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Symbiosis with Dinoflagellates in Two Pelagic Flatworms, *Amphiscolops* sp. and *Haplodiscus* sp.

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Abstract

Two pelagic acoelous flatworms, *Amphiscolops* sp. and *Haplodiscus* sp. have been investigated. Reinfection experiments conducted on juvenile and adult *Amphiscolops* demonstrated that these flatworms are highly selective during the process of symbiont acquisition, the worms always accepting and establishing a symbiosis with *Amphidinium* sp. even though they may, under experimental conditions, take up *Symbiodinium*.

In the case of *Haplodiscus*, two different symbionts were observed to coexist in the same host, and surprisingly, even within the same host's cell. Although the *Amphidinium* sp. is much larger than the *Symbiodinium* sp., the relative biomass of the two symbionts is approximately equal, and apparently is maintained that way. *Haplodiscus* represents the first recorded example wherein two closely related symbiotic algae coexist in the same host.

Keywords: *Amphidinium*, *Symbiodinium*, *Amphiscolops*, *Haplodiscus*, symbiosis, specificity, competitive exclusion

Introduction

One of the remarkable attributes of symbioses between marine invertebrates and microalgae is the specificity of these associations (Trench, 1986). For example, the flatworm *Convoluta convoluta* harbours the diatom *Licmophora* (Apelt, 1969) while *C. roscoffensis* is symbiotic with the green alga *Platymonas convolutae* (Parke and Manton, 1967). Similarly,

the chondrophore *Velella velella* harbours an amphidinioid dinoflagellate, *Amphidinium* (= *Endodinium*) *chattonii*, while invertebrates such as corals and tridacnid bivalves are symbiotic exclusively with gymnodinioid dinoflagellates, *Symbiodinium* spp. One apparent exception to this specificity is demonstrated in the sea anemones *Anthopleura elegantissima* and *A. xanthogrammica* which, along the north-west coast of the United Sates may simultaneously harbour a green alga and a dinoflagellate (Muscatine, 1971; O'Brien, 1978, 1980).

Many marine invertebrates do not pass their symbiotic algae from one sexual generation to the next (Fitt, 1984; Trench, 1986). The larval or juvenile stages acquire the appropriate symbiont from the ambient environment. That the animals always appear to accept and establish a symbiosis with one particular type of alga is indicative of a process of selection. The basis of selection phenomena leading to specificity has been analysed in the jelly-fish *Cassiopeia xamachana* (Trench, 1981; Trench, Colley and Fitt, 1981; Fitt and Trench, 1983; Colley and Trench, 1983, 1985), justifying the conclusion that selection is not effected solely during the initial phase of contact between the algae and the animals' cells, but also by processes that occur after phagocytosis of the algae.

In one of the marine 'lakes' in the Republic of Belau (Western Caroline Is.) the flatworm *Amphiscolops* sp. (*australis ?*) occurs in great abundance. The animals are usually found swimming in a discrete layer about 1 m thick and 0.5 to 1 m above the soft bottom. Irradiance measurements indicated that at high noon in summer, the population perceives about $300\mu mol.m^{-2}s^{-1}$. The flatworm *Haplodiscus* sp. was first observed on the reefs near the Micronesia Mariculture Demonstration Centre (MMDC) off the island of Malakal, on the poritid corals *Porites (= Synaraea) iwayamensis* and *P. andrewsi*. Although initial impressions suggested a specific association, the worms were subsequently observed on a variety of symbiotic stony corals (*Lobophyllia, Echinopora, Turbinaria*), the non-symbiotic coral *Tubastraea micrantha*, and on the Great Barrier Reef of Australia (Lizard Is., Magnetic Is. and Heron Is.) they were found on hard and soft corals.

Our studies indicate that while *Amphiscolops* sp. is highly selective for one species of *Amphidinium* as symbiont, another *Amphidinium* sp. and a *Symbiodinium* sp. coexist in the tissues of *Haplodiscus* sp. The situation found in *Haplodiscus* represents the first observation of two dinoflagellate species coexisting within the same invertebrate host.

Materials and Methods

Collection and maintenance of animals

Amphiscolops sp. were collected from 'flatworm lake' near Malakal. The lake has an area of about 4,300 m^2, is about 4 m deep and is roughly the shape of an isoseles triangle. The population of flatworms occupied an area of about 529 m^2, with worms at a density of $1.28 \times 10^6 m^{-3}$. Temperature was 30.6°C at the surface and 29.3°C at 4 m; salinity at the surface was 31.37°/$_{oo}$ and 32.06°/$_{oo}$ at 4 m. The oxygen profile showed a surface concentration of 4.82 $ml.L^{-1}$, decreasing to 4.1 $ml.L^{-1}$ at 2 m, increasing to 6.19 $ml.L^{-1}$ at 3 m, and decreasing to 4.8 $ml.L^{-1}$ at 3.5 m.

The worms were collected by swimming down to the layer with a closed hand-held plankton net, and towing the opened net through the population. After transport back to the MMDC, they were transferred to plastic containers of filtered sea water and maintained on a 12:12 h light:dark photoperiod with irradiance levels of 100μ $mol.m^{-2}s^{-1}$, and fed every third day on freshly-hatched *Artemia* nauplii. Within a few days after transfer, many of the larger worms produced egg cases, the eggs hatching to produce swimming juveniles within 3 to 4 days. Neither the eggs nor the juveniles contained algal cells; the juveniles were separated from the adults, and were also fed *Artemia* nauplii.

Haplodiscus sp. were collected from the reefs on their various coral "hosts". All attempts to maintain these flatworms in the laboratory were unsuccessful.

Elimination of algae from adult flatworms

Adult *Amphiscolops* sp. were rendered free of algae by exposure to 5$\mu mol.L^{-1}$[3-(3-4-dichlorophenyl)-1,1-dimethylurea] (DCMU) and irradiance of 900$\mu mol.m^{-2}s^{-1}$ for 7 days. Only worms devoid of symbionts, as determined by microscopic examination, were used in infection experiments.

Algae used in infection experiments

Symbiodinium microadriaticum and several *Symbiodinium* spp. (see Blank and Trench, 1985a,b) were employed in this study. These algae have been maintained for several years as previously described (Chang et al., 1984). The algae used (identified by the host species from which they were originally isolated) are as follows:

Algae	Host
Symbiodinium microadriaticum	*Cassiopeia xamachana*
Symbiodinium sp.	*Aiptasia tagetes*
Symbiodinium sp.	*A. pallida*
Symbiodinium sp.	*Bartholomea annulata*
Symbiodinium sp.	*Ragactis (= Heteractis) lucida*
Symbiodinium sp.	*Rhodactis sancti-thomae*
Symbiodinium sp.	*Tridacna gigas*
Symbiodinium sp.	*T. maxima*
Symbiodinium sp.	*Zoanthus sociatus*
Symbiodinium sp.	*Z. solanderi*

In all cases, algae in culture were maintained in the artificial defined medium ASP-8A (Ahles, 1967). The *Amphidinium* sp. isolated from *Amphiscolops* was employed as freshly isolated cells and also as cultured cells after maintenance in ASP-8A for 2 weeks to 1 month. *Amphidinium klebsii* (originally isolated from *Amphiscolops langerhansi* by D.L. Taylor and deposited with L. Provasoli), were also used, as were *A. carterae*, obtained from the Carolina Biological Supply Co.

Infection experiments

Actively swimming 8 day old juvenile *Amphiscolops* were placed in 20 ml filtered sea water in plastic petri dishes at a final density of 3 worms.ml^{-1}. All treatments were conducted in triplicate. In all cases, treatments with only *Artemia* nauplii added served as controls.

Three series of experiments were conducted. First, groups of worms were separately exposed to each type of alga at initial algal cell densities of 10^4ml^{-1}. Second, worms were exposed to mixtures of various *Amphidinium* spp. and *Symbiodinium* spp., with the combined algal cell density being 10^4 ml^{-1} with each algal type being numerically represented approximately equally. Third, each algal type was first fed to *Artemia*, and the nauplii were then separately, and in mixed combinations, provided to the adult worms previously rendered aposymbiotic by treatment with DCMU. In this latter case, the algal cell densities could not be controlled. The nauplii do not digest the algae (Fitt and Trench, 1983), and since the nauplii are preyed upon by the worms, they serve as vectors.

Microscopy

Microscopic observations were made on freshly macerated worms using a compound microscope fitted with Nomarski optics. Use of maceration fluid

such as that employed on coelenterate tissues (Trench, 1981; Colley and Trench, 1983) caused worm cells to rupture. Maceration was effected simply by teasing with fine needles in sea water.

For light and electron microscopic observation, worms were fixed for 3 hr in 4% cacodylate-buffered (pH 7.4) glutaraldehyde in sea water and post-fixed in cacodylate-buffered 1% OsO_4 in sea water. Tissues were then washed in distilled water, dehydrated in serial changes of ethanol and infiltrated and embedded in Spurr's medium. Thin sections were prepared, mounted and observed with either a light microscope (after staining with toluidine blue) or an electron microscope after staining with uranyl acetate and lead citrate.

Results

Studies with Amphiscolops sp.

A. Morphological observations

The general morphology of *Amphiscolops* sp. (from Belau) was not significantly different from that described by Taylor (1971) for *A. langerhansi* (from Miami, Florida) nor from *A. australis* (Dörjes and Young, 1973). Adult worms were up to 7 mm long, swam actively and "hunted" and caught individual *Artemia* nauplii when maintained in the laboratory. Light microscopic examination of thin sections revealed that the algae were located in the region of the peripheral parenchyma below the layer of composite muscle. Microscopic examination of teased cells with Nomarski optics showed that many algae were located in large animal cells, an individual animal cell often containing more than one algal cell (cf. Taylor, 1971). Neither light nor electron microscopy of thin sections readily resolved whether the algae were inter- or intracellular.

The *Amphidinium* sp. observed as symbiont in *Amphiscolops* sp. bore some resemblance to *A. klebsii*, described as the symbiont in *A. langerhansi* (Taylor, 1971), but because there were several points at variance, we have chosen to exercise caution and not assume that the symbionts in the Belauan worms are identical to *A. klebsii*.

The general morphology of the algae in *Amphiscolops* sp. is illustrated in Figs. 1 to 5. As indicated before, the algae are located in the cells of the peripheral parenchyma, but they were not aligned with epicones always directed away from the epithelial layer (cf. Taylor, 1971). Indeed, no particular orientation was observed (Fig. 1). The algal dimensions in profile are about $30 \times 18 \mu m$. The cells are bounded by a thin theca; thecal vesicles are apparent

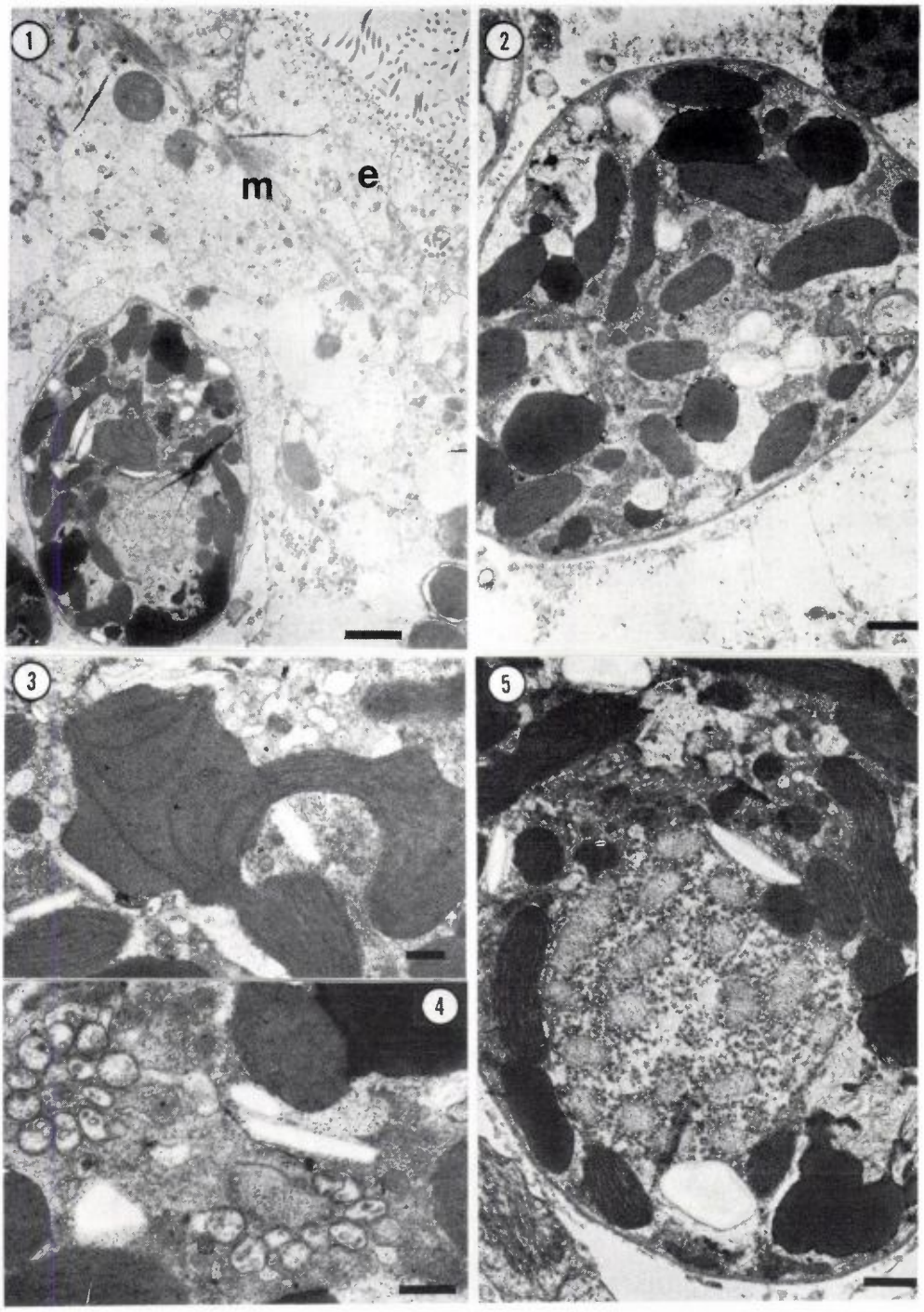
Figure 1. Micrograph of a section through *Amphiscolops* sp. showing the location of *Amphidinium*. The alga is located below the layer of muscle (m) with its epicone directed towards the ciliated epithelium (e). Scale bar, 4.5μm

Figure 2. An electron micrograph of *Amphidinium* sp. in *Amphiscolops*. An animal cell nucleus (upper right-hand corner) is closely appressed to the alga, suggesting an intracellular location for the alga. Scale bar, 2μm.

Figure 3. Details of the pyrenoid in *Amphidinium* sp. in *Amphiscolops*. Note that the thylakoid lamellae from one chloroplast lobe are continuous with the other chloroplast lobe. Scale bar, 1μm.

Figure 4. The flagellar pusules in *Amphidinium* sp. in *Amphiscolops*. Scale bar, 2μm.

Figure 5. The nucleus of *Amphidinum* sp. in *Amphiscolops*. Scale bar, 2μm.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(Fig. 2). No microtubules were observed associated with the theca. The single pyrenoid has several chloroplast lobes radiating from it, and the pyrenoid itself is traversed by chloroplast thylakoid lamellae. Since many thylakoid lamellae were found to be continuous from one chloroplast lobe to another (Fig. 3), it is likely that there is only one multilobed chloroplast present. The chloroplast thylakoids are stacked in groups of three, and the chloroplast is limited by an envelope comprised of three membranes. Although there were clear indications of flagellar pusules (Fig. 4) within cells, flagella actually arising from the algae were not observed. The nuclear envelope is typical of dinoflagellates, with the usual nuclear pores, and the nucleus contains many large chromosomes (Fig. 5). The apparent volume of the nucleoplasm occupied by chromosomes seems much greater than that indicated by Taylor (1971) in *A. klebsii*.

B. Experimental infection studies

When 8 day old aposymbiotic juvenile *Amphiscolops* sp. were separately exposed to cells of various *Amphidinium* and *Symbiodinium* spp., within as brief a period as 2 hr, some worms were observed to have engulfed the algae freshly isolated from *Amphiscolops* sp., those same algae that had been maintained in culture and to a lesser extent *A. klebsii*. Table 1 illustrates that within 48 hr, all worms exposed to freshly isolated and cultured *Amphidinium* sp. had become infected. Achieving the same level of infection with *A. klebsii* took somewhat longer, but within 96 hr, all worms exposed to symbiotic *Amphidinium* were infected. By contrast, the worms exposed to *A. carterae* or any *Symbiodinium* spp., as well as those worms that served as controls, remained uninfected for the duration of the experiment.

In another experiment, juvenile aposymbiotic *Amphiscolops* were simultaneously exposed to combinations of *Amphidinium* sp., *A. carterae* and *Symbiodinium* spp. Table 2 shows that even in the presence of other types of algae, only *Amphidinium* sp. was taken by the juvenile worms.

The observations presented in Tables 1 and 2 suggested high degree of selectivity on the part of the juvenile worms for a particular type of alga. Similar observations were made by Taylor (1971), who suggested that algal size was probably influential in the selection process, since large *Amphidinium* spp. were accepted, and the smaller *A. carterae* and *Symbiodinium* spp. were not.

In an attempt to test the hypothesis that selection of algae by *Amphiscolops* sp. was based on algal size, aposymbiotic juvenile worms were placed in

Table 1. Percentage of juvenile *Amphiscolops* sp. with algae during monospecific exposure to algal cells (f.i.a., freshly isolated algae; c, cultured algae).

Algal species	Elapsed time (h) after start of exposure					
	(24)	(48)	(72)	(96)	1 w	2 w
Amphidinium sp. (f.i.a.)	99	100	100	100	100	100
Amphidinium sp. (c)	98	100	100	100	100	100
A. klebsii (c)	42	50	96	100	100	100
A. carterae (c)	0	0	0	0	0	0
Symbiodinium microadriaticum	0	0	0	0	0	0
Symbiodinium spp. (from all other sources)	0	0	0	0	0	0
Controls	0	0	0	0	0	0

Table 2. Percentage of *Amphiscolops* sp. acquiring algae after 48 hr constant exposure to polyspecific inocula of symbiotic and non-symbiotic algae

Worms exposed to:	% infected with:				
	Amphidinium sp.	*A. klebsii*	*A. carterae*	*S. microadriaticum*	*Symbiodinium* spp.
Amphidinium sp. & *A. carterae*	100		0		
Amphidinium sp. & *S. microadriaticum*	100			0	
Amphidinium sp. & *Symbiodinium* spp. (from *A. tagetes, B. annulata, R. lucida*	100				0
Amphidinium sp. & *Symbiodinium* spp. (from *T. gigas, Z. sociatus, A. pallida*)	100				0
A. klebsii & *Symbiodinium* spp. (from *T. maxima, Z. solanderi, R. sancti-thomae*)		66			0

separate containers with cultures of *S. microadriaticum* (which tends to form clumped aggregates of cells) and *Symbiodinium* sp. (originating from *Aiptasia tagetes* and *Zoanthus sociatus*). The algae from *A. tagetes* were not

clumped, while those from *Z. sociatus* were. The size of the aggregates of cells encompassed that of *Amphidinium* sp. and *A. klebsii*.

The results showed that even after one week exposure, none of the worms had acquired any of the *Symbiodinium* spp. offered to them. When similar experiments were conducted using a large ($35\times22\mu$m) unidentified free-living amphidinioid dinoflagellate also found in flatworm lake, these algae were not accepted by *Amphiscolops* sp. either. These observations indicate that size of the alga is probably not the sole factor regulating selectively by the worms.

In a final series of experiments, *Amphidinium* sp. and several *Symbiodinium* spp. were first separately fed to *Artemia* nauplii. Within about 2 hr the nauplii contained from 25 to 100 algal cells in their guts. These *Artemia*, laden with algae were provided separately and in combination to 7 day-starved adult *Amphiscolops* previously rendered aposymbiotic by DCMU treatment. Exposure of worms to *Artemia* was for 6 hr, after which the worms were transferred to petri dishes of clean sea water. Worms fed *Artemia* without algae served as controls.

Flatworms fed *Artemia* laden with *Symbiodinium* spp. (derived from *A. tagetes* and *Z. sociatus*) acquired algae initially, but were free of algal cells within 24 hr. It appears that the algae were eliminated along with the indigestible remains of the nauplii. Those worms which were fed nauplii containing *S. microadriaticum* retained algae for up to 48 hr, and in many cases the algae remained in the worms even after the remains of the nauplii had been regurgitated. Nevertheless, the algal cells never moved towards the peripheral parenchyma, but remained in the central digestive region of the worms. All *Symbiodinium* spp. were eliminated within 72 hr, and were morphologically intact as judged by light and electron microscopic examination.

In contrast to the results described above, when flatworms were exposed to nauplii laden with only *Amphidinium* sp. or in combinations including *Symbiodinium* spp., all the worms became infected with *Amphidinium* sp. which became established in the peripheral parenchyma. Within 48 hr after initial exposure, there was no evidence of *Symbiodinium* in any of the worms.

2. Studies with Haplodiscus sp.

Failure to maintain *Haplodiscus* under laboratory conditions, precluded any attempt at experimentation. Nonetheless, light and electron microscopic observations revealed some unique characteristics in the context of specificity.

First, it was immediately apparent upon maceration of animal tissues that there were two distinct dinoflagellates present, one a large *Amphidinium* sp. and the other a *Symbiodinium* sp. As unexpected as this observation was, it was even more alarming to discover that both types of symbionts were seen in the same individual animal parenchyma cell (Fig. 6).

Analyses of sectioned tissues revealed that the numerical ratio of *Symbiodinium* to *Amphidinium* was about 13:1 (Fig. 7), but although an individual *Amphidinium* is significantly larger (about 24μm×16μm) than a *Symbiodinium* (about 8μm in diameter), the biomass of the two algae when calculated in terms of relative volumes, was approximately equal. This is true for worms found in Belau as well as those found on the Great Barrier Reef.

The *Amphidinium* sp. is limited by a thin theca with no apparent associated microtubules, but amphiesmal vesicles are clearly present (Figs. 8 and 9). There was clear evidence of flagella arising from the cells (Figs. 8 and 9). Immediately under the theca, the cytoplast appears highly vacuolated, the vacuoles giving the appearance of containing some mucous substance (Fig. 9). At the level of the light microscope, the vacuoles showed strong metachromasia when stained with toluidine blue, consistent with the presence of acid mucopolysaccharide. The general appearance of the highly vacuolated cytoplast is not unlike that described for the freshwater *Amphidinium cryophylum* (Wilcox, et al., 1982). The chloroplast, as in the case of the *Amphidinium* sp. in *Amphiscolops* sp. appears to be single and multilobed, radiating from the single, centrally-located pyrenoid. Chloroplast thylakoid lamellae invade the pyrenoid, and in the chloroplast, are arranged in groups of three. Peripheral lamellae and grana are absent. The chloroplast envelope is comprised of three membranes. Starch grains are found interspersed among the chloroplast lobes and in association with the pyrenoid. The nucleus is relatively large, and contains large chromosomes which appear to occupy much of the nuclear volume (Fig. 10).

The *Symbiodinium* spp. found in *Haplodiscus* conforms to the description applied to that genus. The chloroplast is lobed and peripheral, and is attached to the pyrenoid by at least two stalks (Fig. 11). Many cells give the distinct impression of being gymnodinioid in outline (Figs. 7 and 11) but no evidence of flagella or basal bodies was detected. Cytokinesis occurs in the manner typical of the genus *Symbiodinium* (Fig. 12).

Figure 6. Electron micrograph showing two profiles of *Amphidinium* sp. and several profiles of *Symbiodinium* sp. in *Haplodiscus*. Animal cell nuclei can be seen in close apposition to both algal cell types suggesting their intracellular location. Scale bar, 5μm.

Figure 7. Light micrograph of a section through *Haplodiscus* illustrating the relative abundance of *Symbiodinium* and *Amphidinium*. Scale bar, 8μm.

Figure 8. An electron micrograph of *Amphidinium* sp. in *Haplodiscus*. Note the peripheral mucocytes, the centrally located pyrenoid with lobes of the chloroplast radiating from it, and the many starch grains distributed through the cytoplast. Two flagella (arrow) can be seen in the sulcus. Scale bar, 8μm.

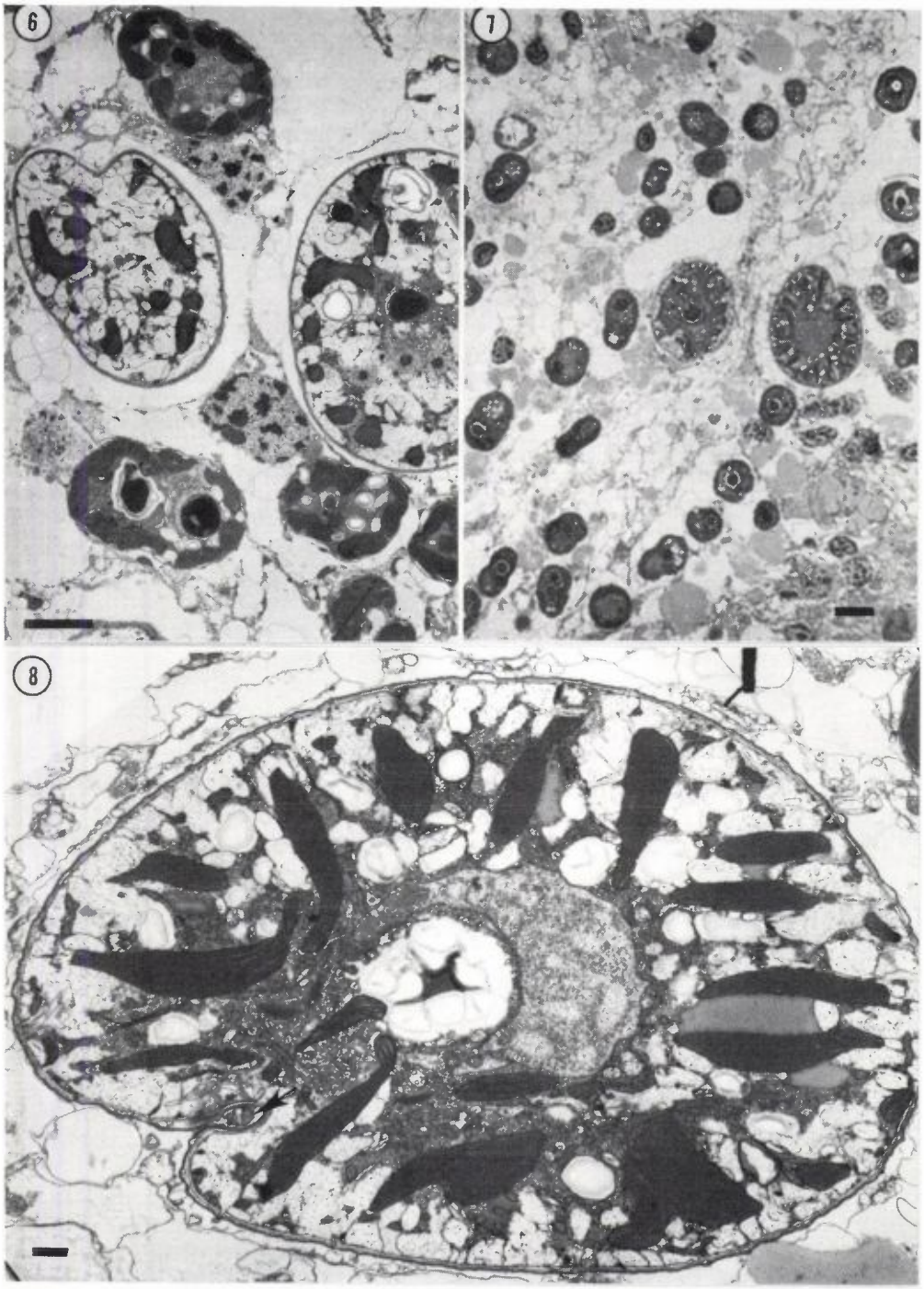
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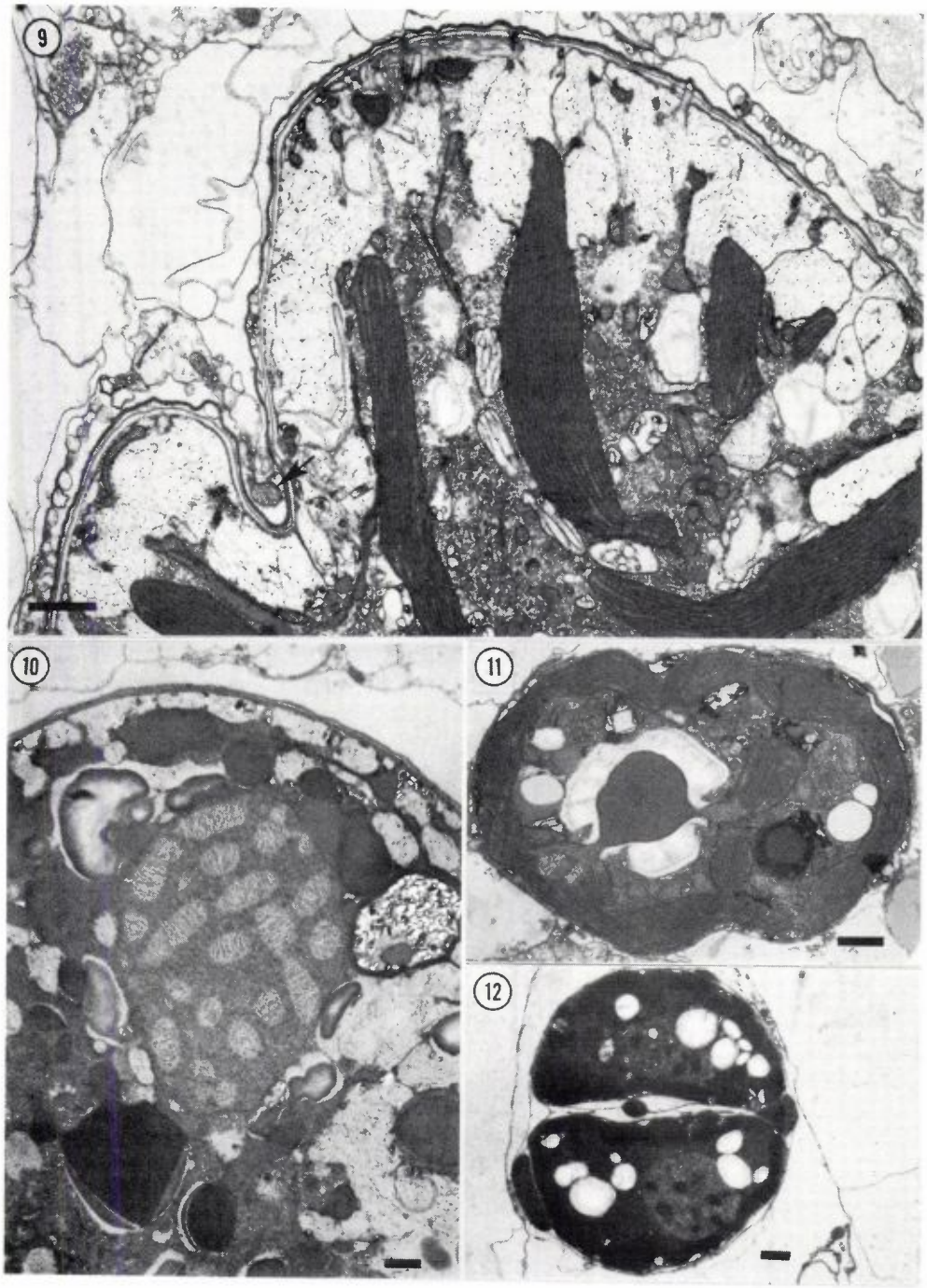
Figure 9. An electron micrograph of a portion of the epicone of *Amphidinium* sp. in *Haplodiscus* indicating the flagellum (arrow) and showing details of the mucocytes in the peripheral cytoplast. Sclae bar, 1μm.

Figure 10. The nucleus of *Amphidinium* sp. in *Haplodiscus*. Scale bar, 1μm.

Figure 11. An electron micrograph of *Symbiodinium* sp. in *Haplodiscus* illustrating the double-stalked pyrenoid without invasive thylakoids. Note that the algal cell appears gymnodiniod rather than coccoid, but no flagella or flagellar basal bodies were observed. Scale bar, 1μm.

Figure 12. An electron micrograph of *Symbiodinium* sp. in *Haplodiscus* illustrating the typical profile of a divided symbiont. Scale bar, 1μm.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Discussion

The observations we have presented in this paper indicate that *Amphiscolops* sp. is highly selective for the symbiont *Amphidinium* sp. Even though the worms do accept and form an association with *A. klebsii* they never form an association with any *Symbiodinium* sp. Because it was not possible to distinguish readily between *A. klebsii* and *Amphidinium* sp. at the level of light microscopy of living cells, we could not directly analyse whether there was any preference by *Amphiscolops* for one or the other alga. However, the relative rapidity with which juvenile worms acquired *Amphidinium* sp. relative to *A. klebsii*, when provided separately (Table 1 and 2) may reflect a preference for the former.

Although we have presented a clear demonstration of the selectivity of *Amphiscolops* for *Amphidinium* sp. the mechanism on which such selectivity is based continues to lie in the realm of speculation. It is clear that in *Amphiscolops*, as in symbiotic coelenterates, selection is not based on recognition between animal and alga which may occur on initial intercellular contact, since *Symbiodinium* may also gain access to worm cells, but they do not persist. As has been recorded for different systems such as the scyphistomae of *Cassiopeia* (Trench et al., 1981; Colley and Trench, 1983), *Hydra* (Smith, 1981) and *Paramecium bursaria* (Karakashian and Karakashian, 1973; Reisser, 1981), selection appears to occur after the initial uptake of the algae by the animal cells (Trench et al., 1981). In this latter context, it is significant to reiterate that in *Amphiscolops* sp. the algae are intracellular, in apparent contrast to the situation in *A. langerhansi* (Taylor, 1971). Whatever the mechanism of recognition may be, we believe that algal size, although it may play some role, is not a critical factor, since the large free-living amphidinioid dinoflagellate found in the same environment as *Amphiscolops* was not accepted.

The novel and significant aspects of the observations on *Haplodiscus* are that (a) the worms simultaneously harbour two closely related dinoflagellates (*Amphidinium* and *Symbiodinium* are both in the family Gymnodiniidae) and (b) that the biomass ratios of the two algae appear to remain constant.

A review of the literature on specificity of symbioses in the marine biosphere (or elsewhere) is totally unwarranted here. Nonetheless, it might be useful to point out that most marine (and freshwater) invertebrates demonstrate a great deal of specificity in their interactions with symbionts. The recent literature on symbioses involving the chemoautotrophic bacteria associated with clams, mussels and tubeworms suggest that, within the limits

of detection, each host has a homogeneous population of one bacterial type, and that the bacteria are different from one host species to another (Lane et al., 1985; H. Felbeck, pers. commun.). The same appears to be true for the HTLV-III/LAV (AIDS) virus (Hahn, et al., 1986). Most symbioses involving phototrophic symbionts (sponges excepted) demonstrate a high degree of specificity (Leutenegger, 1983; Trench, 1979, 1986). In sponges that harbour more than one bacterial or cyanobacterial symbiont, one type is often intracellular and the other intercellular (Wilkinson, 1978; Wilkinson et al., 1981). Although *A. elegantissima* and *A. xanthogrammica* are reported to harbour a dinoflagellate and a green alga simultaneously, a recent analysis of the frequency distribution of the two algae in their hosts indicates a bimodal distribution, with only a very small proportion of the anemone population actually containing both algae simultaneously (L. McCloskey, pers. commun.). Both algae occur in the endodermal cell layer, but whether both occur in the same endodermal cell is unknown. The co-occurrence of two dinoflagellate species in *Haplodiscus* may suggest a lack of specificity. However, it should be emphasized that the same two dinoflagellates, based on their morphological appearance, occur in the worms found in Belau and on the Great Barrier Reef.

The coexistence of two closely related algal species in the same microhabitat raises questions that may be pertinent to ecological thought in the context of competitive exclusion. This concept would be consistent with the observations that only one species of *Symbiodinium* appears to occupy a given host at any one time (Schoenberg and Trench, 1980; Chang and Trench, 1982) and that unnatural symbionts can be displaced by natural ones (*Platymonas convolutae* in *Convoluta roscoffensis*, Provasoli, et al., 1968). The fact that two algae coexist in *Haplodiscus* sp. is clear, but whether this phenomenon is transitory, yielding only one algal type in adult worms, is unknown. All the worms analysed were sexually immature. If it is assumed that both algae persist through the entire life history of the worm, then *a priori*, appropriate conditions to promote such coexistence must prevail. Hence, the concept that these algae may be competing for some limited resource (light, nitrogen, phosphorous) is negated, or at least rendered insignificant.

It has been pointed out (Taylor, 1974) that many pelagic marine invertebrates harbour amphidinioid dinoflagellate symbionts. *Haplodiscus*, although a pelagic worm, obviously spends some portion of its existence in the benthic habitat. It is therefore possible that *Amphidinium* is the natural symbiont, and *Symbiodinium* is acquired from the corals on which the worms settle.

However, no evidence was obtained that the worms fed on any of the corals on which they were found, and the *Symbiodinium* in the poritid corals on which *Haplodiscus* was found in Belau does not correspond morphologically to that found in the worms.

Analysis of the frequency of division as determined by the relative proportion of two-cell stages (Fig. 12) representing divided but not yet separated daughter cells, suggested that the smaller *Symbiodinium* divides (mitotic index about 3%) about ten times faster than the larger *Amphidinium* (mitotic index about 0.2%). In neither instance was any evidence obtained to support the concept of host digestion of algae. One of the outstanding problems in symbioses is understanding the dynamics of control of symbiont populations. The situation in *Haplodiscus* further aggravates the situation, in that the control of two different symbiont populations has to be considered.

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