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黄、渤海区不同生态类型鱼卵卵膜和受精孔区亚显微形态

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摘要：黄、渤海区分布的硬骨鱼类鱼卵几乎涵盖了所有鱼卵生态类型。本研究借助扫描电镜观察技术对近年来黄、渤海渔业资源监测调查期间采集的15种不同生态类型鱼卵卵膜和受精孔区亚显微形态特征进行观察，以阐明其亚显微结构并总结提炼可用于鱼卵形态分类的特征，同时将相应鱼种产卵场环境因子引入到卵膜形态特征的认识中，以揭示其生境适应性。结果显示不同生态类型或不同鱼种卵膜形态不尽相同，浮性鱼卵和口含鱼卵卵膜较薄，沉性鱼卵和具卵膜丝鱼卵卵膜较厚；浮性鱼卵卵膜表面壁孔密度、卵膜外表面呈现的蚀刻或特殊结构以及受精孔形状类型，沉性鱼卵卵膜黏膜层表面结构、受精孔形状类型，具卵膜丝鱼卵卵膜丝数量、着生位置及受精孔形状类型，口含鱼卵受精孔区形状及周边附着丝数量等均可用于鱼卵形态分类。鱼卵卵膜和受精孔区亚显微结构差异与鱼种系统分类地位、产卵场环境和地理分布区相关联。鱼卵卵膜表面结构可以作为区分鱼类科或属的性状，卵膜壁孔密度、表面呈现的蚀刻或奇异结构是鱼卵形态分类的重要依据；受精孔结构则一般具有物种特异性。卵膜结构与厚度可反映鱼卵对不同生境的适应性，而卵膜表面的许多特殊结构和装饰被认为是鱼卵应对产卵场环境的保护和适应性策略。本研究将为黄、渤海区硬骨鱼类早期生活史阶段个体发生和鱼卵形态分类研究积累基础资料。

关键词：鱼卵；卵膜；受精孔；亚显微形态；生态类型；生境适应性

中图分类号：Q 132.1；S 917.4 **文献标志码**：A

黄、渤海属典型陆缘浅海性质，在地域上又受北温带季风区气候影响，且处复杂水文要素作用，形成上兼有多重动物地理学复合区系镶嵌特点(北太平洋温带区系与印度—西太平洋热带区系的混合带)[1]。黄、渤海鱼类的适温性类型中，以暖温性种占优势，暖水性种次之，冷温性种再次，冷水性种最少；从栖所类型来看, 绝大多数为大陆架浅水底层鱼类，大陆架岩礁性、大陆架浅水中上层、大陆架浅水中底层、大陆架大洋洄游性中上层和大洋深水底层鱼类次之[2]。黄、渤海区各鱼种通过不断进化并与产卵场的生态环境长期适应，不同适温类型和栖所类型硬骨鱼类鱼卵形态特征和属性各异，涵盖了几乎所有鱼卵生态类型(浮性、沉性、具卵膜丝、口含、具被囊鱼卵等)。

鱼卵阶段是鱼类生活史中的一个重要环节，在其中存储着鱼类很多早期生活史和生态信息[3]。硬骨鱼类卵膜是鱼卵最外端具有一定厚度的复杂多层蛋白质壳[4]。自鱼卵排出母体受精直至孵化前胚胎成型关键阶段，卵膜和卵周液各自独立或相互结合组成统一屏障保护胚胎正常发育[5]。卵膜可保护鱼卵，防止扭曲和变形[6]；卵分泌物

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质可聚集在卵子周围增加保护作用，以免细菌、病毒的侵害[3]。卵膜表面壁孔可供鱼卵营养物质汲取和代谢物排出[7]，此外亦提供呼吸作用[8]。不同生态类型鱼卵卵膜形态特征各异，因种而异的卵膜结构与厚度可反映鱼卵对不同生态环境的适应性[9-11]，其为各鱼种不断进化并与产卵场生态环境长期适应性共同作用结果[12]。已有学者通过研究发现鱼卵卵膜表面某些特征具物种的特异性，可用于鱼卵形态分类[9-10, 13-14]。受精孔是鱼类无顶体精子通过鱼卵卵膜并完成受精作用的唯一通道[15]。在受精过程中受精孔起着重要的精子识别引导作用，它们的形态则具物种特异性[16-17]。Riehl等[18]在综述前人鱼卵受精孔形态特征研究基础上，根据受精孔形状将其分为3类：Ⅰ型，受精孔具较深受精孔前庭而精孔管较浅；Ⅱ型，受精孔前庭平坦，但精孔管孔道较长；Ⅲ型，受精孔无前庭但精孔管较长，一般外开口增大。利用电子显微镜观察鱼类卵膜、受精孔区亚显微结构已成为进行海洋硬骨鱼类鱼卵种类鉴别的重要手段[19-20]。

截至当前，黄、渤海区分布的绝大多数具经济价值硬骨鱼类鱼卵已被从胚胎发育形态学角度进行过相关报道[21-26]，这些特征已广泛运用于鱼类个体发生和鱼卵形态分类研究中。而借助扫描电镜对黄、渤海区鱼卵卵膜和受精孔区结构进行观察研究较少。已有研究主要涉及如下几方面：精子入卵扫描电镜观察，如卢敏德等[27]研究的暗纹东方鲀(*Takifugu obscurus*)、章龙珍等[28]研究的纹缟鰕虎鱼(*Tridentiger trigonocephalus*)和张涛等[29]研究的日本鳗鲡(*Anguilla japonica*)等精卵形态和受精过程观察；成熟卵膜形态结构比较，如张筱兰等[30]报道的褐牙鲆(*Paralichthys olivaceus*)、黑鲷(*Sparus macrocephalus*)和红鳍东方鲀(*T. rubripes*)，卞晓东等[31]报道的沙氏下鱵鱼(*Hyporhamphus sajori*)和张世奎等[32]报道的3种鲆鲽类卵膜表面形态特征；受精前后卵膜和受精孔区形态变化，如Bian等[33]报道4种鲽亚目(Pleuronectoidei)分批产鲆鲽类鱼卵和Bian等[34]报道的大头鳕(*Gadus macrocephalus*)鱼卵。上述各项研究仅涉及鱼种12种，约占黄、渤海区分布鱼卵种类总数的1/7。

本研究拟借助扫描电镜观察技术对近年来黄、渤海渔业资源监测调查期间采集不同生态类型鱼卵卵膜和受精孔区亚显微形态进行观察，以阐明其亚显微结构，并总结提炼可用于鱼卵形态分类的特征，同时将相应鱼种产卵场环境因子引入到卵膜形态特征的认识中，以揭示其生境适应性。本研究将为黄、渤海区硬骨鱼类早期生活史阶段个体发生和鱼卵形态分类研究积累基础资料。

1 材料与方法

1.1 实验材料

于2010至2015年开展黄、渤海渔业资源监测调查期间同步收集不同生态类型鱼卵样品。本研究所涉及鱼卵种类、生态类型、收集地、收集方法及成鱼适温类型和栖所类型[2]见表1。自然海域浮性鱼卵样品通过大型浮游生物网表层水平拖曳收集；具卵膜丝和沉性鱼卵为调查期间从调查网具上收集；口含细条天竺鱼(*Apogon lineatus*)卵取自细条天竺鱼雄鱼口腔内；高眼鲽(*Cleisthenes herzensteini*)卵为拖网试捕渔获物中选择性成熟的雌鱼，采用轻轻按压鱼腹部的方法获取；大头鳕、褐牙鲆和日本鳗鲡鱼卵采用人工授精方法获取。自然海域收集部分鱼卵用网目0.5 mm筛绢滤掉海水，0.1 mol/L，pH 7.4的磷酸盐缓冲溶液(PBS)冲洗至少3次，然后用0.1 mol/L，pH 7.4的PBS配制2.5%戊二醛溶液对样品进行预固定，4 °C低温储存，带回实验室备用；将采集的同批部分鱼卵样品用95%酒精固定，用于难定种鱼卵的DNA条形码鉴定。采用人工授精法获取的鱼卵用网目0.5 mm 筛绢滤掉海水，0.1 mol/L，pH 7.4的PBS冲洗至少3次，然后用0.1 mol/L，pH 7.4的PBS配制的2.5%戊二醛溶液对样品进行预固定，4 °C低温储存。

1.2 鱼卵电镜样品制作，卵膜表面亚显微形态观察

在光学显微镜下，据鱼卵形态特征对自然海域采集的不同生态类型鱼卵进行种类鉴别，将同种鱼卵并为一组，再将具有一定数量(20粒以上)鱼卵在确定种类后制成扫描电镜样品。人工授精法获取的鱼卵则直接挑选状态好的用于扫描电镜样品制作。扫描电镜样品制作具体步骤：取20~50粒目标鱼卵，用PBS漂洗3次，l%锇酸后固定1 h，酒精梯度脱水，酒精浓度依次为30%、50%、70%、80%、90%、95%和100%，其中用100%浓度乙醇脱水2次，每次10 min；临界点干燥仪干燥，之后用离子溅射仪镀金。借助

扫描电镜(JEOL-JSM-840)观察卵膜表面的形态特征，并拍照记录。观测对象主要包括卵膜表面特殊结构，壁孔排列方式、大小、形状，受精孔前庭和精孔管的形状、大小等。用Image-Pro Plus软件对所拍摄的照片进行分析，测量各结构的大小和长度[34]。

1.3 难定种鱼卵DNA条形码鉴定

对传统形态学方法定种有困难种类，取95%酒精固定鱼卵，单粒鱼卵经蛋白酶K消化，使用传统酚–氯仿法提取鱼卵基因组DNA，置于4 °C冰箱中保存待用[31]。采用正向引物FishF1 (TCAACCAACCACAAAGACATTG GCAC)和反向引物FishR1 (ACTTCA GGGTGACCGAAGAATC AGAA)扩增目标鱼卵线粒体DNA *COI*基因片段序列[35]。

PCR反应体系：*Taq*酶0.25 μL，DNA模板1 μL，正反向引物各1 μL，dNTPs 2 μL，10×PCR buffer 2.5 μL，去离子水17.25 μL。PCR反应条件：94 °C预变性5 min，94 °C变性45 s，52 °C退火45 s，72 °C延伸45 s，循环35次，然后72 °C延伸10 min。PCR产物用琼脂糖凝胶进行电泳检测，用回收试剂盒将目的条带回收和纯化，之后送北京六合华大基因科技股份有限公司进行正反链测序。用DNASTAR软件包(DNASTAR Inc., Madison, USA)对测得序列进行编辑和人工比对。使用NCBI的BLAST比对分析序列，通过MEGA 4.0构建邻接关系树(K2P 模型), 并计算遗传距离，给未定种鱼卵定种[31, 35]。

2 结果

2.1 基于DNA条形码的鱼卵种类鉴别

利用DNA条形码鉴定技术对自然海域采集的3种难定种鱼卵进行鉴别。对难定种鱼卵A扩增得到COI序列长713 bp，BLAST分析相似性最高(99%)的物种是斑翼文鳐鱼(*Hirundichthys affinis*)，经查台湾鱼类资料库(http://fishdb.sinica.edu.tw/eng/home.php)，斑翼文鳐鱼应为尖头燕鳐鱼(*H.*

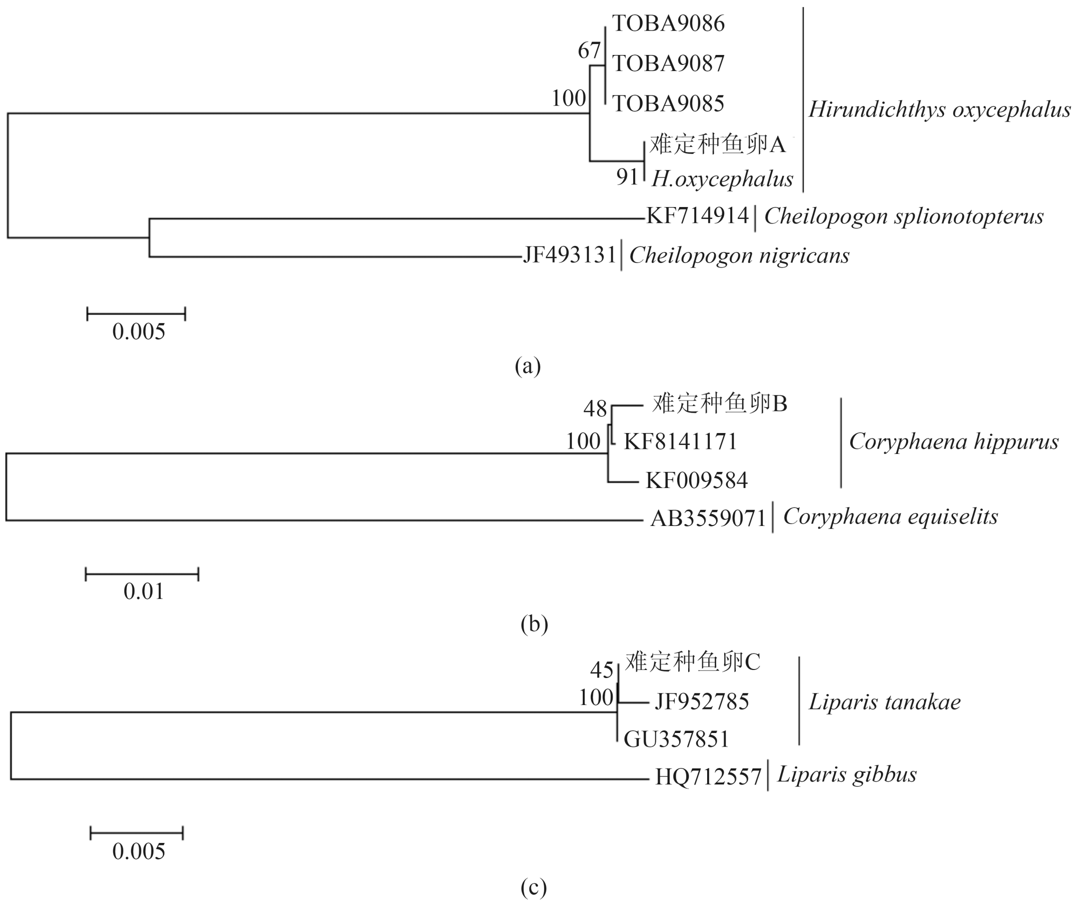


图 1 基于COI 序列构建的难定种鱼卵A~C和相近种邻接关系树

Fig. 1 Neighbor-Joining tree for COI sequences of unidentified eggs A to C and related species

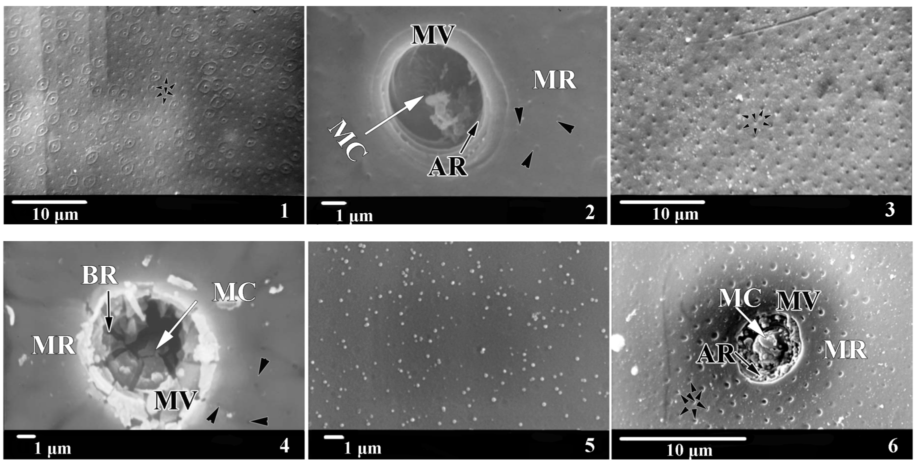
oxycephalus)别名。从台湾鱼类资料库中下载同源序列*H. oxycephalus*，从GenBank中下载序列TOBA9086、TOBA9085和TOBA9087 (*H. affinis*)、JF493131 (*Cheilopogon nigricans*)、KF714914 (*C. spilonotopterus*)进行比对，与难定种鱼卵A的遗传距离依次为0、0.004、0.004、0.004、0.060和0.064。邻接关系树显示，难定种鱼卵A先与*H. oxycephalus*聚为一支后再与TOBA9086、TOBA9085和TOBA9087聚为一支(图1-a)。因此认为难定种鱼卵A是尖头燕鳐鱼。

对难定种鱼卵B扩增得到COI序列长728 bp，BLAST分析相似性最高(99%)的物种是鲯鳅(*Coryphaena hippurus*)，下载序列KF719178、KF814117 (*C. hippurus*)、AB355907 [等棘鲯鳅(*C. equiselis*)]进行比对，与难定种鱼卵B的遗传距离依次为0.006、0.003和0.113。邻接关系树显示，难定种鱼卵B与KF814117和KF719178聚为一支(图1-b)。因此认为难定种鱼卵B是鲯鳅。

对难定种鱼卵C扩增得到COI序列长711 bp，BLAST分析相似性最高(99%)的物种是细纹狮子鱼(*Liparis tanakae*)，下载序列JF952785、GU357851 (*L. tanakae*)、HQ712557 [细尾狮子鱼(*L. gibbus*)]进行比对，与难定种鱼卵C的遗传距离依次为0.002、0.001和0.067。邻接关系树显示，难定种鱼卵C与JF952785和GU357851聚为一支(图1-c)。因此认为难定种鱼卵C是细纹狮子鱼。

2.2 几种浮性鱼卵卵膜和受精孔区亚显微形态

扫描电镜下斑鰶(*Konosirus punctatus*)囊胚期受精卵卵膜平整，表面布满平滑壁孔，呈六角晶格形分布，即每个壁孔被6个相同大小壁孔包



图版 I 扫描电镜下浮性鱼卵卵膜和受精孔区

1. 斑囊胚期卵膜表面，壁孔(前端箭头)呈六角晶格形分布；2. 斑囊胚期卵受精孔区(MR)；前庭区(MV)不明显；精孔管(MC)末端阻塞，内壁自上至末端具单螺旋脊(AR)，受精孔区周缘散布大小不等壁孔(前端箭头)；3. 鲯鳅胚胎期卵膜表面平整，壁孔(前端箭头)呈六角晶格形分布；4. 鲯鳅胚胎期卵受精孔，前庭区(MV)不明显，精孔管内壁自上至末端具5至7块隆起脊状物(BR)，精孔管(MC)末端阻塞；受精孔区(MR)周缘散布着大小不等壁孔(前端箭头)；5. 白姑鱼胚胎期光滑卵膜表面；6. 白姑鱼胚胎期卵受精孔区(MR)，前庭区(MV)不明显，精孔管(MC)末端阻塞；精孔管内壁自上至末端具有单螺旋脊(AR)，受精孔区(MR)周缘散布呈六边形分布的壁孔(前端箭头)

Plate I Ultrastructures of the envelope surface and micropyle of several floating fertilized eggs observed by SEM

1. egg envelope surface with pores (arrowheads) distributed in hexagonal pattern of the blastula stage *K. punctatus* eggs, the shape of the outer surface has a slightly depressed lip as it circumvents the openings of pore canals (arrowheads); 2. micropylar region (MR) of the blastula stage *K. punctatus* eggs with pores and shallow cavities of various sizes (arrowheads) scattered and with no distinguishable micropyle vestibule (MV), the micropylar canal (MC) with its inner lumen completely blocked, consists of counterclockwise arrangements of single spiral-shaped ridges (AR) (from outer to inner); 3. egg envelope surface with flush pores (arrowheads) distributed in hexagonal pattern of the embryonic stage *C. hippurus*; 4. micropylar region (MR) of the embryonic stage *C. hippurus* eggs with no distinguishable micropyle vestibule (MV) and scattered with pores and shallow cavities of various sizes (arrowheads), the micropylar canal (MC) with its inner lumen partially blocked, consists of 5 to 7 pieces of bulged ridge substance (BR); 5. smoothing egg envelope surface of the embryonic stage *P. argentata*; 6. micropylar region (MR) of the embryonic stage *P. argentata* eggs with no distinguishable micropyle vestibule (MV) and scattered with uiniform pores and shallow cavities (arrowheads) distributed in hexagonal pattern, the micropylar canal (MC) with its inner lumen partially blocked, consists of counterclockwise arrangements of single spiral-shaped ridges (AR) (from outer to inner)

表 1　本研究所涉及鱼卵种类、生态类型、采集地、采集方法、卵膜和受精孔区结构特征及其亲体适温类型和栖所类型

Tab. 1　Species names, ecological types, collecting areas, collecting method, ultrastucture of the egg envelope surface and micropyle of the teleosts eggs used in this study with temperature adaptation and habitat types to their broodstock　n=6

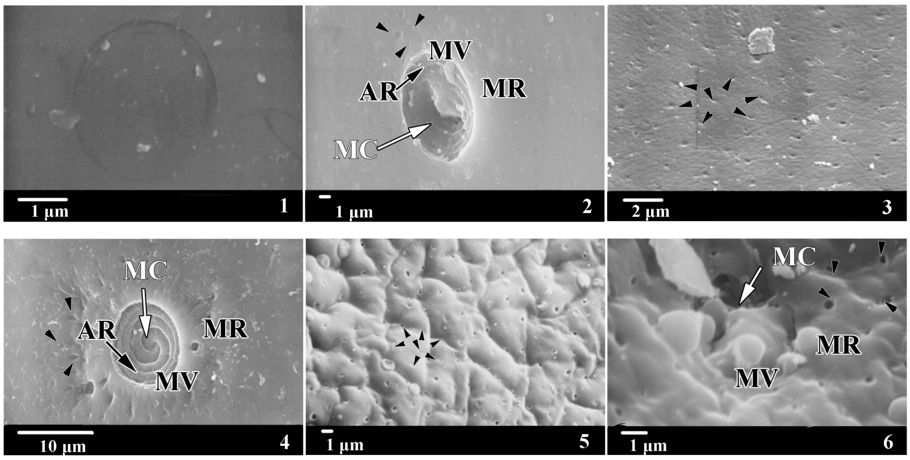
种类 species	卵生态类型 ETE	采集地 CA	采集方法 CM	亲体适温 TAB	亲体栖所 HTB	固定后卵径 DFE	受精孔类型 TM	受精孔径/μm DMO	卵膜结构 COES	结构密度/(个/100 μm²)SDD
大头鳕 *G. macrocephalus*	沉性卵	育苗场	人工授精	冷温种	大陆架浅水底层	1.05±0.03	Ⅰ	18.13±2.03	六角网纹	25.38±6.67
细纹狮子鱼 *L. tanakae*	沉性卵	黄海南部	生产网具	冷温种	大陆架浅水底层	1.44±0.02			蜂窝状粘着层	
大泷六线鱼 *H. otakii*	沉性卵	黄海北部	人工授精	冷温种	大陆架岩礁性	1.18±0.07			蜂窝状粘着层	
细条天竺鱼 *A. lineatus*	口含卵	黄海南部	生产网具	暖温种	大陆架浅水中底层	0.57±0.03	Ⅲ	13.84±1.43	规整壁孔	7.04±4.46
沙氏下鱵鱼 *H. sajori*	卵膜丝卵	黄海南部	生产网具	暖温种	大陆架浅水中上层	2.10±0.14	Ⅱ	11.69±1.27	颗粒状突起	0.44±0.08
尖头燕鳐鱼 *H. oxycephalus*	卵膜丝卵	黄海南部	生产网具	暖水种	大陆架大洋洄游中上层	1.58±0.34	Ⅲ	5.55±0.18	规整壁孔	51.09±12.04
斑 *K. punctatus*	浮性卵	渤海	浮游生物网	暖温种	大陆架浅水中上层	1.35±0.84	Ⅲ	4.39±0.40	规整壁孔	51.79±2.73
尖海龙 *S. acus*	卵胎生卵	黄海南部	生产网具	暖温种	大陆架浅水中底层	0.70±0.03			壁孔不明显	142.17±13.07
鲯鳅 *C. hippurus*	浮性卵	黄海北部	浮游生物网	暖水种	大陆架大洋洄游中上层	1.37±0.05	Ⅲ	5.58±0.28	规整壁孔	26.86±7.16
白姑鱼 *P. argentata*	浮性卵	渤海	浮游生物网	暖温种	大陆架浅水中底层	1.36±0.48	Ⅲ	5.41±0.38	壁孔不明显	
鮻 *L. haematocheila*	浮性卵	渤海	浮游生物网	暖温种	大陆架浅水中底层	1.01±0.05	Ⅲ	7.52±1.26	壁孔不明显	
日本鬼鲉 *I. japonicus*	浮性卵	黄海南部	浮游生物网	暖水种	大陆架浅水底层	1.09±0.05	Ⅲ	9.86±0.70	规整壁孔	91.98±2.34
高眼鲽 *C. herzensteini*	浮性卵	黄海北部	人工授精	冷温种	大陆架浅水底层	0.81±0.03	Ⅲ	4.08±0.32	规整壁孔	27.11±6.59
褐牙鲆 *P. olivaceus*	浮性卵	育苗场	人工授精	暖温种	大陆架浅水底层	0.92±0.01	Ⅲ	4.89±0.29	规整壁孔	33.95±8.95
绯鲔 *C. beniteguri*	浮性卵	渤海	浮游生物网	暖温种	大陆架浅水中底层	0.63±0.07			六角网纹	0.63±0.14
日本鳗鲡 *A. japonica*	浮性卵	育苗场	人工授精	暖水种	大陆架浅水底层	1.05±0.03	Ⅲ	3.02±0.66	镰刀状突起	16.32±3.31

注：COES. 卵膜结构；DFE. 固定后卵径；TM. 受精孔类型；DMO. 受精孔径；SDD. 结构密度

Notes: COES. charactures on the envelope surface; DFE. diameter of the fixed egg in mm, mean±SD (n=6); TM. type of micropyle; DMO. diameter of micropyle opening in μm, mean±SD (n=6); SDD. structure distribution density in per100 μm², ind/100 μm² (n=6)

围(图版Ⅰ-1)。当卵膜经过部分壁孔外端开口时，其呈微凹唇形(图版Ⅰ-1)。卵膜壁孔密度(51.79±2.73) 个/100 μm²(n=6)(表1)。斑鰶受精孔Ⅲ型，前庭区不明显，受精孔区周缘散布大小不等壁孔；胚胎期精孔管末端阻塞，内壁自上至末端具逆时针方向排列单螺旋脊(图版Ⅰ-2)。精孔管外径(4.39±0.40) μm(n=6)(表1)。鲯鳅胚胎期受精卵卵膜表面平整，布满平滑壁孔，呈六角晶格形分布(图版Ⅰ-3)，壁孔密度(26.86±7.16) 个/100 μm²(n=6)(表1)。鲯鳅受精孔Ⅲ型，前庭区不明显，受精孔区周缘散布着大小不等壁孔；胚胎期精孔管末端阻塞，内壁自上至末端具5~7块隆起脊状物(图版Ⅰ-4)。精孔管径(5.58±0.28) μm (n=6)(表1)。白姑鱼(*Pennahia argentata*)胚胎期受精卵卵膜表面光滑平整，卵膜壁孔不明显(图版Ⅰ-5)。白姑鱼受精孔Ⅲ型，前庭区不明显，受精孔周缘散布着明显呈辐射六角晶格状分布大小不等的壁孔(图版Ⅰ-6)。胚胎期精孔管末端阻塞，精孔管内壁自上至末端具有单螺旋脊(图版Ⅰ-6)，精孔管径(5.41±0.38) μm(n=6)(表1)。

扫描电镜下，鮻(*Liza haematocheila*)胚胎期受精卵卵膜平整，表面十分光滑，卵膜壁孔不明显(图版Ⅱ-1)。鮻受精孔Ⅲ型，前庭区不明显，受精孔周缘散布着大小不等壁孔(图版Ⅱ-2)。鮻胚胎期精孔管末端阻塞，精孔管内壁自上至末端具有单螺旋脊(图版Ⅱ-2)，精孔管径(7.52±1.26) μm(n=6)。日本鬼(*Inimicus japonicus*)胚胎期受精卵卵膜平整，布满平滑壁孔，呈六角晶格形分布(图版Ⅱ-3)，壁孔密度(91.98±2.34) 个/100 μm²(n=6)(表1)。日本鬼受精孔Ⅲ型，前庭区不明



图版 II 扫描电镜下几种浮性鱼卵卵膜和受精孔区

1. 鲮胚胎期光滑卵膜表面，壁孔不明显；2. 鲮胚胎期卵受精孔区(MR)，前庭区(MV)不明显；精孔管(MC)末端阻塞，内壁自上至末端具单螺旋脊(AR)，受精孔区周缘散布大小不等壁孔(前端箭头)；3. 日本鬼鲉胚胎期平整卵膜表面，壁孔(前端箭头) 呈六角晶格形分布；4. 日本鬼鲉胚胎期卵受精孔区(MR)，前庭区(MV)不明显，精孔管(MC)末端阻塞，内壁自上至末端具双螺旋脊(AR)；5. 高眼鲽未受精鱼卵明显波纹状卵膜，壁孔(前端箭头) 呈六角晶格形分布；6. 高眼鲽未受精鱼卵受精孔区(MR)，前庭区(MV)不明显；精孔管(MC)为卵周液分泌物阻塞，受精孔区(MR)周缘散布着大小不等壁孔(前端箭头)

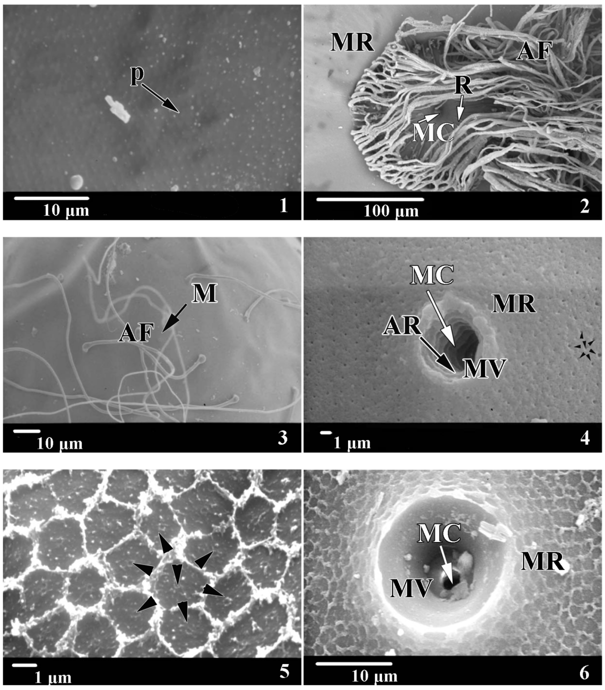
Plate II Ultrastructures of the envelope surface and micropyle of several floating fertilized eggs observed by SEM

1. smoothing egg envelope surface of the embryonic stage *L. haematocheila* egg with indistinct envelope pores; 2. micropylar region (MR) of the embryonic stage *L. haematocheila* eggs with pores and shallow cavities of various sizes(arrowheads)scattered and with indistinct micropyle vestibule (MV), the micropylar canal (MC) with its inner lumen completely blocked, consists of counterclockwise arrangements of single spiral-shaped ridges (AR) (from outer to inner); 3. flatten egg envelope surface of the embryonic stage *I. japonicus* eggs with flush pores (arrowheads) distributed in hexagonal pattern; 4. micropylar region (MR) of the embryonic stage *I. japonicus* eggs with pores and shallow cavities of various sizes(arrowheads)scattered and with no distinguishable micropyle vestibule (MV), the micropylar canal (MC) with its inner lumen completely blocked, consists of counterclockwise arrangements of double spiral-shaped ridges (AR); 5. undulating status of the envelope surface of the unfertilized mature *C. herzensteini* eggs and the pore canals distributed in hexagonal pattern (arrowheads); 6. micropylar region (MR) of the unfertilized mature *C. herzensteini* eggs with no distinguishable micropyle vestibule (MV) and scattered with uiniform pores and shallow cavities (arrowheads) distributed in hexagonal pattern, the micropylar canal (MC) with its inner lumen completely blocked by secretions of the perivitelline fluid

显，受精孔区周缘散布着大小不等壁孔。胚胎期精孔管末端阻塞，内壁自上至末端具有双螺旋脊(图版Ⅱ-4)，精孔管径(9.86±0.70) μm(n=6)(表1)。高眼鲽成熟未受精鱼卵具明显波纹状卵膜，卵膜壁孔明显，呈六角晶格形分布(图版Ⅱ-5)，壁孔密度(27.11±6.59) 个/100 μm^2(n=6)(表1)。高眼鲽受精孔Ⅲ型，前庭区不明显，未受精卵精孔管为卵周液分泌物阻塞，受精孔区周缘散布着大小不等壁孔(图版Ⅱ-6)，精孔管径(4.08±0.32) μm(n=6)(表1)。

2.3 口含卵、具卵膜丝卵和沉性鱼卵卵膜和受精孔区亚显微形态

扫描电镜下，细条天竺鱼口含囊胚期受精卵卵膜表面较平整,卵膜表面壁孔向外突起似颗粒状(图版Ⅲ-1)，壁孔密度(7.04±4.46) 个/100 μm^2 (n=6)(表1)。细条天竺鱼受精孔Ⅲ型，前庭区明显，周缘具50~60条隆起脊，隆起脊末端延长形成游离于卵膜表面粘着卵膜丝(图版Ⅲ-2)；囊胚期精孔管末端阻塞，精孔管径(13.84±1.43) μm (n=6)。尖头燕鳐鱼胚胎期卵膜表面极光滑，在鱼卵动物极端密集着生10~12根细长卵膜丝，同时在植物极所在半球稀疏散布5~6根细长卵膜丝，受精孔位于动物极卵膜丝附着区域(图版Ⅲ-3)，卵膜壁孔不明显，壁孔密度(51.09±12.04) 个/100 μm^2(n=6)(表1)。尖头燕鳐鱼受精孔Ⅲ型，前庭区不明显，胚胎期精孔管呈直筒状，内壁自上至末端具有逆时针分布单螺旋脊(图版Ⅲ-4)，精孔管径(5.55±0.18) μm (n=6)。大头鳕未受精沉性卵卵膜表面壁孔明显，呈六角晶格状分布，每个壁孔周缘具六角形隆起网状花纹(图版Ⅲ-5)，壁孔密度(25.38±6.67) 个/100 μm^2(n=6)(表1)。大头鳕受精孔Ⅰ型，前庭区明显，未受精卵精孔管末端开放通道短(图版Ⅲ-6)，精孔管径



图版 III 口含卵、具卵膜丝卵、沉性鱼卵卵膜和受精孔区结构

1. 细条天竺鱼口含卵囊胚期卵膜表面布满颗粒状突起，壁孔(P)不明显；2. 细条天竺鱼囊胚期卵受精孔区(MR)，受精孔(MC)末端完全阻塞，周缘具50~60条隆起脊(R)，隆起脊末端延长形成游离于卵膜表面的粘着卵膜丝(AF)；3. 尖头燕鳐鱼胚胎期具卵膜丝动物极光滑卵膜表面，受精孔(M)位于卵膜丝(AF)附着区域；4. 尖头燕鳐鱼胚胎期卵受精孔区(MR)，前庭区(MV)不明显，精孔管(MC)内壁自上至末端具单螺旋脊(AR)；5. 大头鳕未受精沉性卵卵膜表面，壁孔(前端箭头)呈六角晶格形分布，每个壁孔周缘具六角形网状花纹；6. 大头鳕未受精沉性卵受精孔区(MR)，前庭区(MV)明显，受精孔(MC)通道短

Plate III Ultrastructures of the envelope surface and micropyle of the mouth brood, adhesive filaments and demersal eggs

1. envelope surface of the blastula stage *A. lineatus* eggs with uniform grain substances and indistinct pores; 2. micropylar region (MR) of the blastula stage *A. lineatus* eggs, the micropylar canal (MC) with its inner lumen completely blocked, ridges (R) arranged in a radial form around the micropyle, adhesive filaments (AF) are attached at the outer end of the ridges; 3. envelope surface of the blastula stage *H. oxycephalus* eggs with adhesive filaments (AF); 4. micropylar region (MR) of the blastula stage *H. oxycephalus* eggs with indistinct micropyle vestibule (MV); the micropylar canal (MC) with its inner lumen completely blocked, consists of counterclockwise arrangements of single spiral-shaped ridges (AR) (from outer to inner); 5. unfertilized mature *G. macrocephalus* egg envelope surface with pores (arrowheads) distributed in hexagonal patterns, the pores were surround with a hexagonal structure; 6. micropylar region (MR) of the unfertilized mature *G. macrocephalus* eggs with distinguishable micropyle vestibule (MV) and a short micropylar canal (MC)

(3.28±0.55) μm(n=6)，受精孔前庭外径可达(18.13±2.03) μm(n=6)。

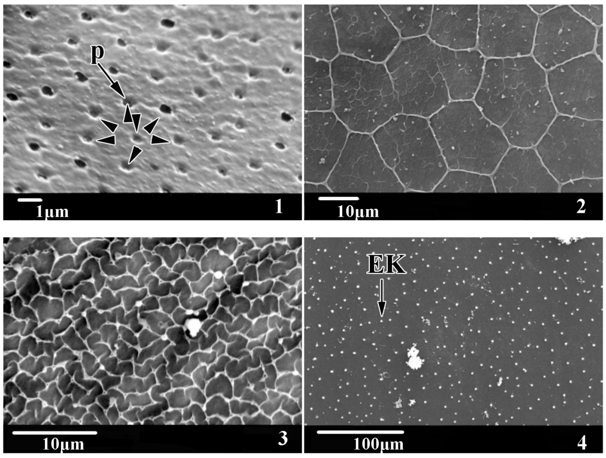
2.4 扫描电镜下几种浮性、具卵膜丝、卵胎生和沉性鱼卵卵膜表面结构

扫描电镜下，褐牙鲆浮性胚胎期卵膜表面平整，壁孔大小均匀，呈六角晶格形分布(图版Ⅳ-1)，壁孔密度(33.99±8.95) 个/100 μm^2(表1)。绯䲗(*Callionymus beniteguri*)胚胎期卵膜表面平整，表面布满精致六角网格状肋状脊(图版Ⅳ-2)，结构密度(0.63±0.14) 个/l00 μm^2(表1)。成熟未受精日本鳗鲡鱼卵卵膜具有明显纵横交错的褶皱，表面具不规整镰刀状突起(图版Ⅳ-3)，结构密度(16.32±3.31) 个/100 μm^2。沙氏下鱵鱼卵膜表面壁孔不明显，表面具规整颗粒状突起(图版Ⅳ-4)，结构密度(0.44±0.08) 个/100 μm^2。扫描电镜下，卵胎生尖海龙(*Syngnathus acus*)未受精卵膜表面较平整，卵膜表面壁孔向外突起似颗粒状(图版Ⅴ-1)，壁孔密度(142.17±13.07) 个/l00 μm^2 (n=6)(表1)；卵膜较薄，仅具薄片层状放射带(图版Ⅴ-2)。细纹狮子鱼沉性囊胚期卵膜较厚，厚度达(48.97±0.08) μm(n=6)。卵膜最外缘具厚胶质蜂窝状粘着层(图版Ⅴ-3)，(27.88±0.63) μm(n=6)，厚度约占鱼卵卵膜总厚度的58%。粘着层内为片层状的放射带，约占卵膜总厚度的42%。放射带又分为厚外放射带层(ZRE，约占整个放射带层厚度90%)和薄内放射带层(ZRI，约占整个放射带层厚度10%)(图版Ⅴ-4)。大泷六线鱼(*Hexagrammos otakii*)沉性未受精鱼卵卵膜较厚(图版Ⅴ-6)，卵膜最外部具厚胶质蜂窝状粘着层(图版Ⅴ-5)。

3 讨论

3.1 不同生态类型鱼卵卵膜亚显微结构及其生境适应性

鱼卵卵膜表面结构差异与鱼种系统分类地位、产卵场环境和地理分布区相关联[9-10]。Ivankov等[10]通过对分属不同系统分类地位及生态类型鱼类卵膜表面结构的比较也得出，绝大多数鱼类卵膜结构能体现该鱼系统分类地位，但鱼类卵膜结构主要是由其保护功效决定。因种而异的卵膜结构与厚度可反映鱼卵对不同生态环境适应性[9-11]。本研究通过对鱼卵受精孔区和破裂卵膜观察发现，海产浮性卵具相对卵径薄、结构简单片层状卵膜结构，但卵膜表面通常具平整卵膜壁孔；具卵膜丝卵卵膜一般较厚[31]，卵膜表面壁孔不明显；多数沉性卵卵膜较厚，且卵膜表面通常包裹一厚胶质粘着层，壁孔不宜观察；卵胎生卵和口含卵卵膜极薄，壁孔不明显。



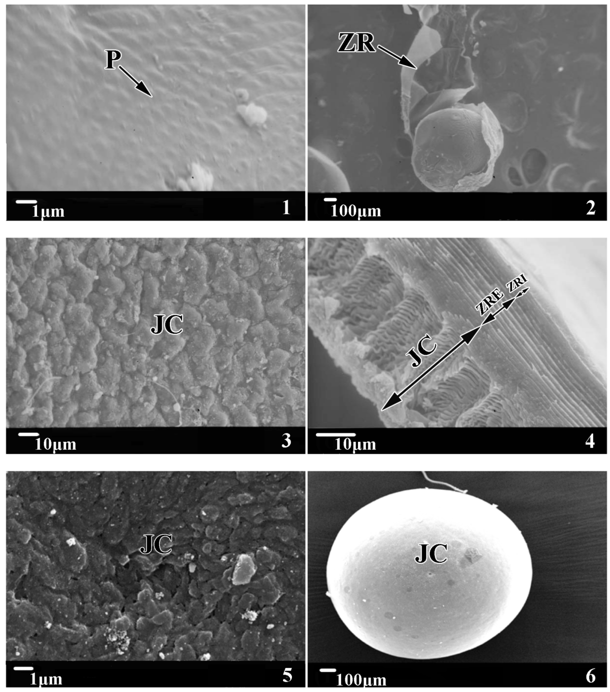
图版 IV 口含卵、具卵膜丝卵、沉性鱼卵卵膜和受精孔区结构

1. 褐牙鲆卵膜表面结构，壁孔(P)(前端箭头)呈规整六角晶格形分布；2. 绯鲔鱼卵表面具精致的六边形网格肋状脊；3. 日本鳗鲡卵膜表面不规整的镰刀状突起；4. 沙氏下鱵鱼卵膜表面规整的颗粒状(EK)突起

Plate IV Envelope surface of some floating eggs

1. envelope surface of the *P. olivaceus* eggs with uiniform pores (P) (arrowheads) distributed in hexagonal pattern; 2. envelope surface of the *C. beniteguri* with elaborate hexagonal ridges; 3. envelope surface of the *A. japonica* eggs with irregular looped structure with spike knobs; 4. envelope surface of the *H. sajori* eggs with elaborate knobs (EK)

海洋硬骨鱼类卵膜发育过程免疫组织化学及亚显微结构研究表明，卵膜是由成卵膜物质分层沉积形成，并且在卵膜形成过程中许多放射状排列小孔穿透卵膜[8, 36-39]，源自卵母细胞或滤泡细胞指状微绒毛或细胞突通过发育卵膜的小孔相互彼此交错结合，并输送外源性卵黄蛋白原及卵膜蛋白原以供卵膜合成和卵母细胞发育之用[40]。鱼卵成熟后从母体排出前，微绒毛或指状细胞突从卵膜壁孔中撤出，在卵膜表面构成辐射状分布壁孔。为满足卵母细胞成熟过程中不同发育需求，同一物种或不同物种卵膜表面特征会发生些许变化[41]。Olivar[42]研究认为卵膜壁孔分布趋势通常不具种或科的特异性。Gwo[41]研究的鲈形目(Perciformes)鲷科(Sparidae)3个鱼种，Bian等[33]研究的鲽亚目4种以及本研究中所有能观察出壁孔分布方式鱼种，壁孔均呈六角晶格形分布，进一步证实该观点。但不同鱼种卵膜壁孔密度不同，其似乎具有种的特异性。此外，具卵膜丝鱼卵卵膜丝着生位置、数量可成为鱼卵种类鉴别主要依据[31, 43]。口含卵则一般借助卵膜受精孔周边附着丝粘附于亲体口腔上皮上发育，受精孔周边的附着丝数量也可作为鱼卵形态分类主要依据[44]。



图版 V 几种卵胎生和沉性鱼卵卵膜结构和厚度

1. 尖海龙未受精卵膜，表面布满颗粒状突起，壁孔(P)不明显；2. 尖海龙未受精卵破碎卵膜表面结构，卵膜具一薄放射带层(ZR)；3. 细纹狮子鱼囊胚期卵蜂窝状的粘着层表面(JC)；4. 细纹狮子鱼卵膜结构，外部具厚胶质粘着层(JC)，内为放射带(ZR)；放射带又分为厚外放射带层(ZRE)和一薄内放射带层(ZRI)；5. 大泷六线鱼鱼卵卵膜蜂窝状粘着层表面(JC)；6. 大泷六线鱼完整卵膜表面，图示胶质粘着层(JC)

Plate V Envelope surface ultrastructures and thickness of ovoviviparous and demersal eggs

1. envelope surface of the unfertilized mature *S. acus* egg with uniform grain substances and indistinct pores (P); 2. rupture unfertilized mature *S. acus* egg, the envelope surface was only with a thin zona radiate (ZR); 3. the outer surface of the gelatinous-honeycomb layer (JC) of *L. tanakae* egg displayed round granular structures; 4. structures of the thick egg envelope of *L. tanakae*: outer surface of thick gelatinous-honeycomb layer (JC), inner zona radiate extra layer (ZRE) and a thin zona radiate inner layer (ZRI); 5. the gelatinous-honeycomb outer layer (JC) of *H. otakii*; 6. the whole egg envelope surface of *H. otakii* and gelatinous-honeycomb layer (JC) was shown in this picture

不同生态类型鱼卵卵膜表面通常呈现突出卵膜表面的蚀刻或奇异结构，其可直接用于鱼卵形态分类[45-46]。如本研究中浮性绯鲔鱼卵卵膜具精致六边形网格状肋状脊；浮性日本鳗鲡鱼卵卵膜具不规整镰刀状突起；沉性大头鳕卵膜则具六角形轮纹，卵膜壁孔位于每个六角形网纹中心；具卵膜丝沙氏下鱵鱼卵膜表面则呈现规整排列颗粒状小突起等。据Wourms[47]研究卵膜表面呈现的蚀刻或奇异结构均是由卵母细胞成熟末期滤泡细胞封闭包装卵膜形成。近年来卵膜

表面特异结构在浮性鱼卵中于水体垂直分布的调节作用和作为卵膜支架对鱼卵在抗击波浪袭扰中的保护作用[48]，在沉性鱼卵中作为粘着器对鱼卵卵膜表面粘性指示[5, 11, 34, 49-51]和在鱼卵粘合部形成间隙便于新鲜海水通透保护胚胎正常发育[34]等生态功效已被人们广泛认知。本研究中浮性绯䲗鱼卵卵膜壁极薄，在扫描电镜样品制作过程中极易破裂，由于其卵子主要分布于近岸海区，卵膜表面精致六边形肋状脊被认为用作骨架支撑卵膜在抗击海水表层波浪袭扰过程中起保护作用；浮性日本鳗鲡鱼卵卵膜具不规整镰刀状突起，因其产卵场位于马里亚纳海山附近水域[52-53]，这种突出卵膜表面特异结构被认为在鱼卵水体垂直分布中起调节作用。多数沉性鱼卵外周有较厚黏膜层[54]，在鱼卵成熟排出体外并受精后沉性卵可利用自身粘性限定在特定区域直至孵化，这一行为策略可与产卵场环境条件相联系，在研究该类鱼卵形态特征时将产卵场环境因子的作用引入并加以讨论将非常有帮助[11]。如细纹狮子鱼与大泷六线鱼产卵场位于受潮汐和海浪影响剧烈的潮下或潮间带，其卵膜均具厚黏膜层，以使鱼卵始终粘着于大型海藻或特殊底质上发育。而大头鳕沉性受精卵则位于水深相对较深[55]，受潮汐和海浪影响较小海域；作为粘着器的表面六角轮纹结构附于相对光滑卵膜表面，预示其粘性较弱，但这足以满足鱼卵于环境相对稳定海底发育的需要[34]。沙氏下鱵鱼和尖头燕鳐鱼等海产具卵膜丝鱼卵通常借助卵膜表面延伸的具粘性卵膜丝附于海面漂浮物体上或是聚集成团一起发育，一般卵膜较厚，卵膜表面壁孔不明显或具颗粒状突起，颗粒状突起在鱼卵接触处形成适当间隙便于新鲜水体通透保护胚胎正常发育。

3.2　不同生态类型鱼卵受精孔区亚显微形态及其在分类鉴定中的运用

已有相关研究报道认为当卵母细胞发育成熟前，受精孔区为呈蘑菇状细胞和细胞突所占据，即受精孔细胞(MPC)或栓状细胞[56-59]。MPC及附近滤泡细胞会对发育过程中的卵母细胞表面施加机械压力，形成受精孔前庭。在卵膜形成过程中，MPC及其细胞状突触对受精孔区域成卵膜物质的沉积起限制性作用，从而形成精孔管。精孔管内壁螺旋脊状结构则主要是由卵膜分层形成[16]。当卵膜完全形成后，受精孔细胞的细胞突在卵母细胞成熟或即将排卵时从精孔管中抽出。成熟卵子即拥有一个开放受精孔。沉性卵受精孔一般位于上半球(卵子沉至水底后，受精孔位于卵子上半部位)；而浮性卵受精孔则一般位于下半球(鱼卵上浮时受精孔位于鱼卵下半部位)。受精过程中受精孔起着精子识别引导的重要作用，它们的形态可能具有物种特异性[16-17]。早在20世纪60年代中期，受精孔形态特征已经被认为是鉴定鱼卵种类最重要依据[19]。截至当前其已广泛应用于多种鱼卵分类鉴别中。包括未受精的7种鲑和4种鳟鱼卵[60]、南极海域分布的6种极地鱼类鱼卵[61]和4种鲷科鱼类鱼卵[17]；自然海域采集的已受精鲈(*Lateolabrax japonicus*)和石鲽(*Kareius bicoloratus*)等9种[62]，形态相近鲽亚科(Pleuronectinae)4种[63]和天竺鲷科(Apogonidae)5种[44]鱼卵的分类鉴定。

本研究中鱼卵受精孔涵盖全部类型，如沉性大头鳕受精孔属Ⅰ型、具卵膜丝沙氏下鱵鱼受精孔属Ⅱ型。Ⅲ型受精孔种类最多，包括浮性日本鳗鲡、鮟鱇、斑鰶、白姑鱼、鮻、日本鬼、高眼鲽和褐牙鲆鱼卵；口含细条天竺鱼卵和具卵膜丝尖头燕鳐鱼卵。受精孔径在不同种类中大小不同，大头鳕鱼卵受精孔在光学显微镜下即清晰可见[34]，细条天竺鱼和沙氏下鱵鱼受精孔也较大，而斑鰶、褐牙鲆、高眼鲽、日本鳗鲡受精孔则较小。精孔管内壁螺旋脊数量和旋转方式也成为鱼卵种类鉴定依据[33]。本研究能够观察到的受精孔种类中，除日本鬼精孔管具双螺旋脊外，其余均为单螺旋脊。需注意的是作为鱼类无顶体精子入卵并完成受精作用唯一通道的受精孔形状在受精后及胚胎发育过程中处在动态变化中[34]。如精孔管由开放变为关闭(本研究中斑鰶、日本鬼、鮻、鮟鱇等各发育期鱼卵)等，这些变化被认为是阻止多余精子和有害有机体进入卵子并保障胚胎正常发育的机械屏障的一部分[64]，但其也使得受精孔某些形态特征如精孔管径、内壁螺旋脊数量等形态特征不再适用于物种分类鉴定。

3.3　鱼卵卵膜和受精孔区亚显微形态在鱼卵种类鉴定中运用评价

近半个世纪来，已有不少学者利用扫描电镜对鱼卵卵膜进行超显微结构观察，发现不同鱼类卵膜超显微结构不尽相同，卵膜许多形态结构可以作为区分鱼类科或属的性状，而受精孔

(区)结构一般具有物种特异性。许多研究表明，鱼卵亚显微结构特征也可用来检测相近种或属，甚至亚科系统发生关系，从而了解其是否与根据鱼卵形态学特征建立的系统发生关系相吻合[17]。如卵膜及受精孔亚显微结构是进行鲻科(Mugilidae)、鲷科、天竺鲷科、鲽亚科鱼卵种类鉴定以及系统发生研究的重要依据[17, 33, 41, 44-45, 65]。需注意的是尽管扫描电镜观测能够获取比光学显微镜观察更多的形态特征，但是由于多数鱼卵形态结构的未知，卵膜形态可能受产卵环境适应性及其系统分类地位双重影响等，仅依靠扫描电镜技术目前仍不能准确将鱼卵鉴定到种[66]；且成熟鱼卵从卵巢中排出后即被激活，鱼卵在不同发育阶段呈现不同形态特征[33-34]，并且鱼卵不同发育期形态结构难以预料，基于此进行的比较和系统发生研究结果可信度较差[41]。因此在将鱼卵卵膜亚显微形态作为个体发生的主要特征而进行系统发生研究时，为得出最准确的结果，应保证各种鱼卵发育期的同步(多数采用成熟未受精卵)。

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参考文献：

[1] 陈大刚. 黄渤海渔业生态学[M]. 北京: 海洋出版社, 1991, 8-31.
Chen D G. Fishery ecology of the Bohai Sea and the Yellow Sea[M]. Beijing: China Ocean Press, 1991, 8-31(in Chinese).

[2] 刘静, 宁平. 黄海鱼类组成、区系特征及历史变迁[J]. 生物多样性, 2011, 19(6): 764-769.
Liu J, Ning P. Species composition and faunal characteristics of fishes in the Yellow Sea[J]. Biodiversity Science, 2011, 19(6): 764-769(in Chinese).

[3] Shao K T, Yang J S, Chen K C, *et al*. An identification guide of marine fish eggs from Taiwan[M] . Taiwan: Institute of Zoology, Academia Sinina, 2001: 1-14.

[4] Iconomidou V A, Chryssikos D G, Gionis V, *et al*. Secondary structure of chorion proteins of the teleostean fish *Dentex dentex* by ATR FT-IR and FT-Raman spectroscopy[J]. Journal of Structural Biology, 2000, 132: 112-122.

[5] Laale H W. The perivitelline space and egg envelopes of bony fishes: A review[J]. Copeia, 1980, 2: 210-226.

[6] Stehr C M, Hawkes J W. The comparative ultrastructure of the egg membrane and associated pore structures in the starry flounder, *Platichthys stellatus*(Pallas), and pink salmon, *Oncorhynchus gorbuscha* (Walbaum)[J]. Cell and Tissue Research, 1979, 202: 347-356.

[7] Ortiz-Delgado J B, Porcelloni S, Fossi C, *et al*. Histochemical characterization of oocytes of the swordfish *Xiphias gladius*[J]. Scientia Marina, 2008, 72: 549-564.

[8] Fausto A M, Picchietti S, Taddei A R, *et al*. Formation of the egg envelope of a teleost, *Dicentrarchus labrax* (L.): immunochemical and cytochemical detection of multiple components[J]. Anatomy and Embryology, 2004, 208: 43-53.

[9] Lönning S. Comparative electronmicroscopic studies of teleostean eggs with special reference to the chorion[J]. Sarsia, 1972, 49: 41-48.

[10] Ivankov V N, Kurdyayeva V P. Systematic differences and the ecological importance of the membranes in fish eggs[J]. Journal ofJ Ichthyology, 1973, 13: 864-873.

[11] Huysentruyt F, Adriaens D. Adhesive structures in the eggs of *Corydoras aeneus*(Gill, 1858; Callichthyidae)[J]. Journal of Fish Biology, 2005, 66: 871-876.

[12] Mekkawy I A A, Osman A G M. Ultrastructural studies of the morphological variations of the egg surface and envelopes of the African catfish *Clarias gariepinus* (Burchell, 1822) before and after fertilisation, with a discussion of the fertilisation mechanism[J]. Scientia Marina, 2006, 70(S2): 23-40.

[13] Hagström B E, Lönning S. Electron microscopic studies of unfertilized and fertilized eggs from marine teleosts[J]. Sarsia, 1968, 33: 73-80.

[14] Hosokawa K. Electron microscopic observation of chorion formation in the teleost, *Navodon modestus*[J]. Zoological Science, 1985, 2: 513-522.

[15] Otani S, Iwai T, Nakahata S, *et al*. Artificial fertilization by intracytoplasmic sperm injection in a teleost fish, the Medaka (*Oryzias latipes*)[J]. Biology of Reproduction, 2009, 80: 175-183.

[16] Kobayashi W, Yamamoto T S. Fine structure of the micropylar apparatus of the chum salmon egg, with a

discussion of the mechanism for blocking polyspermy[J]. Journal of Experimental Biology, 1981, 217: 265-275.

[17] Chen K C, Shao K T, Yang J S. Using micropylar ultrastructure for species identification and phylogenetic inference among four species of Sparidae[J]. Journal of Fish Biology, 1999, 55: 288-300.

[18] Riehl R, Götting K J. Zu Struktur und Vorkommen der Mikropyle an Eizellen und Eiern von Knochenfischen (Teleosti)[J]. Archiv für Hydrobiologie, 1974, 74: 393-402.

[19] Riehl R, Schulte E. Bestimmungsschlüssel der wichtigsten deutschen Süsswasser Teleosteer anhand ihrer Eier[J]. Archiv für Hydrobiologie, 1978, 83: 200-212.

[20] Riehl R. The ecological significance of the egg envelope in teleosts with special reference to limnic species[J]. Limnologica, 1996, 26: 183-189.

[21] 赵传絪, 张仁斋, 陆惠芬, 等. 中国近海鱼卵与仔鱼[M]. 上海: 上海科学技术出版社, 1985.
Zhao C Y, Zhang R Z, Lu H F, *et al*. Fish eggs and larvae in the offshore waters of China[M]. Shanghai: Shanghai Science and Technology Press, 1985(in Chinese).

[22] 姜言伟, 万瑞景. 渤海半滑舌鳎早期形态及发育特征的研究[J]. 海洋水产研究, 1988, 9: 121-149.
Jiang Y W, Wan R J. Reproductive behavior and spawning ecology of *Cynoglossus semilaevis* Günther in the Bohai Sea[J]. Marine Fishery Research, 1988, 9: 121-149(in Chinese).

[23] 万瑞景, 陈瑞盛. 渤海鲈鱼的生殖习性及早期发育特征的研究[J]. 海洋水产研究, 1988, 9: 203-211.
Wan R J, Chen R S. Reproductive behavior and early development of *Lateolabrax japonicas* (Cuvier Valenciennes) in the Bohai Sea[J]. Marine Fishery Research, 1988, 9: 203-211(in Chinese).

[24] 万瑞景. 多鳞早期发育形态[J]. 海洋水产研究, 1996, 17(1): 35-41.
Wan R J. Morphology of early development of *Sillago sihama* (Forskål)[J]. Marine Fishery Research, 1996, 17(1): 35-41(in Chinese).

[25] 陈四清, 高天翔, 王琛, 等. 圆斑星鲽早期发育特征的研究[J]. 中国海洋大学学报, 2006, 36: 281-286.
Chen S Q, Gao T X, Wang C, *et al*. Study on developmental characters in early stage of spotted halibut *Verasper variegates*[J]. Periodical of Ocean University of China, 2006, 36: 281-286(in Chinese).

[26] 肖志忠, 郑炯, 于道德, 等. 条石鲷早期发育的形态特征[J]. 海洋科学, 2008, 11(2): 25-30.
Xiao Z Z, Zheng J, Yu D D, *et al*. Developmental characters at the early stayes of the Japanese parrotfish (*Oplegnathus fasciatus*)[J]. Marine Sciences, 2008, 11(2): 25-30(in Chinese).

[27] 卢敏德, 葛志亮, 倪建国, 等. 暗纹东方鲀精、卵超微结构及精子入卵早期电镜观察[J]. 中国水产科学, 1999, 6(2): 5-8.
Lu M D, Ge Z L, Ni J G, *et al*. Ultrastructural observation on sperm egg and early sperm-penetration in *Fugu obscures*[J]. Journal of Fishery Sciences of China, 1999, 6(2): 5-8(in Chinese).

[28] 章龙珍, 陈丽慧, 庄平, 等. 长江口纹缟虾虎鱼精子、卵子及受精过程扫描电镜观察[J]. 海洋渔业, 2008, 30(4): 308-313.
Zhang L Z, Chen L H, Zhuang P, *et al*. Observation on spermatozoa, egg and sperm penetration of *Tridentiger trigonocephalus* by means of scanning electron microscopy[J]. Marine Fisheries, 2008, 30(4): 308-313(in Chinese).

[29] 张涛, 柳凌, 张洁明, 等. 日本鳗鲡精卵的超微结构以及受精过程观察[J]. 水生生物学报, 2010, 34(4): 769-778.
Zhang T, Liu L, Zhang J M, *et al*. Ultrastructure of spermatozoa and fertilized eggs of *Anguilla japonica* and observation on the fertilization process[J]. Acta Hydrobiologica Sinica, 2010, 34(4): 769-778(in Chinese).

[30] 张筱兰, 郭恩棉, 王昭萍, 等. 3种海产经济鱼类成熟卵膜形态的比较研究[J]. 海洋科学, 1999, 6: 48-51.
Zhang Y L, Guo E M, Wang Z P, *et al*. The morphorology of mature chorions in three species of marine fish[J]. Marine Sciences, 1999, 6: 48-51(in Chinese).

[31] 卞晓东, 张秀梅, 肖永双, 等. 沙氏下鱵鱼(*Hyporhamphus sajori*)卵的形态学及遗传学鉴别研究[J]. 水产学报, 2008, 32(3): 342-353.
Bian X D, Zhang X M, Xiao Y X, *et al*. Morphological and genetic identification of Japanese halfbeak (*Hyporhamphus sajori*) eggs[J]. Journal of Fisheries of China, 2008, 32(3): 342-353(in Chinese).

[32] 张世奎, 刘海金, 李忠红, 等. 3种鲆鲽鱼精子和卵子表面形态结构的比较研究[J]. 水产科学, 2010, 29(2): 63-68.
Zhang S K, Liu H J, Li Z H, *et al*. A comparative study on surface morphology of sperm and mature egg in three species flatfish[J]. Fisheries Science, 2010, 29(2): 63-68(in Chinese).

[33] Bian X, Zhang X, Gao T, *et al*. Morphology of unfertilized mature and fertilized developing marine pelagic eggs in four types of multiple spawning flounders[J]. Ichthyological Research, 2010, 57: 343-357.

[34] Bian X, Zhang X, Sakurai Y, *et al*. Envelope surface ultrastructure and specific gravity of artificially fertilized Pacific cod *Gadus macrocephalus* eggs[J]. Journal of Fish Biology, 2014, 84: 403-421.

[35] 纪东平, 卞晓东, 宋娜, 等. 荣成俚岛大泷六线鱼摄食生态研究[J]. 水产学报, 2014, 38(9): 1399-1409.
Ji D P, Bian X D, Song N, *et al*. Feeding ecology of *Hexagrammos otakii* in Lidao Rongcheng[J]. Journal of Fisheries of China, 2014, 38(9): 1399-1409(in Chinese).

[36] Ravaglia M A, Maggese M C. Ovarian follicle ultrastructure in the teleost *Synbranchus marmoratus* (Bloch, 1795), with special reference to the vitelline envelope development[J]. Tissue and Cell, 2003, 35: 9-17.

[37] Meloni S, Mazzini M, Fausto A, *et al*. Egg envelope organization in the icefish *Chionodraco hamatus*[J]. Polar Biology, 2004, 27: 586-594.

[38] Francisco J A, Medina A. Ultrastructure of oogenesis in the bluefin tuna, *Thunnus thynnus*[J]. Journal of Morphology, 2005, 264: 149-160.

[39] Shabanipour N, Hossayni S N. Histological and ultrastructural study of Zona Rodiata in oocyte of comman carp *Cyprinus carpio* (Linnaeus 1758)[J]. Micron, 2010, 41(7): 877-881.

[40] Arukwe A, Goksøyr A. Eggshell and egg yolk proteins in fish: hepatic proteins for the next generation: oogenetic, population, and evolutionary implications of endocrine disruption[J]. Comparative Hepatology, 2003, 2: 4.

[41] Gwo H H. Morphology of the fertilizable mature egg in the *Acanthopagrus latus*, *A. schlegeli* and *Sparus sarba* (Teleostei: perciformes: sparidae)[J]. Journal of Microscopy, 2008, 232: 442-452.

[42] Olivar M P. Chorion ultrastructure of some fish eggs from the southeast Atlantic[J]. South African Journal of Marine Science, 1987, 5: 659-671.

[43] Ahlstrom E, Moser H. Characters useful in identification of pelagic marine fish eggs[J]. California Cooperative Oceanic Fisheries Investigations Reports, 1980, 21: 121-131.

[44] Chen C H, Wu C C, Shao K T. Chorion microstructure for identifying five fish eggs of Apogonidae[J]. Journal of Fish Biology, 2007, 71: 913-919.

[45] Riehl R. Surface morphology and micropyle as a tool for identifying fish eggs by scanning electron microscopy[J]. Microscopy and Analysis, 1993, 5: 29-31.

[46] Merrett N R, Barnes S H. Preliminary survey of egg envelope morphology in the macrouridae and the possible implications of its ornamentation[J]. Journal of Fish Biology, 1996, 48: 101-119.

[47] Wourms J. Annual fish oogenesis I. differentiation of the mature oocyte and formation of the primary envelope[J]. Developmental Biology, 1976, 50: 338-354.

[48] Robertson D A. Possible functions of surface structure and size in some planktonic eggs of marine fishes[J]. New Zealand Journal of Marine and Freshwater Research, 1981, 15(2): 147-153.

[49] Patzner R A, Glechner R. Attaching structures in eggs of native fishes[J]. Limnologica, 1996, 26: 179-182.

[50] Riehl R, Patzner R A. Minireview: the modes of egg attachment in teleost fishes[J]. Italian Journal of Zoology, 1998, 65(Sl): 415-420.

[51] Rizzo E, Sato Y, Barreto B P, *et al*. Adhesiveness and surface patterns of eggs in neotropical freshwater teleosts[J]. Journal of Fish Biology, 2002, 61: 615-632.

[52] Tsukamoto K. Discovery of the spawning area for Japanese eel[J]. Nature, 1992, 356: 789-791.

[53] Tsukamoto K. Spawning of eels near a seamount[J]. Nature, 2006, 439: 929.

[54] Park J, Kim I. Fine structure of the oocyte envelopes of three related cobitid species in the genus *Ikookimia* (Cobitidae)[J]. Ichthyological Research, 2001, 48: 71-75.

[55] Alderdice D F, Forrester C R. Effects of salinity, temperature, and dissolved oxygen on early development of the Pacific cod (*Gadus macrocephalus*)[J]. Journal of the Fisheries Research Board of Canada, 1971, 28: 883-

902.

[56] Takano K, Ohta H. Ultrastructure of micropylar cells in the ovarian follicles of the pond smelt, *Hypomesus transpacificus nipponemis*[J]. Bulletin of the Faculty of Fisheries Hokkaido University, 1982, 33: 65-78.

[57] Kobayashi W, Yamamoto T S. Fine structure of the micropylar cell and its change during oocyte maturation in the chum salmon, *Oncorhynchus keta*[J]. Journal of Morphology, 1985, 184: 263-276.

[58] Iwamatsu T, Nakashima S, Onitake K. Spiral patterns in the micropylar wall and filaments on the chorion in eggs of the medaka, *Oryzias latipes*[J]. Journal of Experimental Zoology, 1993, 267: 225-232.

[59] Nakashima S, Iwamatsu T. Ultrastructural changes in micropylar and granulosa cells during *in vitro* oocyte maturation in the medaka, *Oryzias latipes*[J]. Journal of Experimental Zoology, 1994, 270: 547-556.

[60] Riehl R. Micropyle of some salmonins and coregonins[J]. Environmental Biology of Fishes, 1980, 5(1): 59-66.

[61] Riehl R, Kock K H. The surface structure of Antarctic fish eggs and its use in identifying fish eggs from the southern ocean[J]. Polar Biology, 1989, 9: 197-203.

[62] Hirai A. Fine structures of the micropyles of pelagic eggs of some marine fishes[J]. Japanese Journal of Ichthyology, 1988, 35: 351-357.

[63] Hirai A. Fine structure of the egg membranes in four species of Pleuronectinae[J]. Japanese Journal of Ichthyology, 1993, 40: 227-235.

[64] Yamamoto T S, Kobayashi W. Closure of the micropyle during embryonic development of some pelagic fish eggs[J]. Journal of Fish Biology, 1992, 40: 225-241.

[65] Li Y H, Wu C C, Yang J S. Comparative ultrastructural studies of the zona radiata of marine fish eggs in three genera in Perciformes[J]. Journal of Fish Biology, 2000, 56: 615-621.

[66] Shao K T, Chen K C, Wu J H. Identification of marine fish eggs in Taiwan using light microscope, scanning electric microscope and mtDNA sequencing[J]. Marine and Freshwater Research, 2002, 53: 355-365.

Morphological diversities in envelope surface and micropyle of marine teleosts eggs with different ecological types in the Bohai Sea and the Yellow Sea

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Abstract: Marine teleosts eggs distributed in the Yellow Sea and the Bohai Sea cover almost all the egg ecological types. Scanning electron microscope (SEM) observation was conducted to examine the ultrastructure of external egg membrane and micropyle of the 15 species with different egg ecological types which have been collected during the fisheries resource surveys in recent years. Taxonomic diagnostic ultrastructures have been accumulated, also habitat suitability of some related ultrastructures have been discussed. Current SEM studies have demonstrated that ultrastructure of the external egg membrane varies among egg ecological types or even among species. The external egg membrane of the floating eggs and mouth-breeding eggs was thin, but was thick in the demersal eggs and the eggs with adhesive egg filaments. The pores distribution density, sculptured patterns and peculiar elaborations of the external egg membrane and type of the micropyle in the floating eggs; fine structure of the external egg membrane and type of the micropyle in the demersal eggs; number and location of membrane thread or filament of the external egg membrane and type of the micropyle in the adhensive eggs; number and arrangement of the filaments and surface ridges in the micropyle region in the mouth-breeding eggs could be used as the diagnostic characters in taxonomy of fish eggs. Untrastructural differences in envelope surface and micropyle between species were associated with its systematic groups, spawning environment and geographic differences. Envelope surface ultrastructures were considered to be taxonomically useful features for identifying fish eggs to the family or genus level, with the pores distribution density, sculptured patterns and peculiar elaborations of the external egg membrane may be possible to assign the eggs to a species. While morphology of the micropyle was usually species-specific. Egg envelope structure and thickness of various teleosts often reflect the ecological challenges a species is faced with during its embryonic life stages. The sculptured patterns and peculiar elaborations on the outer egg membrane were considered as biological response to different habitat environments. The present study would accumulate basic data on ontogeny and morphological classification features during its embryonic life stages of the teleost fishes distributed in the Yellow Sea and the Bohai Sea.

Key words: fish eggs; envelope; micropyle; ultrastructure; ecological type; habitat suitability

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