The functional anatomy of the male reproductive system in *Penaeus indicus*

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Received 23 April 1986; accepted 29 May 1987

A histological reconstruction of the main anatomical features of the male reproductive system in *Penaeus indicus* is presented. The passage of spermatozoa and associated secretions from the testis to the terminal ampoule is described, as is the formation of the spermatophoric mass. The origins and possible functions of elements making up the spermatophoric mass are discussed with reference to their destination in the inseminated thelycum.

'n Histologiese rekonstruksie van die belangrikste anatomiese eienskappe van die manlike voorplantingstelsel van *Penaeus indicus* word beskryf. Die pad van die spermatozoa en verwante sekresies vanaf die testis tot by die terminale ampul, asook die vorming van die spermatofoormassa, word beskryf. Die oorsprong en moontlike funksies van elemente wat verantwoordelik is vir die vorming van die spermatofoormassa, word bespreek met verwysing na hul bestemming in die bevrugte thelycum.

Introduction

Penaeus indicus is the most important commercial nearshore prawn species on the South African coast (Champion 1970; de Freitas 1980). As such it has been the subject of local biological, as well as culture, studies (Champion 1985). An aspect of this research which has received emphasis, is the reproductive biology (Joubert & Davies 1966; Champion 1970; de Freitas 1980; Emmerson 1980). Whereas the reproductive development of the female is reasonably well understood, determinations of maturity in male penaeids in general, are problematic (Cummings 1961; Hall 1962; Cheung 1963).

One of the criteria which has been used to determine male maturity is the anatomy of the reproductive organs (King 1948; Shaikhmahmud & Tembe 1958; Subrahmanyam 1965; Tuma 1967). In this regard, Subrahmanyam (1965) provides the only relevant study on *P. indicus* but his account was found to warrant clarification. Apart from questions of interpretation, Subrahmanyam (1965) does not refer to important features which are an integral part of the male reproductive system. Similarly, studies on other species such as *Penaeus setiferus* (King 1948), *Parapenaeopsis stylifera* (Shaikmahmud & Tembe 1958) and *Penaeus merguiensis* (Tuma 1967), while providing a sound background to the investigation, do not describe elements which are apparent in *P. indicus*.

The purpose of this submission is to contribute to a better understanding of the internal structure of the male reproductive system in *P. indicus* and to suggest the functions of the more obvious reproductive products. The cytology of the different tissues and glands was not investigated.

Methods

Material selected for histological study was dissected from live specimens and preserved in Bouin's solution. Serial transverse sections 5 – 8 μm thick were stained with either Heidenhain's iron-haematoxylin or Erlich's haematoxylin and eosin. They were then inspected by light microscopy. The testis, vas deferens, terminal ampoule and extruded spermatophoric mass were investigated in four males ranging in size from 28,5 mm to 39,6 mm carapace length (CL), or 146 mm to 179 mm total length (TL). The inseminated thelycum of a 40 mm CL (178 mm TL) female was also studied.

Difficulty was experienced in obtaining sections free of distortion, as was found by King (1948) and Shaikhmahmud & Tembe (1958). Photographs of these sections are therefore interpreted with the aid of scale diagrams.

Gross anatomy of the internal sex organs

A schematic diagram of the male reproductive system is shown in Figure 1, together with the approximate positions of nine successive transverse sections used to illustrate the internal organization.

The paired testes lie dorsolaterally in the thoracic cavity, one on each side of the cardiac region. The testes are subdivided into a number of lobes. Each testis gives rise to a vas deferens, the distal portion of which penetrates the lateral cephalothoracic musculature and terminates ventrally in a bulbous terminal ampoule. The terminal ampoules are situated medially in the coxae of the fifth pair of pereiopods. They are ventrally enclosed by a fine, transparent membrane through which they are apparent as white spherical bodies, once developed. When mating takes place, the contents of the terminal ampoules are simultaneously extruded and deposited as a combined spermatophoric mass in the thelycum of the female.

King (1948) characterizes the vas deferens in *P. setiferus* as having four parts: a proximal region connected to the testis, followed by a dilated medial section which is recurved and leads to a narrow, tubular, distal section which terminates in an expanded spherical ampoule. The same arrangement is evident in *P. indicus* (Figure 1). However, Subrahmanyam (1965) in his study of *P. indicus*, differentiates the proximal and recurved medial portions from the rest, referring to them as the tubular portion. He thus limits the vas deferens to the

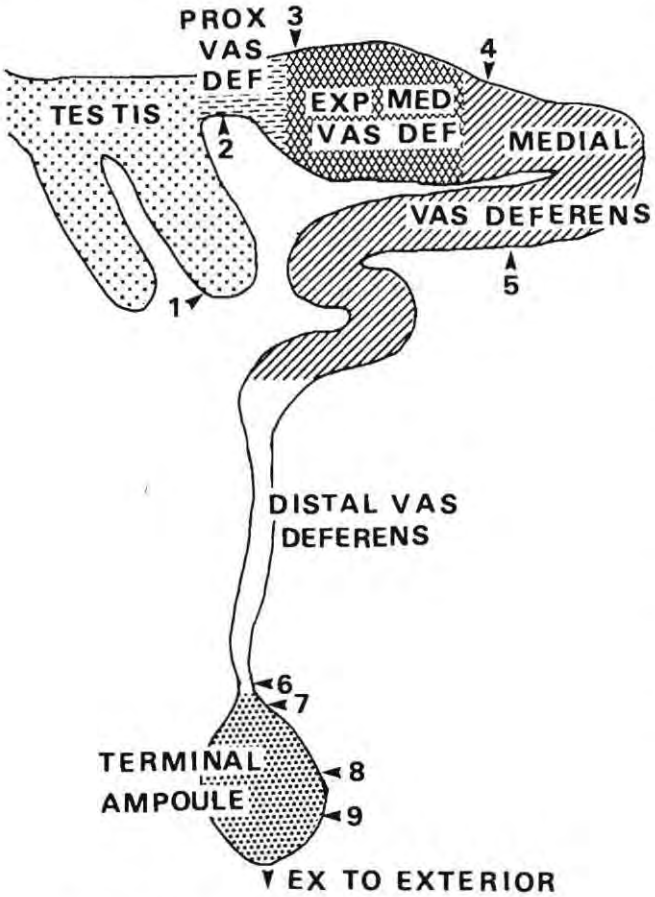

Figure 1 Schematic diagram of the internal male reproductive system of *Penaeus indicus* (not to scale). Pointers 1 to 9 show approximate positions of transverse sections featured in Figures 2.1 to 2.9. PROX VAS DEF = proximal vas deferens; EXP MED VAS DEF = expanded medial vas deferens; EX TO EXTERIOR = zone of extrusion to exterior.

narrow distal portion and terminal ampoule. His rationale is that generative tissue, reflecting spermatogenesis, occurs in the recurved section up to a specimen size of 140 mm TL. Consequently its function is considered to be analogous to that of the testis. He reports that once 170 mm TL is attained, the generative function is no longer evident and the recurved section retrogresses to serving as a conduit for mature spermatozoa, as in the conventional vas deferens.

In the present study, which was limited to specimens of 146 mm TL and larger, no evidence of the generative tissue referred to by Subrahmanyam (1965) could be found. The terminology used by King (1948) for *P. setiferus*, Shaikhmahmud & Tembe (1958) for *P. stylifera* and Tuma (1967) for *P. merguiensis*, was found to be appropriate.

Testis and vasa efferentia

Cross sections through lobes of the testis revealed the different stages of spermatogenesis, from spermatogonia with a diameter of approximately 14 μm, to spermatids and spermatozoa of 3 – 4 μm. The spermatogenic cells are densely packed in clusters, bordered by interstitial connective tissue (Figure 2.1A, INT CON TIS). Subrahmanyam (1965) refers to these as follicles, whereas Shaikhmahmud & Tembe (1958) call them lobules. Observation, however, strongly supports King's (1948) interpretation that they represent a mass of highly convoluted seminiferous tubules (Figure 2.1A, SEM TUB). These tubules give rise to vasa efferentia, one of which is seen at the periphery of a testis lobe in Figure 2.1. The vas efferens consists of an epithelium of glandular cells (SEC EP) and an outer layer of connective tissue (Figure 2.1B), enclosing a lumen which is densely packed with spermatozoa (SP). The glandular cells are presumed to secrete seminal fluid to facilitate both nutrition of the gametes and their movement down the tube. Subrahmanyam (1965) does not comment on these structures though Shaikhmahmud & Tembe (1958) referred to them in *P stylifera*. The vasa efferentia coalesce, ultimately feeding into the vas deferens which leaves the testis.

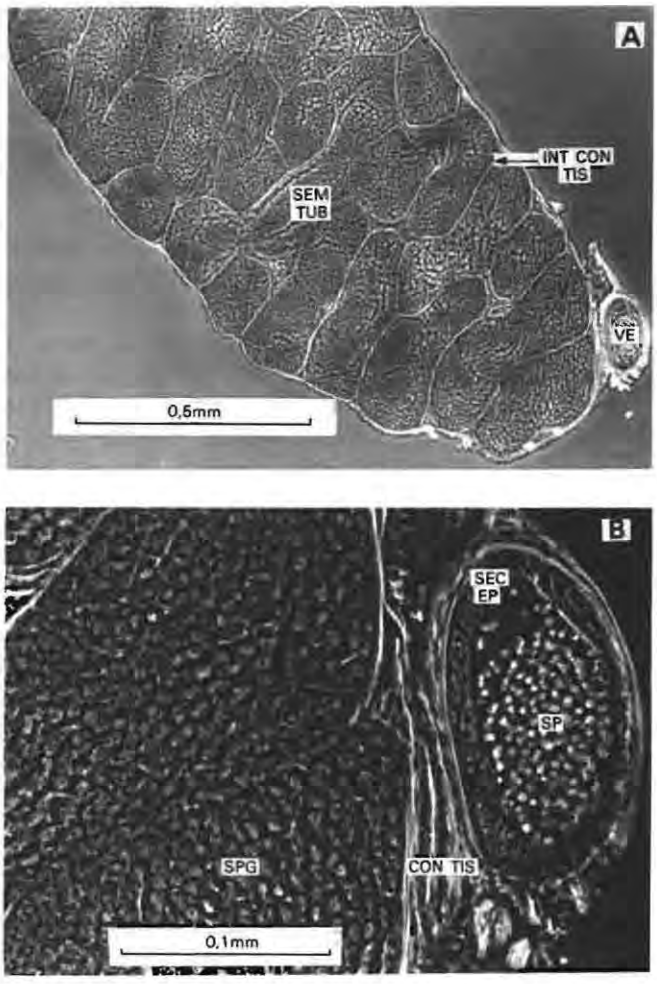

Figure 2.1 Photographs of a transverse section through a lobe of the testis of *P. indicus* showing A, the relative size and position of the vas efferens and B, the internal detail of the vas efferens. CON TIS = connective tissue; INT CON TIS = interstitial connective tissue; SEC EP = secretory epithelium; SEM TUB = seminiferous tubule; SP = spermatozoa; SPG = spermatogonia; VE = vas efferens.

Proximal vas deferens

Close to its emergence from the testis, the proximal part of the vas deferens appeared as a grouping of three closely associated channels, one of them much larger than the other two (Figure 2.2). Each channel is surrounded by a thick layer of secretory cells (SEC EP) which in turn are enclosed by a layer of connective tissue (CON TIS) comprising collagen-like fibres.

The lumen of the largest tube (CH3) shows dense aggregates of spermatozoa (SP) surrounded by a secretion (SM) given off from its epithelium. The contents of the other two channels (CH1, CH2) could not be determined.

Progressing towards the medial vas deferens, the dimensions of the tubes become greatly expanded. Serial sections also suggest that the two smaller channels become progressively wider until they merge more or less at the commencement of the medial vas deferens (Figure 2.3). Associated with the latter development, the aggregates of sperm cells (SP) increase in number within their surrounding fluid matrix (SM).

The junction between the proximal and medial vas deferens is thus characterized by a change in arrangement from three to two closely allied tubes of unequal size. They are lined with expanded layers of secretory tissue (SEC EP), each producing secretions which appear to be different in composition. The secretion of the larger channel is invested with dense clusters of spermatozoa (SP), whereas the other has no apparent inclusions. The two secretions are evident throughout the length of the vas deferens. They are referred to respectively as the wing matrix (WM), after King (1948), and the spermatophoric matrix (SM). The lens-like aggregates of spermatozoa (SP) seen in cross-section within the spermatophoric matrix (SM), take a form suggestive of a tightly-packed, continuous, convoluted column (Figure 2.3). Burkenroad (1934) and Pèrez Farfante (1975) refer to a column of spermatozoa within a gelatinous sheath in *P. setiferus,* while Tuma (1967) describes discrete macroscopic columns of spermatozoa in gelatinous material in *P. merguiensis.*

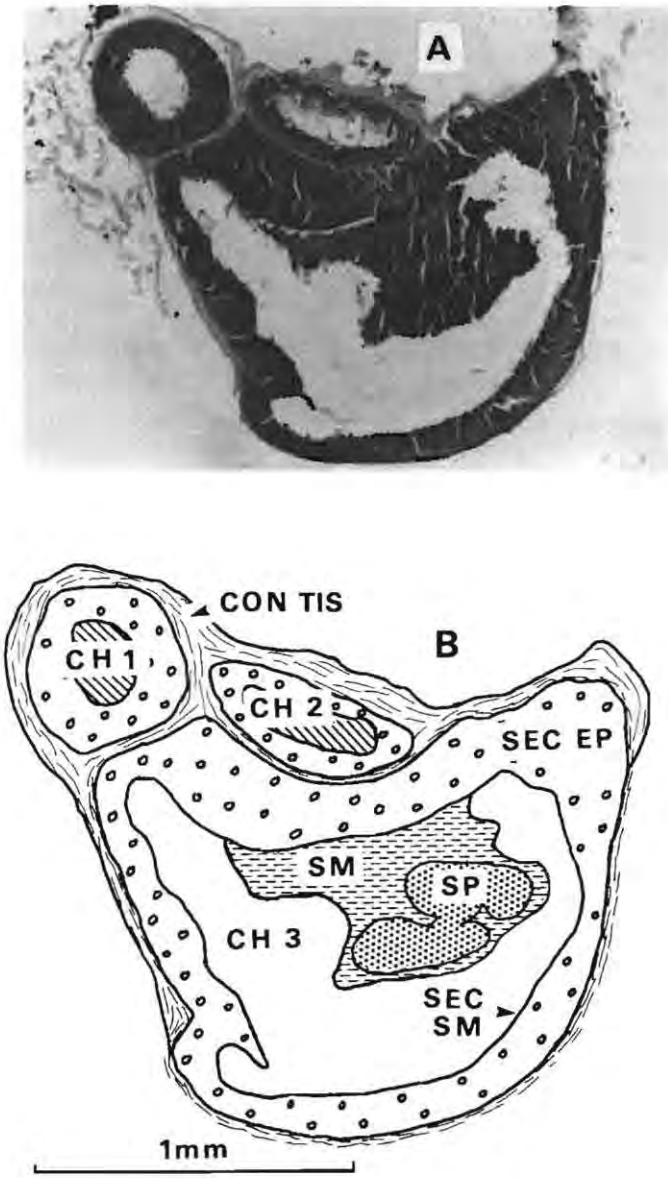

Figure 2.2 Photograph (A) and reconstruction (B) of a transverse section through the proximal vas deferens of *P. indicus* at position 2 in Figure 1. CH 1 = channel 1; CH 2 = channel 2; CH 3 = lumen of channel secreting spermatophoric matrix; CON TIS = connective tissue; SEC EP = secretory epithelium; SEC SM = spermatophoric matrix secretions; SM = spermatophoric matrix; SP = spermatozoa.

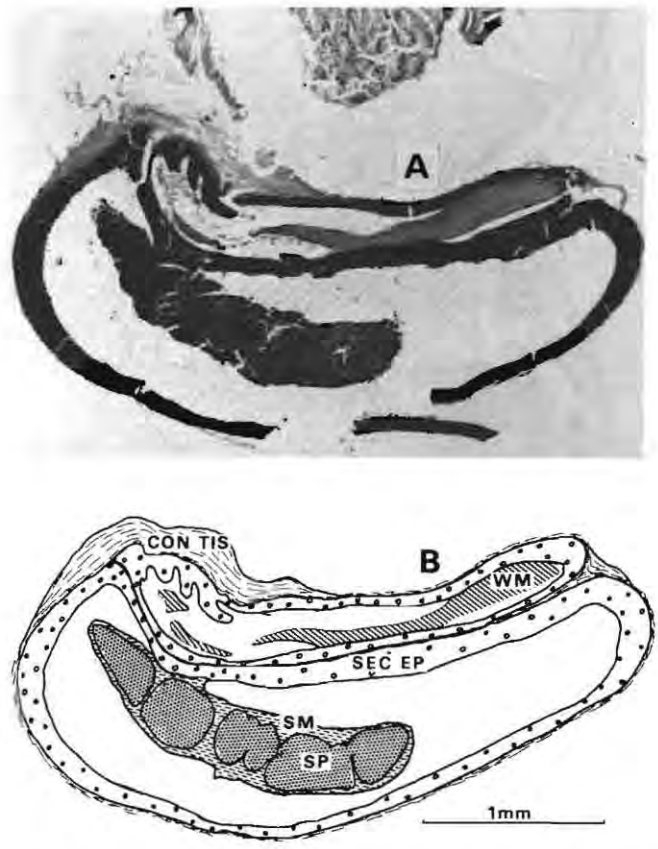

Figure 2.3 Photograph (A) and reconstruction (B) of a transverse section through the expanded medial vas deferens of *P. indicus* at position 3 in Figure 1. CON TIS = connective tissue; SEC EP = secretory epithelium; SM = spermatophoric matrix; SP = spermatozoa; WM = wing matrix.

King (1948) cites the presence of cilia attached to the epithelial lining in the proximal vas deferens and parts of the terminal ampoule in *P. setiferus*. Although elements similar to those illustrated by King were observed in *P. indicus* (Figure 2.2, SEC SM), they are not regarded as cilia. They are believed to be distortions of the inner epithelial lining, arising from their strong secretory function.

Medial vas deferens

The medial recurved part of the vas deferens is characterized by a triple flexure before it leads on to the relatively thin and straight distal portion connecting with the terminal ampoule. Its maximum dimension is achieved from a point immediately after the proximal vas deferens to just before the first flexure. The functional anatomy of this portion, as will be discussed later, motivates its differentiation from the rest of the medial vas deferens by the term 'expanded medial vas deferens' (Figure 1). The internal arrangement of the expanded medial vas deferens is illustrated in Figure 2.3.

Figure 2.4 shows a section taken distally to the expanded medial vas deferens, where the diameter is becoming progressively reduced prior to the first flexure (Figure 1). A consolidated oval structure comprising two chambers of nearly equal size is evident. The chambers are separated by a narrow partition consisting of the closely allied walls of the two channels. The thick glandular linings evident in the expanded medial vas deferens (Figure 2.3) have become reduced to thin epithelial layers (EP) of single cell thickness in places. Muscular tissue (M) appears for the first time in

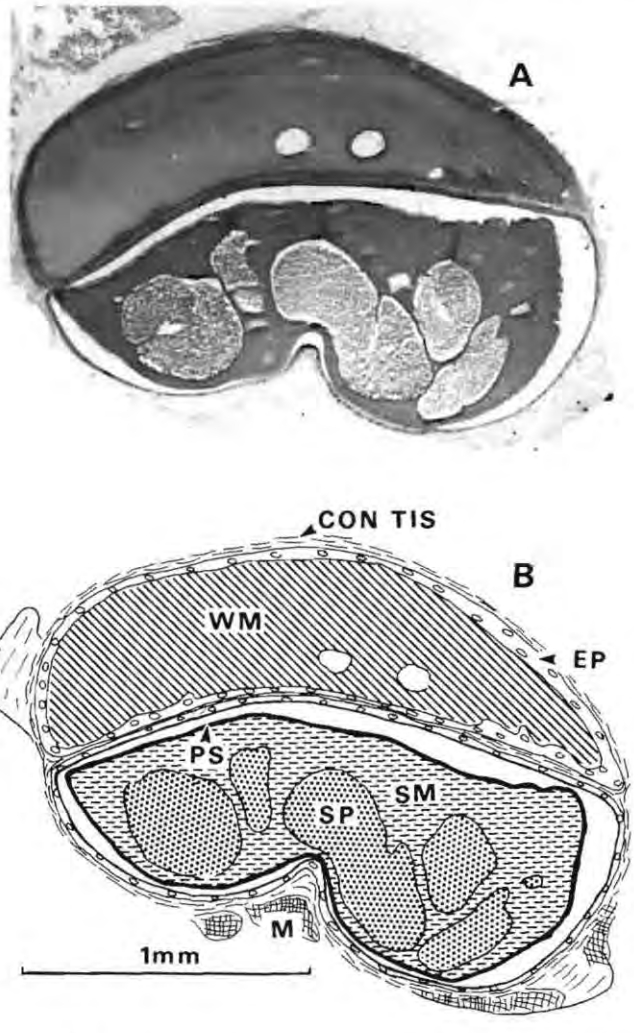

Figure 2.4 Photograph (A) and reconstruction (B) of a transverse section through the medial vas deferens of *P. indicus* at position 4 in Figure 1. CON TIS = connective tissue; EP = epithelium; M = muscle tissue; PS = peripheral secretion; SM = spermatophoric matrix; SP = spermatozoa; WM = wing matrix.

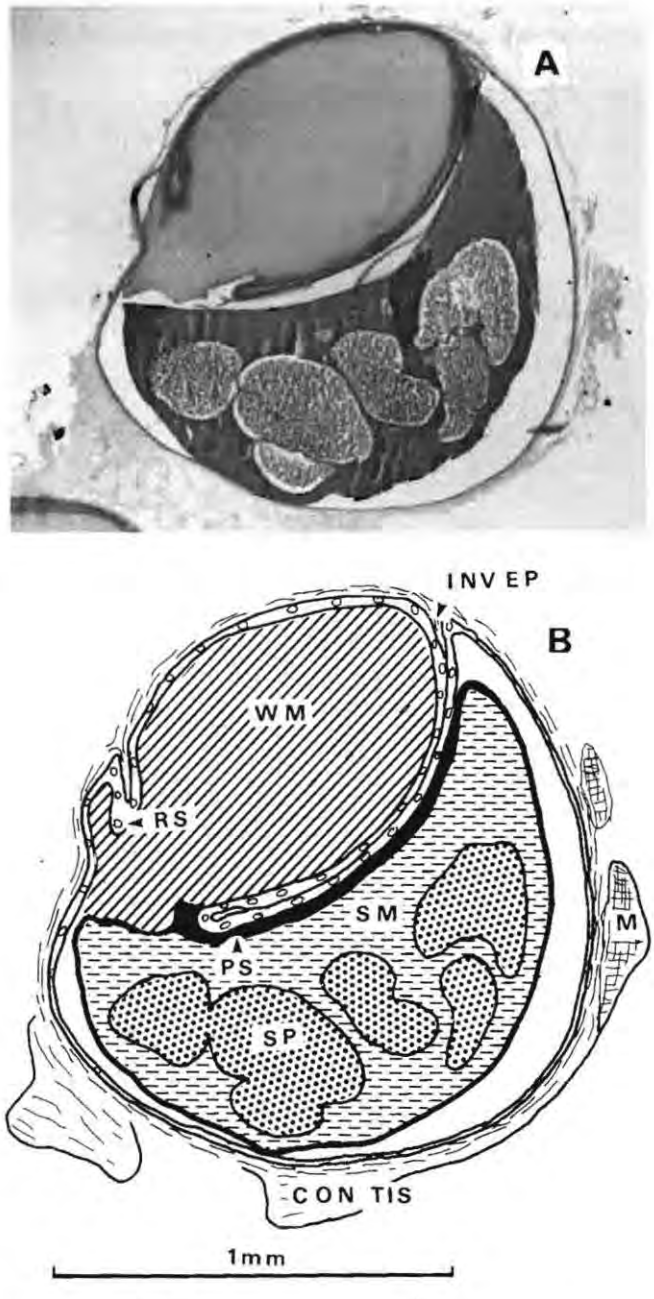

Figure 2.5 Photograph (A) and reconstruction (B) of a transverse section through the medial vas deferens at position 5 in Figure 1. CON TIS = connective tissue; INV EP = invaginated epithelium; M = muscle tissue; PS = peripheral secretion; RS = remnant of septum; SM = spermatophoric matrix; SP = spermatozoa; WM = wing matrix.

association with the outer layer of connective tissue (CON TIS). The wing and spermatophoric matrices (WM, SM) now fully occupy the two lumens, lenses of densely packed spermatozoa (SP) being prominent in the latter. In addition, a narrow differentially stained zone appears at the periphery of the spermatophoric matrix. It could not be established whether this was an artifact or a different substance but for discussion purposes it is referred to as a peripheral substance (PS).

After flexure, as is evident from Figure 2.5, the double-walled septum dividing the vas deferens into two, has parted at one side. The former septum thus changes into a long, thin invagination (INV EP), referred to by Subrahmanyam (1965) as a typhlosole. It subdivides what has now become a common lumen, permitting contact between the two matrices at its apex. Beyond the apex of the invagination where the septum separated, a stubby remnant of the other end of the wall protrudes into the wing matrix (RS). This remnant persists into the terminal ampoule (Figure 2.8), indicating that it takes the form of a continuous ridge. It is consequently postulated that the septum appears intact down the length of the vas deferens in juveniles, and that separation takes place simultaneously down the entire length during development towards maturity. Investigation of the juvenile reproductive system is needed to establish whether this interpretation is correct.

No mixing between the spermatophoric and wing matrices (SM, WM) takes place once the septum is breached, implying a difference in consistency. There does, however, appear to be contact between the peripheral substance (PS) and wing matrix (WM). It was not possible to determine on visual evidence whether these two substances were the same, although this is suspected.

Subrahmanyam (1965) apparently did not observe either the internal arrangement described here, or the different secretions in his *P. indicus* material. He also found no evidence of muscle tissue. The description given by King (1948) for *P. setiferus* is in broad agreement with current findings, except that he is imprecise about the different secretions, provides no detail on the separation of the dividing septum and refers to the presence of cilia. The detailed arrangement described by Shaikhmahmud & Tembe (1958) for *P. stylifera* is completely different.

Distal vas deferens

Progressing down the distal vas deferens, the lenses of spermatozoa reduce to a single aggregate, suggesting a straightening of the previously convoluted column as the

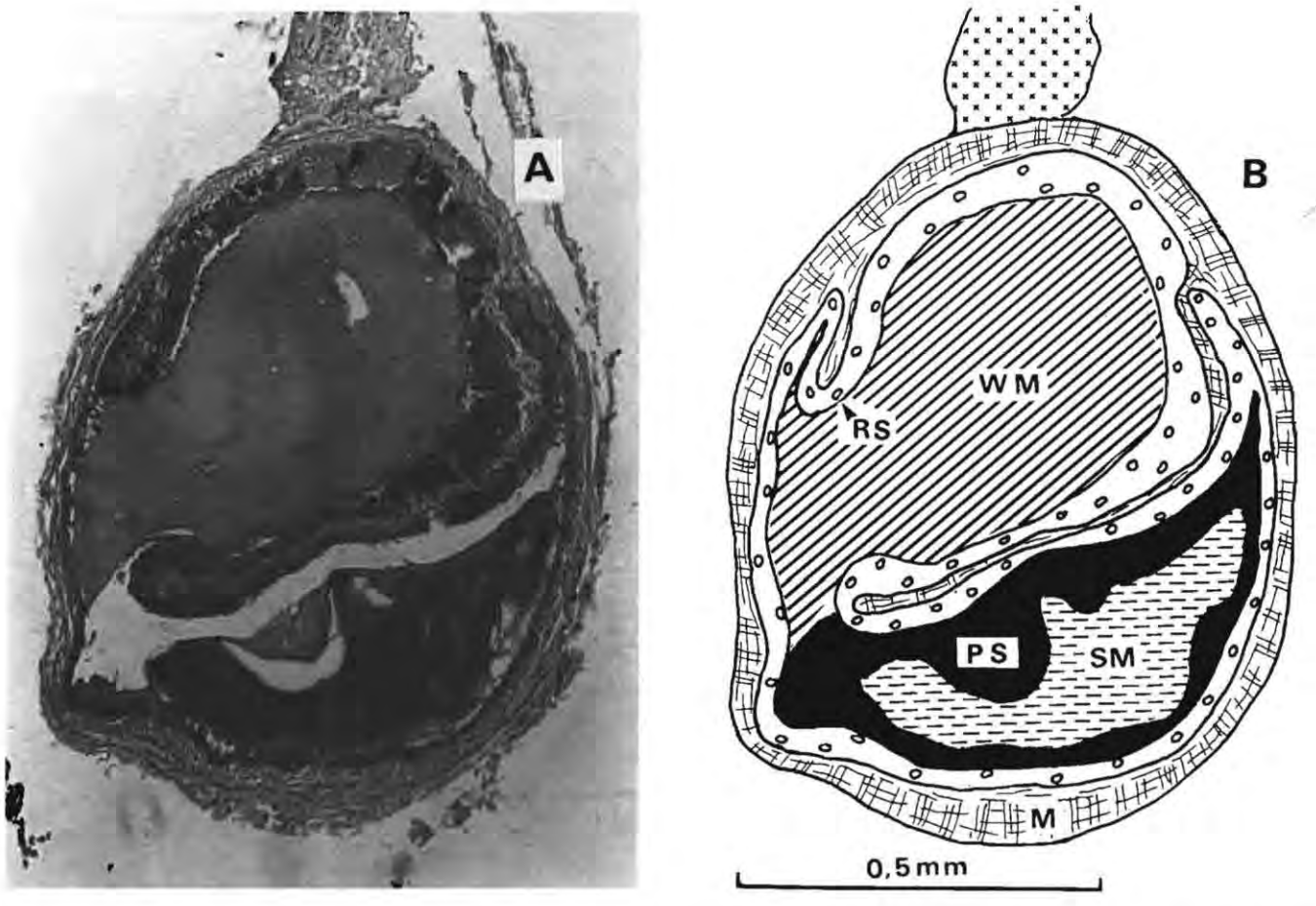

Figure 2.6 Photograph (A) and reconstruction (B) of a transverse section through the distal vas deferens at position 6 in Figure 1. M = muscle tissue; PS = peripheral secretion; RS = remnant of septum; SM = spermatophoric matrix; WM = wing matrix.

vas deferens becomes thinner. Near the junction with the terminal ampoule, the inclusion of spermatozoa disappears. The sperm column is thus interrupted in this region, becoming sealed off within the spermatophoric matrix (Figure 2.6). Muscle tissue (M) now forms a broad, prominent band which completely surrounds the vas deferens. The peripheral substance (PS) enveloping the spermatophoric matrix (SM) is also prominent. This substance is thought to be continuous with the wing matrix as shown in the reconstruction (Figure 2.6).

Terminal ampoule

The distal vas deferens terminates in a greatly expanded, spherical ampoule. Figure 2.7 is a cross-section immediately following the narrow neck connecting the terminal ampoule to the distal vas deferens. Most significant is the recurrence of the column of spermatozoa (SP), appearing as a few small aggregates in the spermatophoric matrix. The epithelial lining is somewhat expanded and is contained within a heavy outer layer of muscle tissue (M). Of further significance is the appearance of a new secretion emanating from the epithelium round the spermatophore. This secretion becomes progressively more copious, particularly in the region of the invagination which partially sub-divides the lumen (Figures 2.8, 2.9). It contains numerous inclusions comprising discrete, irregularly shaped globules of varying size, giving rise to the term globular matrix (GM).

The globular matrix completely surrounds the spermatophoric matrix (SM) and its lining of pheripheral substance. Contact with the globular matrix appears to catalyse the gelling of this lining into a dense, hyaline protective barrier (HL), isolating the spermatophoric matrix from its surroundings. Apart from protection, a further function of the hyaline layer is clearly evident: at the apex of the tongue of tissue partially dividing the lumen (where the contents of the two channels make contact) it interacts with the wing matrix in a series of complex folds and strand-like intrusions (AT), thus tying the wing matrix to the spermatophore (Figures 2.7 – 2.9).

Figure 2.8 shows a section approaching the mid-region of the terminal ampoule and Figure 2.9, indicates the position immediately after the mid-region, progressing towards the distal end where extrusion takes place. The significant expansion of the terminal ampoule is evident, as are the increasingly convoluted form of the sperm column (SP), the attachment role of the hyaline layer (HL) and the increased secretion of globular matrix (GM). The prominent outer layer of muscle tissue (M) is evident throughout the series, penetrating between adjacent epithelial layers where invaginations occur.

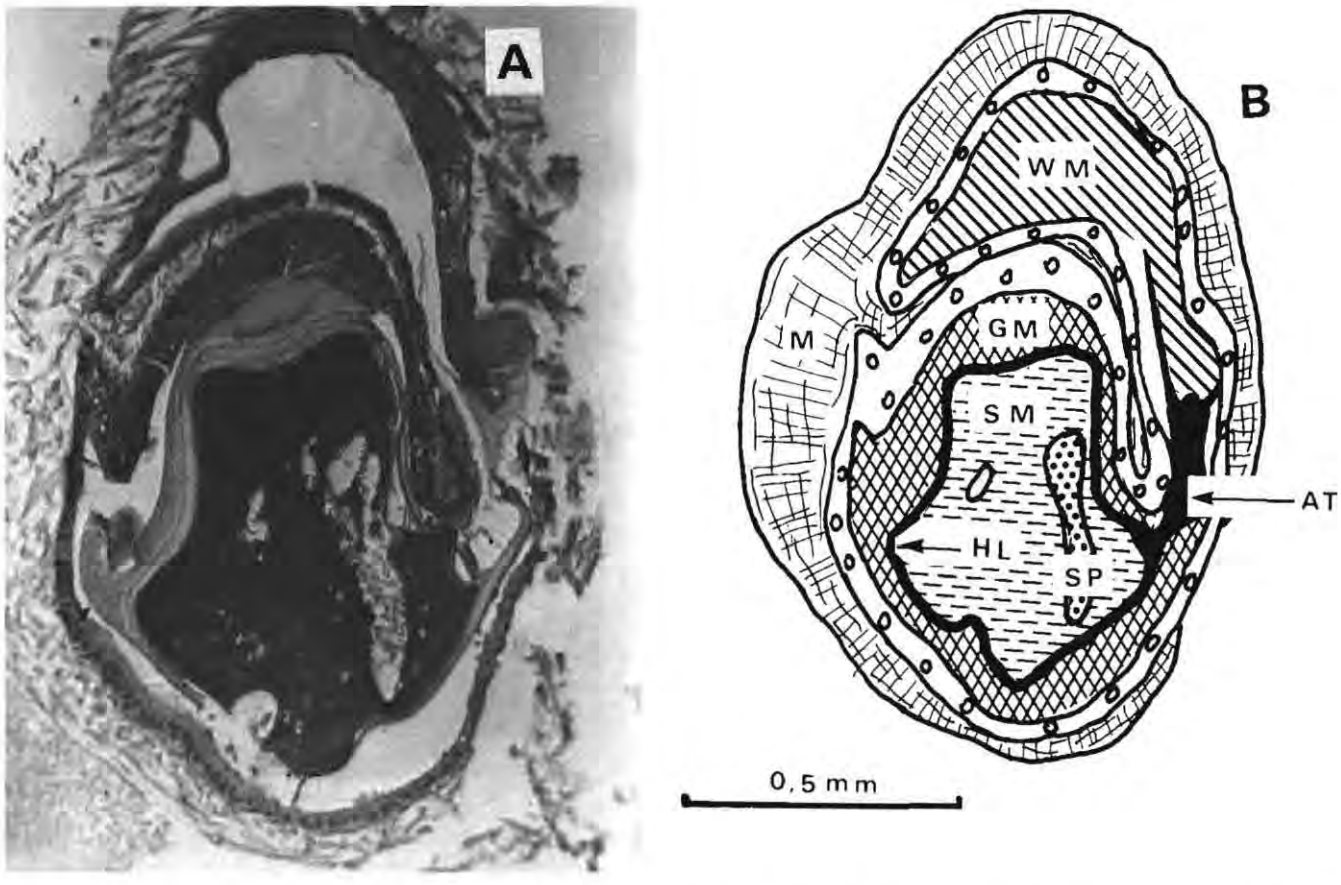

Figure 2.7 Photograph (A) and reconstruction (B) of a transverse section through the terminal ampoule at position 7 in Figure 1. AT = attachment of spermatophore to wing matrix by hyaline layer; GM = globular matrix; M = muscle tissue; SM = spermatophoric matrix; SP = spermatozoa; WM = wing matrix.

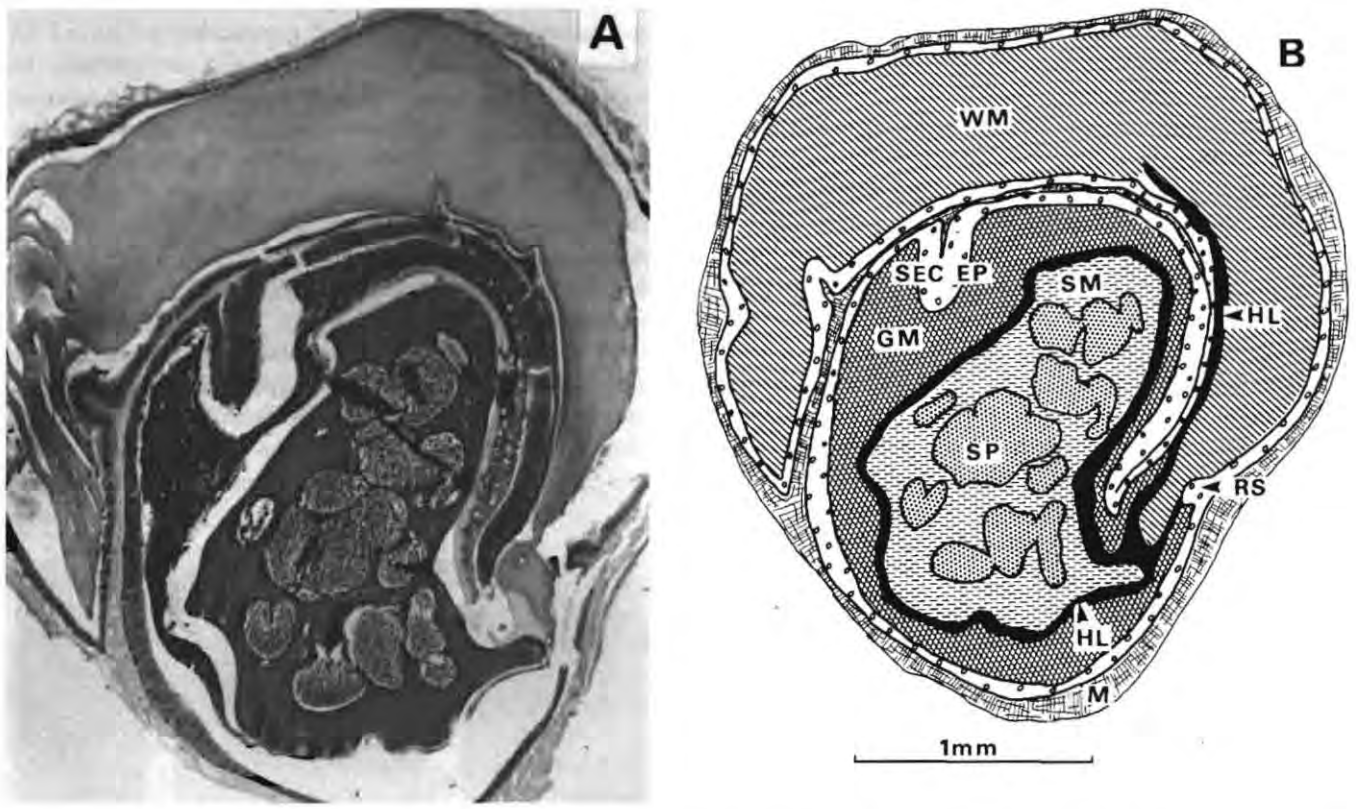

Figure 2.8 Photograph (A) and reconstruction (B) of a transverse section through the terminal ampoule at position 8 in Figure 1. GM = globular matrix; HL = hyaline layer; M = muscle tissue; RS = remnant of septum; SEC EP = secretory epithelium; SM = spermatophoric matrix; SP = spermatozoa; WM = wing matrix.

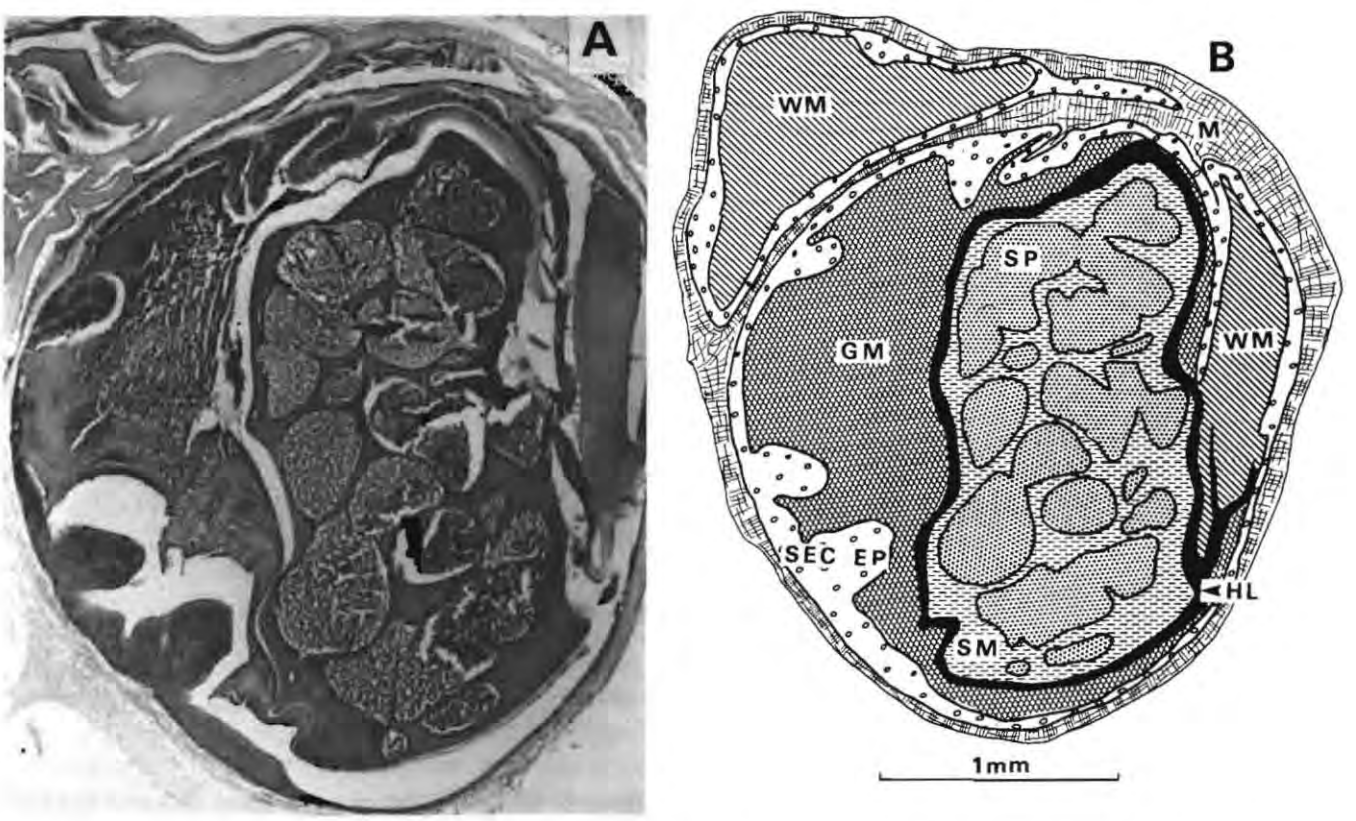

Figure 2.9 Photograph (A) and reconstruction (B) of a transverse section through the terminal ampoule at position 9 in Figure 1. GM = globular matrix; HL = hyaline layer; M = muscle tissue; SEC EP = secretory epithelium; SM = spermatophoric matrix; SP = spermatozoa; WM = wing matrix.

A change in the relative proportions of the contents may also be seen. As the mid-region of the terminal ampoule is approached from the proximal end (Figures 2.7, 2.8), the wing and globular matrices (WM, GM) become progressively more copious. Secondary invaginations of the epithelial lining develop, possibly to enhance secretion (Figure 2.8). Beyond the mid-region, however, the area occupied by the wing matrix (WM) reduces rapidly and folds form in the epithelial lining, giving the impression that portions of the wing matrix are isolated (Figure 2.9). Conversely, the area occupied by the spermatophoric and globular matrices (SM, GM) increases greatly.

The above arrangement is logical since, on extrusion, the spermatophoric matrix emerges first, trailing progressively more wing material behind it. This permits the spermatophore to precede the wing material into the thelycum during copulation, enabling the latter to serve as an outer protective plug.

No gonopore through which the spermatophore might exit was found. It is assumed, therefore, that the muscle tissue is arranged in such a way to permit easy extrusion at the lower end. Mechanical extrusion is readily achieved in mature specimens by gently squeezing the animal in the vicinity of the fifth pair of pereiopods. The spermatophoric material simply erupts from the terminal ampoule.

Extruded spermatophoric mass

A spermatophore is conventionally known as a capsule of albuminous matter containing a number of spermatozoa (Kenneth 1963). In the present context, the spermatophore is therefore confined to the spermatophoric matrix and spermatozoa, encapsulated by the protective hyaline layer. The entire extruded mass, including the wing and globular matrices, is termed the spermatophoric mass.

To the naked eye, three elements are readily discernible in the extruded spermatophoric mass: a tapering, viscous, white matrix (WM), attached to an oval, translucent, gelatinous matrix within which a convoluted white thread of matter resides (Figure 3). The latter is obviously the column of densely-packed spermatozoa, thus confirming the form this element takes in the vas deferens.

A transverse section through the extruded spermatophoric mass reveals all the components seen in the terminal ampoule (Figure 4). The sticky wing matrix (WM) is attached to the spermatophore by a complicated extension of the hyaline layer (AT) at one end. This layer (HL) encapsulates the spermatophoric matrix (SM). The convoluted column of spermatozoa (SP) shows up as lens-like aggregates within the spermatophoric matrix. Finally, a portion of the globular matrix (GM) is evident as a layer between the spermatophore and wing.

Spermatozoa

Individual spermatozoa comprise a spherical head and slender tail. The largest portion of the cell is occupied by a central nucleus which stains dark blue with haematoxylin. The surrounding area and tail stain light red with eosin. The spermatozoon is not globular as suggested by Subrahmanyam (1965), nor does it have an expanded middle portion preceding a short thick tail as was found for *P. setiferus* by King (1948). It is similar to the spermatozoon of *Penaeus japonicus* (Hudinaga 1942), *Penaeus duorarum* (Eldred 1958) and *P. merguiensis* (Tuma 1967) respectively, but differs from that of *P. stylifera* (Shaikhmahmud & Tembe 1958; Tirmizi 1968).

Spermatozoa were measured from the vasa deferentia and terminal ampoules of four specimens measuring 29 mm, 31,2 mm, 37,4 mm and 39,6 mm CL. As expected, there was no significant difference in spermatozoon size between specimens or the two anatomical localities. The

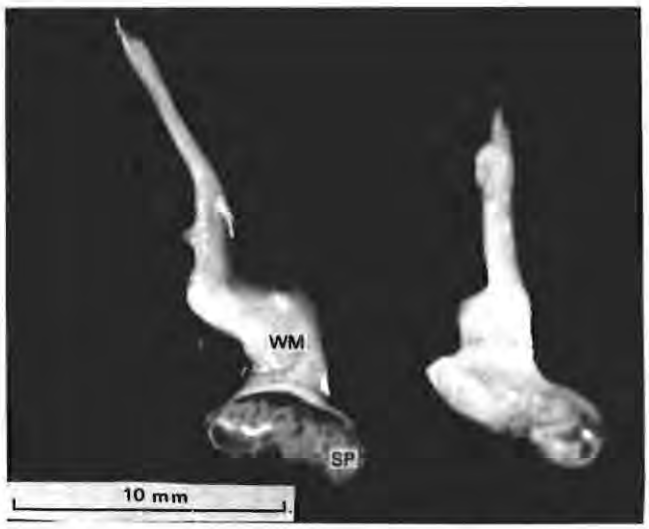

Figure 3 Photograph of the extruded spermatophoric masses from the left and right terminal ampoules of *P. indicus*. The viscous white wing matrix (WM) is attached to an oval, semi-translucent spermatophore (SP).

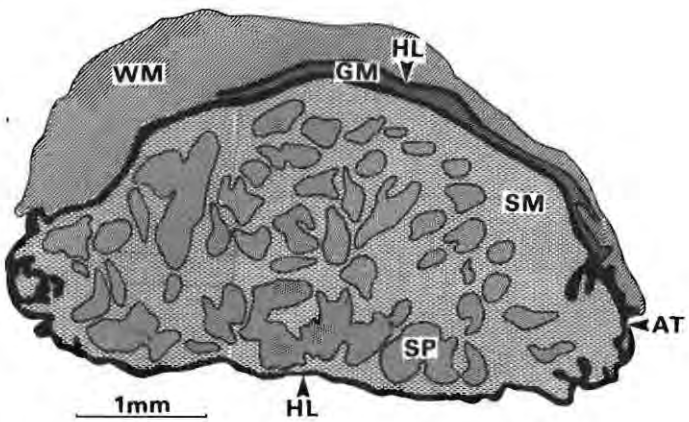

Figure 4 Reconstruction of a transverse section through an extruded spermatophoric mass. AT = attachment between wing and spermatophore; GM = globular matrix; HL = hyaline layer; SM = spermatophoric matrix; SP = spermatozoa; WM = wing matrix.

mean diameter of the head from 107 measurements was 2,8 μm (standard error 0,7 μm; range 2,0 – 4,0 μm) and the length of the tail from 43 measurements was 2,3 μm (standard error 0,9 μm; range 1,1 – 4,0 μm). These measurements are subject to the limitations imposed by light microscopy and the tails in particular, posed problems, since the entire length was not always in the measuring plane. The values are nevertheless considerably lower than Subrahmanyam's (1965) mean figure of 9,6 μm. The measurements given for *P. japonicus* are head and tail, 5,0 – 5,3 μm each (Hudinaga 1942), *P. merguiensis,* head 3,1 μm, tail 5,0 μm (Tuma 1967), while King (1948) depicts a total length of 3,1 μm for *P. setiferus*.

Functional anatomy

Having illustrated the basic organization of the internal reproductive organs and identified their more obvious products, it is possible to speculate briefly on functions.

Spermatocytogenesis takes place in the testis. The subsequent process of spermiogenesis may take place in the early part of the vas efferens, though the section examined (Figure 2.1) showed what appeared to be fully developed spermatozoa. Be that as it may, a flow of spermatozoa into the proximal vas deferens, regulated largely by mating activity, is postulated. The spermatozoa are densely packed in seminal fluid secreted in the vasa efferentia and project into the proximal vas deferens in a compact column. On entry, the integrity of the column is maintained by a liberal secretion from the epithelial lining which surrounds it. The column becomes convoluted in this spermatophoric matrix, which not only supports its form, but is also believed to supply nutrients to the spermatozoa.

At the same time, secretory tissue of the proximal vas deferens gives rise to the formation of a second, closely allied channel in which a further secretion, the wing matrix is invested. The channel is fully formed by the stage that the proximal vas deferens meets the expanded medial vas deferens (Figures 2.2, 2.3).

Secretion of both the spermatophoric and wing matrices continues in the expanded medial vas deferens, but ceases where the diameter diminishes prior to its first 180° flexure (Figure 2.4). Thus it is concluded that the primary function of the proximal and expanded medial vas deferens (Figure 1) is the formation of the spermatophoric and wing matrices. They are believed to depart this region complete, other than for possible incidental supplementation of the wing matrix in subsequent sections. Burkenroad (1934) states that the gelatinous part of the spermatophore in *P. setiferus* is secreted in the middle portion of the vas deferens where it enfolds a column of spermatozoa. His subsequent statement, however, that the 'terminal ampoule adds a further sperm-free gelatinous substance, whereupon the spermatophore is complete', is only in agreement with the current findings if he is referring to the globular and not the wing matrix.

As the diameter of the expanded medial vas deferens diminishes prior to first flexure, the epithelial linings become much thinner indicating their reduced secretory role. At this point, what appears to be a differentially stained layer round the spermatophoric matrix becomes apparent. It is tentatively termed the peripheral substance (Figure 2.4, PS). If the peripheral substance is an artifact, reference to it implies the periphery of the spermatophoric matrix. The peripheral substance later gels into the hyaline barrier which forms in the terminal ampoule (Figures 2.7 – 2.9).

After flexure, contact between the contents of the two halves of the vas deferens becomes possible owing to a break developing near one end of the sub-dividing septum (Figure 2.5). Mixing is, however, limited to the peripheral substance and the wing matrix. No obvious differentiation is evident between them, suggesting that they may comprise the same substance. (If the peripheral substance is an artifact, the intrusion of wing matrix to form a film round the spermatophoric matrix appears to take place.) As has already been indicated, the parting of the dividing septum is believed to occur simultaneously down the succeeding length of the vas deferens at some time during the approach to maturity. Arising from this, a question which immediately poses itself is why such a development should take place? The need to tie the wing to the spermatophore only arises much lower down in the terminal ampoule. The answer may be that contact between the two halves all the way up to the first flexure in the medial vas deferens, facilitates the synchronous movement of products down the channels. Once the wing and spermatophoric matrices have been produced in the expanded medial vas deferens, the epithelial linings lose their primary secretory function, become much thinner and approximate more the role of linings to two conduits. The break in the dividing wall at this point enables the contents of the two channels to move to the terminal ampoule together in a common lumen, their separate identities, however, being preserved by the invaginated tongue of tissue which remains after the break. The latter half of the medial and the distal vas deferens thus appears to serve primarily as a conduit but also as a store of mature gonadal products.

Discontinuity of the sperm column in the narrow neck connecting the distal vas deferens with the terminal ampoule (Figure 2.6), may serve the purpose of ensuring that the column is not breached when extrusion takes place. The intermediate core of matrix separating the spermatozoa in the terminal ampoule from those in the distal vas deferens, is located in the most likely position for a break to occur when muscular contraction causes extrusion of the spermatophore (Figure 1 position 6).

In the terminal ampoule a new secretion, the globular matrix, appears. On contact with this matrix, the film surrounding the spermatophore becomes transformed into a dense, hyaline barrier. It would appear that a biochemical reaction takes place which extends into the passage giving access to the wing matrix, resulting in the complex series of hyaline folds and strand-like intrusions effectively tying the wing to the spermatophore (Figures 2.7 to 2.9). It is surmised that the reaction itself seals off the passage to the wing matrix, preventing excessive

penetration of globular matrix which is consequently retained as an enveloping layer adhering to the spermatophore.

The terminal ampoule thus fulfils both storage and secretory functions. The wing and spermatophore are stored there in preparation for mating while globular matrix is secreted. Part of the function of the globular matrix is to facilitate attachment between the wing and spermatophore. Its absence from any other part of the reproductive system ensures that only those elements which are to be extruded from the terminal ampoule at mating are tied together.

After discharge on mating, the terminal ampoule is replenished with spermatophoric material from the preceding distal and medial vas deferens. New sperm cells are in turn fed from the testis into the proximal vas deferens which, together with the expanded medial vas deferens, secretes replacement wing and spermatophoric matrices. The convoluted nature of the sperm column presumably confers flexibility in accommodating movement down the vas deferens. In addition, this arrangement ensures more even distribution of sperm cells through the spermatophoric matrix, enabling more efficient transfer of nutrients. The manner in which the column appears to be interrupted at the neck of the terminal ampoule is a matter for conjecture: it could be a result of enforced redistribution of the sperm cells through muscular compression once the terminal ampoule is full, or be due to a break effected in the column higher up the vas deferens during transfer. Musculature is absent in the proximal and expanded medial vas deferens. While being present in the rest of the medial vas deferens, it only really becomes prominent in the distal vas deferens. Thus movement of spermatophoric material is possible by peristalsis in the distal vas deferens. In the event, preceding material is probably drawn down with the aid of weaker musculature present in the latter part of the medial vas deferens.

Speculation on the purpose of the wing matrix and the additional functions of the globular matrix requires assessment of the positions they occupy in the thelycum after mating. Figure 5 shows a transverse section through an inseminated thelycum. The two spermatophoric masses can be seen lying side by side in the thelycal pouch. The wing matrix (WM) is confined to the median longitudinal opening of the thelycum, where it forms a protective plug against the external environment. Tuma (1967) refers to the wing matrix in *P. merguiensis* as a hygroscopic, sperm-free substance, which expands into a plug on contact with sea water. It was not established whether the wing matrix of *P. indicus* is hygroscopic, but if it is, its expansion could assist in bedding down the spermatophore in the thelycum.

The function of the globular matrix (GM) is less clear. It is confined to the interior of the thelycum where it completely envelops the spermatophore and is invested in all the irregular crevices, channels and depressions of the interior surface (Figure 5). In doing so, it ensures that the interior is completely filled while protecting the spermatophore from excessive distortion. To this extent,

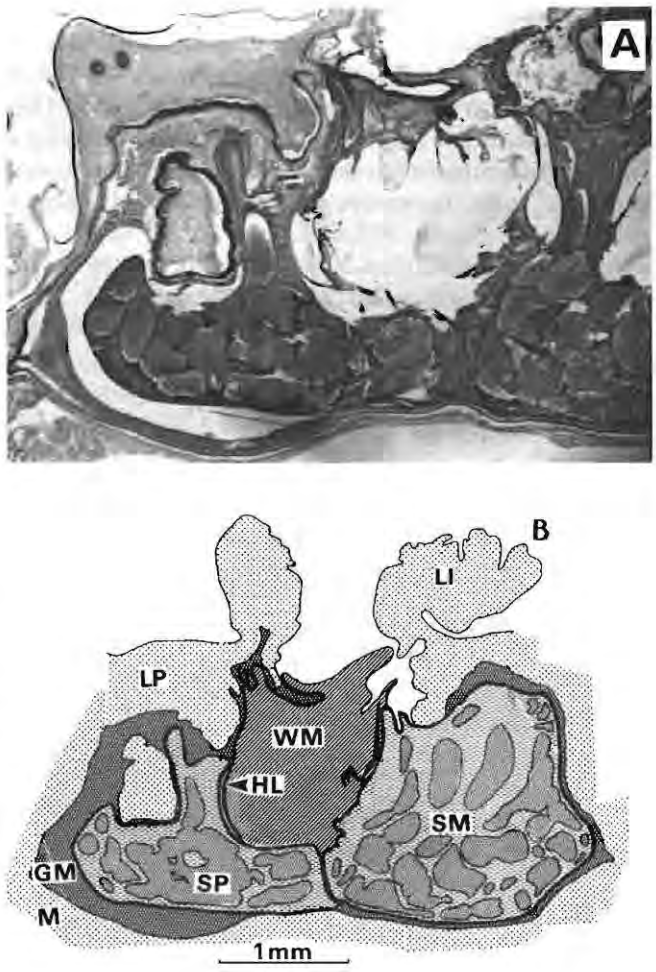

Figure 5 Photograph (A) and reconstruction (B) of a transverse section through the mid-region of an inseminated *P. indicus* thelycum. GM = globular matrix; HL = hyaline layer; LI = lip of thelycum; LP = lateral plate; M = muscle; SM = spermatophoric matrix; SP = spermatozoa; WM = wing matrix.

it is believed to fulfil an important hydraulic function. Its ready investment of the interior irregularities suggests a fluid consistency. Consequently, the globular matrix is also thought to have a lubricating function whereby it could help to ease the spermatophores into position during insertion. Finally, its close adherence to the interior surface of the seminal receptacle implies adhesive properties, in which case it would serve to maintain the spermatophore securely in position. The possibility of a nutritional role cannot be excluded, particularly in view of the globular inclusions which might be packets of nutrients. The dense hyaline wall it helped to form round the spermatophore, as well as its irregular distribution within the receptacle, however, provide reasons to doubt such a function. It is therefore tentatively concluded that the globular inclusions relate to the hydraulic role of this fluid and that nutrition of the spermatozoa is a function of the spermatophoric matrix.

Concluding comment

The smallest *P. indicus* male investigated in this study measured 28,5 mm CL and the largest, 39,6 mm CL.

This size range unfortunately excludes immature males lacking spermatozoa in their terminal ampoules. Anatomical changes reflecting progress towards maturity could therefore not be observed.

In a comparable study of Indian material, Subrahmanyam (1965) observed free spermatozoa in the vasa deferentia for the first time at 120 mm TL, the local size equivalent of which is 22,8 mm CL. He found that the spermatophore became well developed at 130 mm TL (24,9 mm CL) and concluded that the fully mature condition was reached at 140 mm TL (27 mm CL).

The results of this investigation thus support Subrahmanyam's contention to the extent that males of 28,5 mm CL and larger, all possessed fully developed spermatozoa in their vasa deferentia and terminal ampoules; anatomically, they appeared to be sexually mature.

Acknowledgements

The collection of material took place under the auspices of the Oceanographic Research Institute, Durban. This aspect was funded by the C.S.I.R. Histological preparation took place at the University of Natal, Durban. The contribution forms part of a Master of Science thesis awarded by the Rand Afrikaans University. The thesis supervisor, Prof. H. J. Schoonbee, is thanked for his critical comment.

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