *Macrobrachium rosenbergii* (de Man) (Crustacea: Palaemonidae), with first evidence of induced feminization in a nonhermophroditic decapod, *Gen. Comp. Endocr.*, 1980, **41**, 423–441.
11. RAO, CH. N., KATRE SHAKUNTALA AND RAVICHANDRA REDDY, S. — Further observations on the annual breeding pattern of the freshwater prawn *Macrobrachium lanchesteri* (de Man) (Natantia: Palaemonidae), (under preparation).
12. RAO, CH. N., KATRE SHAKUNTALA AND RAVICHANDRA REDDY, S. — Annual reproductive cycle and breeding pattern in the freshwater prawn *Macrobrachium lanchesteri* (de Man) (Natantia: Palaemonidae), (under preparation).

13. GHARAGOZLOV-VAN GINNEKEN, I. D. — Sécrétion et organisation de la paroi stratifieé du spermatophore chez quelques copépodes: Ultrastructure et cytochimie, *Cytobiologie*, 1978, **18**, 231–243.

14. GHARAGOZLOV-VAN GINNEKEN, I. D. AND POCHON-MASSON, J. — Infrastructure nature et formation des grains de sécrétion dans le canal deferent de *Tisbe holothuriae* (Copépode: Harpacticoide), *Arch. Biol. (Bruxelles)*, 1979, **90**, 59–81.

15. BLADES, M. F. AND YOUNGBLUTH, M. J. — Ultrastructure of the male reproductive system and spermatophore formation in *Labidocera aestiva* (Crustacea: Decapoda), *Zoomorphology*, 1981, **98**, 1–21.

16. ADIYODI, K. G. AND ADIYODI, R. G. — Morphology and cytology of the accessory sex glands in invertebrates, *Int. Rev. Cytol.*, 1975, **43**, 353–398.

17. CHARNIAUX-COTTON, H., ZERBIB, C. AND MEUSY, J. J. — Monographic de la glande androgène des crustacès superieurs, *Crustaceana*, 1966, **10**, 113–136.

18. NAGAMINE, C., KNOGHT, A. W., MAGGENTI, A. AND PAXMAN, G. — Masculinization of female *Macrobrachium rosenbergii* (de Man) (Decapoda: Palaemonidae) by androgenic gland implantation, *Gen. Comp. Endocr.*, 1980, **41**, 442–457.

19. CHARNIAUX-COTTON, H. — Sex determination. In *The physiology of crustacea*, Vol. I. (Waterman, T. H., Ed.), Academic Press, New York, 1960, p. 670.

20. CHARNIAUX-COTTON, H. — Androgenic gland of crustaceans, *Gen. Comp. Endocr. (Suppl.)*, 1962, **1**, 241–247.