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Systematic Study on a Bivalve-Inhabiting Hydroid *Eucheilota intermedia* Kubota from Central Japan

By

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(With 5 Text-figures, 10 Tables, and 1 Plate)

As the specific name shows *Eucheilota intermedia* Kubota, 1984 was designated as a form that fills the morphological gap between *Eutima japonica* Uchida, 1925 and *Eugymnanthea inquilina japonica* Kubota, 1979 (Kubota 1984). Recently collection of the bivalve-inhabiting hydroids was made again at Zagashima Is., the type locality of the present species, where *Euchei. intermedia* was found in some other bivalves than *Mytilus edulis galloprovincialis* with which *Eugym. i. japonica* was dominantly associated (Kubota 1985).

Through the rearing of a part of the materials collected on that occasion, some unknown character states are revealed in every life stage of the present species, especially in the mature medusa, generally the most indispensable life stage for the taxonomy of bivalve-inhabiting hydroids. In the present paper, they are described in detail together with the discussion on their significance in taxonomy. Moreover, the chromosome number was determined, and the crossability test with the related species was conducted and the offspring viability was checked to demonstrate their genealogical closeness.

Materials and Methods

The hydroid materials used were associated with six specimens of *Barbatia virescens* (Nos. 1, 5, 7, 9, 10, 12) collected from the intertidal region of the sea and one specimen of *Mytilus edulis galloprovincialis* (specimen A) attached to a rope hanging down from a raft within several meters in depth. These bivalve specimens were collected at Zagashima Is., in Ago Bay, Mie Prefecture on September 11 and 13, 1984. After dissecting them except for two specimens of *B. virescens* many polyps were picked up. When these host specimens were collected, the medusa-buds were produced in only polyps associated with *M. e. galloprovincialis*.

Table 1. Materials obtained, reared, and examined their morphology. Hosts are *Barbatia virescens* (Nos. 1, 5, 7, 9, 10, 12) and *Mytilus edulis galloprovincialis* (specimen A).

Host no.	Host size in mm[1)]	No. of the youngest (within 1-day-old) medusae				No. of mature medusa (abnormal ones)[3)] obtained
		liberated (abnormal ones)	examined (see Table 2)	tried to rear until mature[2)]	reared at (±1°C)	
1	34	4 (0)	2	3	22	2♀♀ (0)
5	47	2 (0)	2	1	22	1♀ (0)
7	42	3 (0)	1	2	22	2♀♀ (0)
12	39	27 (0)	24	16	22	13♀♀ (2)
9	43	9 (0)	8	8	22	8♂♂ (0)
10	39	37 (1)	25	21	22	19♂♂ (1)
12	39	5 (0)	4	5	14.5	2♀♀ (0)
12	39	3 (0)	2	3	12.5	1♂ (0)
10	39	1 (0)	1	1	12.5	0 (0)
A	53	8 (0)	8	5	22	4♀♀ (0)

1) In *B. virescens* shell length was measured and in *M. e. galloprovincialis* antero-posterior axis was measured.

2) Other specimens reared for examination of chromosome were not included.

3) All such specimens had reticulated radial canals.

In the other polyps the medusa-buds were produced afterwards in laboratory.

The two bivalves harboring the present species (Nos. 10 and 12) had been reared for more than eight months in laboratory, and many polyps or immature medusae came out from them. Most of the newly liberated medusae as well as the polyps were reared at 22±1°C in a small vessel in which the filtered seawater supplied from Oshoro Bay was filled and they were fed with many newly hatched *Artemia* nauplii *per se* and pieces of them torn off by needles. Only a small number of medusae were reared at 14.5±1°C or 12.5±1°C. The number of medusae obtained, reared, and examined their morphology is summarized in Table 1.

The low water temperature seems to be unfavorable for the development of the medusa as was noticed in the previous study (Kubota 1984). Including abnormally developed ones (only three in number) 52 out of 56 specimens survived at 22±1°C, but only three out of nine specimens survived at the low temperature (see Table 1) and the six specimens degenerated within eight days after liberation. This might be due to a sudden change of water temperature from 22±1°C to 14.5±1 or 12.5±1°C. On the contrary as one of the reasons why some specimens survived at the low temperature, the daily supply of *Artemia* nauplii

torn off by hands as a portion of food could be considered. It should be mentioned here that 10 female specimens, seven reared at 14.5±1 or 12.5±1°C and three reared at 22±1°C (Nos. 12-13a-j), were liberated from several polyps asexually reproduced from one polyp.

Mature medusae of only one sex liberate from each host specimen examined (Table 1), and simultaneous association of *Eugym. i. japonica* was not found. This indicates that a clone of *Euchei. intermedia* is usually associated with each host specimen as is seen in the other bivalve-inhabiting hydroids (cf. Kubota 1983, '85).

All the measurements were taken from the living specimens without narcotization in general and are shown in text in the following form: mean±SD (range, the number of individuals examined). The description in text is based on the specimens associated with *B. virescens*, and if the specimens associated with *M. e. galloprovincialis* were used they are expressed plainly. As to a small number of medusae found within two days after liberation, the age was counted from 1.5 days. Only Fig. 5 was drawn with the aid of a drawing apparatus.

The methods as to the chromosomal observation, the crossability test, and the examination of offspring viability are described in each section.

Polyp

Only a small number of specimens were examined in detail. The polyp is up to 4.3 mm in length, with up to 34 tentacles and up to three medusa-buds per specimen. The daughter polyp asexually reproduced has 10-17 tentacles, measuring about 0.5-0.6 mm in length. One specimen produced two different stalks of medusa-bud at the same position of the hydrocaulus, keeping at a short distance with each other; and each stalk bore one medusa-bud. Such a formation of medusa-buds, though it might be an extraordinary one, has never been found in other related bivalve-inhabiting hydroids.

The nematocyst equipment of polyp was examined in three specimens including a daughter polyp. There are two types of basitrichous isorhizes in every specimen. The large type, which is found on the pedal disk and the hydrocaulus, was absent in the previous study. The measurements of nematocysts (in μm) on three body portions of polyp are as follows: small type on tentacles, 8.1±0.6 (6.6-9.0)×1.9±0.2 (1.8-2.2), 39; small type on hydrocaulus (examined in only one specimen), 7.8±0.3 (7.4-8.2)×1.8±0 (1.6-1.8), 10; large type on hydrocaulus, 8.0±0.5 (7.2-9.0)×3.4±0.3 (3.2-4.0), 29; large type on pedal disk, 7.4±0.9 (6.0-8.8)×3.3±0.5 (2.4-4.0), 22.

The movement of polyp on the surface of the rearing vessel was observed. A polyp does not go straight on, but moves around very slowly. The details of such movement will be reported elsewhere.

Medusa

The Youngest Medusa

The gross morphology of the youngest medusae within one day after liberation was examined in 69 specimens (35 ♀♀ + 34 ♂♂). Of these three female specimens could be measured when they were immediately after liberation from their polyps and at the time just one day passed. The youngest medusa has eight marginal swellings and eight statocysts. It is 0.75-1.63 mm in umbrellar diameter and the following variation is found (Table 2): (1) Most of the specimens (78%) have two tentacles while the others have three or four tentacles; (2) Most of the specimens (71%) have rudimental gonads, and the gonads slightly develop in some male ones; (3) When a specimen has three or four tentacles the rudimental gonads are always produced, and such a specimen was not found in the previous study; (4) Smaller specimens tend to have two tentacles, and any sign of gonads does not appear in them; (5) The male has usually (71% of the specimens examined) two tentacles and rudimental gonads, whereas the female has two tentacles but without gonads (45% of the specimens examined); (6) The ring canal is green in some specimens, while colorless in some specimens; (7) On the abaxial side of the perradial marginal swelling a black band is present apparently or obscurely, while absent in some specimens. In 11 youngest medusae (6 ♀♀ + 5 ♂♂) whose umbrellar diameter is 0.78-1.56 mm the number of statoliths and that of cirri (abaxial cirri plus lateral ones) were counted. There are 12±4 (8-22) statoliths and 29±5 (20-38) cirri per specimen, and up to three statoliths per statocyst and up to six cirri per marginal swelling. The number of cirri on the perradial marginal swelling is more than that on the interradial one even if the

Table 2. Umbrellar diameter (in mm) of four types of the youngest medusae within 1 day old classified by the character states of tentacles and gonads. Sexes of most of the specimens were determined afterwards by rearing.

Characters	Two tentacles		Three tentacles	Four tentacles
	without gonads	with gonads	with gonads	with gonads
♀*	0.88±0.07 (0.75-1.00, 17)	1.14±0.17 (0.94-1.44, 11)	1.56	1.25±0.16 (0.94-1.50, 9)
♂	0.96±0.11 (0.81-1.03, 4)	1.21±0.18 (1.00-1.63, 24)	1.44	1.21±0.26 (0.94-1.50, 5)
♀ + ♂	0.90±0.08 (0.75-1.03, 21)	1.19±0.18 (0.94-1.63, 35)	1.50±0.09 (1.44-1.56, 2)	1.24±0.19 (0.94-1.50, 14)

* Three specimens were examined twice, at the stage just detached from the polyp and at another stage just one day passed after liberation.

abaxial cirri are included. There is one abaxial cirrus on every interradial marginal wart of the above specimens except for only one marginal wart in a male medusa. In these two characters sexual difference is found. The number of cirri is more in male than in female: the number of cirri per specimen is 31-38, that per perradial marginal swelling is 3-6 (usually 4 or 5), and that per interradial marginal swelling is 3-5 (usually 3 or 4) in male, while it is 20-29 per specimen, 2-5 per perradial marginal swelling (usually 3 or 4), and 1-4 per interradial one (usually 2 or 3) in female. The number of statoliths per specimen is also more in male than female: 9-22 in male and 8-10 in female. This sexual difference may be due to the more rapid development in male than in female.

From one specimen of *Mytilus edulis galloprovincialis* six youngest medusae were obtained. They have two tentacles, and the smaller ones, below 1.0 mm in umbrellar diameter, have no gonads while the larger ones, above 1.1 mm in diameter, have rudimental gonads. The youngest medusae with three or four tentacles have never been found in the material associated with this bivalve (as far as 50 or more specimens have so far been examined, cf. Kubota 1984).

The external morphology of the young medusa less than one day old is summarized as follows, including the previous description (hosts are *Barbatia virescens* and *Mytilus edulis galloprovincialis*): the umbrellar diameter is 0.63-1.63 mm, with eight marginal swellings including none to four (usually two) tentacles, eight statocysts, up to three statoliths per statocyst, up to six cirri per marginal swelling, and with or without rudimental gonads.

Early Developmental Stage of Medusa

Early metamorphosis from the youngest medusa to the earliest developmental stage of the mature medusa could be examined in detail in one female specimen (No. 12-14), measuring various meristic characters every day after narcotizing it by $MgCl_2$ solution. During the period the umbrellar diameter increased day by day, 0.4-0.5 mm per day regularly (Fig. 1, A). The disappearance of the abaxial cirri began on the second day (Fig. 1, B: AC). Several small marginal warts were produced beside the statocyst on the 4th day and the number of marginal swellings increased (Fig. 1, B: MS). On this day the immature gonads were slightly separated from the stomach (Fig. 1, A: S). All the other characters began to change on the 3rd day: the number of lateral cirri, the perradial tentacles, and of the statoliths were increased (Fig. 1, B: LC, TE, ST). This specimen matured on the 7th day, though a quadrant of the umbrella slightly degenerated on the 6th day, and the number of lateral cirri decreased on these two days. The life-span of this medusa was about 24 days.

As described above, some youngest medusae of the present material have already four tentacles. Including such specimens the morphology of the earliest developmental stage of medusa with four tentacles was revealed in 24 females and 30 males. The medusa assumes such a form within five days after liberation. At this developmental stage the umbrella is under 3.0 mm in diameter, there are up

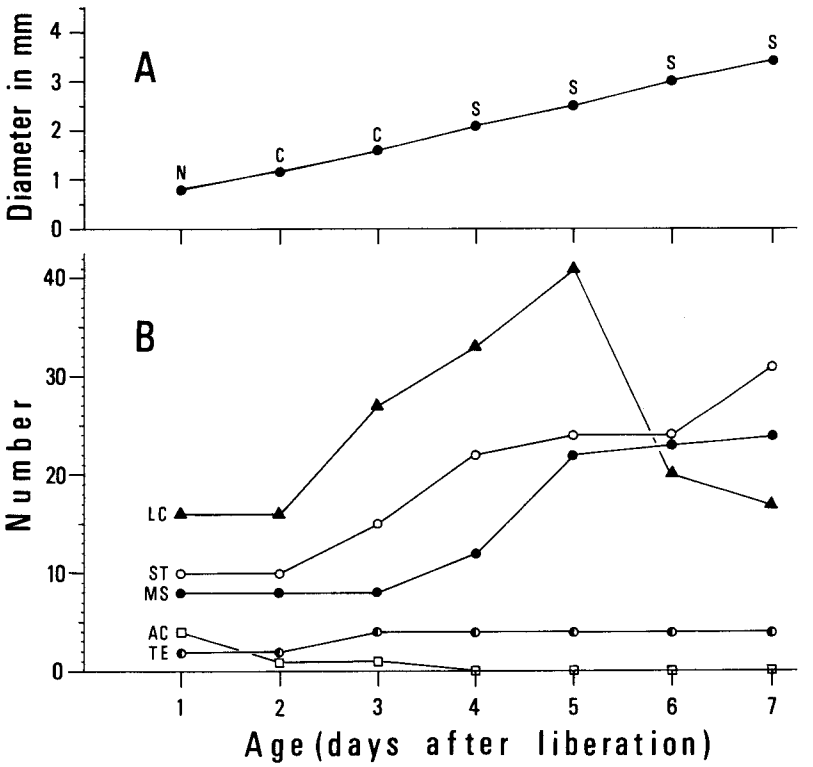

Fig. 1. Early metamorphosis of a female specimen (No. 12-14) until maturation. A: growth of umbrellar diameter and change of gonadal condition (N: without gonads; C: with gonads connected with stomach; S: with gonads separated from stomach). B: developmental change of five meristic characters such as number of abaxial cirri per specimen (AC), number of lateral cirri per specimen (LC), number of marginal swellings (MS), number of statoliths (ST), and number of tentacles (TE).

to 20 marginal swellings, and maturation of gonads is observed in some specimens (8 out of 41 ones examined). On the average it takes 2 ± 1 days to attain this stage in female and 3 ± 1 days in male, when the average diameter is 1.7 ± 0.5 mm (1.1-3.0 mm, 23) in female and 1.9 ± 0.4 mm (0.94-2.5 mm, 29) in male. One female specimen reared at 12.5 ± 1°C (No. 12-13 i) had four tentacles on the 14th day, when the umbrellar diameter was 2.9 mm and only eight marginal swellings were present. This specimen had two tentacles when liberated from the polyp.

The gonad appears as a rudiment on the radial canal just beside the stomach, and as the medusa grows it develops soon and is separated from the stomach (cf. Fig. 1, A). In 11 females and 17 males originated from four host specimens, at what stage such separation occurs was examined (Table 3). It takes place within

Table 3. Age and morphology of two developmental stages of medusae decided from the position of gonads: one developmental stage whose gonads are connected with stomach (shown in left side of each item) and the other whose gonads are separated from stomach (right side of each item). When both the sides of item are filled, the oldest stage of the former and the youngest stage of the latter is clarified by the daily observation of the specimen.

Sex	Host Specimen no. no.	Age (days)	Umbrellar diameter (mm)	No. of marginal swellings	Gonadal condition[1]
♀	12- 6	2 -3	1.8 -2.3	12 -17	IM -(M)
	12-14	3 -4	1.6 -2.1	8 -12	IM -IM
	12-11	4 -5	2.2 -2.5>	18 -18	M - M
	12- 3	4 -5	2.8 -2.9	17 -17	(M) - M
	12-25[2]	4 -5	2.6 -3.1	16 -18	(M) - M
	1- 3	4	2.7	21	M
	12- 9	7	3.0	22	M
	12-12[2]	6	3.4	22	M
	12- 1	4	2.3	18	IM
	12- 8	6	2.9	21	M
	12-10	7	4.0	24	M
♂	10-27	2[3] -3	1.6[3] -2.2	8[3] -18>	IM[3] -(M)
	10-23	2 -3	2.0 -2.4	8? -12	(M) - M
	10-22	3 -4	2.2 -2.8	? -16	(M) - M
	10- 5	3.5 -4.5	2.9 -3.1	18 -24	M - M
	10-21e	4 -5	2.3 -2.4	15 -15	IM -IM
	10- 9	4 -5	2.5 -2.8	17 -17	(M) - M
	10-11a	4 -5	2.3 -2.7	17 -18	(M) - M
	10-32	5 -6	2.0 -2.4	14 -16	M - M
	10-20	5 -6[4]	2.0 -2.0[4]	11 -12[4]	M - M[4]
	9- 5	3	1.9	11	M
	9- 4	3	2.1	9	M
	10-11b	3.5	2.3	18	(M)
	10-11b	6.5	2.8	21	M
	9- 7	5	2.7	24	M
	9- 1b	5	2.8	17	M
	10- 2	4	2.1	17>	(M)
	10-17	4	2.3	16	(M)
	10-25	5	2.4	18	M

1) IM: immature; (M): nearly mature; M: mature.
2) Only two gonads are connected with the stomach.
3) Number of tentacles is two only in this specimen, and in the others four tentacles are found.
4) Three gonads are separated from the stomach.

seven days after liberation, when the medusa is 2.6±0.5 mm (2.1-4.0 mm, 20) in diameter and 17±3 (12-24, 19) marginal swellings are present. The oldest developmental stage of medusae whose gonads and stomach are connected are 2.4±0.5 mm (1.6-3.4 mm, 22) in diamter and 16±5 (8-24, 20) marginal swellings are present. Whether the gonads are separated from the stomach or not, the gonads mature. In one specimen reared at 14.5±1°C (No. 12-13 g) the morphology at such two developmental stages is the same as that in the above specimens reared at 22±1°C: the oldest developmental stage of this specimen (6-day-old) whose gonads (immature ones) and stomach are connected is 1.9 mm in diameter and 10 marginal swellings including four tentacular bulbs are present; the earliest developmental stage (7-day-old) whose gonads (still immature ones) and stomach are separated is 2.1 mm in diameter and 12 marginal swellings are present. In one specimen reared at 12.5±1°C (No. 12-13 i) it took more time (nearly twice) to attain such two developmental stages and the number of marginal swellings including two tentacular bulbs is only eight: the diameter is 2.4 mm on the 12th day and 2.7 mm on the 13th day. In three specimens originated from one specimen of *Mytilus edulis galloprovincialis* the early development is the same as that in the specimens reared at 22±1°C.

Mature and Spent Medusa

As was observed in the previous study, the present medusa becomes mature soon after liberation. The specimens reared at 22±1°C mature on the 3rd to 6th day, and as described above the gonads of some of the mature medusae are separated from the stomach. The measurements of mature medusa in the earliest developmental stage is summarized in Table 4, examining 36 specimens in which the above-described specimen (No. 12-14) was not included because a part of the umbrella degenerated and the maturation was slightly delayed. The maturation was delayed in the female specimens survived at 14.5±1 or 12.5±1°C and their total number of marginal swellings is fewer than that of the female specimens reared at 22±1°C, though this number is the same as that in male medusa reared at 22±1°C (Table 4). All the mature medusae of this develop-

Table 4. Age and morphology of the earliest developmental stage of mature medusa reared at different water temperatures.

Sex	Water temperature reared (°C)	Age (days)	Umbrellar diameter (mm)	No. of marginal swellings	No. of specimens examined
♀	22 ±1	5±1(4-6)	2.8±0.3(2.2-3.4)	20±2(17-23)	14
	14.5±1	10, 13	2.6, 2.9	13, 16	2
	12.5±1	17	3.4	12	1
♂	22 ±1	4±1(2.5-5)	2.3±0.3(1.9-2.8)	15±4(8-20)	19

Table 5. Frequency distribution of life-span of medusa reared at 22±1°C, represented by individual number of medusa. The life-span is arbitrarily divided into categories at intervals of 15 days.

Life-span (days)	1-14	15-29	30-44	45-59	60-74	75-89
♀	3	7	4	0	1	1
♂	0	8	5	3	0	0

mental stage have four tentacles and many cirri. The mature medusa with two tentacles as described before was not found in the present material.

As the specimens reared at 18-20°C described before, the life-span is not long in the present specimens reared at 22±1°C even if they were carefully reared. The life-span greatly differs among individuals (31±16 days in 32 specimens) (Table 5), though it is nearly the same in both sexes: 30±20 days (10-81 days, 16) in female, 32±13 days (15-56 days, 16) in male. On the other hand, three female specimens reared at low temperature lived for at least 50, 53, and 74 days, respectively. If specimens were survived at the low temperature, the life-span seems to be longer than that at the high temperature. In these specimens the development of gonads and formation of eggs took place normally.

During the above-described life-span of medusa reared at 22±1°C, the maturation of gonads takes place only once in most of the specimens (11 ♀♀ +12 ♂♂ out of 14 ♀♀ +16 ♂♂ examined). The period of maturation of such medusae is ascertained in 13 specimens: in six females it is 19, 14, 12, 12, 4, 3 days and in seven males 52, 42, 20, 19, 8, 7, 4 days, respectively. Two females (Nos. 12-6, 13 d) and three males (Nos. 10-5, 15, 21 b) matured twice, and one female (No. 1-3) and one male (No. 9-3) matured three times during the life. In the two female specimens reared at 14.5±1°C (Nos. 12-13e, g), the unfertilized eggs had been discharged for a long time, 55 and 43 days, respectively, though the number of eggs discharged per day was variable. In the one female specimen reared at 12.5±1°C (No. 12-13 i) the discharge of eggs had been observed for 34 days, during which no eggs were discharged three times, one or two days in each time.

On and after the earliest mature stage described above, the medusa continues to grow for a given period, and both the diameter and the number of marginal swellings increase. Two types of the growth are distinguished as shown in Fig. 2 in which only some examples are drawn: one is that the growth stops earlier in life and degeneration sometimes occurs (Nos. 1-3; 10-5, 11a; 12-6); the other is that the growth lasts nearly throughout the life (Nos. 9-3, 4, 7; 12-13 e, i). Consequently a wide morphological variation appears. Such a variation showing in the form of the relationship between the umbrellar diameter and the number of marginal swellings of the medusa from the earliest mature stage to an older stage near degeneration is summarized in Fig. 3. This figure was drawn from the data

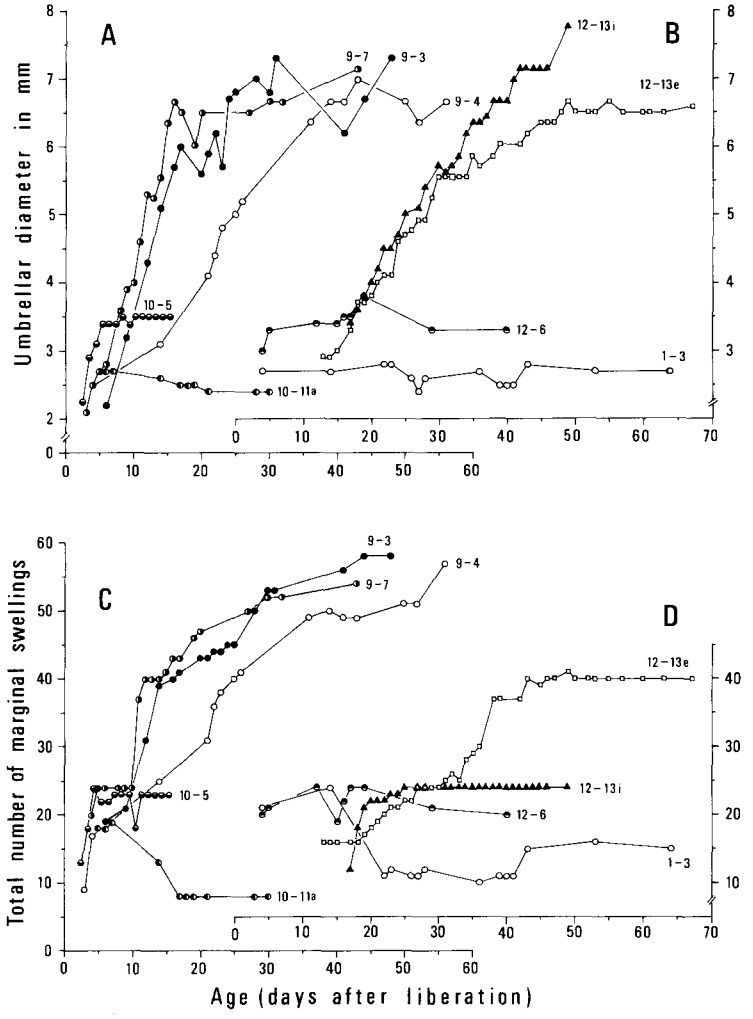

Fig. 2. Growth of medusa after first maturation in male (A, C) and in female (B,D). Figures are specimen numbers. Circles are data obtained from seven specimens reared at 22 ± 1°C, quadrangles from one specimen reared at 14.5 ± 1°C, and triangles from one specimen reared at 12.5 ± 1°C.

obtained from many specimens (22 ♀♀ +26 ♂♂), most of which examined several to many times at different developmental stages. The variation range in female medusa is 2.2-7.8 mm in diameter and 12-41 in the number of marginal swellings. It is noteworthy that this wide range is due to the existence of the specimens survived at the low temperature which are larger than the specimens reared at the

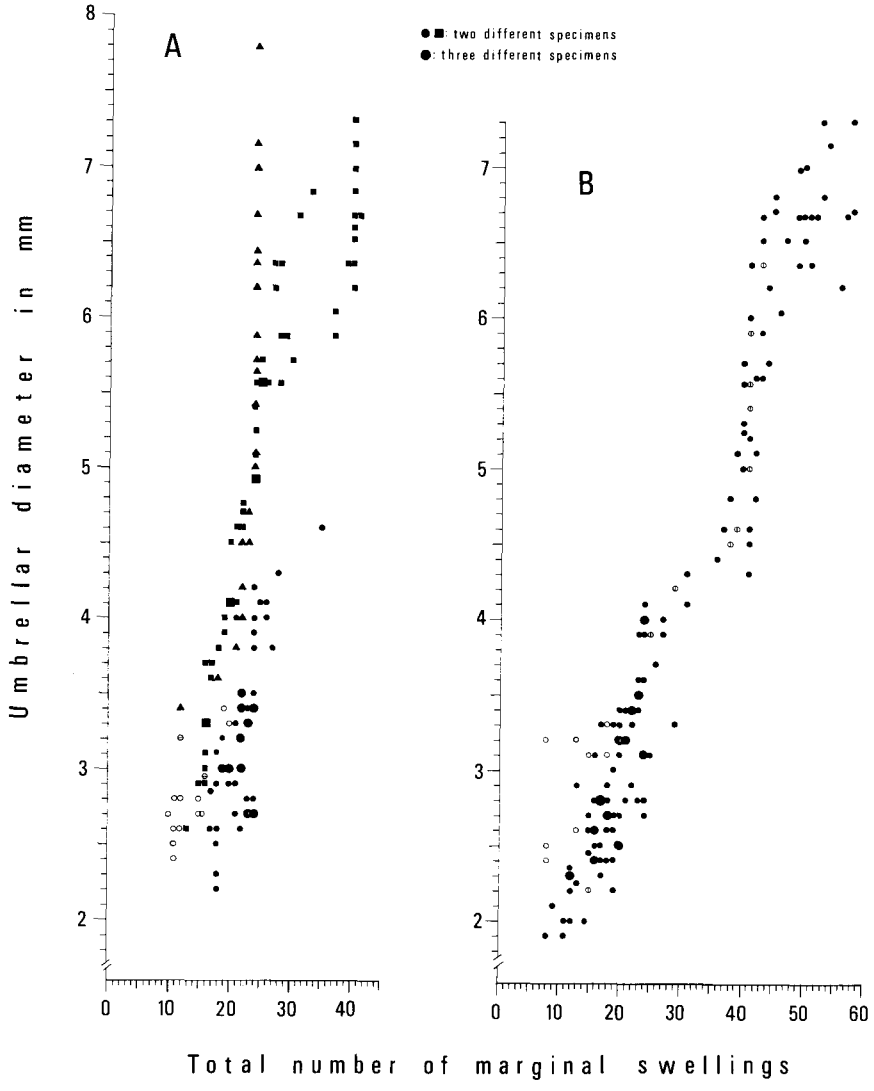

Fig. 3. Relationship between the total number of marginal swellings and the umbrellar diameter in female mature or spent medusa (A) and in male mature or spent medusa (B). Circles are data obtained from 19 female and 26 male specimens reared at 22 ± 1°C, quadrangles from two female ones reared at 14.5 ± 1°C, and triangles from one female specimen reared at 12.5 ± 1°C. Open circles are remarkably degenerated specimens, and the other kinds of circles are abnormal specimens regarding their radial canals (in A) or shape of gonads (in B). The same measurements obtained at different ages of a certain specimen are not included.

Table 6. Age and morphology of the earliest developmental stage of medusae with peduncle reared at three different temperatures, and their maximum values of measurements in three characters throughout the life-span.

Water temperature reared (±1°C)	Specimen no.	Sex	Age (days)	Umbrellar diameter (mm)	Total no. of marginal swellings	Maximum value of measurements:		
						length of peduncle (mm)	length of manubrium (mm)	no. of tentacles
22	9- 2[1)]	♂	21	4.3	41	0.16	—	4
22	9- 3[2)]	♂	20	5.6	43	0.5	1.0	6
22	9- 4	♂	26	5.2	41	0.24	1.0	6
22	9- 7	♂	15	6.4	41	0.5	0.8	4
14.5	12-13e[3)]	♀	32	5.6	26	0.48	1.6	4
14.5	12-13g	♀	22	5.6	25	0.48	1.3	4
12.5	12-13i[4)]	♀	35	6.3	24	0.48	2.4	4

1) With three oral lips.
2) With nine statocysts afterwards (41-day-old).
3) Manubrium reached umbrellar aperture afterwarts (47-day-old).
4) With four oral lips once frilled afterwards (45-day-old).

high temperature (Fig. 3, quadrangles and triangles). If the data obtained from the spent medusae were added to the above (cf. Fig. 3), the range slightly widens (10-41 in the number of marginal swellings). On the other hand, the male medusa is 1.9-7.3 mm in diameter and 8-58 in the number of marginal swellings. Several male specimens grow well even if they are reared at the high temperature, and the diameter is over 4.6 mm, the maximum value of the female medusa reared at the high temperature, being the same range as the female medusa in the umbrellar diameter, though more in the number of marginal swellings.

Such a wide variation range exceeds the former one described. In addition to this, some unknown character states are found in this life stage. In four male specimens reared at 22±1°C and three female ones reared at 14.5±1 or 12.5±1°C a peduncle is produced (Table 6). A very short peduncle begins to form on the 15th to 35th day, when the umbrellar diameter is above 4.3 mm in them. The peduncle does not develop well thereafter and remains short throughout the life, measuring up to 0.5 mm in length (see Fig. 4; Plate I, C). The presence of such a short peduncle is often difficult to recognize when the medusa is contracted. It is noteworthy that the peduncle is produced only in a well-grown medusa, but the marginal swellings in the female medusa reared at the low temperature is not many, 24-26 in number, (Table 6; cf. Figs. 2, 3). It is also noteworthy that all the female specimens reared at 22±1°C produced no peduncle, though four of

which attained to 4.0-4.6 mm in diameter. Despite of the presence of the peduncle, the manubrium is found within the subumbrellar cavity except for one female specimen (No. 12-13e) in which the manubrium reached the umbrellar aperture (see Fig. 4; Plate I).

Among the above specimens with peduncle two males (Nos. 9-3, 4) have up to six tentacles and one of them (No. 9-3) has nine statocysts. These are other unknown character states. As an exceptional case, one male medusa (No. 9-6) had oval gonads connected with the stomach during the period from 4 to 14 days after liberation. Its umbrella was 2.2-5.0 mm in diameter and 15-41 marginal swellings were present during the period. Although it attained to 6.4 mm in diameter and had 43 marginal swellings, no peduncle was produced, moreover on the 29th day eight tentacles were produced, when the manubrium was completely absent (cf. Fig. 3, B; Table 7).

One of other unknown character states is an elongated gonads (see Fig. 4; Plate I). The oval or linear gonads, which are produced on the proximal portions

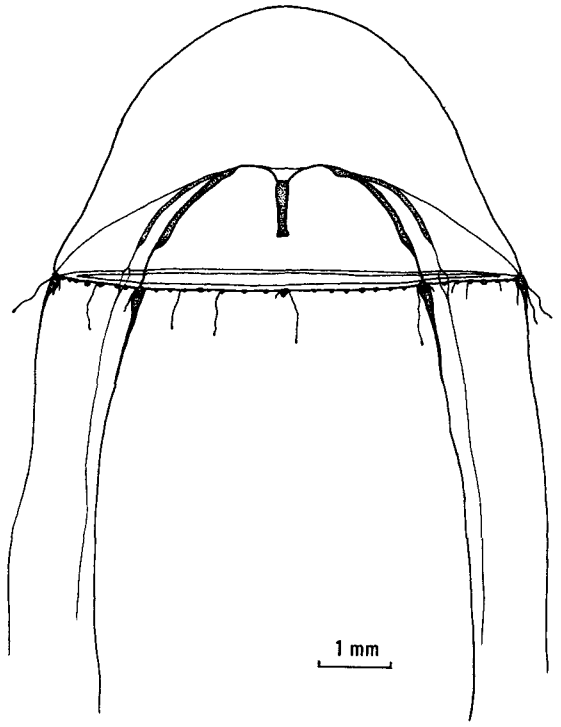

Fig. 4. Side view of a spent male medusa 56 days old (No. 9-4). Note the presence of a short peduncle, the linear gonads extended along the most parts of the radial canals, and six tentacles.

Table 7. Measurements (in mm) of various body portions of mature medusae of different ages reared at three different temperatures.

Water temperature reared (±1°C)	22	22	22	22	22	14.5	14.5	12.5
Specimen no.	5-1	12-14	9-7	9-6	9-4	12-13e	12-13g	12-13i
Sex	♀	♀	♂	♂	♂	♀	♀	♀
Age in days	5	5	27	29	56	32	40	45
Umbrellar diameter	2.6	2.5	6.5	6.4	6.7	5.6	7.0	7.1
Umbrellar height	—	—	4.3	—	3.8	4.8	5.1	5.7
Thickness of jelly at umbrellar apex	—	—	2.4	—	2.2	2.1	2.7	2.7
Length of peduncle	0	0	0.16	—	0.16	0.16	0.48	0.48
Length of manubrium	—	—	0.95	0	0.79	0.95	1.3	2.4
Width of stomach	—	—	0.32	—	0.16	0.44	—	—
Length of SG[1)]	—	—	0.63	—	0.56	0.63	—	—
Breadth of velum	—	—	—	—	0.29	0.71	—	0.79
Length of gonads	—	—	2.1	—	1.6	1.7	—	—
Width of gonads	—	—	0.14	—	—	0.19	—	—
No. of tentacles	4	4	4	8	6	4	4	4
No. of marginal swellings	17	22	50	43	57	26	40	24
Max. MS/Q[2)]	5	6	14	11	15	8	10	6
No. of statoliths per statocyst	3-4	3	—	8-10	6-8	? 4-7	? 9-14**	? 5-10*
No. of statoliths	25	24	—	70	59	? 46	? 92**	? 55*
Max. C/MS[3)]	5	6	—	3	3	2	2**	2*
No. of cirri	37	41	—	34	31	25	17**	7*

1) Length from center of stomach to proximal portion of gonads.
2) Maximum number of marginal swellings per quadrant.
3) Maximum number of cirri per marginal swelling.
* Measured at 50-day-old. ** Measured at 54-day-old.

of the radial canals at first, extends more and found to be along the most parts of the radial canals in a well-grown medusa of both sexes.

The other unknown character state is concerning the shape of oral lips. The oral lips are crusiform, but in one female specimen (No. 12-13i) it was frilled once. As an exceptional case, one male specimen (No. 9-1) had three oral lips.

It is noteworthy that the female medusa reared at 14.5±1 or 12.5±1°C tends to have a longer manubrium (Table 6). Accordingly it could be said that the

well-grown female medusae reared at different water temperatures show different external morphology.

The lateral cirri are produced, though not always, on the marginal warts of the second or more sets. The black band or pigmentation is a variable character. On the tentacular bulbs or the interradial marginal warts the pigmentation is usually found, but sometimes absent. On the manubrium it is usually absent, but sometimes present.

As an example of the above-described wide morphological variation of mature to spent medusa, the measurements of various body portions in eight specimens of 5-56 days old reared at different temperatures are shown in Table 7. It was observed that the number of statoliths was difficult to count in the specimens reared at the low temperature.

Three female mature specimens 5 or 6 days old originated from one specimen of *Mytilus edulis galloprovincialis* were examined in detail, and the number of cirri is found to be more than that described before (see Kubota 1984): the number of cirri per specimen attains to 46 and that per marginal swelling attains to eight (both are the maximum values of cirri in the present species). Several cirri were present on a free margin of the umbrella. This is also an undescribed character state.

It is true that several specimens mature two or three times during their short life-span, but the development of the present species may not be an example of dissogony which is said to be observed in other cnidarians such as siphonophores and scyphozoans as well as ctenophores.

The nematocyst equipment of mature medusa on various body portions except for exumbrella was examined in six male specimens of 4-56 days old originated from the two host specimens. The measurements (in μm) are as follows: large type of atrichous isorhizes on tentacles, 9.6 ± 0.8 (8.0-11.2)$\times4.2\pm$ 0.5 (3.4-5.6), (examined in 67 nematocysts in five specimens); medium type of atrichous isorhizes on tentacles, 6.3 ± 0.7 (4.8-7.6)$\times2.3\pm0.4$ (1.8-3.2), (48, 5); small type of atrichous isorhizes on cirri, 5.2 ± 0.3 (4.6-5.8)$\times1.8\pm0.1$ (1.6-2.0), (20, 2); larger type of basitrichous isorhizes on tentacles, 8.2 ± 0.4 (7.6-9.0)$\times2.6\pm0.2$ (2.4-3.0), (31, 5); smaller type of basitrichous isorhizes on tentacles, 6.8 ± 0.5 (5.8-7.8)$\times2.2\pm0.2$ (1.8-2.6), (59, 5); larger type of basitrichous isorhizes on oral lips, 7.8 ± 0.5 (7.2-9.2)$\times2.5\pm0.1$ (2.2-2.8), (21, 2); merotrichous isorhizes on cirri, $11.6\pm$ 0.7 (9.8-12.8)$\times4.0\pm0.3$ (3.4-4.6), (22, 2). Compared these measurements with the previously described ones, the large type of atrichous isorhizes on tentacles and merotrichous isorhizes on cirri tend to be larger. As to basitrichous isorhizes on tentacles, two types are distinguished as described above, and the larger type tends to be larger and the smaller type tend to be smaller than the nematocysts of the same kind described before. The sizes of the other types of nematocysts are the same as those described before. It was observed that the number of merotrichous isorhizes per cirrus was 22, 25, and 27 in respective three cirri of one 4-day-old male specimen. It is more in number than before.

The external morphology of mature to spent medusa is summarized as follows including the previous data (hosts are *Barbatia virescens* and *Mytilus edulis galloprovincialis*): the umbrellar diameter is 1.5-7.8 mm, with eight to 58 marginal swellings including two to six (usually four, extraordinary eight) tentacular bulbs, eight (rarely nine) statocysts, up to 14 statoliths per statocyst, oval to elongated gonads, several (up to eight) cirri per marginal swelling, crusiform (rarely once frilled) oral lips, and with or without short (up to 0.5 mm in length) peduncle.

Gametes

The diameter of unfertilized eggs within 1 day old discharged from one specimen reared at 22±1°C (No. 12-13a) is 68±3 μm (64-72 μm, 27). The diameter of unfertilized eggs just discharged from another specimen reared at 22±1°C (No. 12-14) and one specimen reared at 14.5±1°C (No. 12-13g) is 80±3 μm (72-88 μm, 44). Accordingly the newly discharged eggs are larger than the eggs within 1 day old. Although such a size difference is noticed, the range of the diameter of eggs in the present material is nearly the same as that previously described.

The size of sperm was measured in five male specimens. The length of head and middle piece together is 2.6±0.1 μm (2.4-2.8 μm, 40), and the greatest width is 2.1±0.2 μm (1.8-2.4 μm, 37). The length of tail was measured in three of the above specimens: 52±2 μm (49-56 μm, 27).

Early Developmental Stages

Combined 10 female specimens originated from three host specimens of *Barbatia virescens* with 10 male specimens from two host specimens of *B. virescens*, 12 pairs were prepared to obtain their offspring (Table 8), in which several specimens were used twice or three times. The other one pair was also prepared, combining one male specimen originated from *B. virescens* with one female specimen (specimen A-5) originated from *Mytilus edulis galloprovincialis*.

The medusae of both sexes are able to reproduce on and after the 4th day, and even a 58-day-old female medusa whose gonads matured again after once spent has ability to reproduce. But when the medusa is in the spent stage, as an example of the two pairs (one of which two spent females were used), no larvae appeared.

Some early developmental stages from the unfertilized egg to the blastula originated from one pair are shown in Fig. 5, A-F.

The size (length×maximum width, in μm) of planulae two to four days old obtained from three pairs (in one pair the female medusa is originated from *M. e. galloprovincialis*) is 82.4±12.8 (64.0-104.0)×58.0±4.8 (52.0-64.8), 9 in a well-extended condition, while in a contracted condition it is 68.0-74.0×56.0-68.8, 3. The size of planulae about 8 days old is 92.8-98.0×56.0 μm, 2. The cilia is about

32-36 μm in length. The nematocyst equipment of planula was examined in one planula about 1 day old, in two planulae about 2 days old originated from two pairs, and in one planula about 3 days old (Fig. 5, G) whose female parent is originated from *M. e. galloprovincialis*. One kind of nematocysts (? basitrichous isorhizes, Fig 5, H, I) is found and the measurements of the nematocysts (in μm) in the above respective three different ages are as follows: 5.1 ± 0.3 (4.8-5.8)$\times$ 1.8, 11; 5.4 ± 0.6 (4.2-6.4)$\times$ 2.1 ± 0.3 (1.6-2.4), 19; 5.3 ± 0.7 (4.2-6.2) $\times2.2\pm0.1$ (2.0-2.4), 11.

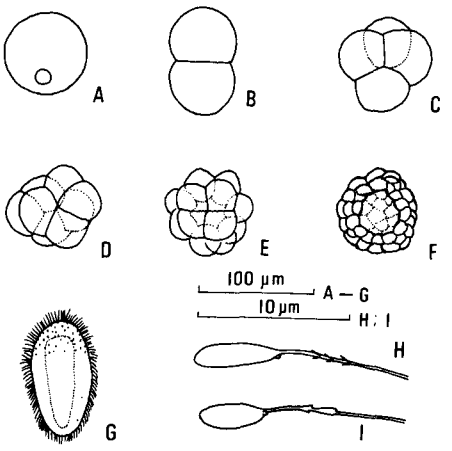

Fig. 5. An unfertilized egg (A), embryos (B-F), a planula larva within three days old (G), and two discharged nematocysts of a planula within three days old (H, I).

Only four out of 40 planulae within 2 days old metamorphosed into the primary polyp in one small specimen of *M. e. galloprovincialis* from Mie Prefecture (Table 8). They attached to the left mantle and two were found on the mantle just beneath the labial palp. In a slightly contracted condition their length and the maximum width of pedal disk is 80-104 μm and 56-72 μm, respectively and the total length except for thickness of pedal disk is up to 64 μm, and the maximum width of hydrocaulus is 48 μm.

Chromosome

As a preliminary chromosomal analysis, the chromosomal number was determined by an ordinary method (a kind of air-drying one). Only the difference is that the time for treating colchicine solution is longer than usual: whole specimens (medusae of different ages) were kept in about 0.005-0.01% colchicine solution in the filtered seawater for up to several days.

The chromosome number could be determined in only three specimens (1 ♀ + 2 ♂♂) among many specimens examined. In one female specimen 5-6 days old (No. 5-2), most of the gonadal cells (18 out of 23) show $2n=30$, though some (5 out of 23) show $2n=28$ or 29. In each of the two male specimens 3-5 days old (Nos. 10-12, 13), the chromosome number could be counted in only one gonadal cell and it is $2n=28$, respectively.

Table 8. Conspecific crossing in 11 pairs used 17 specimens of different ages originated from five specimens of *Barbatia virescens* (Nos. 1, 7, 9, 10, 12) and one specimen (No. A-5) of *Mytilus edulis galloprovincialis*, with the check of offspring viability. Arabic figures show the number of times crossed.

Female specimen number (age in days)			×	Male specimen number (age in days)			Appearance of planula	Size of host prepared for settlement (mm)[1)]	No. of planulae put into each host
7- 3	(8)		×	9- 2	(9)**		yes	—	—
1- 3	(4)	I	×	9- 4	(4)**		yes	—	—
1- 3	(45)	II	×	10-16	(5)	I	yes	31, 32	3, 7
12- 6	(24)	I	×	10-11b	(14.5)	II*	yes	—	—
12- 9	(7)		×	10-16	(22)	III	yes	22, 22, 26, 39	10, 10, 10, 20
12- 8	(7)		×	10-17	(13)	II*	yes	28	4
12-13a	(4)		×	10-21b	(14)		yes	—	—
12-13d	(30)	I	×	10-21e	(16)		yes	30	10
12-13d	(58)	II	×	10-27	(11)		yes	7+10	10
1- 3	(54)	III							
12- 6	(30)	II	×	10-16	(14)		no		
12-23	(13)	I	×	10-32	(10)	I	yes	—	—
12-22	(18)								
12-23	(18)	II	×	10-32	(15)	II	yes	17[≒2)]	40
12-25	(5)								
A - 5	(5.5)	I	×	9- 1	(4)	I	yes	—	—
A - 5	(26.5)	II	×	9- 1	(20)	II	no		

* These specimens are also used for crossing with *Eutima japonica* (see Table 9).

** These specimens have a short peduncle afterwards (see Table 6).

1) Antero-posterior axes of *Mytilus edulis galloprovincialis* from Oshoro Bay (≒ from Gokasho Bay, Mie Pref. collected on May 1, '85).

2) Only to this host four planulae attached, for this host about 1.5 days were given to settle.

Crossability Test with *Eutima japonica*

Seven well-grown laboratory-reared mature medusae of both sexes as shown in Table 6, which are originated from *Barbatia virescens*, appear as an intermediate form between almost all of the specimens of *Eucheilota intermedia* associated with *Mytilus edulis galloprovincialis* and *B. virescens* and the northern form of *Eutima japonica* (see Kubota 1983), because they have a peduncle, many (up to 58) marginal swellings, elongated gonads, sometimes six or eight tentacles, rarely

frilled oral lips. In order to know the degree of the reproductive isolation between the two species which are also different in the generic level, the reciprocal cross was conducted, and if the offspring were produced its viability (in this case a capability of settlement to the host) was checked.

For the materials of *Eutima japonica* the laboratory-reared mature medusae originated from *Mytilus edulis* from Oshoro, Hokkaido were used. Combining seven male specimens of *Eucheilota intermedia* with five female specimens of *Eutima japonica*, nine pairs were prepared, in which several specimens of both species were used up to five times (Table 9). Except for one pair whose female (No. 1-3) was spent at that age, all the pairs produce their offsprings as the conspecific cross described above.

The size (length×maximum width, in μm) of such a planula about three or four days old was measured in 14 specimens: 85.2±14.0 (70.0-108.0)×57.6±6.0 (48.0-66.0), 14 in a well-developed condition, while 64.0-92.0×56.0-76.0, 4 in a contracted condition. The size of planula about 6 days old is 64.0-84.0×48.0-56.0 μm, 4, which is slightly smaller than the above younger one. The

Table 9. Crossability test between the male medusa of *Eucheilota intermedia* and the female medusa of *Eutima japonica*, with the check of offspring viability. Arabic figures show the number of times crossed.

Female specimen number (age in days)		×	Male specimen number (age in days)		Appearance of planula	Size of host prepared for settlement (mm)[1)]	No. of planulae put into each host
1-1 (21)		×	9- 3 (25)**		yes	—	—
1-2 (21)		×	9- 6 (16)		yes	39	9
1-3 (23)	I	×	10- 9 (5)		yes	42, 43, 44	10, 15, 10
3-1 (17)	I	×	10-15 (6.5)	II	yes	27	4
1-4 (43)	I	×	10-11b (8.5)	I*	yes	33, 36	10, 5
1-3 (43)	III	×	9- 7 (33)	I**	yes	35, 37	10, 10
1-3 (50) 1-4 (50) 3-1 (28)	IV II II	×	9- 7 (40)	II**	yes	—	—
1-3 (55) 1-4 (55) 3-1 (33)	V III III	×	10-17 (5)	I*	yes	25	6
1-3 (37)	II	×	10-15 (4.5)	I	no		

* Also used for conspecific crossing (see Table 8).

** These specimens have a short peduncle at these developmental stages (cf. Table 6).

1) Antero-posterior axes of *Mytilus edulis* from Oshoro.

Table 10. Crossability test between the female medusa of *Eucheilota intermedia* and the male medusa of *Eutima japonica*, with the check of offspring viability.

Female specimen number (age in days)	×	Male specimen number (age in days)	Appearance of planula	Size of host prepared for settlement (mm)[1)]	No. of planulae put into each host
12- 3 (6)	×	2-1 (11)	yes	—	—
12-10 (7)	×	4-1 (10)	yes	19, 34	10, 10
12-11 (7)	×	4-2 (13)	yes	26	1
12-12 (6)	×	4-3 (13)	yes	18, 23, 32	10, 10, 8

nematocyst equipment of the 'hybrid' planula was examined. All the specimens examined, 16 in number, produce nematocysts, and the ones older than the 3-day-old have two types of nematocysts. The measurements of nematocysts (in μm) of planulae at different ages are as follows: 5.4 ± 0.5 (5.0-6.4) $\times 2.2\pm0.2$ (1.8-2.2), 10, examined in one planula 1 day old; 6.2 ± 0.8 (5.0-8.0) $\times 2.4\pm0.2$ (2.2-2.8), 14, in two planulae 2 days old; large type, 6.5 ± 0.3 (5.8-7.2) $\times 2.8\pm0.3$ (2.4-3.4), 12, in three planulae 3 or 4 days old; small type, 5.9 ± 0.4 (5.0-6.8) $\times 2.1\pm0.3$ (1.4-2.6), 61, in four planulae 3 or 4 days old; large type, 6.4 ± 0.5 (5.0-7.2) $\times 2.6\pm0.5$ (2.0-3.4), 21, in three planulae 6 days old; small type, 5.9 ± 0.3 (5.6-6.4) $\times 1.7\pm$ 0.1 (1.6-2.0), 20, in three planulae 6 days old.

Such a hybrid planula is the same size as the planula reproduced from the conspecific cross described above. However, the nematocysts of the hybrid planula are larger than those of the latter planula of the same or different ages.

Besides the above nine pairs, other four pairs were prepared, combining four female specimens of *Eucheilota intermedia* with four male specimens of *Eutima japonica* (Table 10). All these pairs can also produce similar planulae described above.

A small number of planulae within several days old, which were produced from a specified pair, was put into a small vessel in which one specimen of *M. edulis* was reared at 22 ± 1°C. The bivalve, which attached to buoys or rock, was collected from Oshoro, Hokkaido on October or November, 1984 and kept in laboratory for a short period. One day was given for the larva to settle on each host. After exchanging the seawater the bivalve was preserved by formalin solution on the second day after narcotization. After dissecting each host, the number of planulae settled was counted. But no primary polyps were found on any body portion of the host. The same result was obtained in the other viability test of the larvae reproduced from the conspecific crossing (control experiment) except for one trial in which the host was collected from Gokasho Bay, adjacent to Ago Bay from which the present material was collected (Table 8). Therefore the degree of offspring viability of the hybrid planula could not be clarified. The

reason why most of planulae failed to settle is unknown, but in one possibility larvae might be eaten up by most of the host.

In conclusion, despite of the wide morphological variation revealed by the present study, the external morphology of *Eucheilota intermedia* is clearly distinguished from that of *Eutima japonica*, and there is no reproductive isolation between them as demonstrated above. The lack of distinct morphological difference in the gametes of these two species seems to be related to this phenomenon. Such facts indicate that these two species are phylogenetically very close, supporting an opinion that they constitute one monophyletic group (see Kubota 1984) and at least their familial level is not different. Moreover, a taxonomic problem arises: "Are these hydroids truly two separate species ?"

Summary

Eucheilota intermedia Kubota associated with six specimens of *Barbatia virescens* and one specimen of *Mytilus edulis galloprovincialis* from Zagashima Is., Mie Prefecture is described in the life stages such as gametes, embryos, larva, polyp (from the primary polyp to a well-developed one with medusa-bud), and medusa (from the youngest to the spent one) through the rearing of the material in laboratory. Besides the age variation, other types of the variation such as individual or intraclonal variation, sexual variation, accidental or teratological variation, and the variation induced by different water temperature are particularly paid attention to. The present material shows a wide morphological variation which exceeds the variation range in the original description. This is especially ascribed to the existence of seven medusae which grow into forms with the undescribed characteristics such as (1) a short peduncle, measuring up to 0.5 mm in length, (2) usually four tentacles, but up to six, extraordinary eight ones, (3) an elongated gonads produced along the most parts of the radial canals, (4) a crusiform oral lips but rarely once frilled ones, (5) many (up to 58) marginal swellings, and (6) a large body size, up to 7.8 mm in diameter. They resemble the mature and spent medusa of *Eutima japonica* Uchida. The reciprocal cross between these two species demonstrates that no reproductive isolation is found between them despite of their distinct morphology, indicating at least their familial level is not different.

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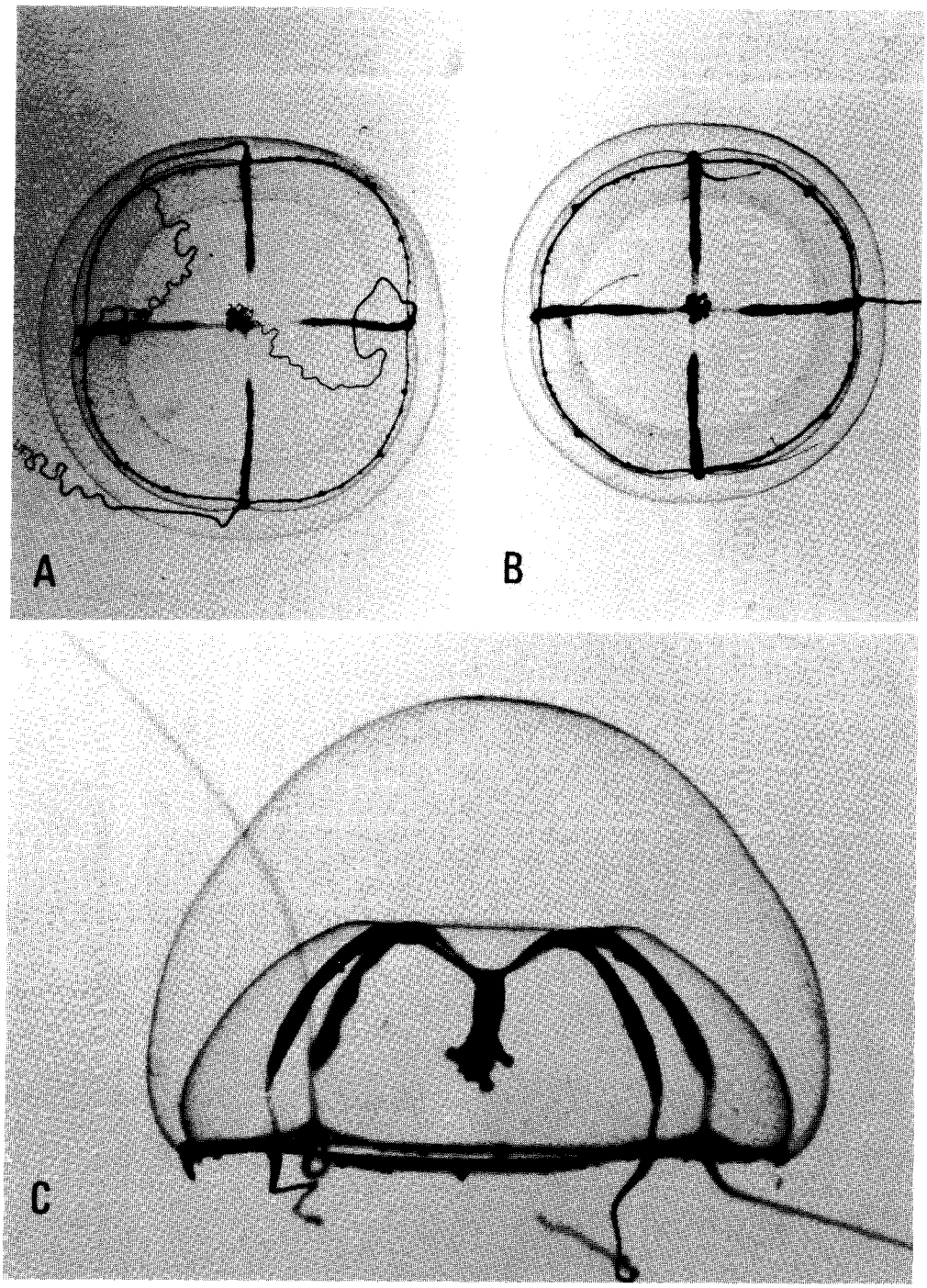

S. Kubota: Systematic Study on Eucheilota intermedia

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Explanation of Plate I

A: a female medusa 54 days old reared at 14.5±1°C (No. 12-13g), ca. 6.8 mm in diameter. Note 40 marginal swellings with cirri. B, C: a female medusa 50 days old reared at 12.5±1°C (No. 12-13i), ca. 7.1 mm in diameter. Note only 24 marginal swellings and a short peduncle.