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Changes during late-stage embryonic development from egg-juvenile to free-living hatchling in Chinese freshwater crab *Sinopotamon yangtsekiense* (Decapoda, Brachyura, Potamidae)*

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Abstract This study expands on recent reports that direct development in the Chinese potamid freshwater crab *Sinopotamon yangtsekiense* involves the completion of all brachyuran larval stages (nauplius, zoea, and megalopa) inside the egg case during embryonic development. Detailed studies of embryonic development in this species revealed the presence of an additional larval stage (the egg-juvenile) between the megalopa and the free-living hatchling crab. We described and compared the appendages of the head, thorax, and abdomen of the egg-juvenile with those of the hatchling crab in *S. yangtsekiense*. Significant differences were found between most of the appendages of these two stages with a soft exoskeleton in the egg-juvenile, no joint articulation, a slimmer appearance, and a lack of setae when compared with the newly emerged free-living hatchling crab. These modifications of the appendages are related to the confinement within the egg case of the egg-megalopa and egg-juvenile during direct development, and the need for the free-living hatchling freshwater crab to move, feed, and respire. In marine crabs, the megalopa gives rise to the first crab stage whereas in freshwater crabs the egg-juvenile follows the megalopa and immediately precedes the free-living first crab stage.

Keyword: *Sinopotamon yangtsekiense*; freshwater crab; embryonic development; egg-juvenile; hatchling crab; appendage

1 INTRODUCTION

Freshwater and marine crabs (Crustacea, Decapoda, Brachyura) differ significantly in one main aspect of their lives: the adaptation of their life cycle for reproduction. The vast majority of marine brachyuran crabs are capable of producing large numbers of small eggs (Ng et al., 2008; Yeo et al., 2008). Their freshly spawned fertilized eggs are attached initially to the female's abdominal pleopods where they remain for a short time, after which they are released into the surface waters of the ocean. Once marine crab larvae have been released, they undergo a series of postembryonic developmental stages while floating in the plankton. Marine crab larval development typically includes two to six larval stages, comprising the nauplius, zoea, and megalopa. They settle on the sea floor until final metamorphosis from the megalopa to the first-living juvenile crab (Warner, 1977; Giménez and Anger, 2003; Anger, 2005). In contrast, more than 1 300 species of true freshwater crabs (Potamidae, Potamonautidae, Gecarcinucidae, Pseudothelphusidae, and Trichodactylidae) are well adapted to freshwater and complete their life cycle entirely independent of salt water (Ng et al., 2008; Yeo et al., 2008; Cumberlidge et al., 2009; Wu et al., 2010; Xue et al., 2010). Unlike their marine relatives,

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all true freshwater crabs exhibit direct development, whereby their entire larval development is embryonic and is completed inside the egg case (Cumberlidge, 1999; Sternberg et al., 1999; Wu et al., 2010; Xue et al., 2010). Once the free-living hatchling crab has emerged from the egg case, all future growth would be achieved by molting from the juvenile to sub-adult and adult crab in a similar manner to their marine crab relatives (Cumberlidge, 1999).

Previous studies on embryonic development in the Chinese potamid *Sinopotamon yangtsekiense* Bott 1967 (Xue et al., 2010) confirmed the presence inside the developing egg case of the same larval stages that are seen in marine crabs. However, the presence of an additional embryonic larval stage, i.e. the egg-juvenile, has also been noted in freshwater crabs. This stage is the final embryonic stage seen immediately before the free-living hatchling crab emerges from the egg case (Wu et al., 2010; Xue et al., 2010). In this study, the detailed morphology of the carapace and appendages of the egg-juvenile and free-living hatchling crab of *S. yangtsekiense* are described and compared. Since morphological changes of appendages have direct effects on movement, feeding, sense and defense of the egg-juvenile and free-living hatchling crab, the evolutionary and ecological importance is also considered and discussed in this paper.

2 MATERIAL AND METHOD

Freshwater crabs (*S. yangtsekiense*) were collected between July and September 2002 from the Shouchang River, a third-level tributary to the Qiantang River, Zhejiang, China. Crabs were kept in laboratory in aquaria (0.50 m×0.30 m×0.35 m) supplied with freshwater at a temperature of 25±1°C, fitted with a water purifier (MF-1 Filter) to purify the aquaculture water, and freshwater was replaced every 2 days. Crabs were fed every evening with mealworms (*Tenebrio molitor*) and each aquarium was partially covered with black strawboard to create a shaded area over part of the tank. Three pairs of adult crabs were initially cultured in each aquarium until the female crabs spawned, at which point they were separated and cultured in individual aquaria. Ovigerous females were checked every day from spawning until hatching, and two to four eggs were removed each day at 8:00 am and fixed in 70% ethanol for analysis. Free-living hatchling crabs that emerged from the eggs were fixed immediately in 70% ethanol. The fixed eggs and free-living hatchling crabs were subsequently rehydrated to distilled water in a series of steps (70% ethanol → 50% ethanol for 30 min → 30% ethanol for 30 min → distilled water for 30 min). Rehydrated eggs (whose egg membranes had been digested) and hatchling crabs were placed in 5% HNO_3 for 15 min to remove the calcium from their exoskeleton. Appendages and abdomens peeled from egg-juvenile and hatchling crabs were dehydrated to 95% ethanol in a series of steps (distilled water → 50% ethanol for 15 min → 70% ethanol for 30 min → 80% ethanol for 30 min → 95% ethanol for 30 min). Dehydrated appendages and abdomens were then stained with eosin for 20 min and further dehydrated twice for 30 min in absolute alcohol before being treating with methyl salicylate for 24 h to enhance their transparency (Wu et al., 2010; Xue et al., 2010). They were then transferred to dimethylbenzene for 10 min. Finally, whole slices of appendages and abdomens were sealed with resimene, dried at 24°C, and photographed using a digital camera fitted to a stereomicroscope.

3 RESULT

The body plan and appendages of egg-juvenile and hatchling crabs of *S. yangtsekiense* described here follow the same general arrangement as those of juveniles and adults of this species (and indeed of all freshwater crabs) (Ng, 1988; Cumberlidge, 1999). This general freshwater crab body plan consists of 20 somites plus the telson divided into two main regions, the cephalothorax (with 14 somites) and the abdomen (with six somites plus the telson). The cephalothorax is subdivided into the head (with six somites and five pairs of appendages) and the thorax (with eight somites and eight pairs of appendages). The different pairs of appendages of the cephalothorax and abdomen of freshwater crabs are each specialized for a variety of functions including feeding, locomotion, reproduction, and sensing the environment. The appendages show one of two basic structural plans. They are either biramous or uniramous. Biramous appendages consist of a peduncle (the subcoxa, coxa and the basis) with two branches, an inner endopod and an outer exopod, while uniramous appendages have a peduncle (the subcoxa, coxa and the basis) and an endopod, but they lack an exopod. The endopod consists of five segments (the ischium, merus, carpus, propodus, and dactylus), while the exopod is usually shorter and has fewer segments. Males have abdominal appendages only on the first two abdominal segments, while females only have appendages on four (the second to fifth) abdominal segments. The sixth abdominal segment and the telson always lack appendages in both sexes (Ng, 1988; Cumberlidge, 1999).

3.1 Head appendages

3.1.1 First antenna (antennule) (Fig.1a, b)

The antennule of the egg-juvenile crab is biramous with a peduncle (the subcoxa, coxa and the basis), an endopod and an exopod. The subcoxa of the egg-juvenile is sub-square shaped, recessed on the inner lateral side, and lacks setae, whereas the subcoxa of the hatchling crab is round, bulges at the joint with the coxa, and has a spine on the inside margin. The coxa and basis of the egg-juvenile are rectangular- and palmate-shaped, respectively, and each is similar to that found in hatchling crabs. The endopods of both the egg-juvenile and the hatchling crab are finger-like and lack setae, and the exopods of both stages are flat with the inside margin being shorter than the outside margin. However, the end of the exopod of the egg-juvenile has two buds, whereas these buds are replaced by spines in the hatchling crab. Furthermore, the joint between the coxa and the basis of the egg-juvenile is not movable, whereas in hatchlings this joint is fully articulated. The muscles in all of the segments of the antennule of the egg-juvenile are not developed, whereas the muscles of the coxa are well developed in the hatchling crab, although those of the other segments are not.

3.1.2 Antenna (Fig.1c, d)

The antenna of the egg-juvenile crab is uniramous, long, and slender. The subcoxa of the antennal peduncle is small, oval-shaped, and fused to the epistome, while the coxa forms the basal joint of the antenna. The endopod of the egg-juvenile comprises two articulated segments. The second is expanded basally, whereas this segment is narrow in the hatchling crab. The distal part of the endopod consists of a short flagellum of small segments. The last segment of the flagellum of the egg-juvenile has two completely separate setae. The muscles in the antennae of both the egg-juvenile and the hatchling crab are not developed.

3.1.3 Mandible (Fig.1e, f)

The mandible is uniramous with a mandibular corpus comprising a fused mandibular process and plate and a mandibular palp in both the egg-juvenile and hatchling crab. The mandibular palp consists of three segments and there are prominent setae on the last segment in both the egg-juvenile and hatchling crab. The protractor muscles of the mandibular process that control the movement of the mandible are not well developed in the egg-juvenile, whereas these muscles are well developed in the hatchling crab.

3.1.4 First maxilla (Fig.1g, h)

The first maxilla, consisting of the peduncle plus the endopod, exopod, and epipod, is morphologically similar in both the egg-juvenile and the hatchling crab. This appendage is uniramous and comprises the fused coxa and basis, and a cone-like, two-segmented endopod that aids in feeding and respiration. The distal part of the median lacinia of the basis has about 12 thorn-like setae. The distal part of the external lacinia of the endopod is finger-like with two thorn-like setae, and the muscles are poorly developed in both the egg-juvenile and the hatchling crab.

3.1.5 Second maxilla (maxillule) (Fig.2a, b)

The biramous maxillule consists of the peduncle (the coxa or internal lacinia, and basis, or median lacinia), plus the endopod (maxillary palp) and exopod (scaphognathite). The morphology of the maxillule of the egg-juvenile is quite different from that of the hatchling crab. For example, the internal and median lacinia and the maxillary palp are poorly developed and lack setae in the egg-juvenile, whereas these structures are all better developed and setose in the hatchling crab. In addition, the scaphognathite is flat, lacks setae, and has no visible muscles in the egg-juvenile, whereas this structure is flat, broadened, highly setose and has well-developed muscles in the hatchling.

3.2 Thoracic appendages

Each of the eight thoracic segments of both the egg-juvenile and hatchling crab has a pair of appendages: three anterior pairs (the maxillipeds) and five posterior pairs (the pereiopods, P1–P5).

3.2.1 First maxilliped (Fig.2c, d)

The first maxilliped is biramous and consists of the peduncle plus an endopod, exopod, and a well-developed epipod. The basis is quadrate and the coxa is cone-shaped in the egg-juvenile, while the basis is thumb-shaped and the coxa is flattened in the hatchling. The endopod is narrow in the egg-juvenile but expands to form a broad, flattened surface that functions as the movable floor of the exhalent

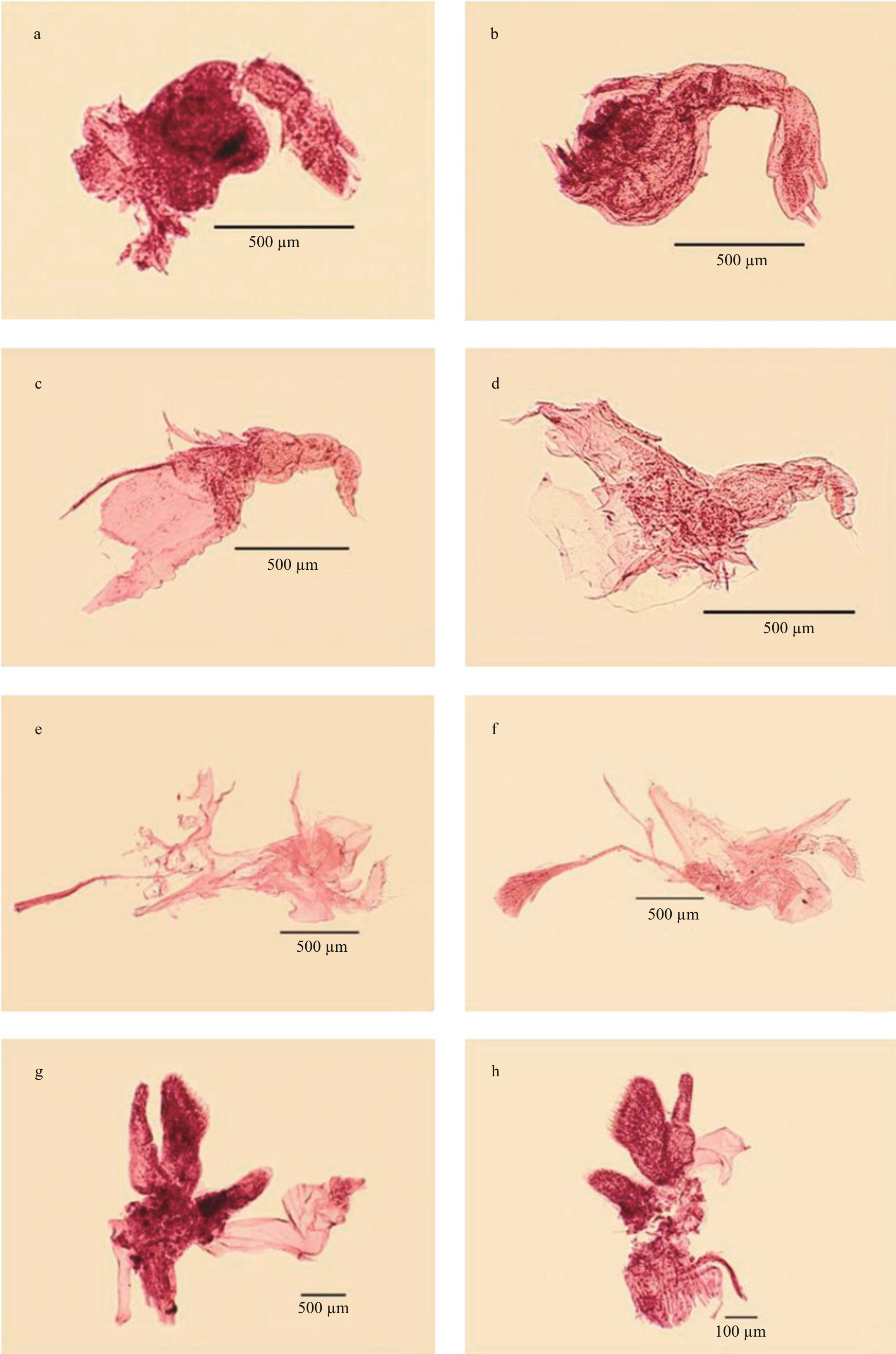

Fig.1 *Sinopotamon yangtsekiense* from China

Comparison of the morphology of the head appendages of the egg-juvenile (a, c, e, g) and free-living hatchling crab (b, d, f, h). a. antennule, egg-juvenile; b. antennule, free-living hatchling crab; c. antenna, egg-juvenile; d. antenna, free-living hatchling crab; e. mandible, egg-juvenile; f. mandible, free-living hatchling crab; g. maxillule, egg-juvenile; h. maxillule, free-living hatchling crab.

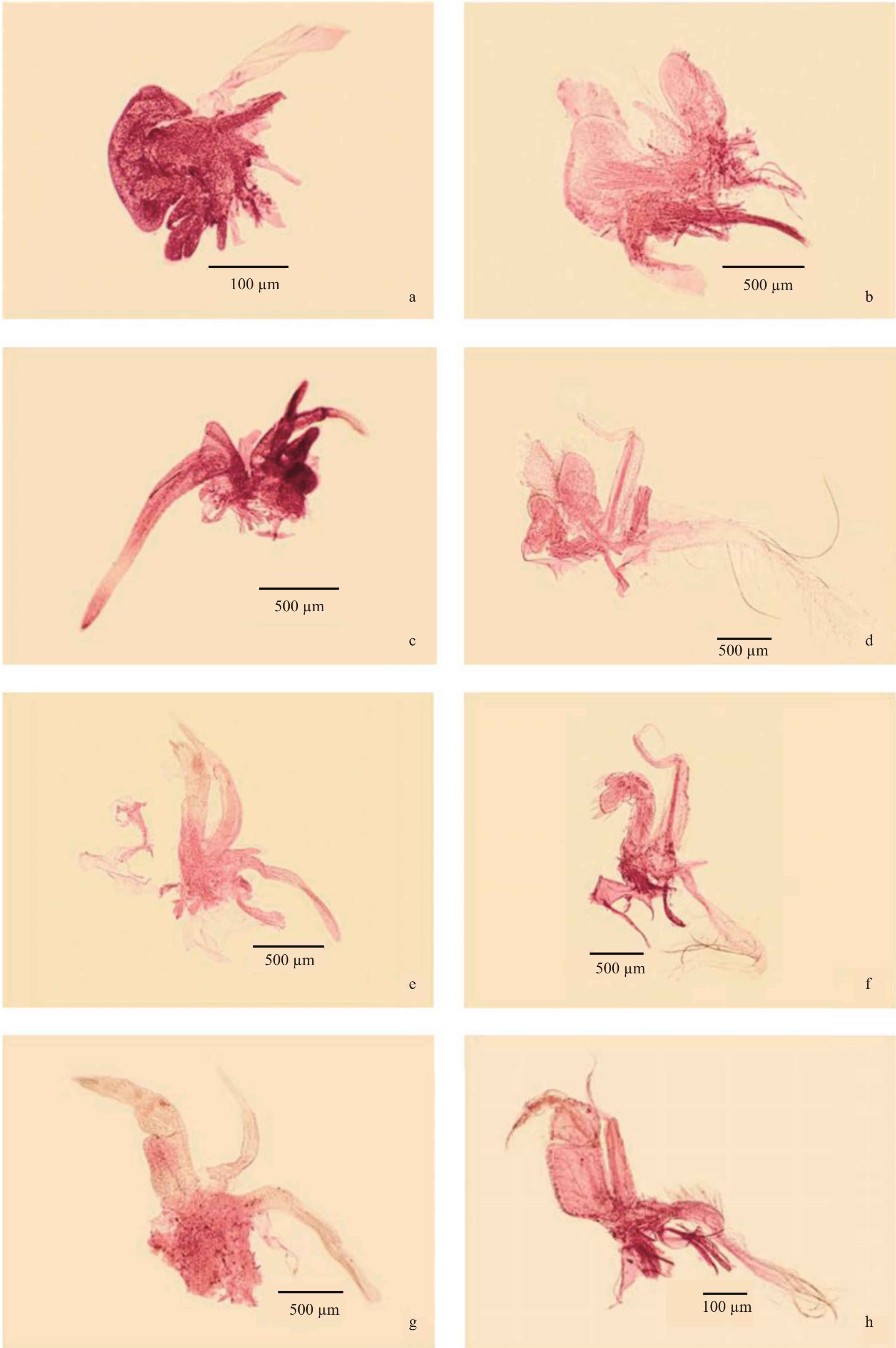

Fig.2 *Sinopotamon yangtsekiense* from China

Comparison of the morphology of the head and thoracic appendages of the egg-juvenile (a, c, e, g) and free-living hatchling crab (b, d, f, h). a. maxilla, egg-juvenile; b. maxilla, free-living hatchling crab; c. first maxilliped, egg-juvenile; d. first maxilliped, free-living hatchling crab; e. second maxilliped, egg-juvenile; f. second maxilliped, free-living hatchling crab; g. third maxilliped, egg-juvenile; h. third maxilliped, free-living hatchling crab.

respiratory chamber in the hatchling crab (Cumberlidge, 1999). The exopod is slim in the egg-juvenile but is thick with a shorter flagellum in the hatchling crab. There are no setae on any part of the first maxilliped in the egg-juvenile, whereas there are distinct setae on the endopod, exopod and epipod in the hatchling crab, and those on the epipod are noticeably long. The muscles of the exopod are not visible in the egg-juvenile, but are well developed in the hatchling crab.

3.2.2 Second maxilliped (Fig.2e, f)

The second maxilliped is biramous and consists of the peduncle plus an endopod, exopod, and a long curving epipod. The carpus, propodus, and dactylus of both the endopod and exopod are slim and straight in the egg-juvenile, whereas all the segments of the endopod and exopod are broad in the hatchling, and the carpus, propodus and dactylus are flexed. The second maxilliped completely lacks setae in the egg-juvenile, whereas there are setae on the tip of the exopod flagellum and on the propodus and dactylus of the endopod in the hatchling, and there are long setae at the tip of the epipod. The muscles of the exopod in the egg-juvenile are not visible whereas these muscles are well developed in the hatchling crab.

3.2.3 Third maxilliped (Fig.2g, h)

The third maxilliped is biramous and consists of the peduncle plus an exopod, a long curving epipod, and an endopod with five segments (the ischium, merus, carpus, propodus, and dactylus). Significant changes occur in the morphology of the third maxilliped during development from the egg-juvenile to the hatchling crab. The ischium and merus of the endopod are slim in the egg-juvenile, whereas the ischium and merus are wide in the hatchling crab and together cover the buccal frame. In addition, the three terminal joints of the endopod (the carpus, propodus, and dactylus) of the egg-juvenile together form a straight palp whereas these three joints are large, movable and flex downward in the hatchling crab, resting on the superior and medial margins of the merus. The endopod, exopod, and epipod all lack setae in the egg-juvenile, but these structures are setose in the hatchling, particularly the epipod (the mastigobranch), which has long, dense, setae. There are no muscles visible in the third maxilliped of the egg-juvenile, whereas the muscles of the ischium and merus of the endopod and the exopod are all well developed in the hatchling crab.

3.2.4 Pereiopods P1–P5

The five pereiopods of both the egg-juvenile and the hatchling crab are all morphologically similar. Each one is uniramous, comprising the coxa, basis, and the endopod with five segments, the ischium, merus, carpus, propodus, and dactylus.

3.2.4.1 Cheliped (pereiopod P1) (Fig.3a, b)

Significant changes occur in the morphology of the cheliped during development from the egg-juvenile to the hatchling crab. In the egg-juvenile the base of the propodus is slim and widened at its base, and tapers to a long, thin, fixed finger that lacks teeth, while the dactylus (the movable finger) is slim, elongated, and also lacks teeth. In the hatchling crab the cheliped develops six small rounded teeth on the inner margins between the fixed and movable fingers that form the pincers, which are used for ingesting food and for defense. Furthermore, the cheliped of the egg-juvenile lacks setae and there are no visible muscles in each segment of the cheliped, and the dactylus (movable finger) cannot move. In contrast, the hatchling crab has setae on the surface of the merus, carpus, and propodus, and there are visible muscles in each segment, especially those muscles that control the movement of the dactylus (movable finger), carpus, propodus, and merus.

3.2.4.2 Ambulatory legs (pereiopods P2–P5) (Fig.3c–h, Fig.4a–b)

All four ambulatory legs are uniramous and each comprises seven segments: the basis, coxa, ischum, merus, carpus, propodus, and dactylus. In the egg-juvenile P2–P5 all lack visible muscle development and setae, and the limbs are straight because the joints of the legs are in line and unable to move. In contrast, in the hatchling crab P2–P5 have visible and well-developed muscles in all segments (except for the dactylus), and setae are visible on the surface of each of the legs. In addition, the joints between the coxa and the merus, between the merus and the carpus, and between the propodus and the dactylus are flexed at an angle in the hatchling crab.

3.3 Abdomen and abdominal appendages (Fig.4c, d)

In the egg-juvenile the abdomen of both sexes is narrow, finger-like and flat, and is held flexed against the sternum in the sterno-abdominal cavity. The abdominal appendages are visible but only as buds. In the hatchling crab, the abdomen of both males and

Fig.3 *Sinopotamon yangtsekiense* from China

Comparison of the morphology of pereiopods 1-4 of the egg-juvenile (a, c, e, g) and free-living hatchling crab (b, d, f, h). a. Cheliped (pereiopod 1), egg-juvenile; b. cheliped, free-living hatchling crab; c. pereiopod 2, egg-juvenile; d. pereiopod 2, free-living hatchling crab; e. pereiopod 3, egg-juvenile; f. pereiopod 3, free-living hatchling crab; g. pereiopod 4, egg-juvenile; h. pereiopod 4, free-living hatchling crab.

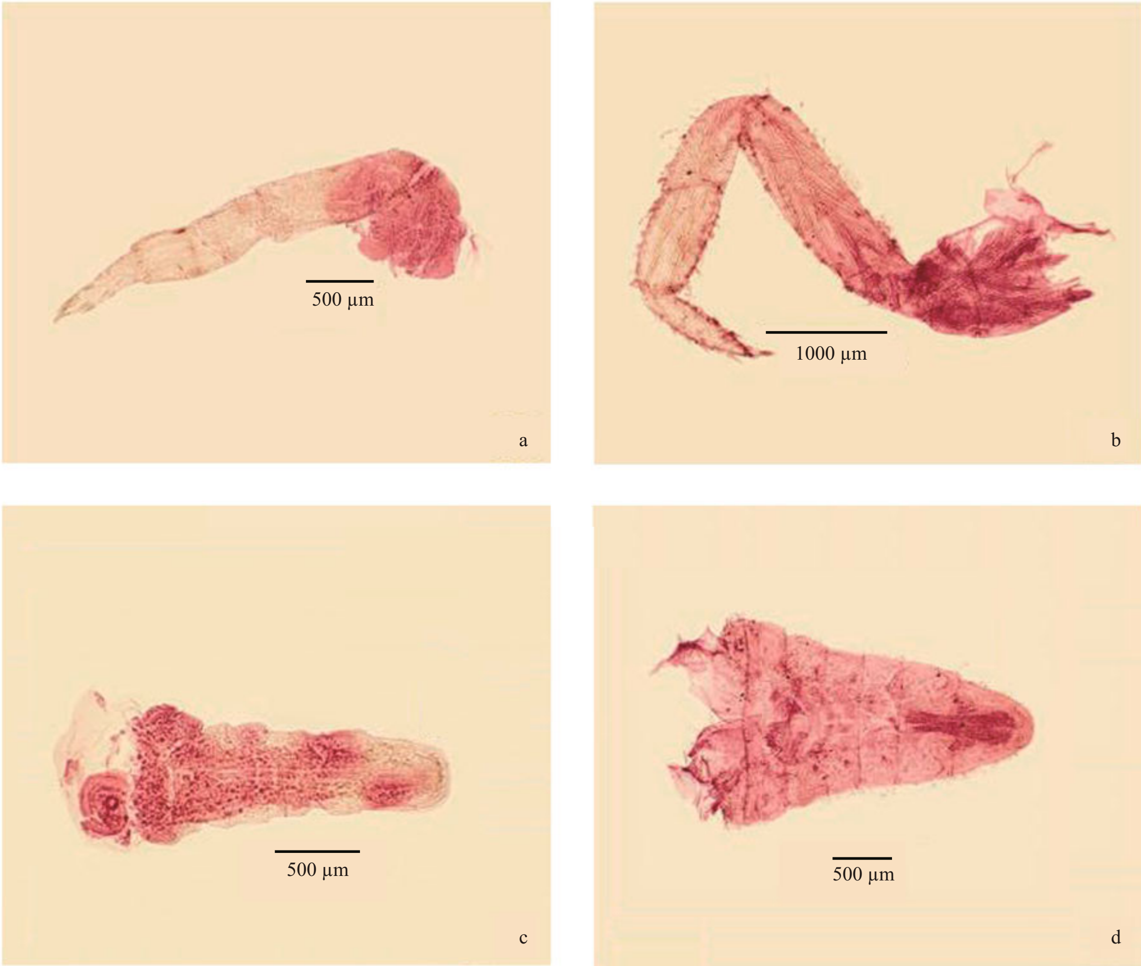

Fig.4 *Sinopotamon yangtsekiense* from China

Comparison of the morphology of pereiopod 5 and abdomen of the egg-juvenile (a, c) and free-living hatchling crab (b, d). a. pereiopod 5, egg-juvenile; b. pereiopod 5, free-living hatchling crab; c. abdomen, egg-juvenile; d. abdomen, free-living hatchling crab.

females is wide basally becoming triangular distally, and is held flexed against the sternum in the sterno-abdominal cavity. The jointed abdominal appendages of the hatchling crab are visible (in males there are uniramous appendages on the first and second segments, and in females there are biramous appendages on the second to fifth segments), although these structures are still poorly developed and their mobility is limited.

4 DISCUSSION

In typical brachyuran development the megalopa is the last larval stage that precedes metamorphosis into the first juvenile crab stage (Warner, 1977). The megalopa of marine crabs lives in the surface waters of the ocean where it uses its backward-projecting abdomen and well-developed limbs for propulsion, and its well-developed mouthparts for feeding. Following metamorphosis, the first juvenile crab stage sinks down in the water column to begin its life on the seabed. In contrast to marine crabs, direct development in *Sinopotamon* takes place inside the egg case where the egg-megalopa neither moves nor feeds (Pace et al., 1976; Wu et al., 2010; Xue et al., 2010). Direct development in these freshwater crabs also includes an extra larval stage, the egg-juvenile, which is produced after the egg-megalopa and immediately before the hatchling crab (Xue et al., 2010). The freshwater crab egg-juvenile inside the egg case is isolated from the external environment, and does not feed, move, ventilate its gills, reproduce, or sense its surroundings. The limited needs of the egg-juvenile are reflected in its poorly developed setae, immovable joints, soft exoskeleton, weak ambulatory limbs, undefined muscles, undeveloped feeding mouthparts (e.g., mandibles, maxillae, and maxillipeds) and crab-like non-propulsory abdomen held flexed against the sternum (Table 1) (Xue et al., 2010).

Table 1 A list of appendage differences for *Sinopotamon yangtsekiense* between the egg-juvenile crab and free-living hatchling crab

Characters	Egg-juvenile crab	Free-living crab
ANTENNULE	Fig.1a	Fig.1b
Shape of the subcoxa	Sub-square	Round
Number of subcoxa spines	Absent	1 spine
End of the exopod	2 buds	2 spines
Muscles in all of the segments	Not developed	Only well developed in the coxa
ANTENNA	Fig.1c	Fig.1d
Second segment of the endopod	Expanded basally	Narrow
MANDIBLE	Fig.1e	Fig.1f
Protractor muscles	Poor developed	Well developed
MAXILLULE	Fig.2a	Fig.2b
Internal and median lacinia	Poor developed and lack setae	Well developed and setose
Maxillary palp	Poor developed and lack setae	Well developed and setose
Scaphognathite	Flat, lack setae, no visible muscles	Broadened, highly setose, well developed muscles
FIRST MAXILLIPED	Fig.2c	Fig.2d
Shape of the basis	Quadrate	Thumb-shaped
Shape of the coxa	Cone-shaped	Flat
Endopod	Narrow	Form a broad flattened surface
Exopod	Slim	Thick with a shorter flagellum
Setae	Absent	Distinct on the endopod, exopod and epipod
Muscles of the exopod	Not developed	Well developed
SECOND MAXILLIPED	Fig.2e	Fig.2f
All the segments of the endopod and exopod	Slim and straight	Broad and flexed
Setae	Absent	On the tip of exopod flagellum and on the propodus and dactylus of the endopod
Muscles of the exopod	Not developed	Well developed
THIRD MAXILLIPED	Fig.2g	Fig.2h
Ischium and merus of the endopod	Slim	Wide
Three terminal joints of the endopod	Together form a straight palp	Large, moveable and flex downward
Setae of endopod, exopod and epipod	Absent	Setose
Muscles of the endopod and exopod	Not developed	Well developed
CHELIPED	Fig.3a	Fig.3b
Tooth between the fixed and movable fingers	Absent	6 small round teeth
Setae in each segment and dactylus	Absent	Setose
Muscles in each segment and dactylus	Not developed	Well developed
AMBULATORY LEGS	Fig.3c, e, g, Fig.4a	Fig.3d, f, h, Fig.4 b
Setae on surface of each leg	absent	Setose
Muscles in all segment of each leg	Not developed	Well developed
Joints between the coxa and the merus, between the merus and the carpus, and between the propodus and the dactylus	In line	Flexed at an angle
ABDOMEN AND ABDOMINAL APPENDAGES	Fig.4c	Fig.4d
Shape of the abdomen	Narrow, finger-like and flat	Wide basally becoming triangular distally

Once the egg-juvenile has completed its development inside the egg case it changes into a hatchling crab just before it emerges. On emergence of the hatchling, long setae appear on its appendages, its exoskeleton, thoracic appendages, and the joints between the segments are hardened, and the chelipeds and walking legs are functional. These changes are accompanied by general muscular development, especially in the articulated segments of the endopods of the maxillipeds and the pereiopods. Further, the broadened and hardened gill bailers (scaphognathites) enable rapid rowing movements that maintain a flow of respiratory water (or air) through the gill chambers (Cumberlidge, 1999). In addition, the hatchling crab has a crab-like abdomen with developing abdominal appendages that is held flexed against the sternum.

Continuous live monitoring of the egg-juvenile to the point of emergence of the hatchling crab from the egg case revealed the absence of a final molt, in the sense that the old carapace was not observed to be shed and the body did not increase in size. This latter point was confirmed by measurements of the carapace width and carapace length (4.025 mm and 3.221 mm for egg-juvenile crab, 4.049 mm and 3.025 mm for hatchling crab) (Wu et al., 2010), where no size differences between the egg-juvenile and hatchling crab were found. The observed morphological changes from egg-juvenile to hatchling crab may be the result of expansion by water uptake that results in the unfolding of the thoracic and abdominal appendages of the egg-juvenile, plus physiological changes that result in the development of muscles fibers and in the calcification of the exoskeleton. The hardened exoskeleton supports movements by the jointed legs brought about by the now-developed muscles, and the sensitive setae on the mouthpart appendages detect changes in the external environment. It is this suite of morphological changes from egg-juvenile to free-living hatchling crab of *S. yangtsekiense* that support its life in freshwater by enabling it to ventilate its gills, move around, and detect alterations in the conditions of its external environment.

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