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近江蛏精子超微形态结构观察及与缢蛏精子的比较

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摘要: 利用扫描电镜和透射电镜分别观察了近江蛏和缢蛏成熟精子的超微形态结构。发现近江蛏精子与缢蛏精子在超微形态结构上差异很大。近江蛏精子为典型的原生型,成熟精子由头部、中段和尾部组成,头部包括顶体和细胞核。近江蛏精子与缢蛏精子顶体均为保龄球状,但近江蛏精子顶体全长比缢蛏短。近江蛏精子细胞核略扁圆形,外缘具8~9个圆弧形凸起;缢蛏精子细胞核则呈圆球状。近江蛏精子中段由线粒体和中心粒复合体构成,线粒体一般5个,个别4~6个;缢蛏线粒体5个或6个。近江蛏精子尾部为细长的鞭毛,轴丝为"9+2"结构,与缢蛏的相同。从近江蛏与缢蛏的精子形态差异看,两者应属于种间差异。

关键词: 近江蛏; 缢蛏; 精子; 超微形态结构
中图分类号: Q 174; S 917　　**文献标识码**:A

近江蛏(*Sinonovacula rivularis* sp. *nov*.)发现于福建省闽江口海域,经研究认定为一新种[1],隶属于双壳纲(Bivalvia)、灯塔蛤科(Pharellidae)、缢蛏属(*Sinonovacula*)。近江蛏贝壳外形特征与缢蛏[*Sinonovacula constricta* (Lamarck,1818)]相似,贝壳上亦具有凹陷沟,容易被认为是缢蛏,有可能与缢蛏具有较近的亲缘关系。与缢蛏比较,近江蛏壳长与壳高的比值较大,并且具有生长较快、更适应低盐度水的特性,当养殖海区因洪水盐度降低、缢蛏不能存活时,近江蛏仍能生存。2009年、2010年近江蛏生产性人工育苗获得成功,已经在福建等沿海河口低盐度海域推广养殖。基于上述优点,近江蛏是一种很有发展前途的河口经济养殖贝类,有望成为我国海水双壳类养殖新品种,但目前对近江蛏的生物学研究报道还很少。

动物精子的形态结构是生殖生物学研究的重要内容之一。由于动物精子的形态结构具有种的特异性,因此,通过研究和比较亲缘关系较近的物种精子的超微结构可以显示出种间差异,同时可以作为分类的辅助依据[2-6]。本研究利用扫描电镜和透射电镜分别观察了近江蛏和缢蛏成熟精子的超微形态结构,旨在为近江蛏的生殖生物学研究提供一定的基础资料,并为近江蛏和缢蛏的区分提供有力证据。

1 材料与方法

1.1 材料

近江蛏和缢蛏样品于繁殖季节(11月)采集。近江蛏取自福建省长乐市梅花镇海区,样品20个,平均壳长72.04 mm(68.84~80.28 mm),平均壳高20.74 mm(19.74~21.82 mm)。缢蛏取自厦门市场,样品20个,平均壳长70.83 mm(62.20~79.60 mm),平均壳高22.39 mm(2.00~24.60 mm)。

1.2 方法

扫描电镜制样　将成熟亲贝解剖,用吸管取精子以2.5%(*V*/*V*)的戊二醛固定。取固定、浓缩后的样品1~5 mL,过滤至1.8 μm的核孔滤膜上。用灭菌海水、蒸馏水分别洗3次,每次10 min;用0%、50%、70%、90%、95%的酒精梯度脱水各1次,然后用无水乙醇重复脱水2次,每个梯

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度 10 min。样品放置在 CO_2 临界点干燥仪中干燥;干燥后的样品喷金 25 s,LEO1530 场发射扫描电镜观察与摄影。

透射电镜制样　　取性腺发育饱满的雄性近江蛏,用吸管吸取精巢组织,迅速地放置于前固定液(2% 的多聚甲醇 + 2.5% 的戊二醛 + 0.05% 的 $CaCl_2$,pH 7.6、0.1 MPBS),并冷藏于 4 ℃的环境中固定。经脱水、包埋,用 NOVA 型号超薄切片机切片,3% 醋酸铀 - 枸橼酸铅双染色;JEM - 2100HC 型高衬度透射电子显微镜观察和摄影。

2　结果

2.1　扫描电镜的观察

近江蛏的精子结构具有双壳类典型的原生型(primitive type)精子的特征,成熟精子由头部、中段和尾部组成(图版Ⅰ-1),精子全长 57 ~ 58 μm。成熟精子头部由顶体和细胞核两部分组成。顶体由顶体头部和顶体柄构成,顶体头部呈保龄球状,长约 0.97 ~ 1.0 μm,最宽处直径约 0.2 μm;顶体柄长 1.6 μm,直径 0.12 μm 顶体全长约为细胞核长的 2.5 倍(图版Ⅰ-2)。精子细胞核长度 0.8 μm,直径 2.0 ~ 2.2 μm,直径大于长度;细胞核外缘具 8 ~ 9 个圆弧状凸起,个别 7 个(图版Ⅰ-2,3,4)。精子中段具 5 个球形线粒体(偶见 4 个)(图版Ⅰ-3,4),直径 0.4 ~ 0.6 μm。精子尾部长约 54 μm,直径 0.15 μm。缢蛏精子亦为原生型,细胞核扁圆形,细胞核长度约 1 μm,直径约 1.8 μm(图版Ⅰ-5,6),精子具 5 个球形线粒体。顶体全长 6.0 ~ 6.1 μm,顶体头部亦呈保龄球状,长约 1.1 μm,顶体柄长约 5.0 μm。顶体全长约为细胞核长的 6 倍(图版Ⅰ-7,8)。

2.2　透射电镜的观察

细胞核外缘圆弧状凸起间的缺刻深(图版Ⅱ-1,2),核内染色质凝集而使核成为一致密物。核后端有浅的核后窝,为近端中心粒嵌入细胞核的部位(图版Ⅱ-3)。

精子中段由线粒体和中心粒复合体(图版Ⅱ-4,5)构成。横切面观有 5 个或 6 个线粒体(图版Ⅱ-5 ~ 7)围绕在远端中心粒周围(图版Ⅱ-6,7),线粒体卵圆形,双层膜组成,其内膜向内折叠形成明显的皱褶嵴突(图版Ⅱ-3)。

精子尾部为细长的鞭毛,由远端中心粒伸出,横切面可见由轴丝及外包轴丝的质膜组成,轴丝为“9 +2”结构,由中央的 2 个单微管和周围的 9 个微管组成(图版Ⅱ-8,9)。

缢蛏精子细胞核扁圆形(图版Ⅲ-1),直径1.7 μm,长度 0.1 μm。线粒体 5 个或 6 个(图版Ⅲ-2,3)。

3　讨论

根据精子形态和受精方式的不同,软体动物的精子分为两大类:原生型(primitive type)精子和修饰型(modified type)精子。绝大多数营体外受精的双壳类软体动物的精子均为原生型精子[7]。近江蛏和缢蛏的精子均为原生型结构,由头部、中段和尾部组成。对双壳类软体动物精子超微结构进行比较分析,有助于对双壳类软体动物开展系统分类及亲缘关系分析[8-9]。不同种的双壳贝类精子形态各不相同,主要取决于精子顶体、细胞核以及线粒体等结构。HEALY 等[3]认为双壳类精子头部的顶体和细胞核的形态可以作为研究不同种类间差异的依据。近江蛏精子与缢蛏精子的超微形态结构具一定相似性,但也存在明显的差异,如表 1 所示。

3.1　精子顶体

近江蛏精子顶体头部与缢蛏精子顶体头部形状相似,均为保龄球状,顶体头部与细胞核之间连接着顶体柄,这与其它双壳类精子的顶体结构具有明显的差异。近江蛏精子的顶体全长约 2.6 μm,顶体头部长约 1.0 μm,顶体柄长约 1.6 μm;缢蛏精子顶体全长约 6.1 μm,顶体头部长约 1.1 μm,顶体柄长约 5.0 μm。近江蛏精子的顶体长度比缢蛏短,缢蛏顶体长度约为近江蛏顶体长度的 2.3 ~ 2.7 倍。

3.2　精子细胞核

两者的精子细胞核形态差异显著。近江蛏精子的细胞核呈扁圆形,直径 2.0 ~ 2.2 μm,长约 0.8 μm。外缘具 8 ~ 9 个圆弧形凸起;缢蛏精子细胞核呈圆球状,直径约 1.8,长约 1.0 μm。本文观察的近江蛏的精子形态与分类地位相近的缢蛏的精子形态存在较大的差异。缢蛏[1,10-11]、长竹蛏(*Solen strictus*)[12]、淡水蛏(*Novaculina chinensis*)[13]的精子细胞核均为圆球形。在双壳类精子中,细胞核外缘呈多个圆弧形凸起的尚未见报道。

表 1 近江蛏与缢蛏精子超微结构的比较

Tab. 1 The comparison of sperm's ultrastructure between *S. rivularis* sp. *nov.* and *S. constricta* (Lamarck)

项目 item		近江蛏 *S. rivularis* sp. *nov.*	缢蛏 *S. constricta*	缢蛏 *S. constricta*
顶体 acrosome	全长(μm) full length	2.6	6.1	6.1~7.1
	头部长(μm) head length	1.0	1.1	1.1
	顶体柄长(μm) length of acrosome handle	1.6	5.0	5.0~6.0
细胞核 nucleus	长度(μm) length	0.8	1.0	1.3
	直径(μm) diameter	2.0~2.2	1.8	1.8
	外缘弧状突起数 the number of outer arc-shaped protrusions	8~9	–	–
精子头部长(μm) sperm's head length		3.4	7.1	7.4~8.4
顶体/头部* acrosome/ head		76.5	85.9	82.4~84.5
顶体柄/头部* acrosome handle/ head		47.1	70.4	67.6~71.4
顶体/细胞核* acrosome/ nucleus		3.3	6.1	4.7~5.5
顶体头部形状 acrosome shape of the head		保龄球状 bowling shape	保龄球状 bowling shape	保龄球状 bowling shape
细胞核形状 nuclear shape		呈扁圆形 oblate	圆球状 spherical	矮圆桶 short barrel
线粒体数量(个) mitochondria(piece)		5(偶见 4 或 6) 5(occasionally 4 or 6)	5~6	6

注:顶体/头部. 顶体全长与精子头部长度的百分比;顶体柄/头部. 顶体柄长度与精子头部长度的百分比;顶体/细胞核. 顶体全长与精子细胞核长之比。

Notes: acrosome/head. the percentage between acrosome length and sperm-head length; acrosome handle/head. the percentage between the length of acrosome handle and sperm-head; acrosome/nucleus. the ratio between acrosome length and sperm nucleus length.

3.3 线粒体

近江蛏精子线粒体一般 5 个,偶见 4 或 6 个。缢蛏精子线粒体一般为 5~6 个。竺俊全等[10]亦报道缢蛏具 6 个线粒体。

综上所述,近江蛏精子细胞与缢蛏的精子细胞在超微形态结构上具显著差异。精子超微形态结构是动物分类的依据之一,也是分析不同动物类群之间亲缘关系的主要依据[8]。"顶体与细胞核的形态是区分不同物种的主要依据"[14],"不同种类,顶体与细胞核的形状、大小和结构也不同","顶体在大小和形状上都有'种'的特征"[15]。本研究认为,软体动物精子的超微形态结构差异可作为分类的依据,近江蛏与缢蛏应分别属于不同的种类。

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Comparative ultrastructure of spermatozoa of *Sinonovacula rivularis* sp. *nov.* and *Sinonovacula constricta*

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Abstract: *Sinonovacula rivularis* sp. *nov.*, found in Minjiang Estuary Waters of Fujian Province, was identified as a new species after study, and it belonged to *Sinonovacula*, Pharellidae, Bivalvia. The outer shape for the shell of *S. rivularis* sp. *nov.* was similar to that of *S. constricta* (Lamarck, 1818), and it was likely that *S. rivularis* sp. *nov.* and *S. constricta* had a relatively closer affinity. Because the morphological structure of spermatozoon had the specificity of species, the differences between the species could be shown by studying and comparing the ultrastructure of spermatozoa with relatively closer affinity and could be used as the auxiliary evidence for related classification. This article observed the ultrastructure of mature sperms of *S. rivularis* sp. *nov.* and *S. constricta* respectively by scanning electron microscope and transmission electron microscopy aiming at providing a certain basic data and related figures for the reproductive biology research of *S. rivularis* sp. *nov.* and also providing strong evidences for differentiating *S. rivularis* sp. *nov.* and *S. constricta*. The samples of *S. rivularis* sp. *nov.* and *S. constricta* were collected in mating season (in November). Samples of *S. rivularis* sp. *nov.* were collected from coastal waters of Meihua town, Changle city, Fujian, while samples of *S. constricta* were collected from Xiamen Seafood Market. The sample preparation method of scanning electron microscope: dissecting the mature brood stock, extracting the sperms by suction tube and fixating by glutaric dialdehyde of 2.5% (*V*/*V*); taking 1 – 5 mL of the fixated and concentrated sample, and filtering it into the nuclear porous filter membrane at the size of 1.8 μm; then washing them three times with sterilized sea water and distilled water respectively, each time 10 minutes, dehydrate it once using 0%, 50%, 70%, 90% and 95% graded ethanol respectively; and then dehydrate it twice using absolute ethyl alcohol, 10 minutes for each grade. The sample is to be dried in CO_2 Critical Point Dryer; the sample after being dried is to be sprayed with metal for 25 s; and using LEO1530 field emission scanning electron microscope to observe and take photographs. The sample preparation method of transmission electron microscopy: selecting male *S. rivularis* sp. *nov.* with full and well-developed gonad,

absorbing spermary by the suction tube, and quickly placing it into the fixative solution (2% paraformaldehyde +2.5% glutaraldehyde +0.05% $CaCl_2$, pH 7.6, 0.1 MPBS), and then fixating it in an environment of 4 ℃, after dehydration and embedding, and then cutting it into slices by NOVA ultramicrotome; dyeing it with 3% uranyl acetate-lead citrate; using JEM-2100HC High contrast TEM to observe and take photographs. The ultrastructures of spermatozoa of *Sinonovacula rivularis* sp. *nov*. and *Sinonovacula constricta* were examined by transmission electron microscopy (TEM) and scanning electron microscopy (SEM). The results showed that there were a great deal of differences between *S. rivularis* sp. *nov*. and *S. constricta*. The mature sperm of *S. rivularis* sp. *nov*. was a typical primitive type, consisting of head, middle piece and tail. *S. rivularis* sp. *nov*. and *S. constricta* both have ellipse-shaped acrosome, but the length of acrosome for *S. rivularis* sp. *nov*. was shorter than *S. constricta*. The nucleus of *S. rivularis* sp. *nov*. was oblate with 8-9 arc-tubes in outer margin, whereas the nucleus of *S. constricta* was spherical. The middle piece of *S. rivularis* sp. *nov*. contained five mitochondria, and typically a proximal and a distal centriole. Few mature sperm of *S. rivularis* sp. *nov*. contained 4 or 6 mitochondria. There were 5 or 6 mitochondria of *S. constricta* based on published information. The flagellum exhibited the typical 9 + 2 microtubule structure in both species.

Key words: *Sinonovacula rivularis* sp. *nov*.; *Sinonovacula constricta*; spermatozoon; ultrastructure

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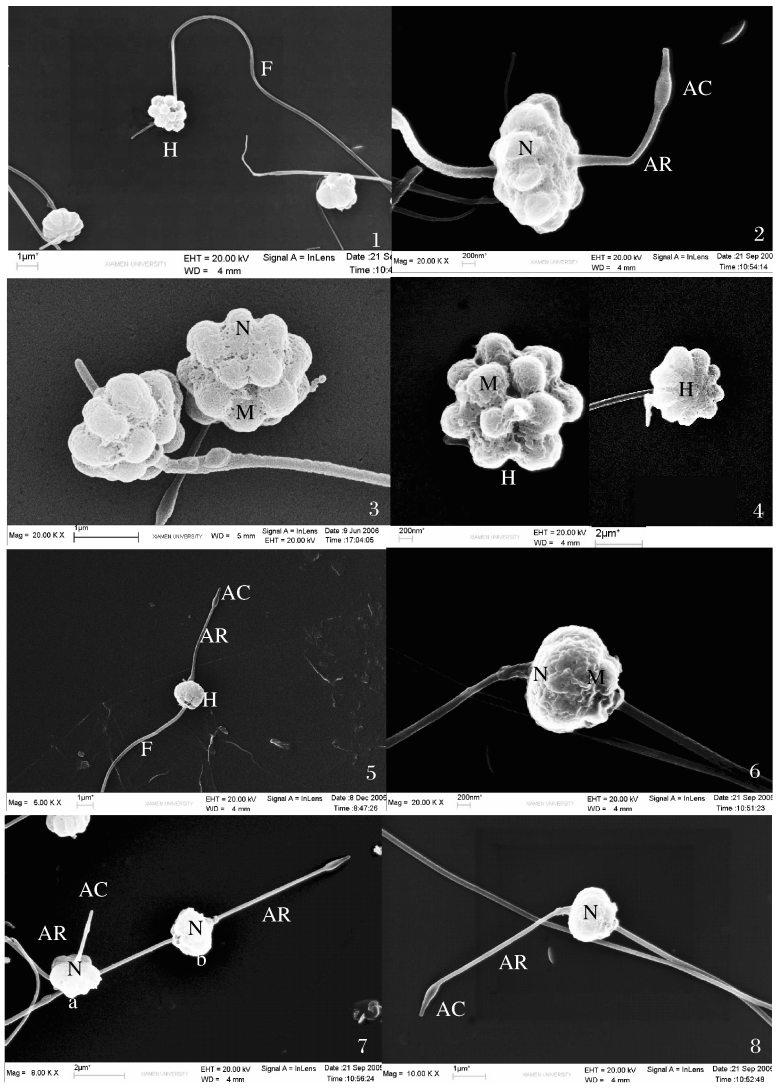
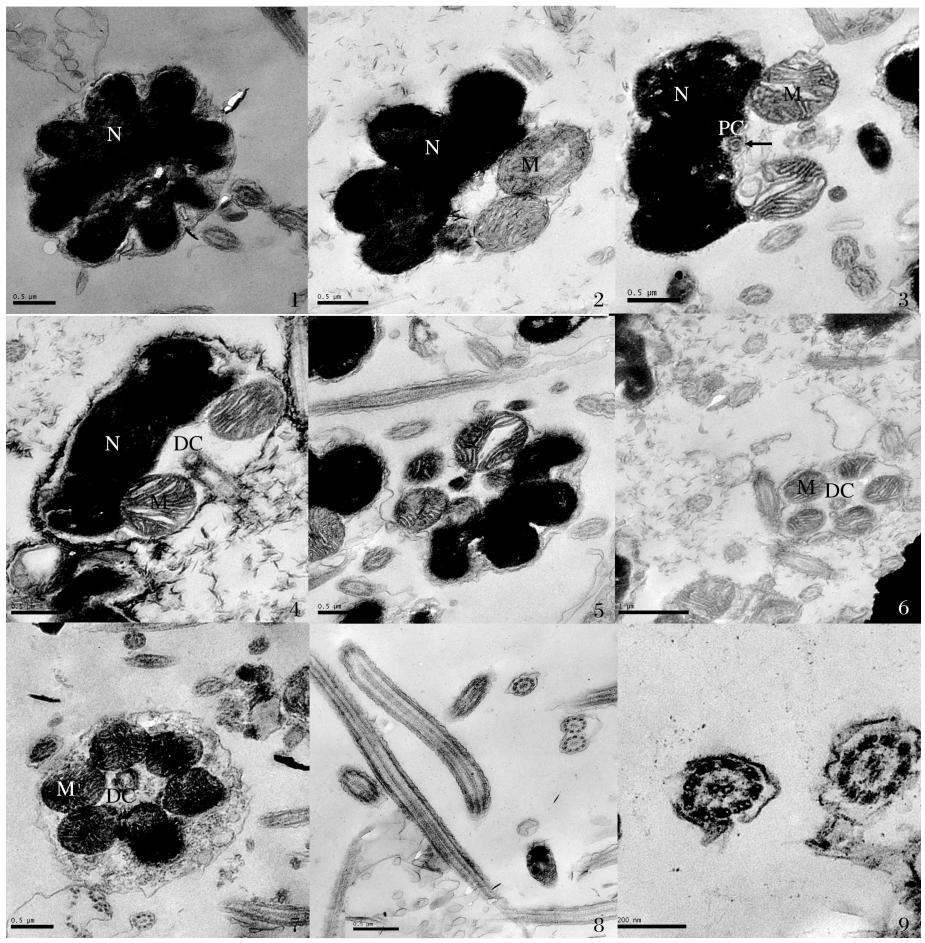


图版 I　近江蛏和缢蛏的精子外部形态

1. 近江蛏精子; 2. 近江蛏精子头部和顶体; 3. 近江蛏精子细胞核和线粒体; 4. 示近江精子 4 个线粒体; 5. 缢蛏精子; 6. 缢蛏精子细胞核; 7. 近江蛏与缢蛏精子顶体和细胞核的比较(a. 近江蛏,b. 缢蛏); 8. 缢蛏精子顶体。

Plate I　The ultrastructure of spermatozoa of *S. rivularis* sp. *nov*. and *S. constricta*

1. Spermatozoon of *S. rivularis* sp. *nov*. ; 2. Head and acrosome of *S. rivularis* sp. *nov*. ; 3. nucleus and mitochondria of *S. rivularis* sp. *nov*. ; 4. Transverse section through midpiece, showing four mitochondria of *S. rivularis* sp. *nov*. ; 5. Spermatozoon of *S. constricta*; 6. nucleus of *S. constricta*; 7. Ultrastructure comparison of acrosome and nucleus of *S. rivularis* sp. *nov*. and *S. constricta*(a. *S. rivularis* sp. *nov*. , b. *S. constricta*); 8. acrosome of *S. constricta*.

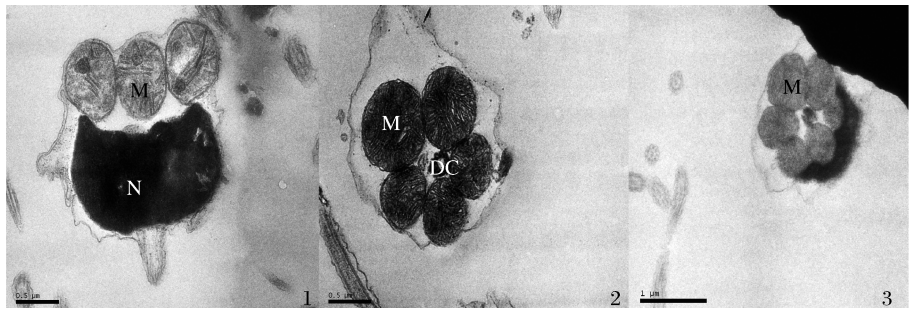


图版Ⅱ 近江蛏的精子切面形态结构

1. 近江蛏精子细胞横切面；2. 近江蛏精子线粒体横切面；3. 示近端中心粒；4. 示远端示中心粒；5～6. 示5个线粒体；7. 示6个线粒体；8～9. 近江蛏精子尾部横切面和纵切面。

Plate Ⅱ Longitudinal and transverse section of spermatozoon of *S. rivularis* sp. *nov*.

1. Transverse section of spermatozoon of *S. rivularis* sp. *nov*. ; 2. Transverse section through mitochondria of *S. rivularis* sp. *nov*. ; 3. Longitudinal section through midpiece, showing proximal centriole; 4. Longitudinal section through midpiece, showing distal centriole; 5 – 6. Transverse section through midpiece, showing five mitochondria; 7. Transverse section through midpiece, showing six mitochondria; 8 – 9 Longitudinal and transverse section through flagellum of *S. rivularis* sp. *nov*.



图版Ⅲ　缢蛏的精子切面形态结构

1. 缢蛏精子细胞纵切面; 2. 示 5 个线粒体; 3. 示 6 个线粒体。

Plate Ⅲ　Longitudinal and transverse section of spermatozoon of *S. constricta*

1. Longitudinal section of spermatozoon of *S. constricta*; 2. Transverse section through midpiece, showing five mitochondria; 3. Transverse section through midpiece, showing six mitochondria.