Observations on the embryonic development of the mud crab, *Scylla paramamosain*

Li-Kun Xu, Ke-Yi Ma, Chun-Yan Ma, Zhi-Qiang Liu

Background

The mud crab *Scylla paramamosain* (Crustacea, Decapoda, Portunidae, *Scylla*) is one of the most important marine crab species widely cultured along the coasts of southern China. However, despite its high economic value, the production of S. *paramamosain* has not yet been able to meet the growing demand of the consumer market. Therefore, there is an urgent need to further increase the production. Until now, there are still many scientific and technological problems that have not yet been effectively solved in *S. paramamosain* culture. Embryo development is a key step in the crab breeding process and has significant effect on the growth and development of the crab individual. In the context of the urgent need for upgrading and expansion of the *S. paramamosain* culture industry, as well as the urgent need to break through the problems of low survival rate and unstable yield in nurseries, it is necessary to systematically study the embryonic development of *S.* ***paramamosain***.

Results

1. Egg color change of Ovigerous crabs

During embryonic development, the egg color exhibited a progressive transition, shifting from orange to reddish-orange, then to brown, before ultimately darkening to black.

2. Fertilized eggs and cleavage stage

The fertilized eggs, each about 280 μm in diameter, were spherical in shape, medium-yolked, smooth and without cracks. The first cleavage of the embryo was an inward depression of the fertilized egg, splitting the embryo into two cells, or blastomeres, of different sizes (Figure 1C), The following 2nd to 6th rounds of division were in the form of spiral oogenesis, dividing the embryo sequentially into 4, 8, 16, 32 and 64 cells (Figure 1E, 1G and 1I, and Figure 2A and 2C). The 7th to 8th rounds of division changed from spiral oogenesis to embryonic surface oogenesis.

3.Blastoderm stage

After the 8th cleavage, the *S. paramamosain* embryo entered the blastocyst stage, in which many nuclei were distributed on the surface of the embryo (Figure 2H). Additionally, the cleavage furrow on the embryo surface was difficult to visualize (Figure 2G, 2H, 2I, 2J). At the late blastocyst stage, the uniformly distributed nuclei began to recess inward along the major axis of the embryo (Figure 2J).

4. Gastrulation stage

In the early stage of gastrulation, the blastopore was formed because of the migration of some cells into the endosomes. Due to the dense aggregation of cells, the yolk around the blastopore was rapidly consumed in comparison to the areas where the cells were more dispersed. Hence, a transparent area appeared where the blastopore was located (Figure 3A). It could be clearly observed that the blastopore had taken shape, based on the small pore formed by cell aggregation in the embryo (Figure 3B). With further development, the transparent region was enlarged and inverted triangularly concave toward the inner part of the embryo (Figure 3C). At this period, a large number of cells around the blastopore were found to be aggregated into clusters on the surface of the embryo, and the nuclei of the cells in the region were small and densely packed, whereas the cells in the region outside the blastopore were larger and more loosely arranged (Figures 3D). After the blastopore's appearance, the transparent region of the embryo was elongated toward both ends into a crescent shape, and the yolk color above the concavity of the transparent area became lighter, showing that there were cell clusters in this area (Figure3E).

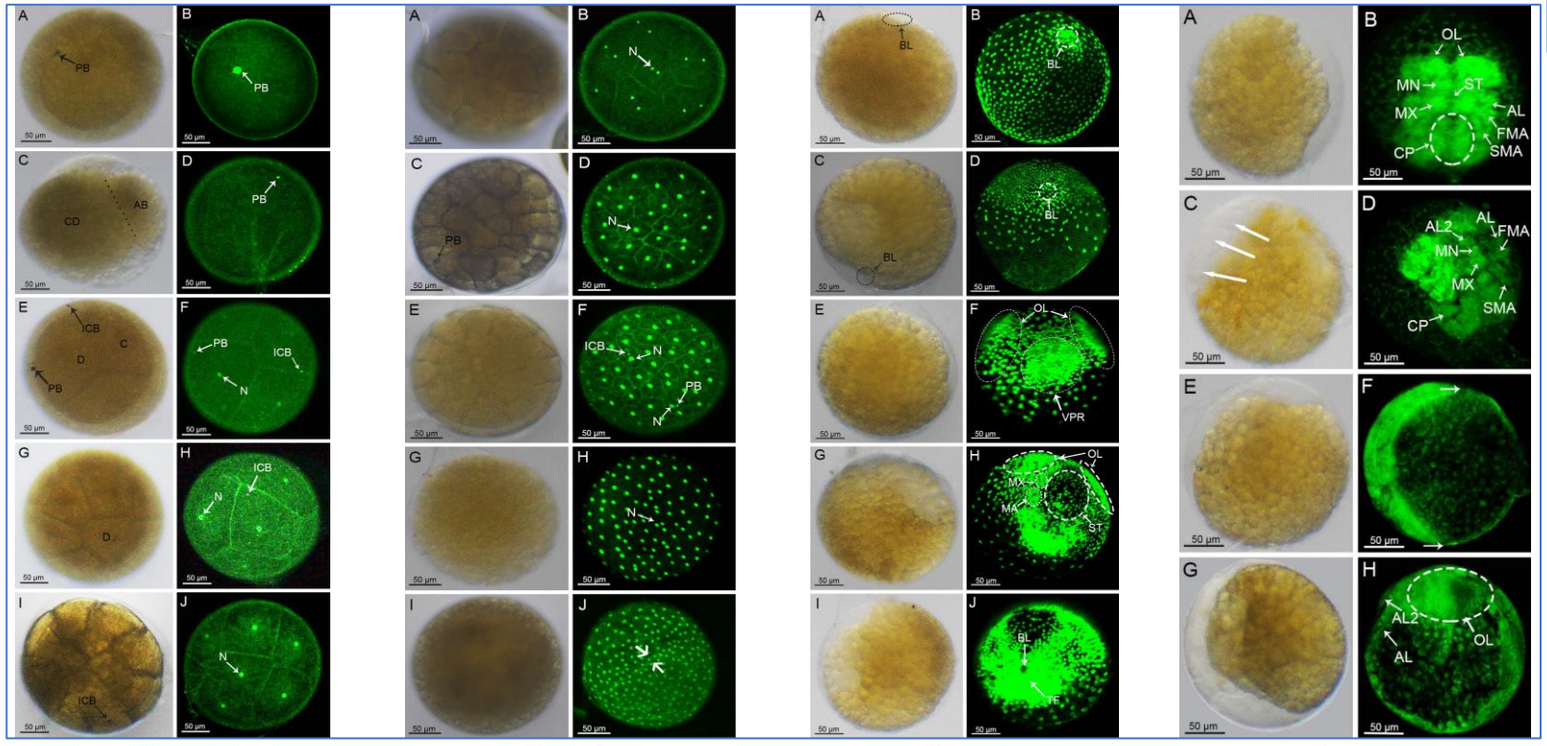

Figure1 Figure2 Figure 3 Figure 4

5.Nauplius stage and abdominal limb formation stage

the embryonic development entered the Nauplius stage, the overall size of the embryo increased significantly compared with the previous developmental stage. The transparent area of the embryo was crescent-shaped and accounted for about 1/5 of the egg. Part of the semicircular ventral limb primordia could be seen within the transparent area (Figure 4A). Moreover, distinct zonation occurred on the TF and evolved into distinct protostomes. From the top to bottom as shown in Figure 4B, these were the OL, MN, MX, AL, first maxilliped primordia (FMA), second maxilliped primordia (SMA), and caudal papilla (CP). Thereafter, the transparent region of the embryo occupied about 1/4 of its volume, and wavy projections appeared within the transparent region (Figure 4C). The elongated ends of the FMA and SMA diverged, and the CP lengthened to evolve a ventral segmental partition, with an inwardly curved end (Figure 4D). Later, the nauplius wrapped around the yolk in an arc shape and extended to both ends (Figure 4E and 4F).

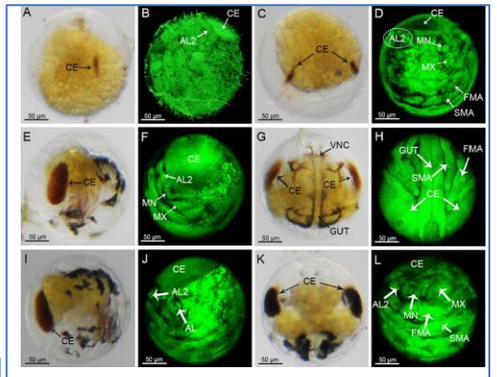

Figure5

conclusion

In the current study, the whole embryonic development of *S. paramamosain* has been observed, focusing on the overall color of all the eggs, the morphological changes of individual embryos, and the changes of nuclei migration. The first cleavage of the embryo was an unequal cleavage, and the 2nd-6th cleavages were spiral oogenesis. The larger D cell during the 4-cell period exhibited delayed division relative to the smaller crown cells surrounding it. The 7th-8th cleavages were surface cleavage, and the cleavage furrow was difficult to observe after the sixth oocyte cleavage. In the early gastrulation stage, the embryo concaved inwards to form the blastopore. The cells gathered around the blastopore to further differentiate into different appendage primordia. This work fills a significant gap in our knowledge about the embryonic development of *S. paramamosain* at the nucleus level, and the data obtained will help in the determination of embryo development status and also potentially in the obtaining of high-quality mud crab seedlings.