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Egg colony and early development of *Pterosagitta draco* (Chaetognatha) collected from Kuroshio front

TAISHIN SHIMOTORI[1], TAICHIRO GOTO[1] & MAKOTO TERAZAKI[2]

[1]*Department of Biology, Faculty of Education, Mie University, Tsu, Mie 514, Japan*
[2]*Ocean Research Institute, University of Tokyo, 1–15–1 Minamidai, Nakano-ku, Tokyo 164, Japan*

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Abstract: On 31 May 1996, an egg colony packed in a gelatinous mass was found in a plankton sample collected from the surface layer at a station (36°32'N, 143°22'E) where the Kuroshio front was formed. The eggs were in the process of development and it was evident from the cleavage pattern that they were chaetognath eggs. Considering the formation of the gelatinous mass covering the eggs and the structure of the juveniles, we identified the eggs as those of *Pterosagitta draco*. We followed their development and examined details of the juveniles up to 5 days after hatching. The egg colony was irregular in shape and was 20 mm along its major axis, 15 mm along its minor axis and 5 mm deep. It consisted of approximately 200 eggs and the egg diameter was 300 μm. The embryos were in the blastula stage when we began observation. Body elongation and hatching occurred at 6 h and 30 h after the beginning of observation, respectively. The newly hatched young were 1.24 mm in body length. The newly hatched young remained in the gelatinous material for 4 days after hatching and then moved out into the surrounding sea water. Remarkable growth occurred on the first day after hatching when the body length became 1.61 mm (1.3 times that of the previous day). The pigmented spot in the eye appeared and the lateral and tail fins developed. Two days after hatching, hooks appeared and the caudal transverse septum was in the progress of formation. Two pairs of primordial germ cells were found in the lateral portion of the septum. Three days after hatching, the hooks were fully developed and the mouth was clearly observable. The caudal transverse septum was completely formed, and the primordial germ cells and anus were readily evident. Three paired processes on the lateral side of the trunk, which are regarded as a defining character in *P. draco*, developed. The results are discussed in comparison with the first description of a *P. draco* egg colony.

Key words: *Pterosagitta draco*, chaetognath, arrowworm, egg colony, early development

Introduction

Pterosagitta draco is an oceanic, epiplanktonic, cosmopolitan species of warm temperate regions. In the Pacific, *P. draco* has been observed from 40°N to 45°S in one report (Alvariño 1964) and from about 42°N in the western Pacific, 28°N on the eastern side, to about 30°S in

another (Bieri 1959). However, the species indeed does extend, on the north-eastern side of the Pacific, to the latitude reported by Alvariño according to her data from ten cruises in that region. The species has also been reported from the Sea of Japan (Furuhashi 1953; Terazaki 1993), the Seto Inland Sea (Murakami 1959), Suruga Bay (Nagasawa & Marumo 1982), Sagami Bay (Nagasawa & Marumo 1977), Otsuchi Bay (Terazaki & Marumo 1982) and a Kuroshio warm-core ring (Terazaki 1992). Of particular interests is Mori & Kuroda's study (1983) in which they reported the distribution pattern of *P. draco* in the waters around the Izu Islands, south of Japan. The mean density of this species during the monthly observations was high in June, July, November and December, and low from February to April. This species was distributed mainly in the offshore area south of the 15°C isotherm at 200-m depth. According to Mori & Kuroda (1983), the body length of each maturity stage was as follows; 0-stage (juvenile): 2.7–6.3 mm; I-stage: 4.9–7.7 mm; II-stage: 6.0–8.3 mm; III-stage: 5.9–9.6 mm; and IV-stage (spawning group): 6.2–8.1 mm. The mean number of eggs in the ovary was 31, ranging from 21 to 46, and their diameter ranged from 0.13 to 0.30 mm. The spawning season of *P. draco* in the waters around the Izu Islands is April to December, chiefly from June to August.

In the present study we describe the eggs and early development of *P. draco*. The eggs were found in a plankton sample collected from the Kuroshio front during Cruise KH–96–2 of the R/V *Hakuho Maru*. The collection of eggs and early development have been reported by Sanzo (1937) who first found an egg colony in a gelatinous mass in a sample in the Straits of Messina in the Mediterranean Sea. However, eggs have not been recorded to date in samples from near Japan.

Materials and Methods

On 31 May 1996, adults of *Pterosagitta draco* and an egg colony packed in a gelatinous mass were found in a plankton sample collected from the surface layer at Stn K 14 (36°32′N, 143°22′E) at night, using an ORI net (Omori 1965) with a 0.69-mm mesh size during Cruise KH–96–2. Water temperature and salinity were monitored with a conductivity-temperature-depth measurement system (CTD) over a 0–500 m depth range.

The adults of *P. draco* were fixed with 4% paraformaldehyde in 0.1 M cacodylate buffer (pH 7.4) containing 0.4 M sucrose.

The egg colony was placed in a dish (12 cm in diameter) and the development of the eggs was observed at room temperature (22°C) and photographed under a stereoscopic microscope with a Fujichrome–100 film in the laboratory of the R/V *Hakuho Maru*.

Several of the newly-hatched individuals were fixed every day up to 4 days after hatching with the same fixative described above. They were observed under a conventional light microscope and drawn with a camera lucida. To observe the primordial germ cells, young chaetognaths were stained with trypan blue. They were also observed under a microscope equipped with Normarski optics and photographed with a Kodak Tri–X film.

Results

Five adults of *Pterosagitta draco* were collected from the surface layer. Body length ranged from 5.5 to 8.2 mm. One of the specimens had mature eggs in oviducts (Fig. 1a). The eggs in the oviducts were arranged in two linear rows at the dorsal and ventral sides. The number of eggs in each row was 20. Thus the total number of eggs was about 80.

An egg colony packed in a gelatinous mass was found in a sample collected at 2200 h on 31 May 1996 at Stn K 14, where the Kuroshio front was formed (Sainz-Trapaga, pers. comm.). Surface water temperature and salinity at the station was 17.8°C and 34.37 PSU, respectively. The gelatinous mass was irregular in shape and was 20 mm along its major axis, 15 mm along its minor axis and 5 mm deep. The egg colony consisted of approximately 200 eggs and the egg diameter was 300 μm. A part of the colony is shown in Fig. 1b. The egg was almost completely transparent as in other *Sagitta* species. The embryos were in the blastula stage when we began observation (2400 h). Body elongation and hatching occurred 6 h and 30 h after the beginning of observation, respectively. Figure 1c is an example showing elongated embryos of 17 h after the beginning of observation. Hatching occurred synchronously and hatchability was more than 90% (Fig. 1d). Newly hatched young individuals moved weakly within the

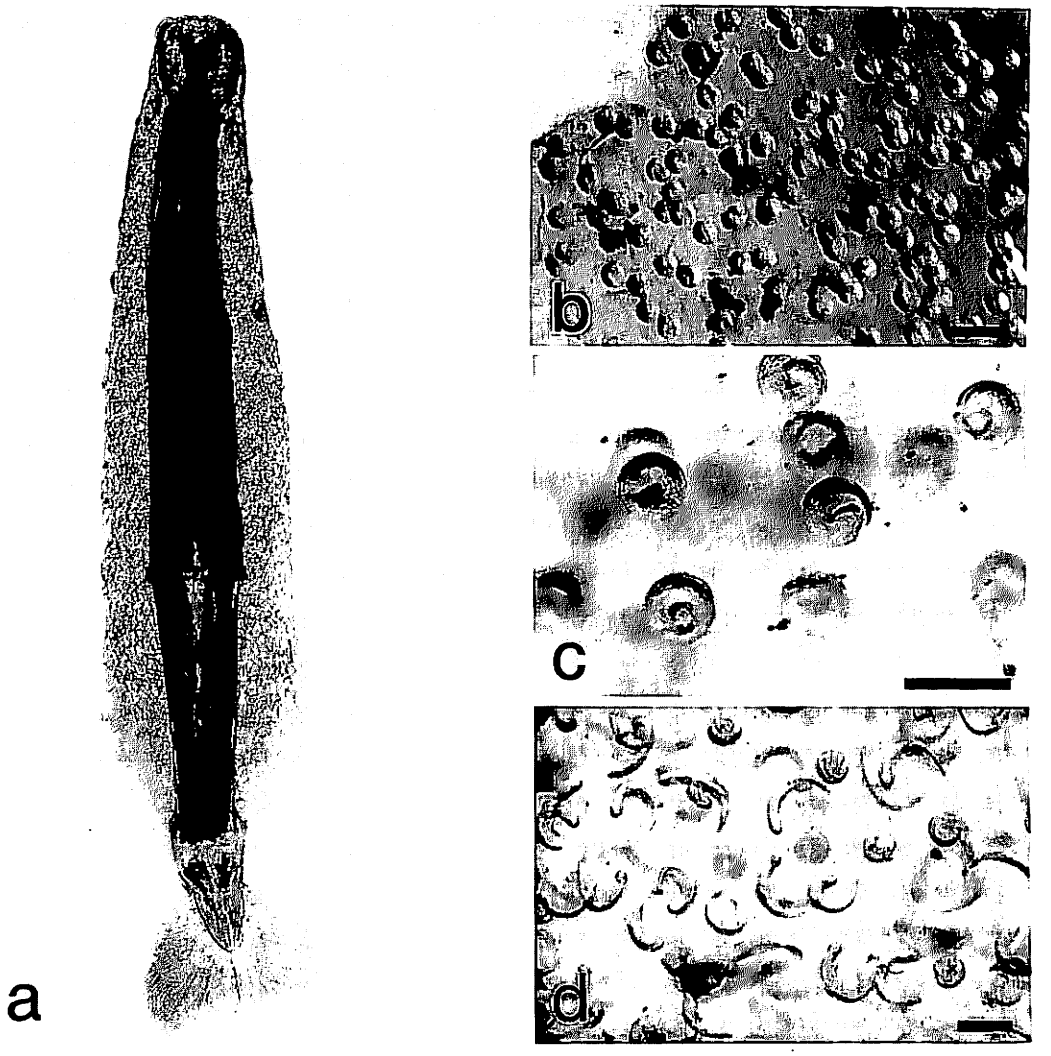

Fig. 1. **a.** Adult of *Pterosagitta draco* (fixed preparation); body length is 8.2 mm. **b.** Egg colony of *P. draco*; eggs are packed in a gelatinous mass. **c.** Embryos at body elongation. **d.** Newly hatched young *P. draco* in the gelatinous mass. Bar in b=1 mm, bars in c and d=0.5 mm.

Table 1. Early development of *Pterosagitta draco*.

days after hatching	body length	tail ratio	hooks	eye pigment	primordial germ cell	caudal transverse septum
0	1.24	—	absent	absent	unclear	absent
1	1.61	—	absent	present	unclear	absent
2	1.73	0.37	present	present	two pairs	appeared
3	1.93	0.36	present	present	two pairs	present
4	2.00	0.34	present	present	two pairs	present
5	2.23	0.34	present	present	two pairs	present

gelatinous mass and were unable to emerge easily from it. It took about one day for some of the young to reach the outside. Even 4 days after hatching more than half of the young remained within the gelatinous mass. The newly hatched young were 1.24 mm in body length. The young floated in sea water and their movement was very weak.

Several of the young individuals were fixed every day for further observation. Morphological characteristics during growth were noted, especially the developmental stage of the fins, hooks, eyes, the caudal transverse septum, and the primordial germ cells (Table 1). Figure 2 shows the appearance of the newly hatched young through to 5 days after hatching. The newly hatched young had no eyes or hooks (Fig. 3a), only the tail fin (Fig. 2a). The caudal transverse septum was not yet formed and it was difficult to recognize the primordial germ cells. Remarkable growth occurred in the first day after hatching when the body length became 1.61 mm (1.3 times that of the previous day). The pigmented spot in the eye appeared (Fig. 3b) and the lateral and tail fins developed (Fig. 2b). The caudal transverse septum was not yet formed and primordial germ cells were undetectable. Two days after hatching, hooks appeared (Fig. 3c) and the caudal transverse septum was in the process of formation. Two pairs of primordial germ cells were found in the lateral portion of the septum (arrowheads in Fig. 4a). Three days after hatching, the hooks developed and the mouth was clearly observable (Fig. 3d). The caudal transverse septum was completely formed, and the primordial germ cells and anus were readily recognizable (Fig. 4b). The primordial germ cells were separated into the anterior and caudal sections by the septum. Body length was 1.93 mm and the percentage of the body length comprised by the caudal section was 36%. Three paired ciliary fence organs developed on the lateral side of the body (arrowheads in Fig. 5a). The ciliary fence was covered with a thin layer which we regarded as a collarette (Fig. 5b). Young individual 5 days after hatching had fully developed hooks of total number 10 (Fig. 3f). The shape of the pigmented spot in the eye was similar to that seen in the adult (Fig. 3e). The number of primordial germ cells did not increase (Fig. 4c). Survival rate was more than 90% up until 5 days after hatching.

Discussion

Record of Egg Colony of Pterosagitta draco

The egg colony of *Pterosagitta draco* has been recorded for the first time in plankton samples taken near Japan. There is only a single report dealing with the eggs of this species, written by Sanzo (1937), who first found an egg colony in a gelatinous mass in a sample from the Straits of Messina in the Mediterranean Sea in 1917. It looked like a fish egg colony but he

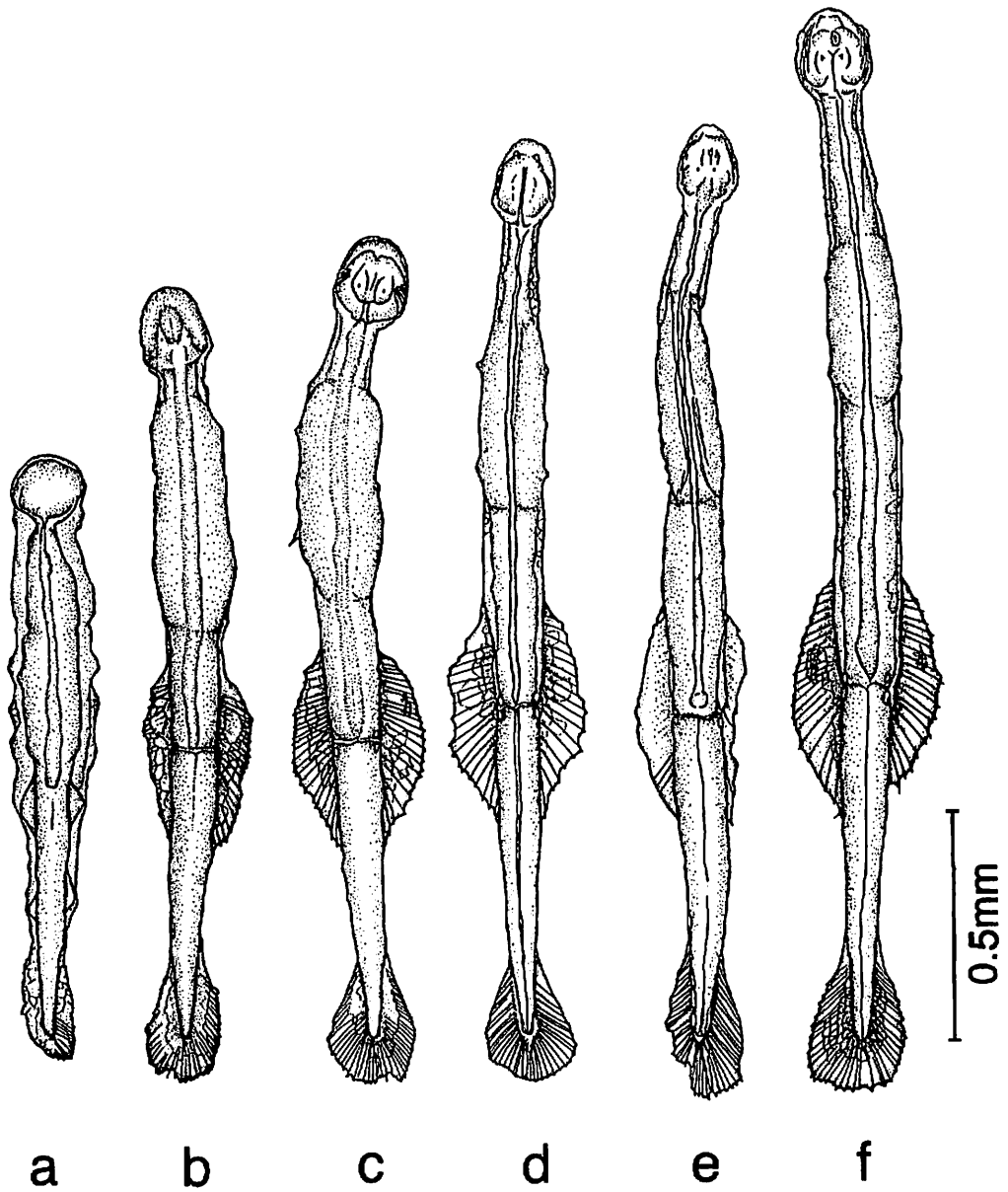

Fig. 2. Young individuals of *P. draco*. **a.** Newly hatched young. **b.** One day after hatching. **c.** Two days after hatching. **d.** Three days after hatching. **e.** Four days after hatching. **f.** Five days after hatching.

found that arrowworms hatched out. He collected other egg colonies in February 1936 and observed the development and growth of the arrowworms. As described below, he concluded that the egg colony was produced by *P. draco* according to the morphological characteristics of the juveniles. As the egg colony collected in the present samples was similar to the description by Sanzo, we identified it as that of *P. draco*. Mature individuals of *P. draco* were also collected in the present samples. The other chaetognath collected was *Sagitta nagae* and some of these were mature individuals. However, this species does not lay eggs as a colony. These facts also support our view that the egg colony was derived from *P. draco*.

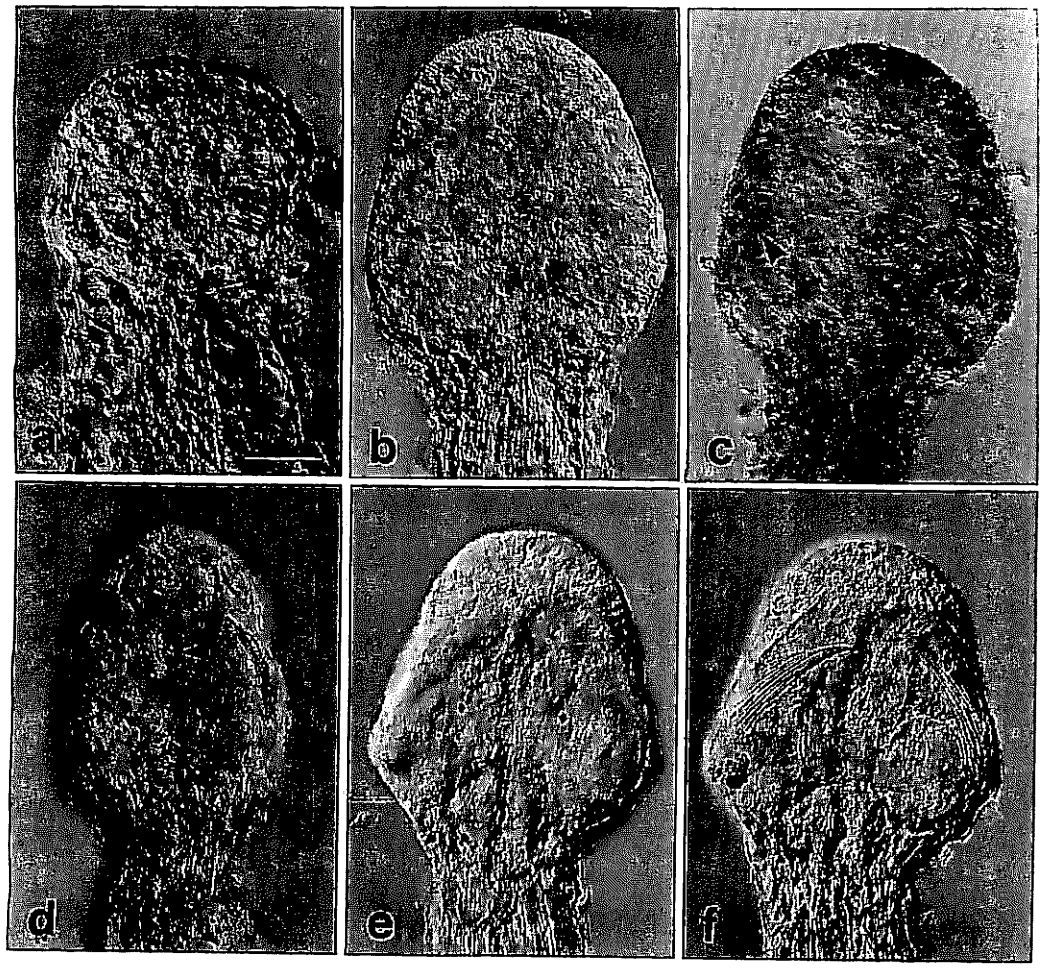

Fig. 3. Head regions of young *P. draco*. **a.** Newly hatched young (dorsal view). **b.** One day after hatching (dorsal view). **c.** Two days after hatching (ventral view); *arrowheads* indicate hooks. **d.** Three days after hatching (ventral view); an *arrowhead* indicates the mouth. **e.** Five days after hatching (dorsal view). **f.** Five days after hatching (ventral view). Bar=50 μm.

P. draco has a thick collarette layer which is very fragile, causing external structure of collected individuals to be extremely damaged in most samples. The present sample, which contained adult *P. draco* and the egg colony, was collected by towing a net in the surface layer at 1-m depth for 2 min, and this ensured *P. draco* (Fig. 1a) and the fragile egg colony (Fig. 1b) were collected in good condition.

Though the egg colony and adults of *P. draco* were collected at Stn K 14, *P. draco* was rare in this area. The dominant chaetognath species at the sampling station was *Sagitta nagae* (Terazaki, unpublished data).

Size of Egg Colony

The egg colony collected by Sanzo was oval with dimensions of 6–8 mm×5–7 mm× 0.6 mm deep. There were 200–300 eggs in the colony and the egg diameter was 0.36 to 0.4 mm (Table 2). This size was rather smaller than ours, but it is interesting that the number of eggs contained was similar. The gelatinous mass might be produced during egg laying by a

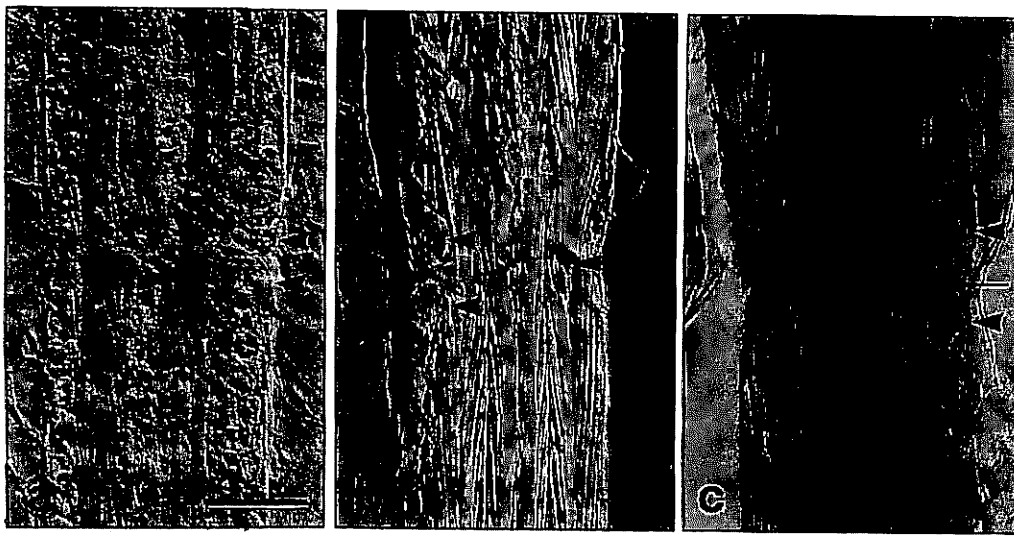

Fig. 4. Primordial germ cells (*arrowheads*) located near the transverse septum (*arrows*) between the body and tail. **a.** Two days after hatching. **b.** Three days after hatching. **c.** Five days after hatching. Bar=50 μm.

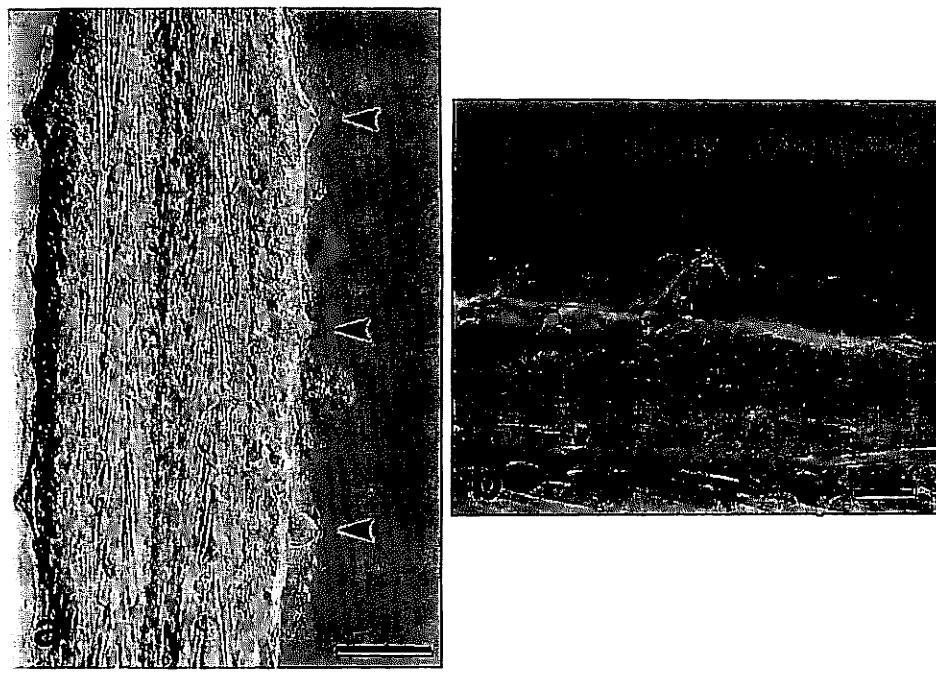

Fig. 5. Ciliary fence organs appeared in young at three days after hatching. **a.** Three paired ciliary fence organs on the body (*arrowheads* indicate the organs on the right side). **b.** View of one of the ciliary fence organs at higher magnification. Bars in a=50 μm, bar in b=20 μm.

Table 2. Principal differential chatacteristics of the egg colony and early development of *Pterosagitta draco* compared with the report by Sanzo (1937).

	present study	Sanzo (1937)
sampling season	May	February
sea water temperature at the sampling station	17.8°C	—
temperature at the observation	22°C	—
shape of the egg colony	irregular	oval
size of the egg colony	20 mm×15 mm ×5 mm deep	6–8 mm×5–7 mm ×0.6 mm deep
number of eggs in a colony	200	200–300
size of eggs	0.3 mm	0.36–0.4 mm
time to hatch out	less than 40 h	3–4 days
size at hatch out	1.24 mm	1.4 mm
time to reach 2 mm in length	4 days after hatch out	8–9 days after hatch out
appearance of eye pigment	1 day after hatch out	11 days after hatch out
appearance of hooks	3 days after hatch out	8–9 days after hatch out
formation of transverse septum	3 days after hatch out	8–9 days after hatch out

secreted substance which is hydrated to form gelatinous material. The difference in size between Sanzo's preparation and ours may be due to variations in this kind of substance.

Duration from Egg Laying to Hatching

The early development of chaetognaths including time courses have been reported for a few species (Murakami 1959; Kuhl & Kuhl 1965). It takes 3.5 h to reach the blastula stage at 16–18°C in *Sagitta crassa* (Murakami 1959). In *Paraspadella gotoi* it is 5 h at 23°C and 10 h at 17°C (Goto, unpublished data). Duration from egg laying to hatching is 24 h at 16–18°C in *Sagitta crassa* (Murakami 1959) and 2 days at 23°C and 4 days at 17°C in *Paraspadella gotoi* (Goto, unpublished data). In *P. draco* the duration from blastula to hatching was 30 h at 22°C. This time course was earlier than that of *P. gotoi* at 23°C. Therefore, it would be less than 40 h from egg laying to hatching at 22°C. The developmental stage of the eggs collected by Sanzo (1937) was gastrula and they took 3–4 days to hatch of an unknown temperature (Table 2). As described below, it took longer for the eggs proceed to the next stage in Sanzo's description, suggesting that the eggs were incubated in colder conditions. His observation was made in February, so ocean sea water temperature and laboratory room temperature was probably colder than that in the present study.

Morphological Characteristics of Young Pterosagitta draco

Growth of young *P. draco* as described by Sanzo (1937) is as follows (Table 2). The size of newly hatched young was 1.4 mm in length and 0.24 mm in width. Three pairs of processes were evident on the lateral sides of the body. The young remained in the gelatinous material for 3–4 days after hatching and then emerged into the surrounding sea water. The young at 8–9 days after hatching reached 2 mm in length and developed with hooks. Lateral fins were not developed but the body was divided into three portions; head, trunk, and tail. The young at 11 days after hatching were 2.16 mm in length and the eyes, anus, and lateral fins appeared at this stage. Twenty days after hatching they developed a pair of bristles, which is a defining character in *P. draco*. Further development was unable to be followed.

In contrast to Sanzo's description, the present study describes an earlier appearance of the eye in *P. draco* (Table 2). From observations on *Sagitta* and *Spadella*, the appearance of the eye is followed by that of the hooks (cf. Goto et al. 1992). Three pairs of processes on the lateral sides of the trunk (Fig. 5), which are not found in *Sagitta* and *Spadella*, are regarded as a defining character for *P. draco*, as suggested by Sanzo (1937).

Problem on the Number of Eggs

As Sanzo (1937) pointed out, it would be expected to be difficult for mature individuals of *P. draco*, which is just 10–11 mm in length, to lay 200–300 eggs. In order to produce this number of eggs, body length would have to be at least 20 mm. In the present study, we obtained an adult specimen containing mature eggs in its oviducts and the total number of eggs it carried was only 80. The egg colony was produced by a single egg laying and never thought to be the result of successive egg layings. Because, in chaetognaths, mature eggs to be laid are produced at intervals of several days and fertilization occurs internally. The fertilized eggs in the oviducts are laid by a single egg laying and there are intervals between egg layings (cf. Goto 1995; Goto & Yoshida 1997). Synchronization of developing embryos in the egg colony is a result of a single egg laying. The contradiction on the number of eggs in the egg colony and that in a mature individual of *P. draco* should be resolved by further study.

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