

## Comparative Cytology and Morphology of Seminal Vesicles in Male Gobiid Fishes

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**Abstract** Anatomical, histological and EM studies are presented on the male seminal vesicles of 111 species of gobies (Gobiidae, Teleostei). These vesicles, attached to the sperm-ducts, are lined with an excretory epithelium composed of three types of cells: 1) columnar cells with giant Golgi cisterns in the form of large rings apical to the nucleus; 2) excretory cells with remarkably interdigitative basal lamina, that resemble sperm-duct cells; and 3) groups of interstitial, Leydig-type cells that possibly form a part of the mesorchial gland. Morphologically the vesicles appear to be taxa specific, the simplest one in the form of a few tubules on the sperm duct, and the most elaborate one forming large, wing-like structures, with an elaborate mass of passages. In many species the final form of the vesicles develops during the fish ontogenesis; in others, the form of this organ does not change during the lifetime of the fish. With the onset of reproduction, secretion and sperm accumulate in the tubule of the seminal vesicle and are expelled from there onto the eggs during fertilization. The comparative morphology, as well as possible ecological and physiological role of this secretion is discussed.

Various types of accessory glandular structures, attached to testes of teleost fishes, are known from a number of families (Blennidae, Gobiidae, Claridae, Bagridae, Tripterygiidae). They are termed "accessory glands", "seminal glands", or "seminal vesicles". According to Weisel (1949), at least in some fish, they are not derived from the Wolffian duct, and for this reason, they are not homologous to the mammalian accessory glands of the testis.

The first detailed description of these organs was given by Eggert (1931) for some blennies and gobies. Following this, these two fish families have served for numerous studies: the Blennidae by Eggert (1931) and recently by Seiwald and Patzner (1987), and Gobiidae by Vivien (1938), Weisel (1949), Pilati (1950), Egami (1960) and Miller (1963, 1984). Some studies attempted to correlate the physiology of these vesicles with the observed seasonal cycles of the testes (Colombo and Burighel, 1974; Colombo et al., 1982). Egami (1960) was the first to try to compare the gross morphology of some of those vesicles in several species of gobies.

The present study provides data on comparative morphology and cytology of the seminal vesicles in 111 species of gobies from almost 200 taxa that were studied. An attempt was made to find some macro- and microevolutionary similarities between various species groups of these fish.

### Materials and methods

The gobies for this study were collected live by the author in the Red Sea proper, as well as in the Gulf of Suez and Gulf of Aqaba (Israel), Hawaii and California (USA), Miyake Jima Is. (Japan), Oban (Scotland), Plymouth (England), Durban (S. Africa) and along the shores of the Isle of Man (UK). Preserved material was obtained by dissecting gobies from collections of various museums in Europe, Japan, Australia and the USA. Altogether, a total of about 800 specimens of 200 taxa were dissected, of which 111 species served for this detailed morphological and cytological study, as well as for the study on bisexuality in gobies (Fishelson, 1989).

All selected fish were opened along the venter and the testes were exposed, including the attached seminal vesicles. These two parts were extracted intact, together with the urogenital papilla. Following this, the organs were measured and drawings of them were prepared on millimeter paper using a dissection microscope and also often a camera lucida. Fixation in Bouin's solution for slide preparations of total vesicles, or parts of them, was performed as described elsewhere (Fishelson, 1989). For electron microscopic studies pieces of gonads and vesicles were fixed in 3.5% glutaraldehyde,

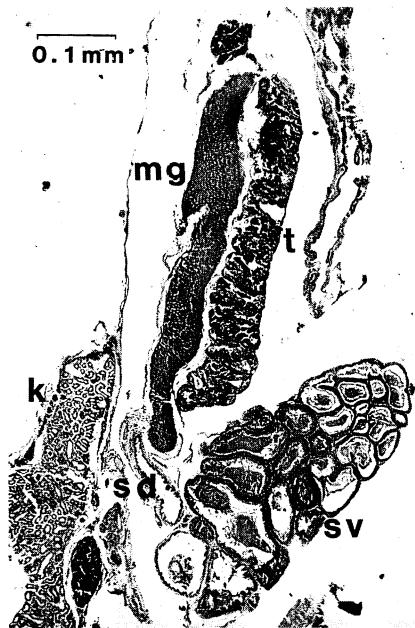


Fig. 1. Longitudinal section of male reproductive organ of *Gobius niger*. mg, mesorchial gland; t, testis; sv, seminal vesicle; sd, sperm duct; k, kidney.

buffered with cacodylate pH 7.2, then stained in 1% osmium tetroxyde, post-washed and embedded in Epon. From these blocks, micro-sections were studied with a Jelco 9 EM.

## Results

**General structure.** The testes of gobiids are paired organs, narrow and elongated, and as shown by various authors (see reference list in Miller, 1984, 1986; Fishelson, 1989), their dimensions vary with seasonality in the reproductive cycle. Each testis also includes a dorsally-situated sperm-duct and also the so-called "mesorchial gland", an oblong glandular structure formed by an accumulation of androgen-producing Leydig-type cells (Colombo et al., 1982, Fishelson, 1989). In some species of *Bathygobius* spp. and *Gobius* spp. this gland is brownish in appearance, differing from the whitish testis. Posteriorly, each testis continues with sperm-ducts that separately or via a short joint duct, open at the terminal end of the genital papilla. At the termination of the testicular tissue, on the sperm duct, the s.c. seminal vesicles appear as paired organs (Fig. 1). Only in some species were such vesicles not detected (Eggert, 1931, and this study). In *Buenia jeffreysii*

and *Acentrogobius cyanomus*, these are very small structures of 3–5 mm, much smaller than the respective testes; whereas in *Gobius cobitis* and *Bathygobius fuscus*, they are usually much larger than the testis (Fig. 15).

The anatomical relationship between the epithelial tissues of the three parts of the male system (testicular, vesicular and that of the sperm-ducts) differs in different species. Thus, for example, in *Buenia jeffreysii* (Scotland) and *Pterogobius virgo* (Japan), the testis and gland tissue mingle at their point of junction with the sperm-duct (Fig. 2). In *Eleotris dormitatrix* (Puerto Rico), *Eleotris* spp. (St. Thomas) and some of the *Gobius niger* (Plymouth), the transition from the testicular to the glandular structure along the sperm-ducts is a gradual one. In *Knipowitschia caucasica*, the vesicular gland cells occur as an extension of a group of loose cells on the basal portion of the testis (Fig. 11).

As observed in a series of fish of various ages and sizes in different species, the ontogenic structural development of the vesicles follows two characteristic lines: in one group of species (for example, the genera *Bathygobius*, *Pomatoschistus* and *Gobius*), the vesicles, from their first appearance in the smallest male up to the largest one, have a constant form typical for the species and only their dimensions change with growth (Figs. 15, 16). The second group includes numerous eleotrin genera (*Callogobius*, *Cryptocentrus*, *Eleotris*, *Periophthalmus*), in which the vesicle morphology alters with growth, attaining its final structure in full-grown males (see below).

Histologically, the seminal vesicles are glandular structures of smooth or lobulated form that, within the abdominal cavity, partly envelop the latero-ventral side of the testis. The wall of the vesicles has a tunic of connective tissue, penetrated by muscle fibers and blood capillaries (Fig. 3). As described for several species of gobiids (Miller, 1963; Moiseyeva and Ponomareva, 1973; Colombo et al., 1980), the secretory, internal cell-layer of the vesicles forms ducts-tubular passages, sometimes producing a very contorted network of epithelium, supported at the cell bases by a strong, basal lamina. In some species, as for example *Gobius paganellus* and *G. buccichichi*, groups of interstitial Leydig-type cells occur between the tubules, embedded into the connective tissue of the boundary layers (Stanley et al., 1965). During reproduction, these Leydig cells multiply, often widely separating the tubules from each other (Fig.

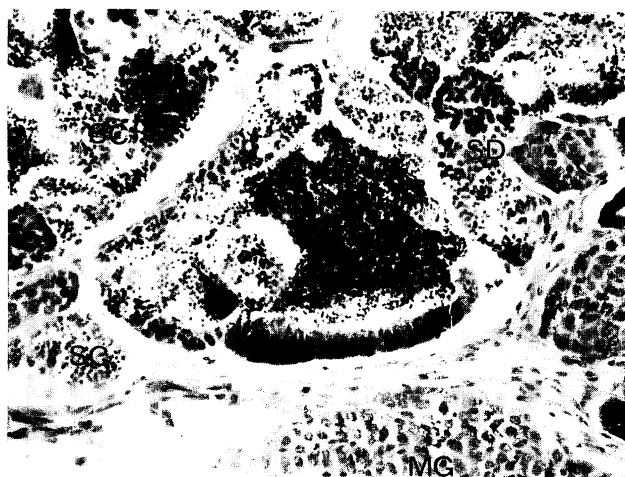


Fig. 2. Cross section of testis of *Beunia jeffreysii* on the junction with the seminal vesicle. SD, spermatids; SC, spermatocytes; SV, seminal vesicle epithelium; MG, mesorchial gland.  $\times 170$ .

4). It seems that in such species, these interstitial cell islands are parts of the mesorchial gland.

In some species, such as *Pomatoschistus microps* (Miller, 1963) and *Gobiusculus flavescens*, such organized mesorchial glands are absent and their function seems totally dependent on the interstitial cells embedded in the seminal vesicles. In other species, like the hermaphroditic genera *Paragobiodon* and *Gobiodon*, this gland, which is absent in the female, develops with sex change from female to male stages (Fishelson, 1989). Bonnin (1975) demonstrated that the mesorchial cells are activated by extracts of pituitary homogenate in cell culture and according to Colombo et al. (1970), these cells produce 5-reduced androgen conjugates, that act as pheromones during reproduction. The same cells also produce non-conjugatable 11-oxygenated androgens, as androsterone and 11-hydroxandrostenedion, acting as sex hormones.

The secretory epithelium of the seminal vesicle is formed by a monolayer of cells that change dimensions and shape at various stages of their activity (also Moiseyeva and Ponomareva, 1973). During the preparatory stages, the cells are columnar, 23–36  $\mu\text{m}$  high and 6.5–11.5  $\mu\text{m}$  wide (Fig. 5). The subcentrally located nucleus divides the cell's interior into two parts: the basal section of 6–10  $\mu\text{m}$  and the distal section of 20–28  $\mu\text{m}$ . The nucleus is usually oval or oblong-irregular, 6.5–7.5  $\mu\text{m}$  along the axis, 3–4 times longer than wide, and possesses a granulated nucleoplasm, a very dense, regular karyosome

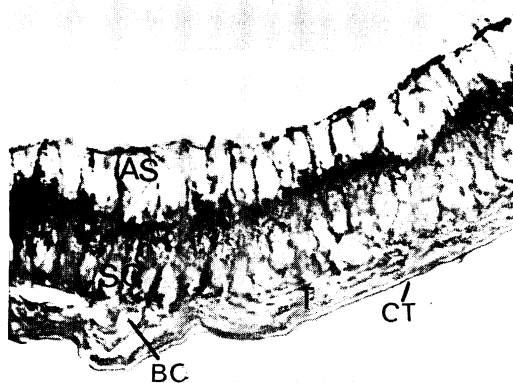


Fig. 3. External aspect of the seminal vesicle (*Bathygobius cyclopterus*). T, tunic of connective tissue; CT, external cover tunic; BC, blood capillary; SC, inside secretory epithelium; AS, accumulation of secretion at the apical parts of secretory cells.  $\times 230$ .

along the nuclear membrane and a centrally located nucleolus (Fig. 6). The distal ends of these cells extend in the tubule lumens, laterally connected to the neighboring cells, with their bases strongly affixed to the basal membrane (Fig. 5). The most distinctive organelle in the apical parts of these cells is the very large Golgi complex shaped like a ring that forms a circular structure of 5–8  $\mu\text{m}$  diameter above the nucleus. Each such Golgi ring is com-

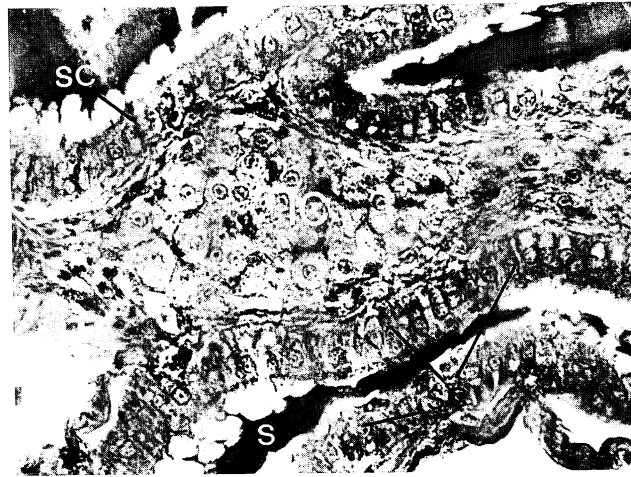


Fig. 4. Longitudinal section of the seminal vesicle of *Gobius cobitis*. IC, aggregation of interstitial cells; SC, secretory epithelium; S, PAS-positive secretory substance.  $\times 250$ .

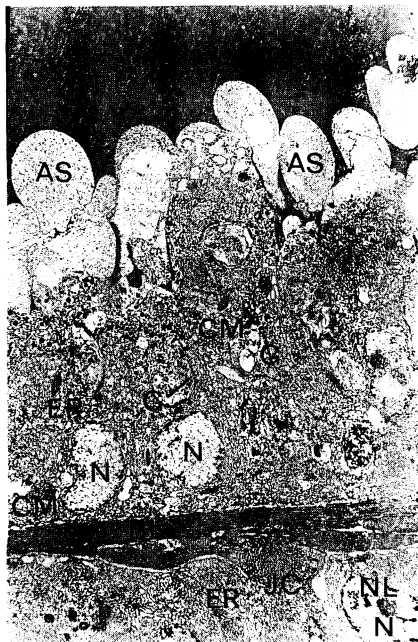


Fig. 5. Micrograph of the seminal vesicle secretory epithelium (*Gobius cobitis*). N, nucleus; NL, nucleolus; G, Golgi ring of cisterns; ER, rough endoplasmic reticulum; CM, cell membrane; AS, accumulation of granular secretion; TL, tubule lumen with secretion; BL, basal lamina; JC, juvenile secretory cells.  $\times 2,500$ .

posed of 5–6 compressed and parallel running tubules of the smooth endoplasmic reticulum (SER) (Fig. 6). The entire cellular cytoplasm is penetrated by a very dense network of rough endoplasmic reticulum (RER), encrusted with numerous, dark zymogen granules with prominent limiting membranes in and around the Golgi apparatus. These are especially dense at the distal portion of the cells (Fig. 6). The cell membranes have only a few digitations with neighboring cells, interconnected with desmosomes and tight junctions, especially numerous near their apical regions.

In secreting cells, the apical free endings of the secretory cells become rounded, and on these sites the cell membrane becomes fragmented and the secretion, together with granular cytoplasm and parts of the cell organelles are led out into the tubule lumen (Fig. 7). Concomitantly, the junctions between the cell-endings also disappear and secretion is found on these sites. This separation can be so prominent that it becomes visible even in light-microscopy sections (Fig. 8).

In addition to these types of secretory cells, in some parts of the vesicles, and especially close to the sperm-duct, a different type of cell is found that we shall tentatively call B-cells. These are also columnar, with a very large, irregular nucleus and prominent nucleolus. Differing from the former described secretory cells, they lack the large Golgi apparatus and their basal cell membrane, as well as the basal lamina below them, are very deeply interdigitated



Fig. 6. Micrograph of a secretory cell from the vesicle of *Gobiusculus flavescens*. CM, cell membrane; D, desmosomes; N, nucleus; NL, nucleolus; ZG, zymogene granules; M, mitochondria; RER, rough endoplasmic reticulum; G, Golgi rings (a part inserted enlarged  $\times 25,000$ ); TL, tubule lumen; V, vesicles; BL, basal lamina.  $\times 9,800$ .

(Fig. 9).

B-cells, occurring on the transition site between the vesicles and sperm-duct, strongly resemble the sperm-duct cells. With the onset of reproduction and the initiation of secretory activity in the seminal vesicles, their tubules begin to fill up with the secretion, as well as with sperm from the testis (Fig.

8). Following this increase in pressure, the secretory cells of this epithelium become shorter, cuboid and, at a later stage, greatly extended and flat (Fig. 10). Finally, during the last stages of filling, the walls of the tubules tiny and burst open on many sites, making way for the sperm-secretion mixture towards ejaculation. Such collapsing partitions are almost

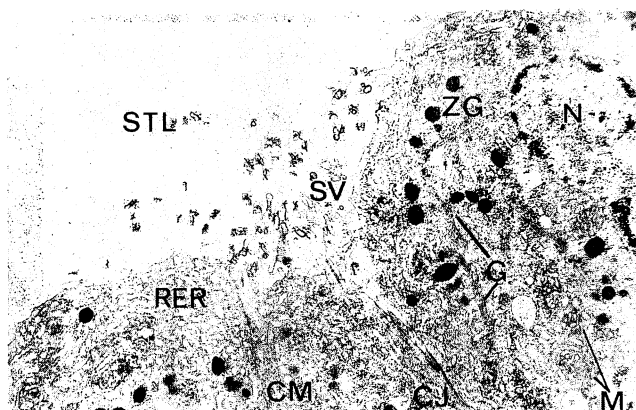


Fig. 7. Apical zones of secretory cells from vesicles at high activity. SV, secretory microvesicles in the tubule; STL, secretory granules in the tubule lumen; N, nucleus; G, Golgi fragments; ZG, zymogene granules; M, mitochondria; CM, cell membranes; CJ, cell tight junctions.  $\times 9,800$ .

denuded of the secretory epithelium, and are mainly formed by remnants of connective tissue and intermediate cells (Figs. 8, 10).

**Types of seminal vesicles.** As already shown by Egami (1960), in various species of gobies the vesicles differ in form. The comparison of the general structure and internal complexity of the seminal vesicles, as well as their relation to the testis in the studied species of gobies enables their grouping into several separate types:

**Type A:** The seminal vesicle is either a part or small extension of the testis or sperm-duct and is formed by a relatively small number of cells or a few tubules (Fig. 11). Within this category lie *Knipowitchia caucasica*, *Ptereleotris microlepis*, *Apocryptichthys contortis*, *Aphia pellucida*, *A. minuta*, *Acentrogobius cyanomus*, *Apocryptus bato*, *Stigmatogobius sadanundo*, *Odondebuania bolearica* and some others (Table 1). In some of these species, e.g. *A. cyanomus*, the vesicular cells form a ridge within the sperm-duct wall; in *K. caucasica* and *Aphia* spp., the vesicular cells mingle with the gametogenic ones. In more advanced species, such as *Ptereleotris microlepis*, the vesicle is formed by only 6–8 tubules (Fig. 11); in *Apocryptichthys contortis* by 8–9 tubules, and in *Pseudoapocryptes lanceolatus* by 10–12 tubules. In *Magurunda m. magurunda*, the seminal vesicle consists of 2–3 long crypts divided by a few transversal septa. The seminal vesicles of *Periophthalmus papilio*, *P. sobrinus* and *P. schlosseri* that are of Type A, are slightly more advanced (Fig. 12). In these fish the number of longitudinal crypts reaches up to 20, and the transversal septa are more numerous.

**Type B:** The seminal vesicles in these species (Table 2) are much richer in tubule and crypts but form an integral part of the enlarged sperm-duct. In *Eleotris dormitator* and *Philypnos obscura* the vesicles extend throughout the sperm-ducts (Fig. 13). In *Pterogobius virgo* the testicular section graduates into the vesicular one (Fig. 13)—a phenomenon also observed in hermaphroditic gobies, such as *Gobiodon* spp. and *Paragobiodon* spp. (Fishelson, 1989), in which the vesicles develop with the transition of females to the male phase (Fig. 8c).

**Type C:** The seminal vesicles are long, finger-like extensions that, in some instances, such as *Brachygobius xanthozona* and adult *Bathygobius soporator*, are longer than the testis (Fig. 14). *Bathygobius fuscus*, *B. lineatus* and *Fussigobius* spp. also belong to this category. In some of the species (Table 3), such as *Istigobius decoratus*, distally, each of the oblong vesicles split in two from the middle on (Fig. 14).

**Type D:** The vesicles are flat and wing-like, and in several species, such as *Gobius cobitis* (Fig. 15), they grow with age while continuing to preserve their original form. In gobies of this type (Table 4) the number of tubules in each vesicle is very high, forming within a dense and complex spongy structure. In many species, such as *Gobius platycephalus*, *G. guttatus*, *Coryphopterus nickolsii* and *Gobiusculus flavescens* (Fig. 16), their dimensions can be as great as, or even greater than those of their testes.

These 4 types of seminal vesicles can each be divided also into several sub-types according to their form and relationship to the sperm-ducts.

**Subtype 1:** The vesicles open into the sperm-duct

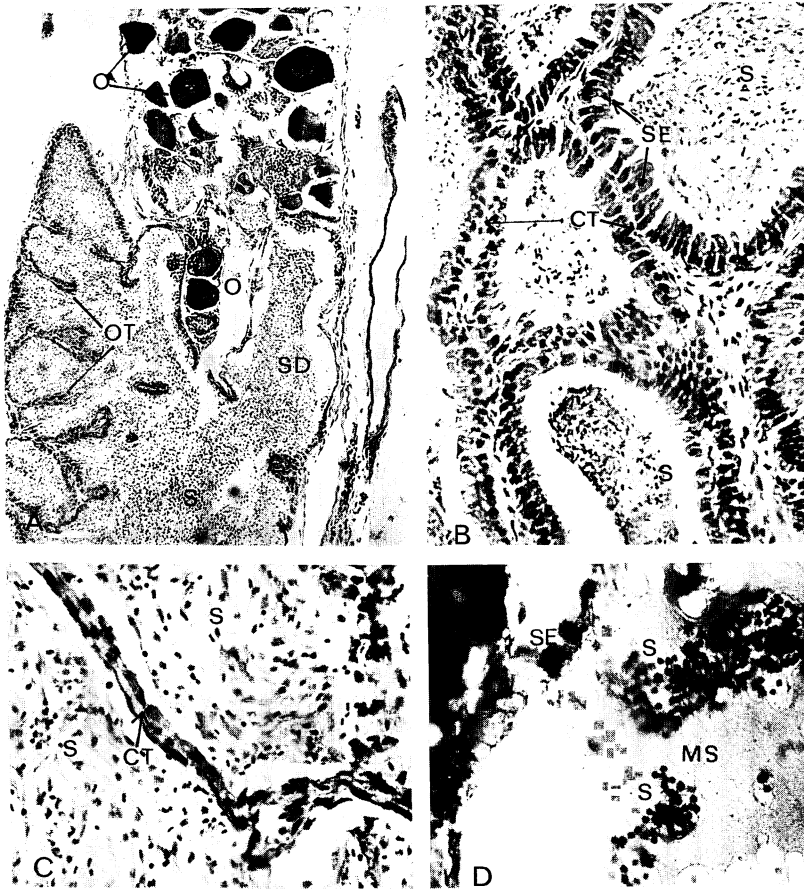


Fig. 8. Sections of gonads and seminal vesicles. A, *Gobiodon rivulatus*, a sex-changed fish in male stage ( $\times 30$ ); B, seminal vesicle of *Gobius paganellus* during reproduction ( $\times 130$ ); C, the same of *Gobius bucchichi* post reproduction ( $\times 180$ ); D, *Paragobiodon xanthosoma*, sperm in the seminal vesicle ( $\times 280$ ). CT, connective tissue of tubule; O, luteinized egg cells; S, sperm; SD, sperm duct (secondary); SE, secretory cells separated from each other; OT, open tubule at the time of reproduction; MS, mucotic secretion around groups of sperm.

immediately after the end of the testis, very close to the genital papilla. This is prominent in *Gobius cobitis* (Fig. 15), *Callogobius* spp., *Pomatoschistus microps* and *Ophiocara* spp. (Fig. 17). In most instances, on this site of attachment, the secretory and gametogenic cells occur side by side.

Subtype 2: The vesicles are attached to elongated parts of the sperm-ducts free of testicular tissue, as in the organs of *Gobius platycephalus* (Fig. 16), *Glossogobius giuris*, *Philypnus maculatus* and *Cryptocentrus* spp. (Fig. 18).

Subtype 3: The testicular and vesicular parts of the system merge and partly mingle, covering the entire length of the sperm-duct, up to the genital

papilla. This is prominent in *Benthophilus stellatus*, *Gobius guttatus*, *Eleotris oxycephalus*, partly in *Pomatoschistus norvegicus* and *Gobiusculus flavescens* (Fig. 19).

An additional dividing character of the seminal vesicles in various species is the form of their distal margins: In some instances, as in *Pomatoschistus* spp. and *Eleotris* (Fig. 19), along the margins are several finger-like extensions, or only slight lobulations. In *Periophthalmus barbatus*, *Glossogobius giuris* and *Dormitator (Eleotris) maculatus*, these extensions and lobulations become very prominent with age (growth) and, as in *Eleotris oxycephalus*, divide the vesicle into several parts (Fig. 19). Such lobulation

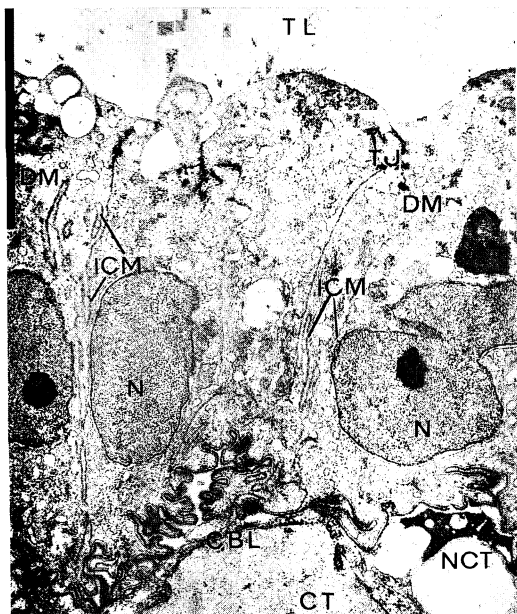


Fig. 9. The Type B-cell from the vesicle of *Lesiurogobius friesii*. N, nucleus; CBL, convoluted basal lamina; CT, connective tissue; ICM, interdigitating cell membranes; TJ, tight junctions; DM, desmosomes; NCT, nucleus of connective tissue of the basal membrane; TL, tubule lumen.  $\times 9,600$ .

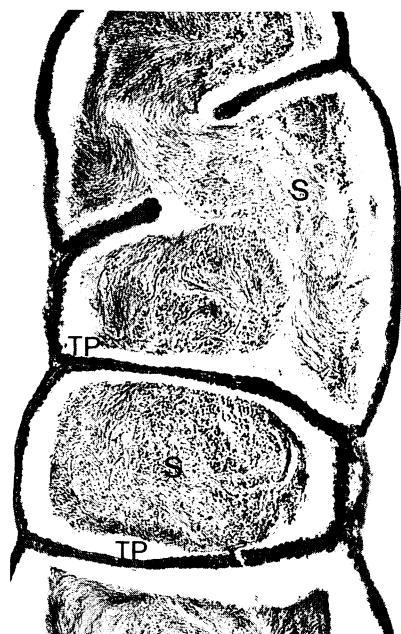


Fig. 10. Seminal vesicle of *Gobius niger* loaded with sperm (S). TP, tubule partition.  $\times 180$ .

was also observed in *Tridentiger* spp. (see also Egami, 1960; Arai, 1964). Contrary to this, in *Gobius cobitis* (Fig. 16), the wing-like seminal vesicles possess smooth or almost smooth margins and do not change in form with growth.

### Discussion

Beginning with the study of Eggert (1931), Vivien (1938) and Egami (1960) and up until the present, it was agreed that although the secretion by the seminal vesicles performs an integral role in the sexual performance of the male goby, it is not directly involved in the process of sperm ripening and fertilization (Miller, 1984). More recent studies, however (Fishelson, 1989), reveal that this seems not to be the case. Artificial sperm-milking from *Gobius paganellus*, *G. buchichi* and *Bathygobius fuscus* during spawning, invariably produced an outflow of gametes within mucotic fluid produced by the semi-

Table 1. Species of gobies with Type A seminal vesicles.

<i>Gobiosoma bosci</i> (Lacepède)
<i>Chaenogobius laevis</i> (Steindachner)
<i>C. urotaenia</i> (Hilgendorf)
<i>C. isaza</i> Tanaka
<i>C. castaneus</i> (Shaughnessy)
<i>Magurnda magurnda</i> Richardson
<i>Apocryptichthys contortis</i> (Day)
<i>Gobiomorus maculatus</i> (Lacepède)
<i>Gobioides broussonneti</i> (Lacepède)
<i>Knipowitchia caucasica</i> (Krawrajsky)
<i>Sicyopterus micrurus</i> (Bleeker)
<i>S. cynocephalus</i> (Valen.)
<i>Odontobutis (Eleotris) obscura</i> (Temme. et Schl.)
<i>Stiphodon elegans</i> (Steindachner)
<i>Stenogobius genivittatus</i> (Valen.)
<i>Stigmatogobius sadanundo</i> (Temme. et Schl.)
<i>Pseudocaprocites lanceolatus</i> (Bl. et Schn.)
<i>Ptereleotris microlepis</i> (Bleeker)
<i>Buenia jeffreysii</i> (Günther)
<i>Cryptocentrus caeruleopunctatus</i> (Valen.)
<i>C. steinitzi</i> Klausewitz
<i>Gobiopsis quenquecincta</i> (H. M. Smith)
<i>Chasmichthys guolus</i> (Guichenot)
<i>Chloea mororanus</i> (Jordan et Snyder)
<i>Crystallogobius linearis</i> (Duben)
<i>Lophogobius cyprinoides</i> (Valen.)
<i>Ophiocara porecephala</i> (Cuv. et Valen.)
<i>O. ophioccephalus</i> (Cuv. et Valen.)
<i>O. aporos</i> (Bleeker)
<i>Phylipnus (Eleotris) dormitator</i> (Bailey)
<i>P. (Eleotris) maculatus</i> (Günther)



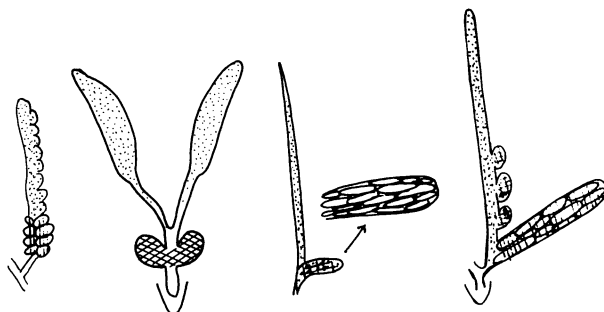


Fig. 11. Testis (punctuated) and Type A seminal vesicles of (left to right) *Knipowitchia caucasica* ( $\times 1.8$ ), *Aphia pellucida*, *Apocryptichthys contortis* and *Ptereleotris microlepis* ( $\times 1.6$ ).

nal vesicle. Histological sections of male organs of *Gobius paganellus*; *G. niger*; *Pomatoschistus* spp.; *Gobiodon* spp.; *Paragobiodon* spp.; and several others, revealed that with the onset of reproduction, the tubules of the seminal vesicles are loaded with sperm, embedded in secretion produced by the surrounding epithelium (Fig. 8). In the bisexual gobies, such as *Coryphopterus nickolsii* (Cole and Robertson, 1988), and in *Paragobiodon* spp. and *Gobiodon* spp.

Table 2. Species of gobies with Type B seminal vesicles.

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<i>Periophthalmus papilio</i> (Pall.)
<i>P. schlosseri</i> (Pall.)
<i>P. sobrinus</i> (Eggert)
<i>P. barbarus</i> (Bleeker)
<i>P. kohlreutheri</i> (Rüppell)
<i>Odontobutis obscurus</i> (Temm. et Schl.)
<i>Odondebeunia bolearica</i> (Pellegrin et Fage)
<i>Pterogobius virgo</i> (Temm. et Schl.)
<i>Aphia minuta</i> Risso
<i>Ptereleotris tricolor</i> (J. L. B. Smith)
<i>Brachygobius xanthozona</i>
<i>Gobiodon rivulatus</i> (Rüppell)
<i>G. citrinus</i> (Rüppell)
<i>Paragobiodon xanthosoma</i> (Bleeker)
<i>P. echinocephalus</i> (Rüppell)
<i>Bryaninops natans</i> Larson
<i>B. amplus</i> Larson
<i>B. yongei</i> (Davis et Cohen)
<i>B. isis</i> Larson
<i>Priolepis cinctus</i> (Rygen)
<i>Luposicya lupus</i> (J. L. B. Smith)
<i>Heteroleotris zonatus</i> (Fowler)
<i>H. diadematus</i> (Rüppell)
<i>H. vulgaris</i> (Klunzinger)
<i>Fusigobius neophytes</i> (Günther)
<i>Trimma mendelssohni</i> Goren
<i>Eviota distigma</i> (Jordan et Seale)

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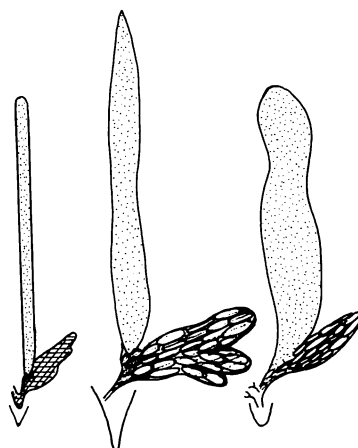


Fig. 12. Testis (punctuated) and Type B seminal vesicles of *Periophthalmus* of (left to right) *P. papilio* ( $\times 2.6$ ), *P. schlosseri* ( $\times 3.4$ ) and *P. sobrinus* ( $\times 2.6$ ).

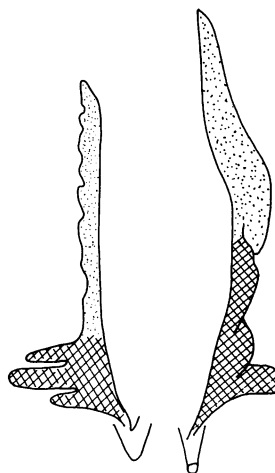


Fig. 13. Seminal vesicles of *Philypnus obscura* (left) and *Pterogobius virgo*.  $\times 1.2$ .

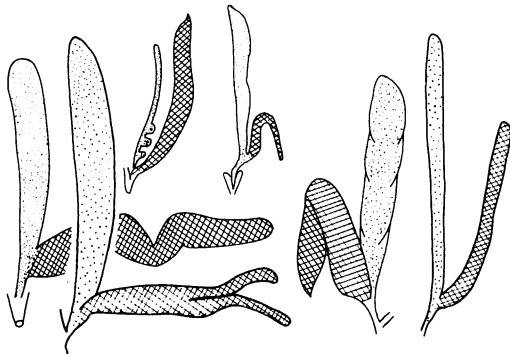


Fig. 14. Type C seminal vesicles of (left to right) *Bathygobius fuscus* ( $\times 1.4$ ), *Istigobius ornatus* ( $\times 1.4$ ), *Brachygobius xanthozona* ( $\times 2.7$ ), *Bathygobius lineatus* ( $\times 1.4$ ), *B. soporator* ( $\times 1.0$ ) and *Fussigobius* sp. ( $\times 2.7$ ).

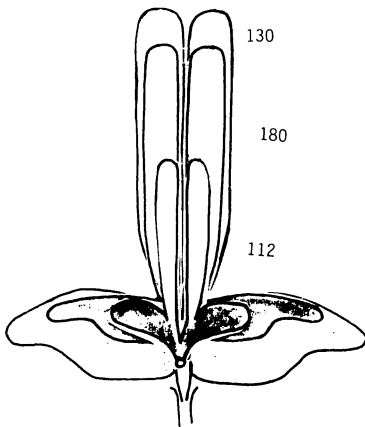


Fig. 15. Type D seminal vesicles of *Gobius cobitis*. Various ages superimposed over each other. Dimensions of fish given in numbers alongside (mmTL).  $\times 1.2$ .

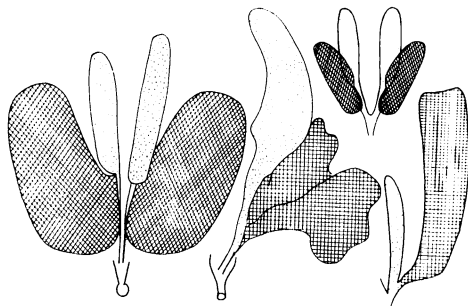


Fig. 16. Type D seminal vesicles of (left to right) *Gobius platycephalus* ( $\times 1.0$ ), *G. guttatus* ( $\times 0.8$ ), *Gobioculus flavescens* ( $\times 0.8$ ) and *Coryphopterus nicholsii* ( $\times 1.0$ ).

(Fishelson, 1989), the vesicles begin to grow and function as the gonads transform from a female one to male. In several species of the gobies studied, such as *Bathygobius fuscus* and *Gobius bucchichi*, ripe sperm was found not only in the basal portion of the vesicles, but also in their distal endings.

In spite of some very important studies dealing with the structure and function of the seminal vesicles or accessory glands in male gobies, very little is known about the microstructure and form in a larger number of species. Weisel (1947) provided the first microscopy description of these organs in *Gillichthys mirabilis*, and Colombo and Burighel (1974) the first electron-microscopy details of the secreting cells in *Gobius jazo*.

Our study showed that in most of the gobies studied three different types of secretory cells form the epithelium of the seminal vesicles: One type is prominent by a very large Golgi ring, composed of several SER cisterns and dominant in most tubules of the vesicles. The second type of cells (B-type) is observed on the merging sites of the vesicles with the sperm-duct. In those cells the Golgi is small, and with this character and the convoluted junction of the cell membrane with the underlying basal membrane, these cells bear a strong resemblance to the secretory cells of the sperm-duct.

During activation the vesicular cells go through a process of ripening and secretion opening into the lumen of the vesicles' tubule. At this stage of development, the Golgi apparatus becomes fragmented and disappears. This seems to be the stage at which

Table 3. Species of gobies with Type C seminal vesicles.

<i>Boleophthalmus pectinirostris</i> (L.)
<i>B. baddaerti</i> (Pall.)
<i>Drombus</i> sp.
<i>Glossogobius giuris</i> (Ham.-Buch.)
<i>Bathygobius soporator</i> (Ham.-Buch.)
<i>B. fuscus</i> (Rüppell)
<i>B. lineatus</i> (Valen.)
<i>B. fishelsoni</i> Goren
<i>B. cocosensis</i> (Bleeker)
<i>B. cyclopterus</i> (Valen.)
<i>Chaeturichthys hexanema</i> (Temm. et Schl.)
<i>Istigobius ornatus</i> (Rüppell)
<i>Exyrias bellissimus</i> (Smith)
<i>Callogobius irasus</i> (Smith)
<i>C. flavobrunneus</i> (Smith)
<i>Amblygobius albimaculatus</i> (Rüppell)
<i>Typhlogobius californicus</i> (Steindachner)

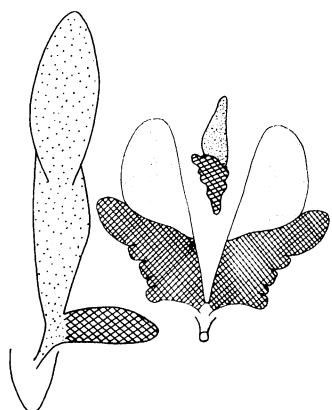


Fig. 17. Seminal vesicles of *Pomatoschistus microps* (right) with a juvenile vesicle in middle ( $\times 5$ ), and of *Ophiocara aporos* ( $\times 1$ ).

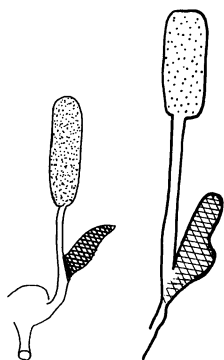


Fig. 18. Seminal vesicles of *Glossogobius giuris* (left) and *Cryptocentrus caeruleopunctatus* ( $\times 1.3$ ).

Colombo and Burighel (1974) described the cells of the seminal vesicles, missing the large Golgi.

According to various authors (summary in Miller, 1984, and recently in Cinquetti and Rinaldi, 1987) a small, additional vesicle or additional gland is situated at the junction between the main seminal vesicles and the sperm-duct. The EM study shows that, in fact, we are dealing here with a transition zone between two secretory epithelia of the seminal vesicle, one characterized by the very large Golgi apparatus, and the secretory cells of the sperm-duct that have the same shape but without the prominent Golgi rings. In addition to this, the cells of the "sperm-duct type" have a very convoluted basal membrane compared to the almost smooth one of the normal secretory cells of seminal vesicles. This type of convolution is also observed in the sperm-ducts.

The third type of cells, named "Leydig-type" by

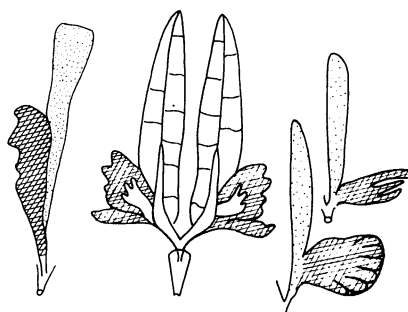


Fig. 19. Seminal vesicles of (left to right) *Benthophilus stellatus* ( $\times 1.0$ ), *Eleotris oxycephalus* ( $\times 0.8$ ), *Pomatoschistus norvegicus* ( $\times 1.2$ ), and *P. minutus* ( $\times 1.2$ ).

Table 4. Species of gobies with Type D seminal vesicles.

<i>Gobius batrachocephalus</i> (Pall.)
<i>G. platycephalus</i> (Kessler)
<i>G. cruentatus</i> (L.)
<i>G. personatus</i> (Pall.)
<i>G. ponticola</i> (Kessler)
<i>G. (Proteorhinus) marmoratus</i> (L.)
<i>G. fluviatilis</i> (Pall.)
<i>G. ophioccephalus</i> (Pall.)
<i>G. guttatus</i> (Pall.)
<i>G. cobitis</i> (Pall.)
<i>G. buccicci</i> (L.)
<i>G. gymnotrachelus</i> (Kessler)
<i>G. paganellus</i> (L.)
<i>G. niger</i> (L.)
<i>G. syrman</i> Norman
<i>G. capito</i> (L.)
<i>G. ramosus</i> (Ginsburg)
<i>Psammogobius knysnensis</i> (J. L. B. Smith)
<i>Rhinogobius giurineus</i> (Rutter)
<i>Pomatoschistus microps</i> (Krøyer)
<i>P. norvegicus</i> (Collet)
<i>P. minutus</i> (Pall.)
<i>P. pictus</i> (Malm)
<i>Gobiusculus flavescens</i> (Fabricius)
<i>Lesuerigobius friesii</i> (Collet)
<i>Tridentiger brevispinis</i> Katsuyama, Arai et Nakamura
<i>T. kuroiwae</i> Jordan et Tanaka
<i>Asteropteryx semipunctatus</i> (Rüppell)
<i>Eleotris oxycephala</i> (Temm. et Schl.)
<i>E. vittata</i> Dumeril
<i>Gobionellus baleosoma</i> (St. Vincent et Jordan)
<i>G. microdon</i> (Gilbert)
<i>Coryphopterus nickolsii</i> (Gilbert)
<i>Dormitator maculatus</i> (Bloch)
<i>Benthophilus stellatus</i> (Sauvage)
<i>Awaous grammepomus</i> (Bleeker)

Colombo and Burighel (1974), are found interstitially within the connective tissues and between the tubule. In some species they occur dispersed as single cells, in others they form islands—aggregations between the convoluted excretory tubule, and in still others they form a compact organ along the testis. These cells are very rich in mitochondria, a typical marker for Leydig cells. As mentioned by Colombo et al. (1980), their number and structure changes in various stages of the gonadal cycle as well as after the experimental addition of hormones. According to Colombo et al. (1980), these cells, as well as the cells of the mesorchial gland, situated within the testis, are very alike and produce androgen-derivates, that serve as pheromones during reproduction.

During reproduction and related activities of male gobies, the secretion of all types of cells within the seminal vesicles is released into the tubule and envelops aggregations of spermatozoa (Fishelson, 1989). As observed in this study this secretion, that fills out the tubule, is PAS-positive, and when fresh, forms a dense liquid. Following fixation, the tubule content looks foamy, fibrous or uniform.

Despite numerous studies describing the seminal vesicles, no clear-cut evidence exists as to their function. Are they storage sites of secretion, as postulated by Miller (1984) and others, or do they improve the viability of the spermatozoa, as discussed by Eggert (1931) and Sundararaj and Nayyar (1967), and so optimize fertilization?

During spawning of *Bathygobius fuscus*, *Gobius paganellus*, *G. cruentatus*, *Paragobiodon* spp. both in nature and aquaria (Fishelson, pers. observ.), the territory-holding males glide over the spawned eggs adhering in their “nest”, rubbing the swollen urogenital papilla on the spawn. During this, the emission of a dense “mucotic” substance is often observed, and analysis taken of this material disclosed sperm and PAS-positive substance. Is this a way of “attaching” the sperm to the egg-capsules? Is it a kind of lubrication with repugnant substances that protects the eggs from parasites or, possibly attracts the additional females to spawn?

A preliminary experiment with cultures of bacteria demonstrated that strips of paper that were impregnated with fluid from seminal vesicles of spawning *Bathygobius cyclopterus* remained clean for 15 days, whilst those not impregnated became invaded within 48 hours. From aquaria observation on spawns without care-taking males, we know that in most

instances the eggs are attacked by bacteria and protozoa and finally degraded. Is this protection the function of the seminal vesicles? These questions remain to be studied.

As for the morphology of the seminal vesicles, the only papers that tried to compare this structure in a group of species are those of Egami (1960) and Arai (1964) with some additional data in Miller (1986). This study showed that within the gobies, several types of seminal vesicles are found, beginning with the simplest one as small or integrated parts of the sperm-duct or testis, and ending with the well-separated wing-like structures, like those of *Gobius cobitis* (Fig. 15).

In numerous species of gobies the ontogenic development of the vesicles starts as accumulations of cells forming a primordium on the sperm-duct (Cole and Robertson, 1988) that with growth enlarges and changes form. De Vlaming (1972) already showed that in *Gillichthys mirabilis*, the growth and development of the sperm-duct and seminal vesicles follows the increase in sex-steroid production. As also shown by Egami (1960) for *Pterogobius zonoleucus* and in this study, in numerous species of gobies the vesicles remain primordial in their structure (Type A, Table 1) also in adults. In *Chasmichthys* spp. and many others, they remain integrated parts of the sperm-duct. In most species, the primordial form is a passing one in subadults and from this, more convoluted and advanced structures develop. The results show that in some taxa, as for example in the genus *Gobius*, the wing-like vesicles (Type D) and their attachment to the sperm-duct, are very uniform and specific for the genus, and in this way they may possess a systematic value. Contrary to this, in all species of *Bathygobius*, the vesicles are elongated, finger-like structures. Lacking this uniformity, in other taxa, as in *Eleotris*, the form of the vesicles is very variable among different species, and one wonders how this can be related to ecology or physiology of reproduction of the different taxa. No cardinal differences were found between the fresh-water, brackish water or marine species. But it is interesting to note that in species digging deep nests in mud, such as *Periophthalmus* spp, *Baleophthalmus*, *Odonotobius*, *Cryptocentrus* and *Ophiocara*, the vesicles are very small compared to the gonads, whereas in species living and breeding in surf-zones, such as *Gobius cobitis* and other ecological related species, the vesicles are large, sometimes larger than the testis. Does this show that the gobies breeding in well-sheltered

nests, where the flow of water is a minimal one, need less secretion for sperm attachment, compared to gobies breeding in turbulent sites where the wash-out of the male product is much more likely?

Additional and specially designed experiments are required in order to elucidate this problem.

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#### ハゼ科魚類の貯精嚢の比較細胞学および形態学的研究

Lev Fishelson

ハゼ科魚類 111 種の貯精嚢を肉眼解剖, 組織的ならびに電子

顕微鏡によって調べた。貯精嚢は、精管に付いており、次の3型の細胞から構成されている分泌上皮によって裏打ちされている: 1) 核の上方に輪状の大きなゴルジ槽をもつ円柱細胞, 2) 複雑に入り組んだ基底板をもつ精管細胞に似た分泌細胞, 3) 精果間膜の一部を構成する間質ライディッヒ細胞群。貯精嚢の形態は、分類群ごとに特異性を示している。最も単純なのは、精管上にある数本の小管であり、最も複雑なのは錯綜した通路の果塊を包んだ大きな翼状形であった。貯精嚢の形態は、発育過程で変るものと、生涯変らないものがあった。繁殖期には、嚢内に精子と分泌物が貯えられ、受精に際し卵群へ放出される。この分泌物の生態学的、生理学的役割について考察した。