STUDIES OF JAPANESE COMPOUND STYELID ASCIDIANS
I. TWO NEW SPECIES OF *BOTRYLLUS* FROM THE VICINITY OF SHIMODA[1)]

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With Text-figures 1–5

Since 1951, the compound ascidians have been a part of the main materials for physiological, morphogenetical and immunological studies carried out at the Marine Biological Station of Tokyo Kyoiku University, that is situated in Shimoda near the southern end of Izu Peninsula, Middle Japan, and is known at present as the Shimoda Marine Research Center of the University of Tsukuba. Clarification, exact and in detail, of the life history in respective species experimented with is requested for these studies. Thus, the culture method of material ascidians has been established (Oka and Usui, 1944) and this has made clear in those ascidians many features such as the structure of gonads, the morphology of larvae, and the mode of reproduction by budding. Recently, a part of researches at the Station, and later at the Center, has been focussed on the studies of the actual mode and mechanism of asexual reproduction manifested so diversely in botryllid and polyzoic ascidians of the family Styelidae. On the other hand, most species of botryllids and polyzoic styelids known from the Japanese waters have been established on the morphology mainly of zooids of preserved specimens. Necessity of observations on larvae and mature gonads and of confirmation of the details of asexual reproduction in those has been noted but hardly fulfilled so far as the preserved specimens are treated with. Culture of such ascidians has clarified many of these points in them. Further, the stability of some minor morphological differences has been confirmed by culture throughout several generations. All these have contributed much to make the configuration of some species distinct, but at the same time have suggested the necessity to split some other known, probably polyphyletic species into a few to several

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ones and to establish new species. The taxonomic works for this object have progressed slowly but steadily, fortunately being supported by encouragement and advices given from specialists in the country, who are fully aware of the necessity of the details of life history in the taxonomy of compound styelids and seemingly have left the revision of these ascidians to the group of researchers working on live ascidians at this marine research center. Results of these taxonomic studies are to be published successively in several papers. And, in the present first paper, the descriptions of two new species of genus *Botryllus* are given, together with the details of their life history.

Before going further, the authors want to present their 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, and to the former for his kindness in reading the manuscript critically.

Botryllus sexiens Saito and Watanabe n. sp.

(Figs. 1–2)

In 1973, some colonies of a botryllid were found in the sea water tank of the Shimoda Marine Research Center, the University of Tsukuba. These colonies stripped off by spatula were fastened on glass slides and then cultured in boxes immersed in the water of Nabeta Bay, a small cove adjoining the ground of the research center. Later, colonies of the same botryllid were collected from the surface of stones on the muddy floor of the same cove.

Description: Colonies are usually encrusting, varying from small discs of a few millimeters in diameter to large sheets about 10 centimeters across; usually 2.5–3.0 mm in thickness, but only 1.5–2.0 mm in the "resting" phase when the alternation of generation of zooids takes place. The colony surface is generally flat and free from any foreign matter. The gelatinous test is extremely soft, transparent and colorless. The colonies may look brown, orange or yellow when alive. Such colorations are attributed to the pigment cells deposited around the branchial siphon and on the atrial languet of respective zooids and frequently representing altogether some patterns on the colony surface. The periphery of the colony is fringed by sausage-shaped vascular ampullae about 700 μ in length and 200 μ in width.

Zooids (Fig. 1a) are arranged in ladder systems with several common cloacal apertures, though the systems become often obscured by crowding of zooids, and are always connected one another by a common vascular system. They are 2.5–3.0 mm in length and situated more or less vertically standing, though obliquely in the periphery. Branchial tentacles consist of 6 larger and 6 smaller ones alternating regularly. There are 9 to 10 rows of stigmata on each side; the second row never reaches the dorso-median line. Around the middle of the branchial sac, stigmata are arranged between the 3 inner longitudinal bars as follows: dorsal lamina

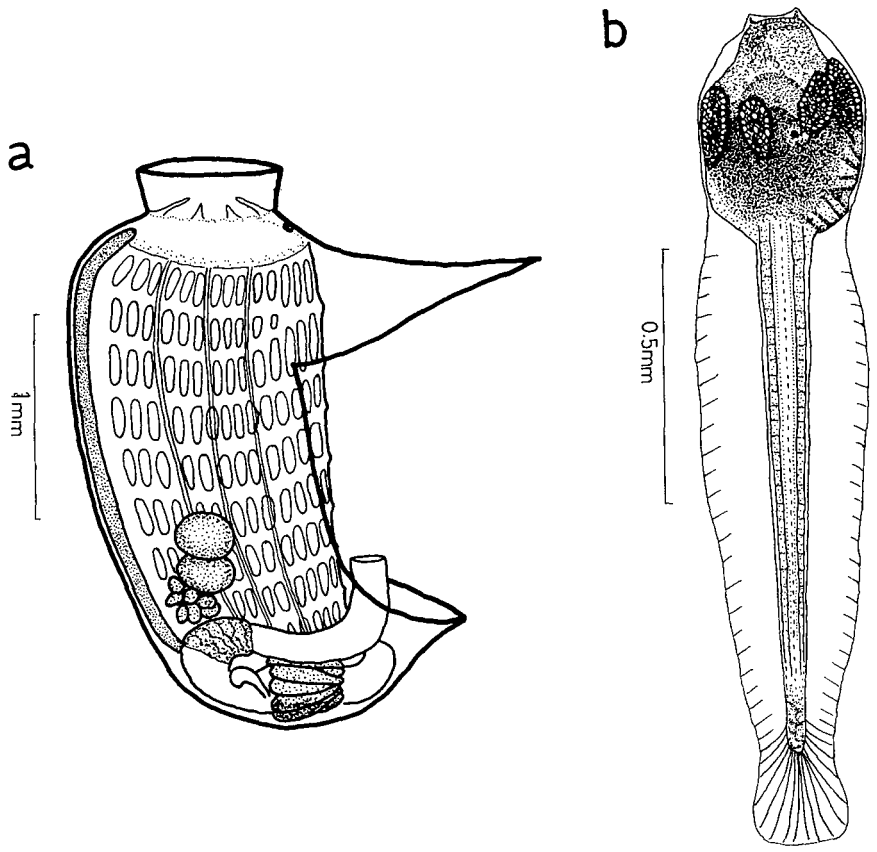

Fig. 1. *Botryllus sexiens* n.sp. a, a zooid, from left side. b, a larva, from left side.

5·3·2–3·4 endostyle. The anterior edge of the intestinal loop attains anteriorly the level of the 8th transverse vessel and the anus opens at the level of the 7th transverse vessel. The most part of the stomach is exposed posterior to the rear end of the branchial sac. The stomach is furnished with 11 longitudinal plications and a very elongate pyloric coecum. In fresh specimens, it is generally colored orange, though always white pigments are deposited densely along its posterior edge.

The asexual reproduction, which is called "peribranchial budding", can be observed all through the year. Each zooid usually produces a single bud on each side of the body. The sexual reproduction can be observed only from July to December, with a peak in August. The testis is situated along the anterior edge of the circum-intestinal gland area on the left side and at the level of the 9th row of stigmata on the right side, and posterior to the ovary. It consists of several lobes forming a rosette. Usually two eggs are matured on each side, they are yellow in color and approximately 150 μ in diameter. The development of fertilized eggs always takes place in the peribranchial cavity. The larva (Fig. 1b) is about 1.5–1.8 mm in total length and light yellow in color when alive. The trunk is oval in outline and provided with 6 ampullae; three attachment processes are arranged in a triangle.

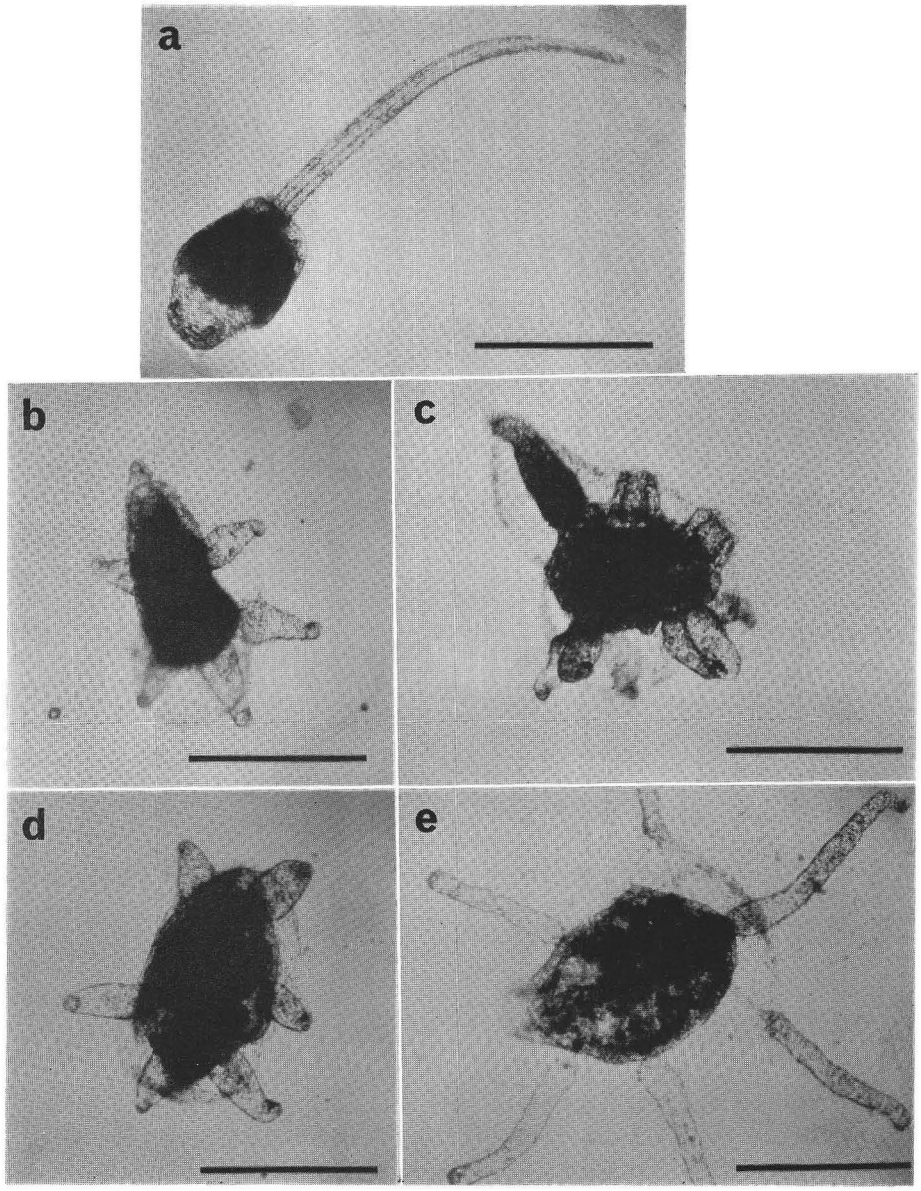

Fig. 2. *Botryllus sexiens* n. sp. Stages in the metamorphosis from larva to oozooid. a, a swimming larva. b, an oozooid, 8 hours after the attachment of larva; ventral view. Six ampullae are elongated. c, the same, from right side. Siphons are open, though the tail absorption is not yet completed. d, the same, 12 hours after the attachment; ventral view. e, the same, 20 hours after the attachment; ventral view. Each scale bar indicates 500μ.

Metamorphosis (Fig. 2): Immediately after the attachment to the substratum, 6 ampullae begin to elongate, then the siphons open and thus the attached individual becomes functional before the tail absorption is completed. The first bud always appears after the metamorphosis is completed.

Remarks: The present species resembles closely *Myxobotrus japonicus* Oka, 1931 in having extremely soft test and general structure of zooids similar to that of the latter. In the present species, however, zooids are arranged in ladder systems and connected one another by a common vascular system, whereas in *M. japonicus* neither system in arrangement of zooids nor common vascular system is described. Further, in the present species, there are always 6 ampullae on larvae, that are elongated after the attachment of the larva. In addition, in the branchial tentacular ring 6 larger tentacles and the same number of smaller ones are alternating. It is quite exceptional in the species of *Botryllus,* that the first ampullae seen on the larva and the tentacles are six or a multiple of six, respectively. Evidently, the present form can be defined as a new species even by this feature only, and this is the reason for the present nomenclature.

Botryllus scalaris Saito and Mukai n. sp.

(Figs. 3–5)

The present species was first found in the sea water tank of the research center together with the preceding new species and later collected from the natural environments, such as the surface of rocks and stones in shallow water or sometimes the under surface of floats in still water.

Description: Colonies are usually encrusting and sometimes attain a size about 10 centimeters across, usually 1.0–1.5 mm in thickness. The colony surface is flat and free from any foreign matter. The test is soft gelatinous and transparent. When alive, they are colored yellowish-brown, sometimes with orange, white or purple pigment cells deposited around the branchial siphon and on the atrial languet of respective zooids. The periphery of the colony is fringed by sausage-shaped vascular ampullae about 800 μ long and 160 μ wide.

Zooids (Fig. 3a) are about 1.5 mm in length, situated obliquely in the test and arranged in ladder systems with several common cloacal apertures. They are connected one another by a common vascular system. Branchial tentacles consist of 4 larger and 4 smaller ones alternating regularly. There are 8 rows of stigmata on each side; the second row never reaches the dorso-median line. Around the middle of the branchial sac, stigmata are arranged between the 3 inner longitudinal bars as follows: dorsal lamina 4·3·2–3·3–4 endostyle. Many blood cells colored orange are deposited along each side of the endostyle in the range from the second to the sixth stigmatal row. The anterior edge of the intestinal loop attains anteriorly the 6th transverse vessel, the anus is open at the level of the 6th stigmatal row, and the stomach is wholly exposed posterior to the rear end of the branchial sac. The stomach is orange in color in fresh specimens and is furnished with 8–9 longitudinal plications and a very elongate prominent pyloric coecum.

The asexual peribranchial budding can be observed all through the year. Each zooid always produces a single bud on each side of the body. The sexual reproduc-

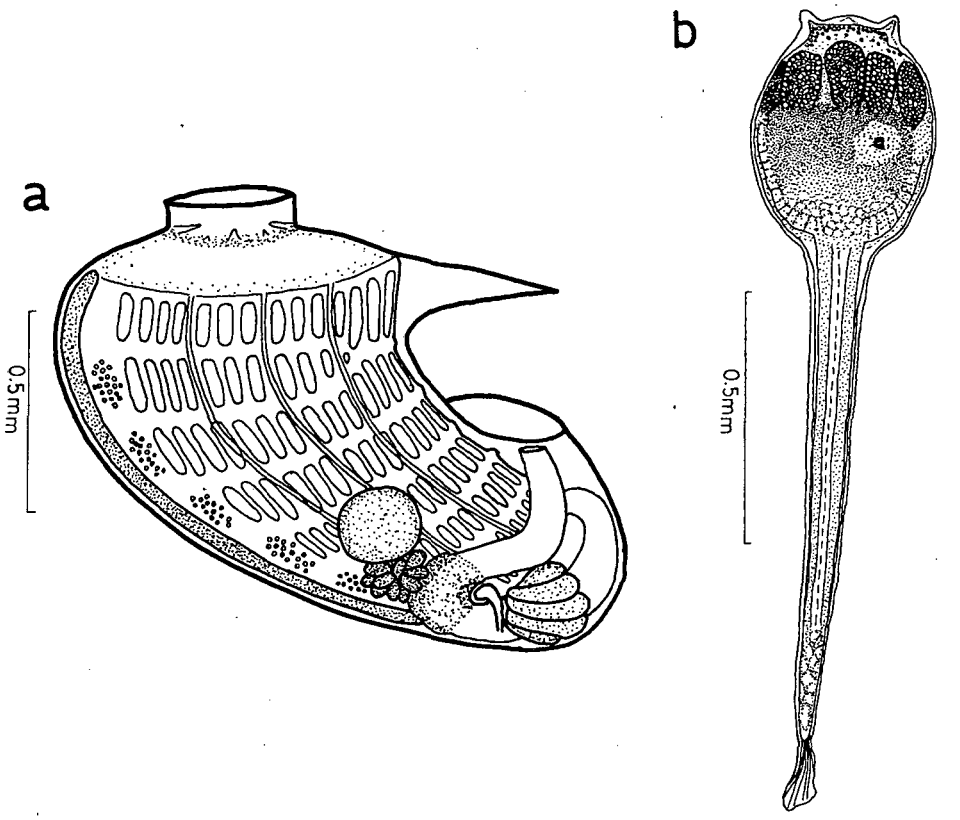

Fig. 3. *Botryllus scalaris* n. sp. a, a zooid, from left side. b, a larva, from dorsal side.

tion can be observed from June to November, with a peak in August. The testis consisting of several oval lobes in a rosette group is situated along the anterior edge of the circum-intestinal gland area on the left side and at the level of the 7th or 8th stigmatal row on the right side, and ventrolaterally to the ovary. Usually a single and sometimes two eggs are matured on each side, they are orange and about 220 μ in diameter. The fertilized eggs always develop in the peribranchial cavity and larvae leave the colony before their parent zooids degenerate. Larvae (Fig. 3b) are about 1.5 mm in total length and colored orange when alive. The trunk is about 400 μ long, oval in outline and includes a large statolith typical to botryllids, three attachment processes are arranged in a triangle and eight ampullae are arranged to form a circular ampullar band surrounding the anterior part of the trunk.

Metamorphosis (Fig. 4): Larvae are released from the colony in the morning from 10:00 to 12:00 and become attached to the substratum 2–4 hours after liberation by extension of 8 ampullae. Usually the apertures open and then feeding is started about 20 hours after release, and the first bud appears 2 days after the attachment.

Remarks: The present new species is closely related to *Botryllus schlosseri* in the structure of zooid. However, the stellate systems characteristic to the colony of *B. schlosseri* (Berrill, 1950; Tokioka, 1953) are never formed in the present species, ex-

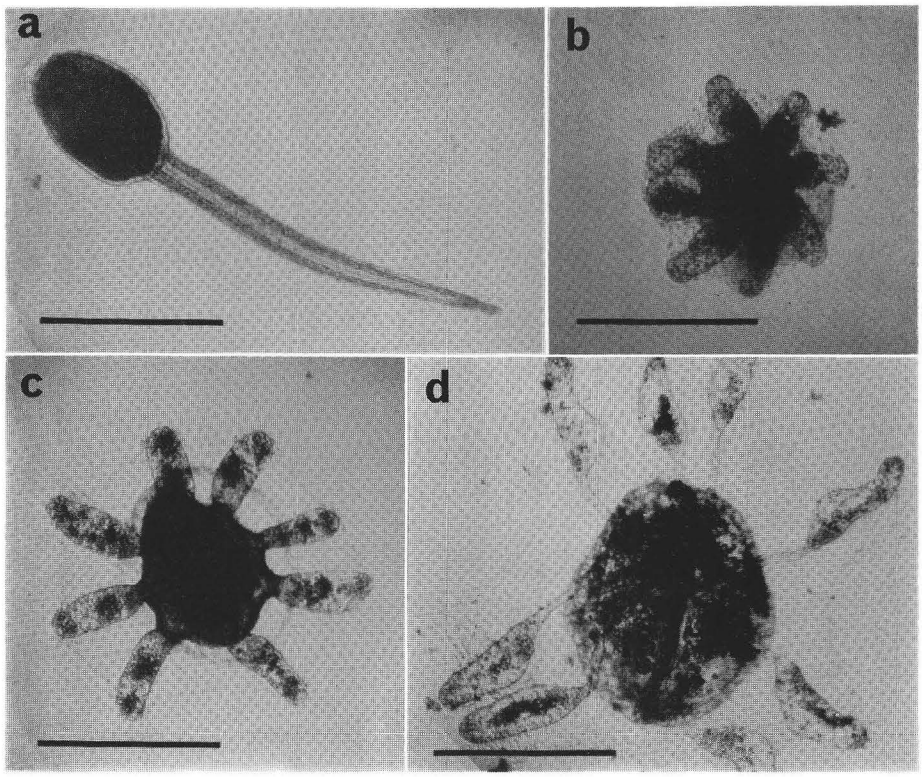

Fig. 4. *Botryllus scalaris* n. sp. Stages in the metamorphosis from larva to oozooid. a, a swimming larva. b, an oozooid, 4 hours after the attachment of larva; ventral view. c, the same, 10 hours after the attachment; ventral view. d, the same, 24 hours after the attachment; ventral view. Each scale bar indicates 500 μ.

cept in very small colonies including only 5–15 zooids. Generally the ladder systems are formed in this new species. Mature eggs are fewer and smaller in the present species than in *B. schlosseri,* in the latter mature eggs are about 450 μ in diameter and 1–4 on each side. In *B. schlosseri,* the mature eggs pass through the very short oviduct and come to rest each in a cup-like extension of the peribranchial wall (Fig. 5a), where they are fertilized and stay to develop to the tadpole that will leave there by rupture of the egg membrane (Berrill, 1950). On the contrary, in the present new species, the cup-like extensions mentioned above are never formed and therefore the ovulated eggs are always free in the peribranchial cavity (Fig. 5b).

Very fortunately, some colonies of *B. schlosseri* were collected by Mr. Hiromichi Koyama near the Asamushi Marine Biological Station, Tohoku University, in autumn, 1979, and brought to us. Since then, these colonies have been cultured by the first author at the Research Center. Thus, the whole life history and morphology of living colonies have been compared very exactly between the present species and *B. schlosseri.* Even by careful observations, it was impossible to distinguish these two species from each other in their young stage when colonies were composed of only 5 to 15 zooids, but in larger colonies respective species were easily identified by the

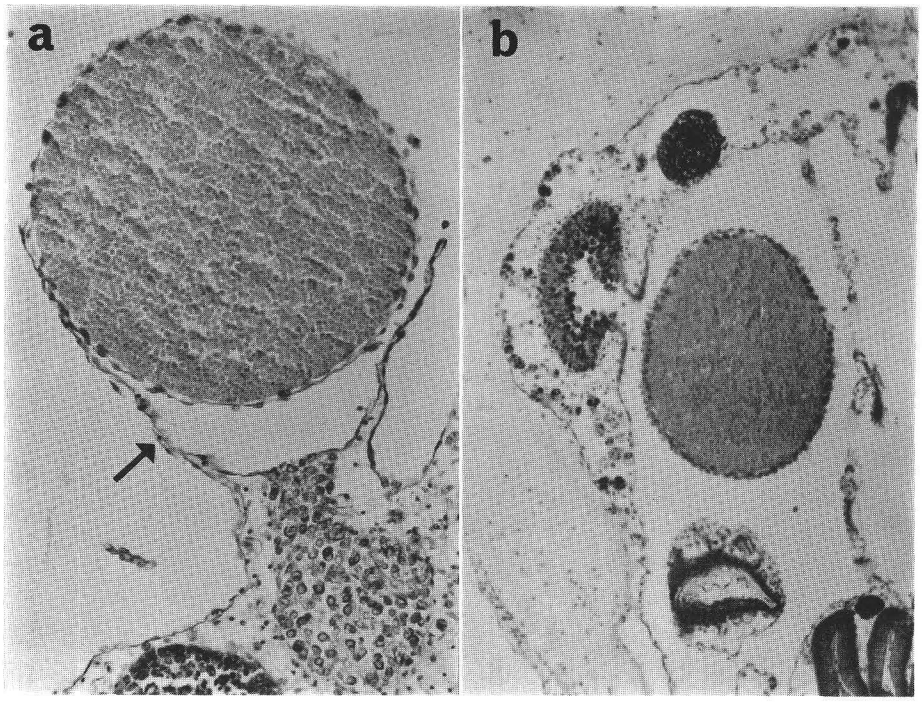

Fig. 5. Cross section of an embryo in the peribranchial cavity. a, in *B. schlosseri*. The ovidual cup (pointed by arrow) is formed distinctly. b, in *B. scalaris*. No ovidual cup is formed.

difference in the arrangement system of zooids. Further, in *B. schlosseri* the sexual reproduction takes place throughout the year. Three or 4 months are required for the growth from an oozooid to a sexual colony, and the colony will come to the end of life after releasing larvae 2 or 3 times. In contrast with this, in the present species the breeding season is limited to the warm water season, when even young colonies will bear mature gonads. Furthermore, the colonies usually survive after releasing larvae several times, though all the zooids in the colony may sometimes wholly disappear.

The present species resembles also *Botryllus planus* (Van Name) of the West Indies in the feature of systems on the colony and in the structure of zooids. But, the stigmatal rows are fewer in the former than in the latter (11–13), and only a single mature egg is found on each side in *B. planus*. Probably the present species may safely be defined as a new species related most closely to cosmopolitan *B. schlosseri* and is named after the feature of ladder systems on the colony. Lastly, it is very possible that some colonies, including no zooids with developed gonad, of the present new species and also of the preceding new species might be included in the descriptions or records of *Botrylloides violaceus* Oka from the Japanese waters, because of the ladder system of zooids common to these species.

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