Studies on Japanese Compound Styelid Ascidians IV. Three New Species of the Genus *Botrylloides* from the Vicinity of Shimoda

By

Yasunori Saito[1] and Hiroshi Watanabe

Shimoda Marine Research Center, The University of Tsukuba, Shimoda, Shizuoka 415, Japan

With Text-figures 1–9, Table 1

In 1981, we reported three new species of botryllid ascidians and redescribed *Botrylloides violaceus* Oka, 1927 in two papers. In those papers, we pointed out that there had been some confusion in the taxonomy of botryllid ascidians, and proposed the necessity of the observations on the details of their life histories to avoid the confusion in the taxonomy.

Really, most of Japanese botryllids seem to resemble one another in morphology of their colonies and zooids. Therefore, it is difficult to distinguish respective species exactly on the preserved specimens that are devoid of gonads in zooids. Thus, we have cultured several botryllids collected from the vicinity of the Shimoda Marine Research Center, the University of Tsukuba, all through the year in the cove near the Center, and made observations on the details of their life histories. This work has enabled us to classify them into some distinct species inclusive of new species.

In this paper, we are going to present three new species of the genus *Botrylloides* and to describe their morphology and life histories in detail. Further, discussions in comparing these three new species and the two already established species, *B. violaceus* and *Botrylloides simodensis* Saito et Watanabe, 1981, one another will be made at the end of the paper to see the taxonomic significance of especially the manner of the sexual reproduction in botryllid ascidians.

The type-series are deposited at the Shimoda Marine Research Center, the University of Tsukuba, Shimoda. This article is Contributions from the Shimoda Marine Research Center, No. 449.

Before going further, we want to present our sincere thanks to Dr. Takasi Tokioka, Professor Emeritus of Kyoto University, and Mr. Teruaki Nishikawa of the College of General Education, Nagoya University, for their suggestions and advices in taxonomy.

1) Present address: Hopkins Marine Station, Stanford University, Pacific Grove, California 93950, U.S.A.

Publ. Seto Mar. Biol. Lab., 30(4/6), 227–240, 1985. (Article 10)

Botrylloides lentus n. sp.

(Figs 1–2)

Type-series. A large colony, which was collected from a tide pool facing the open sea at the Noroshi Point near the Center and has been reared in a box kept immersed in Nabeta Bay, is designated as the holotype (K. Hashimoto *leg.*, May, 1979). Another colony collected from the same locality is designated as paratype (Y. Saito *leg.*, July, 1983). Type locality: Shimoda, Japan.

Description: Colonies are usually found encrusting the surface of rocks at about 1 meter depth in that tide pool, and sometimes attain a large size, about 20 cm across; usually 3.0–3.5 mm in thickness, though sometimes more than 4 mm.

The colony surface is generally flat and free from any foreign matter. The test is soft gelatinous and translucent. When alive, colonies are colored orange. The periphery of the colony is fringed with sausage-shaped vascular ampullae about 1000 μm long and 150 μm wide.

Blastozooids (Fig. 1a) are arranged in ladder systems with several common cloacal apertures, and always connected one another by common vascular vessels. They are 3.1–3.5 mm in length and situated almost standing vertically, though obliquely in the peripheral portion. Branchial tentacles consist of four larger and

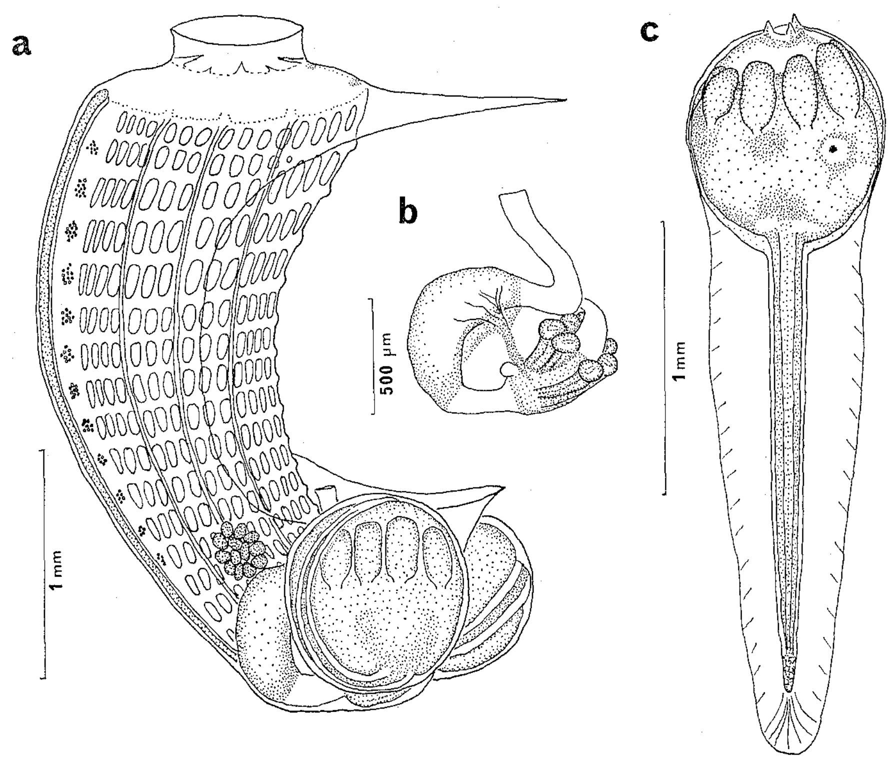

Fig. 1. *Botrylloides lentus* n. sp. a, a zooid, from left side. b, a digestive organ of a zooid. c, a larva, from left side.

four smaller ones regularly alternating, additionally with one or two minute ones in some interspaces. There are 18 rows of stigmata on each side in zooids of a fully grown colony, though only 15–16 rows are counted in zooids of some young colonies. The second stigmatal row never reaches the dorso-median line. Around the middle of the branchial sac, stigmata are arranged between the three inner longitudinal bars as follows: dorsal lamina 4·3·3·4 endostyle. Many blood cells are deposited along each side of the endostyle in the range from the second to the twelfth or thirteenth stigmatal row. The anterior edge of the intestinal loop attains anteriorly the level of the fifteenth stigmatal row and the anus opens at the level of the thirteenth row. The most part of the stomach is exposed posterior to the rear end of the branchial sac. The stomach (Fig. 1b) is orange in fresh specimens and furnished with nine longitudinal plications and a small pyloric coecum. The pyloric corner of the stomach, at just the base of the coecum, is connected to circum-intestinal gland region by a thick duct.

The testis is situated anteriorly to the ovary along the anterior edge of the circum-intestinal gland region on the left side of the body and at the level of the fifteenth to eighteenth stigmatal row on the right side. It consists of 12–15 round lobes gathered in a rosette, and is colored grayish. Usually a single egg is matured on each side; it is orange and attains approximately 300–330 μm in diameter just before ovulation. The incubatory pouch is a sac-like outgrowth of the bodywall formed near the ovary and the development of fertilized eggs takes place within it. The embryo in the pouch grows to about 1000–1100 μm in diameter.

The larva (Fig. 1c) is about 2.5–2.7 mm in total length and orange in color when alive. The trunk is about 800 μm long, oval in outline and includes a single photolith as typical to botryllids; three attachment processes are arranged in a triangle at the anterior edge of the trunk and eight ampullae are surrounding the anterior half of the trunk; neither branchial nor atrial aperture is open yet.

Life history: The asexual peribranchial budding can be observed all through the year; usually a single bud is produced on each side of the body. The intervals between the alternation of generations are usually 7–9 days, though may be prolonged to 10–12 days when zooids are keeping embryos in their pouches. The sexual reproduction is observed in July to August, but an exception of a colony that released larvae in December, 1982.

The ovarian egg in buds will be ovulated soon after the buds are grown to be adult zooids taking the place of their degenerated parent zooids. The egg at this time is about 300–330 μm in diameter. The ovulated egg goes into the incubatory pouch and there the embryogenesis is started. About 2 days after ovulation the embryo starts gastrulation, and 4 days after that it starts to elongate the tail. By this stage, the embryo increases its size up to 500 μm in diameter. Seven days after ovulation the tail formation is completed, when the embryo has reached already the size of about 800 μm in diameter. Finally the embryo grows up to the full grown larva of a size 1000–1100 μm in diameter about 10–12 days after ovulation.

Full grown larvae are pushed out from their incubatory pouches by the

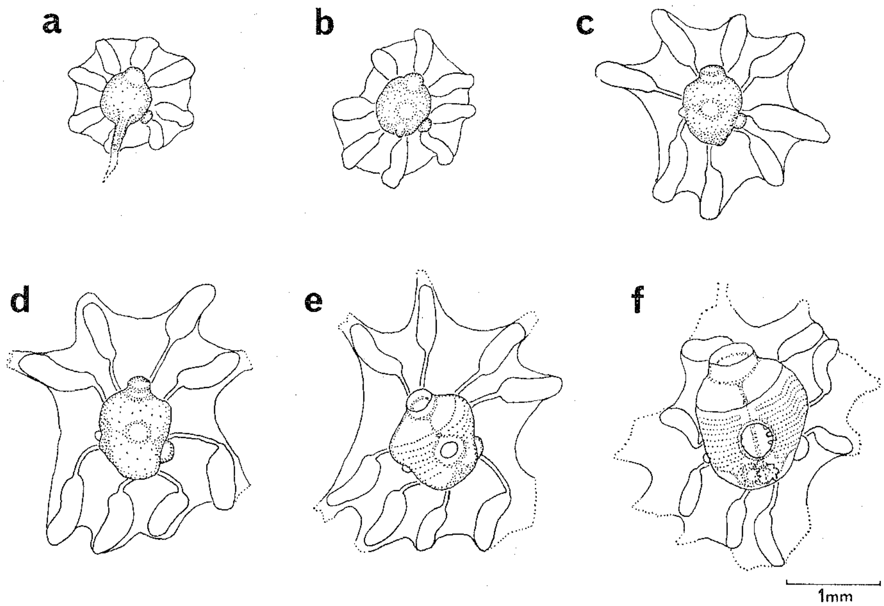

Fig. 2. *Botrylloides lentus* n. sp. Stages in metamorphosis from larval attachment to a functional oozooid. a, an oozooid, 0.5 hours after larval attachment. Tail absorption in progress. The first bud recognized on the right side of body. b, the same, 2 hours after attachment. Tail absorption almostly completed. c, the same, 8 hours after attachment. A small bud appearing on the left side of body. d, the same, 20 hours after attachment. e, the same, 30 hours after attachment. Both branchial and atrial siphons open, then completion of a functional oozooid. f, the same, 3 days after attachment.

contraction of the pouch itself just before the degeneration of their parent zooids. About 15 min. after release, the larvae expand their tails and swim out from the common cloacal aperture of their parent colony. The larval release is not synchronized in a colony; it takes more than 12 hours to release all larvae within a colony. Three to 6 hours after liberation, larvae become attached to the substratum by extension of eight ampullae, and the metamorphosis (Fig. 2) is started. As the tail absorption is proceeding, the outer layer of larval test is taken off as pointed out by Oka (1960). The first bud always appears on the right side of the body of each oozooid soon after larval attachment, and 8 hours later another bud appears on the left side. Both branchial and atrial apertures open about 30 hours after attachment. This functional oozooid lives for about a week.

Remarks. Colonies of the present species are found limitedly at the Noroshi Point near the Shimoda Marine Research Center, but none has ever been found at any other parts in the vicinity. The external appearance of living colonies very closely resembles those of *B. fuscus* which will be stated later and *B. violaceus*. However, the colonies are usually thicker in this species than in latter two, hence we prefer at present to treat this specimen under a specific name, *B. lentus*. As to the life history including the morphology of zooid, see Table 1.

Botrylloides fuscus n. sp.

(Figs 3–5)

Type-series. Two colonies collected from rocks in the intertidal zone of Shimoda are designated as the holotype and paratype (Y. Saito *leg.*, September, 1982). Type locality: Shimoda, Japan.

Description: Colonies are usually encrusting and sometimes reaching a large size about 10 cm across. They are 2.5–3.0 mm in thickness, sometimes attaining more than 3.0 mm. The colony surface is flat and free from any foreign matter. The test is soft gelatinous and translucent. When alive, the colonies are colored dark red, red brown or dark brown as a whole. In addition, yellow or white pigment cells are sometimes deposited around the branchial siphon and on the atrial languet of respective zooids. Thus, somewhat different color patterns may appear on the colony surface. The periphery of the colony is fringed with sausage-shaped vascular ampullae about 1000 μm long and 200 μm wide.

Zooids (Fig. 3a) are arranged in ladder systems with several common cloacal apertures, and always connected one another in a common vascular system. They are 2.2–2.7 mm in length and nearly standing vertically, though obliquely in peripheral parts. Branchial tentacles consist of four larger and four smaller ones, regularly alternating. There are usually nine rows of stigmata on each side, though the zooids in younger colonies are provided with seven or eight rows on each side. The second row never reaches the dorso-median line. Around the middle of the

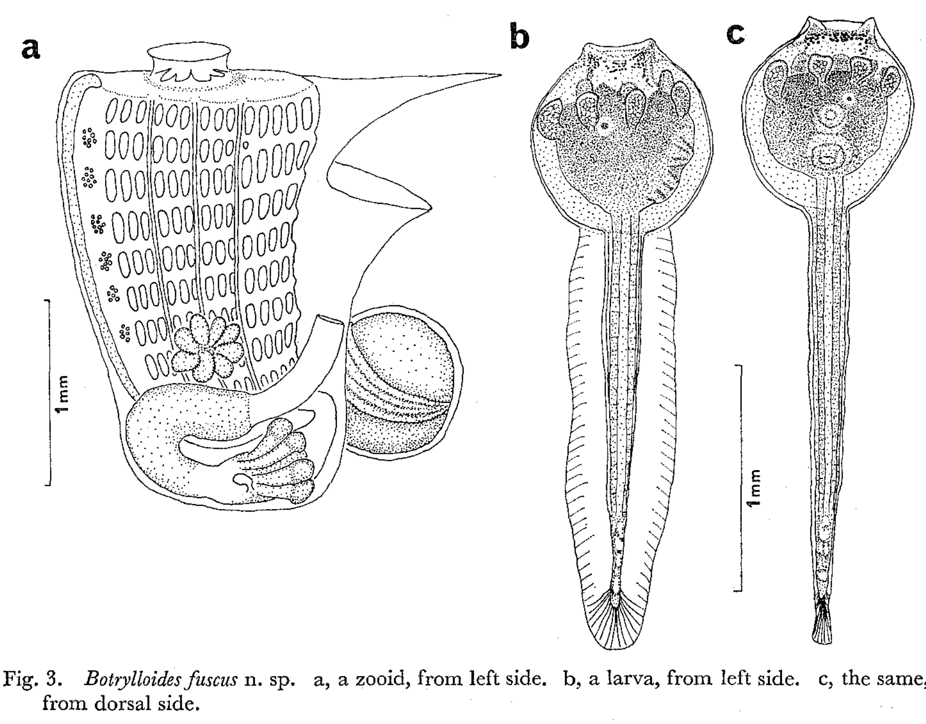

Fig. 3. *Botrylloides fuscus* n. sp. a, a zooid, from left side. b, a larva, from left side. c, the same, from dorsal side.

branchial sac, stigmata are arranged between the three inner longitudinal bars as follows: dorsal lamina 5·3·2–3·2–3 endostyle. Many blood cells are deposited along each side of the endostyle in the range from the second to the seventh stigmatal row. The anterior edge of the intestinal loop attains anteriorly the level of the eighth transverse vessel and the anus opens at the level of the seventh transverse vessel. The most part of the stomach is exposed posterior to the rear end of the branchial sac. The stomach is orange in fresh specimens and furnished with eight longitudinal plications and a small pyloric coecum.

The testis is situated anterior to the ovary along the anterior edge of the circum-intestinal gland region on the left side and at the level of the eighth stigmatal row on the right side. It consists of several lobes forming a rosette, and is colored grayish. Usually a single egg is matured on the right side, but on the left side the egg maturation is rarely achieved. Mature eggs are orange and approximately 160–200 μm in diameter. The incubatory pouch is a sac-like outgrowth of the body-wall formed near the ovary on the right side and the development of the fertilized egg takes place within the pouch. The embryo grows in the pouch up to about 800–1000 μm in diameter.

The larvae (Fig. 3b, c) are about 2.6–2.7 mm in total length and bright orange when alive. The trunk is about 900 μm long, oval in outline and includes a single photolith as typical to botryllids. Three attachment processes are arranged in a triangle in the anterior end of the trunk, and eight ampullae are arranged in a circular ampullar band surrounding the anterior half of the trunk, though respective ampullae are smaller than in the preceding species and set apart more widely. Both the branchial and atrial siphons are discernible, but no apertures are opened yet.

Life history: The asexual peribranchial budding can be observed all through the year. Each zooid usually produces a single bud on each side of the body. The cycle of the alternation of generations is about 7–9 days. The sexual reproduction is limited to the season of higher water temperature, from July to September, with a peak in August. In the breeding season, each bud of zooids produces gonads on both sides, but usually oocytes will not grow and finally become degenerated in the ovary on the left side. On the other hand, one of the oocytes in the ovary on the right side grows into a mature egg about 160–200 μm in diameter and then is ovulated into the incubatory pouch soon after the alternation of generations. The embryogenesis and growing of the embryo size take place inside the pouch. Seven to 9 days after ovulation, zooids begin to degenerate, leaving only the epithelium forming the incubatory pouch (Fig. 4). Embryos at this time are 800–1000 μm in diameter and already provided with the tail. About 4 or 5 days after the degeneration of the parent zooids, most of larvae swim out of the pouch in the morning from 10.00 to 12.00, but their hatching is not always synchronized.

Two to 10 hours after liberation, larvae become attached to the substratum by extension of eight ampullae, and the metamorphosis (Fig. 5) is started. The first bud appears on each side of respective individuals about 2 hours after attachment.

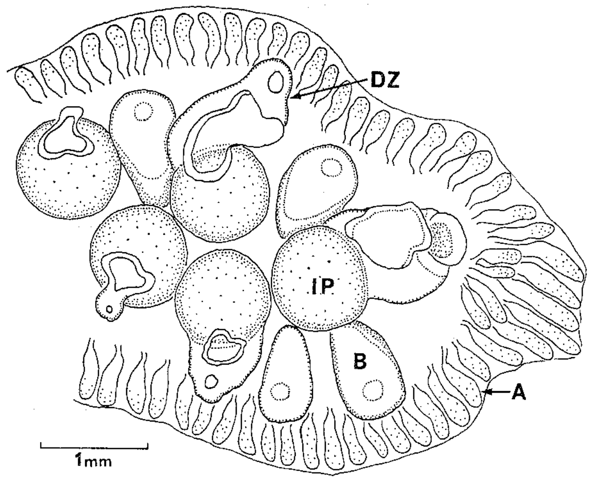

Fig. 4. *Botrylloides fuscus* n. sp. Degenerating zooids, with embryos in incubatory pouches. A: ampulla. B: bud. DZ: degenerating zooid. IP: incubatory pouch containing an embryo.

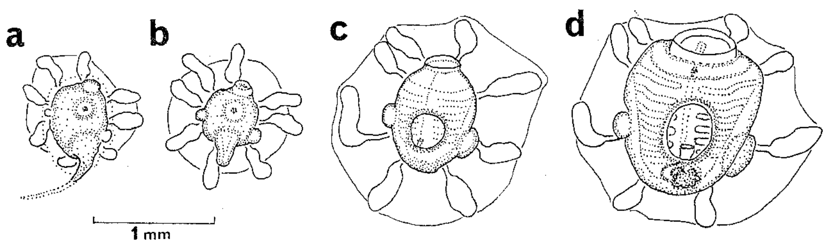

Fig. 5. *Botrylloides fuscus* n. sp. Stages in metamorphosis from larval attachment to a functional oozooid. a, an oozooid, 2 hours after larval attachment. Tail absorption in progress, and the first bud appearing on each side of body. b, the same, 4 hours after attachment. Tail absorption not completed. c, the same, 12 hours after attachment. Both branchial and atrial siphons open, and completion of a functional oozooid. d, the same, 60 hours after attachment.

The bud on the right side is always larger than that on the left side. Both the branchial and atrial apertures open about 12 hours after attachment. This functional oozooid will live for a week before the alternation of generations takes place.

Remarks. The present new species is found commonly on the stone or rock surface in the lower intertidal zone on the rocky shore around Shimoda. The morphology of zooid of the present species closely resembles that of *B. simodensis*. However, the external appearance such as the coloration of living colonies is different between these two species. Therefore, we are inclined to consider that this species might be treated under a specific name, *B. fuscus*. As for the life history inclusive of the morphology of zooid, see Table 1.

Botrylloides lenis n. sp.

(Figs 6–9)

Type-series. Two colonies collected from the stones in the intertidal zone of Shimoda are designated as the holotype and paratype (Y. Saito *leg.*, July, 1980). Type locality: Shimoda, Japan.

Description: Colonies are usually encrusting and sometimes attaining to a large size about 10 cm across; usually 1.7–2.0 mm in thickness. The colony surface is flat and free from any foreign matter. The test is very soft, gelatinous and transparent. When alive, the colonies are colored as a whole yellow, orange, brown, dark brown or red brown. In some colonies, dark pigment cells are deposited around the branchial siphons and on the atrial languets. The periphery of the colony is fringed with sausage-shaped vascular ampullae about 600 μm long and 170 μm wide. Very strangely, some colonial parts of significant sizes including only the vascular system, but none of zooids may often be extended out from the colony margin (Fig. 6).

Zooids (Fig. 7a) are arranged in ladder systems with several common cloacal apertures, and always connected one another in a common vascular system. They are 2.0–2.3 mm in length and situated obliquely. Branchial tentacles consist of four larger and four smaller ones regularly alternating. There are eight rows of stigmata on the left side and nine rows on the right side: the second row never reaches the dorso-median line. Around the middle of the branchial sac, stigmata are arranged between the three inner longitudinal bars as follows: dorsal lamina 3–4·2·2·3 endostyle. The anterior edge of the intestinal loop attains anteriorly the level of the seventh row of stigmata and the anus also opens at the level of the same stigmatal row. The most part of the stomach is exposed posterior to the rear end of the branchial sac. The stomach is orange in fresh specimens and furnished with eight longitudinal plications and a small transparent pyloric coecum.

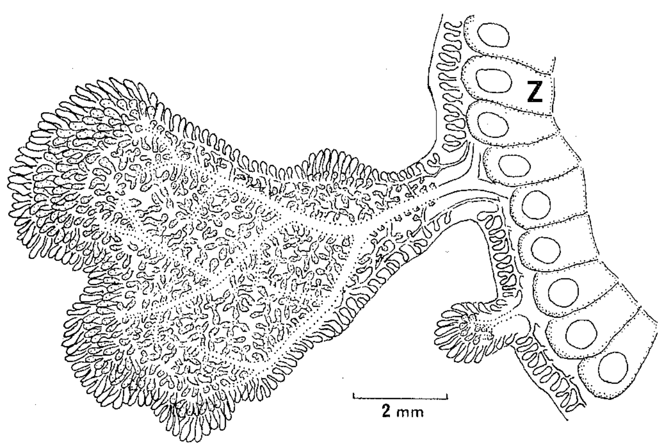

Fig. 6. *Botrylloides lenis* n. sp. Extended colony part including only the vascular system but none of zooids. Ampullae are very crowded, and internal vascular network is rather obscure. Z: zooid.

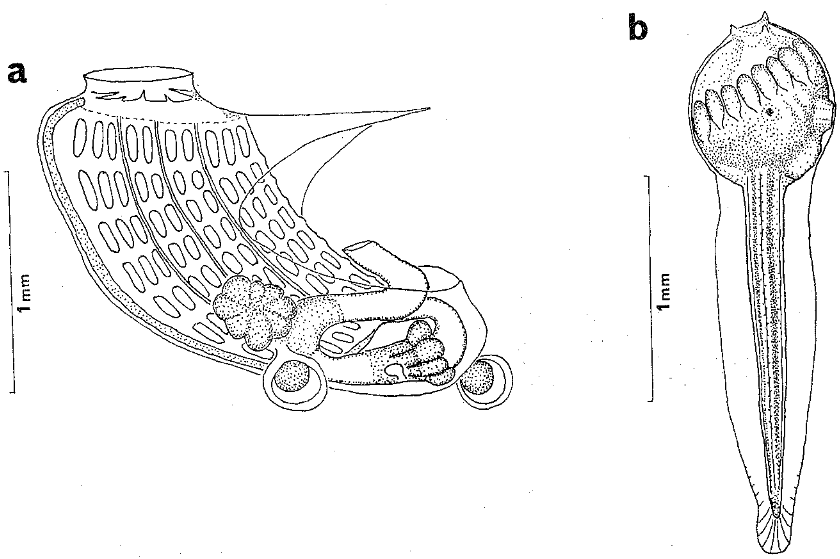

Fig. 7. *Botrylloides lenis* n. sp. a, a zooid, from left side. b, a larva, from left side.

The testis is situated anterior to the ovary along the anterior edge of the circum-intestinal gland region on the left side and at the level of the ninth stigmatal row on the right side. It consists of several lobes forming a rosette and is colored grayish. Usually only a single egg is matured in the ovary on each side; mature eggs are pinkish or orange and approximately 90–100 μm in diameter. The incubatory pouch is a sac-like outgrowth of the bodywall formed near the ovary.

Larvae (Fig. 7b) are about 2.2–2.4 mm in total length and yellowish white when alive. The trunk is about 700 μm long and oval in outline; three attachment processes are arranged in a triangle and 14–24 ampullae are arranged to form a circular ampullar band surrounding the anterior part of the trunk. The number of ampullae is variable even among larvae derived from the same colony. The differentiation of both siphons can be seen on the dorsal side of the trunk.

Life history: The asexual peribranchial budding can be observed all through the year. Each zooid usually produces one bud on each side of the body. The cycle of the alternation of generations is about one week. However, buds will always stop their growing or even degenerate when their parent zooids are keeping embryos in their incubatory pouches.

The sexual reproduction can be observed from July to October with a peak in August. The ovarian eggs in respective buds will be ovulated soon after the buds are grown to be the adult zooids and taking the place of their degenerated parent zooids. The eggs at this time are 90–100 μm in diameter, colored pinkish or orange, and opaque. The ovulated eggs go respectively into the incubatory pouch which is about 200 μm in the outer diameter, but 150–170 μm in the inner diameter. Therefore, considerable space is left between the fertilized egg and the pouch wall. About a week after ovulation, the embryo grows up to 300–380 μm

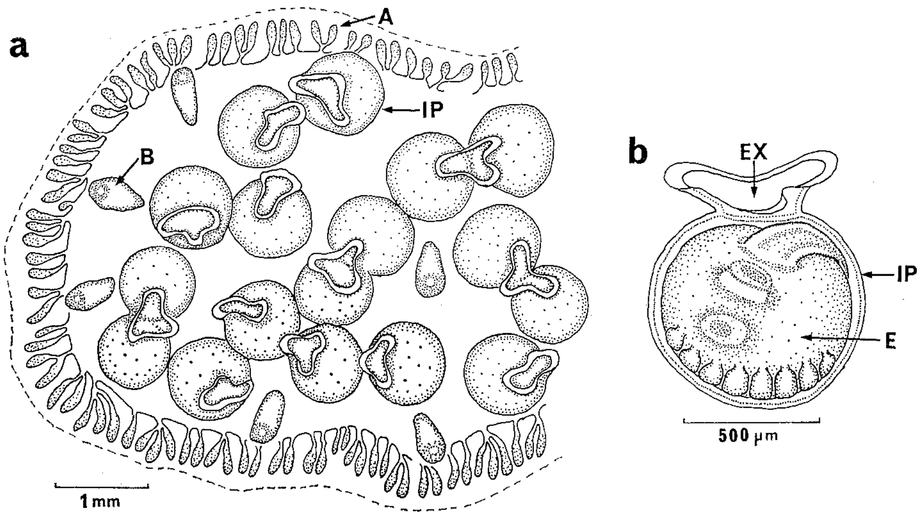

Fig. 8. *Botrylloides lenis* n. sp. a, incubatory pouches involving embryos, degeneration of zooids already over. A: ampulla. B: bud. IP: incubatory pouch. b, an incubatory pouch involving an embryo. E: embryo having finished embryogenesis. EX: exit for larval releasing. IP: wall of incubatory pouch.

in diameter and becomes transparent, when the above-mentioned space between the embryo and the pouch wall will disappear. The parent zooids begin to degenerate at this time, leaving the epithelium forming the incubatory pouch and the exit for the tadpole larva (Fig. 8a, b). One week after the degeneration of parent zooids, namely about 2 weeks after ovulation, the embryos in the pouch

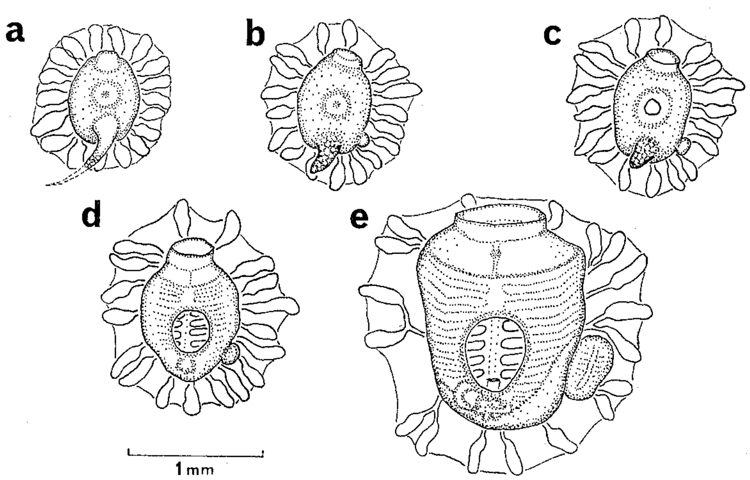

Fig. 9. *Botrylloides lenis* n. sp. Stages in metamorphosis from larval attachment to a functional oozooid. a, an oozooid, 1 hour after larval attachment. b, the same, 4 hours after attachment. The first bud appearing on the right side of body. c, the same, 8 hours after attachment. Both branchial and atrial siphons open, but tail absorption not completed. d, the same, 1 day after attachment. e, the same, 4 days after attachment.

attain the full size of 750–800 μm in diameter and the tail formation has been achieved by this time. About 20 days after ovulation, larvae hatch out from the incubatory pouch. Most of them escape from the pouch in the morning from 9.00 to 12.00, but hatching is not always synchronized.

Two to 5 hours after liberation, the larvae become attached to the substratum by extension of 14–24 ampullae. The metamorphosis (Fig. 9) goes so fast that both the branchial and atrial siphons are opened about 6–8 hours after attachment. A single bud first appears on the right side of the body 4 or 5 hours after attachment. The functional oozooids are provided with eight rows of protostigmata on each side, and live for a week.

Remarks. This new species is found commonly on the surface of stones, rocks or sea weeds, together with *B. simodensis* and *Botryllus scalaris* Saito et Mukai, 1981, in the lower intertidal zone on the rocky shore near the Shimoda Marine Research Center. Colonies of this species are thinner and softer than those of other four species, such as *B. simodensis*, *B. lentus*, *B. fuscus* and *B. violaceus*. Hence it is given the specific name *lenis*. As to the life history inclusive of the morphology of zooid, see Table 1.

Discussion

As noted already (for instance, Van Name, 1945), it is difficult to identify exactly the specimens of botryllids that are devoid of developed gonads. The superficial characteristics, such as the coloration or the system of zooidal arrangement, can not be the decisive clue even to distinguish the two genera, *Botryllus* and *Botrylloides*. The morphology of zooids is quite uniform in these genera, if the features of well developed gonads are inaccessible. Therefore, the detailed observations of the life history, inclusive of the morphology of gonads, are indispensable for the precise identification of the ascidians belonging to the Botryllidae.

The characteristics in morphology and life history of five Japanese species of *Botrylloides*, *B. simodensis* (Saito et al., 1981b), *B. violaceus* (Oka, 1927; Tokioka, 1953; Saito et al., 1981b), *B. lentus*, *B. fuscus*, and *B. lenis*, are summarized in Table 1. It will be seen rather easily on this table that the morphological characteristics closely resemble one another among these species that are, however, separable clearly from one another, if the details of their life histories are compared carefully.

As stated before, the external appearance of living colonies of *B. lentus* closely resembles those of *B. fuscus* and *B. violaceus*. However, the colonies are usually thicker in *B. lentus* than in the latter two, and the zooids are provided with more stigmatal rows in the former than in the latter two. In *Botrylloides tyreum* Herdman, 1886 (Tokioka, 1967) and the specimens described under the name of *Botrylloides violaceus marginatus* Tokioka, 1967 (Tokioka, 1967; 1968), the zooids have almost the same number of stigmatal rows as in *B. lentus*. In zooids of *B. tyreum*, however, the second stigmatal row reaches the dorso-median line of the branchial sac unlike the case in *B. lentus*. The remarkable color pattern, which is shown in the

Table 1. Characteristics in morphology and life history in five Japanese species of *Botrylloides*.

	B. simodensis	*B. lentus*	*B. fuscus*	*B. lenis*	*B. violaceus*
Colony					
system	ladder	ladder	ladder	ladder	ladder
thickness (mm)	2.0–3.0	3.0–3.5	2.5–3.0	1.7–2.0	2.0–3.0
test	soft, translucent	soft, translucent	soft, translucent	very soft, transparent	soft, translucent
Zooid					
length (mm)	2.0–2.5	3.1–3.5	2.2–2.7	2.0–2.3	2.5–3.0
number of stigmatal rows	11	18	9	R9, L8	10–11
number of tentacles (large), (small), (minute)	4(L), 4(S), 1–2(M)	4(L), 4(S), 1–2(M)	4(L), 4(S)	4(L), 4(S)	4(L), 4(S), 2–3(M)
number of stomach plications	9	9	8	9	8
pyloric coecum	small	small	small	small	small
Asexual reproduction					
budding	all seasons	all seasons	all seasons	all seasons	all seasons
number of buds in each zooid	R1, L1	R1, L1	R1, L1	R1, L1	R2, L2
cycle of blastozooid generations	5–6 days	7–9 days	7–9 days	6–7 days	5–6 days
Sexual reproduction					
breeding season	Jul.–Sep.	Jul.–Aug.	Jul.–Sep.	Jul.–Oct.	May-Aug.
production of larvae	ovoviviparous	viviparous	viviparous	viviparous	viviparous
mature egg diameter (μm)	250	300–330	160–200	90–100	90
embryo size in incubatory pouch	hardly increasing	grow larger	grow larger	grow larger	grow larger
embryo size just before hatching (μm)	300	1000–1100	800–1000	750–800	1000–1200
development to larva	in 4–5 days	in 10–12 days	in 12–14 days	in 20 days	more than one month
release of larvae	shortly before degeneration of parent zooids	shortly before degeneration of parent zooids	5 days after degeneration of parent zooids	2 weeks after degeneration of parent zooids	after 6–7 times of alternation of generations of zooids
length of larva (mm)	1.7–2.0	2.5–2.7	2.6–2.7	2.2–2.4	3.0
length of larval trunk (μm)	500	800	900	700	1000
number of larval ampullae	8	8	8	14–24	24–34

specimens of *B. violaceus marginatus* as a very important characteristic to distinguish this subspecies from others, has not yet been found in any specimens of *B. lentus*.

To distinguish *B. fuscus* from *B. violaceus*, the observations on the life history are indispensable as seen clearly on Table 1, because these two species share almost the same external appearance and morphology of both colonies and zooids. Though the morphology of zooids of *B. fuscus* is also similar to that of *B. simodensis*, the external appearance such as the coloration of living colonies is different between these two species.

Colonies of *B. lenis* are thinner and softer than those of other four species, and this can be a clue to distinguish this species from others to the experienced eyes. On the other hand, the colonies of this species colored yellow very closely resemble the colonies of *B. scalaris* (Saito et al., 1981a). Furthermore, the colonies of these two species are found in the same habitat. Therefore, the morphology of well developed gonads should be applied for the determination of these two species. In *B. lenis*, the colony parts including only the vascular system, but none of zooids are often extending out from the colony margin, and there vascular budding, noted by Oka & Watanabe (1957), occurs naturally. This feature can also be a unique characteristic of the species.

As seen clearly in the above-mentioned comparison, the three species presented here may safely be accepted as new species. The first species, *B. lentus*, forms thick and tough colonies and this feature is the base for the present nomenclature. The second, *B. fuscus*, has few morphological characteristics to distinguish it from other species of *Botrylloides*, but all the colonies of this species are colored darker than in others, and then, the species is named after its darker coloration. The last one, *B. lenis*, has very soft test and is named after this feature of the test.

In zooids of all the species presented in Table 1, the second row of stigmata never reaches the dorso-median line, though this feature was first given by Tokioka (1953) as a unique characteristic of *B. violaceus*. Tokioka's *B. violaceus* can not be a monospecific taxon at that time as noted by himself. Furthermore, the same feature is also recognized later in two species of *Botryllus* (Saito et al., 1981a). Therefore, this feature might be common among a number of botryllid ascidians.

Until now, some observations have been made on details of the sexual reproduction of botryllids, *Botryllus primigenus* Oka, 1928 (Mukai & Watanabe, 1978), *B. scalaris* (Saito et al., 1981a), *Botryllus sexiens* Saito et Watanabe, 1981 (Saito et al., 1981a), *Botryllus schlosseri* Pallas, 1766 (Berrill, 1950), *B. simodensis* (Saito et al., 1981b), *Botrylloides leachi* Savigny, 1816 (Berrill, 1947; 1950), and *B. violaceus* (Saito et al., 1981b). Furthermore, by the presentation of the details of the sexual reproduction of the three new species in this paper, the outline of the evolution of the manner of the sexual reproduction in the Botryllidae seems to become clearer. It may be suggestible from Table 1 that the manner of the sexual reproduction in the genus *Botrylloides* is progressing gradually from ovoviviparity to viviparity. On the other hand, in the genus *Botryllus* all the species, in which the manner of the sexual reproduction has been cleared, are ovoviviparous. These facts might suggest the following course of progress from 1 to 4: (1) ovoviviparity without incubatory pouches as seen in *Botryllus*, (2) ovoviviparity with formation of incubatory pouches, (3) nourishing the embryos and extension of the incubating period, (4) reduction of the correlation between the cycle of the alternation of generations and the development of embryos in the pouches.

Thus, the observations on the details of life history give us evidently an important information as to both the taxonomy and phylogeny of the Botryllidae. However, there are left so many other described species in this family, in which nothing has been given as to their life histories. Further, in mentioning the phylogenetic significance of the features of the sexual or asexual reproduction, it is requested that the crucial comparisons are made between the features of different groups of ascidians and also between those of different animal groups. Needless to mention, the further efforts are needed to clarify those one by one.

References

Berrill, N.J. 1947. The developmental cycle of *Botrylloides*. Quart. Jour. Micr. Sci., 88: 393–407.

————. 1950. The Tunicata. 354pp. The Ray Society, London.

Ger, G., & Y. Zan. 1983. Ascidians of Jiaozou Bay. I. Botryllidae. Jour. Shandong Coll. Oceanol., 13: 93–100.

Kott, P., & I. Goodbody. 1980. The ascidian of Hong Kong. In: B.S. Morton & C.K. Tseng. eds., Proceeding of the First International Marine Biological Workshop: Hong Kong, 1980, pp. 503–554. Hong Kong University Press, Hong Kong.

Mukai, H., & H. Watanabe. 1978. Studies on some aspects of ovulation and embryogenesis in the compound ascidian, *Botryllus prymigenus*. Annot. Zool. Japon., 51: 211–221.

Oka, A. 1927. Zur Kenntnis der japanischen Botryllidae (Vorläufige Mitteilung). Proc. Imp. Acad., 3: 607–609.

Oka, H. 1960. Molting at metamorphosis in ascidians. Bull. Mar. Biol. Sta. Asamushi, Tohoku Univ., 10: 177–180.

————, & H. Watanabe. 1957. Vascular budding, a new type of budding in *Botryllus*. Biol. Bull., 112: 225–240.

Saito, Y., H. Mukai & H. Watanabe. 1981a. Studies on Japanese compound styelid ascidians. I. Two new species of *Botryllus* from the vicinity of Shimoda. Publ. Seto Mar. Biol. Lab., 26: 347–355.

————, ———— & ————. 1981b. Studies of Japanese compound ascidians. II. A new species of the genus *Botrylloides* and redescription of *B. violaceus* Oka. Publ. Seto Mar. Biol. Lab., 26: 357–368.

Tokioka, T. 1953. Ascidians of Sagami Bay. H. Hattori. ed. 315pp. Iwanami Shoten, Tokyo.

————. 1967. Pacific Tunicate of the United States National Museum. U.S. Nat. Mus. Bull., 251: 1–247.

————. 1968. Contributions to Japanese ascidian fauna XXIII. On *Botrylloides violaceus marginatus* Tokioka, 1967. Publ. Seto Mar. Biol. Lab., 16: 141–145.

Van Name, W.G. 1945. The North and South American Ascidians. Bull. American Mus. Nat. Hist., 84: 219–230.