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雄性大弹涂鱼贮精囊的形态结构

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摘要：应用组织学和透射电镜研究雄性大弹涂鱼（*Boleophthalmus pectinirostris*，Linnaeus）贮精囊的形态结构。结果表明：（1）贮精囊是其精巢的1对附属腺体，黑色素分布于翼状贮精囊表面；（2）贮精囊外包结缔组织被膜，并向内伸出结缔组织隔膜，被膜和隔膜中含有间质细胞、平滑肌纤维、毛细血管、成纤维细胞和纤维细胞；（3）结缔组织隔膜将贮精囊分隔成为许多小室腔，被膜和隔膜上排列着单层上皮细胞，不同小室腔的上皮细胞发育并不同步，按外形分为柱形和立方形。小室腔内还有片状分泌物，PAS呈阳性反应，提示分泌物为粘多糖蛋白；（4）电镜显示上皮细胞顶部有微绒毛结构。生殖期间上皮细胞内粗面内质网、管状嵴线粒体和高尔基复合体发达，上皮细胞顶部有无膜包裹的分泌颗粒。排精后细胞器退化，胞质中出现大量的大空泡；（5）在生殖高峰期不论贮精囊的近端、中央或远端均先后贮存大量的精子，并混合在分泌物中。

关键词：大弹涂鱼；贮精囊；形态结构；超微结构
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在某些硬骨鱼类中，雄性精巢具有成对的附属腺体（Accessory gland），它们与精巢后端相连，称之为贮精囊（Seminal vesicles）。目前已知胡子鲇科（Claridae）、鰕虎鱼科（Gobiidae）、鳚科（Blennidae）、鲿科（Bagridae）、三鳍鳚科（Tripterygiidae）和蟾鱼科（Batrachoididae）等有些种类具有贮精囊。国外主要研究胡子鲇科、鰕虎鱼科和鳚科鱼类贮精囊的形态结构[1－6]，也有研究贮精囊的功能[7－9]。而国内关于硬骨鱼类贮精囊形态结构的研究仍是空白。大弹涂鱼（*Boleophthalmus pectinirostris*，Linnaeus）隶属鰕虎鱼亚目（Gobioidei），弹涂鱼科（Periophthalmidae），栖息于沿海河口潮间带滩涂的洞穴中，是我国东南沿海的重要养殖鱼类，其雄性生殖系统中有一对贮精囊。赵卫红等[10]研究表明，大弹涂鱼贮精囊的提取液能诱导雌性大弹涂鱼产卵，但其贮精囊的形态结构尚未了解。为此，本实验对雄性大弹涂鱼贮精囊进行了形态结构的研究。

1　材料与方法

实验用大弹涂鱼于生殖季节（2003年4～6月）采自福建省九龙江口滩涂和泉州湾。体长8.2～11.9 cm，体重9.0～29.6 g。生物学测定后解剖观察尿殖系统。取贮精囊固定于Bouin液，石蜡包埋，切片厚6μm，HE染色，部分贮精囊切片作PAS反应检测。Olympus DX51显微镜下观察，Olympus DP11显微数码照相机拍摄。部分贮精囊组织小块以2.5%戊二醛和1%锇酸双重固定，环氧树脂包埋，超薄切片后醋酸铀和柠檬酸铅双重染色，JEM-100CXⅡ透射电镜观察和摄影。

2　结果

2.1　雄性大弹涂鱼贮精囊形态结构

雄性大弹涂鱼贮精囊是1对精巢的附属腺体，贮精囊从精巢后区基部发育并与精巢伸出的输精管相连结，通过左右输精管与膀胱会合后形成尿殖管（Urinogenital duct），然后开口于尿殖乳突（Urinogenital papilla）。贮精囊呈翼状结构，不透明，表面分布许多黑色素，生殖期间左叶贮精囊基部宽2～3 mm，伸长至9～11 mm；右叶基部宽2.5～4 mm，伸长至8.5～11.5 mm（图版Ⅰ－1）。

2.2　雄性大弹涂鱼贮精囊的组织结构

光镜下，贮精囊外包结缔组织被膜（Connective tissue capsula），并向内伸出结缔组织隔膜（Connective tissue partition），结缔组织隔膜连接成网状，将

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贮精囊分隔成为许多小室腔(Locule lumina)(图版Ⅰ-2,3)。小室腔内含有片状分泌物,其部分边缘呈纤维丝状,并且与上皮细胞相连(图版Ⅰ-4)。小室腔大小不一,越靠近中部,小室腔越大。贮精囊横切面的小室17~33个,小室腔的直径为66.1~426 μm。结缔组织被膜和隔膜中含有间质细胞、平滑肌纤维、毛细血管、成纤维细胞和纤维细胞。隔膜和被膜的厚度分别为3.5~20.9 μm和6.9~10.4 μm。间质细胞单个或成群分布于结缔组织被膜和隔膜中,而且主要分布在结缔组织隔膜内(图版Ⅰ-5,6,7)。

各小室的结缔组织被膜和隔膜上均排列着单层的分泌上皮细胞(Secretory epithelial cell,简称上皮细胞)。按其外形可分为柱形和立方形。柱形上皮细胞高22.6~37.2 μm,宽4.5~5.3 μm,胞核呈椭圆形,其位置不定。立方形上皮细胞高13.3~16.9 μm,宽3.9~4.3 μm,胞核圆形或椭圆形,位于上皮细胞的中央(图版Ⅰ-4,8)。

生殖期间上皮细胞顶端出现小泡状分泌物(图版Ⅰ-8,9)。小室腔内的片状分泌物PAS阳性反应呈深紫红色的,提示分泌物为粘多糖蛋白(Mucopolysaccharide-mucin)(图版Ⅰ-10)。生殖高峰期,贮精囊外形饱满,贮精囊的近端、中部和远端的小室腔内均含有精子和分泌物,而且两者混合存在(图版Ⅰ-11)。

2.3　雄性大弹涂鱼贮精囊超微结构

电镜显示生殖期间贮精囊上皮细胞顶部具有微绒毛结构,其长度为1.37~1.85 μm,直径为33~41 nm(图版Ⅱ-1)。上皮细胞胞质粗面内质网发达,线粒体数量较多,其内嵴致密(图版Ⅱ-2,3)。高尔基复合体位于胞核的附近(图版Ⅱ-4)。上皮细胞顶部聚集许多无膜包裹的分泌颗粒,颗粒内电子密度稀疏(图版Ⅱ-1,7)。排精后的上皮细胞出现老化现象,胞质和核质的电子密度增加,细胞体积变小。胞质中的细胞器已退化,线粒体较少,且多不完整,还有一些粗面内质网。细胞中出现大空泡,而且数量较多,可观察到髓样小体(图版Ⅱ-5,6,7,8)。

贮精囊被膜和隔膜中,均有平滑肌纤维、成纤维细胞(Fibroblast)和纤维细胞(Fibrocyte)分布。平滑肌纤维平行地排列,其胞核呈长椭圆形(图版Ⅱ-8,9)。梭形的成纤维细胞,胞核呈长椭圆形,核仁1个,胞质内含有粗面内质网、游离核糖体和高尔基复合体,具有合成和分泌胶原蛋白的细胞特征。胶原蛋白聚合成为胶原原纤维(Collagen fibril),又聚合为胶原纤维(Collagenous fiber),沿其长轴具有等间隔的横纹结构。成纤维细胞在分泌胶原蛋白后呈静止状态,并转变为纤维细胞,呈长梭形,其体积比成纤维细胞小。纤维细胞电子密度增加,胞质减少,细胞器退化。小室腔内的分泌物也含有胶原纤维(图版Ⅱ-10,11,12)。

2.4　生殖过程贮精囊细胞组织的形态变化

恢复期、生殖期间和排精后,大弹涂鱼贮精囊上皮细胞的形态和贮精囊的部分组织有明显的变化。恢复期,贮精囊上皮细胞多呈立方形,细胞核位于细胞中部,小室腔较小,内含分泌物。结缔组织被膜和隔膜厚度较薄,厚度分别为6.96~10.44 μm和3.48~6.96 μm。生殖期间,贮精囊上皮细胞逐渐由立方形变为柱形,胞核的位置多在细胞顶部,细胞顶端外部有纤维丝状分泌物与腔内分泌物连成一体。小室腔的体积不断变大,且分泌物含量增多,分泌物中包围着精子群。部分小室腔之间的隔膜破裂,使相邻的小室腔联合成为更大的小室腔(图版Ⅰ-3)。结缔组织被膜和隔膜不断增厚,厚度分别为6.96~10.44 μm和13.92~20.88 μm,与恢复期相比,结缔组织隔膜增厚较大。排精后,上皮细胞多变为立方形,小室腔内分泌物很少,结缔组织被膜和隔膜的厚度与恢复期相近。

3　讨论

活体解剖观察大弹涂鱼贮精囊,其分泌物为无色半透明,这与中华乌塘鳢贮精囊的分泌物相似。通过光镜观察大弹涂鱼贮精囊切片,其片状分泌物的部分边缘呈纤维丝状,但中华乌塘鳢贮精囊切片,其分泌物不呈片状而呈纤维丝状[1]。

Sundararaj[1]研究雄性异囊鲇(*Heteropneustes fossilis*)贮精囊指出,贮精囊不作为精子贮存之用。Nawar[11]对雄性胡子鲇(*Clarias lazera*)贮精囊的研究表明,贮精囊的近端、中央和远端的分泌物内均有精子群贮存。Rastogi[2]报道生殖期间雄性印度鳗鱼(*Mystus tengara*)贮精囊也有贮存精子的功能。Fichelson等[12]研究雄性非洲鲇(*Clarias gariepinus*)认为贮精囊的远部含精子很少,生殖期间精子只聚积在贮精囊的近端。作者对雄性大弹涂鱼贮精囊的

1)张其永,洪万树,陈仕玺,等. 雄性中华乌塘鳢贮精囊的结构与功能[J]. 动物学报,2004,50(2).

研究表明，在生殖高峰期，贮精囊的近端、中央和远端均能观察到大量精子群混合在分泌物中，因此认为贮精囊在生殖期间起到贮存精子的作用。

对非洲鲇、蜥头鰕虎鱼（*Zosterisessor ophiocephalus*）和异囊鲇贮精囊的研究表明[3,13-14]，贮精囊上皮细胞排精后变成扁平形，终至死亡脱落，在恢复期，结缔组织被膜和隔膜的上皮细胞层通过有丝分裂新生上皮细胞。而大弹涂鱼贮精囊上皮细胞在排精后并未变成扁平形，仍为立方形，也未观察到上皮细胞死亡脱落的现象。

大弹涂鱼贮精囊柱形上皮细胞胞核在胞内的位置与其分泌功能有关。胞核在柱形上皮细胞顶端时，分泌能力较强，能观察到柱形上皮细胞顶端纤维丝状分泌物与小室腔内的片状分泌物相连。而胞核位于中部或底部时，柱形上皮细胞顶端无此现象。因此，推测柱形上皮细胞在分泌粘多糖蛋白过程中，胞核先逐渐上移，分泌完成后，胞核下移到细胞底部。立方形上皮细胞顶端也出现小泡状分泌物，但未见小泡状分泌物与小室腔内的片状分泌物相连。

Fishelson 等[12]对雄性非洲鲇贮精囊研究表明，贮精囊中的间质细胞与精巢中的 Leydig 型间质细胞形态特征相似，因而认为贮精囊中的间质细胞与精巢中产生性类固醇的 Leydig 型间质细胞同源。酶组织化学研究指出[15-20]，非洲鲇贮精囊中的间质细胞是类固醇和葡糖苷酸类固醇合成的场所。色谱质谱分析表明雄性非洲鲇贮精囊液含有各种葡糖苷酸类固醇，在水中完全溶解，可作为性外激素诱发雌性非洲鲇排卵。大弹涂鱼贮精囊组织学切片 PAS 呈阳性反应，提示贮精囊分泌物为粘多糖蛋白，这与中华乌塘鳢贮精囊的分泌物相似[1)]。赵卫红等在陶瓷管道内吊挂大弹涂鱼成熟雄鱼的贮精囊提取液，发现贮精囊提取液有诱导雌亲鱼进入管道排卵的作用，因而认为大弹涂鱼贮精囊液含有性外激素[10]。赵卫红等[21]检测了性成熟大弹涂鱼贮精囊和精巢提取液中的 17α－羟基孕酮和前列腺素水平，研究显示，性成熟大弹涂鱼贮精囊提取液中的 17α－P、PGE_2 和 $PGF_{2\alpha}$ 含量均高于精巢提取液，而且 $PGF_{2\alpha}$ 的含量又高于 PGE_2。上述研究表明大弹涂鱼贮精囊液确实含有性外激素，而且比精巢液的含量高。本研究揭示了大弹涂鱼贮精囊的分泌上皮细胞以及成纤维细胞具备合成分泌的细胞特征，证实了生殖高峰期大弹涂鱼的贮精囊含有精子和分泌物，并能释放性外激素。

1）张其永，洪万树，陈仕玺，等. 雄性中华乌塘鳢贮精囊的结构与功能[J]. 动物学报，2004，50(2).

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Morphology and structure of the seminal vesicle in male *Boleophthalmus pectinirostris*

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Abstract: The mudskipper (*Boleophthalmus pectinirostris*), which inhabits intertidal zones, is an economically important species of marine fish cultured along the coastal areas in southeastern China. However, the supply of fry was completely dependent on capture from intertidal zones, and the number of wild fry has been gradually declining. Therefore, the establishment of a method of artificial propagation for the mudskipper was needed to stabilize the supply. Maturation of the 4th phase oocytes of mudskipper was induced in vitro by pheromones in the extract of the seminal vesicles. The present study attempted to describe the morphological and structural characteristics of the seminal vesicles in male mudskipper by means of histology and transmission electron microscope. Live specimens of male mudskipper, 8.2 – 11.9 cm in body lengths and 9.0 – 29.6 g in body weigths, were collected from the intertidal zones of Jiulong River and Quanzhou Bay, Fujian Province. The animals were dissected to remove the testes and seminal vesicles together with the sperm duct. The lengths and weigths of these organs were measured and taken photos by a SONY-digital camera. Some of the seminal vesicles were fixed in Bouins solution for light microscopy. For electron microscopy, other seminal vesicles were fixed in 2.5% phosphate buffered glutaraldehyde, followed by post-fixation in osmium tetroxide and embedding in Epon. Light microscopic sections were observed under an Olympus DX51 microscope and photographed by an Olympus DP11 digital camera, whereas the transmission electron micrographs were obtained with a JEM-100CX Ⅱ. The results showed that the seminal vesicles were a paired structure which is situated posterior to the testis along the whole length of the nontesticular part of the sperm ducts. Both sperm ducts join to form an unpaired portion which opened on the genital papilla with bladder. Each vesicle was wing-like in shape, opaque and with melanins on exterior. The maximal widths of the left part and the right part were 2 – 3 mm and 2.5 – 4 mm, respectively. The lengths of the left part and the right part were 9 – 11 mm and 8.5 – 11.5 mm, respectively. Histological sections of the seminal vesicles revealed that the organ was enclosed by connective tissue capsula, and connective tissue partitions were prolonged inside. Both capsula and partition were consisted of smooth muscle fibers, blood capillaries, fibroblasts, fibrocytes and interstitial cells. The thickness of the connective tissue partitions and connective tissue capsula was 3.5 – 20.9 μm and 6.9 – 10.4 μm, respec-

tively. The seminal vesicle was divided into many locule lumina, which were 66.1 – 426 μm in diameter, by the connective tissue partitions. Single epithelial cells, which were columnar and cuboidal in shape, arranged along the partition. Bubble-like secretion, which connected to slice-secretion, were at top of the epithelial cells. The columnar cells were 22.6 – 37.2 μm in heights and 4.5 – 5.3 μm in widths. The cuboidal cells were 13.3 – 16.9 μm in heights and 3.9 – 4.3 μm in widths. Slice-like secretion of the seminal vesicles showed PAS positive reaction in dark purple-red colour, suggesting that the secretion should be mucopolysaccharide-mucin. Electron microscopic observation showed that there were microvilli at top of the epithelial cells. The microvilli were 1.37 – 1.85 μm in lengths and 33 – 41 nm in diameters. Many secretory granules without membranes accumulated to the apical portion of the epithelial cell. During the spawning season, rough endoplasmic reticulum, tubular cristae mitochondria and Golgi complex were well developed in the epithelial cells. The cell organelles degenerated and large vacuoles appeared after the seretory granules were released. During the breeding season the seminal vesicles were turgid, more or less conical in shape and the connective tissue partitions and connective tissue capsula were thinner than those in the post-breeding season. At the peak period of spawning, a large number of spermatozoa, which were mixed with the secretion, appeared in proximal, middle and distal parts of the seminal vesicles. This phenomenon suggested that the seminal vesicles played a role in storage of spermatozoa during the breeding season.

Key words: *Boleophthalmus pectinirostris*; seminal vesicles; morphological structure; ultrastructure

图版 I 说明

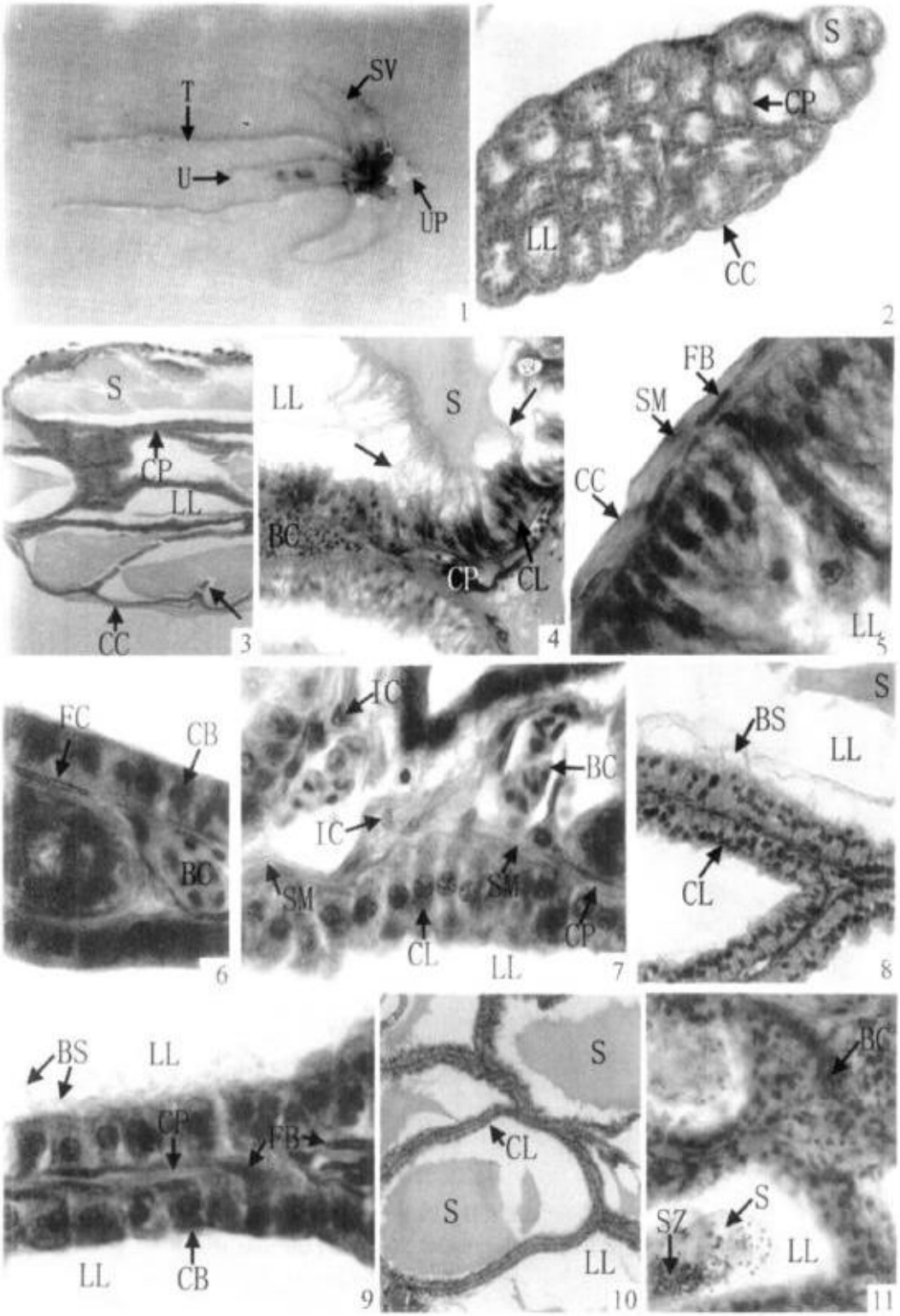
1. 雄性大弹涂鱼贮精囊、精巢、膀胱和尿殖乳突，×100。2. 贮精囊横切面，×100。3. 贮精囊纵切面，示结缔组织隔膜破裂（↑），×40。4. 贮精囊分泌物，其部分边缘呈纤维丝状（↑），×400。5. 结缔组织被膜中的平滑肌纤维和成纤维细胞，×1 000。6. 结缔组织隔膜中的纤维细胞和毛细血管，×1 000。7. 结缔组织隔膜中的间质细胞和平滑肌纤维，×1 000。8. 柱形上皮细胞和小泡状分泌物，×400。9. 立方形上皮细胞和小泡状分泌物，×1 000。10. 贮精囊分泌物呈深紫红色 PAS 阳性反应，×100。11. 精子混合在贮精囊分泌物中，×400。

BC—毛细血管；BS—小泡状分泌物；CB—立方形上皮细胞；CC—结缔组织被膜；CL—柱形上皮细胞；CP—结缔组织隔膜；FB—成纤维细胞；FC—纤维细胞；LL—小室腔；S—分泌物；SM—平滑肌纤维；SV—贮精囊；SZ—精子；T—精巢；U—膀胱；UP—尿殖乳突

Explanation of Plate I

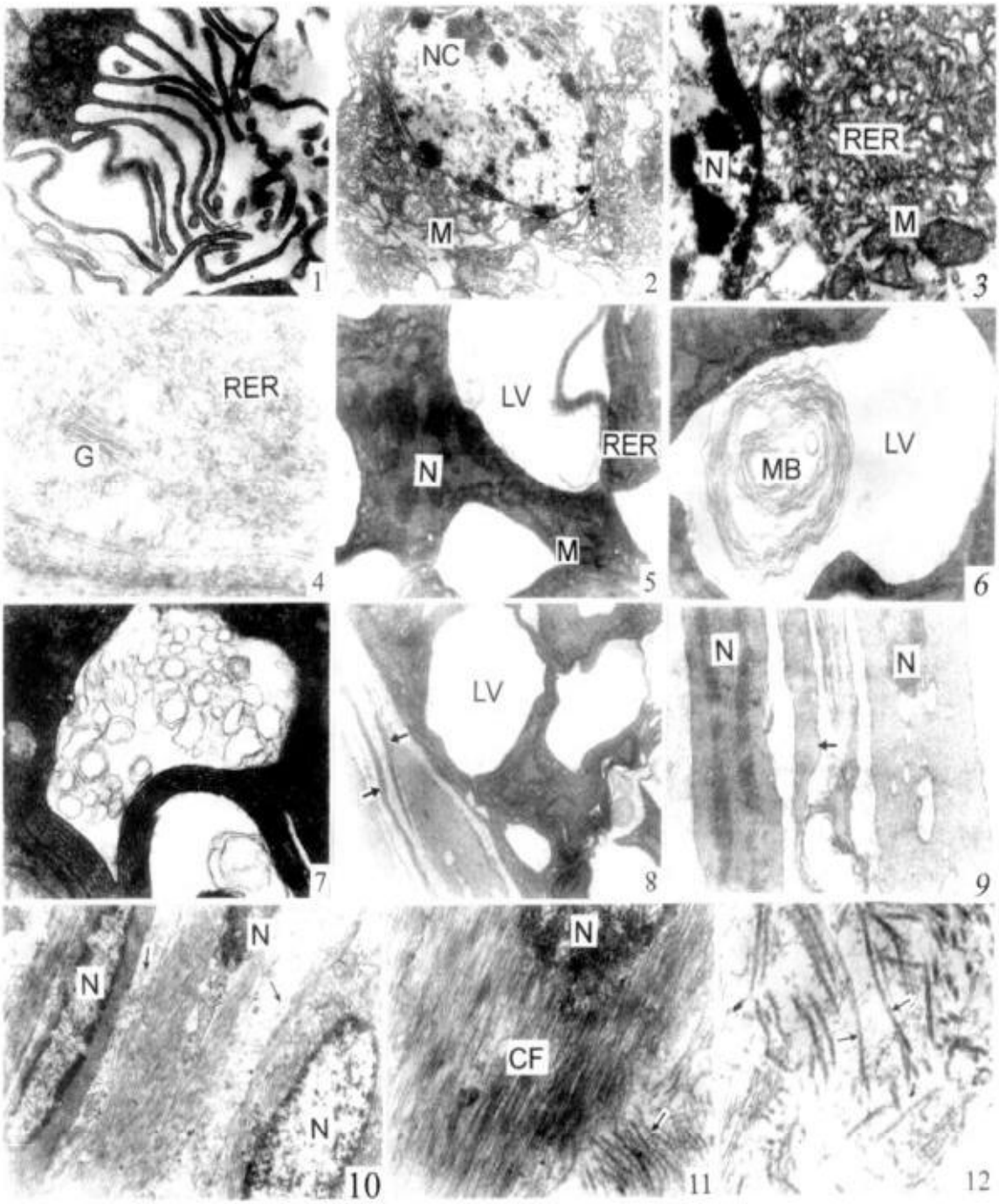
1. The seminal vesicles, testis, urinary bladder and urogenital papilla in male *Boleophthalmus pectinirostris*. ×100.
2. Transverse section of the seminal vesicle. ×100.
3. Longitudinal section of the seminal vesicle, showing collapsing partition(↑). ×40.
4. The secretion of the seminal vesicle, showing fiber-like secretion(↑) around the partial margin of secretion. ×400.
5. Smooth muscle fibers and fibroblasts in the connective tissue capsula. ×1 000.
6. Fibrocytes and blood capillary in the connective tissue partition. ×1 000.
7. The interstitial cells and smooth muscle fibers in the connective tissue partition. ×1 000.
8. Columnar epithelial cells and the bubble-like secretion. ×400.
9. Cuboidal epithelial cells and the bubble-like secretion. ×1 000.
10. The secretion of seminal vesicle showed PAS positive reaction in dark purple-red colour. ×100.
11. The spermatozoa were mixed with the secretion in the seminal vesicle. ×400.

BC—Blood capillary; BS—Bubble-like secretion; CB—Cuboidal epithelial cell; CC—Connective tissue capsule; CL—Columnar epithelial cell; CP—Connective tissue partition; FB—Fibroblast; FC—Fibrocyte; LL—Locule lumina; S—Secretion; SM—Smooth muscle fiber; SV—Seminal vesicle; SZ—Spermatozoa; T—Testis; U—Urinary bladde; UP—Urogenital papilla.



图版 I　Plate I

(图版说明见文末　Explanation of Plate I at the end of the text)



图版 II Plate II

(图版说明见下页 Explanation of Plate II at the next page)

图版 II 说明

1. 贮精囊上皮细胞顶部微绒毛和分泌颗粒(↑),×27 000。2. 贮精囊上皮细胞,示粗面内质网、管状嵴线粒体和核仁,×10 000。3. 贮精囊上皮细胞,示管状嵴线粒体和粗面内质网,×20 000。4. 贮精囊上皮细胞,示高尔基复合体和粗面内质网,×50 000。5. 贮精囊上皮细胞,示大空泡、线粒体和粗面内质网,×10 000。6. 贮精囊上皮细胞,示大空泡和髓样小体,×14 000。7. 贮精囊上皮细胞,示大空泡、粗面内质网和分泌颗粒(↑),×27 000。8. 贮精囊上皮细胞,示大空泡、平滑肌纤维和纤维细胞(⬆),×5 000。9. 贮精囊结缔组织隔膜中的平滑肌纤维和纤维细胞(⬆),×20 000。10. 贮精囊成纤维细胞和纤维细胞,示胶原原纤维胶原纤维(↑),×10 000。11. 贮精囊结缔组织隔膜中的成纤维细胞,示胶原原纤维和胶原纤维(↑),×27 000。12. 贮精囊小室腔内的胶原纤维(↑),×40 000。

CF—胶原原纤维;FB—成纤维细胞;FC—纤维细胞;G—高尔基复合体;LL—小室腔;LV—大空泡;M—线粒体;MB—髓样小体;MV—微绒毛;N—胞核;NC—核仁;RER—粗面内质网;SM—平滑肌纤维

Explaination of Plate II

1. The microvilli and secretory granules(↑) in epithelial cell on the top of the seminal vesicle. ×27 000.

2. Epithelial cell of the seminal vesicle, showing rough endoplasmic reticulum, tubular cristae mitochondria and nucleolus. ×10 000.

3. Epithelial cell of the seminal vesicle, showing tubular cristae mitochondria and rough endoplasmic reticulum. ×20 000.

4. Epithelial cell of the seminal vesicle, showing Golgi complex and rough endoplasmic reticulum. ×50 000.

5. Epithelial cell of the seminal vesicle, showing large vacuoles, mitochondria and rough endoplasmic reticulum. ×10 000.

6. Epithelial cell of the seminal vesicle, showing large vacuoles and myelin body. ×14 000.

7. Epithelial cell of the seminal vesicle, showing large vacuoles, rough endoplasmic reticulum and secretory granules(↑). ×27 000.

8. Epithelial cell of the seminal vesicle, showing large vacuoles, smooth muscle fibers and fibrocytes(⬆). ×5 000.

9. Smooth muscle fibers and fibrocytes(⬆) in the connective tissue partition of the seminal vesicle. ×20 000.

10. Fibroblast and fibrocytes in the connective tissue partition of the seminal vesicle, showing collagen fibrils and collagenous fibers(↑). ×10 000.

11. Fibroblast in the connective tissue partition of the seminal vesicle, showing collagen fibrils and collagenous fibers(↑). ×27 000.

12. Collagenous fibers(↑) in the locule lumina. ×40 000.

CF—Collagen fibril; FB—Fibroblast; FC—Fibrocyte; G—Golgi complex; LL—Locule lumina; LV—Large vacuole; M—Mitochondria; MB—Myelin body; MV—Microvillus; N—Nucleus; NC—nucleolus; RER—Rough endoplasmic reticulum; SM—Smooth muscle fiber.