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Morphological Studies on the Bathyal Ascidian, *Megalodicopia hians* Oka 1918 (Octacnemidae, Phlebobranchia), with Remarks on Feeding and Tunic Morphology

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ABSTRACT—*Megalodicopia hians* Oka is a solitary ascidian belonging to the family Octacnemidae inhabiting the bathyal /abyssal zone as well as other octacnemid ascidians. The phylogenetic relationship of octacnemids is open to argument because of its extraordinary morphological features due to habitat adaptation, e.g., a pharynx lacking ciliated stigmata. Aggregations of *M. hians* were discovered by the manned submersible *Shinkai 2000* in the bathyal seafloor of Toyama Bay, Japan Sea, in 2000; this was the first *in situ* observation of *M. hians* in the Japanese coastal waters. In 2001, a total of 36 *M. hians* specimens were collected from the bay (592~978 m deep). *In situ* observation indicated that *M. hians* usually opens its large oral apertures to engulf the drifting food particles in the water current. Microscopical observation of the gut contents also showed that *M. hians* is a non selective macrophagous feeding on small crustaceans, diatoms, detritus, and so on. Along with the position of the intestinal loop and gonad, the morphological characteristics of the tunic (integument of ascidians) suggest that *M. hians* is closely related to Cionidae and/or Corellidae. Some symbiotic/parasitic organisms were occasionally found in the tunic, including rod-shaped bacteria, fungi-like multicellular structure, and spawns of unknown animals.

Key words: octacnemid, food habit, parasites, *Shinkai 2000* (JAMSTEC), deep sea

INTRODUCTION

Megalodicopia hians Oka is a solitary ascidian belonging to the family Octacnemidae (Phlebobranchia; Enterogona). Because the members of this family exclusively inhabit the deep sea, many aspects of their life are undisclosed to date. Unlike most of the other ascidians, an octacnemid species has an extraordinarily large oral aperture and its pharynx lacks ciliated perforations (stigmata). This unique morphology is thought to be an adaptation to life in the deep sea (Berrill, 1950; Kott, 1969). In general, it is difficult to obtain undamaged specimens of soft-bodied benthos of the bathyal /abyssal zone for histological and ultrastructural investigation because they are usually collected by means of dredging. In July 2001, live specimens of *M. hians* were collected by the submersible *Shinkai 2000* of the Japan Marine Science and Technology Center (JAMSTEC) in three dives (Dives 1278, 1279, and 1280) at Toyama Bay, Japan. Some of the specimens were immediately fixed on board the support vessel, *Natsushima*. Using these undamaged specimens, we studied the morphology of *M. hians* with special reference to the contents of the alimentary canal and tunic.

Most ascidians always filter-feed; ciliated perforations of the branchial wall (stigmata) generate water current into the branchial basket, and the mucus net catches the small particles and organisms such as microalgae (Cf. Monniot *et al*., 1991). By contrast, octacnemids do not have ciliated stigmata and they might directly engulf large particles and organisms such as small invertebrates (Monniot and Monniot, 1978), and they are therefore thought to be macropha-

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gous (Millar, 1959). Microscopic investigations on the gut contents thus seem to be essential to obtaining a better understanding of the feeding habits of these species.

Integumentary tissue has a variety of protective functions as an interface between the internal animal body and the external environment. The tunic is a unique integument of the ascidian containing both cellulosic components and free mesenchyme-like cells called tunic cells (Cf. Burighel and Cloney, 1997). The ascidian tunic always contains several types of tunic cells supporting the various functions of the tunic. In *M. hians*, histological study of the tunic could disclose the special adaptation to the bathyal /abyssal environment. Moreover, knowledge of the tunic morphology would likely provide valuable information with regard to consideration of ascidian phylogeny. The phylogenetic position of Octacnemidae is still a matter of controversy. Although the morphology of the branchial basket is an important factor in classification in ascidians, that of octacnemids differs too much different from that of other families of ascidians to discuss the phylogenetic relationships. On the other hand, the tunic is a typical synapomorphy of ascidians and thaliaceans, and we have attempted to consider ascidian phylogeny based on tunic morphology. For this purpose, a comprehensive survey was made of the fine structures of the tunic cuticle and the presence of some types of tunic cells, with the results revealing the general stability of the character-state distribution of the examined characteristic forms (Hirose et al, 1997; Hirose, 2001). However, no octacnemids could be examined in the previous studies because undamaged specimens were not available.

The present study describes the habitat, gut contents, and tunic morphology (tunic cuticle and tunic cells) of *M. hians* with regard to a discussion of its feeding and phylogenetic relationships to other ascidians. As for tunic morphology, we also report on the bacterial inclusions, fungi-like structures, and spawning of other animals in the tunic.

MATERIALS AND METHODS

Animal collection

Animals were collected at ten sites in Toyama Bay, Japan during the deep-sea dives by the manned submersible *Shinkai 2000* (JAMSTEC) (Table 1). *In situ* habitation and behavior of *M. hians* in response to disturbance by the submersible were observed and partly video-recorded. A total of 36 live specimens were collected by gentle grab with the submersible's manipulator at depths ranging from 592 m-978 m as summarized in the table. The specimens were fixed with 10% formalin-seawater or 2.5% glutaraldehyde-seawater on board the support vessel.

Light and electron microscopy

Some fixed tunic pieces were cut into slices approximately 0.5 mm thick with a razor blade and observed with a light microscope equipped with Nomarski differential interference contrast optics (DIC).

For the paraffin sections, the formalin-fixed specimens were dehydrated through an ethanol-xylol or butanol series and embedded in paraffin. Serial sections of 7–10 μm thick were stained with Delafield's hematoxylin and eosin.

For the resin sections, the glutar-fixed specimens were post-fixed with 1% osmium tetroxide-0.1 M sodium cacodylate (pH 7.4) for 2 hr, dehydrated through an ethanol series, cleared with *n*-butyl glycidyl ether and embedded in low-viscosity epoxy resin. Sections of 1 μm thick were stained with toluidine blue for light microscopy. Thin sections were doubly stained and observed in a transmission electron microscope (JEM-1010, JEOL) at 80 kV.

RESULTS

General morphology and habitat

Megalodicopia hians is composed of a body that looks like a ripe pomegranate and a long peduncle protruding from the body (Fig.1A). The body contains the large oral aperture and main organs of the ascidian such as the branchial basket, stomach, heart, and gonads. The oral aperture has huge anterior and posterior lobes. Each lobe has many parallel circular muscle fibers, and longitudinal muscles run across them. The muscle fibers are crowded at the oral aperture and make a broad muscular band at the margin of the lobes. These muscle fibers become more thinly spaced as they are apart from the lobe margin. Branchial basket is unfolded and has some indistinct transverse and longitudinal vessels. There are numerous oral tentacles of about 1mm long. The short endostyle, about 1 cm long, is present along the median line in the ventral side of the branchial basket. The unciliated branchial perforations (stigmata) are rectangular and 200–300 μm in diagonal. They are larger and more densely scattered in the anterior region of the branchial basket than in the posterior. A small atrial siphon opens at the antero-dorsal edge of the body. The gut forms a simple loop and is positioned on the right side of the body. The stomach is oval and irregularly folded on its surface. Gonads are in the gut-loop, and when the organism is alive, it is white in color. The ovary forms a spherical mass and it is situated anterior to the hemisphere-shaped testis. In the collected specimens, the length from the top of the body to the end of peduncle was found to be 5~26 cm (Table 1). The lengths did not show any correlation with the depths of the collection sites. The tunic was found to be very thin, and its surface was often attached with hydrozoans. Some other solitary ascidians adhered to the bodies of the eight specimens collected from the same station at a depth of 700 m (1280 B-1c, 1d, 1e, 1g, 1i, 1j, 3d, 3e; 37°07.846'N, 137°09.680'E).

M. hians was attached on a rock bed of the slope with the posterior end of the peduncle. The peduncle is provided with a pair of thick longitudinal muscular bands in the ventral part. These muscular bands begin from the posterior end of the body. The rock bed was covered with muddy sediment. At the collection sites, water temperature was approximately 0.3°C, dissolved oxygen was 4.6~4.7 ml/L, and the velocity of the water current was 0.03~0.32 m/s. Although the current direction at the *M. hians* aggregations was not precisely determined, up-slope currents at <850 m deep have previously been reported (Odamaki and Kikuchi, 1985). Most of the *M. hians* individuals oriented the wide-opened oral aper-

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Table 1.

Dive #	Location	Depth (m)	Sample #	body length (cm)
1278	37°04.805'N, 137°09.374'E	841	1278 B-1a	16.0
			1278 B-1b	16.4
	37°04.909'N, 137°09.144'E	643	1278 B-2a	18.2
			1278 B-2b	14.8
			1278 B-2c	12.4
			1278 B-2d	7.8
	37°04.930'N, 137°09.116'E	600	1278 B-3a	11.7
			1278 B-3b	11.1
			1278 B-3c	12.7
1279	37°09.648'N, 137°12.366'E	978	1279 B-1a	13.8
			1279 B-1b	15.9
	37°09.678'N, 137°12.310'E	942	1279 B-1c	15.8
			1279 B-1d	14.4
	37°10.146'N, 137°11.458'E	614	1279 B-1e	15.3
	37°10.149'N, 137°11.376'E	592	1279 B-2a	
1280	37°07.846'N, 137°09.680'E	700	1280 B-1a	26.2
			1280 B-1b	14.3
			1280 B-1c	12.8
			1280 B-1d	17.6
			1280 B-1e	17.1
			1280 B-1f	
			1280 B-1g	18.8
			1280 B-1h	5.0
			1280 B-1i	19.2
			1280 B-1j	
			1280 B-1k	
			1280 B-1l	
	37°07.846'N, 137°09.664'E	693	1280 B-2a	12.5
			1280 B-2b	9.0
			1280 B-2c	16.0
			1280 B-2d	
	37°07.846'N, 137°09.680'E	700	1280 B-3a	14.2
			1280 B-3b	
			1280 B-3c	
			1280 B-3d	10.0
			1280 B-3e	9.1

We could not measure some samples because of being torn off at the collection.

ture to down-slope direction, *i.e.*, in the counter-direction to the presumed upwelling currents (Fig.1B, C). Tens of individuals attached to hard substrates such as a thinly sedimented rock bed, forming a patchy aggregation of ranging from approximately a few to several m^2. Patchy aggregations were scattered along the rock-exposure area of the slope, with several patches occurring over the migrated rocks on the soft sediment floor. The *M. hians* individuals showed quick (>5 sec) protection behavior during the submersible's approach to the animals. They closed their mouth apertures and exhibited a crouching action in response to sampling by the manipulator. It was likely that the organisms were reacting to the turbulence caused by the manipulator rather than the light and noise disturbance caused by the submersible.

We could see many small invertebrates such as crusta-

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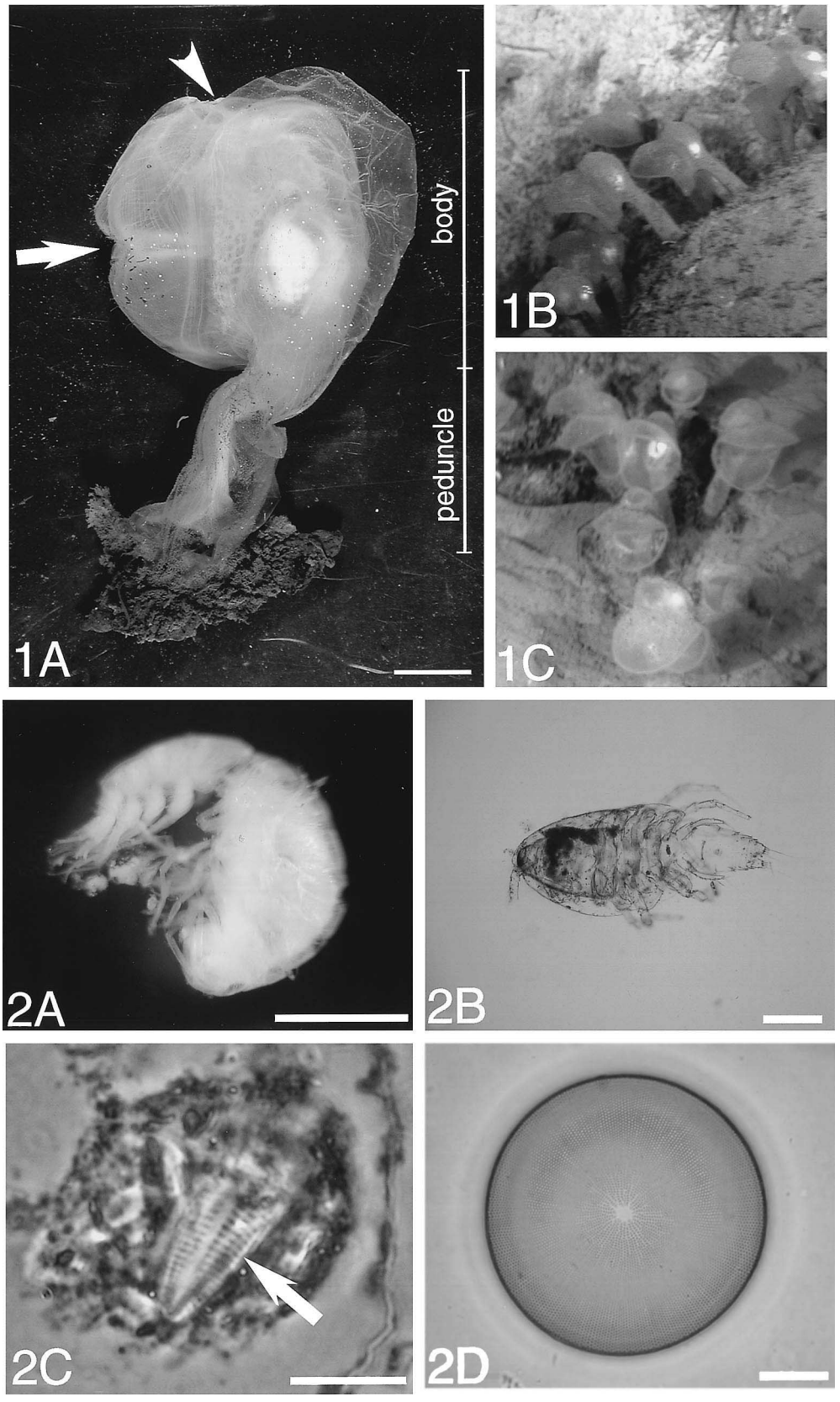


Fig. 1. The fixed specimens *Megalodicopia hians* Oka (A). Arrow indicates the oral aperture, and arrowhead indicates the atrial aperture. This ascidian is consisted of body and the peduncle. Scale bar, 2cm. Aggregation of *M. hians* on the slope (B and C). Animals face to the same direction (B, lower left corner, C, lower side). **Fig. 2.** Diet of *M. hians*. Small crustaceans found in the oral aperture (A) and feces (B). Diatoms found in the gut (C) and feces (D). Scale bars; (A) 1mm, (B) 200 μm, (C) 10 μm, (D) 200 μm.

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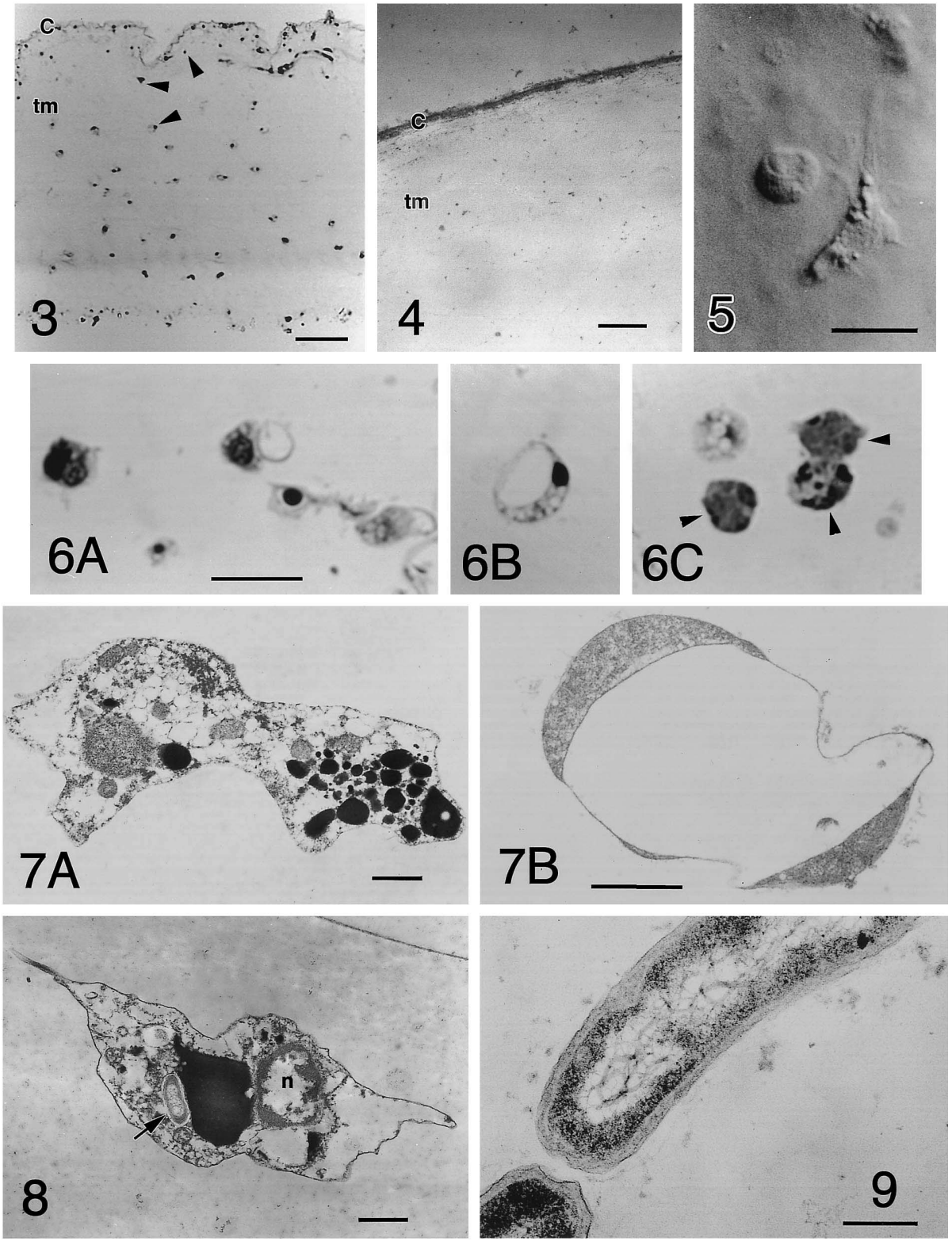


Fig. 3. A cross-section of the tunic (paraffin section stained with hematoxylin and eosin). Tunic cuticle (c) covers tunic matrix (tm). Arrowheads indicate some tunic cells distributed in the tunic matrix. Scale bar, 50 μm. **Fig. 4.** Electron micrograph of the tunic cuticle (c) covering tunic matrix (tm). Scale bar, 0.2 μm. **Fig. 5.** Tunic cells observed with DIC optics. Scale bar, 10 μm. **Fig. 6.** Tunic cells in resin sections of the tunic (stained with toluidine blue). Arrowheads in C indicate the tunic cells containing metachromatic granules. Scale bar, 10 μm. **Fig. 7.** Electron micrographs of tunic cells. An amoeboid-shaped cell with electron-dense granules and vesicles (A) and a spherical cell with a large vacuole (B). Scale bars, 1 μm. **Fig. 8.** Tunic cell containing a bacterium (arrow). n, nucleus of the tunic cell. Scale bar, 1 μm. **Fig. 9.** Rod-shaped bacteria distributed in the tunic matrix. Scale bar, 0.2 μm.

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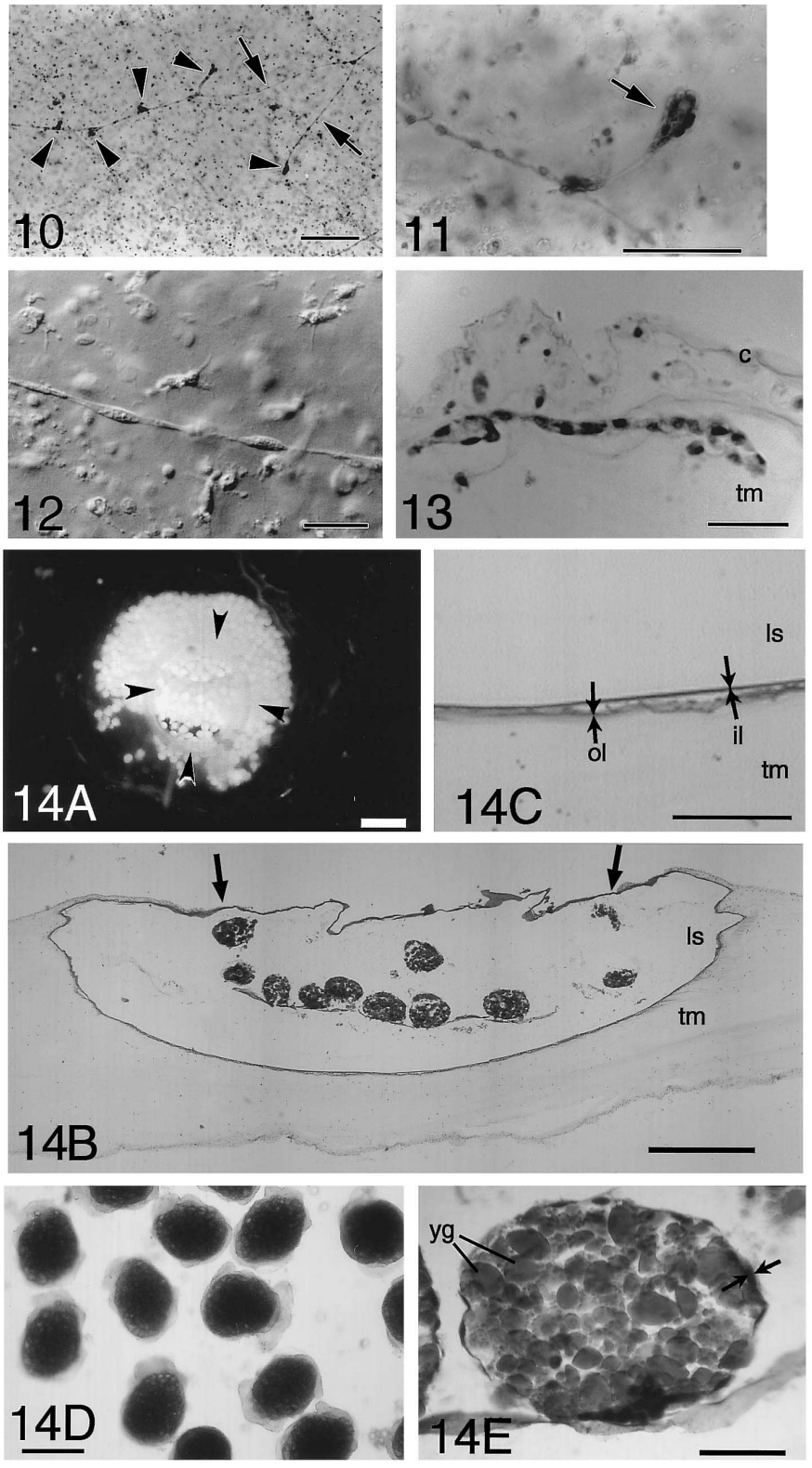


Fig. 10. Multicellular structure (arrows) in the tunic. Arrowheads indicate branchlets. Scale bar, 100 μm. **Fig. 11.** The terminus of the sac-shaped branchlet (arrow) protrudes outside the tunic cuticle. Scale bar, 50 μm. **Fig. 12.** Multicellular cord in the tunic observed with DIC optics. Scale bar, 20 μm. **Fig. 13.** Multicellular cord in the tunic matrix (tm) (paraffin section). c, cuticle. Scale bar, 20 μm. **Fig. 14.** Sac-like structure containing spawn in ascidian tunic. (A) The sac observed through the tunic surface. Arrowheads indicate the edge of the tunic covering. (B) Cross -section of the sac. Arrows indicate the periphery of the tunic. (C) Enlargement of the shell of the sac. (D) Eggs /embryos in the sac. (E) Histological section of an egg. Arrows indicate the ectodermal layer. il, inner layer; ls, lumen of the sac; ol, outer layer; tm, tunic matrix; yg, yolk granule. Scale bars; (A) 1mm, (B) 500 μm, (C) 100 μm, (D) 200 μm, (E) 50 μm.

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ceans, chaetognaths, and so on, swimming around the lights of the submersible. They also swam around the ascidians. Many fragments of crustaceans and diatoms were found in the gut and feces of *M. hians*. We observed a crustacean caught in the lobe of the oral aperture of one specimen (1278 B-2b; Fig. 2A). The sizes of the fragments were various, and whole shells of crustaceans were sometimes found in the feces (Fig. 2B). Many diatoms were also found in the gut (Fig. 2C) and feces (Fig. 2D).

Tunic morphology

The tunic of *M. hians* is gelatinous and semi transparent. The tunic matrix is overlaid with a thin layer of tunic cuticle (Fig. 3). Tunic cells were found throughout the tunic matrix, and many of them were distributed with bias just beneath the tunic cuticle. There were no blood vessels found in the tunic. The tunic cuticle was found to be approximately 50 nm thick or less and composed of electron-dense, fibrous materials (Fig. 4). The cuticular surface was found to be flat with no protuberant structures.

There appear to be several types of tunic cells; some spherical-shaped and others amoeboid-shaped (Fig. 5). Amoeboid-shaped cells were found to contain granules and vesicles (Fig. 6A). Some spherical cells have a large vacuole (Fig. 6B), and some contain metachromatic granules (Fig. 6C). Although electron micrographs showed granular and vesicular inclusion in the cytoplasm, the fine structures of organelles were not preserved well (Fig. 7).

Bacteria were occasionally found in the cytoplasm of tunic cells (arrow in Fig. 8). Because the bacteria appeared to be enclosed in the membrane, they were probably endocytized by the tunic cell. The bacteria were also found in the tunic matrix (Fig. 9). Many of them seemed to be distributed in the sub cuticular area. All bacteria both inside and outside the cells were of the same morphological type.

Multicellular structures were often found beneath the tunic cuticle (Fig. 10), though these structures were not distributed in any other part of the tunic. A long multicellular cord was found to have several sac-shaped branchlets (arrowhead in Fig. 10), and the termini of the branchlets protruded out through the tunic cuticle (arrow in Fig. 11). The cord sometimes branched but connected with neither other cords nor any other structures. The histological sections showed that the cord consists of spindle-shaped cells forming a line and runs parallel to the tunic surface (Fig. 12, 13).

Spawn in the tunic

Sac-like structures of 5-6 mm in diameter were found in the tunic of the two specimens collected at a depth of 978 m (1279 B-1a; 37°09.648'N, 137°12.366'E) and 942 m (1279 B-1d; 37°09.678'N, 137°12.310'E), respectively. These structures were exclusively found in the right-side tunic of the ascidian body. One specimen (1279 B-1a) had six sacs, and the other (1279 B-1d) had four. These sacs were adjacent to one another, and contained many eggs / embryos (Fig. 14A, 14B). The sac was almost embedded in the tunic, except for the center of the upper side of the sac (Fig. 14B). The edge of the tunic covering the sac was circular (arrowheads in Fig. 14A). The eggs /embryos were white and spherical, approximately 200 μm in diameter (Fig. 14B, 14D). In the histological observation, the shell of this sac consisted of two layers (Fig. 14C). The outer layer was continuous with the tunic matrix, but it had no direct connection with the inner layer. The inner layer was well stained with hematoxylin. The center of the upper side of the sac was not covered by either the outer layer or the tunic. In the histological section of the eggs /embryos, a layer of ectoderm entirely covered the ellipsoidal yolk granules (Fig. 14E).

DISCUSSION

Taxonomical Remarks

The morphologic characteristics of specimens collected in this study are consistent with the original description of *Megalodicopia hians* made by Oka (1918) and the record by Tokioka (1953). These records are based on specimens collected from the Japan Sea at a depth of 360 m and from Sagami Bay (Japan), which faces to the Pacific Ocean at 350 m, respectively. Sanamyan (1998) has observed the morphology of *M. hians* collected from the South Kurile Islands and redescribed the record of this species from the Weddell Sea, Antarctica by Kott (1969). The specimen collected from South Kurile Islands was "gradually tapering to the base of the peduncle. Peduncle not distinctly demarcated from body." However, the bodies were obviously constricted at the junction with the peduncles in the specimens investigated here and in the specimens described by Oka (1918) and Tokioka (1953). Therefore, it might be possible that the specimen from the South Kurile Islands is not of the same species as the Japanese *M. hians*.

Food habit

Most of the ascidians are filter-feeders. Water enters the branchial basket from the branchial aperture, and the captured particulate materials are carried by the mucus towards the oesophagus. The water current is made by the ciliary movement of stigmata. However, because *M. hians* does not have ciliated stigmata, it is not able to produce water current by itself. *In situ* observation showed that most of the animals face their oral apertures in the same direction, keeping their apertures open. This finding may suggest that *M. hians* utilizes the water current for feeding. Because there is a water current (0.03~0.32 m/s) in their habitat, this ascidian might catch relatively large materials drifting in the water currents and engulf them without distinction. Various sizes and kinds of animal fragments and diatoms were observed in the gut of this ascidian. These findings also support other evidence of the nonselective feeding of this species. Based on a previous study, Monniot and Monniot (1978) have also suggested the presence of weak filtration and a mixed diet such as one including fine organic ele-

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ments and small crustaceans in octacnemid species based on the morphology of the branchial basket. In another abyssal group, the family Hexacrobylidae, the animals are thought to catch small invertebrate with their 6-lobed oral siphon (Cf. Monniot *et al*., 1975). The present species, however, does not have large tentacle-like structures, and such catching-behavior was not recorded in the present *in situ* observation.

Tunic morphology and parasitic inclusions

The tunic of *M. hians* is basically the same as those of other ascidians. In *M. hians*, the tunic is not a tough integument, because the tunic cuticle is very thin and the tunic matrix is gelatinous. If the animal needs to be protected from predators and/or infectious organisms, cellular or chemical defenses should be available in the tunic. The biased distribution of tunic cells around the tunic surface may indicate their defensive function. Although there are several types of tunic cells, the function of each type is uncertain. Some amoeboid-shaped tunic cells containing granules and vesicles are similar in morphology to tunic phagocytes in *Aplidium yamazii* (Hirose *et al*., 1994) and some other species. In the present study, the fine structures of the tunic cells were not well-preserved; some vacuoles appeared to have swelled, and the cell membranes were sometimes discontinuous. Deformation and puncture of tunic cells might have been caused by the rapid change in hydraulic pressure during animal collection.

The ascidian tunic usually contains rod-shaped bacteria of one or two morphological types, and the tunic of *M. hians* also contains bacteria that are mostly distributed beneath the cuticle. Bacteria are also found in the vesicles of the amoeboid-shaped cells, and the bacteria would be endocytized by tunic cells. It is unknown whether the bacteria are symbionts for the ascidians.

The multi-cellular structure in the subcuticular area seems to be hyphae of fungi. Although this is the first time we have found fungi-like structures in the tunic matrix, the occurrence of algal and fungal filaments have been reported in some ascidians (Lambert *et al*., 1996).

Sac-like structures contained spawn were found in the tunic of *M. hians*. The shell of the sac was found to consist of two layers. The outer layer was continuous to the tunic matrix and seemed to be a tunic cuticle that was restored after spawning. No direct connection was detected between the inner layer and tissue of ascidian, and, as such, the inner layer might be derived from the spawner. Although there is no doubt that this sac and spawn are not derived from the ascidian, the spawner is uncertain. This is the first report regarding the occurrence of spawn in the ascidian tunic to our best knowledge. In the bathyal fauna, because there are few materials such as seaweed and rocks protruding from the rock bed covered by muddy sediment, the animal might utilize the ascidian as a substratum for spawning.

Phylogenetic consideration based on tunic morphology

It is generally agreed that Octacnemidae is a member of the suborder Phlebobranchia (Cf. Tokioka, 1953; Monniot *et al*., 1991; Kott, 1985), but its phylogenetic relationship with other phlebobranchian families remains controversial. For example, Tokioka (1953) puts *M. hians* in “Aberrant forms” with some other genera. He states that these genera undoubtedly belong to Phlebobranchia but that the relationships among them and with other families are uncertain. While most members of Phlebobranchia have the gut-loop on the left side of their body, the gut-loop is situated on the right side of the body in the family Corellidae and Octacnemidae families, possibly suggesting that Octacnemidae is closely related to Corellidae (Kott, 1985). On the other hand, Millar (1956; 1959; 1966) have pointed that the octacnemid species has evolved from an ancestral form of ascidians like *Ciona* based on observation of *Octacnemus bythius*, and that octacnemids might be related to the Cionidae.

Here, we provide a brief phylogenetic discussion of *M. hians* based on the tunic morphology. The fine structures of the tunic cuticle were investigated in 116 species covering the all families and subfamilies of the class Ascidiacea except for Octacnemidae, Plurellidae, and Dimeatidae, which are all bathyal or abyssal species (Cf. Hirose *et al*., 1997). The tunic cuticles of some ascidians have minute protrusions, and there is a general tendency for the protrusions occurs in limited families. Of the 27 phlebobranchians, cuticular protrusions were exclusively found in *Pterygascidia longa* (Cionidae) and *Adagnesia vesiculiphora* (Agneziidae). In *M. hians*, the tunic cuticle has a flat surface and no protrusions, and this character-state is the same as that of most other phlebobranchians species.

The presence or absence of the two types of tunic cells (tunic bladder cells and tunic net cells) was surveyed in 65 ascidian species covering 11 families (Hirose, 2001). As for phlebobranchians, the survey did not cover Agneziidae, Octacnemidae, Plurellidae, and Dimeatidae. Of the 14 phlebobranchian species, tunic bladder cells were exclusively distributed in Diazoninae and Ascidiidae, while tunic net cells were not found in any phlebobranchians. The tunic of *M. hians* has neither tunic bladder cells nor tunic net cells. This character-state may suggest some affinity to Cioninae, Corellidae, or Perophoridae, although 3 other phlebobranchian families have not yet been examined. Some corellids and ascidiids are known to have giant cells that are very large hemocytes containing sulfuric acid (Wright, 1981). The hemocytes found in the branchial wall of *M. hians* did not include giant cells (data not shown).

In the present report we first described the histological and fine structures of the tunic in Octacnemidae. Here, we could deal with only one species, *M. hians*, and it is uncertain whether the present observations can represent all octacnemids. Moreover, other bathyal or abyssal families might have independently originated from other lineages of ascidians. Although deep-sea ascidians have been poorly studied because of their limited availability, a further accu-

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mulation of their data is necessary to more fully disclose details of their life, phylogeny, and environmental adaptation.

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REFERENCES

Berrill NJ (1950) The Tunicata. 354pp The Ray Society, London

Burighel P, Cloney RA (1997) Urochordata: Ascidiacea. In "Microscopic Anatomy of Invertebrates Vol. 15" Ed by FW Harrison and EE Ruppert, Wiley-Liss, New York, pp 221–347

Hirose E, Ishii T, Saito Y, Taneda Y (1994) Phagocytic activity of tunic cells in the colonial ascidian *Aplidium yamazii* (Polyclinidae, Aplousobranchia). Zool Sci 11: 203–208

Hirose E, Lambert G, Kusakabe T, Nishikawa T (1997) Tunic cuticular protrusions in ascidians (Chordata, Tunicata): A perspective of their character-state distribution. Zool Sci 14: 683–689

Hirose E (2001) Acid containers and cellular networks in the ascidian tunic with special remarks on ascidian phylogeny. Zool Sci 18: 723–731

Kott P (1969) Antarctic Ascidiacea. Antarct Res Ser 13: 1–239

Kott P (1985) The Australian Ascidiacea Part 1, Phlebobranchia and Stolidobranchia. Mem Qd Mus 23: 1–440

Lambert G, Lambert C, Waaland JR (1996) Algal symbionts in the tunics of six New Zealand ascidians (Chordata, Ascidiacea). Invertebr Biol 115: 67–78

Millar RH (1956) Structure of the ascidian *Octacnemus* Moseley. Nature 178: 703–704

Millar RH (1959) Ascidiacea. Galathea Rep 1: 189–209

Millar RH (1966) Evolution in ascidians. In "Some contemporary studies in marine science" Ed by Harold Barnes, pp 519–534

Monniot C, Monniot F, Gaill F (1975) Les Sorberacea: Une nouvelle classe de tuniciers. Arch Zool Exp Gén 116: 77–122

Monniot C, Monniot F (1978) Recent work on the deep-sea tunicates. Oceanogr Mar Biol Ann Rev 16: 181–228

Monniot C, Monniot F, Laboute F (1991) Coral reef ascidians of New Caledonia. ORSTOM Paris, pp 247

Odamaki M, Kikuchi S (1985) Water movement and fine topographic structure in Toyama deep sea bottom. JAMSTEC Deep Sea Res 1: 17–22 (in Japanese with English abstract)

Oka A (1918) *Megalodicopia hians* n. g., n. sp., eine sehr merwurdige Ascidia aus dem japonischen Meere. Annot Zool Japon 9: 339–406

Sanamyan K (1998) Ascidians from the north-western pacific region. 5. Phlebobranchia. Ophelia 49: 97–116

Tokioka T (1953) Ascidians of Sagami Bay. Iwanami Shoten, Tokyo, pp 315

Wright RK (1981) Urochordates. In "Invertebrate blood cells Vol. 2" Ed by NA Ratcliffe and AF Rowley, Academic Press, New York, pp 565–626

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