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罗氏沼虾胚胎附着机制的初步研究

姚俊杰,赵云龙,段晓伟,崔丽丽
(华东师范大学 生命科学学院,上海 200062)

摘要:甲壳动物十足目的大多数种类,其受精卵自产出后就附着于亲体腹部,并完成胚胎发育过程直至孵化,这对于胚胎的发育、保护和幼体散布有重要的生物学意义。胚胎的附着机制较为复杂,与刚毛、卵柄、卵索、卵膜和黏液腺有关:卵柄和卵索的形成决定了胚胎发育的顺利进行;卵膜的变化影响着胚胎的附着;黏液腺在胚胎附着过程中形成卵柄和外层卵膜,胚胎是依靠卵柄或卵索附着于腹肢的刚毛上,进而完成胚胎发育过程。本研究采用常规组织学和扫描电镜的方法研究了罗氏沼虾(*Macrobrachium rosenbergii*)胚胎及其附着结构。结果表明,胚胎附着与母体腹肢的结构、黏液腺分泌的黏液相关,也与胚胎表面结构、次级卵膜的形态和结构密切相关。原肢底节和内肢刚毛密而长,基部有细小的分支,有利于胚胎的附着,外肢刚毛羽状则有利于胚胎的托附和保护。抱卵雌虾腹肢的原肢和内肢上布满腹肢黏液腺分泌的黏液;携卵刚毛中空、壁厚,刚毛外壁由微绒毛状结构组成,扩大了携卵刚毛的表面积,以利胚胎附着。胚胎外表面由初级卵膜和次级卵膜组成,次级卵膜主要由卵巢黏液腺分泌的黏液参与形成。排卵时,受精卵依靠坚韧的次级卵膜相互粘连,同时,部分受精卵在刚毛上滑动、旋转,并借助次级卵膜逐渐形成了卵柄或卵索固着在腹肢刚毛上。从受精卵到胚胎孵化,胚胎之间最主要的连接方式是依靠次级卵膜的连接。胚胎的表面有许多褶皱和黏液,利于胚胎的附着以及胚胎对溶氧和小分子物质等的通透。以上结构与附着相适应,保证了胚胎发育的顺利进行和幼体的正常孵化。本研究旨为进一步认识甲壳动物胚胎附着机制、胚胎发育和人工育苗的研究提供参考。[中国水产科学,2007,14(1):67-73]

关键词:罗氏沼虾;胚胎;附着
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虾、蟹在繁殖过程中大多存在抱卵现象,除了对虾科种类的受精卵直接散落水中外,其余种类的受精卵产出后依靠特殊的结构附着于雌体腹肢的刚毛上,完成胚胎发育。虾蟹抱卵的附着结构及附着机制是动物繁殖中一种特别的现象,它与胚胎发育是否顺畅密切相关,因此,多年来一直引起学者们的关注。Fisher 等[1]研究了巨指长臂虾(*Palaemon macrodactylus*)外层卵膜的形成;Johnson 等[2]报道了美洲螯龙虾(*Homarus americanus*)抱卵雌虾黏液腺在胚胎附着中的作用;杨万喜等[3]报道了日本沼虾(*Macrobrachium nipponense*)外骨骼黏液腺的分泌与胚胎附着的关系。相关研究进一步表明,卵柄和卵索的形成保证了胚胎发育的顺利进行[4-6];卵膜的变化影响着胚胎的附着[7];黏液腺在胚胎附着过程中形成卵柄和外层卵膜,这是胚胎附着中的重要因素[8]。以上这些是人们对胚胎的附着机制的初步认识,但由于抱卵虾蟹胚胎的附着机制较为复杂,仍需进一步深入研究。

罗氏沼虾(*Macrobrachium rosenbergii*)是中国重要的经济养殖虾之一。其个体平均每次抱卵量为6 000~10 000粒,在28 ℃水温下,受精卵附着在雌体腹肢刚毛上发育至卵内幼体孵化约需480 h[9],因此有必要对罗氏沼虾胚胎的附着结构以及附着关系进行深入研究,了解十足类虾、蟹胚胎的附着机制,为抱卵经济虾、蟹的人工育苗及品种改良提供相关基础资料。

1 材料与方法

1.1 材料

实验用罗氏沼虾亲虾取自江苏省吴江市八都振兴水产良种场,该虾于2003年10月由浙江南太湖淡水水产种业有限公司(浙江湖州市省级罗氏沼虾

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作者简介:姚俊杰(1968—),男,博士研究生,从事水生动物发育生物学研究. E-mail:Junjieyao@163.com
通讯作者:赵云龙. E-mail:ylzhao@bio.ecnu.edu.cn

良种场)从缅甸引进。将亲虾分成 3 组,每组 10 只雌虾、5 只雄虾。共 45 只亲虾分别饲养于 3 个室内水泥池中,日换水量 1/6～1/10;28 ℃人工调控恒温;充气泵增氧;每天在 8∶00 和 17∶00 左右投喂螺蛳和配合饲料,并及时清除残饵和粪便等。亲虾交配后,将雌虾移至水泥池内的小网箱(1.0 m×1.0 m×0.5 m)中饲养,小网箱内 10 只抱卵雌虾的体长为(8.57±0.80)cm,体质量为(14.69±5.33)g。雌虾抱卵后,取抱卵虾附有胚胎的腹肢内肢,切成小段,根据组织切片和扫描电镜制片的要求,取部分在解剖镜下观察,其余部分分别用 Bouin's 液和 3%戊二醛固定。

1.2 组织切片

将附有胚胎的内肢材料用 Bouin's 液固定 24 h 后,系列乙醇梯度脱水,水杨酸甲酯与二甲苯透明,常规石蜡包埋,AO-B20 切片机切成 6 μm 的连续切片,苏木精-伊红(HE)染色,Olympus 显微镜观察并拍照。

1.3 扫描电镜制片

将附有胚胎的内肢材料,用 0.1 mol/L 磷酸缓冲液(pH 7.4)配制的 3%戊二醛及 1%锇酸进行双重固定,系列乙醇梯度脱水,用醋酸异戊酯置换,CO_2 临界点干燥,离子溅射仪喷金,Hitachi S-450 型扫描电镜观察并拍照。

2 结果与分析

2.1 抱卵附肢

罗氏沼虾雌虾腹肢 6 对,双肢型,原肢(Protopodite)底节(Basipodite)、内肢(Endopodite)和外肢(Exopodite)均具致密的刚毛。胚胎附着于原肢底节和内肢的刚毛上,刚毛长而密(图版Ⅰ—1)。外肢的刚毛有羽状分支,无胚胎附着(图版Ⅰ—2)。抱卵雌虾的前 4 对腹肢为携卵肢,每次排出的约 10 000 个受精卵靠卵柄或卵索均匀地附着分布于这 4 对携卵肢的刚毛上,直到完成整个胚胎发育孵化出幼体。扫描电镜观察,在刚毛囊及刚毛基部有大量黏液腺分泌的白色分泌物(图版Ⅰ—7)。

2.2 携卵刚毛

刚毛簇生于携卵附肢上的刚毛囊中(图版Ⅰ—7),刚毛基部有细小分支(图版Ⅰ—6),外壁有许多黏液附着(图版Ⅰ—8)。横切面显示,腹肢刚毛中空(图版Ⅱ—1),刚毛基部的一端有内含物(图版Ⅰ—3),末端则没有内含物。刚毛的内外壁皆无膜物质覆盖,而由一定厚度的纤维状物构成(图版Ⅰ—8;图版Ⅱ—1)。在电镜下观察,刚毛外壁凹凸不平,均由微绒毛状结构组成(图版Ⅱ—2),刚毛末端具较多分支。

2.3 胚胎

罗氏沼虾排出的受精卵由次级卵膜相互粘连(图版Ⅱ—3),胚胎发育至幼体孵化时,次级卵膜形成的胚胎间连接依然很强(图版Ⅱ—4)。图版Ⅰ—4 示受精卵的次级卵膜,次级卵膜使受精卵相互粘连(图版Ⅰ—5)。母体排出的受精卵随着在虾体腹部移动、旋转、缠绕形成卵索或卵柄(图版Ⅱ—7),胚胎就依靠卵索或卵柄牢牢地粘附在充满微绒毛状突起的刚毛上。胚胎附着在一根刚毛上或多根刚毛上,也有一些胚胎与刚毛基部相连,刚毛连接于次级卵膜内(图版Ⅱ—5、6)。胚胎表面有许多褶皱,布满黏液腺分泌的黏液(图版Ⅱ—7、8),胚胎之间在相对位置改变的过程中,也会形成卵索。

3 讨论

3.1 黏液腺分泌的黏液与胚胎附着

黏液腺的活动与胚胎的附着有密切的关系。随着抱卵虾蟹的个体发育,黏液腺的生理活动也会发生变化,其数量与分泌的黏液量不断增加,且通过分泌导管输送到腹肢表面,为胚胎的附着奠定基础[1,3,10]。当雌体进入抱卵阶段,其外骨骼上出现大量分泌导管和刚毛囊,黏液腺分泌的黏液能顺利通过分泌导管达到腹部的携卵刚毛[6],为胚胎的附着提供条件。

在受精卵的形成过程中,随着精子入卵后引发皮层反应,卵巢黏液腺分泌的黏液参与了受精卵次级卵膜的形成。杨万喜等[3]在研究日本沼虾的附着结构后也认为,外层卵膜和卵柄是由黏液腺的分泌物形成。本研究对罗氏沼虾的实验观察也显示,腹肢原肢底节和内肢有黏液腺存在,其产生的黏液分泌到腹肢表面,并在卵子附着过程中形成卵柄、卵索等附着结构。因此,黏液腺分泌的黏液不仅形成了胚胎的次级卵膜,也形成了胚胎的附着结构,它们是十足类抱卵虾蟹胚胎附着的物质基础。

3.2 腹肢刚毛、次级卵膜与胚胎附着

罗氏沼虾抱卵腹肢外肢的刚毛呈羽状,有利于对胚胎的保护,同时在运动中能激起水流,有利于胚胎对溶氧的吸收。原肢底节和内肢刚毛较长,非羽状,这些刚毛的基部具较多的细小分支,在刚毛的末

端也有较多的分支,它们与胚胎的次级卵膜相连接,因此,原肢底节和内肢刚毛作为携卵刚毛,其结构有利于胚胎的附着,是对较大的胚胎附着量的适应。罗氏沼虾腹肢刚毛呈现出两方面的功能,一是胚胎的附着;二是使附着的胚胎存在一定的活动范围。伴随着母体的运动,大量密集的胚胎处于一定范围内的波动中,避免水中污物、尘渣等吸附在胚胎表面,同时保持胚胎表面具有对溶氧、小分子物质等的通透性,从而保证胚胎发育的顺利进行。

雌虾排卵时,将储存在纳精囊中的精子排出。精子通过顶体反应入卵并将卵子激活,卵子发生皮层反应,形成初级卵膜,同时卵巢分泌的黏液包被在初级卵膜之外形成次级卵膜。两层外膜为胚胎提供了良好的发育环境,使胚胎免受外界物质侵扰,利于胚胎与外界进行氧气、物质交换等。在受精卵排出体外后不久,雌虾孵化室中出现由母体腹肢黏液腺分泌的黏液。携卵刚毛不断摆动将黏液涂在胚胎的表面,使胚胎表面具有黏性,在胚胎表面形成次级卵膜。次级卵膜在受重力和胚胎旋转的影响下逐渐成为一条扭转的卵柄。在日本沼虾(*Macrobrachium nipponense*)[3]、红螯相手蟹(*Sesarma haematocheir*)[5]中也观察到相似的现象。在结构上,携卵刚毛中空且具一定的厚度,有较大的韧性和灵活性,利于大量胚胎的附着以及在母体游泳运动中保持其附着的牢固性。携卵刚毛中空但内外壁均无膜覆盖,说明携卵刚毛不具备运送物质的能力,母体不能通过携卵刚毛为胚胎提供营养,携卵刚毛的生理功能就是胚胎的附着。刚毛的外壁由微绒毛状结构组成并有细小分支,这就最大限度地保证了胚胎的附着。

前人的研究表明,黏液腺分泌的黏液与胚胎的附着有关[6,11-12];对三疣梭子蟹(*Portunus trituberculatus*)卵附着机制的研究也显示了原肢底节和内肢黏液腺的存在,其所分泌的黏液在胚胎附着过程中能形成卵柄和外层卵膜[8];卵膜具有溶胶性和延展性[7]。在胚胎表面,次级卵膜形成许多褶皱,分布有较多的黏液,利于胚胎与腹肢刚毛以及胚胎相互间的附着,使胚胎相互粘连,并依靠卵柄及卵膜牢固地附着在抱卵雌虾的携卵腹肢上。

胚胎之间最主要的连接方式是依靠次级卵膜的连接。从受精卵期到[illegible]状幼体期,胚胎依靠坚韧的次级卵膜相互集聚在一起,不至于被母体运动时产生的水流冲散,甚至当胚胎孵化时,[illegible]状幼体破膜而出,而卵膜之间仍然相互连接。刚排出的胚胎中一部分凭借形成的卵柄、卵索等紧紧连结在腹肢的携卵刚毛上,同时借助于胚胎的相互黏连,从而实现所有胚胎对母体的附着。

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Mechanism of embryo attachment in freshwater prawn, *Macrobrachium rosenbergii*

YAO Jun-jie, ZHAO Yun-long, DUAN Xiao-wei, CUI Li-li
(School of Life Science, East China Normal University, Shanghai 200062, China)

Abstract: Fertilized eggs of most decapods in crustacean attach to female abdomen and finish their embryonic development. Embryo attachment has important biological significance in embryo protecting and scattering of larvae. The mechanism of egg-attachment is complex. People realized that embryo attachment has relevance to seta, egg stalk, egg funiculus, egg membrane and also mucus gland. Embryonic development depends on the formation of egg stalk and egg funiculus. Embryo attachment was affected by the differentiation of egg membrane. Egg stalk and outer egg membrane were formed from mucus during development. Embryos attached to female pleopods by peculiar structure, such as egg stalk, egg funiculus. In the present study, the structural features of embryo attachment systems of the freshwater prawn, *Macrobrachium rosenbergii* were investigated by scanning electron microscopy and histological methods. The results showed that embryo attachment have relevance to not only structure of pleopods, mucus that mucus gland excretive, but also characteristics embryo surface, morphology and structure of the secondary membrane. Embryos adhered to the setae of pleopodal basipodite and endopodite for hatching followed by spawning but no embryo attached on the setae of exopodite. The setae of pleopodal basipodite and endopodite were smooth for embryo attachment while the setae of exopodite were branched and suitable for collecting and protecting embryo. Lots of mucus secreted by pleopod mucus gland was distributed on pleopodal basipodite and endopodite. Ovigerous setae were hollow and thick. The surface of setae was full of microvillus. These structures enlarged the surface of setae, which benefited to attachment of embryos. The surface of embryos was composed of primary egg membrane and secondary egg membrane. The secondary egg membrane was formed mainly by mucus which from ovary mucus gland. When eggs were discharged, fertilized eggs were connected to each other by means of the secondary membrane. Egg stalk and funiculus were formed gradually from the secondary membrane when fertilized eggs gliding and rotating on the setae. From fertilized egg stage to late embryonic development stage, the main connection between embryos was the secondary membrane. A lot of wrinkles and mucus were found on the surface of embryos. These structures not only benefit the attachment for embryos, but also benefit the absorbing of oxygen and the passage for some substance. The characteristics of these embryo attachments ensure embryonic development and hatching successfully. The results obtained in the present study help know better on mechanism of egg—attachment, embryonic development and larva culture of *Macrobrachium rosenbergii*, and also other species of crustacean. [Journal of Fishery Sciences of China, 2007, 14(1): 67—73]

Key words: *Macrobrachium rosenbergii*; embryo; attachment

Corresponding author: ZHAO Yun-long. E-mail: ylzhao@bio.ecnu.edu.cn

图版说明

图版Ⅰ　罗氏沼虾胚胎的附着结构

1. 携卵附肢,示大量胚胎附着于腹肢的刚毛上,×30.
2. 外肢上的羽状刚毛,×100.
3. 刚毛基部横切,示基部有内含物,×200.
4. 一个胚胎纵切,示卵黄颗粒、初级卵膜和次级卵膜,×200.
5. 胚胎间的连接,示卵黄颗粒和初级卵膜,×400.
6. 原肢上簇生的刚毛,示刚毛基部有细小分支,×230.
7. 一根刚毛基部,示刚毛囊、黏液,×2 000.
8. 刚毛表面,示纤维状结构和黏液,×3 500.

En:内肢. Ex:外肢. Ey:胚胎. FM:初级卵膜. SM:次级卵膜. Fu:卵索. Mu:黏液. Se:刚毛. SF:刚毛囊. YG:卵黄颗粒.

图版Ⅱ　罗氏沼虾胚胎的附着结构

1. 刚毛横切,示中空的刚毛和纤维状结构.
2. 刚毛外壁,示微绒毛状结构.
3. 受精卵,示受精卵之间的卵膜连接,×50.
4. 蚤状幼体期胚胎,示胚胎间的卵膜连接,×40.
5. 连接于刚毛的一个胚胎,示刚毛和次级卵膜.
6. 胚胎的卵柄和刚毛连接处.
7. 胚胎的卵柄放大,×1 200.
8. 胚胎表面,示皱褶、黏液,×1 500.

Ey:胚胎. FM:初级卵膜. Mu:黏液. Se:刚毛. SM:次级卵膜

Explanation of Plates

Plate Ⅰ　Attachment structures of the embryos of *Macrobrachium rosenbergii*

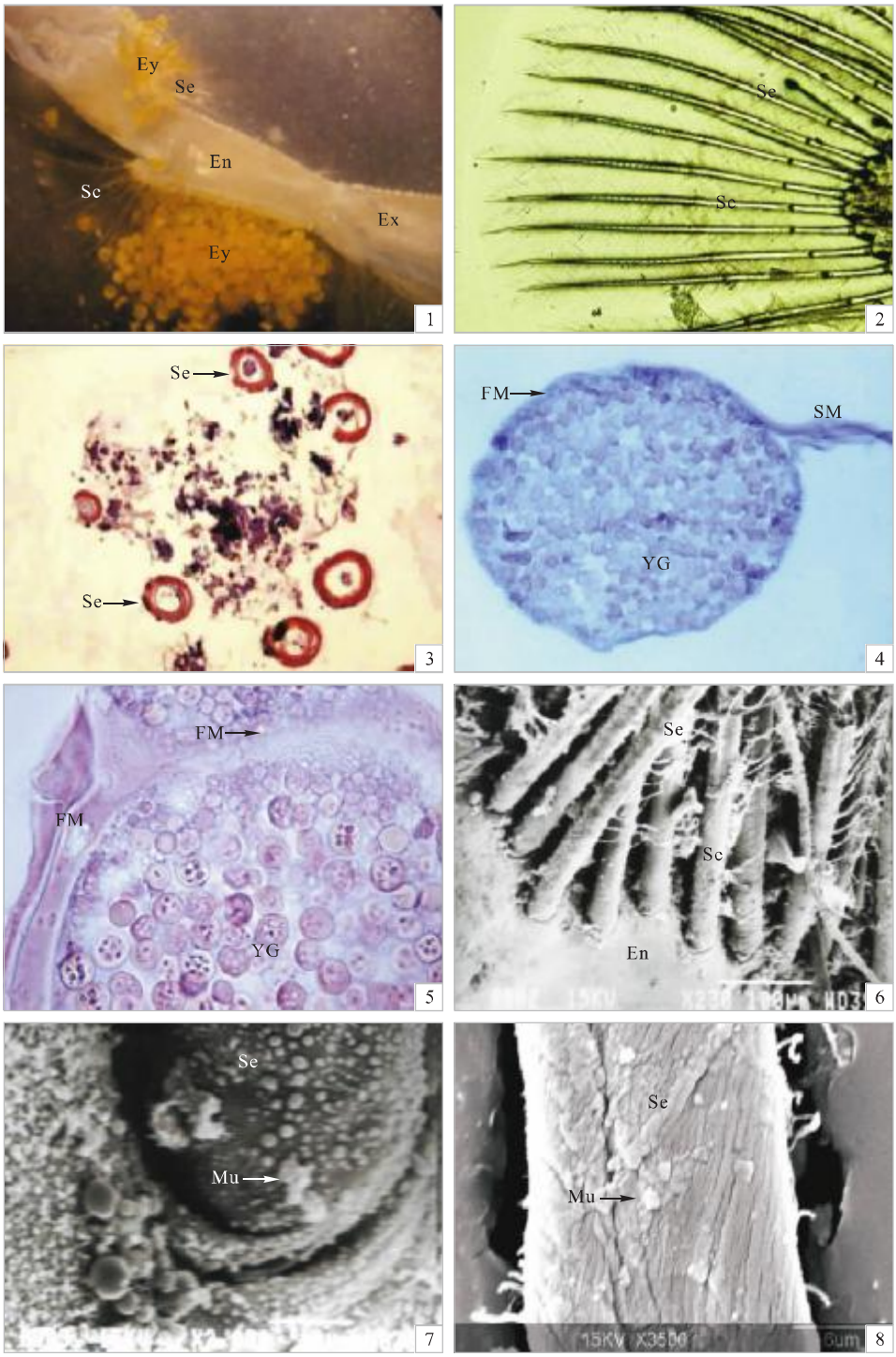
1. Ovigerous appendage, showing numerous embryos attaching to the pleopod setae, ×30.
2. Bilimbate setae in exopodite, ×100.
3. Setae basis transaction, showing inclusion in it, ×200.
4. An embryo frontal section, showing yolk granules and funiculus, ×200.
5. Joint between embryos, showing yolk granules and first egg membrane, ×400.
6. Setae on protopodite, showing small branches at the base of setae, ×230.
7. Seta basis, showing seta follicle and mucus, ×2 000.
8. Surface of a seta, showing fiber structure and mucus, ×3 500.

En: Endopodite. Ex: Exopodite. Ey: Embryo. FM: First egg membrane. SM: Secondary egg membrane. Fu: Funiculus. Mu: Mucus. Se: Seta. SF: Seta follicle. YG: Yolk granule.

Plate Ⅱ　Attachment structures of the embryos of *Macrobrachium rosenbergii*

1. Seta transaction, showing hollow seta and fiber structure.
2. Seta surface, showing microvilli structure.
3. Fertilized egg stage, showing links among fertilized eggs, ×50.
4. Zoea stage, showing links among embryos, ×40.
5. An embryo attaches to a seta, showing setae and second egg membrane.
6. Egg stalk and seta which joint to an embryo.
7. Egg stalk magnify, ×1 200.
8. Surface of an embryo, showing wrinkle and mucus, ×1 500.

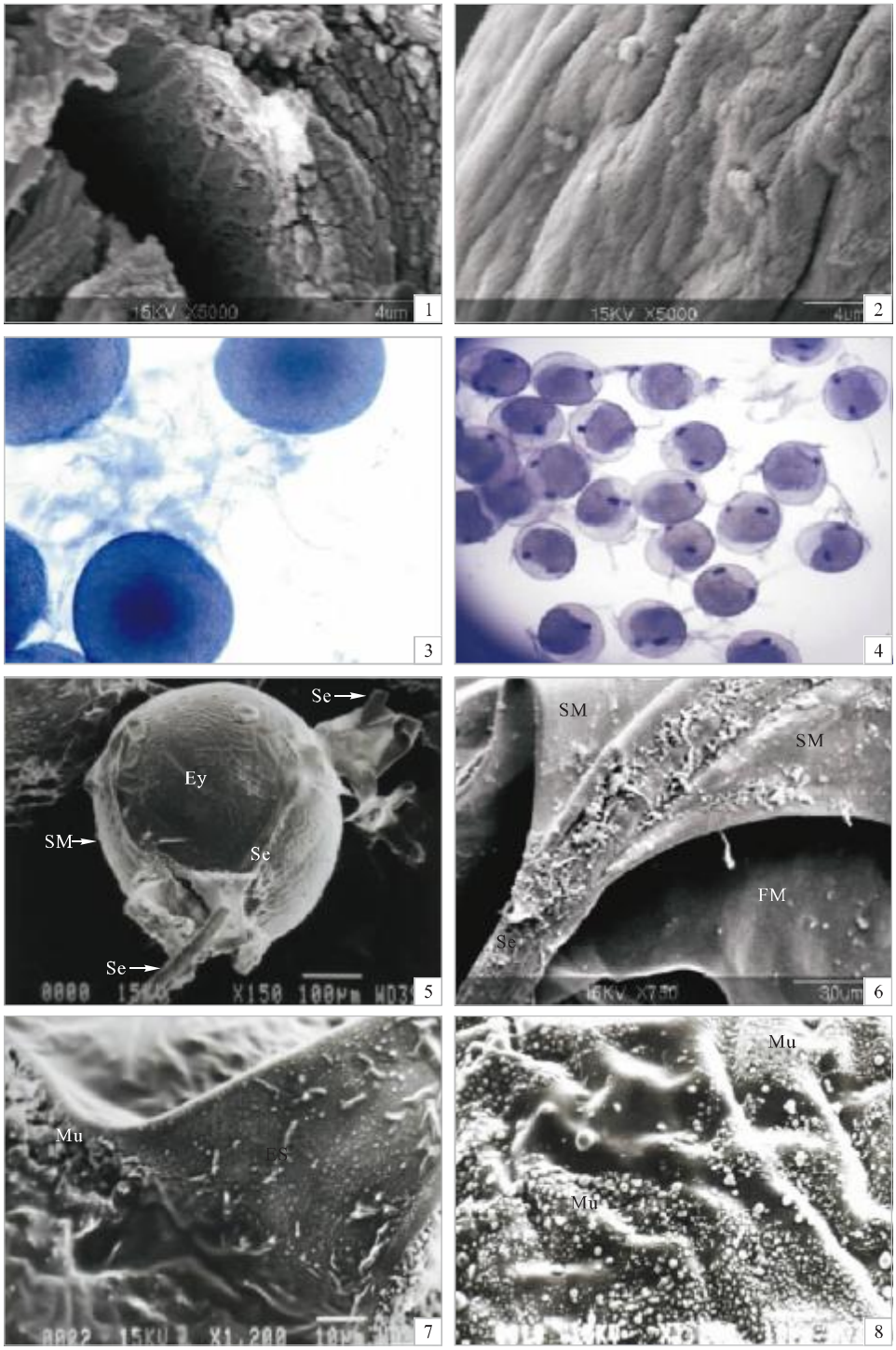
Ey: Embryo. FM: First egg membrane. Mu: Mucus. Se: Seta. SM: Secondary egg membrane



图版Ⅰ　罗氏沼虾胚胎的附着结构

Plate Ⅰ　Attachment structure of the embryos of *Macrobrachium rosenbergii*

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



图版Ⅱ　罗氏沼虾胚胎的附着结构

Plate Ⅱ　Attachment structure of the embryos of *Macrobrachium rosenbergii*

(图版Ⅱ说明见文末　**Explanation of Plate Ⅱ at the end of the text**)