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An extra embryonic phase in the true freshwater crab *Sinopotamon yangtsekiense* Bott, 1967 (Decapoda, Potamidae)*

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Abstract The true freshwater crabs (Crustacea, Decapoda, Brachyura) are highly adapted to life in freshwater and complete their life cycle entirely independently of sea water. All true freshwater crabs exhibit direct development and lack the free-living larval forms (zoea and megalopa) typical of most other brachyurans. After a prolonged embryonic period (during which they pass through the typical brachyuran larval forms embryologically) the eggs of true freshwater crabs hatch to produce juvenile (hatchling) crabs. We provide here the first report and description of the continuous record of embryonic development from egg-laying up to hatching in the Chinese true freshwater crab *Sinopotamon yangtsekiense* Bott, 1967 (Potamoidea, Potamidae). Direct development (complete secondary embryonization) in *S. yangtsekiense* was observed to take 77 days and to include an additional embryonic phase (termed here the egg-juvenile-crab) that occurs in the embryo between the imprisoned megalopa and the newly-emerged juvenile (hatchling) crab. This is significant because the only other freshwater crab whose embryonic development has been studied in detail is *Potamon fluviatilis* (Potamidae) which takes 45–47 days and involves only nine embryonic stages.

Keyword: true freshwater crab; *Sinopotamon yangtsekiense*; embryonic development; egg larvae

1 INTRODUCTION

Most species of brachyuran crabs (Decapoda, Crustacea) are marine and produce large numbers of small eggs and have a series of free-living planktonic larval stages. Postembryonic development following egg hatching in marine crabs typically includes two larval phase (the zoea and the megalopa), and between 2 and 6 zoeal stages followed by a megalopa (the settlement phase) that metamorphoses into a juvenile crab (Anger, 2005; Giménez et al., 2003). In contrast, the more than 1 000 species of true freshwater crabs (Potamoidea, Pseudothelphusoidea, Gecarcinucoidea, Trichodactylidae, *sensu* Martin et al., 2001) exhibit direct development whereby their entire larval development is embryonic and is completed inside the egg. Freshwater crabs are highly adapted to life in fresh water and produce small numbers of large yolky (macrolecithal) eggs that remain attached to the mother's pleopods throughout development, and emerge from the egg as juvenile crabs (hatchlings). The hatchlings of freshwater crabs are further retained in the female's abdominal brood pouch for several days before they are finally released as free-living crabs. Pace et al. (1976) described embryonic development within the macrolecithal eggs of the Mediterranean potamid freshwater crab *Potamon fluviatilis* (Herbst, 1785) over the 45–47 days period between spawning to hatching. Those authors reported that embryonic development inside the eggs of P. *fluviatilis* passes through a total of nine stages from cleavage (stage I, day 1) to the imprisoned megalopa (stage IX, days 35–46) (Pace et al., 1976). Embryonic development in *P. fluviatilis* includes recognizable larval forms

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such as the egg-zoea and the egg-megalopa (imprisoned megalopa) that correspond to the free-living zoea and megalopa forms seen in most brachyurans. The megalopae must metamorphose into first juvenile crab with molting in the postembryonic development of free-living larval crab (Arshad et al., 2006; Jeng et al., 2004; Simith et al., 2008). We discovered the juvenile crab of *Sinopotamon yangtsekiense* hatched out from the egg and absorbed water to expand its body from imprisoned larvae to first stage free living juvenile crab directly. We supported there might be an imprisoned juvenile following imprisoned megalopa during the embryonic development of the true freshwater crab. Therefore, we observed the entire period of embryonic development from egg-laying up to hatching and discovered an extra embryonic phase (egg-juvenile-crab) in the Chinese true freshwater crab. The results completed the developmental theory and let us understand the embryonic development mechanism of direct development of true freshwater crab.

2 METHODS

The samples (*Sinopotamon yangtsekiense*) were collected from the Qiantang River, Zhejiang, China, between July and September 2002, and were maintained in the laboratory in aquaria (0.50 m×0.30 m×0.35 m) supplied with fresh water at a water temperature of 25±1°C and fitted with a water purifier (MF-1 Filter) that was replaced every two days. The samples 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 crabs (3 males and 3 females) were initially cultured in each aquarium, until the female crabs spawned, at which point they were separated and cultured in individual aquaria. Ovigerous crabs were observed every day from spawning until hatching, and 2 to 4 eggs were removed each day at 8:00 am and fixed in 70% ethanol for analysis. Embryos were carefully peeled away from the yolk under a stereomicroscope, and egg-larval stages were identified, recorded, and photographed using a digital camera fitted to the microscope. Free-living (hatchling) crabs that emerged from the eggs were fixed immediately in 70% ethanol, and subsequently rehydrated to distilled water in a series of steps (70% ethanol → 50% ethanol for 30 minutes → 30% ethanol for 30 minutes → distilled water for 30 minutes). Rehydrated eggs and hatchling crabs were placed in 5% HNO_3 for 15 min in order to dissolve away the calcium in their exoskeleton. Abdomens peeled from the imprisoned megalopae, egg-juveniles, 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 abdomens were then dyed with eosin for 20 min and further dehydrated twice for 30 min in absolute alcohol before being treating with methyl salicylate for 10 min to enhance their transparency. Finally, whole slices of abdomens were sealed up with resinene, dried at 24°C, and photographed using a digital camera fitted to a stereomicroscope.

3 RESULTS

3.1 Egg-larval forms

Embryonic development in *S. yangtsekiense* was found to pass through the same nine stages from cleavage (I) up to the imprisoned megalopa (IX) that were described for *P. fluviatilis* by Pace et al. (1976). It took a total of 77 days for larval metamorphosis in *S. yangtsekiense* to be completed embryonically in the laboratory. Changes in egg-larval morphology during this embryonic period showed there to be four main larval forms: the egg-nauplius (Fig.1a), the egg-zoea (Fig.1b), the imprisoned megalopa (Fig.1c), and the egg-juvenile-crab (Fig.1d).

Differences between these forms included the size of the embryo, the numbers of pairs of pereiopods, and the shape of the abdomen. The total length of each of the embryonic forms measured along the longitudinal axis was 0.325 mm (egg-nauplius), 0.825 mm (egg-zoea), 1.867 mm (imprisoned megalopa), and 3.250 mm (hatching larvae).

For each of these four main larval forms the total number of pairs of appendages on the cephalothorax was 3 pairs (egg-nauplius, Fig.1a), 7 pairs (egg-zoea, Fig.1b), 13 pairs (imprisoned megalopa, Fig.1c), and 13 pairs (egg-juvenile-crab stage, Fig.1d). Both the imprisoned megalopa and the egg-juvenile-crab had 5 pairs of pereiopods corresponding to those of the free-living hatchling crab, but the pereiopods of the imprisoned megalopa were bud-like (Fig.1e) while those of the egg-juvenile-crab were complete miniature crab limbs (Fig.1f). The abdomen of the egg-nauplius was short with a forked telson with 2 equal-sized finger-like processes each as long as the abdomen, and separated by a narrow space (Fig.1a). The abdomen of the egg-zoea was long and trapezoid-shaped with a wide base tapering to a short,

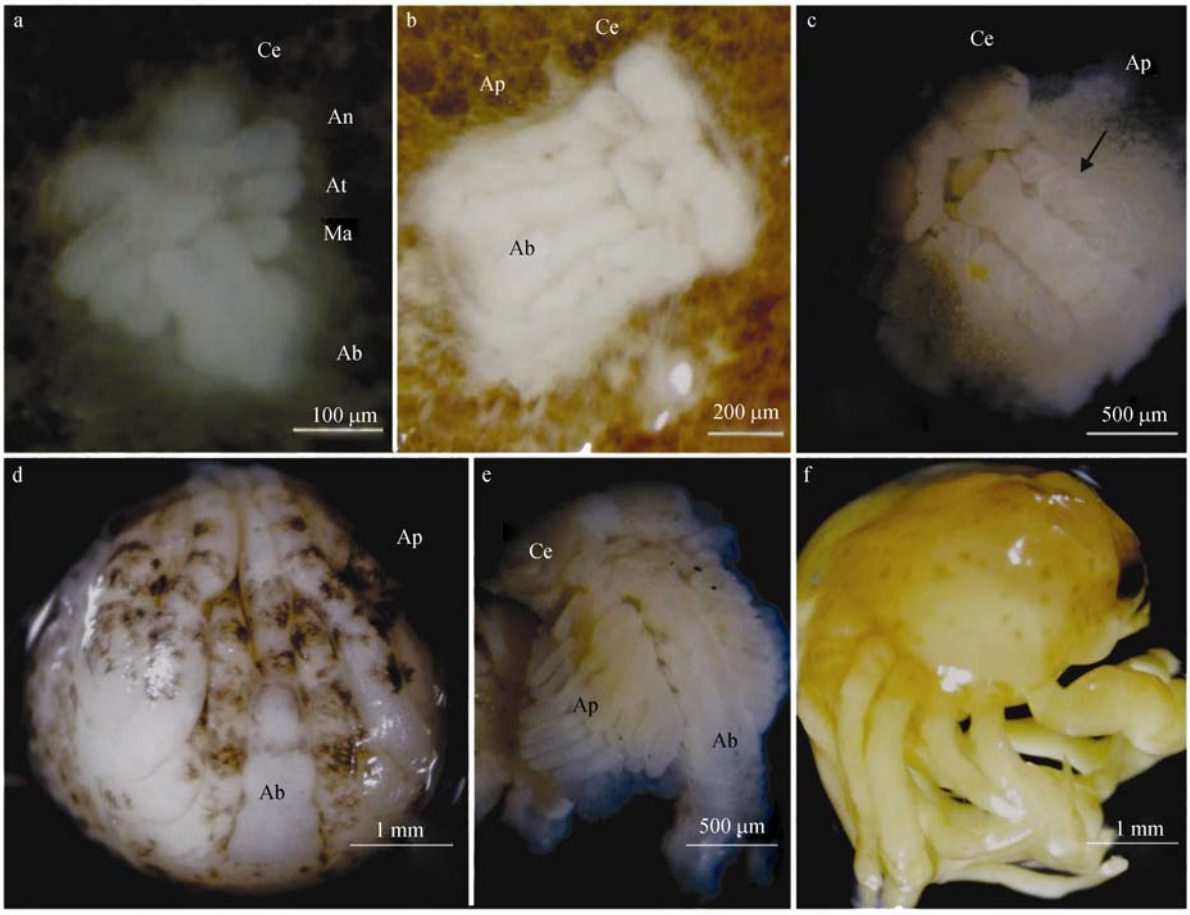

Fig.1 The embryonic larval forms of the Chinese freshwater crab *Sinopotamon yangtsekiense*

a. Egg-nauplius; b. Egg-zoea; c. Imprisoned megalopa; d. Egg-juvenile-crab; e. Imprisoned megalopa; f. Egg-juvenile-crab
Ab: Abdomen; An: antennule; Ap: Appendages; At: antenna; Ce: Compound eye; Ma: mandible

narrow telson with 2 equal-sized fingerlike processes separated by a broad space (Fig.1b). The abdomen of the imprisoned megalopa was narrow and long, with a rounded leaf-like telson with finger-like processes that cling to each other (Fig.1c). The abdomen of the egg-juvenile-crab was short, thin, and triangular with a rounded telson that lacked fork-like processes (Fig.1d).

3.2 Hatching of the egg-juvenile crab

The last embryonic larval form in *P. fluviatilis* immediately before hatching is the imprisoned megalopa (Pace et al., 1976). If this were the case in *Sinopotamon yangtsekiense*, then molting and metamorphosis from megalopa to the hatchling crab would be expected to be observed either before, or during, the final hatching of the egg, because it is the juvenile (hatchling) crab that emerges from the egg. However, in *S. yangtsekiense* the last embryonic form immediately before hatching was observed to be an imprisoned crab-like form, termed here the egg-juvenile-crab (Fig.2a–c), which would constitute stage X using the terminology coined by Pace et al. (1976). The stage X egg-juvenile-crab of *S. yangtsekiense* develops after the imprisoned megalopa (stage IX), and represents the final larval stage in this species. The last embryonic stage X is clearly not an imprisoned megalopa because the abdomen of the imprisoned megalopa (stage IX) is stick-like with a rounded telson that has two leaf-like processes (Fig.3a), while the abdomen of the egg-juvenile-crab (stage X) is a broad-based triangle that tapers to a triangular telson lacking a pair of forked processes (Fig.3b, d), and is folded underneath the cephalothorax and fitted tightly into the sterno-abdominal cavity (Fig.2a–c). The egg-juvenile-crab (stage X) of *Sinopotamon yangtsekiense* is similar to free-living hatchling crabs but the former has a more elongated carapace outline and a much narrower abdomen. During hatching, the crab-like pre-hatchling that emerges from the egg has curly pereiopods and a soft exoskeleton which soon becomes hardened and calcified from the deposition of calcium salts (Fig.2d–f) so producing a free-living

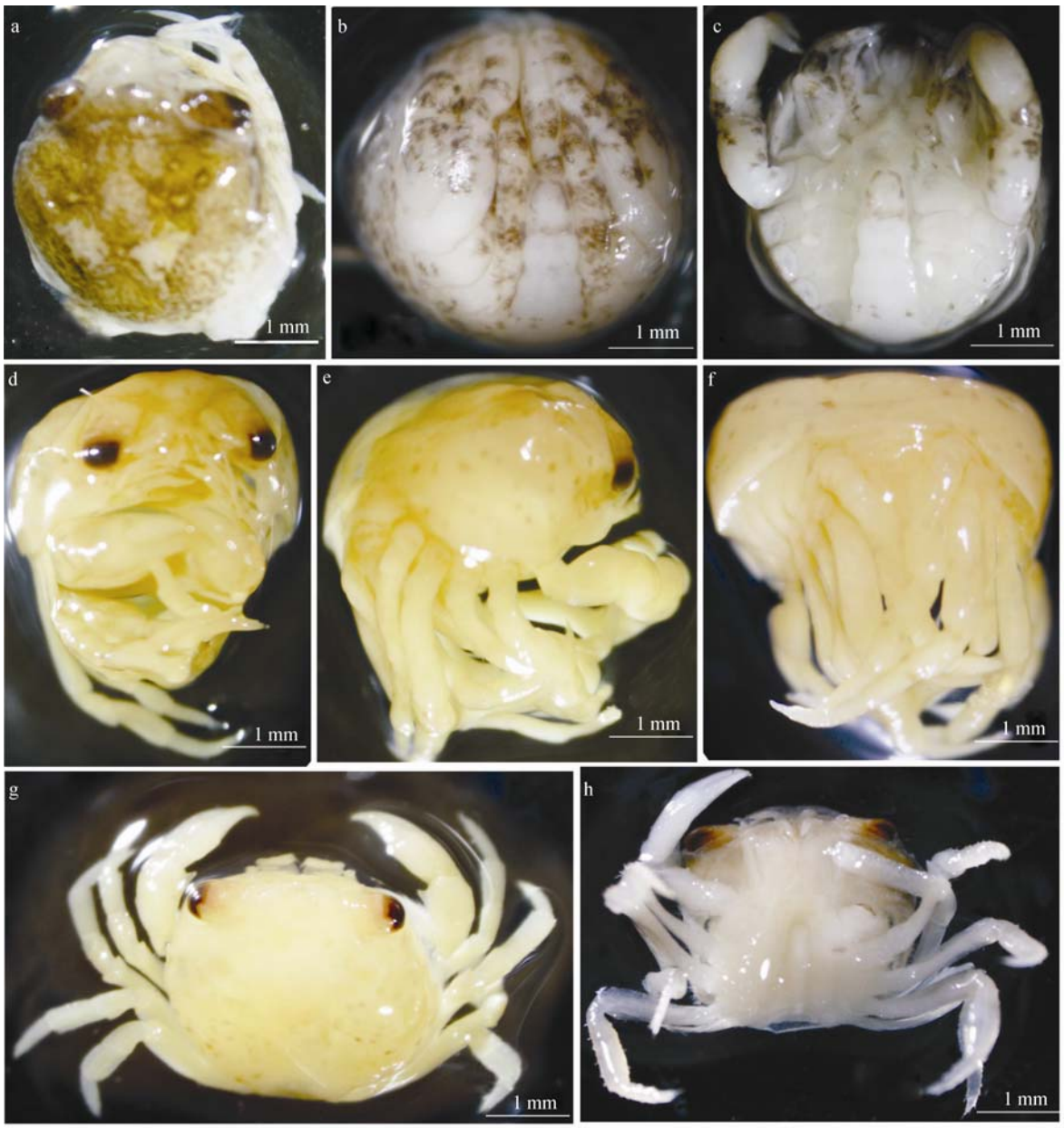

Fig.2 The process of the emergence of juvenile crabs from the eggs of *Sinopotamon yangtsekiense* Bott, 1967

The egg-juvenile crab stage (a–c), the moment of hatching from the egg (d–f), and the free-living juvenile (hatchling) crabs (g–h); a. Dorsal view of the egg-juvenile crab; b. Ventral view of the egg-juvenile crab; c. Ventral view of the egg-juvenile crab (with appendages removed); d. Anterior view of a crab hatching from an egg; e. Lateral view of a crab hatching from an egg; f. Posterior view of a crab hatching from an egg; g. Dorsal view of a free-living juvenile (hatchling) crab; h. Ventral view of a free-living juvenile (hatchling) crab

hatchling crab (Fig.2g–h).

Direct development (complete secondary embryonization) in *S. yangtsekiense* takes 77 days and includes an additional embryonic stage (termed here stage X, the egg-juvenile-crab). This is a significant finding, because the only other freshwater crab whose embryonic development has been studied in detail is *Potamon fluviatilis* (Potamidae) which takes 45–47 days, and involves nine embryonic stages (Pace et al., 1976). In this species there is an additional embryonic form after the megalopa (the egg-juvenile-crab stage) that takes an extra 20 days to develop. At present, it is not known whether the longer development time and additional embryonic stage (stage X) are seen only in this species, or whether embryonic development follows that seen in *Potamon* in other families of freshwater crabs.

4 DISCUSSION

Direct development in freshwater crabs, whereby

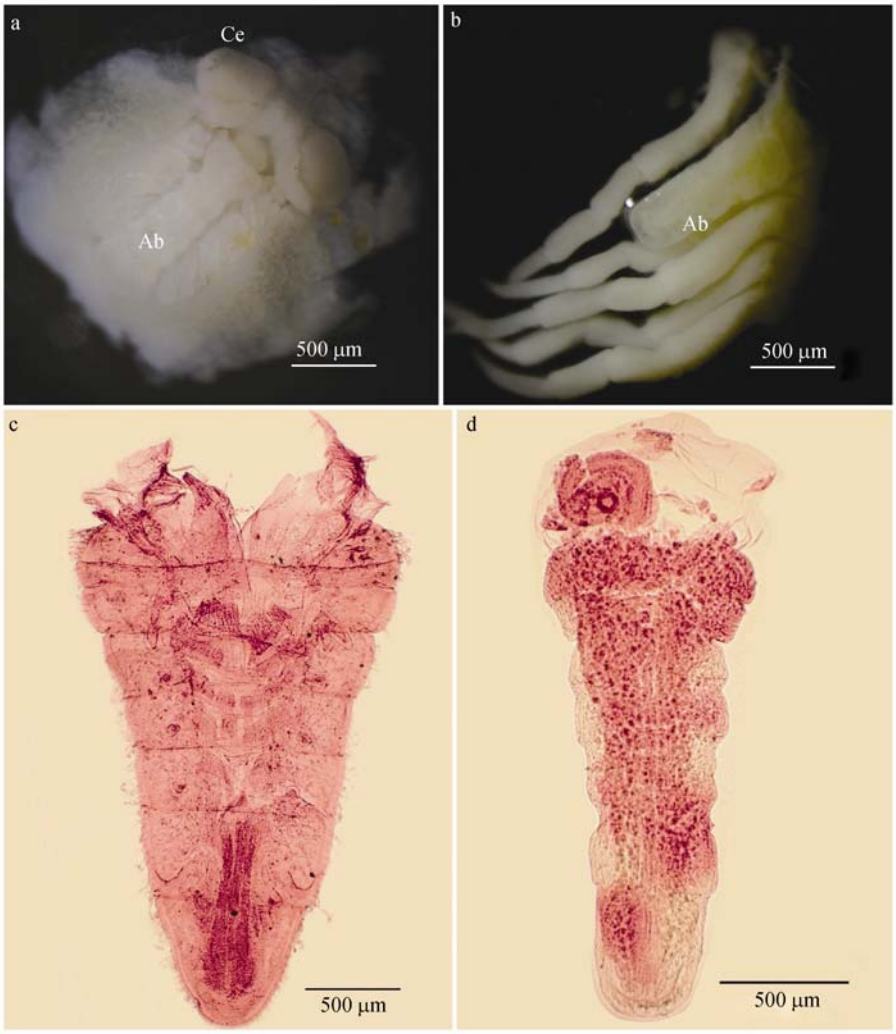

Fig.3 The abdomens of the egg-juvenile crab and the egg larvae

a. Imprisoned megalopa stage; b. Egg-juvenile crab stage; c. The first free-living juvenile crab; d. Egg-juvenile crab
Ab: Abdomen; Ce: Compound eye

the entire larval development takes place embryologically, represents the most advanced stage in the trend towards extended secondary embryonization within Brachyurans that live in freshwater habitats. Differences in the embryological retention of larval forms are most dramatic between species of crabs that inhabit marine and freshwater habitats. All crabs retain the nauplius, metanauplius and protozoea stages within the developing egg and all have two free-living larval phases, the zoea and the megalopa [except for the true freshwater crabs, and species such as the false spider crab *Amarinus lacustris* (Hymenosomatidae) (Richer de Forges et al., 1997, Ng et al., 2008), and *Geosesarma notophorum* (Sesarmidae) (Ng et al., 1995)]. For example, the marine swimming crab *Portunus Pelagicus* has 4 planktonic zoeal larval stages followed by a megalopa that settles and molts into a juvenile crab (Arshad et al., 2006) whereas freshwater-living sesarmid crabs from Jamaica have only 2 or 3 zoeal larval stages followed by a megalopa that molts into a juvenile crab (Diesel et al., 2000).

Increases in secondary embryonization are usually accompanied by increases in the amount of yolk in each egg and by a reduction in the number of eggs laid. Increased secondary embryonization (known as abbreviated development) has been reported to occur in a number of New World freshwater sesarmids (e.g., *Sesarma fossarum*, *S. ayatum*, *S. verleyi*, *S. jarvisi*, *Metopaulias depressus* (Diesel et al., 2000). In the case of these freshwater species, crabs produce small numbers of large (macrolecithal) eggs, retain one or more zoeal stages within the egg, have a long (>40 to 60 days) embryonic phase, and have fewer, more short-lived, free-living larval stages (abbreviated

development) (Diesel et al., 1995; Schuh et al., 1995a, 1995b; Cuesta et al., 1999; Diesel et al., 2000). True freshwater crabs such as species of the genera *Potamon* and *Sinopotamon* are similar in that they also produce small numbers of large (macrolecithal) eggs, have a long (>40 to 77 days) embryonic phase, but differ in that they completely lack free-living larval stages which implies a longer evolutionary history in freshwater habitats. Despite this interesting adaptation of the life cycle of freshwater crabs, until the present study, the study by Pace et al. (1976) on *Potamon fluviatilis* (Potamidae) represents the only detailed embryological study that has been undertaken on any species of true freshwater crab to date.

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