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Embryogenesis of *Crangonyx floridanus* (Crustacea: Crangonyctidae)*

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Arthropoda is one of the most prosperous groups in terms of the specific diversity. Within this group, crustaceans specialized their appendages morphologically and functionally (*e. g.*, antennae, mandibles, maxillae, maxillipeds, gnathopods, pereopods, pleopods and uropods), whilst retaining some of their ancestral body plan of arthropods (*e. g.*, biramous appendages). Thus, crustaceans exhibit characteristics of the original archetype along with subsequent adaptions to the original body plan of the arthropod. Crustacea is a particularly useful taxon to elucidate the path of evolution and the diversification of Arthropoda, and in attempting to clarify the morphogenetic and evolutionary shift process from the basic body plan of the Arthropoda.

For such evolutionary discussion, the comparative embryological approach is one of the most promising methods. With respect to the shift process, the embryogenesis of crustaceans has been studied recently by Wolff and Scholtz (2002), Browne *et al*. (2005) and Ungerer and Wolff (2005), but crustacean embryology still remains unclear, as crustaceans are a diversified group in marine habitats where study areas are very restricted to coastal areas. Moreover, because many crustaceans have unique life cycles with multiple metamorphic stages developing through many unique larval stages, such as the nauplius, zoea, and misis larvae, it is difficult to progress their comparative embryological study.

In such a context, much attention has been paid to freshwater amphipod crustaceans in terms of comparative embryological study (*e. g.*, Gerberding *et al*., 2002; Browne *et al*., 2005; Ungerer and Wolff, 2005), because they develop simply without metamorphosis (*i. e*., 'direct development'). We have started an embryological study of an amphipod crustacean using *Crangonyx floridanus*. This amphipod is not a native species to Japan, but recently, they were introduced to and have become widely distributed across Japan (Kanada *et al*., 2007). As such we can collect them easily as specimens for comparative embryology. From our observations in the field and laboratory, we found a large range of favorable characteristics making them well suited for study. We can breed them very easily in small containers, and also maintain strains. Also, they can mate and reproduce throughout the season in the laboratory (not only in the laboratory, but also in the field even with large temperature variations). We can always obtain embryological materials. Moreover, due to their pale semi-transparent body, we can externally observe their egg maturation. As a result, we can easily control the timing of their mating, and also can retrieve promptly their newly oviposited eggs (*i. e*., at the 0 h of embryogenesis).

We outline the embryogenesis of this amphipod species. (1) The embryogenesis takes about 10 days to reach completion when conducted at 20℃ (10.6 ± 0.57 days, N = 15). (2) The embryonic cleavage pattern is initially holoblastic (Fig. 1), but the cleavages from 32-cell stage onward are superficially performed, due to the retardation of cytoplasmic division (Fig. 2). In the superficial cleavages, egg nuclei migrate to the surface of the egg, and the boundaries of blastomeres disappear. (3) Embryonic and extra-embryonic areas become distinguishable (*i. e*., germ disc formation), the germ band forms on the ventral side of the egg, and then extends towards the posterior. (4) Anlages of appendages appear from anterior to posterior (Fig. 3). (5) A single pair of knob-like structures appear (*i. e*., presumptive 'paragnathal' swellings), developing in the mandibular segment in the later stage (Pg in Fig. 4), and they do not commence formation concurrently with the other appendage anlages of cephalic segments (*i. e*., antennal and gnathal appendages). Also of note is that they were formed in the inner region of the mandibular anlages and

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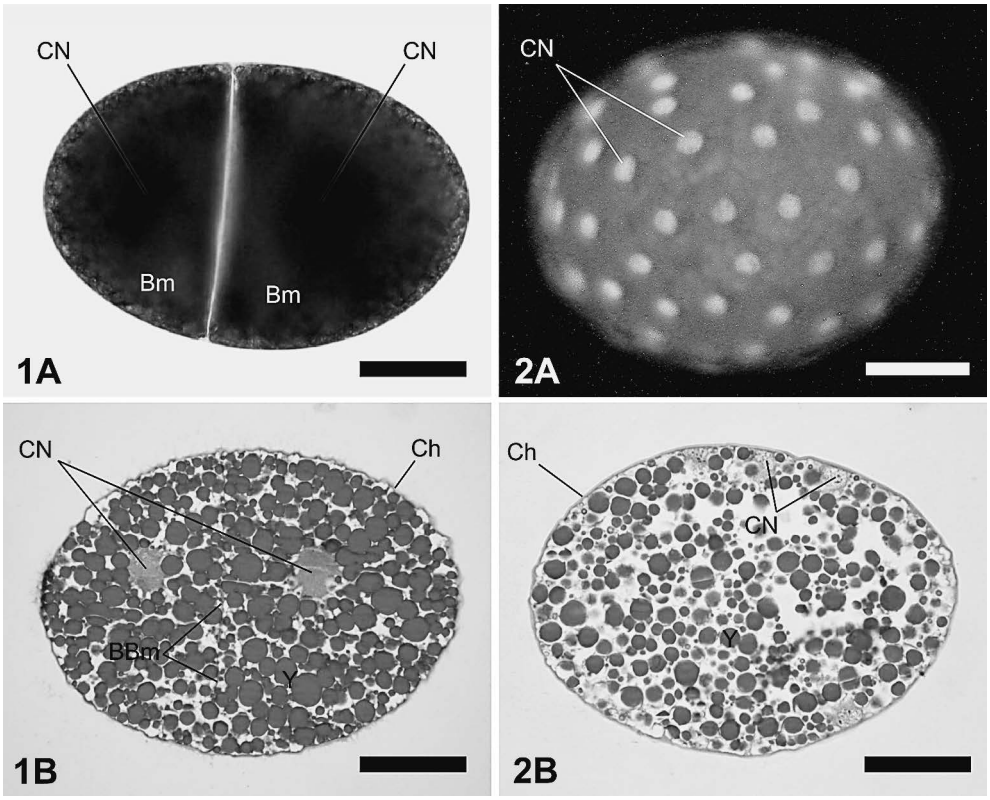
the other cephalic appendages (Figs. 4, 5). That is, the 'paragnath' is not appendicular but sternal in its origin. (6) A 'germ band split' is not observed in this amphipod *Crangonyx floridanus* (Fig. 5), though it is observed during the appendage elongation stage of some other talitrid amphipods' embryogenesis (*e. g.*, *Orchestia cavimana* in the family Talitridae; Ungerer and Wolff, 2005).

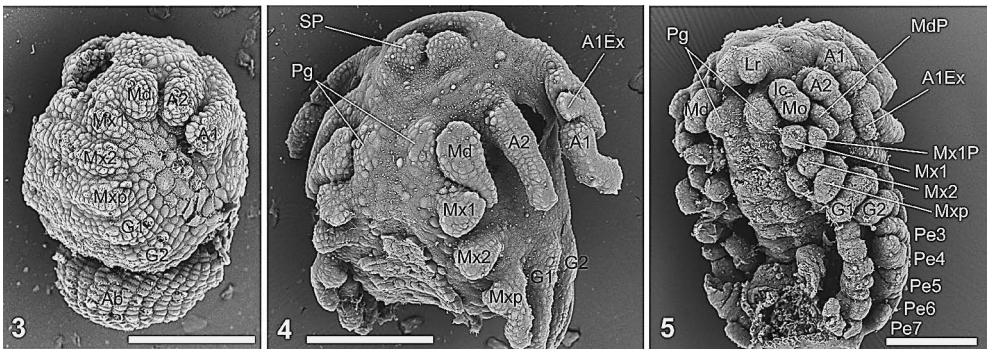


Figs. 1, 2 Eggs of *Crangonyx floridanus* in cleavage.

Fig. 1 A lateral view (A) and longitudinal section (B) of the 2-cell stage eggs (several hours after oviposition). The egg is divided into blastomeres, *i. e.*, it is in the holoblastic cleavage phase. Each cleavage nucleus can be seen at the center of each blastomere.

Fig. 2 A lateral view (A) and longitudinal section (B) of eggs in the superficial cleavage phase. An egg of about 1.5 days after oviposition (A), stained with DAPI, and observed using fluorescence microscopy (UV excitation). An egg of about 2 days after oviposition (B), in which cleavage nuclei can be seen just under the surface of the egg.

BBm: boundary of blastomeres, Bm: blastomere, Ch: chorion, CN: cleavage nucleus, Y: yolk. Scales = 100 μm.



Figs. 3–5 Embryos of *Crangonyx floridanus* in appendages developing stage (SEMs).

Fig. 3 Ventro-lateral view of a dissected embryo. Embryo acquires an S-shape, with its folded posterior (predominantly being the abdominal region). The segmentation of embryo commences in the cephalic and thoracic regions, and appendage anlages appear in the cephalic and thoracic segments.

Fig. 4 Ventro-lateral view of a dissected embryo. The posterior part of the thorax and abdomen were removed. A single pair of presumptive 'paragnathal' swellings appear in the mandibular segment.

Fig. 5 Ventro-lateral view of a dissected embryo. The abdomen was removed. Paragnaths can be observed developing to knob-like structures.

A1, 2: first and second antenna, A1Ex: exopod of the first antenna, Ab: abdomen, G1, 2: first and second gnathopods, Ic: incisor, Lr: labrum, Md: mandible, MdP: mandibular palp, Mo: molar, Mx1, 2: first and second maxillae, Mx1P: first maxillary palp, Mxp: maxilliped, Pe3–7: third to seventh pereopods, Pg: paragnath, SP: stomodaeal projection. Scales = 100 μm.

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