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First report of developmental changes inside the eggs of the Chinese freshwater crab, *Sinopotamon yangtsekiense* Bott, 1967 (Potamoidea, Potamidae), with comments on its evolutionary significance

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Abstract

This paper focuses on the developmental changes that take place inside the eggs of the semi-terrestrial freshwater crab, *Sinopotamon yangtsekiense*, from Qiantang River in Zhejiang Province, China. The egg consists of two layers, a thick outer membrane and a thin inner membrane that encloses the fluid-filled embryonic sac. Development in this species took up to 77 days, after which the free-living juvenile hatchling crab emerged from the egg. During development the embryo underwent a series of morphological changes that corresponded to the free-living larval stages of marine crabs, and the yolk mass decreased in size and changed color (from creamy pale yellow, to orange, and finally grey). The eggs remained attached to the pleopods in the female's abdominal brood pouch during development and showed a great deal of independence from water. Embryos developed normally whether they were immersed in water or in air. The implications of this adaptation for freshwater crab evolution are discussed.

Contents

Introduction

Most of the more than 6800 species of brachyuran crabs (Decapoda, Crustacea) are marine and typically produce large numbers of small eggs that hatch to release planktonic larvae which pass through a series of stages while floating in the surface waters of the oceans (Ng *et al.*, 2008; Yeo *et al.*, 2008). In contrast, the more than 1280 species of true freshwater crabs (Potamoidea, Trichodactyloidea) are highly adapted to life in fresh water and complete their life cycle entirely independently of seawater, producing small numbers of large eggs that hatch directly into juvenile crabs (Cumberlidge *et al.*, 2008; Yeo *et al.*, 2008; Cumberlidge *et al.*, 2009; Wu *et al.*, 2010). Although some groups of crabs have successfully invaded land, most species, with a few exceptions (Anger *et al.*, 1990; Diesel *et al.*, 2000), still need to return to the sea to reproduce. All true freshwater crabs, regardless of habitat preference, show direct development whereby all free-living larval stages (typical of marine crabs) are lacking, and the maturing eggs remain attached to the female's pleopods for over 70 days before hatching and releasing juvenile crabs (Cumberlidge, 1999; Sternberg and Cumberlidge, 2001; Wu *et al.*, 2010).

This reproductive strategy of direct development is common to all families of true freshwater crabs (Cumberlidge and Ng, 2009), and is also seen in a few species of semi-terrestrial crabs in the family Sesarmidae (Anger *et al.*, 1990; Anger, 1995, 2005; Schubart and Diesel, 1999; Diesel *et al.*, 2000; Anger and Schubart, 2005; Anger *et al.*, 2007). True freshwater crabs demonstrate a broad range of habitat preferences (Yeo *et al.*, 2008; Cumberlidge *et al.*, 2009). Many species of true freshwater crabs are fully aquatic and spend their entire life cycle in water, but there are a significant number of species (including *Sinopotamon yangtsekiense* Bott, 1967) that are semi-terrestrial and divide their time between water and land, even when the females are ovigerous (*e.g.* Liu and Li, 2000). There are also species of freshwater crabs (such as the Liberian tree hole crab, *Globonautes macropus* Rathbun, 1898, the northern Nigerian savanna crab, *Sudanonautes monodi* Balss, 1929, and species of Indochinese terrestrial crabs (*Pudaengon* and *Terrapotamon*) that live most of

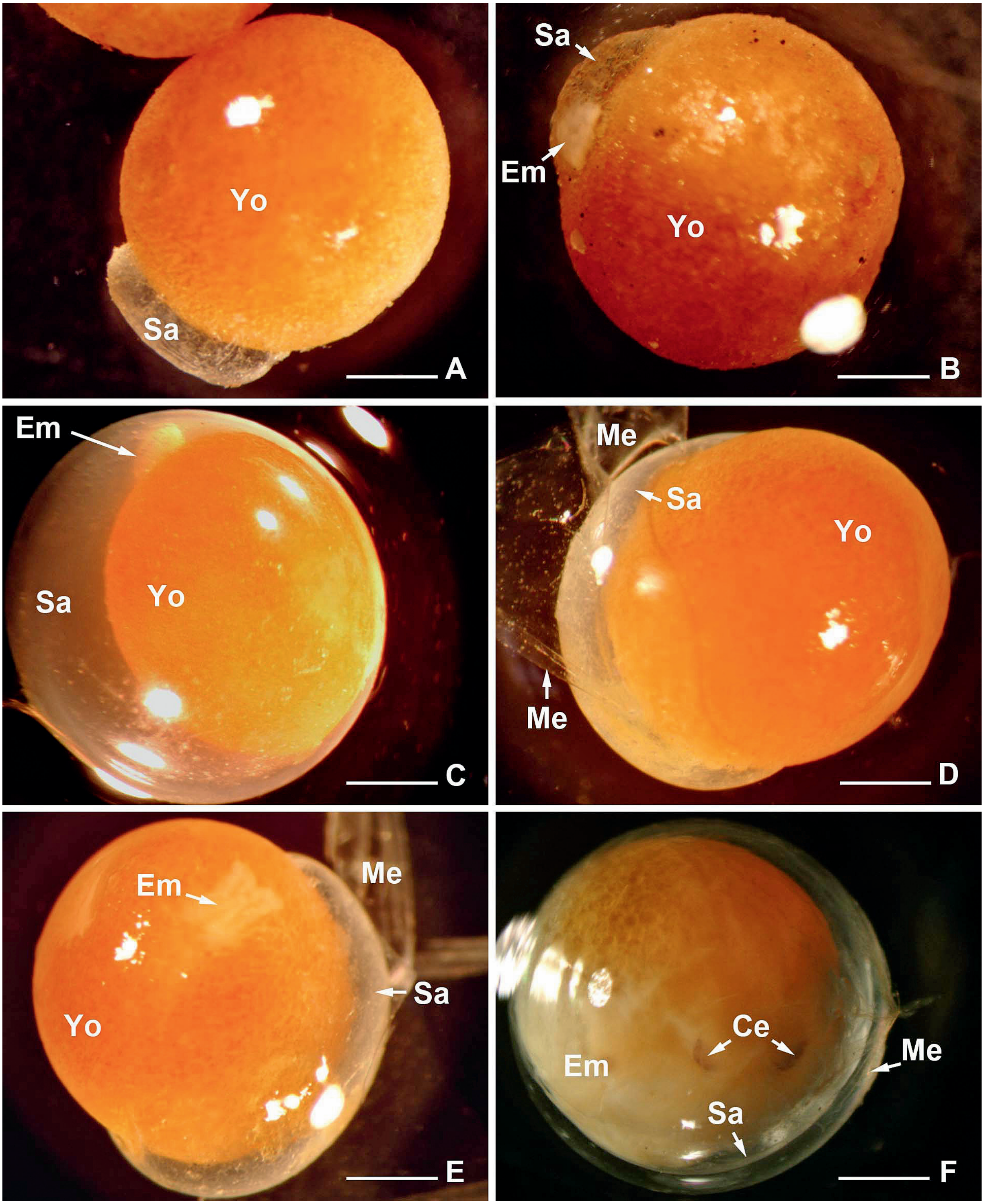

Fig. 1. Changes in the developing eggs of the potamid freshwater crab, *Sinopotamon yangtsekiense* from China. A-B) after 13 days; C-E) after 19 days; F) after 65 days. Yo = yolk; Sa = inner membrane a fluid-filled sac; Em = developing embryo; Me = outer membrane; Ce = compound eye of developing crab. Scale bar = 1 mm.

their lives out of water and are functionally land crabs that rarely (if ever) enter rivers and streams (Cumberlidge, 1986, 1999; Ng and Naiyanetr, 1995, 1998).

The present work focuses on the development of the fertilized eggs of *S. yangtsekiense* a common species of freshwater crab found in and around the mountain streams and rivers of the Yangtze River basin in Zhejiang, Anhui, Henan, Hubei, Jiangsu, and Jiangxi Provinces in China (Chen *et al*., 1994; Dai, 1999; Esser and Cumberlidge, 2008). This species hides under stones on the stream bed during the day and forages either in the stream or on land at night. During the early breeding season (June to August) females carrying between 70 and 100 eggs move into shallow water and typically feed on land at night; later in the season (July to October) females carrying juveniles in their abdominal brood pouches appear (Chen *et al*., 1994).

Sinopotamon yangtsekiense was selected to investigate changes that occur inside the developing embryos of a semi-terrestrial species of freshwater crab. This aspect of freshwater crabs' biology has largely been overlooked in the past and there are only a few studies on two potamid species, one European and one Chinese (Pace *et al*., 1976; Wu *et al*., 2010). The embryos of *S. yangtsekiense* are frequently not submerged for much of the time (more than 70-days) that it takes for them to mature to the point when they release a juvenile crab. The changes that take place inside the developing embryos over time are described and the evolutionary significance of this independence from water discussed.

Material and methods

Freshwater crabs (*S. yangtsekiense*) were collected from the Changhua stream of Fenshui River, a tributary of the Qiantang River in Zhejiang Province, China, between July and September 2002. Eight crabs were maintained in the laboratory in one aquaria (0.50×0.30×0.35 m) supplied with shallow fresh water that did not completely immerse the crabs, and with a raised platform that allowed crabs to spend time out of water if they chose to do so. The water was maintained at 25±1°C, leached with a water purifier (MF-1 Filter), and replaced every two days. Crabs were fed every evening with mealworms (larva of *Tenebrio molitor* Linnaeus, 1758) (Zhao,1963) and each aquarium was 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. Twelve ovigerous females were observed every day from spawning until hatching of the eggs. Crabs divided their time equally between periods of immersion in shallow water and periods when they were resting out of the water. Two to four eggs were removed daily at 8:00 am from each female and fixed in 70% ethanol for analysis. A stereomicroscope was used to aid with the careful peeling away of the developing embryos from the inner membrane, and the embryos were photographed using a digital camera fitted to the microscope.

Results

Batches (6-8) of newly laid fertilized eggs were removed from the pleopods of the twelve ovigerous females of *S. yangtsekiense*. Eggs were large (3.5-4.1 mm diameter), round, and a uniform creamy pale yellow in color. Each embryo was found to be completely surrounded by a two layered membrane - a thick outer membrane and a thinner inner membrane that formed a fluid-filled sac around the developing embryo and yolk mass. Newly laid eggs consisted almost entirely of creamy pale yellow yolk (Fig. 1A). When vitellogenesis had proceeded for 13 days the yolk became dark yellow and a small fluid-filled transparent sac (the inner membrane) was visible that contained a small white mass (the developing embryo) on one side of the egg capsule (Fig. 1A-B) (Wu *et al*., 2010). After 19 days the fluid-filled sac became further enlarged and now covered about one-half of the yolk surface, and the developing embryo was visible on one side of the sac as a large white mass (Fig. 1C-E). After 35 days, the fluid-filled sac had expanded to cover the entire yolk surface with the developing embryo now covering most of the yolk. After 65 days, the yolk had changed color from bright orange to light brown-grey and there was a narrow, but distinct space between the developing embryo (which now had recognizable larval features such as compound eyes) and the fluid-filled sac (Fig. 1 F). After 77 days, the embryo had developed to a stage that resembled a juvenile crab (termed the 'egg-juvenile crab' by Wu *et al*., 2010) and both the outer and inner membranes of the embryo burst to release a free-living hatchling crab. The complete developmental process inside the egg case was therefore observed to take about 77 days from fertilized egg to free-living hatchling crab.

Discussion

All true freshwater crabs complete their entire life cycles in fresh water or terrestrial habitats and never enter the sea at any stage of their life (in fact, they actively avoid salt water environments) (Cumberlidge, 2008). This option is possible in true freshwater crabs because their reproductive strategy is modified so that their embryos hatch directly to produce juvenile crabs. It has been shown that in true freshwater crabs all of the normal brachyuran larval stages (*e.g.* nauplius, zoea, megalopa) are passed through during a lengthy embryonic development within the egg resulting in young hatchling crabs emerging directly out of mature eggs (Pace *et al.*, 1976; Wu *et al.*, 2010). This strategy adopted by freshwater crabs replaces that used by the majority of marine crabs, in which eggs hatch in seawater and release a free-living aquatic larva that metamorphoses while floating in the plankton. The semi-terrestrial behavior of ovigerous females of *S. yangtsekiense* indicates that the eggs of freshwater crabs (containing developing embryos) do not require sustained immersion in freshwater for embryonic development to proceed normally.

Independence from aquatic environments is of tremendous evolutionary significance to these animals because it has allowed them to colonize not only inland fresh waters, but also the more terrestrial parts of their habitats, in places where competition from their aquatic congeners is reduced (Yeo *et al.*, 2008). It is safe to assume that the ancestors of true freshwater crabs were marine crabs that acquired the ability to osmoregulate in brackish and fresh water environments (Cumberlidge and Ng, 2009). Without adaptations of their reproductive system these ancestral crabs would have still needed seawater for the development of their embryos and larvae. The need to breed in sea water is still seen in species of land crabs of the families Gecarcinidae and Sesarmidae that live either on land, in burrows, or in phytotelmic habitats which means that they must migrate back to the ocean in the breeding season to release their eggs in seawater (Burggren and McMahon, 1988; Gilchrist, 1988; Cuesta and Anger, 2005; Cumberlidge *et al.*, 2005; Melo *et al.*, 2006). The evolution of fluid-filled waterproof egg cases, however, has allowed direct development to take place in the true freshwater crabs and has freed them from having to return to the sea to complete their life cycle.

The novel evolutionary adaptation that made direct development possible in freshwater crabs was the switch from small eggs with little yolk and a single thin outer membrane typical of marine crabs, to large-diameter eggs with a massive yolk supply surrounded by two membranes, a thick outer one that is resistant to dehydration and a thinner inner membrane that creates a fluid-filled environment for the developing embryo. The double-membrane around the developing embryos of freshwater crabs provides an aquatic environment inside the egg case throughout development. This adaptation imparts independence from water because it means that embryonic development in these species is possible without any contact with water in the external environment (regardless of whether it is salty or fresh). This was likely a key evolutionary innovation that has contributed to their success in colonizing fresh waters and the nearby land. At the same time the enlarged yolk mass provides enough nutrition for the entire extended duration of enclosed larval development (cf. marine crab eggs with less yolk, in which only the early larval stages are lecithotrophic, while the later larval stages can feed themselves). The increase in egg size in the freshwater crabs has been accompanied by a reduction in the overall number of eggs, from many thousands to a few hundred, and represents a switch from an r-selected to a K-selected strategy (Ng, 1988; Cumberlidge, 1999). Significantly, this adaptation also removed the need for true freshwater crabs to return to sea water to complete their life cycle which is necessary in the many other brachyurans that also live in freshwater habitats (*e.g.*, species of Sesarmidae, Varunidae, Grapsidae, Portunidae, and Xanthidae) (Anger *et al.*, 1990; Anger, 1995, 2005; Schubart and Diesel, 1999; Diesel *et al.*, 2000; Anger and Schubart, 2005; Anger *et al.*, 2007). Similarly, the embryos and larvae of land crabs (Gecarcinidae) need to develop in salt water (Cuesta *et al.*, 1999) and this restricts these species to the coastal areas of the world and is the reason that although these crabs are often found inland, they are never very far from the sea.

Interestingly, some species of insects have eggs with two extra-embryonic membranes, a thick outer one (the serosa) and a thin inner one (the amnion) that forms a fluid-filled sac around the developing embryo (Schmidt-Ott, 2000; Uchifune and Machida, 2005). Freshwater crab eggs are also functionally analogous to the eggs of amniote vertebrates (reptiles, birds, and mammals) whose developing embryo is nourished by yolk and bathed in amniotic fluid in a space created by four membranous sacs that surround and protect it (Stewart, 1997).

The waterproof eggs of freshwater crabs were most likely an adaptation that followed after the ancestral freshwater crabs had developed the osmoregulatory abilities to survive in low salt environments and had moved from sea water into fresh water (Cumberlidge and Ng, 2009). It is possible that the early ancestors of freshwater crabs still needed to return to the sea to breed, as do present-day species of *Sesarma* (Sesarmidae), *Eriocheir*, and *Varuna* (both Varunidae). Direct development in freshwater crabs may have evolved after colonization of fresh waters near the coast, but once available, it would have freed these crabs from the need to return to the sea, thereby allowing them to radiate into all available niches in inland fresh waters worldwide, including associated terrestrial habitats. The evolution of fluid-filled freshwater crab eggs with a thick outer membrane severed the need for embryonic development in aquatic environments, and probably first evolved for reproduction in fresh water. The fact that this adaptation also freed freshwater crabs from any kind of water for their reproductive success probably opened the door for their subsequent conquest of drier and more terrestrial environments.

It would appear that fluid-filled waterproof eggs are a necessary prerequisite for reproduction on land in a number of different animal groups, including reptiles, birds, mammals, insects, and semi-terrestrial brachyuran crustaceans such as the true freshwater crabs.

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References

Anger K. 1995. The conquest of freshwater and land by marine crabs: adaptations in life-history patterns and larval bioenergetics. *Journal of Experimental Marine Biology and Ecology* 193: 119-145.

Anger K. 2005. The early life history of *Sesarma fossarum*, an endemic freshwater crab from Jamaica. *Invertebrate Reproduction and Development* 47: 63-72.

Anger K, Schubart CD. 2005. Experimental evidence of food independent larval development in endemic Jamaican freshwater breeding crabs. *Physiological and Biochemical Zoology* 78: 246-258.

Anger K, Harms J, Montú M, Bakker C. 1990. Effects of salinity on the larval development of a semiterrestrial tropical crab, *Sesarma angustipes* (Decapoda: Grapsidae). *Marine Ecology Progress Series* 62: 89-94.

Anger K, Torres G, Nettelmann U. 2007. Adaptive traits in ecology, reproduction and early life history of *Sesarma meridies*, an endemic stream crab from Jamaica. *Marine and Freshwater Research* 58: 743-755.

Balss H. 1929. Potamonidae au Cameroon. Pp. 115-129 in: Contribution a l'étude de la faune du Cameroun. *Faune Colonies Françaises*, 3.

Bott R. 1967. Potamiden aus Ost-Asien (*Parapotamon* De Man, *Sinopotamon* n. gen., *Candidiopotamon* n. gen., *Geothelphusa* Stimpson) (Crustacea, Decapoda). *Senckenbergiana biologica* 48: 203-220.

Burggren WW, McMahon BR. 1988. Biology of the land crabs: an introduction. Pp 1-5 in: Burggren WW, McMahon BR, eds., *Biology of the Land Crabs*. Cambridge, New York: Cambridge University Press.

Chen T, Lai W, Du NS. 1994. Growth, reproduction and population structure of the freshwater crab *Sinopotamon yangtsekiense* Bott, 1967, from Zhejiang, China. *Chinese Journal of Oceanology and Limnology* 12: 84-90.

Cuesta JA, Anger KK. 2005. Larval morphology and salinity tolerance of a land crab from West Africa, *Cardisoma armatum* (Brachyura: Grapsoidea: Gecarcinidae). *Journal of Crustacean Biology* 25: 640-654.

Cuesta JA, Schuh M, Diesel R, Schubart CD. 1999. Abbreviated development of *Armases miersii* (Grapsidae: Sesarminae), a crab that breeds in supralittoral rock pools. *Journal of Crustacean Biology* 19: 26-41.

Cumberlidge N. 1986. Ventilation of the branchial chambers in the amphibious West African fresh-water crab *Sudanonautes (Convexonautes) aubryi monodi* (Balss, 1929) (Brachyura: Potamonautidae). *Hydrobiologia* 134: 53-65.

Cumberlidge N. 1999. The freshwater crabs of West Africa. Family Potamonautidae. *Faune et Flore Tropicales* 35. Paris: Institut de recherche pour le développement (IRD, ex-ORSTOM).

Cumberlidge N. 2008. Insular species of Afrotropical freshwater crabs (Crustacea: Decapoda: Brachyura: Potamonautidae and Potamidae) with special reference to Madagascar and the Seychelles. *Contributions to Zoology* 77: 71-81.

Cumberlidge N, Ng PKL. 2009. Systematics, evolution and biogeography of the freshwater crabs. Pp. 491-504 in: Martin JW, Crandall KA, Felder D, eds., *Crustacean Issues: Decapod Crustacean Phylogenetics*. Leiden: CRC.

Cumberlidge N, Sternberg RV, Daniels SR. 2008. A revision of the higher taxonomy of the Afrotropical freshwater crabs (Decapoda: Brachyura) with a discussion of their biogeography. *Biological Journal of the Linnean Society* 93: 399-413.

Cumberlidge N, Fenolio DB, Walvoord ME, Stout J. 2005. Tree-climbing crabs (Potamonautidae and Sesarmidae) from phytotelmic microhabitats in rainforest canopy in Madagascar. *Journal of Crustacean Biology* 25: 302-308.

Cumberlidge N, Ng PKL, Yeo DCJ, Magalhaes C, Campos MR, Alvarez F, Naruse T, Daniels SR, Esser LJ, Attipoe FYK, Clotilde-Ba FL, Darwall W, McIvor A, Ram M, Collen B. 2009. Freshwater crabs and the biodiversity crisis: importance, threats, status, and conservation challenges. *Biological Conservation* 142: 1665-1673.

Dai AY. 1999. *Fauna Sinica (Arthropoda: Crustacea: Malacostraca: Decapoda: Parathelphusidae, Potamidae)*. Editorial Committee of Fauna Sinica. Beijing: Science Press.

Diesel R, Schubart CD, Schuh M. 2000. A reconstruction of the invasion of land by Jamaican crabs (Grapsidae: Sesarminae). *Journal of Zoology* 250: 141-160.

Esser L, Cumberlidge N. 2008. *Sinopotamon yangtsekiense*. In: IUCN Red List of Threatened Species, version 2009.2. Available on www.iucnredlist.org [visited 20 February 2010].

Gilchrist SL. 1988. Appendix: Natural histories of selected terrestrial crabs. Pp. 382-390 in: Burggren WW, McMahon BR, eds., *Biology of the Land Crabs*. Cambridge, New York: Cambridge University Press.

Liu HC, Li HW. 2000. Reproduction in the fresh-water crab *Candidiopotamon rathbunae* (Brachyura: Potamidae) in Taiwan. *Journal of Crustacean Biology* 20: 89-99.

Melo MA, Abrunhosa F, Sampaio I. 2006. The morphology of the foregut of larvae and postlarva of *Sesarma curacaoense* De Man, 1892: a species with facultative lecithotrophy during larval development. *Acta Amazonica* 36: 375-380.

Ng, PKL. 1988. *The Freshwater Crabs of Peninsular Malaysia and Singapore*. Singapore: Shinglee Press.

Ng PKL, Guinot D, Davie P. 2008. Systema Brachyuorum: Part I. An annotated checklist of extant Brachyuran crabs of the world. *Raffles Bulletin of Zoology* 17: 1-286.

Rathbun M. 1898. Descriptions of three species of freshwater crabs of the genus *Potamon*. *Proceedings of the Biological Society of Washington* 12: 27-30.

Schmidt-Ott U. 2000. The amnioserosa is an apomorphic character of cyclorrhaphan flies. *Development Genes and Evolution* 210: 373-376.

Schubart CD, Diesel R. 1999. Osmoregulation and the transition from marine to freshwater and terrestrial life: a comparative study of Jamaican crabs of the genus *Sesarma*. *Archiv für Hydrobiologie* 145: 331-347.

Sternberg RV, Cumberlidge N. 2001. Notes on the position of the true freshwater crabs within the brachyrhynchan Eubrachyura (Crustacea: Decapoda: Brachyura). *Hydrobiologia* 449: 21-39.

Stewart JR. 1997. Morphology and evolution of the egg of oviparous amniotes. Pp. 291-326 in: Sumida S, Martin K, eds., *Amniote Origins-Completing the Transition to Land*. London: Academic Press.

Uchifune T, Machida R. 2005. Embryonic development of *Galloisiana yuasai* Asahina, with special reference to external morphology (Insecta: Grylloblattodea). *Journal of Morphology* 266: 182-207.

Wu HX, Xue JZ, Cumberlidge N. 2010. An extra embryonic phase in the true freshwater crab *Sinopotamon yangtsekiense* Bott, 1967 (Decapoda, Potamidae). *Chinese Journal of Oceanology and Limology* 28: 725-730.

Yeo DCJ, Ng PKL, Cumberlidge N, Magalhaes C, Daniels SR, Campos MR. 2008. Global diversity of crabs (Crustacea: Decapoda: Brachyura) in freshwater. *Hydrobiologia* 595: 275-286.

Zhao YC. 1963. Economic insect fauna of China 4: 1-63. Beijing: Science Press.

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