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# Diet of a Notodelphyid Copepod Inhabiting in an Algal-bearing Didemnid Ascidian *Diplosoma virens*

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**ABSTRACT**—A notodelphyid species inhabits in the colonial ascidian, *Diplosoma virens* that contains photosynthetic symbionts *Prochloron* sp. in the cloacal canal. The gut contents of the notodelphyids were examined by light and electron microscopy to estimate their diet. Since the gut contents are almost homogeneous, the notodelphyids are probably monophagous. The histochemical stainability of the gut contents suggests that they mainly feed on the host tunic. Although the host faeces and the fragments of the algal symbionts are sometimes found in the gut contents, they would be accidentally ingested by the notodelphyids.

## INTRODUCTION

Notodelphyids are parasitic or commensal copepods of ascidians and octocorals. In ascidians, notodelphyids usually inhabit in peribranchial cavity, common cloacal canals, pharyngeal lumen, intestinal lumen, or circulatory system (reviewed in Gotto, 1979; Monniot, 1990). The diet of each species is probably specialized depending on their habitat: e.g., faecal material, mucus, contents of the host's alimentary canal, blood cell and blood fluid.

Prokaryotic algae, *Prochloron* spp., are exclusively distributed in some tropical ascidians of the family *Didemnidae*. *Prochloron* spp. are regarded as obligate symbionts of host ascidians because of following reasons; 1) none are known to be free-living, 2) nutrient exchange has been reported (Pardy and Lewin, 1981; Griffiths and Thinh, 1983), 3) the host's larvae form a special tissue holding the algae for vertical transmission of the symbionts (Kott, 1982; Hirose, 2000). This symbiosis is usually extracellular, and the algae occur on the surface or in peribranchial/cloacal cavities of the host colonies. To date, the intracellular distribution of *Prochloron* cells is only known in *Lissoclinum punctatum* (Hirose *et al.*, 1996).

*Diplosoma virens* (Hartmeyer, 1909) is a didemnid ascidian that forms a sheet-like colony. The colonies always contain large amount of *Prochloron* cells in the common cloacal canal. While notodelphyids inhabiting algal-bearing didemnids have been poorly studied to date (Cf. Table 1 in Ooishi, 1998), notodelphyids are sometimes found in the cloacal canal of the host colony. The taxonomic position of this notodelphyid species is unknown at present. Since the adult notodelphyids have reduced appendages no longer natatory,

they never leave the host. They, therefore, need to obtain the foods in the cloacal canal, and the possible candidates of the diets would be ascidian tissue, faecal pellets or *Prochloron* cells. The diet of the notodelphyids is an essential key to understand how this parasitic (or commensal) copepod effects on the ascidian-*Prochloron* symbiosis system. In the present study, the contents of the gut of the notodelphyids were examined with light and electron microscopy to estimate their diet.

## MATERIALS AND METHODS

A total of fifteen adult females of the notodelphyid inhabiting in the colonies were used for histological or electron microscopical observations. The colonies of *Diplosoma virens* were collected in the vicinity of Maeda Point (Okinawa, Japan) on June 12 and July 3 in 1999. The colonies grow on the branch of the dead coral in the shaded sub-tidal area. Voucher specimens of the host colonies containing unidentified notodelphyids were deposited in the Zoological Collection, Faculty of Science, University of the Ryukyus (URB-CRU0010).

The colonies containing the notodelphyids were fixed by 10% formalin in seawater. The fixed colonies were dehydrated through a butanol series and embedded in paraffin. Serial sections of 8 µm thick were prepared and stained with Delafield's hematoxylin and eosin; additional sections were stained with alcian blue at pH 2.5 and/or periodic acid Schiff (PAS) stain for histochemistry of polysaccharides.

Some formalin fixed colonies were dehydrated through an ethanol series and embedded in styrene resin. The sections of 1 µm thick were stained with 1% toluidine blue for light microscopy. For scanning electron microscopy (SEM), the specimens embedded in styrene resin were sectioned until the desired structures were exposed, and then the resin was removed from the specimens in acetone (1 hr, 2 times). Then the specimens were immersed in hexamethyldisilazane (HMDS; 30 min, 2 times). They were air-dried and sputter coated with gold-palladium, and examined in a Hitachi S-570 scanning electron microscope at 10–20 kV.

For transmission electron microscopy (TEM), some unfixed colonies were dissected to dig out the notodelphyids with forceps and a razor blade. The notodelphyids were fixed in 2.5% glutaraldehyde-

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0.45 M sucrose-0.1M cacodylate buffer (pH 7.4). After a brief rinse with 0.45 M sucrose-0.1M cacodylate buffer, the specimens were postfixed with 1% osmium tetroxide-0.1M cacodylate buffer. They were dehydrated through an ethanol series, cleared with *n*-butyl glycidyl ether, and embedded in a modified Spurr's resin (Kushida, 1980). Thin sections were stained with uranyl acetate and lead nitrate and examined in a transmission electron microscope JEOL 2000EX at 80 kV.

## RESULTS

In the colony of *Diplosoma virens*, reddish spheres (about 1–1.5 mm in diameter) are sometimes found through the transparent tunic (Fig. 1). They are notodelphyid copepods. Since the density of zooids and *Prochloron* cells are usually lower around the notodelphyids than in the other parts of the colony, there are often white patches around the notodelphyids. The notodelphyids of various stages inhabit in the common cloacal cavity (Fig. 2). The individual of about 0.7 mm long has a cylindrical body with relatively large legs, suggesting its active motility (Fig. 2A). On the other hand, the adults have an enlarged fourth metasome that is full of oocytes or embryos (Fig. 2C), and their body size is much larger than the common cloacal opening of the host colony, indicating they can not go out of the host cavity.

In the host colony, the ascidian zooids are sparsely embedded in the sheet-like tunic that occupies the major part of the colony. The tunic is a transparent tissue that consists of gelatinous matrices and free cells. The common cloacal canal runs throughout the colony and anastomoses the peribranchial space of each zooid. The algal symbionts, *Prochloron*, are distributed in the canal. The notodelphyids have a narrow space in the canal (Fig. 3, 4). The mouth closely faces the wall of the cloacal canal (Fig. 5). The mouth parts are not specialized and have several setae pointing to the mouth. The masticatory lamella of mandible is serrated. The wall of the canal consists of tunic in which two (or more) types of tunic cells are distributed; tunic bladder cells and amoeboid tunic cells (asterisks and arrows in Fig. 5). The gut of the notodelphyids is a simple duct. In the histological sections, the contents of the gut are homogeneously stained with hematoxylin and toluidine blue (Fig. 6). While elliptical, heterogeneous materials are occasionally found (Fig. 7), they are similar in shape and structure to the host's faecal pellets (Fig. 8).

In the histochemistry with alcian blue at pH 2.5, the tunic matrix and the contents of the notodelphyid's gut are selectively stained, while the other structures, including the ascidian zooids and *Prochloron* cells, are poorly stained (Fig. 9). In alcian blue-PAS staining, the tunic and the notodelphyid's gut contents are purple, and the other structures are red.

In TEM, the gut wall of the notodelphyids is a simple epithelium that extends numerous numbers of long microvilli to the gut lumen (Fig. 10). The gut contents that are mostly homogeneous materials of fuzzy structure (Fig. 11). Occasionally, stratified structures are also found in the gut contents (arrows in Fig. 12). So far examined here, bacterial

inclusions could not be found in the gut lumen by light and electron microscopy.

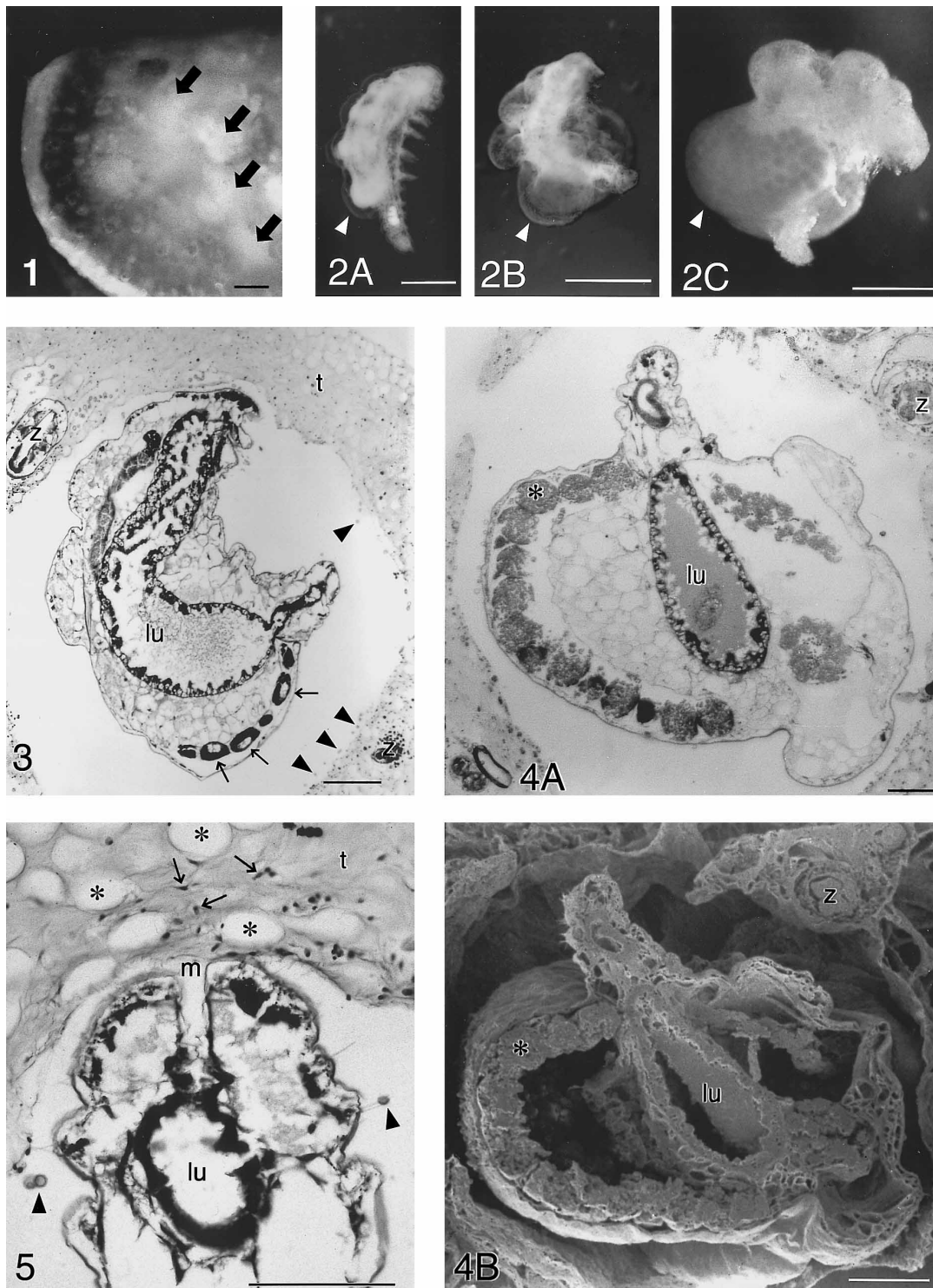
## DISCUSSION

The notodelphyids of various growth stages are found in the colony of *Diplosoma virens*, and some large ones are much larger than the cloacal opening of the colony. Since these notodelphyids can not leave the host, they need to obtain food in the colony to grow and brood the embryos. In the cloacal canal, their head faces to the canal wall, i. e., tunic. The notodelphyids may collect food particles with setae of the mouth parts or they may directly nibble at the host tissue with the serrated masticatory lamella of mandible. In general, the notodelphyids are thought to feed on the food particles and mucus of the host, the faeces of the host, or the host tissues (Cf. Gotto, 1979). Moreover, the algal symbionts should be added to the candidates of the diet in the present case.

Histological sections showed that the gut contents of the notodelphyids are mostly homogeneous. This suggests that their major diet is a particular material of homogeneous structures. The host animal is a filter-feeder, and it ingests various small particles in seawater. As shown in the section of the host's faecal pellets (Fig. 8), the host's diet is very heterogeneous, and thus, the major diet of the notodelphyids seems to be different from the host diet. The notodelphyids would not feed on the host faeces and not steal the host's food. Whereas the host's faeces-like structures are sometimes found in the gut contents, the notodelphyids may accidentally ingest the host's faeces that released into the cloacal canal. In any case, the faeces-like structures are minor components of the gut contents of the notodelphyids. Alcian blue staining and alcian blue-PAS staining showed that the gut contents of the notodelphyids are very similar in stainability to the tunic but different from the zooids, the host's faeces and the symbiotic algae.

In TEM, the major part of the gut contents are fuzzy materials, and this is consistent with the results of histological observations. The microvilli extending into the gut contents indicate that the gut epithelia really absorb the nutrient from the homogeneous gut contents. On the other hand, striated structures are sometimes found in the gut contents (Fig. 12). These structures are probably thylakoids of the symbiotic algae *Prochloron*, suggesting the notodelphyids (accidentally?) ingest the algae. However, the stainability of the *Prochloron* cell is different from that of gut contents in the histochemical observation.

The tunic matrix has a homogeneous structure, and it is well stained with alcian blue. These characteristics are consistent with those of the major parts of the gut contents. On the contrary, the other possible candidates for the diet (zooids, host faeces, and *Prochloron* cells) are not stained with alcian blue, and they are not similar in structure to the major gut contents in histological and electron microscopical observation. These results suggest that the major part of the gut contents are the tunic, and the notodelphyids feed on the



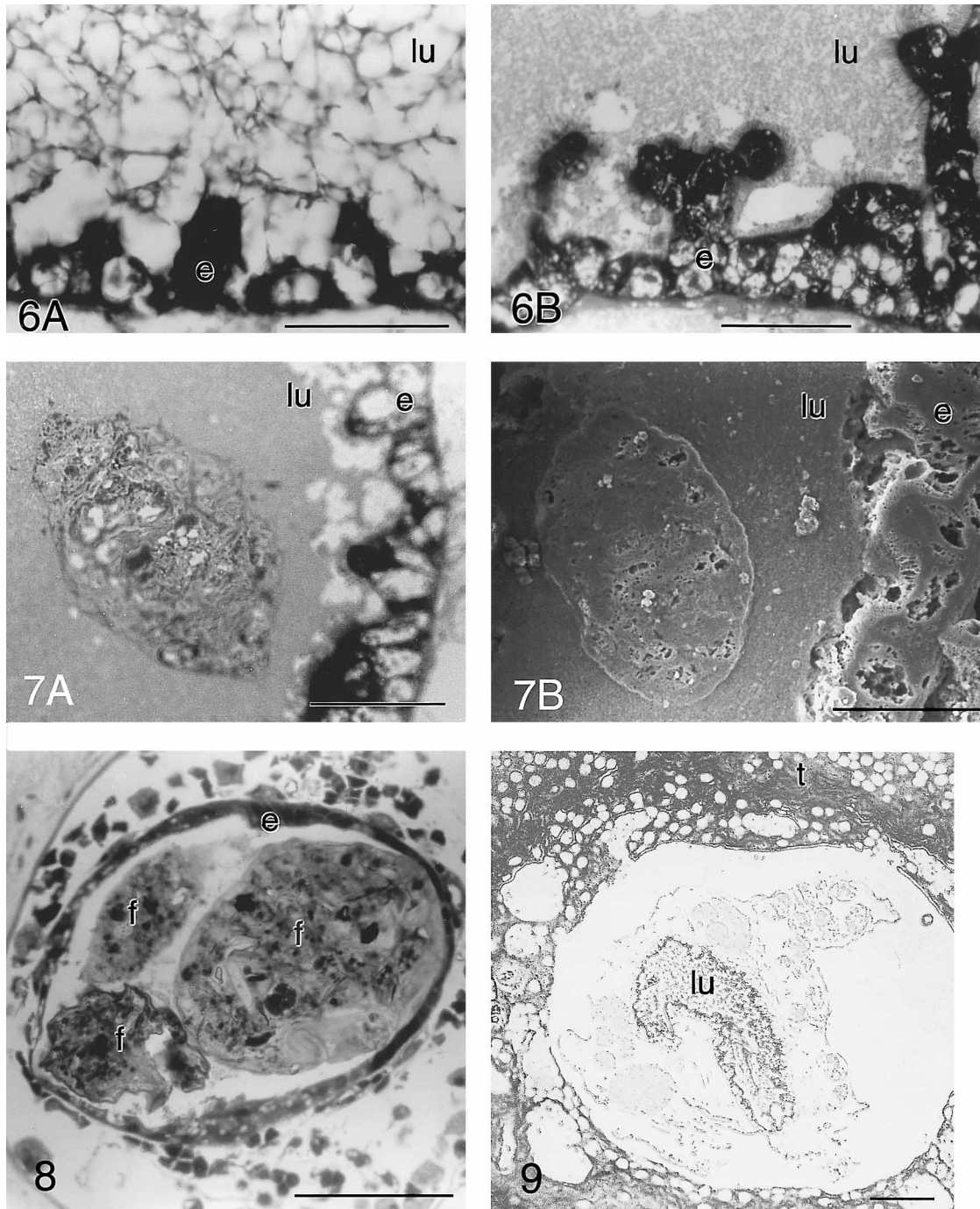
**Fig. 1.** A colony of *D. virens*. Arrows indicate the notodelphyids inhabiting in the colony. Scale, 1 mm.

**Fig. 2.** The notodelphyids dug out from the host colony. The fourth metasome (arrowheads) is not enlarged in A, is enlarged but not contains oocytes in B, and is filled with embryos in C. Scale, 0.2 mm for A; 0.5 mm for B and C.

**Fig. 3.** Vertical section of the notodelphyid in the host colony (paraffin section stained with hematoxylin-eosin). Arrows indicate some of the oocytes in the fourth metasome, and arrowheads indicate some of the *Prochloron* cells in the cloacal canal. lu, lumen of the gut; t, tunic of the host colony; z, zooid of the host colony. Scale, 200  $\mu$ m.

**Fig. 4.** A paired image of the styrene resin section (A) and SEM (B) of the full-matured notodelphyid in the host colony. Asterisks indicate one of the brooded embryos. lu, lumen of the gut; z, zooid. Scale, 200  $\mu$ m.

**Fig. 5.** Enlargement of the head facing the wall of host cloacal canal. Arrows indicate some of the amoeboid tunic cells, arrowheads indicate some of the *Prochloron* cells in the canal, and asterisks indicate some of the vacuolar lumen of the tunic bladder cells. lu, lumen of the gut; m, mouth opening; t, tunic. 100  $\mu$ m.



**Fig. 6.** Contents of the gut of the notodelphyd (A, paraffin section stained with hematoxylin-eosin; B, styrene section stained with toluidine blue). e, epithelial wall of the gut; lu, lumen of the gut. Scale, 50  $\mu$ m.

**Fig. 7.** Heterogeneous component embedded in the homogeneous gut contents (A styrene section; B, SEM). e, epithelial wall of the gut; lu, lumen of the gut. Scale, 50  $\mu$ m.

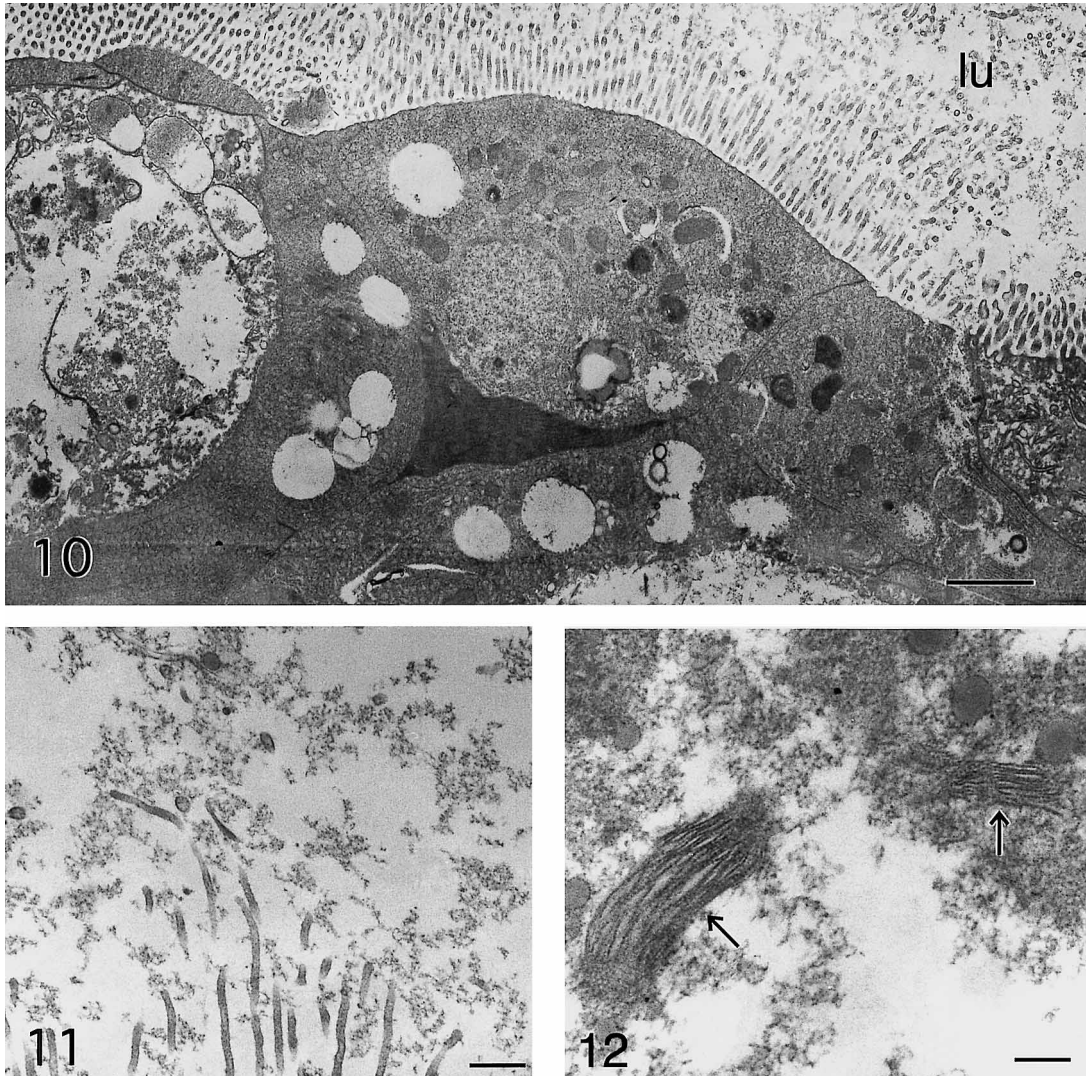
**Fig. 8.** Faecal pellets (f) in the intestine of the host zoid (styrene section). e, epithelial wall of the intestine. Scale, 50  $\mu$ m.

**Fig. 9.** Histochemistry of the notodelphyd in the colony (paraffin section stained with alcian blue at pH 2.5, phase contrast). Alcian blue stains only the tunic and the gut contents. lu, lumen of the gut; t, tunic of the host colony. Scale, 200  $\mu$ m.

gelatinous wall of the cloacal canal as a food. The host faeces and *Prochloron* cells are minor component of the gut contents and they may be accidentally ingested. The tunic contains cellulose fibers linked with protein (De Leo *et al.*, 1977), and it may be nutritious if the notodelphyd can digest the cellulose

and/or linking materials.

Some types of tunic cells are distributed in the tunic of *D. virens*, and one of them is the tunic bladder cells that have large vacuoles (asterisks in Fig. 5). The tunic bladder cells are known to contain acid (<pH 2) in their vacuoles (Cf.



**Fig. 10.** The epithelial wall of the gut (TEM). Numerous microvilli extend to the gut lumen (lu). Scale, 2  $\mu$ m.

**Fig. 11.** The gut contents and microvilli (TEM). Scale, 0.5  $\mu$ m.

**Fig. 12.** Fuzzy homogeneous materials and stratified structure in the gut lumen (arrows) (TEM). Scale, 0.2  $\mu$ m.

Stoecker, 1980), and the tunic bladder cells in *D. virens* also contain acid (pH 1 or more acidic: Hirose, unpublished). If the notodelphyids feed on the tunic, they may accidentally puncture the tunic bladder cells and be suffered by the acid. They should be resistant against the acid, or they can nibble at the tunic without puncturing the tunic bladder cell.

It is uncertain why the notodelphyids do not positively eat the *Prochloron* cells. Many antibiotic, anti-viral, or cytotoxic compounds have been isolated from algal-bearing didemnids, and *Prochloron* cells seem to be involved in the synthesis of some compounds (Sings and Rinehart, 1996). Therefore, *Prochloron* cells may contain toxic compounds to avoid positive predation. In some diatoms, Poulet *et al.* (1994) showed the presence of inhibitory compound(s) detrimental to the reproduction of copepod. On the other hand, the present study deals with only adult females, and thus, it would be possible that *Prochloron* cells are eaten by the males or young animals.

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