Received: 12 May 2021 | Revised: 11 December 2021 | Accepted: 6 January 2022

DOI: 10.1111/are.15748

Aquaculture Research WILEY

ORIGINAL ARTICLE

Morphology of the complete embryonic and larval development of commercially important mud crab *Scylla paramamosain*

Jishan Li[1] | Wanlei Qiu[1] | Hua Hao[1,2,3] | Fangyi Chen[1,2,3] | Ke-Jian Wang[1,2,3]

[1]State Key Laboratory of Marine Environmental Science, College of Ocean & Earth Sciences, Xiamen University, Xiamen, Fujian, China

[2]State-Province Joint Engineering Laboratory of Marine Bioproducts and Technology, College of Ocean & Earth Sciences, Xiamen University, Xiamen, Fujian, China

[3]Fujian Innovation Research Institute for Marine Biological Antimicrobial Peptide Industrial Technology, College of Ocean & Earth Sciences, Xiamen University, Xiamen, Fujian, China

Correspondence
Ke-Jian Wang, State Key Laboratory of Marine Environmental Science, College of Ocean & Earth Sciences, Xiamen University, Xiamen 361102, Fujian, China.
Email: wkjian@xmu.edu.cn

Funding information
Fundamental Research Funds for the Central Universities, Grant/Award Number: 20720190109; National Natural Science Foundation of China, Grant/Award Number: U1805233; Fujian Marine Economic Development Subsidy Fund Project from the Fujian Ocean and Fisheries Department, Grant/Award Number: FJHJF-L-2019-1; Xiamen Science and Technology Planning Project, Grant/Award Number: 3502Z20203012

Abstract

Mud crab, *Scylla paramamosain*, is an important aquaculture species. In recent decades, with the development of mud crab farming, seed production has become more and more important. Therefore, a precise understanding of embryonic and larval development is required for future study or application. In this study, we described the complete embryonic and larval development of *S. paramamosain* under the following conditions: temperature 28°C–31°C, salinity 26,000–30,000 mg/L, pH 7.9–8.2, and dissolved oxygen 4–5 mg/L. The embryonic development was divided into five stages: cleavage, blastula, appendage, eyespot-heartbeat, and prehatching. The entire embryonic period spanned 9–11 days. The larval development consisted of six stages: zoea I (Z1), zoea II (Z2), zoea III (Z3), zoea IV (Z4), zoea V (Z5), and the megalopal stage. The duration of the larval stage was about 22–24 days, and it took about 3 days for each stage of Z1–Z4, about 4–5 days for Z5, and about 5–6 days for the megalopal stage. In general, in this study, we presented a comprehensive description of both embryonic and larval development with massive image information, which would help diagnose different developmental stages and provide an important guidance for future study or application.

KEYWORDS
embryonic development, larval development, morphological features, *Scylla paramamosain*

1 | INTRODUCTION

Mud crab, *Scylla* spp., is a commercially important aquaculture species (Azra & Ikhwanuddin, 2016), which is commonly distributed in the Indo-Pacific region (Ates et al., 2012; Zeng, 2007). The genus includes four different species: *Scylla serrata* (Forskal, 1775), *Scylla olivacea* (Herbst, 1796), *Scylla tranquebarica* (Fabricius, 1798), and *Scylla paramamosain* (Estampador, 1949) (Keenan et al., 1998; Waiho et al., 2018; Zeng, 2007). In recent years, the scale of mud crab farming has greatly expanded (Waiho et al., 2018). FAO statistics showed that the global mud crab aquaculture production was 79,261 tons in 2014, 82,514 tons in 2015, and 89,390 tons in 2016 with a growth rate of about 4–8% (http://www.fao.org/fishery/culturedspecies/Scylla_serrata/en), which has brought considerable income to farmers.

The development of mud crab farming requires a large amount of mud crab seeds. However, it mainly depends on the supply of wild sources (Ikhwanuddin et al., 2015; Waiho et al., 2018; Ye et al., 2010), and due to seasonal resource limitations (Ikhwanuddin et al., 2015; Zeng, 2007), indiscriminate collection (Samuel & Soundarapandian,

2010), and inconsistent supply (Ikhwanuddin et al., 2015), the quantity of natural crab seeds is insufficient, which will hinder the development of mud crab farming. Seed production is a great source of mud crab seeds and has been conducted in several countries, such as Japan (Hamasaki et al., 2011), the Philippines (Quinitio et al., 2011), Malaysia (Ikhwanuddin et al., 2015), and India (Samuel & Soundarapandian, 2010). However, the Z5 stage to the megalopal stage has a low survival rate of about 10% for *S. paramamosain* (Hamasaki et al., 2011) and about 2% for *S. serrata* (Quinitio et al., 2011), making it difficult to meet market demand (Hamasaki et al., 2011; Quinitio et al., 2011). Therefore, there is an urgent need for research on seed production technologies, including embryonic development and larval development. A comprehensive understanding of these technologies will help obtain sufficient and high-quality mud crab seeds.

S. paramamosain, one of the four mud crab species, has been found in the southeastern coastal area of China (Ye et al., 2010; Zeng et al., 2004) and other Asian countries, such as Japan (Hamasaki, 2002), Vietnam (Petersen et al., 2013), the Philippines (Zeng et al., 2004), and Indonesia (Zeng et al., 2004). In studies conducted before 2007, the dominant species of mud crab along the coast of China was described as *S. serrata*, however, subsequent work based on morphology and genetics showed that it was actually *S. paramamosian* (Lin, 2008; Lin et al., 2007). In recent decades, with the increasing interest in seed production of *S. paramamosain*, many studies of embryonic and larval development have been carried out (Li & Wang, 2007). As for the embryonic development, the effect of temperature on the incubation period has been investigated, and the results showed that the incubation period decreased as the temperature increased (Zeng, 2007). Some studies have also provided information on biochemical composition (Wang et al., 1995) and hydrolytic enzyme activities (Li et al., 1995) during different embryonic development stages. As for the larval development, intensive studies were carried out, which mainly focused on rearing techniques, nutrition, and physico-biochemical characteristics. For instance, temperature (Zeng & Li, 1992), salinity (Wang et al., 1998), starvation (Li & Zeng, 2001; Weng et al., 2002), and diet (Lin et al., 2001; Wang et al., 1994) were reported to have great effects on the growth, development, metamorphosis, and survival of larvae.

Regarding the morphology of embryonic development of *S. paramamosain*, ten stages (fertilized egg, cleavage, blastula, gastrula, nauplius, five pairs of appendages, seven pairs of appendages, eye-pigment formation, prehatching, and hatching) have been identified, and the morphological characteristics of each stage were described (Chen, 2005; Zeng, 2007). The division into too many developmental stages was not conducive to further molecular mechanism research. To describe the morphology of larval development of *S. paramamosain*, five zoeal stages and one megalopal stage have been studied (Huang & Li, 1965; Zeng et al., 2001). Due to difficulty in taking photos and the poor imaging system in the early stage, previous morphological information about larval development was mainly displayed in the form of drawings, which was less accessible to production workers. Therefore, in this study, we shortened the embryonic development to five stages and described the main features in detail. In addition, the larval development was systematically illustrated and a large amount of image information was provided.

2 | MATERIALS AND METHODS

2.1 | Animal feeding

The mature female crabs, ovigerous female crabs, and larvae used for morphological observation were fed during the artificial breeding period in Guangxi Institute of Oceanology (Guangxi Province, China) from 2018 to 2019. Animal care and experimental procedures were performed in accordance with the guidelines for the use of laboratory animals of Xiamen University.

Healthy and mature female crabs (*S. paramamosain*) with an average weight of more than 250 g were purchased from a local farm and raised with approximately 10–20 crabs in each cement pond (4 m in length, 4 m in width, 1.1 m in height, and 0.5 m in water level). After spawning, newly ovigerous female crabs were transferred from the cement pond to a single round tank (1 m in diameter, 0.7 m in height, and 0.6 m in water level). Crabs were fed with fresh oyster meat. Water was changed every morning to clean excess food and excreta.

When hatching occurred, the larvae were transferred from the round tank to a cement pond (4 m in length, 4 m in width, 1.1 m in height, and 0.9 m in water level). *Brachionus plicatilis* was fed to larvae in Z1–Z2, and *Artemia salina* was fed to larvae in Z3, Z4, Z5, and the megalopal stage and the first juvenile crab. About one fifth of the water was changed every day.

Seawater used in aquaculture was transferred from the nearby sea areas and treated with sand filter, sedimentation, cotton filtration, and ultraviolet disinfection. All the experimental animals were maintained under the following conditions: temperature 28°C–31°C, salinity 26,000–30,000 mg/L, pH 7.9–8.2, and dissolved oxygen 4–5 mg/L.

2.2 | Sample collection and preparation

Abdominal setae and egg stalks (es) of female crabs were collected and fixed with 4% paraformaldehyde in PBS (NaCl 137 mM, KCl 2.7 mM, Na_2HPO_4 10 mM, KH_2PO_4 2 mM, and pH7.4) for at least 24 h and rinsed with PBS three times, followed by dehydration with an ethanol gradient: 30% ethanol for 5 min, 50% ethanol for 5 min, 70% ethanol for 15 min, 80% ethanol for 15 min, 95% ethanol for 15 min, and 100% ethanol for 15 min. Subsequently, the samples were dried with a critical point drier (EM CPD300, Leica) and covered with a gold layer using a sputter coater (108 Auto, Cressington) and finally examined by a scanning electron microscope (SUPRA 55, Carl Zeiss).

To study embryonic and larval development, a total of 13 ovigerous female crabs and 13 pond larvae were tracked for morphological observation. A small group of embryos were collected every

24 h in the morning, and a dish of larvae in different developmental stages were collected and fixed with 4% paraformaldehyde. Due to diapause of embryos, molting failure of larvae, pathogen infection, and other adverse conditions, only five ovigerous female crabs and three pond larvae were completely and continuously sampled for morphological observation.

2.3 | Sample observation

The description of embryonic development was based on 100 embryos ($n = 100$) from five ovigerous female crabs (20 embryos from each). The terminology used for embryonic development description was guided by previous studies (Ates et al., 2012; Garcia-Guerrero & Hendrickx, 2004; Ikhwanuddin et al., 2015; Sarker et al., 2012).

The description of larval development was based on 60 larvae ($n = 60$) hatched from three ovigerous female crabs (20 larvae from each). The terminology used for larval development description was guided by previous studies (Kornienko & Korn, 2011; Kornienko et al., 2008; Ocampo et al., 2011; Santana et al., 2016). The description of zoeal stages was limited to easily observable characteristics, including the cephalothorax, abdomen, antennule, antenna, first maxilliped, and second maxilliped. For the megalopal stage and the first juvenile crab, only the cephalothorax and the carapace were described, respectively.

2.4 | Sample measurements

Considering individual differences of female crabs, temperature fluctuations, and some other factors, which made it difficult to estimate the corresponding development degree of embryos or larvae among different female crabs, the continuous measurements of the egg size, the heart rate of embryos, and the larval size were based on 20 embryos or 20 larvae from one ovigerous female crab. Both embryonic and larval development was measured using an optical microscope (BX51, OLYMPUS) equipped with a digital camera (MS60, MSHOT).

As for embryonic development, the egg size of the elliptical embryo was defined as the average of long and short diameters. To determine the heart rate of each embryo, the heartbeat was counted manually five times in one minute and averaged.

As for larval development, the measurements were defined as follows: the size of larvae (anterior margin of the eye to posterior of the telson); the size of megalopa (tip of the rostral spine to posterior of the telson); the cephalothorax length of megalopa (tip of the rostral spine to median posterior of the cephalothorax); the cephalothorax width of megalopa (widest part of the cephalothorax); the abdomen length of megalopa (anterior margin to posterior margin of the abdomen); the abdomen width of megalopa (widest part of the abdomen); the carapace length of the first juvenile crab (anterior margin of the forehead to posterior margin of the carapace); and the carapace width of the first juvenile crab (widest part of the carapace).

2.5 | Statistical analysis

Statistical analysis was performed using PASW Statistics ver.18.0 software (IBM). Data were analyzed by one-way ANOVA followed by Tukey's *post hoc* test (when the variances was homogeneous) or Games–Howell *post hoc* test (when the variances was heterogeneous). Statistical significance was defined as $p < 0.05$. The data were shown as mean and standard deviation.

3 | RESULTS

3.1 | The morphological features of ovigerous female crabs, abdominal setae, and egg stalks

In brachyuran crabs, females incubate their embryos under the abdomen from spawning to hatching (Garcia-Guerrero & Hendrickx, 2004; Sarker et al., 2012), and *S. paramamosain* exhibited similar features (Figure 1A–a). In *S. paramamosain*, female crabs contained 16 pleopods (Figure 1A-b,c) with two different microstructures, plumose setae (Figure 1B) and hair-like setae (Figure 1C). The plumose setae in the eight external pleopods, without embryo attachment, played an important role in protecting the embryos during the incubation and were called protective setae (ps) (Figure 1B). The hair-like setae in the eight internal pleopods, with embryo attachment through egg stalks, served as supporting tissue and were called ovigerous setae (os) (Figure 1C). During female crab oviposition, the egg mass would attach to the ovigerous setae, similar to a bunch of grapes (Figure 1D–a). Under the actions of water flow and abdominal pumping, egg stalks assumed different shapes, forming ribbons (Figure 1D–b) or strings (Figure 1D–c).

3.2 | Embryonic development

The embryo incubation period of *S. paramamosain* was 9–11 days (Figure 2), and the embryonic development was divided into five stages, including cleavage, blastula, appendage, eyespot-heartbeat, and prehatching (Figure 3a). The main features of each stage were described in detail as follows (Figures 2 and 3):

3.2.1 | Cleavage

The cleavage stage was observed on day 1. The egg mass showed orange colour. The diameter of the embryos was 298.94 ± 6.97 μm. When the first division occurred, the embryos reached the cleavage stage. Then, the embryos underwent several cleavages and divided into 2, 4, 8, 16, 32, 64, and 128 blastomeres. The

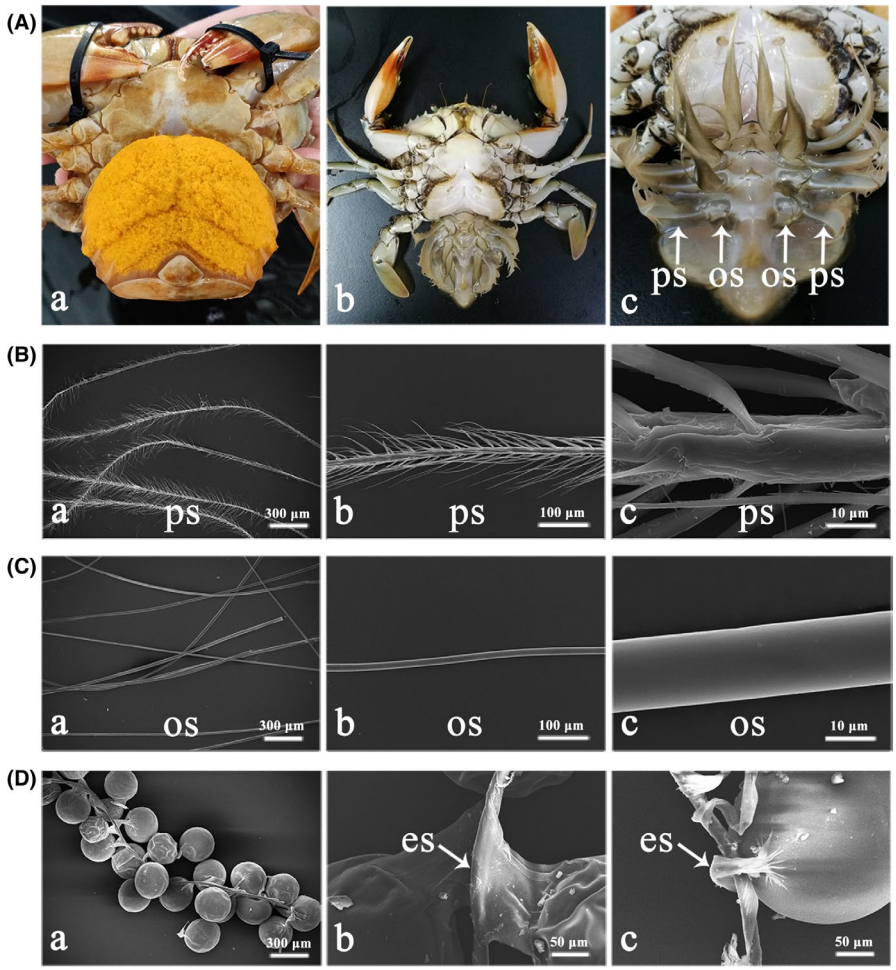

FIGURE 1 Morphological features of ovigerous female crabs, abdominal setae, and egg stalks. (A) Morphological features of ovigerous female crabs (a) and pleopods (b and c); (B) SEM pictures of protective setae (a–c) at different magnifications; (C) SEM pictures of ovigerous setae (a–c) at different magnifications; (D) SEM pictures of the egg cluster (a) and egg stalks (b and c). The scale bars were shown in the figures. ps, protective setae; os, ovigerous setae; es, egg stalks

cleavage stage was characterized by clear cleavage furrows between blastomeres.

3.2.2 | Blastula

The embryos reached the blastula stage on day 2. The egg mass showed orange colour. The diameter of the embryos was 298.10 ± 6.64 μm. After the embryos underwent eight cleavages, 256 blastomeres were produced, and the embryos reached the blastula stage. In the later blastula stage, it was not easy to distinguish individual blastomeres and cleavage furrows disappeared, which made their appearance similar to undivided embryos.

3.2.3 | Appendage

The appendage stage occurred on day 3–5. The egg mass showed orange colour. The diameter of the embryos was 304.23 ± 6.43 μm on day 3 and gradually increased to 307.82 ± 6.02 μm on day 4 and to 320.49 ± 5.86 μm on day 5. The characteristics of this stage were the appearance of different pairs of appendages, tissue differentiation, and organogenesis. On day 3, due to cell migration under the influence of gastrulation, a small yolk-free portion was observed. During this period, the buds of the optic lobe, antennule, antenna, and mandible were observed in the lateroventral position. On day 4, the yolk-free portion continued to increase and occupied 1/5 of the egg volume. With the use of egg yolk and other energy compounds, embryos gradually became transparent. During this period, the buds of maxillule and maxilla were visible, and the size increased. The rudiments of previous three pairs of appendages (antennule, antenna, and mandible) were also more elongated and differentiated. On day 5, with further differentiation and growth, the yolk-free portion increased to 1/4 of the egg volume, and two pairs of maxilliped were obvious. By then, a total of seven pairs of appendages (antennule, antenna, mandible, maxillule, maxilla, first maxilliped, and second maxilliped) had been formed, along with larger and more developed tissues and organs.

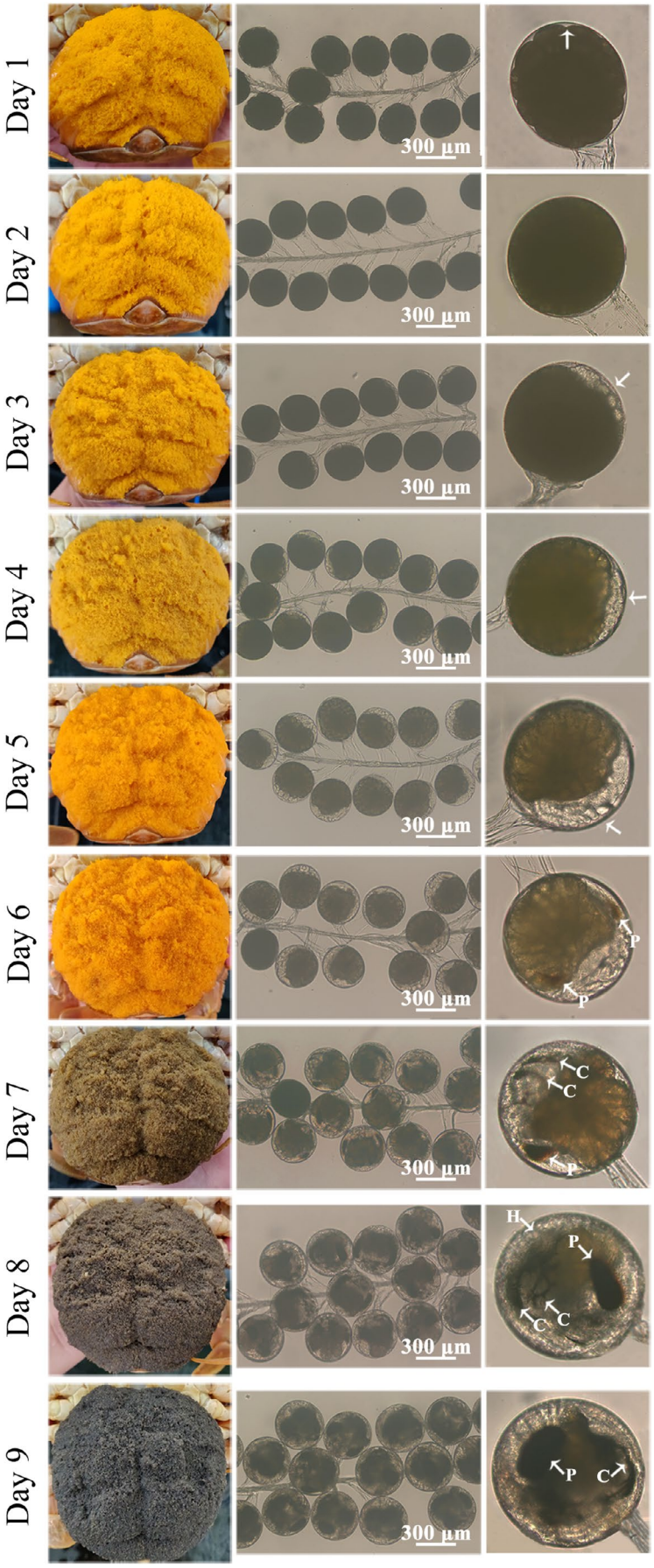

FIGURE 2 Embryonic development and morphological features. The arrows indicated the changes during embryonic development. Scale bar = 300 μm. P, pigments; C, chromatophores; H, heart

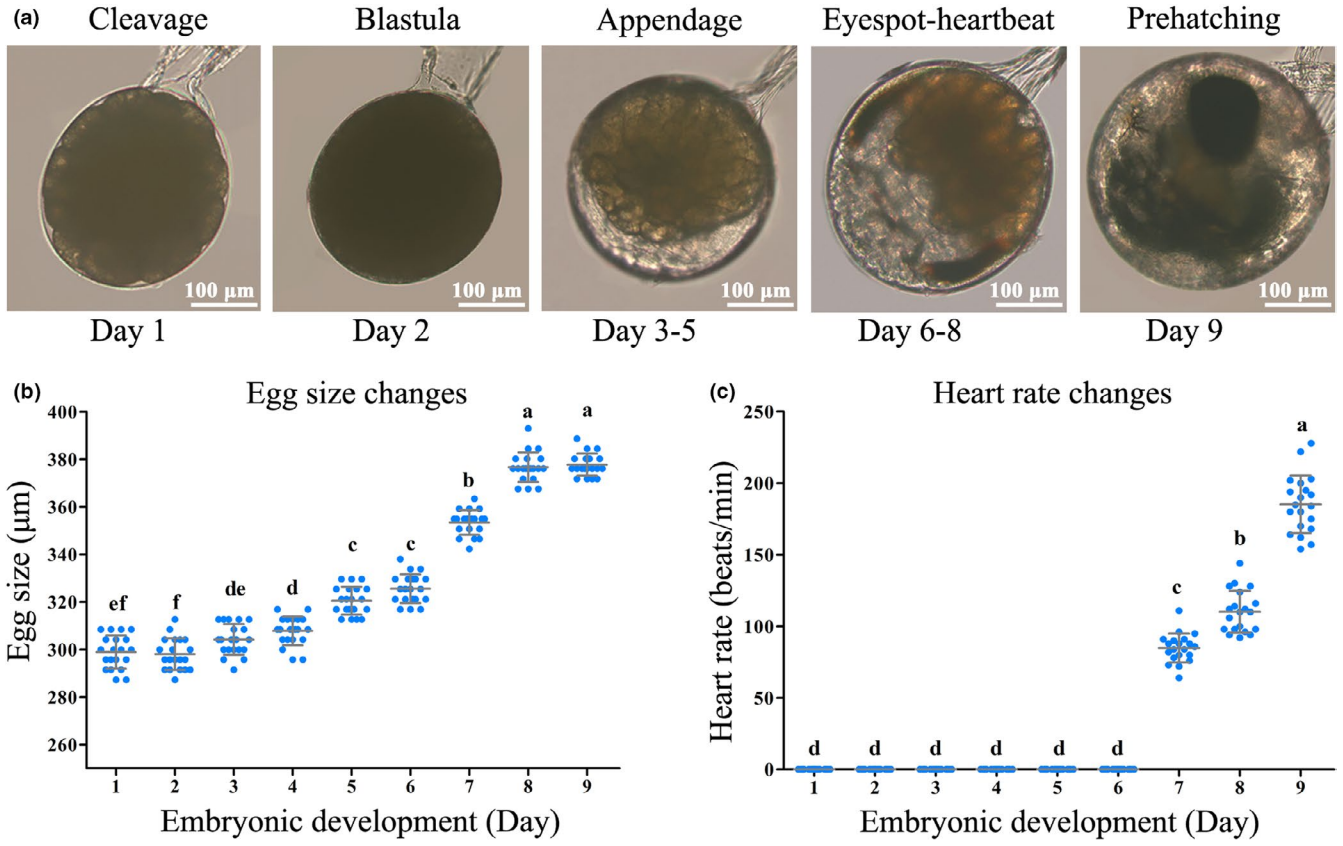

FIGURE 3 Embryonic development and morphological features. (a) Embryonic stages and morphological changes; (b) Egg size changes during embryonic development; (c) Heart rate changes during embryonic development. Vertical bar represented the mean and standard deviation. Statistical significance was defined as $p < 0.05$

3.2.4 | Eyespot-heartbeat

The eyespot-heartbeat stage occurred on day 6–8. The egg mass colour changed from orange to brown, then dark brown. The diameter of the embryos was 325.56 ± 6.05 μm on day 6 and gradually increased to 353.45 ± 5.18 μm on day 7 and to 376.69 ± 6.17 μm on day 8. The first appearance of eye pigments indicated that the embryos reached the eyespot-heartbeat stage. On day 6, the observed eyespots were scarlet crescents. All appendages were larger and incompletely segmented. On day 7, the egg yolk was further consumed, and the remaining egg yolk components were distributed among the four lobes. The cephalothorax and abdomen were separated, and the outlines were observable. During this period, it was noticed that the abdomen was enlarged and divided into segments, with two rows of chromatophores on it. The eye pigments became more intense and formed triangles. In addition, a large portion of the embryos developed heartbeats that beat weakly and irregularly at long intervals in the dorsal region of the cephalothorax. The heart rate was about 60–110 beats/min (Video S1). On day 8, the egg yolk was nearly exhausted. All appendages were well-developed, and the abdominal segmentation was completed. Eye pigments were larger, and the chromatophores continued to expand to the appendages and appeared branched. The heart rate was faster than during the previous stages, reaching about 90–140 beats/min (Video S2).

3.2.5 | Prehatching

The embryos reached the prehatching stage on day 9. The egg mass showed dark grey colour. The diameter of the embryos reached the largest size and was 377.75 ± 4.63 μm. The egg yolk was depleted with the remaining small yolk droplets located in the cephalothorax. The eyes with an oval shape were completely differentiated and fully pigmented. The number of chromatophores increased, and the colour intensity was higher. The heartbeat was more vigorous and regular, reaching about 150–230 beats/min (Video S3). At this stage, the cephalothorax, abdomen, and appendages were well-differentiated. The embryos moved violently, and occasionally twitching was observed (Video S4). The hatching occurred on day 10.

3.2.6 | Changes in the egg size

The diameters of the embryos were measured daily, and the results showed that as the embryo developed, the egg size gradually

increased (Figure 3b). The average egg diameter on the first day was 298.94 ± 6.97 μm, and it increased to 377.75 ± 4.63 μm on the ninth day, an absolute increase of 79 μm in average diameter.

3.2.7 | Changes in the heart rate

The heartbeat occurred on the seventh day, and as the embryo developed, the heartbeat became more vigorous and regular (Figure 3c and Video S1–S4). The heart rates increased from 60–110 beats/min to 90–140 beats/min, then to 150–230 beats/min, and the heart rates of some embryos could not be accurately counted before hatching.

3.3 | Zoeal stages

The larval development of *S. paramamosain* usually comprised five zoeal stages and one megalopal stage, before molting to the first juvenile crab (Video S5–S12). The duration of the larval stage was about 22–24 days, and it took about 3 days for each stage of Z1–Z4, about 4–5 days for Z5, and about 5–6 days for the megalopal stage. The main changes of five zoeal stages were described in detail as follows:

3.3.1 | Size

The total body length of larvae during Z1–Z5 were 1.26 ± 0.06 mm, 1.60 ± 0.05 mm, 2.14 ± 0.11 mm, 2.69 ± 0.19 mm, and 3.40 ± 0.22 mm, respectively (Figure 4a). The body length increased with the development of larvae. In addition, the larvae hatched from different ovigerous crabs showed differences in body length.

3.3.2 | Cephalothorax

The larval body of zoeal stages consisted of the cephalothorax and the abdomen (Figure 4a). The cephalothorax possessed seven appendages, including antennule, antenna, mandible, maxillule, maxilla, first maxilliped, and second maxilliped. In addition, the cephalothorax had a dorsal spine, a rostral spine, and a pair of lateral spines. The dorsal spine was slightly longer than the rostral spine. In Z1, the eyes were sessile, and in Z2–Z5, the eyes were stalked and movable.

3.3.3 | Abdomen

In Z1–Z2, the abdomen comprised six somites (telson included), and in Z3–Z5, the abdomen had seven somites (telson included; Figure 4b,c). Somite 2 had two lateral knobs directed anteriorly, and somite 3 had two hooks directed posteriorly (Figure 4c). Somites 3–5 each had a pair of posterolateral spines, and their lengths elongated with the larval development (Figure 4c). In Z3, the pleopod buds on somites 2–5 and the uropod buds on somite 6 occurred, and in Z4–Z5, the length of four pairs of pleopods and one pair of uropods increased (Figure 4b).

3.3.4 | Telson

In Z1, the telson fork had three pairs of plumose setae on the posterior margin, and each furcal shaft bore a lateral spine and a tiny dorsal spine (Figure 5a). In Z2–Z3, another pair of setae appeared in the median cleft of the posterior margin, and then in Z4–Z5, one seta occurred within the median cleft (Figure 5a).

3.3.5 | Antennule

The antennule was conical, and the amount of aesthetascs at the terminal of Z1–Z5 were 2–3, 5–6, 6–7, 8, and 9–10, respectively (Figure 5b).

3.3.6 | Antenna

The antenna consisted of protopod, exopod, and endopod (Figure 5c). The long protopod bore two rows of spinules along the tip part, and the exopod had one long and one small seta at the terminal. As for the endopod, the bud-like rudiment presented in Z3 and expanded to about half of the length of the exopod in Z4. The endopod was more developed and was as long as or slightly shorter than the protopod in Z5.

3.3.7 | First maxilliped

The first maxilliped included the coxa, endopod, and exopod (Figure 6a,c). The coxa bore several setae. There were five segments in the endopod, with 2, 2, 0, 2, and 5 setae in Z1–Z2 from proximal to distal segments, and 2, 2, 1, 2, and 5 setae in Z3, and 2, 2, 1, 2, and 6 setae in Z4–Z5. The exopod had two segments, and the number of plumose natatory setae in Z1–Z5 were 4, 6, 8, 10–11, and 12–13, respectively.

3.3.8 | Second maxilliped

The second maxilliped consisted of the coxa, endopod, and exopod (Figure 6b). The coxa bore several setae. The endopod had three segments bearing 1, 1, and 5 setae in Z1–Z5 from proximal to distal segments. The exopod had two segments, and the number of plumose natatory setae in Z1–Z5 were 4, 6, 8–9, 12–13, and 14–16, respectively.

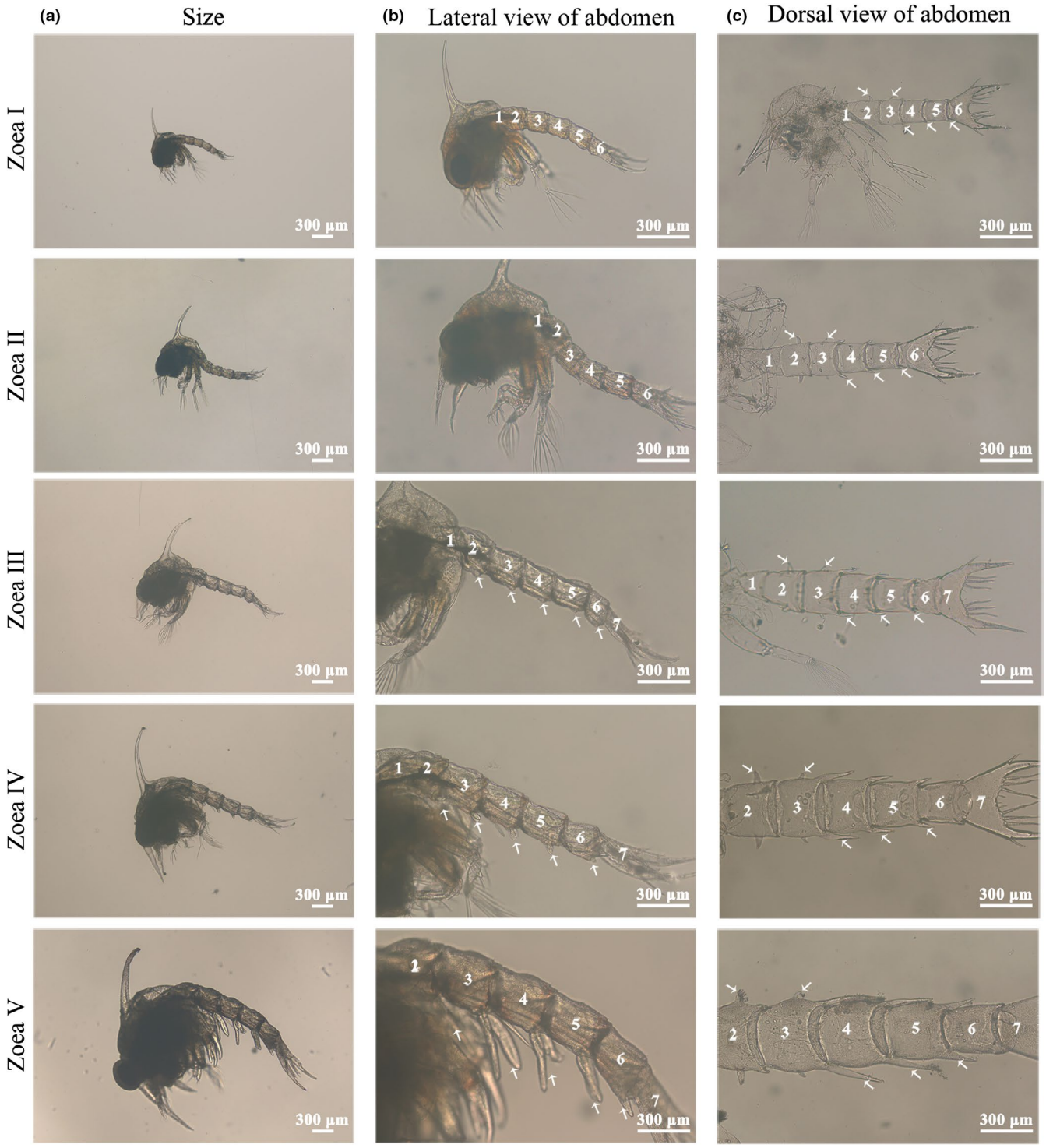

FIGURE 4 Morphological changes of size and the abdomen of zoeal stages. (a) The size changes from zoea I to zoea V; (b) The lateral view of the abdomen from zoea I to zoea V; (c) The dorsal view of the abdomen from zoea I to zoea V. The arrows indicated the body changes during larval development, and the numbers presented the somites. Scale bar = 300 μm.

3.4 | The megalopal stage and the first juvenile crab

The megalopa was characterized by flat body, long abdomen, and long eye stalks, and the pereiopods were similar to juvenile crabs (Figure 7a). As for the first juvenile crab, its shape was the same as the adult crab (Figure 7a). The main features of the megalopal stage and the first juvenile crab were described as follows:

The megalopa had a total body length of 4.04 ± 0.26 mm (the cephalothorax length of 2.55 ± 0.13 mm and width of 1.42 ± 0.12 mm, the abdomen length of 1.49 ± 0.13 mm and width of 0.56 ± 0.10 mm). In the megalopal stage, the dorsal and lateral spines disappeared, leaving

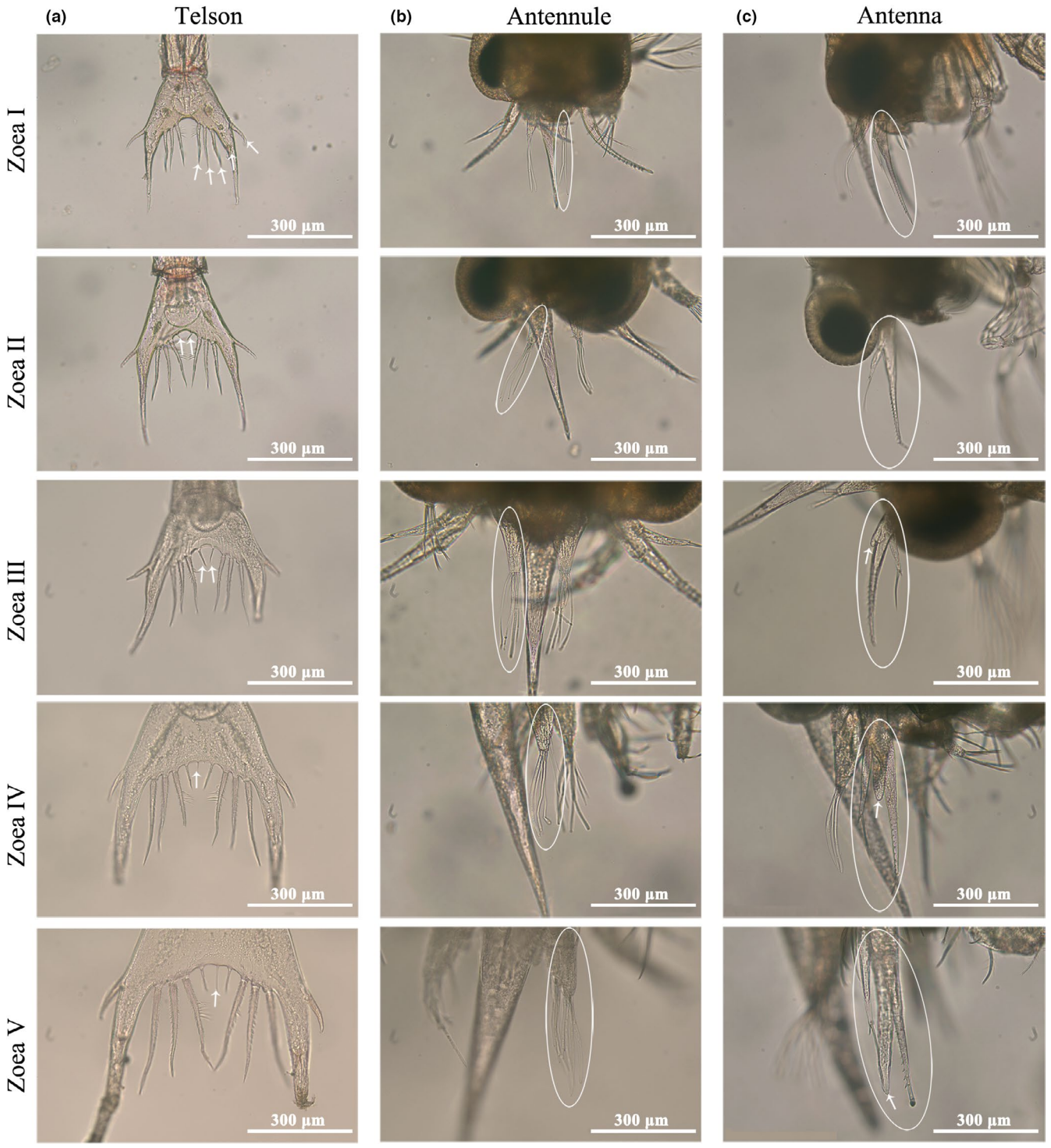

FIGURE 5 Morphological changes of the telson, antennule, and antenna of zoeal stages. (a) Telson changes from zoea I to zoea V; (b) The antennule changes from zoea I to zoea V; (c) The antenna changes from zoea I to zoea V. The arrows indicated the body changes during larval development, and the described parts of the body are highlighted with circles. Scale bar = 300 μm.

only a rostral spine, and the eye stalks were elongated and movable (Figure 7a). As for the first juvenile crab, the rostral spine disappeared, and the carapace was 2.57 ± 0.17 mm in length and 2.42 ± 0.19 mm in width with a row of minute sharp spines in the front margin (Figure 7a). The morphology of the antenna, first pereiopod, fourth pereiopod, fifth pereiopod, and abdomen was shown in Figure 7.

4 | DISCUSSION

In recent decades, with the rapid development of mud crab farming of *S. paramamosain* and the shortage of natural crab seeds, there is an urgent need for research on seed production (Waiho et al., 2018). Although mud crab seeds have been successfully cultivated,

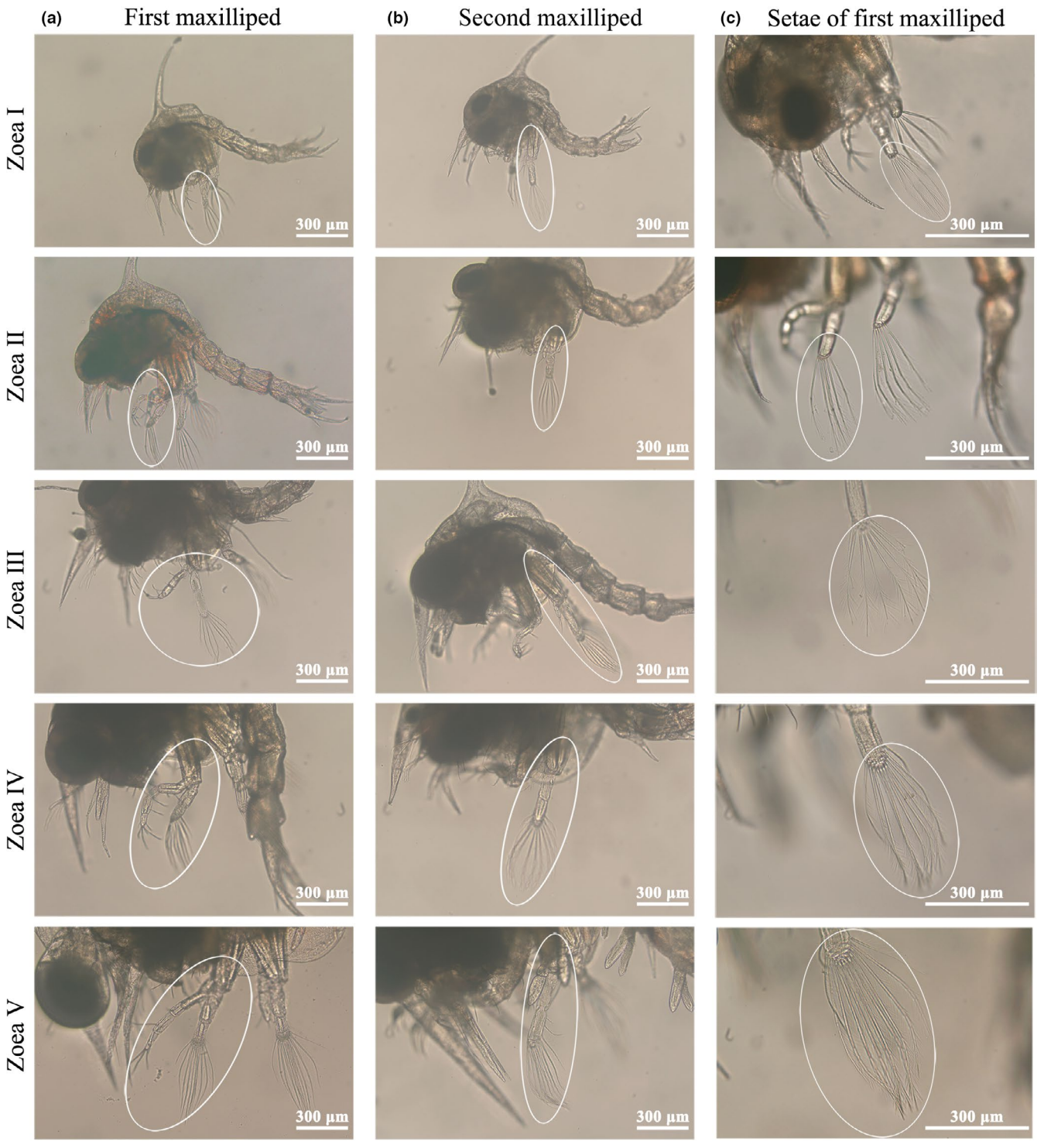

FIGURE 6 Morphological changes of first maxilliped, second maxilliped, and plumose natatory setae of zoeal stages. (a) First maxilliped changes from zoea I to zoea V; (b) The second maxilliped changes from zoea I to zoea V; (c) The plumose natatory setae changes of the first maxilliped from zoea I to zoea V. The described parts of the body are highlighted in circles. Scale bar = 300 μm.

large-scale seed production is still unstable due to many existing problems (Hamasaki et al., 2011), including deficiency of embryonic development in the early stages, high mortality rate of larvae, and pathogen infection during seed production (Chen, 2005; Hamasaki et al., 2011). However, we know little about the pathological characteristics and pathogenic mechanisms. Therefore, it is necessary to carry out fundamental research, including correctly documenting the morphology of embryonic development and larval development, to provide basic information that will help identify solutions to the mentioned bottlenecks of crab seed production.

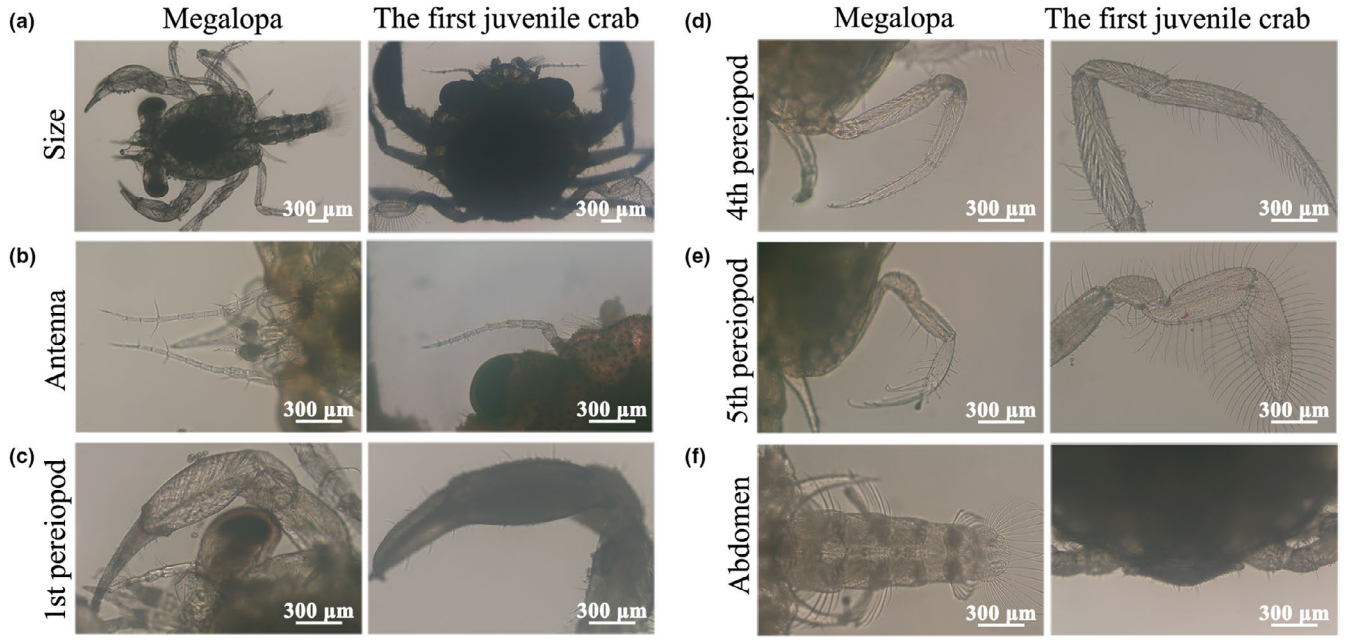

FIGURE 7 Morphological features of megalopa and the first juvenile crab. (a) Body changes from megalopa to the first juvenile crab; (b) Antenna changes from megalopa to the first juvenile crab; (c) First pereiopod changes from megalopa to the first juvenile crab; (d) Fourth pereiopod changes from megalopa to the first juvenile crab; (e) Fifth pereiopod changes from megalopa to the first juvenile crab; (f) Tail changes from megalopa to the first juvenile crab. Scale bar = 300μm.

4.1 | Embryonic development

Embryological studies have been performed in several brachyuran species, and Table 1 showed the comparison of water temperature, incubation period, egg colour changes, egg diameter changes, and embryonic stages of 15 brachyuran crabs. As reported, during embryonic development, the incubation period was related to environmental conditions, especially water temperature (Wear, 1974). In many crustaceans, there is a significant positive correlation between water temperature and development rate (Table 1). In *S. paramamosain*, the incubation periods at 20°C, 25°C, and 30°C were 29 days, 15 days, and 9.5 days, respectively, which indicated that higher temperature resulted in shorter embryo incubation period (Zeng, 2007). Similar results were obtained for *S. serrata* (Hamasaki, 2003) and *Portunus trituberculatus* (Hamasaki et al., 2003). In *S. serrata*, when the temperature ranged from 20.3°C–30.0°C, the incubation period decreased from 30 days to 10 days (Hamasaki, 2003). In *P. trituberculatus*, the duration lasted for nearly 50 days at 15.5°C, but only about 9 days at 28.3°C (Hamasaki et al., 2003). At very low temperature, the incubation period was greatly extended. For *Paralithodes platypus*, the larvae were hatched on day 374, and the hatching lasted for about 36 days at the average temperature of 5.2°C ± 1.2°C (Stevens, 2006); for *Erimacrus isenbeckii*, the incubation period was about 12 months at the average temperature of 5.7°C ± 1.6°C (Nagao et al., 1999); and for *Chionoecetes opilio*, it took about 12–13.5 months (about 365–410 days) to hatch when the ovigerous females were kept at the temperature of 1.8°C–3.8°C (Moriyasu & Lanteigne, 1998). According to Belehradek's equation $D = a(T - \alpha)^b$ (1) (where D is incubation period, T is incubation temperature, α is biological zero, and a and b are parameters; Belehradek, 1957; Belehradek, 1935; Wear, 1974), several equation models were proposed to simulate the relationship between temperature and incubation period in crabs, including $D = 522(T - 11.7)^{-1.38}$ (2) (Zeng, 2007) and $D = 434.9(T - 12.19)^{-1.335}$ (3) (Hamasaki, 2002) for *S. paramamosain*, $D = 275.1(T - 13.93)^{-1.203}$ (4) (Hamasaki, 2003) for *S. serrata*, and $D = 6250(T - 5.331)^{-2.088}$ (5) (Hamasaki et al., 2003) for *P. trituberculatus*. In our study, the duration of embryonic development of *S. paramamosain* at 28°C–31°C was 9–11 days, corresponding to equation (2) and (3).

During embryonic development, the colour of the egg mass changes due to the consumption of egg yolk and the appearance of eye pigments and chromatophores (Ikhwanuddin et al., 2015; Soundarapandian et al., 2013). Alterations of egg colour have been observed in many brachyuran crabs, including *Charybdis feriata* (Soundarapandian et al., 2013), *S. olivacea* (Ikhwanuddin et al., 2015), *Ucides cordatus* (Antonio et al., 2003), *S. serrata* (Chen, 2005), *Portunus pelagicus* (Liao et al., 2011), and *C. opilio* (Moriyasu & Lanteigne, 1998) (Table 1). The colour of the egg mass corresponds to the developmental pattern, which will help identify the embryonic stages and predict the hatching time without optical instruments. In the present study, the colour of the egg mass was orange after spawning, and gradually changed to brown then dark brown, and finally turned dark grey before hatching.

Many scientists attempt to distinguish embryonic stages based on morphological features (Garcia-Guerrero & Hendrickx, 2004; Sarker et al., 2012), morphometric measurements (Stevens, 2006),

TABLE 1 Comparison of embryonic development of 15 brachyuran crabs which were ranked by incubation temperature.

Comparison	*Scylla paramamosain*	*Charybdis feriata*	*Scylla olivacea*	*Uca lactea*	*Ucides cordatus*
Reference	Present study	(Soundarapandian et al., 2013)	(Ikhwanuddin et al., 2015)	(Yamaguchi, 2001)	(Antonio et al., 2003)
Temperature	28°C–31°C	28°C–31°C	28°C–30°C	28°C	27 ± 1°C
Incubation period	About 9–11 days	8–11 days	8 days	About 15.4 days	19 days
Colour changes of egg mass	Orange	Golden yellow	Yellow/Orange	-	Dark bordeaux
	Brown	Deep yellow, or yellowish orange	Brown	-	Dark bright bordeaux
	Dark brown	Orange	Grey	-	Dark ochre
	Dark grey	Brown	Dark grey	-	Light ochre
	-	Dark brown or black	-	-	-
Diameter changes of embryos	298.94 ± 6.97 μm	360–370 μm	329.91 ± 6.62 μm	240 μm	432.0 ± 14.4 μm
	377.75 ± 4.63 μm	440–450 μm	377.26 ± 11.50 μm	320 μm	492.0 ± 9.0 μm
Embryonic stages	Cleavage	Stage-I (Blastula)	Day 1 (Blastula stage)	Stage I (Day 1)	Day 1 (Precleavage stage)
	Blastula	Stage-II (Gastrula)	Day 2(Gastrula stage)	Stage II (Day 2)	Day 2 (From cleavage to blastula)
	Appendage	Stage-III (Eye placode)	Day 3	Stage III (Day 2–3)	Day 5 (Naupliar stage)
	Eyespot-heartbeat	Stage-IV (Pigment)	Day 4	Stage IV (Day 3–4)	Day 8 (Metanaupliar stage)
	Prehatching	Stage-V (Heart beat)	Day 5	Stage V (Day 4–5)	Day 10 (Pigmented stage)
	-	Stage-VI (Newly hatched first zoea)	Day 6	Stage VI (Day 5–6)	Day 14 (Double chromatophoric bridge stage)
	-	-	Day 7	Stage VII (Day 6–7)	Day 16 (Pre-hatching stage)
	-	-	Day 8 (Hatching)	Stage VIII (Day 7–8)	Day 19 (Hatching stage)
	-	-	-	Stage IX (Day 8–9)	-
	-	-	-	Stage X (Day 9–10)	-
	-	-	-	Stage XI (Day 10–11)	-
	-	-	-	Stage XII (Day 11–12)	-
	-	-	-	Stage XIII (Day 12–13)	-
	-	-	-	Stage XIV (Day 13–14)	-
	-	-	-	Stage XV (Day 14–15)	-

TABLE 1 (Continued)

Comparison	*Aratus pisonii*	*Scylla Serrata*[a]	*Portunus pelagicus*	*Neosarmatium indicum*	*Charybdis japonica*	*Scylla Serrata*[b]
Reference	(Garcia-Guerrero & Hendrickx, 2004)	(Chen, 2005)	(Liao et al., 2011)	(Sarker et al., 2012)	(Yan et al., 1998)	(Wu, 1991)
Temperature	26°C–28°C	25.5°C–28.0°C	25°C–26°C	25°C	23.4°C–26.3°C	19.5°C–22.6°C
Incubation period	14 days	About 14 days	About 13 days	16 days	About 13 days	21 days
Colour changes of egg mass	-	Light orange	Light yellow	-	-	-
	-	Orange	Sallow	-	-	-
	-	Light orangered	grey	-	-	-
	-	Orangered	Dark grey	-	-	-
	-	Brown	Black	-	-	-
	-	Grey	-	-	-	-
	-	Grey-black	-	-	-	-
Diameter changes of embryos	570 ± 17.1 μm	320 μm	-	368.76 ± 8.48 μm	280–294 μm	280 μm
	620 ± 16.8 μm	385 μm	-	471.29 ± 8.19 μm	350–364 μm	320 μm
Embryonic stages	Period 1 (Recently laid eggs)	Fertilized egg	Cleavage	Stage-1 (Fertilized egg)	Fertilized egg	Pre-eyespot
	Period 2 (Day 2)	Cleavage	Blastula	Stage-2 (Cleavage)	Cleavage	Eyespot
	Period 3 (Day 4)	Blastula	Gastrula	Stage-3 (Blastula)	Blastula	Heartbeat
	Period 4 (Day 6)	Gastrula	Nauplius	Stage-4 (Gastrula)	Gastrula	-
	Period 5 (Day 8)	Nauplius	Metanauplius	Stage-5–14 (Developing embryos)	Nauplius	-
	Period 6 (Day 10)	5 pairs of appendages	Protozoea	Stage-15 (Before hatching)	Metanauplius	-
	Period 7 (Day 12)	7 pairs of appendages	-	Stage-Zoea (Hatching)	Protozoea	-
	Period 8 (Day 14)	Eye-pigment formation	-	-	Metazoea	-
	-	Prehatching	-	-	-	-
	-	Hatching	-	-	-	-

(Continues)

TABLE 1 (Continued)

Comparison	*Portunus trituberculatus*	*Eriocheir sinensis*	*Paralithodes platypus*	*Chionoecetes opilio*
Reference	(Xue et al., 1998)	(Du et al., 1992)	(Stevens, 2006)	(Moriyasu & Lanteigne, 1998)
Temperature	12.0°C–19.8°C	7°C–17°C	5.2°C ± 1.2°C	1.8°C–3.8°C
Incubation period	About 29 days	About 37 days	About 374–410 days	365–410 days
Colour changes of egg mass	-	-	-	Clear orange
	-	-	-	Orange
	-	-	-	Dark orange
	-	-	-	Brown to dark brown
Diameter changes of embryos	-	-	1170 μm	644.4 ± 21.0 μm
	-	-	1374 μm	772.1 ± 31.5 μm
Embryonic stages	Pre-cleavage	Cleavage	Stage 1 (Cleavage)	Stage 1 (Prefuniculus formation)
	Cleavage	Blastula	Stage 2 (Blastula-Gastrula)	Stage 2 (Funiculus formation)
	Blastula	Gastrula	Stage 3 (V-embryo)	Stage 3 (Cleavage and blastula)
	Gastrulation	Egg-nauplius (First egg-nauplius)	Stage 4 (Pre-nauplius)	Stage 4 (Gastrula)
	Egg-nauplius	Egg-metanauplius (Second egg-nauplius)	Stage 5 (Meta-nauplius)	Stage 5 (Lateral ectodermal band)
	Egg-zoea stage	Protozoea	Stage 6 (Eye formation)	Stage 6 (Prenauplius)
	-	-	Stage 7 (Chromatophore formation)	Stage 7 (Nauplius)
	-	-	Stage 8 (Diapause)	Stage 8 (Maxilliped formation)
	-	-	Stage 9 (Eye enlargement)	Stage 9 (Metanauplius)
	-	-	Stage 10 (Rapid growth)	Stage 10 (Late metanauplius)
	-	-	Stage 11 (Yolk depletion)	Stage 11 (Eye-pigment formation)
	-	-	Stage 12 (Hatching)	Stage 12 (Chromatophore formation)
	-	-	-	Stage 13 (Reduced yolk)
	-	-	-	Stage 14 (Prehatching)

Note: [a,b]In studies conducted before 2007, the dominant species of mud crab along the coast of China was described as *Scylla serrata*, however, subsequent work based on morphology and genetics showed that it was actually *Scylla paramamosian* (Lin, 2008; Lin et al., 2007).

[Correction added on 31 January 2022, after first online publication: Table 1 layout has been modified in this version.]

daily progress (Yamaguchi, 2001), or by a combination of different methods (Antonio et al., 2003). However, there is still no clear standard for brachyuran crabs. Some researchers divided the embryonic development into several stages, while others reported up to fifteen stages (Table 1), which indicated the diversity of divisions and the difficulty of comparison among species. Previous studies divided the embryonic development of *S. paramamosain* into ten stages (Chen, 2005; Zeng, 2007). In the present study, based on a series of complex biological events, including cleavage, blastula, yolk utilization, appendage formation, eye pigment appearance, chromatophore distribution, and heartbeat occurrence, the embryonic development of *S. paramamosain* was shortened to five stages, including cleavage, blastula, appendage, eyespot-heartbeat, and prehatching (Figure 8).

4.2 | Larval development

The larval development of many brachyuran crabs has been described, and Table 2 showed the comparison of water temperature, developmental duration, and larval stages of 15 brachyuran crabs. The larval development consists of several zoeal stages and one megalopal stage. And, the number of zoeal stages varies from species to species, ranging from two to six stages. The larvae with fewer zoeal stages show a relatively shorter life history. For instance, at 23°C–25°C, the duration of *Notolopas brasiliensis* with two zoeal stages (Santana et al., 2006) was 8 days, while the duration of *Charybdis japonica* with six zoeal stages (Yan et al., 1989) was 21–23 days at 23°C–27°C. In our study, the larval development of *S. paramamosain* contained five zoeal stages and one megalopal

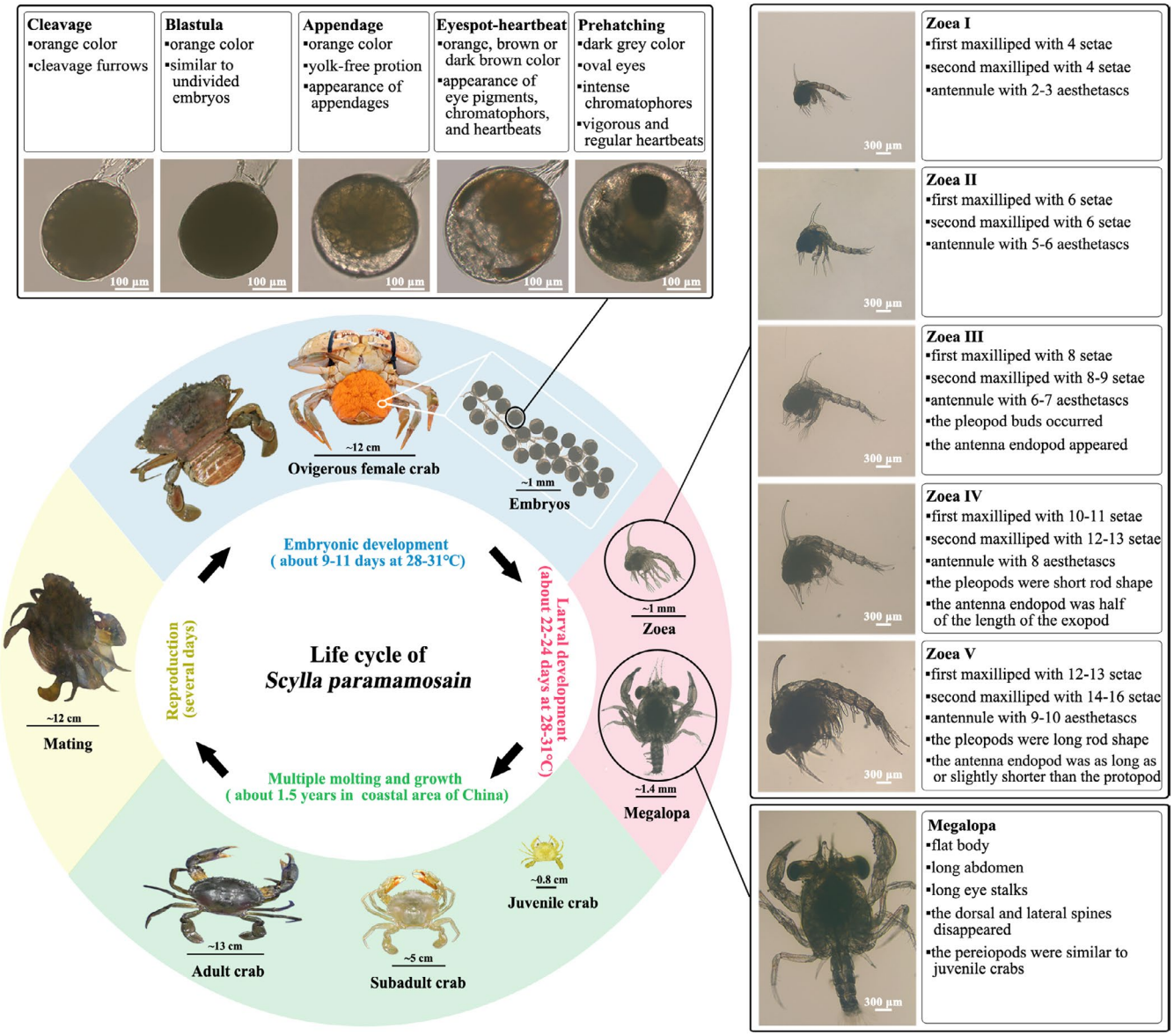

FIGURE 8 Life cycle of *Scylla paramamosain* and the key morphological features of embryonic and larval development. The scale bars were shown in the figures.

TABLE 2 Comparison of larval development of 15 brachyuran crabs which were ranked by the number of stages.

Comparison	*Pitho aculeata*	*Notolopas brasiliensis*	*Sakaina yokoyai*	*Epixanthus frontalis*	*Portunus trituberculatus*	*Perisesarma bidens*	*Portunus pelagicus*
Reference	(Santana et al., 2016)	(Santana et al., 2006)	(Kornienko & Korn, 2011)	(Al-Aidaroos et al., 2014)	(Sun et al., 1984)	(Islam & Shokita, 2000)	(Liao et al., 2001)
Temperature	24°C ± 2°C	24°C ± 1°C	20°C–22°C	28°C	22°C–25°C	21.3°C–23.5°C	27.4°C–28.2°C
Duration of zoeal stages	Total 6–9 days	8 days	15 days	16 days	10–12 days	-	10–11 days
Duration of megalopa		-	-	-	5–6 days	-	4 days
Larval stages	First zoea	First zoea	First zoea	Zoea I	1st zoeal stage	Zoea I	Zoeal stage I
	Second zoea	Second zoea	Second zoea	Zoea II	2nd zoeal stage	Zoea II	Zoeal stage II
	Megalopa	Megalopa	Third zoea	Zoea III	3rd zoeal stage	Zoea III	Zoeal stage III
	-	-	Megalopa	Zoea IV	4th zoeal stage	Zoea IV	Zoeal stage IV
	-	-	-	Megalopa	Megalopa stage	Megalopa	Zoeal stage V
	-	-	-	-	-	-	Megalopa

Comparison	*Scylla paramamosain*	*Scylla Serrata*[a]	*Scylla Serrata*[b]	*Hemigrapsus sanguineus*	*Calyptraeotheres garthi*	*Eriocheir sinensis*	*Charybdis feriatus*	*Charybdis japonica*
Reference	Present study	(Zeng, Li, & Zeng, 2001)	(Huang & Li, 1965)	(Kornienko et al., 2008)	(Ocampo et al., 2011)	(Liang et al., 1974)	(Liao et al., 2010)	(Yan et al., 1989)
Temperature	28°C–31°C	-	25.7°C–29.2°C	20°C–22°C	20°C	11°C–22°C	28.5°C–29.5°C	23°C–27°C
Duration of zoeal stages	16–17 days	-	16–17 days	Total 27 days	30 days	30 days	Total 20–23 days	21–23 days
Duration of megalopa	5–6 days	-	6–7 days		-	9–10 days		5–6 days
Larval stages	Zoea I	Zoea I	First zoeae	Zoea I	Zoea I	First zoeal stage	Zoeal stage I	Zoeal stage I
	Zoea II	Zoea II	Second zoeae	Zoea II	Zoea II	Second zoeal stage	Zoeal stage II	Zoeal stage II
	Zoea III	Zoea III	Third zoeae	Zoea III	Zoea III	Third zoeal stage	Zoeal stage III	Zoeal stage III
	Zoea IV	Zoea IV	Fourth zoeae	Zoea IV	Zoea IV	Fourth zoeal stage	Zoeal stage IV	Zoeal stage IV
	Zoea V	Zoea V	Fifth zoeae	Zoea V	Zoea V	Fifth zoeal stage	Zoeal stage V	Zoeal stage V
	Megalopa	Megalopa	Megalopa	Megalopa	Megalopa	Megalopa stage	Zoeal stage VI	Zoeal stage VI
	-	-	-	-	-	-	Megalopa	Megalopa

Note: [a,b]In studies conducted before 2007, the dominant species of mud crab along the coast of China was described as *Scylla serrata*, however, subsequent work based on morphology and genetics showed that it was actually *Scylla paramamosian* (Lin, 2008; Lin et al., 2007).

stage, which was consistent with previous studies (Huang & Li, 1965; Zeng et al., 2001). However, it is worth noting that occasionally an additional zoeal stage and megalopal stage could be observed in this study. Variations in the larval developmental stages have also been reported in other crabs, including *Eriocheir sinensis* (Montu et al., 1996), *Uca tangeri* (Spivak & Cuesta, 2009), *Epixanthus frontalis* (Al-Aidaroos et al., 2014), and *Chiromantes ortmanni* (Guerao et al., 2012). In addition, *Callinectes sapidus* (Costlow, 1965) was reported to skip some larval stages and reached the megalopal stage. Abnormal larval development was thought to be associated with adverse conditions, such as extreme temperature, low salinity, poor diet, unfavourable photoperiodicity, and high-dose antibacterial agents (Al-Aidaroos et al., 2014; Zeng et al., 2004).

Zoeal stages of *S. paramamosain* could be distinguished based on morphological features, including the number of plumose natatory setae on the maxilliped exopod (first maxilliped with 4, 6, 8, 10–11, and 12–13 setae in Z1–Z5, respectively; second maxilliped with 4, 6, 8–9, 12–13, and14–16 setae in Z1–Z5, respectively), the number of aesthetacs on the antennule (antennule with 2–3, 5–6, 6–7, 8, and 9–10 aesthetacs in Z1–Z5, respectively), the length of pleopod (the pleopod buds occurred in Z3, and showed as a short rod shape in Z4, and increased to a long rod shape in Z5), and the length of the antenna endopod (the endopod buds appeared in Z3, expanded to about half of the length of the exopod in Z4, and were as long as or slightly shorter than the protopod in Z5; Figure 8). During the process of seed production, it is necessary to accurately distinguish the different developmental stages in order to determine an appropriate diet to benefit the growth, development, metamorphosis, and survival of larvae. Previous studies indicated that larvae fed with rotifers (*Brachionus plicatilis*) at early stages and fed with *Artemia* at late stages performed better (Islam et al., 2017). In this study, *Brachionus plicatilis* was fed to larvae in Z1–Z2, and *Artemia salina* was fed to larvae in Z3, Z4, Z5, the megalopal stage, and the first juvenile crab.

5 | CONCLUSION

The life cycle of *S. paramamosain* includes reproduction, embryonic development, larval development, molting, and growth (Figure 8). In our study, the complete embryonic and larval development of mud crab *S. paramamosain* was described. The embryonic development was divided into five stages: cleavage, blastula, appendage, eyespot-heartbeat, and prehatching, and the larval development consisted of five zoeal stages and one megalopal stage before molting to the first juvenile crab (Figure 8). We presented a comprehensive description of both embryonic and larval development with massive image information, which would help diagnose different developmental stages and provide an important guidance for future study or application.

ACKNOWLEDGEMENTS

This study was supported by the National Natural Science Foundation of China (grant # U1805233), the Fundamental Research Funds for the Central Universities (grant # 20720190109), the Fujian Marine Economic Development Subsidy Fund Project from the Fujian Ocean and Fisheries Department (grant # FJHJF-L-2019-1), and the Xiamen Science and Technology Planning Project (grant # 3502Z20203012). We are grateful to laboratory colleagues including Ming Xiong, Yangzhou Liu, Xuewu Zhu, Yanchao Chen, Yuqi Bai, Hui Wei, Weibin Zhang, Ying Zhou, and Su Long for their kind assistance during sample collection. We thank laboratory colleague Dr. Wenfeng Li for providing the valuable picture of reproduction of *Scylla paramamosain*. We also thank staffs of Guangxi Institute of Oceanology (Guangxi Province, China) for their help during the field work.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

AUTHOR CONTRIBUTIONS

Jishan Li: Conceptualization, investigation, data curation, visualization, writing—Original Draft, and writing—Review and Editing. **Wanlei Qiu:** Investigation. **Hua Hao:** Resources. **Fangyi Chen:** Conceptualization, supervision, writing—Review and editing, project administration, and funding acquisition. **Ke-jian Wang:** Conceptualization, supervision, writing—review and editing, project administration, and funding acquisition.

ETHICAL APPROVAL

Animal care and experimental procedures were performed in accordance with the guidelines for the use of laboratory animals of Xiamen University.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ORCID

Jishan Li https://orcid.org/0000-0003-2416-6227
Fangyi Chen https://orcid.org/0000-0001-5714-1339

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How to cite this article: Li, J., Qiu, W., Hao, H., Chen, F., & Wang, K.-J. (2022). Morphology of the complete embryonic and larval development of commercially important mud crab *Scylla paramamosain*. *Aquaculture Research*, 53, 2298–2316. https://doi.org/10.1111/are.15748