Embryonic and larval development of *Babylonia formosae habeı* (Altena and Gittenberger, 1981) (Gastropoda: Buccinidae) on China’s coast

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Abstract
The development of embryos and larvae of *Babylonia formosae habeı* living along the southeast coast of China is observed under laboratory conditions. The egg masses are laid by females on hard substrate at night and each capsule contains 100–500 eggs. Each egg is 250–280 mm in diameter. The first two cleavages of the embryo are meridional and equal, and a polar lobe is produced. Larval kidney, which only consists of a single cell, appears during the gastrula stage on each side of the embryo. The right tentacle develops prior to the left one. At 25–27 °C, an intracapsulate veliger stage is reached about 4.5 d after deposition. The larvae hatch on the fifth day as swimming veligers with a shell length of 360–500 mm. The newly hatched larva can ingest suspended algal cells from the water column and remains in the pelagic stage for 8–10 d. The newly settled juveniles are 900–1 200 mm in shell length.

**Key words:** *Babylonia formosae habeı*, embryo, larva, juvenile, development

1 Introduction

*Babylonia formosae habeı*, belonging to the family of Buccinidae, together with *Babylonia formosae formosae* (Sowerby, 1866), used to be supposed to be two subspecies of *B. formosae* (Altena and Gittenberger, 1981). But recent studies on the morphology and allozyme of the two subspecies indicate that they deserve to be recognized as full species: *B. formosae* and *B. habeı* (Liu and Chiu, 1998). *B. formosae habeı*, living in sandy or muddy subtidal areas along the southeast coast of China, is a commercially important gastropod, because its meat is consumed as food and shell as craft material in China.

Early in the 1930s, the reproduction and larval morphology of many species of the prosobranchs were studied by Lebour. The reports are either the detailed descriptions of the larval development of *Nassarius reticulates* and *N. incrassatus* (Lebour, 1931), or the brief descriptions of the eggs, larvae of more than 20 species of prosobranchs in Plymouth (Lebour, 1936). Considerable work has been done in the following decades after his studies. However, the stud-
ies on neogastropods received less research efforts than those on mesogastropods. The recent studies on the larval development of the prosobranches have made remarkable progresses at the aid of some modern technologies and instruments, such as scanning electron microscope (Collin, 2000; Pedersen and Page, 2000), epifluorescence microscope (Miloslavich, 1994; Miloslavish and Penchasazdeh, 1997).

The distribution of the recent members of the genus Balbomina is only found in the Indo-Pacific region (Altena and Gittenberger, 1981). Little is known about the reproduction and development of the reported 12 Balbomina species. B. japonica (Reeve, 1842) is the only species better known due to its commercial importance in Japan (Chiu and Liu, 1994). However, most studies on this species have been focused on reproductive biology (Ino, 1950; Yoshihara, 1957; Kajikawa, 1978a, b). In Taiwan, China, the studies on the reproduction of B. formosae formosae have been carried out in the latest two decades (Wong, 1980; Cheng and Ting, 1981; Chiu and Liu, 1994; Shieie and Liu, 1999). In China’s Mainland, some break through works on B. formosae habeii have been reported, including the histology of gonad and reproductive cycle (Ke and Li, 1991), spermatogenesis and sperm morphology (Ke and Li, 1992), reproduction behaviors (Ke and Li, 1993), toxicology (Wang and Ke, 2002), and the larvae of this species, such as the effects of temperature and salinity on embryonic development (Zheng et al., 2000), the chemical induction of settlement and metamorphosis (Ke et al., 2000). However, up to date, there is no literature describing the embryonic and larval development of B. formosae habeii.

In this paper, the developmental morphology from oviposition to young juvenile B. formosae habeii is presented based on laboratory-cultured conditions.

2 Materials and methods

The adults of Balbomina formosae habeii were collected by either bottom trawling or baited baskets at depths of 10~50 m, from the Fujian coast (25°6' N, 120°30' E), southeast of China, from May to August 2002, the peak spawning period of this species. The mature adults were 4.5 cm or above in shell length.

Adults were reared in laboratory aquaria provided with aerated, non-circulating, sand-filtered seawater. During night the females deposited egg masses on the walls of the aquaria. The egg masses were scraped off and collected daily, and reared in aquaria with 0.45 μm-filtered seawater, without aeration. After hatching, the larvae were transferred to 1 000 mL beakers with 0.45 μm-filtered seawater, without aerating. The density varied from 0.25 to 1.00 inds/mL according to the size of the larvae. The cultured unicellular alga, Isochysis zhanjiangensis, was added to the larval culturing water daily at a concentration of about 10^5 cells/(mL·d). Juveniles that settled on the bottom of the culture beakers were reared with sand-filtered seawater without aeration, and fed with excised Penaeus vannamei.

Throughout the present study, the seawater in the culture system was changed daily, the water temperature ranged from 25 to 27 °C, and the salinity ranged from 26 to 28.

During the experiments, the samples of egg masses, larvae, and juvenile were taken periodically to record the timings and phenomena of the developmental events. The intracapsulate embryos were observed under an Olympus light microscope without removing the transparent capsule. The hatched larvae and juveniles were observed under a dissecting microscope. All of the embryos, larvae and juveniles under observation were not anesthetized. The sizes recorded
were measured with an ocular micrometer.

3 Results

3.1 Morphology of the egg mass

_Babylonia formosae habei_ females deposit internally fertilized eggs within the benthic egg masses. Egg mass is usually an arc-shaped conglomerate of egg capsules. The capsule is transparent, rectangular and gelatinous. It is measured approximately 1 mm in thickness, 0.41–0.55 cm in height, 0.60–0.88 cm in maximum width (width at the base of the capsule) (Fig.1). Capsules are adhered to the substratum by a jellylike cement, which forms a common basal sheet for the egg masses. Each capsule contains 100–500 eggs, which are brown and 250–280 μm in diameter. The eggs form a single layer within the capsule, holding against the capsule wall by the clear gel that fills the lumen.

![Image of an egg capsule](image)

Fig. 1. The egg capsule of _Babylonia formosae habei_, showing the eggs arranged in a single layer between the capsular walls, 100–500 eggs are contained in each capsule.

3.2 Early embryogenesis

Based on the embryos and larvae reared at 25–27 °C, a schedule of development is given in Table 1. The eggs within a capsule develop synchronously. Within 3 h after oviposition, two polar bodies are extruded from the egg successively (see Figs 2a–c: 1pb, 2pb). A polar lobe with minute setae forms during the first two cell divisions (see Figs 2d–k: 1pl, 2 pl), and the polar lobe contracts as a stubby papilla just before the division (see Figs 2f, h, i). Polar lobe may also present during the subsequent divisions, but the blastomeres make it difficult to be seen with any certainty.

The development proceeds in a spiral cleavage pattern. The first two cleavages are meridional and equal (see Figs 2d–k). The third cleavage, which is equatorial and unequal, forms four clear micromeres that are located in the furrows between the macromeres, with the polar bodies on top (see Fig. 2l). The fourth cleavage is alike (see Fig. 2m). Later in cleavage, a mound of clear micromeres, which can be seen at the animal pole, produces a round blastula by epiboly: the proliferation continues until the opaque yolkly macromeres are completely covered by the clear micromeres (see Figs 2n–p). Embryos begin to rotate slowly in capsules during the blastula stage. There are some invaginations on the embryo during the gastrulation (see Figs 2q, r). The larval

<table>
<thead>
<tr>
<th>Time</th>
<th>Developmental events*</th>
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<tbody>
<tr>
<td>0 h</td>
<td>oviposition</td>
</tr>
<tr>
<td>3.8 h</td>
<td>first cleavage (2 cells)</td>
</tr>
<tr>
<td>4.2 h</td>
<td>second cleavage (4 cells)</td>
</tr>
<tr>
<td>7.3 h</td>
<td>third cleavage (8 cells)</td>
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<tr>
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<td>fourth cleavage (16 cells)</td>
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<tr>
<td>12.1 h</td>
<td>late cleavages</td>
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<tr>
<td>2.5 d</td>
<td>morula</td>
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<tr>
<td>3.5 d</td>
<td>blastula</td>
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<tr>
<td>4 d</td>
<td>gastrula</td>
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<tr>
<td>4.5 d</td>
<td>intracapsulate veliger</td>
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<tr>
<td>5 d</td>
<td>early veliger (hatching)</td>
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<tr>
<td>6.5 d</td>
<td>later veliger</td>
</tr>
<tr>
<td>14 d</td>
<td>metamorphically competent veliger</td>
</tr>
<tr>
<td>15 d</td>
<td>carnivorous juvenile</td>
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* Cultured in seawater with salinity 26–28, temperature 25–27 °C.
Fig. 2. Embryonic development of *Babylonia formosae habeI*. a. The zygote; b and c. the polar bodies are extruded successively, showing the first polar body (1pb), the second polar body (2 pb); d–i. the first cleavage, showing the first polar lobe (1pl); j and k. the second cleavage, showing the second polar lobe (2pl); l. the third cleavage; m. the fourth cleavage; n. late cleavage; o. morula stage; p. blastula stage; q and r. gastrula stage, showing the larval kidney (lk); s and t. the introtpecapsule veliger, showing the rudiment of the velum (vr) and the velar cilia (vc).

kidney, which consists of only a single cell, is located on each side of the embryo (Fig. 2q: lk). Accompanied with the elongation of the embryo along the animal-vegetal axis (Fig. 2r), a small, clear ciliated ridge appears (Fig. 2s: vc, vr). The ridge gradually enlarges and differentiates into small larval velum on the fourth to fifth day (Fig. 2t: vr). The transparent foot rudiment and
spoonlike larval shell appear at this stage. The size of the embryo is almost unchanged during the early embryogenesis.

3.3 Structure of early veliger

During this stage, the foot, shell and velum continue to develop. By the fifth day, the transparent, fragile shell of the larva consists of one whorl (see Fig. 3a: sh). The whole larva can be completely withdrawn into the shell when it retracts.

The velum then enlarges into a bilobate structure (see Fig. 3b: v). Around the margin of the velum there is a thick rim edged with large cilia. On the underside and somewhat internal to the margin is another smaller ridge edged with smaller cilia (see Fig. 3a: vc). The two ciliated ridges form a food groove round the margin of the velum (see Fig. 3a: fg). The larval mouth is connected to the food groove at the ventral base of the velum (see Fig. 3b: m). Beating of the velar cilia can be seen apparently. The larva can move freely within the narrow space in the capsule. The velum is initially colorless and then comes some orange spots, which appear around the border, gradually make up a distinctive stripe that runs proximal and parallel to the food groove on each velum (see Fig. 3b: vp).

Prior to the appearance of the left tentacle, the rudiment of the right tentacle protrudes through the velum as a stubby papilla at the apex of the larval body, with a few large cilia on the tip (see Fig. 3b: rt). The left tentacle does not appear until hatching. Black eyespots, which appear prior to the tentacles, are visible through the transparent cephalic epidermis (see Fig. 3a: e).

The small, triangular foot is located just below the larval mouth, projecting out from the shell aperture, and it has already been provided with a clear operculum (see Fig. 3a: f, op). The midline and margin of the foot are pigmented with orange and blue dots (see Fig. 3b: fp). There is a tuft of large cilia on the blunt end of the foot (see Fig. 3b: fc). One well-developed statocyst is located on each side of the ventral base of the foot (see Fig. 3a: st).

The esophagus connected to the mouth is visible (see Fig. 3b: es). The larval heart is a thin-walled vesicle located somewhat to the left of the body's midline, behind the esophagus and near the shell aperture (see Fig. 3b: h). The heart beats regularly at the speed of about 110~130 times per minute. Some reddish pigment aggregation is visible behind the heart near the outer lip of the shell aperture (see Fig. 3a: pa). Intestine and anus can be seen on the right side of the body (see Fig. 3a: in, an). The mantle fold extends along the inside of the shell a little behind the shell margin (see Fig. 3a: mf). Most of the visceral mass is hidden by the opaque yolk (see Fig. 3a: y).

During this stage, the larvae escape from the capsule in succession. With the ciliated velum, the hatchlings are able to collect and ingest suspended algal cells from the water column. The newly hatched larva has a shell consisting of 1~2 whorls, about 360~500 μm across its longest part in length (across from the outer lip of aperture to the opposite side of the shell).

3.4 Structure of later veliger

One to two days after hatching, all the yolk reserves have been completely absorbed. The larva feed on phytoplankton in a manner typical to that for planktotrophic veligers: the cilia on the margin of the velum beat continuously while the larva is swimming; then the algae cells are collected in the food groove and brought into the mouth connected to the groove.

The larvae increase in size and morphological complexity in this stage. There are a large left digestive glands and a small right one, and
Fig. 3. Larval development and metamorphosis of *Babylonia formosae habe*. a. Early veliger (5 d) in the right lateral view, showing velar anus (an), cilia (vc), eye (e), foot (f), food groove (fg), intestine (in), mantle fold (mf), operculum (op), pigment aggregation (pa), statocyst (st), yolk (y); b. early veliger (5 d) in the left lateral view, showing the esophagus (es), foot cilia (fc), foot pigment (fp), heart (h), mouth (m), right tentacle (rt), shell (sh), velum (v), velar pigment (vp); c. later veliger (7 d) in the right lateral view, showing the anterior lobe of the foot (al), anus (an), foot (f), gill rudiment (gr), intestine (in), left digestive gland (ldg), left tentacle (lt), right digestive gland (rdg), right tentacle (rt), stomach (sto), velum (v); d. metamorphically competent veliger (9 d), showing the foot with two horns (f), gill lamellae (gl), operculum (op), shell (sh), siphon (si), tentacles (t); e. metamorphosed larva with reduced velum (rv); f. early juvenile (10 d); scale bar equals 400 mm; g. later juvenile (13 d), showing muscular food (f), operculum (op), shell (sh), siphon (si), tentacles (t).
the bigger left gland occupies the apex of the shell (see Fig. 3c: ldg, rdg). The digestive glands are distinctive in the bodies because of their red color and considerable size. An ellipsoidal stomach, which connects to the esophagus ventrally, lies between the digestive glands. The stomach lumen is divided into two parts with a constriction in the middle (see Fig. 3c: sto). Some food boluses are rotated continuously in the stomach by the beating of the cilia on the inner surface of the stomach wall. The stomach narrows dorsally into the intestine (see Fig. 3c: in) and extends to the anal opening on the right side of the body in the mantle cavity (see Fig. 3c: an). The wall of the gut is slightly pigmented.

The foot grows perceptibly longer and more muscular (see Fig. 3c: f) with an anterior lobe developed in front of the mouth (see Fig. 3c: al). The pigments on the foot then become more conspicuous. The left tentacle appears in this stage and grows so long as the right one (see Fig. 3c: lt, rt). The rudiment of the gill lamellae appears near the outer lip of the shell aperture, just below the reddish pigment aggregation (see Fig. 3c: gr).

At this stage, the larva has a shell about 500–700 μm in length, and the number of the whorls continues to increase. The velum, with an indentation on each side, becomes four-lobed (see Fig. 3b: v), and the width across it seems much greater than the shell length. The larva with an outspread velum looks like a butterfly with outspread wings. The orange stripe on the margin of the velum is still conspicuous.

3.5 Larval metamorphosis and morphology of juvenile

On the 14th day, the larval shell becomes translucent, consisting of 2–3 whorls, with indistinct ribs on the body whorl (see Fig. 3d: sh). The cephalic tentacles are gracile and flexible like those of the adult (see Fig. 3d: t). A long and retractile siphon projects from the shell aperture (see Fig. 3d: si). The gill lamellae are visible through the transparent shell just under the siphon (see Fig. 3d: gj). The foot elongates greatly, the anterior lobe of the foot expands into two horns (see Fig. 3d: f), which indicates that the larva can use its foot for crawling, and becomes metamorphically competent veliger. Before the degradation of the velum, the larva is able to swim with the velum as well as crawl with the remarkable foot, which becomes greatly inflated and forms a broad ventral platform beneath the shell when the larva is crawling. The larval kidney is replaced by adult kidney in this stage, but the details of the change still remain to be proved in some further researches.

The velum is resorbed gradually. A reddish, round ridge and highly reduced velum is left around the base of each tentacle (see Fig. 3e: rv). It then disappears completely on the 10th day (see Fig. 3f). The loss of the velum signals the irreversible change of the mode of larval life from pelagic to benthic, and a different method of feeding comes into use, until finally the velar method is abandoned and the ordinary adult carnivorous method substituted. Some internal organs may also have some changes, which should be investigated in some further researches. The shell length is 900–1 200 μm during this stage.

About 3 d later, the shell of the juvenile becomes opaque, consisting of 3–4 whorls, and the body whorl elongates along the main axis. There are more than two rows of brown, irregularly squarish stripes arrayed in parallel on the yellowish, ivory shell (see Fig. 3g: sh). Almost the whole epidermis is pigmented with light brown dots. The tentacles and siphon grow longer (see Fig. 3g: t, si), and the foot grows longer, wider and much more muscular (see Fig. 3g: f), attached by the brown, translucent operculum (see Fig. 3g: op). The larva now resembles the adult in appearance except
for the sculpture of the shell.

4 Discussion

*Babylonia formosae habeii* has typically lens-shaped egg capsules. The eggs are arranged in a single peripheral layer that facilitates gaseous exchange, like that in other egg-capsules-producing prosobranchs (Strathmann and Chaffee, 1984). The gelatinous slime of the egg capsule presumably functions, not as nutritive material, but as protective layer only (Thorson, 1946), because there is no obvious evidence that the larvae ever absorb the gel in capsules during the intracapsulate stage.

Regarding the early development of *B. formosae habeii*, the similar spiral cleavage pattern has also been reported in *Margarites helicinus* (Holyoak, 1988), *Nassarius reticulatus* and *N. incrassatus* (Lebour, 1931), and *Bulla gouldiana* (Farfan and Ramirez, 1988). This pattern may be one of the embryonic cleavage modes of gastropods, but its generality remains to be verified. Both distinct polar lobe and larval kidney are characteristics of mesogastropods, such as in *Nitidiscala tincta* (Collin, 2000a). The larval kidney of *B. abylonia formosae habeii* only consists of a single cell, which agrees with the descriptions of Collin (2000b) on the species of the family Calyprt-aeidæ (Caenogastropoda): the direct developers have multiple larval kidney cells, whereas the indirect developers have only one cell on each side of the body. The larval kidneys of *B. formosae habeii* disappear before hatching, perhaps as a mean of disposing of waste products, which has also been reported in *Cypraecassis testiculus* (Hughes and Hughes, 1987). The right tentacle of *B. formosae habeii* develops prior to the left one, which is rarely reported in other species.

The comparisons of egg number, egg size, larval life-span among the three babylonias, *B. formosae habeii*, *B. formosae formosae*, *B. japonica*, are given in Table 2. Compared with *B. formosae formosae* and *B. japonica*, the eggs of *B. formosae habeii* are much smaller and more numerous; the larvae of *B. formosae habeii* spend a relatively shorter intracapsulate stage and a longer pelagic stage. They are planktotrophic during their pelagic period, whereas the larvae of the other two species are lecithotrophic (Ino, 1950; Chiu and Liu, 1994). The yolk provided in the eggs of *B. formosae formosae* and *B. japonica* is clearly sufficient for completing the embryonic development without requiring external nutrients. However, the relatively less yolk in the eggs of *B. formosae habeii* is not sufficient for that. Therefore, the pelagic larvae have to ingest suspended algal cells from the water column after hatching, even before the yolk reserve is completely absorbed. Temperature is an important factor in the larval development. Normally, the larvae grow faster in a higher temperature. The larvae of *B. formosae habeii* hatch on the 7th

<table>
<thead>
<tr>
<th>Species</th>
<th><em>B. formosae habeii</em></th>
<th><em>B. formosae formosae</em></th>
<th><em>B. japonica</em></th>
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<tbody>
<tr>
<td>Egg number per capsule</td>
<td>100–500</td>
<td>19.8±8.8</td>
<td>42</td>
</tr>
<tr>
<td>Egg size/μm</td>
<td>250–280</td>
<td>520–570</td>
<td>500</td>
</tr>
<tr>
<td>Intracapsulate period/d</td>
<td>7</td>
<td>15–21</td>
<td>15–20</td>
</tr>
<tr>
<td>Pelagic period/d</td>
<td>10</td>
<td>1–2</td>
<td>2–3</td>
</tr>
<tr>
<td>Culturing temperature/°C</td>
<td>24–26</td>
<td>25–27</td>
<td>21–22</td>
</tr>
<tr>
<td>Nutritional mode</td>
<td>planktotrophic</td>
<td>lecithotrophic</td>
<td>lecithotrophic</td>
</tr>
<tr>
<td>Reference</td>
<td>present study</td>
<td>Chiu and Liu (1994)</td>
<td>Ino (1950)</td>
</tr>
</tbody>
</table>
day at the water temperature of 24–26 °C, whereas on the 5th day at 28.2–29.5 °C (Zheng, et al., 2000).

Although the egg size is within the range of the average size (230–500 μm) of prosobranch species that have non-pelagic larvae (Jablonski and Lutz, 1983), *B. formosae habei* is a species of indirect development, i.e., the larvae hatch as swimming veligers. In contrast, the temperate buccinid species *Engoniophos unicinctus* hatches as a crawling pediveliger, which has reduced velum and never been observed of swimming (Miloslavich, 1994). According to Miloslavich (1994), in temperate buccinids, the presence of nurse eggs in species with intracapsule metamorphosis is a common feature, and the larvae complete their development with the ingestion of nurse eggs or the albumen contained in the egg capsule fluid; but tropical buccinid species mostly have no nurse eggs, and hatch as swimming veligers. Distributing along the tropical and subtropical coasts of China, *B. formosae habei* has no nurse eggs, and almost all of the eggs in each capsule develop and finally hatch as swimming veligers, with the exception of a few unfertilized eggs, which remain intact till hatching. And it has not been observed that the larvae ingest the perivitelline fluid or intracapsule liquid in which they are embedded.

It seems that the evolution of prosobranchs begins with the forms that release eggs directly into the sea. And it is then, from the forms in which the eggs are contained in protective structure or egg capsules (Shuto, 1974) to the forms, that some eggs evolve into nurse eggs, which are ingested by the neighbor eggs to ensure the success of larval development. Miloslavich (1994) suggested that the species with direct development still maintain the intracapsule veliger stage in their embryogenesis, probably for granting the feeding potential to some leciotrophic gastropod larvae. However, when the intracapsule veligers of *Fusus syracusanus* are experimentally encapsulated in seawater, they are not only unable to swim but die within 30 min (Fioroni, 1966). In *Engoniophos unicinctus*, when experimentally encapsulated, the veligers can freely move, using the velum as a motile organ (Miloslavich, 1994); and the experimentally encapsulated veligers of *B. formosae habei* can survive and continue the development for a few hours or even a couple of days in filtered seawater (Ke, unpublished data). It would be interesting that some follow-up experiments after the survivorship and development of the encapsulated veligers are conducted in the future.

Common classification of modes and strategies of molluscan development is not as useful as we thought because the range of variations of the development can always be underestimated (Son and Hong, 1994). Up to date, our knowledge about the embryology of the gastropods is limited. The present study describes the general events during the early life history of *B. formosae habei*, which have not been documented previously, and provides an example of pelagic developmental mode in the species of *Babylonia*. In addition, *B. formosae habei* is noteworthy due to its large size and delicious taste, which make it a popular seafood in China. As a result of over harvesting, the population of *B. formosae habei* along the coast of China has reduced rapidly in recent years. To protect it from further depleting and develop a culture of this species, investigations into its early life history are absolutely necessary, plus that can provide indispensable information to the further studies on the biology of this species.

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