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Symbiosis between a pelagic flatworm and a dinoflagellate from a tropical area: structural observations

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Abstract

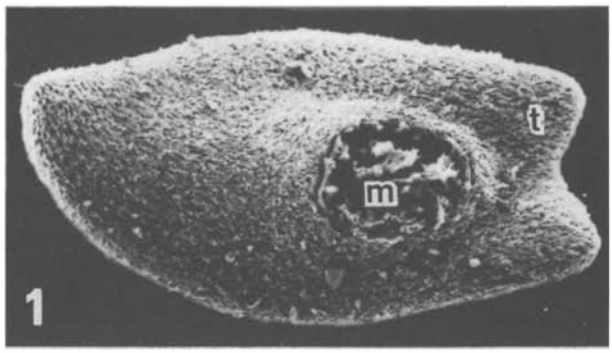
A morphological account on the endosymbiosis between *Amphiscolops* sp and *Amphidinium* sp is given, based on scanning and transmission electron microscopy observations. The algal symbionts (15–20 μm in diameter) are found among cells of the peripheral parenchyma. *Amphidinium* sp. has a single pyrenoid of the multiple-stalked type, with several chloroplast lobes radiating from it. A comparison with *A. klebsii* is made. Our observations reinforce the assumption of selectivity of *Amphiscolops* for the symbiotic genus *Amphidinium*.

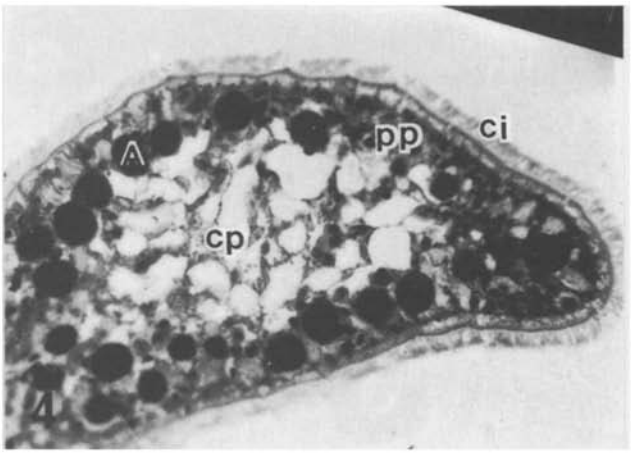
Introduction

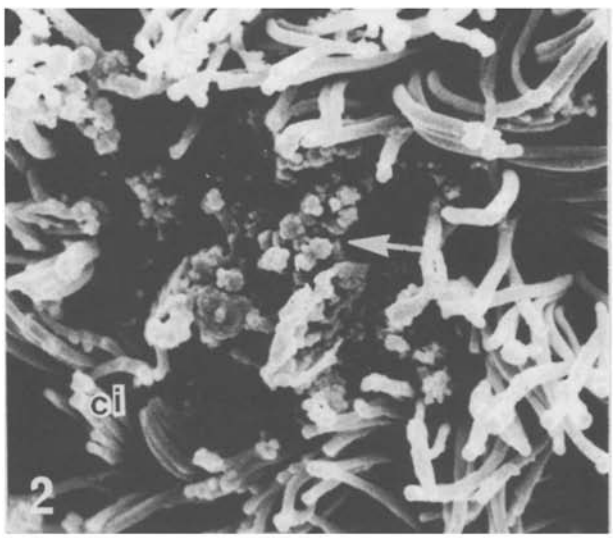
Endosymbiotic associations between algae and marine pelagic invertebrates have been widely documented. The algal symbionts occur within the body of the non-photosynthetic host, usually enclosed within internal vacuoles bounded by a membrane of host origin (Douglas, 1988). Algal partners are mainly dinophyceans, prasinophyceans, cryptophyceans, and prymnesiophyceans. Up to 200 genera of planktonic hosts have been reported, including heterotrophic protists, cnidarians and turbellarians (Fenchel, 1987; Muscatine *et al.*, 1986; Trench, 1986). These associations are of great ecological significance in the marine environment, since heterotrophs are converted into partial or fully functional autotrophs through the acquisition of photobionts (Taylor, 1982). Oceanic mixotrophic organisms are thought to be important in the plankton food web as sites of enhanced primary production and as grazers and predators (Anderson, 1983; Michaels, 1988; Spero & Parker, 1985; Stoecker, 1991; Swamberg, 1983).

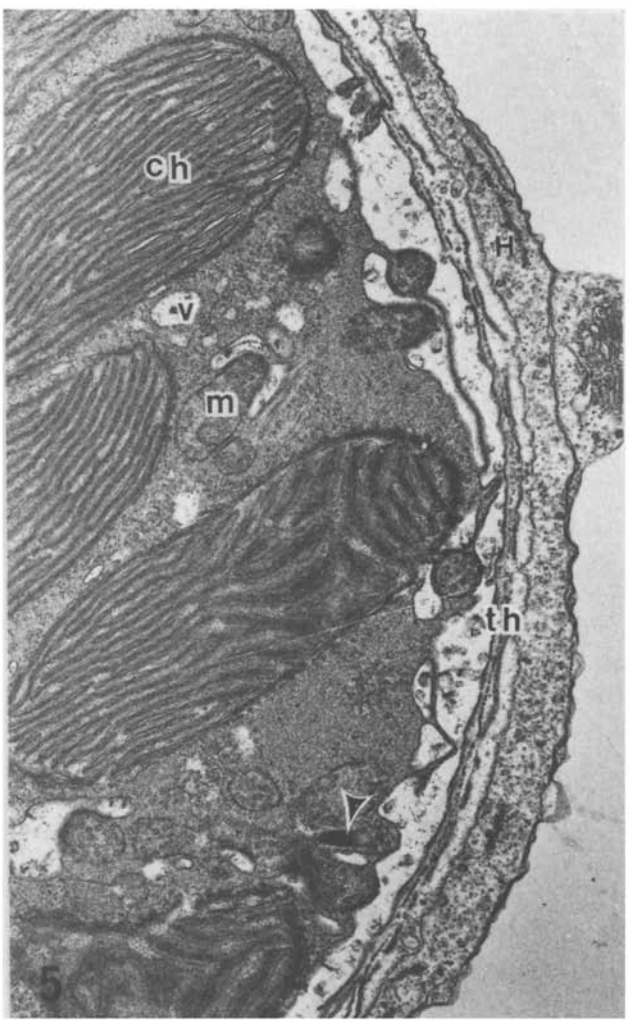
A number of morphological and physiological studies have been made on the intertidal acoel turbellarian *Convoluta roscoffensis* (e.g. Douglas, 1983 a, b, 1985; Douglas & Gooday, 1982; Provasoli *et al.*, 1968); in contrast, comparatively few information is available on symbiotic associations among planktonic algae and pelagic mixotrophic flatworms (Stoecker *et al.*, 1989).

Pelagic acoels frequently appear in population blooms in estuarine and coastal waters of southeastern Brazil (Gaeta *et al.*, 1990; Lopes, in press; Lopes *et al.*, 1986; Por *et al.*, 1984). Despite









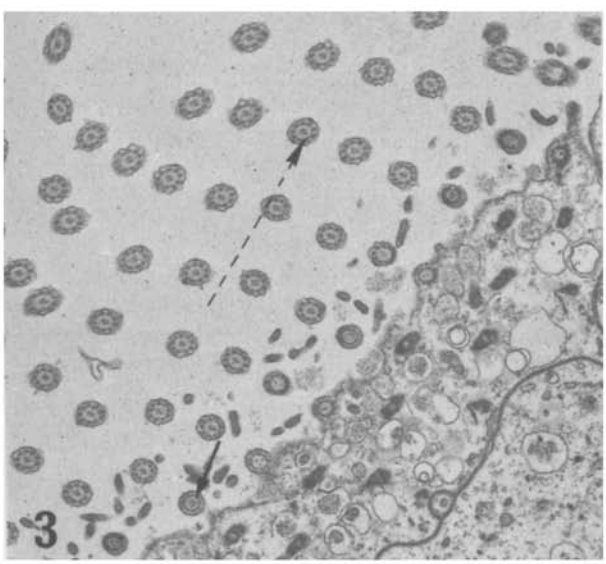


Fig. 1. Ventral view of a juvenile *Amphiscolops* sp., with wide mouth opening (m). Note dense ciliary covering and bilobed tail (t). SEM, × 100.

Fig. 2. Fore end, seen in SEM: droplets of secretion (arrow) from frontal organs emerge among cilia (Ci). × 17000.

extensive taxonomic studies on littoral acoels by Marcus (1950, and references there in), there is no report of a detailed investigation on planktonic algal-acoel symbiosis in this area. Here we describe optical and electron microscopic observations on algal-acoel endosymbiosis based on samples collected at inshore waters of São Paulo State, Brazil.

Material and methods

During February 1991, several oblique and horizontal tows using a conical plankton net (64 micrometers mesh aperture) were carried out off Picinguaba beach, Ubatuba (23° 22′ S, 44° 45′ W). Each sample was diluted into a 20 l polyethylene carboy, and brought to the laboratory. Aliquots were poured into a 1 liter beaker, kept under lateral illumination. Acoels displayed a strong positive phototaxis, and were easily sorted with a wide-mouth pipette. Several flatworms were transferred into a 20 l polyethylene carboy containing unfiltered sea water from the collecting site, and brought to the Electron Microscopy Laboratory at the University of São Paulo. The worms were kept alive for 1 mo. in aerated aquaria, without either water exchange or food additions. For ultrastructural studies (transmission electron microscopy, TEM), the worms were fixed in 2% glutaraldehyde in 0.1 M sodium cacodylate buffer, pH 7.2, with 0.25 M sucrose added (A. E. Douglas, in litt.), washed, post-fixed in 2% osmium tetroxide, dehydrated and embedded in Polybed resin. Sections were stained with uranyl acetate/lead citrate and examined in a Siemens Elmiskop 101 electron microscope. Some sections were stained according to Thiéry (1967) and Namimatsu (1992).

For scanning electron microscopy (SEM), animals fixed as above, were critical point-dried, sputter-coated with gold and examined in a Jeol JSM 840-A electron microscope. Semithin plastic sections were stained with toluidine blue, for light microscopy observation.

Results

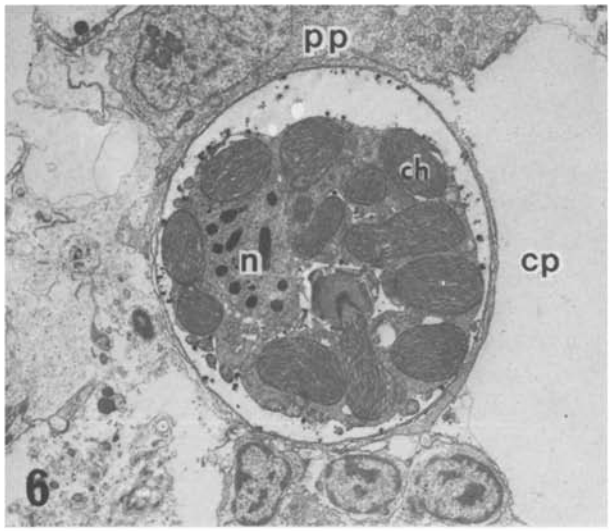
The acoel, tentatively identified as *Amphiscolops sp*, is about 1.0–1.5 mm long, pointed anteriorly, having a somewhat bilobed caudal end (Fig. 1). The mouth stays at about 3/4 from the anterior end; it is frequently studded with particulate material, including diatoms. At the foremost end of the worm, secretory material from the frontal organ forms clumps among the cilia (Fig. 2). The outer ciliated epithelium has insunk nuclei; both in thin sections (Fig. 3) and in SEM views (Fig. 11), the cilia are oriented in rows, indicative of metachronal beating. They are about 5 μm long; their basal bodies contain glycoprotein granules (arrow, Fig. 3), which stain positively following the procedure of Thiéry. In overall fine structure, ciliary details conform to the 'acoelan type' (e.g., Rohde *et al.*, 1988; Tyler, 1979).

All worms observed, including the smallest individuals, harbored numerous symbiotic algae of the genus *Amphidinium* in the peripheral parenchyma. Some 10–30 algae are present in any median, transverse section (Fig. 4). Some algae penetrate deep into this tissue, others are positioned at its borderline, bulging towards the central (digestive) parenchyma (Figs 6 and 12). These algae are partially bound by a very thin cytoplasmic layer, likely of host origin, which contains ribosomes, microtubules and occasional Golgi lamellae (Fig. 5). No particular orientation of the individual algae was ever observed. In each host, there are algae with epicones directed away from the epithelium, and others aligned in the opposite direction.

Fig. 3. Rows of transversely sectioned, epidermal cilia oriented perpendicularly to the cell surface. Dashed arrow indicates direction of effective stroke. Ciliary basal bodies (arrow) contain dense granules. × 3750.

Fig. 4. Low power light microscopy view of a semithin, transverse section of the acoel. Spherical symbionts (A) are associated with the peripheral parenchyma (pp). Ciliated epidermis (Ci); central parenchyma (cp). Toluidine blue staining. × 650.

Fig. 5. Detail of algal symbiont enclosed by a thin layer of host cytoplasm (H). Underneath thecal membranes (th) one sees: chloroplasts (ch), mitochondria (m), vesicles (v) and one trichocyst (arrow). × 54000.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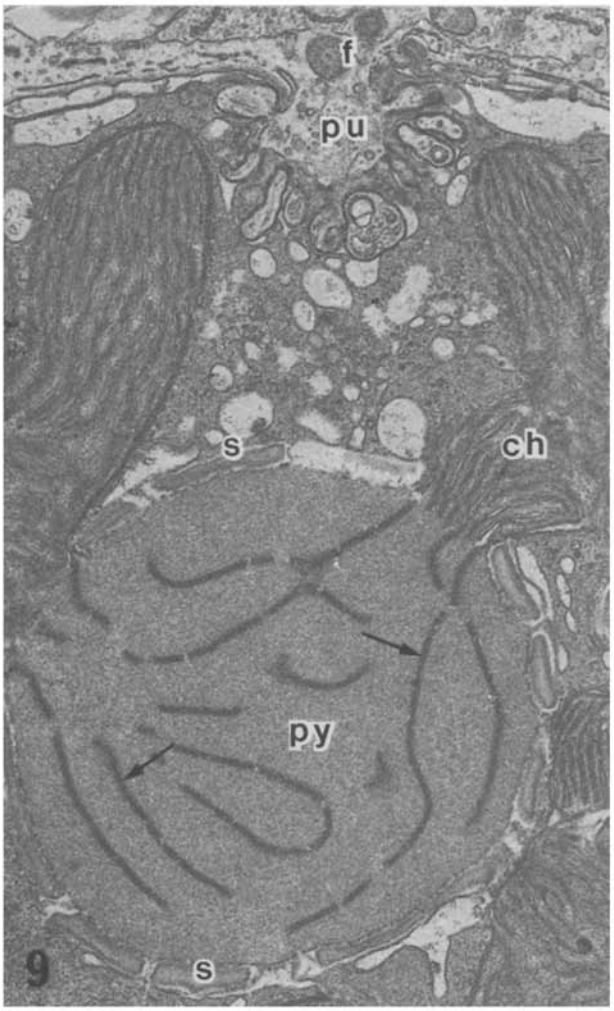
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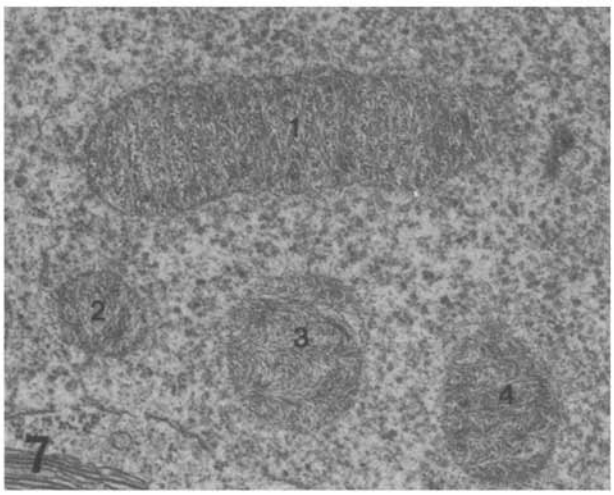
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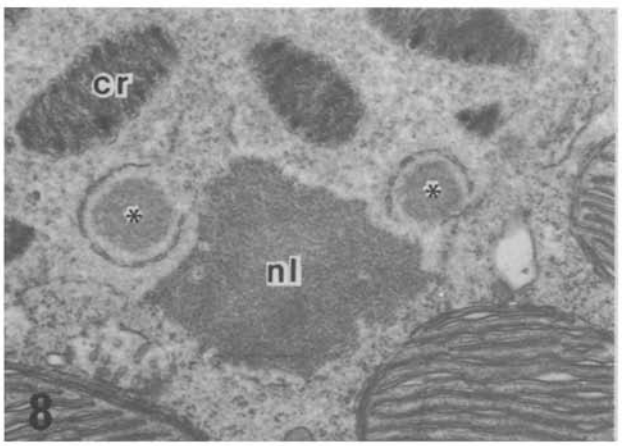
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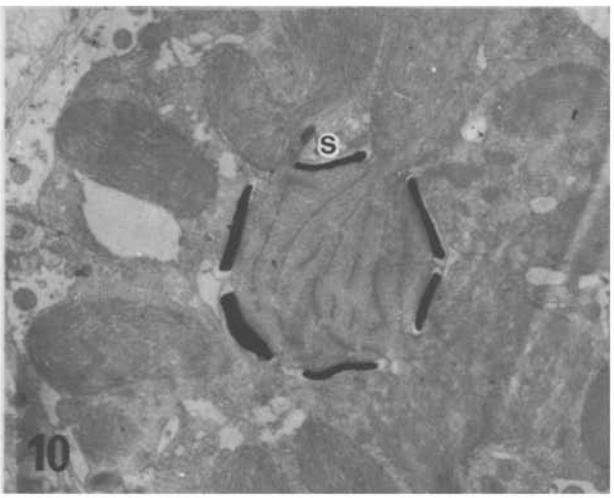
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The alga is about 15–20 μm in diameter; its theca consists of 3 layers of membranes (Fig. 5). Thecal vesicles appear at irregular intervals. The outermost membrane is usually separated from the animal tissues by a thin space (Fig. 5). The intra- or extracellular status of the symbiosis remains undetermined in our material.

The core of the algal cell is occupied by a large pyrenoid of the multiple-stalked type (Dodge & Crawford, 1971), with several chloroplast lobes radiating from it (Figs 6 and 9). Probably a few, or even just one, multilobed chloroplast is present. Many thylakoid lamellae traverse the pyrenoid in different directions, from one chloroplast lobe to another (Fig. 9). The outer surface of the pyrenoid is flanked with starch plates, that stain neatly with silver (Fig. 10). Besides starch, only the discrete trichocysts (Fig. 5, arrow) were observed to react with silver stains.

The pusule in *Amphidinium* sp (Fig. 9) shows a number of membrane outpocketings, ribosomes and small vesicles. Only one flagellum has been observed so far, having the usual '9 + 2' arrangement (Fig. 9, arrow).

Mitochondria are pleomorphic and predominate at the cell periphery (Fig. 5). They have tubular cristae, quite distinct from those of the host.

The nucleus contains many chromosomes in permanent condensed state (Figs 6–8), as typical for dinophyceans (Dodge & Greuet, 1986; Soyer & Haapala, 1974), and a prominent nucleolus, having a homogeneous density. Occasional masses of a non-identified dense material, similar to that of the nucleolus, were noted nearby, partially enclosed by a double membrane (Fig. 8).

Discussion

This paper reports aspects of the symbiosis between a dinophycean and a pelagic acoel flatworm, found in a coastal area off Brazil. Based on TEM studies, the algae were readily identified as *Amphidinium* sp. They share common characters with *Amphidinium klebsii* (Kofoid & Swezy, 1921), which has been described as the symbiont of the acoel *Amphiscolops langerhansi* (Taylor, 1971). The most conspicuous resemblance is their radiating chloroplast lobes, derived from the single pyrenoid, as opposed to the peripheral chloroplasts of *Amphidinium carteri* (Dodge & Crawford, 1968). Also the location of the mitochondria, in the cortical area between theca and chloroplast lobes, is another character distinctive for *Amphidinium klebsii* (Taylor, 1971). Despite these similarities, we prefer to assign our material as *Amphidinium* '*klebsii*-like', for the following reasons: First, the structure of the epicone could not yet be adequately interpreted. Attempts at obtaining good squash preparations were not successful, as an additional procedure to the electron microscopy study of the algae. Second, *Amphidinium klebsii* has no trichocysts (Taylor, 1971), which however are frequent in the *Amphidinium* sp from Ubatuba. In addition, *Amphidinium klebsii* presents a regular orientation within the host acoel, while our specimens lack this pattern. Trench & Winsor (1987) also found no particular orientation of the algae in a *Amphidinium* – *Amphiscolops* symbiosis from Micronesia.

Nucleolar organization, permanence of nuclear envelope during cell cycle, and chromosomal pattern, are important characters for discussions on

Fig. 6. Whole view of an *Amphidinium* cell protruding into the vacuolated central parenchyma (cp). Extensions of peripheral parenchymal cells (pp) hold the alga in place. Chloroplast lobes (ch) and nucleus (n) can be identified in the alga. × 6240.

Fig. 7. Sections of condensed chromosomes (1–4) within the nucleoplasm of *Amphidinium*. Note banded, wavy pattern of DNA fibrils. × 13800.

Fig. 8. Detail of nucleus of *Amphidinium*, containing a nucleolus (nl), chromosomes (cr) and 2 bodies (*) of nucleolar-like material enclosed by discontinuous membranes. × 21600.

Fig. 9. Epicone region of *Amphidinium*, including a flagellum (f),in the pusule (pu). Chloroplast lobes (ch) radiate from the pyrenoid (py). surrounded by starch plates (s). The pyrenoid is traversed by many tylakoid membranes (arrows). × 13400.

Fig. 10. Equivalent section to Fig. 9, after staining with the procedure of Thiéry. Only starch grains reacted positively. × 6800.

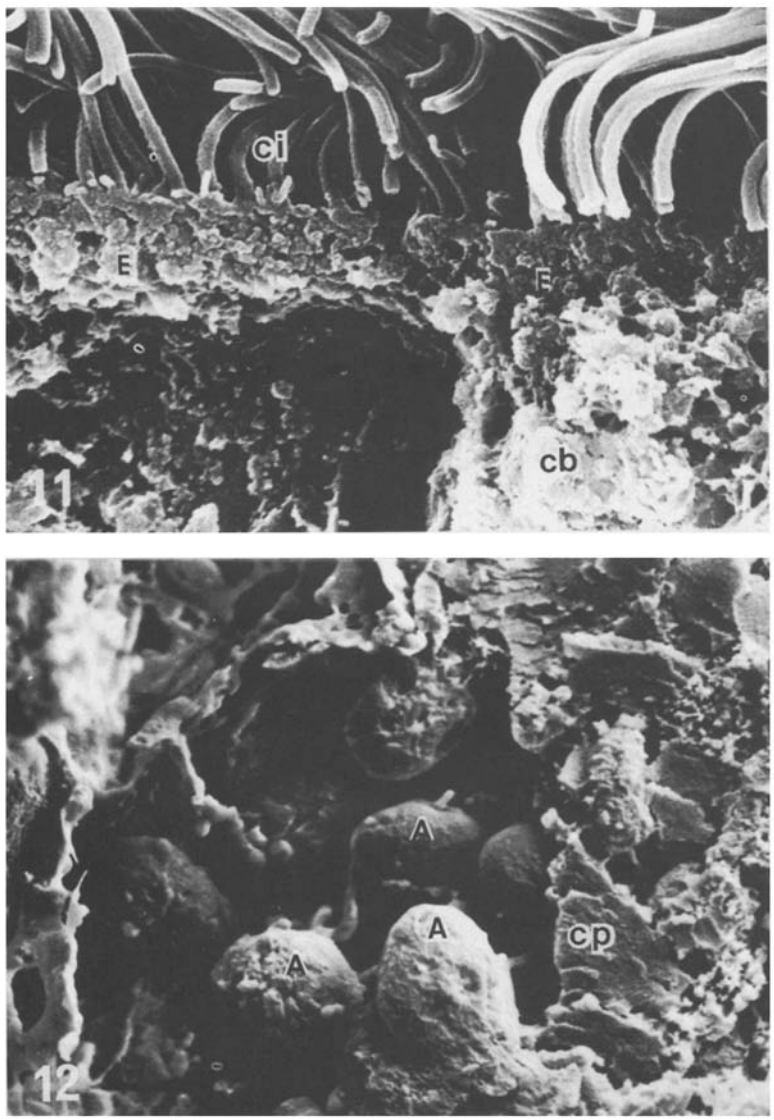

Fig. 11. SEM view of a fractured *Amphiscolops*, to illustrate characters of the ciliated epidermis: the insunk cell body (cb) broadens into the epithelial plate (E). Cilia (ci) and microvilly provide an efficient covering. × 6000.
Fig. 12. SEM view of another fractured acoel, to illustrate a group of presumed algal cells (A) embedded in the parenchyma (cp). × 4500.

cell behavior and phylogenesis of dinophyceans (Soyer-Gobillard & Geraud, 1992). The presence of a prominent nucleolus and associated nucleolar-like material in endosymbiotic *Amphidinium*, as described here, is a significant point to be further investigated.

Sexually mature worms were not available for species identification, as needed, but most characters observed by us point to the genus *Amphiscolops* (Marcus, 1950; Antonius, 1968). This assumption is further reinforced by the fact that symbioses between marine invertebrates and dinoflagellates (among other algae) is a species-specific phenomenon (Trench, 1986; in litt.). All evidence obtained in the present study favor a true endosymbiotic association, whether being of open type or not. However, little is known about the acquisition way of this relationship. Markell

et al. (1992) have recently demonstrated the presence of protein/glycoproteins in cell walls of 4 symbiotic dinoflagellates, questioning their possible rôle as the 'recognition' signal between host-symbiont.

Further morphological and experimental investigations are needed in order to elucidate many unsolved aspects of algal-acoel symbiosis in this area of the South Atlantic.

Acknowledgements

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