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Embryonic development and external morphology of *Amphioctopus aegina* (Gray, 1849) (Cephalopoda: Octopodidae) in Thailand

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ABSTRACT: The embryonic development, morphology of the eggs, and newly hatched paralarvae of the sandbird octopuses (*Amphioctopus aegina*) were described using laboratory-reared specimens. Mature octopuses were collected from an artisanal fishery at Baan Salakeaw, Baan Phe District, Rayong Province, Thailand. The spawned eggs were examined. The non-adhesive eggs were attached to a string to form a cluster, and the average egg size was 2.64 ± 0.13 mm in length and 0.94 ± 0.06 mm in width. Females brooded the eggs in their arms until hatching. The embryonic development period was divided into 27 stages. The first embryo inversion occurred at stage 11, and primordia of major body parts, such as the arms, head, and mantle, also appeared. Eyespots appeared at stage 12, chromatophores appeared on the ventral side at stage 18 and on the dorsal side at stage 19, and the external yolk sac decreased in size until it disappeared at stage 27 (hatching stage). The paralarvae hatched after 18–22 days at 28.0 °C and swam to the surface. The average total length of paralarvae was 3.40 ± 0.24 mm. The paralarvae had a stubby oval shape mantle. Their arms were subequal in length with 5 suckers arranged in a single row per arm, and the eyes became conspicuous. The dorsal side of the head had 2–3 rows of large reddish brown chromatophores with 4 chromatophores in each row. On the funnel, there were 5 chromatophores in two rows each with three and two chromatophores. A single row of chromatophores occurred on the ventral mantle margin.

KEYWORDS: *Amphioctopus aegina*, embryonic development, external morphology, sandbird octopus

INTRODUCTION

The sandbird octopus (*Amphioctopus aegina*) is a moderate-sized (mantle length up to 90 mm) species that occurs in coastal waters in Asia [1]. It has short to moderate arms, which are twice as long as the mantle length. Its lateral arms are the longest (typically $3 = 4 > 2 > 1$) [1]. The arms can regenerate if damaged or lost. A shallow web extends between the dorsal arms, and each arm has two rows of suckers. It has a white, narrow, transverse head bar, and a white longitudinal stripe that extends along the dorsal midline [1]. The third right arm of males is hectocotylized [1, 2]. The average fecundity of mature females is 6895 eggs, and the gonadosomatic index peaks in October and January [2, 3]. There are two spawning seasons (March–May and August–October) [3]; spawning occurs in muddy coastal waters at > 20 m depth. The species is typically found sub-tidally on soft substrates and feeds on live shrimps, crabs, and small fish [1]. The species is mostly caught by large export trawl fisheries and octopus traps, particularly in the Gulf of Thailand and Andaman Sea where it is treated under its junior synonym *Octopus dollfusi* (Chotiyaputta, 1993) [1, 4].

Information on *A. aegina* embryos has been collected from coastal areas of India. The embryonic development period at 31 °C was reported to be 18–22 days [2]. In Thailand, however, knowledge of the early life stages is limited. Hence efforts are being made to study its biological characteristics including its spawning ground and distribution, as well as its fisheries. The present study was carried out to describe the embryonic development and early morphological characteristics of *A. aegina*. The findings from this study will be useful for identifying *A. aegina* hatchlings from plankton samples, thus contributing toward research studies on its spatial distribution, seasonal migration, and predicted spawning grounds.

MATERIALS AND METHODS

Specimen collection

Twenty-five *Amphioctopus aegina* were collected from an artisanal fishery at Baan Salakeaw, Baan Phe District, Rayong Province, and transferred to a laboratory at the Rayong Marine Fisheries Research and Development Center, where they were placed in a circular tank with an open circulation system and filtered seawater. The tank measured 1.5 m in diameter by 1 m in height with volume of approximately 900 l of seawater at an ambient temperature of 28.0 °C, salinity of 30–32 psu, and a 12:12 h light/dark cycle. Each octopus was fed 2–4 live shrimps and/or crabs per day. To maintain the water quality, the tank was cleaned every day and 1/3 of the water was replaced. Males were identified based on the hectocotylized arm, which has smaller suckers than the other arms.

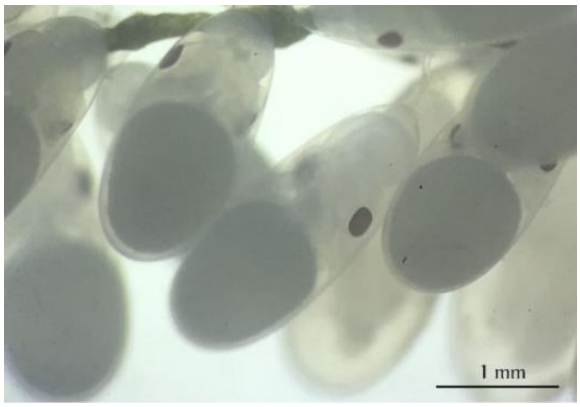

Fig. 1 Developing embryos of *A. aegina* (stage 18).

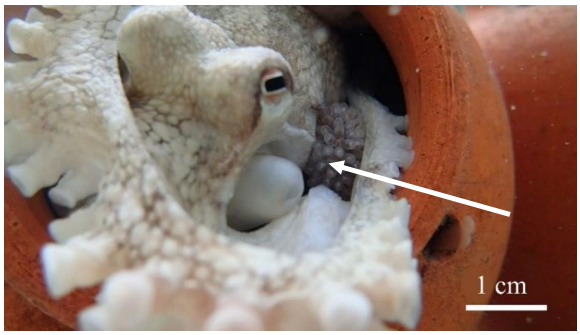

Fig. 2 Parental care: *A. aegina* female brooding her eggs (indicated by arrow).

Eggs and paralarvae measurements

After a female had spawned a cluster (string) of eggs (which were carried in the female's web), the eggs were removed and kept in a separate tank for measurement and observation. About randomly selected 30 eggs from each female were measured (length and width), sterilized with 200 ppm povidone-iodine for 5 min, rinsed with seawater 2–3 times, and kept in a circular 800 l tank [5]. Development was observed under a stereomicroscope until hatching. After hatching, the paralarvae were preserved in 4% formaldehyde, and measurements were made for: dorsal mantle length (ML), total length (TL), mantle width (MW), head length (HL), arm length (AL), and eye diameter (ED). Arm length and web length formula were determined Morphological analyses were conducted on the body shape, number and size of suckers, and number and size of chromatophores on the arms, head, funnel, and mantle [6].

RESULTS

Eggs

Three females spawned 3–5 days after mating, and the eggs were brooded in their arms. Individual eggs are transparent and surrounded by a grape-shaped chorion (Fig. 1). The egg capsules were small, with a mean average size ± SD of 2.64 ± 0.13 mm in length and 0.94 ± 0.06 mm in width. The number of eggs per cluster ranged from 5607 to 13 640 (10 642 ± 4373). The two larger females brooded more eggs than the smaller one (Table 1). After the paralarvae had hatched, they ascended to the surface, suggesting a planktonic lifestyle. The females remained alive for an average of 8 days after hatching eggs and survived 27–30 days without food (Fig. 2).

Embryonic development

The embryonic development is divided into 27 stages (Table 2).

Morphological characteristic of hatchlings

At hatching, the paralarvae are stubby with an oval mantle and finless. The average total length is 3.40 ± 0.24 mm, and Kölliker's organ bristles are present. The mantle length is 56% of the total length, and the mantle width is 44% of the total length. The head, medium size and squarish, represents 49% of the total length with a big eye (12% of the total length in diameter) on each side. All arms are subequal in length and about 35% of the total length. Each arm has one row of 5 suckers with the proximal sucker being the smallest and the middle one the largest. The web is shallow and located between the interbranchial membrane near the base of the arms. The web length is 10% of the arm length, with the web between the first arms the longest and the web between the fourth arms the shortest. The web formula is A.B.C.D (Fig. 4).

Chromatophore patterns in hatchlings

Dorsal side

On the dorsal side of the mantle, chromatophores are distributed on the inner and outer layers (Fig. 5). On the inner layer, there are 8–19 chromatophores formed in a diamond shape; and on the outer layer, there are 23–32 chromatophores in four rows (5–8 per row). On the dorsal side of the head, there are 2–3 rows of large, reddish brown chromatophores with 4 in each row. Each eye has 3 chromatophores on the outer surface layer. There are two rows of 5–8 small, triangular, black or red chromatophores on the aboral side of each arm lining from the arm base to the arm tip.

Ventral side

On the ventral side of the mantle, chromatophores also occur on the inner and outer layers (Fig. 5). In total, there are 29–40 chromatophores of reddish brown in color and round: 10–12 on the inner layer mantle and 19–30 on the outer layer. Chromatophores on the outer layer are arranged in 7 rows (4–5 chromatophores/row). Besides, on the anterior margin

Table 1 Weight, mantle length, egg size, and fecundity of three *A. aegina* females spawning in captivity.

Female ID	Weight (g)	Mantle length (cm)	Egg size (mm)		No. of eggs in cluster
			Length	Width	
1	110.0	6.50	1.54–1.92	0.65–0.77	13 640
2	110.0	6.50	1.78–1.87	0.58–0.72	12 618
3	50.2	4.55	1.54–1.92	0.65–0.77	5 607
Average ± SD	90.07 ± 34.53	5.83 ± 1.13	1.84 ± 0.10	0.66 ± 0.05	10 622 ± 4 373

Table 2 Embryonic development stages of *A. aegina* at 28.0 °C.

Development stage	Description	Hours after spawning
1	Eggs are clear, and cell division starts at the anterior end (animal pole) (Fig. 3 Stage 1)	1–14
2	Cell division is evident at the animal pole (Fig. 3 Stage 2)	15–24
3	Cells develop at the anterior end and small bulging-like cells appear (Fig. 3 Stage 3)	25–52
4	Cell division continues and more bulging-like cells appear at the animal pole (Fig. 3 Stage 4)	53–65
5	Cell division enters cleavage stage (Fig. 3 Stage 5)	66–75
6	Cleavage stage continues (Fig. 3 Stage 6)	76–88
7	Cells enter blastula stage: 10% of egg covered by blastoderm (Fig. 3 Stage 7)	110–136
8	Blastula stage: 30% of egg covered by blastoderm (Fig. 3 Stage 8)	137–161
9	Blastula stage: 60% of egg covered by blastoderm (Fig. 3 Stage 9)	162–186
10	Blastula stage: 90% of egg covered by blastoderm (Fig. 3 Stage 10)	187–205
11	First embryo inversion from vegetal pole to animal pole occurs; primordia of major body parts such as arms, head and mantle appear (Fig. 3 Stage 11)	206–219
12	Eyespots are pale orange (Fig. 3 Stage 12)	220–242
13	Eyespots increase in size and become dark orange; arms are visible (Fig. 3 Stage 13)	243–263
14	Eyespots become amber in color; external yolk sac decreases to about 65% of egg length (Fig. 3 Stage 14)	264–284
15	Eyespots are amber in color; mantle length is about 5% of egg length; suckers appear on each arm; external yolk sac decreases to about 60% of egg length (Fig. 3 Stage 15)	285–305
16	Eyespots are brown; branchial hearts appears at the base of the gills; external yolk sac decreases to about 55% of egg length (Fig. 3 Stage 16)	306–325
17	Eyespots are black; arms and sucker increase in size; buccal mass and funnel are visible; external yolk sac decreases to about 50% of egg length (Fig. 3 Stage 17)	326–346
18	Two chromatophores appear on the ventral head; external yolk sac decreases to about 45% of egg length (Fig. 3 Stage 18)	347–367
19	Chromatophores present on both dorsal and ventral sides; external yolk sac decreases to about 40% of egg length (Fig. 3 Stage 19)	368–386
20	External yolk sac decreases to about 35% of egg length; internal yolk sac is about 40% of mantle length (Fig. 3 Stage 20)	384–406
21	Chromatophores are spread on mantle, head, and arms; arms lengthen and suckers increase in size; external yolk sac decreases to about 30% of egg length; and internal yolk sac is about 45% of mantle length (Fig. 3 Stage 21)	407–425
22	External yolk sac decreases to about 25% of egg length; internal yolk sac is about 50% of mantle length (Fig. 3 Stage 22)	426–443
23	Secondary embryo inversion occurs; external yolk sac decreases to about 25% of egg length; internal yolk sac is about 55% of mantle length (Fig. 3 Stage 23)	444–463
24	External yolk sac decreases to about 10% of egg length; internal yolk sac is about 55% of mantle length (Fig. 3 Stage 24)	464–485
25	External yolk sac decreases to about 5% of egg length; eggs begin hatching if stimulated (Fig. 3 Stage 25)	486–508
26	External yolk sac disappears (Fig. 3 Stage 26)	509–527
27	Paralarvae hatch and swim to the surface (Fig. 3 Stage 27)	528

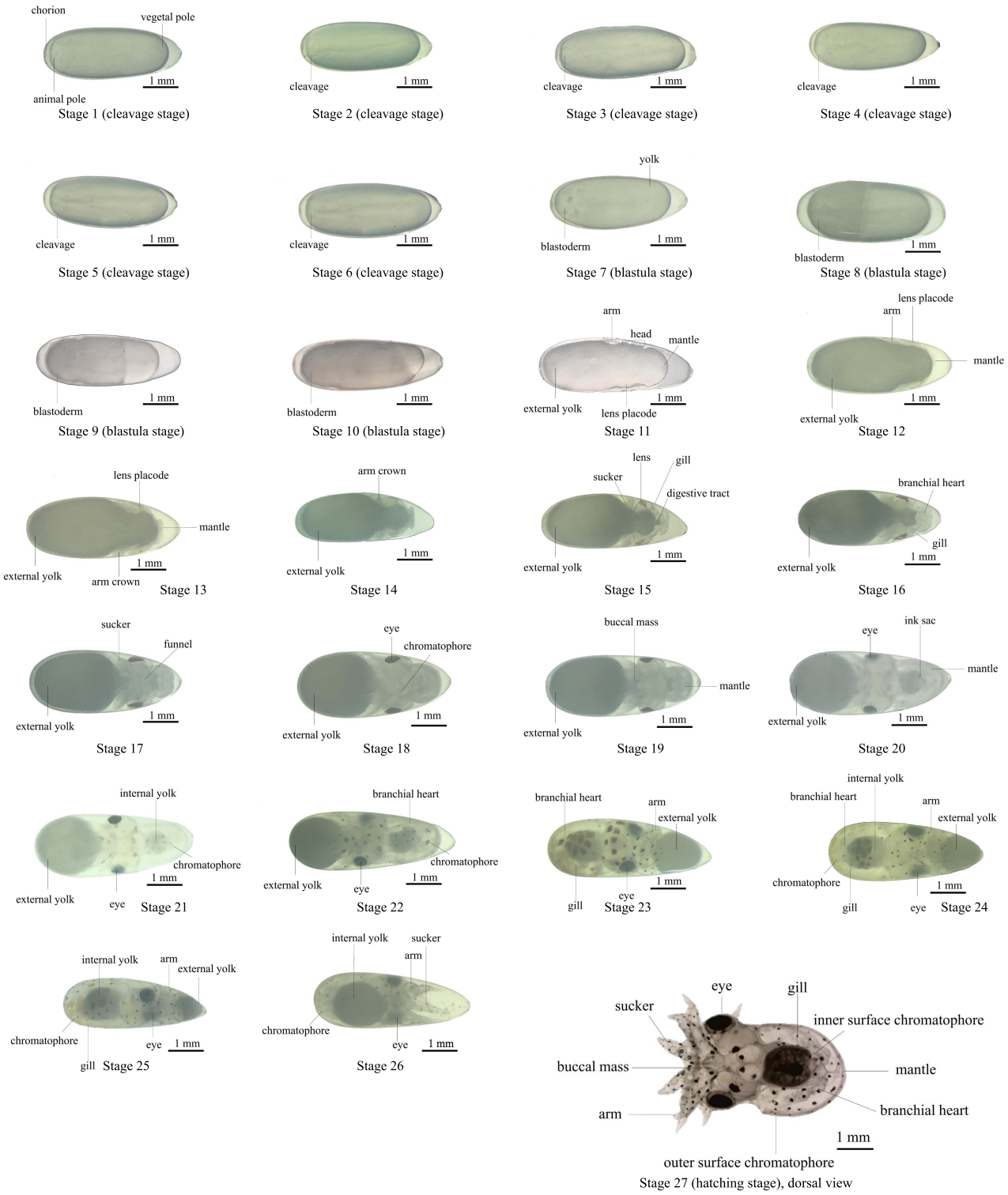

Fig. 3 Embryonic development stages of *A. aegina*.

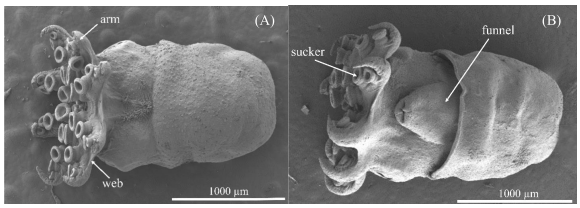

Fig. 4 External morphology of *A. aegina* paralarva: (A) dorsal view; (B) ventral view.

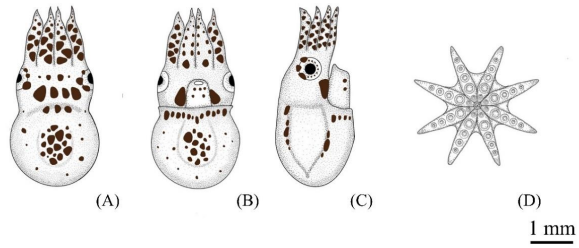

Fig. 5 Chromatophore patterns of hatching stage: (A) dorsal view; (B) ventral view; (C) lateral view (eye); and (D) oral side of arms.

of the mantle, there are 14–18 small regular chromatophores in a single row. There are two large, reddish brown, triangular chromatophores on the ventral side of the head. Each of the eyes has 8–10 chromatophores on the inner surface layer. On the funnel, chromatophores appear in two rows, one with 3 chromatophores and the other with 2. The oral side of each arm has 6–8 small, red chromatophores in 2 rows from the arm base to the arm tip.

DISCUSSION

Eggs of incirrate octopuses (including the family Octopodidae) are similar and look like a sausage or a pear. The egg capsules are covered by chorion membrane to protect the embryos. Normally, the eggs differ in size according to the species [4, 6–8] (Table 3).

Cephalopod development is intrinsically linked to the seawater temperature with increases in mortality and abnormal development at high temperatures [13].

Table 3 Egg sizes of common and commercial Octopodidae species found in Southeast Asia.

Species	Egg length (mm)	Reference
Amphioctopus aegina	2.64 ± 0.13	This report
Amphioctopus aegina	2.40	[2]
Amphioctopus marginatus	3.00	[9]
Amphioctopus rex	3.00	[10]
Amphioctopus siamensis	1.70	[10]
Argonauta hians	1.06 ± 0.11	[11]
Callisoctopus luteus	1.00	[12]
Cistopus indicus	4.50	[12]

Within each species-specific range, temperature is the main factor regulating the development of octopus embryos, which is faster at higher temperatures. Octopus species with small eggs have the fastest development and are found in the tropical-temperate zone [8, 14]. In this study, the embryonic development of sandbird octopus has 27 observed stages and lasted 18–22 days at 28.0 °C. The embryonic development period in *Octopus cyanea* at 27.1 °C is 21 days [15]. In *Octopus oliveri*, the development period is 38 days at 25.14 ± 0.4 °C [16]. *Hapalochaena maculosa* hatches in 25–35 days at 21.0–22.5 °C [17], and *Octopus vulgaris* from Spain hatches in 25 days at 18.0 °C [18]. In contrast, cold-adapted species have a much longer embryonic development. For example, *Octopus bimaculoides* (in Mexico) hatches in 65 days at 18.0 °C [19], and *Eledone cirrhosa* hatches in 90–120 days at 14.0–18.0 °C [20]. The embryonic development period in *Enteroctopus dofleini* at 9.2–13.9 °C is 161 days [8, 21]. The family Octopodidae is the largest contributor to the planktonic octopod fauna. Hatchlings of planktonic paralarvae measure 0.8–2.0 mm in mantle length [8] and are characteristically covered with Kölliker's bristles. The mantle is generally short and round. The mantle length: arm length ratio is typically between 2:1 and 3:1. Muscles in the paralarvae are clear and transparent, and the chromatophore pattern can be used to provide a quick and relatively easy means of visually identifying the species [6, 8].

Sandbird octopus paralarvae have a stubby, round mantle; and their arms are subequal in length. Tegumental chromatophores cover the dorsal and the ventral sides and occur in small triangular, square, or round shape. Arm chromatophores are small and occur in two rows in a zigzag pattern, and there are 5 suckers on each arm in a single row. According to a study of commercially important cephalopods in Thailand [22], this species has a short and oval mantle with medium sized head, which is 56% of the mantle length. Chromatophores are spread across the body, the head, and the arms. Although the chromatophores on the dorsal side are smaller, the numbers on both the dorsal and the ventral sides are similar [22, 23]. The present study revealed many characteristics of the life history of *A. aegina* that differ from other octopus species studied so far [2], including its brooding behavior and embryonic developmental. More studies are required to rear the paralarvae, especially to determine their prey during the planktonic phase. This will be useful for octopus aquaculture in the future.

CONCLUSION

In the present study, female sandbird octopuses brooded eggs in their arms and did not feed until the paralarvae hatched. Eggs occurred in clusters and were non-adhesive with an average size of 2.64 ± 0.13 mm in length and 0.94 ± 0.06 mm in width. The embry-

onic period was divided into 27 stages, and hatching occurred 18–22 days (528 h) after spawning at 28.0 °C. The hatchlings were planktonic, and their average size was 3.40 ± 0.24 mm in total length. Morphological characteristics of *A. aegina* included a stubby oval mantle and subequal arms in length. Each arm had 5 suckers arranged in a single row. The dorsal head had four reddish brown chromatophores arranged in two to three rows. On the mantle's inner surface layer, 8–19 chromatophores formed a diamond shape. On the ventral side of the head, there were two large and reddish-brown chromatophores at the side of the funnel (Table S1).

Appendix A. Supplementary data

Supplementary data associated with this article can be found at http://dx.doi.org/10.2306/scienceasia1513-1874.2022.057.

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Appendix A. Supplementary data

Table S1 Number and position of chromatophores at hatchling stage.

Body part	Side	Number of chromatophores
Arms	Dorsal	5–8 in two rows
	Ventral	6–8 in two rows
Head	Dorsal	4 in 2-3 rows
	Ventral	2 (side of funnel)
Eyes	Dorsal (outer surface layer)	3
	Ventral (inner surface layer)	8–10
Mantle – inner surface layer	Dorsal	8–19
	Ventral	10–12
Mantle – outer surface layer	Dorsal	23–32
	Ventral	19–30
Anterior margin of ventral mantle		14–18
Funnel		5 in two rows