RESEARCH ARTICLE

Three party symbiosis: acoelomorph worms, corals and unicellular algal symbionts in Eilat (Red Sea)

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Abstract Epizoic worms were found to occur on certain coral colonies from reefs off the coast of Eilat (Red Sea). We identified 14 coral species infested by acoelomorph worms at a depth range of 2–50 m. The host corals were all zooxanthellate and included both massive and branching stony corals and a soft coral. Worms from all hosts were identified as belonging to the genus *Waminoa* and contained two distinct algal symbionts differing in size. The smaller one was identified as *Symbiodinium* sp. and the larger one is presumed to belong to the genus *Amphidinium.* Worm-infested colonies of the soft coral, *Stereonephthya cundabiluensis*, lacked a mucus layer and exhibited distinct cell microvilli, a phenotype not present in colonies lacking *Waminoa* sp. In most cases, both cnidarian and Acoelomorph hosts displayed high specificity for genetically distinctive *Symbiodinium* spp. These observations show that the epizoic worms do not acquire their symbionts from the "host" coral.

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Introduction

Coral reefs are often described as the rain forest of the sea (Connell 1978). As such, they include numerous organisms, which interact with each other in a complex array of symbiotic associations (Paulay 1997). A single coral colony may accommodate a variety of symbiotic organisms including invertebrates and vertebrates, bacteria, and algae, all living in close proximity and utilizing different resources from their host or from each other (Paulay 1997).

The most crucial of these symbioses involves invertebrates and photosynthetic dinoflagellates (Trench 1993). In this system the potential hosts may belong to members of various phyla including Porifera, Cnidaria, Platyhelminthes, and Mollusca (Douglas 1994), as well as Acoelomorpha, a recently designated phylum (Baguna and Riutort 2004). Cnidarian-algal symbiosis is currently being extensively studied and continues to draw greater research interest, especially in light of the frequent coral bleaching episodes occurring worldwide (e.g., Hoegh-Guldberg 1999).

Contrary to the well-studied cnidarians, symbiotic platyhelminth worms, and particularly acoelomorphs are the most understudied group of associations considering the numerical abundance, diversity of taxa, range of habitats, and geographic distribution (McCoy

and Balzer 2002). Recent molecular and morphological evidence suggests that members of the phylum Acoelomorpha are the most basal known triploblastic bilateria (Baguna and Riutort 2004; Ruiz-Trillo et al. 1999). This phylum includes the classes Nemertodermatida and Acoela. The latter, the subject of our study, is a morphologically diverse group of small (∼0.5–10 mm long) and soft-bodied worms (Hooge et al. 2002), lacking a gut cavity and protonephridia (Tyler 2003). To date, there are over 340 known acoel species assigned to 21 families (Tyler et al. 2005). Three of the families contain species with symbiotic unicellular algae (*Convolutidae, Sagittiferidae*, and *Haploposthiidae*) (McCoy and Balzer 2002). The algae that may form a symbiosis with acoels belong to separate lineages, including members of the Bacillariophyta (diatoms), Dinophyceae (dinoflagellates) and Chlorophyta (green algae) (see McCoy and Balzer 2002). Symbiotic marine acoels are common in littoral, sublittoral, and pelagic environments and can be found in temperate as well as tropical habitats (McCoy and Balzer 2002). Among acoel worms, three of the well-documented symbiotic associations are those of *Symsagittifera roscoffensis* with the prasinophyte *Tetraselmis*, *Convoluta convoluta* with the diatom *Licomophora*, and *Amphiscolops* with the dinoflagellate *Amphidinium* (Douglas 1992). Symbiotic acoels that are epizoic on corals include *Haplodiscus* sp. from reefs near Micronesia (Trench and Winsor 1987), *Waminoa litus, Waminoa* sp. 1, 2 and *Convolutriloba hastifera* from North Queensland, Australia (Winsor 1990). *Haplodiscus* sp. contains two distinct algal symbionts within the same host cell. This worm was described as pelagic, but spends part of its lifecycle on the stony coral *Porites* (Trench and Winsor 1987). *W. litus* was found living on the soft coral *Sarcophyton* from Magnetic Island, Australia and containing two species of algal symbionts identified as *Symbiodinium* sp. (8 μm in diameter) and *Amphidinium* sp. (16–24 μm in diameter). *Waminoa* sp. 1 and 2 collected from unspecified corals in the marine aquarium of the Australian Institute of Marine Science (AIMS) and from the stony coral *A. longicyathus* from Pandora reef, Australia, respectively, were similarly associated with two different-sized species of algal symbionts (Winsor 1990). *Convolutriloba hastifera* was collected from a soft coral in the aquarium at AIMS; its symbionts measured 7–12 μm, but were not identified (Winsor 1990). Interestingly, coral reef aquarium hobbyists often encounter infestations by these worms. They report that the worms have been observed on damaged corals, feeding on their tissues and incorporating the algal symbionts (Delbeek and Sprung 1994).

Most studies on symbiotic acoels primarily address the morphology of the worms (Winsor 1990; Trench and Winsor 1987). The nature of the symbiotic association i.e., the possible benefits or disadvantages of each of the three associated partners–namely worms, corals and algal symbionts, are relatively unexplored.

The occurrence of *Symbiodinium* in both corals and worms (Barneah et al. 2004) and the close physical encounter of the worms with their coral hosts gave rise to the following working hypothesis: *Waminoa* sp. worms acquire their dinoflagellate algal symbionts from their coral hosts. We thus decided to test this hypothesis using genetic methods in order to shed light on the nature of this unique three-party symbiosis between corals, worms and dinoflagellate algae.

"Spotted" coral colonies in Eilat (Gulf of Eilat, northern Red Sea) were already noted in 1992 (Brickner, personal observations) yet only recently has this phenomenon been attributed to infestation by acoel worms of the genus *Waminoa*. A recent systematic study of *Waminoa* from Eilat identified one new species, *W. brickneri* (Ogunlana et al. 2005).

The present study deals with the diversity of coral hosts harboring acoel worms in Eilat, examining the worms' morphology, the structure of their symbionts, and the possible effects of the worms on their coral hosts; and compares the genetic identities of *Symbiodinium* spp. populations living in corals and of their epizoic worms.

Materials and methods

Field observations and collection of animals

Fieldwork was performed by SCUBA diving on three reef patches in Eilat, including the reef across from the Inter University Institute for Marine Sciences (IUI), near the Underwater Observatory, and at the oil jetties at a depth range of 2–50 m. Our observations indicated that seven species of stony corals most commonly harbor the worms (see Results). In order to examine the abundance of the worms on a given coral host, surveys were conducted at the IUI along a 70 m belt transect at three depth zones: 2–3, 7–8, and 18–20 m. Each colony of these seven species was carefully counted and checked for the presence of worms. Acoelomorph worms were collected during May, July, October, and December 2003 and February, and March 2004, and were photographed in situ associated with their coral hosts. Worms were collected with a fragment of their host colony, placed into a plastic container and kept in running sea water at the IUI until further observations

and analysis. Four coral species were chosen for in-depth study: The stony corals *Acropora hemprichi, Plesiastrea laxa*, and *Stylophora pistillata*, and the soft coral *Stereonephthya cundabiluensis*. In addition, worms were removed from *A. hemprichi, P. laxa* and *S. pistillata* ($n = 10$ from each coral host), preserved in 2.5% glutaraldehyde in sea water and measured under a dissecting microscope.

Scanning electron microscopy of worms

In order to study the morphological features of the worms we removed 10 worms from each of the above-mentioned coral hosts. Specimens were preserved in 2.5% glutaraldehyde in sea water, dehydrated through a graded series of ethanol concentrations, critically point-dried with liquid CO_2, coated with gold and examined under a JEOL JSM 840 scanning electron microscope.

Scanning electron microscopy of coral surface

In order to compare the surface of infected coral hosts versus non-infected ones, we cut branches (3 cm long) from colonies of *S. cundabiluensis*, with and without worms. Worms were removed from the respective branches using a mild water stream produced by a plastic pipette, and the two types of fragments were preserved and treated as described above.

Transmission electron microscopy of worms

In order to study the structure of the worms and features of their symbionts as well as the position of the latter inside their host, worms preserved in 2.5% glutaraldehyde in sea water were rinsed in buffer phosphate, stained with 1% OsO_4, dehydrated through a graded ethanol series and embedded in Epon. Sections were cut with a diamond knife, stained with lead citrate, and viewed with JEOL 1,200 EX transmission electron microscope.

Genetic analysis of *Symbiodinium* spp. symbionts from corals and resident worms

Symbiodinium populations were freshly isolated from 3 to 6 different colonies of *Turbinaria* sp., *Plesiastrea laxa*, *Acropora hemprichi*, and *Stylophora pistillata*. Tissue was stripped from the skeleton of the stony coral species using a scalpel, homogenized with a plastic pestle, and then centrifuged for 10 min at 2,000 rpm in order to pellet the algae. A piece from the soft coral *Stereonephthya cundabiluensis* was homogenized and

centrifuged as described above. Resident worm populations (30–50 individuals) were collected from each coral colony using a plastic pipette and transferred to microfuge tubes and subsequently frozen.

Extractions of zooxanthellae DNA

DNA extractions were performed using Invisorb Spin Plant Mini Kit (Invitek) according to the manufacturer instructions. Algal pellets derived from coral tissues were ground in a microfuge tube with a small plastic pestle in the presence of lysis buffer. Entire worm specimens were ground and total genomic DNA was isolated from both the worm and its symbionts.

Denaturing-gradient gel electrophoresis (DGGE)

PCR-DGGE was used to analyze the ITS2 of nuclear ribosomal RNA genes (LaJeunesse 2001, 2002). PCR-DGGE analyses were conducted using the forward primer "ITSinfor2" (5′-GAATTGCAGA ACTCC GTG-3′) (LaJeunesse and Trench 2000), which anneals to a "Symbiodinium-conserved" region in the middle of the 5.8S ribosomal gene and the highly conserved reverse primer that anneals to the LSU "ITS2CLAMP" (5′-CGCCCGCCGC GCCCCGCG CC CGTCCCGCCG CCCCCGCCC GGGATCCA TA TGCTTAAGTT CAGCGGGT-3′), an ITS-reverse universal primer modified with a 39 bp GC clamp (LaJeunesse and Trench 2000). A "touchdown" amplification protocol with annealing conditions 10°C above the final annealing temperature of 52°C was used to ensure PCR specificity. The annealing temperature was decreased by 0.5°C after each of the 20 cycles. Once the annealing temperature reached 52°C, it was maintained at that setting for another 20 cycles. Samples were loaded onto an 8% polyacrylamide denaturing gradient gel (45–80% urea-formamide gradient: 100% consists of 7 mol l^{-1} urea and 40% deionized formamide) and separated by electrophoresis for 9.5 h at 150 V at a constant temperature of 60°C (LaJeunesse 2002). The gel was stained with Sybr Green (Molecular probes) for 25 min according to the manufacturer's specifications and photographed using 667 Polaroid film.

Results

Field surveys revealed that 14 coral hosts were infested by acoel worms at a depth range of 2–50 m. The 13 stony corals species that were infected belong to six different families (Acroporidae, Pocilloporidae, Faviidae,

Mussidae, Siderastreidae and Dendrophylliidae). All host species were zooxanthellate, and included massive, lamellose and branching stony corals and one soft coral (Table 1). These corals showed variable patterns of worm density, and ranged from colonies that were densely populated by worms to others that were only sparsely populated. *Turbinaria* sp. and *Echinophyllia* sp. Colonies despite being rare showed the highest

Table 1 Occurrence of acoelomorph worms on coral hosts in Eilat (*Red Sea*) during August 2004

Species of host coral	Depth range[a]	Prevalence of infested corals[b]
Acropora hemprichi	12–18	4/61
Echinophyllia sp.	2–26	1/5
Favia favus	6–10	6/74
Favites pentagona	8–13	3/40
Plesiastrea laxa	8–21	6/34
Stylophora pistillata	6–40	5/225
Turbinaria sp.	6–50	2/10
Acantastrea echinata	3–21	<1/50
Cyphastrea sp.	12	<1/50
Echinopora sp.	22	<1/50
Montipora sp.	22–27	<1/50
Platygira sp.	20	<1/50
Siderastrea sp.	8–15	<1/50
Stereonephthya cundabiluensis	10–22	<1/50

Infestation rate is given as number of infected colonies versus number of colonies counted

[a] indicates minimal to maximal depths in which infested corals were detected

[b] includes data obtained from quantitative surveys only

infestation rate (Table 1). The common Red Sea coral *Stylophora pistillata* was mildly infected (Table 1). Interestingly, corals occupied by worms showed no visible surface damage or lesions. Worms from all hosts were identified as belonging to the genus *Waminoa*, although a species designation could not be made, as most of the specimens were sexually immature (see Tyler 2003).

Figure 1a–c presents images of *A. hemprichi, S. pistillata*, and *P. laxa* infested by worms. Worms appear all over the coral surface. In *S. pistillata*, we observed expanded polyps during the daytime, surrounded by worms (Fig. 1b). In *P. laxa* worms were present on both the outside and inside of the polyp calyx (Fig. 1c). Worms derived from *A. hemprichi* were 1.18 ± 0.345 mm in length, from *S. pistillata* 1.14 ± 0.13 mm and from *P. laxa* 2.63 ± 0.397 mm ($n = 10$ worms from each coral species). All worms were characterized by a golden brown color, due to the presence of numerous algal symbionts. Their dorsal surface contained white refractile bodies, evenly scattered. Worms removed from *A. hemprichi* possessed genital pores (Fig. 2 a); the male pore at the ventral posterior end of the animal, in the center of a swollen area, and the female pore adjacent to it, but more anterior. In worms removed from *S. pistillata* and *P. laxa* no genital pores could be seen (Fig. 2b, c).

Scanning electron micrograph of a fractured worm isolated from *A. hemprichi* shows dorsal and ventral surfaces, covered with cilia, 5–8 μm in length (Fig. 3). Algal symbionts are scattered inside the worm.

Fig. 1 Stony corals inhabited by *Waminoa* sp. worms (*black arrows*). **a** *Acropora hemprichi* and **b** *Stylophora pistillata* with worms surrounding expanded polyps during daytime. **c** *Plesiastrea laxa*. Scale bar: 3 mm
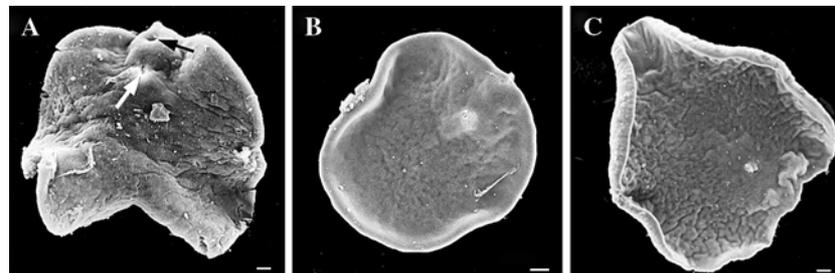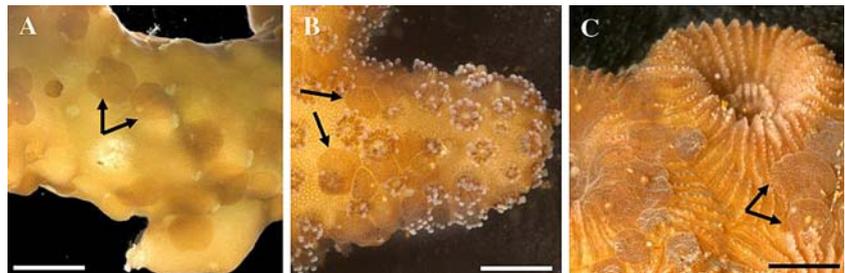

Fig. 2 Scanning electron microscope images of worms isolated from Red Sea stony corals: **a** specimen from *Acropora hemprichi* with a male gonopore (*black arrow*) and female gonopore (*white arrow*); **b** specimen from *Stylophora pistillata* with no visible openings; **c** specimen from *Plesiastrea laxa*. Scale bar: 100 μm
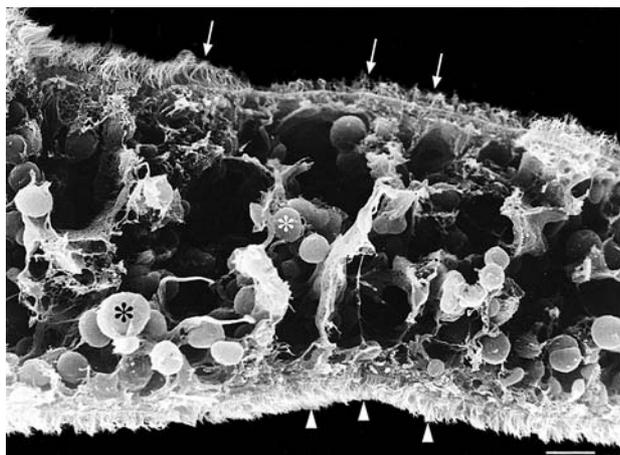
Fig. 3 Scanning electron micrograph of a fractured *Waminoa* sp. worm. Layer of cilia 5–8 μm in length covers the dorsal (*white arrows*) and ventral (*white arrow heads*) surfaces. Numerous small algal symbionts (*white asterisk*) and a few larger ones (*black asterisk*) scattered in the parenchyme. Scale bar: 10 μm

Figure 4a–c presents TEM micrographs of the algal symbionts within the worm. All the worms studied (removed from *A. hemprichi, P. laxa, S. pistillata* and *S. cundabiluensis*) contained algal symbionts of two distinct types: small ones 5–8 μm and larger ones 10–20 μm in diameter, with the former being much more

abundant. Both symbiont types were located within the worms' parenchyme, each engulfed by cytoplasmic processes (Fig. 4 a). The epidermis is ciliated and the parenchymal cell nucleus is in close proximity to the algal symbionts. The smaller symbiont, identified as *Symbiodinium* sp. (Barneah et al. 2004), is characterized by a double-stalked pyrenoid that does not have intruding thylakoid membranes from the chloroplast. The larger algal symbiont appears oval in shape, with an irregular margin line containing surface clefts and ridges (Fig. 4b). The epicone and the hypocone regions (terminology follows Lee 1999) of the cell are distinct, as is the theca, consisting of three layers of membranes (Fig. 4b). The nucleus, situated at the posterior end of the hypocone, contains multiple condensed chromosomes (Fig. 4b). The pyrenoid, situated above the nucleus, shows no evidence of thylakoids invasions. Chloroplasts are scattered around both the cell periphery and in its interior. Figure 4c presents a symbiont with a flagellum within the sulcus, characterized by '9 + 2' arrangement.

The surface of worm-infested *S. cundabiluensis* had distinct cell microvilli (Fig. 5 a), while non-infested colonies possessed a layer of mucus (Fig. 5b). Moreover, these former images indicated that the coral tissue did not contain any lesions or injuries.
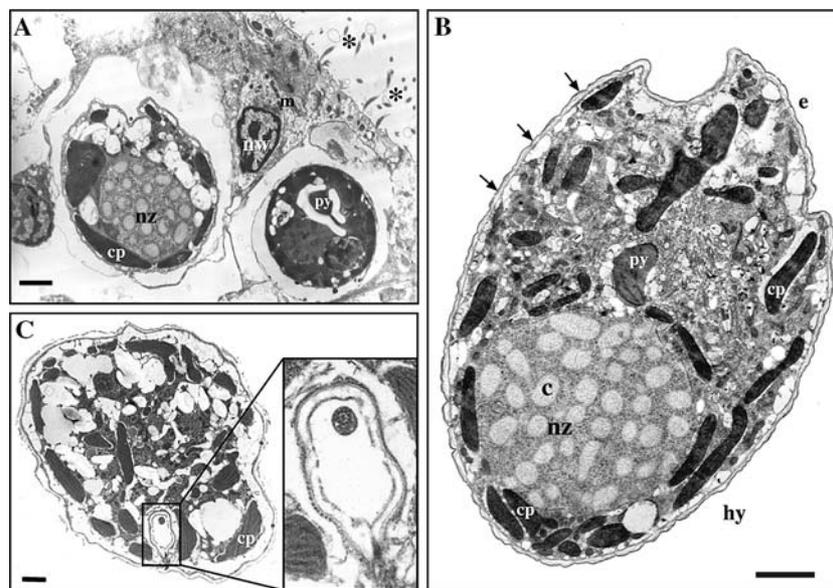

Fig. 4 Transmission electron micrographs of algal symbionts within *Waminoa* sp. **a** A section through a worm isolated from *Acropora hemprichi*. Epidermal cilia in various section orientations (*asterisks*). A nucleus of a parenchymal cell (*nw*) located beneath the muscle layer (*m*) in close vicinity to algal symbionts. On the left side an algal symbiont with a nucleus (*nz*) and chloroplast (*cp*). An *arrow* indicates the smaller symbiont with a double-stalked pyrenoid (*py*). Scale bar 2 μm. **b** The larger symbiont (*Ampidinium* sp.) from *Waminoa* sp. isolated from *Stylophora* *pistillata* with irregular margin line containing clefts and ridges. The epicone (*e*) and the hypocone (*hy*) regions of the cell are distinct. *Dark arrows* indicate the theca, consisting of three layers of membranes. The nucleus is situated at the posterior end of the hypocone (*nz*), containing 38 condensed chromosomes (*c*). Pyrenoid (*py*) is located just above the nucleus. Scattered chloroplasts (*cp*) appear around the periphery and the internal part of the cell. Scale bar 1 μm. **c** Symbiont with a flagellum within the sulcus, with internal '9 + 2' arrangement (*inset*). Scale bar: 2 μm

Fig. 5 Scanning electron micrographs of the surface area of the soft coral *Stereonephthya cundabiluensis*. **a** A fragment inhabited by *Waminoa* sp. appears clean of mucus, shown with protruding cell microvilli. **b** Fragment devoid of worms, covered with a mucus layer. Scale bar: 1 μm
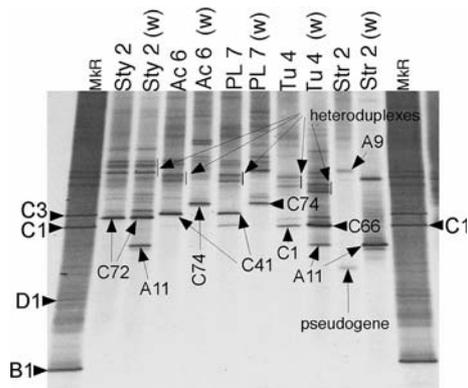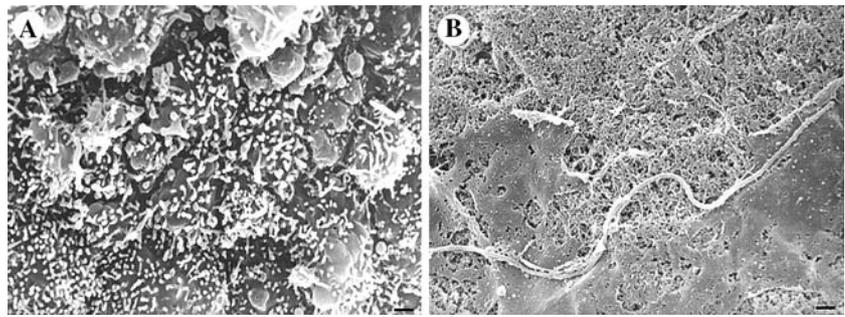

Fig. 6 Representative PCR-DGGE ITS2 fingerprints (*profiles*) of *Symbiodinium* spp. Symbionts observed in coral hosts (Sty2 = *Stylophora pistillata*, Ac6 = *Acropora hemprichi*, PL7 = *Plesiastrea laxa*, Tu4 = *Turbinaria* sp. and Str2 = *Stereonephthya cundabiluensis*) and their resident worms (*w*). *Uppercase letters* indicate lineage or clade, numbers represent ITS type. Examples of heteroduplexes are indicated; they are artifacts of the DGGE-PCR process present in fingerprints of genomes with more than one dominant ITS 2 sequence

The *Symbiodinium* spp. from both the coral and the resident worm population were analyzed using PCR-DGGE fingerprints. In general, the symbiont identified in the coral was not the symbiont found in the worm (Fig. 6). *A. hemprichi* and *Plesiastrea laxa* shared the same *Symbiodinium* type, C41 (clade C type 41), while their resident worms both had type C74. *Turbinaria* sp. possessed C1 while its worms had mixed populations of two different types: C66 occurred consistently and usually in greater relative proportion than type A11. *S. cundabiluensis* possessed A9 while its worms possessed A11. *Stylophora pistillata* and its worms shared C72 symbionts but in a few replicates the worm populations also revealed A11 symbionts.

To demonstrate the consistency and distinctiveness of each kind of algal-invertebrate symbiosis, Fig. 7 presents the PCR-DGGE fingerprints of *Symbiodinium* spp. sampled from six colonies of the stony coral *Turbinaria* sp. and the respective fingerprints of symbionts from their respective resident worms. Four colonies (Tu2-5) harbored C1, one colony (Tu10) harbored
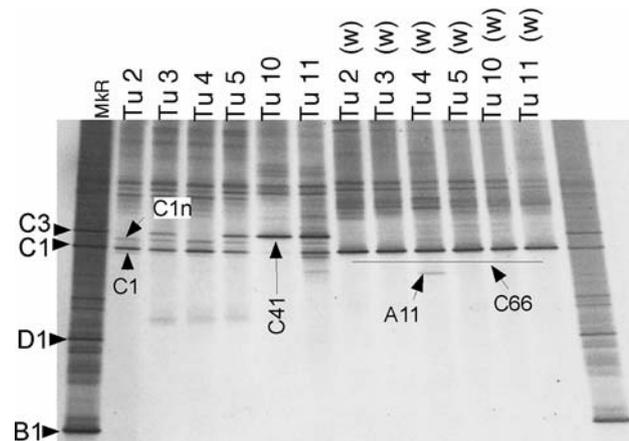

Fig. 7 PCR-DGGE ITS2 fingerprints of endosymbionts obtained from six colonies of the stony coral *Turbinaria* sp. (Tu2, Tu3, Tu4, Tu5, Tu10, and Tu11) and the respective profiles of the endosymbionts obtained from the worms found on each of them (*indicated with the lowercase letter w*)

C41, and another colony (Tu11) harbored both C1 and C41. Their worms all possessed symbionts belonging to type C66 (the migration of C66 during electrophoresis is similar to C1). In addition, the worms on one *Trubinaria* colony (Tu4) also possessed A11 *Symbiodinium*.

Discussion

Numerous coral taxa surveyed in Eilat's reefs were found to sometimes possess dense populations of acoel worms belonging to the genus *Waminoa*, previously described from Australia (Winsor 1990). Sexually mature worms from *Plesiastraea laxa* were identified as a new species named *W. brickneri* (Ogunlana et al. 2005). To date, the only other described species in this genus is *W. litus* (Winsor 1990). Worms isolated from the corals *Acropora hemprichi*, *Stylophora pistillata* and *P. laxa* exhibited variability in respect to size and reproductive state, which might be indicative for the presence of several species of *Waminoa* in Eilat. The classification of acoelomorph species is based on characteristics of their reproductive organs (Tyler 2003). Since most of

the worms collected in the present study were not sexually mature, species identification of worms from different coral hosts was not feasible, and therefore, the specificity between coral host and resident worms could not be resolved. To date, only *W. brickneri* has been identified from Eilat. This new species (see Ogunlana et al. 2005) was found on the stony coral *P. laxa*. Molecular data concerning the genus *Waminoa*, which could be helpful as a systematic tools are still scarce (Ogunlana et al. 2005). In the future, such data will surely aid in the classification of these worms.

The lamellose coral species *Turbinaria* sp. and *Echinophyllia* sp. showed the highest infestation rate by the worms (Table 1). However, it should be noted that in these two species only one colony out of five was infected. On the other hand, in the branching coral *S. pistillata*, which is one of the most abundant stony corals in Eilat (Loya 1972), only five out of 225 sampled colonies were infected. Overall, we found a low infestation rate, which was also very patchy in nature. Hence, whereas one colony of a certain species may be found completely covered with worms, other colonies in its close vicinity (same species or different) may be worm-free. Our quantitative survey took place only at the reef across from the IUI. Undoubtedly, additional surveys of the Red Sea reefs are needed in order to obtain a realistic assessment of the extent of the worms' distribution and prevalence on various coral taxa.

Only worms from *A. hemprichi* contained genital openings and histological sectioning corroborated that these animals were sexually mature. All the other specimens lacked such openings and not even a mouth could be detected. A low occurrence of sexually mature acoelomorph worms has been also encountered in other studies of *Waminoa* sp. and *Haplodiscus* (Trench and Winsor 1987; Winsor 1990). Such findings suggest that sexual reproduction might be seasonal.

The worms contained two distinct algal symbionts. The smaller ones, which possessed a double-stalked pyrenoid (Fig. 4a) were assessed genetically using PCR-DGGE analysis of ITS-2 rDNA (LaJeunesse 2002) and were assigned to the genus *Symbiodinium* (see Barneah et al. 2004). The larger symbionts were characterized by the presence of multiple chloroplasts, an irregular cell margin and the presence of a flagellum (Fig. 4c). The flagellum within the sulcus is most probably the longitudinal flagellum. The irregular margin line, characterized by the presence of clefts and ridges, is presumed to have resulted from section planes cutting through both flagellar canals i.e., the girdle and the sulcus. Several species of *Amphidinium* are known to occur as symbionts in acoelomorph worms, including *W. litus*, *Haplodiscus* sp. (Winsor 1990) and three

different species of *Amphiscolops* (Taylor 1971; Trench and Winsor 1987; Lopes and Silveira 1994). Interestingly, in symbiotic *Amphidinium* species, whether intercellular (Taylor 1971) or intracellular (Trench and Winsor 1987), the algae *in hospite* were found to retain their free-living morphology, including the flagellar apparatus (Trench 1993), similar to our findings (Fig. 4c). *Amphidinium klebsii* symbionts from *Amphiscolops langerhansi* were reported to present a specific and uniform orientation within their host (Taylor 1971), a unique trait that has not been reported in any other symbiotic acoelomorph worms, including *W. brickneri*. The symbionts from *Amphiscolops langerhansi* were characterized by the presence of a spherical pyrenoid, which formed the center of attachment for the chloroplasts. The latter characteristic was valid also for the symbionts isolated from *Haplodiscus* sp. and *Amphiscolops* sp. (Trench and Winsor 1987; Lopes and Silveira 1994). In our study, no evidence of radiating chloroplasts was found. The multiple and condensed chromosomes observed in the larger symbiont of *Waminoa* sp. resemble the situation described in *Amphidinium* sp. from *Haplodiscus* sp. (Trench and Winsor 1987), but differ from that described for *Amphidinium klebsii* in *Amphiscolops langehansi* (Taylor 1971). The presence of a flagellum *in hospite*, and the general size and shape lead us to suggest that the larger symbiont in *Waminoa* sp. is an *Amphidinium*.

Algal symbionts within the worm were each surrounded by cytoplasmic processes originating from the worm's parenchyme cells and found in close proximity to their nuclei, thus implying a possible intracellular position of the symbionts. Coexistence of two species of algal symbionts was previously described in species of the convolutid *Amphiscolops* sp. (Yamasu and Okazaki 1987), in *W. litus* (Winsor 1990) and in *Haplodiscus* sp., where the two co-occurred within the same host cell (Trench and Winsor 1987). Trench and Winsor (1987) speculated that the two symbiont types might be involved in a competitive exclusion process, yielding only one algal type in adult worms, but assume that it is possible for both algal types to persist through the entire life history of the worm. Since sexually mature specimens of *Haplodiscus* sp. were lacking, it was impossible to confirm this assumption. In our study sexually mature specimens contained both symbiont types, thus negating the occurrence of competitive exclusion. Trench and Winsor (1987) additionally speculated that the *Amphidinium* was the natural symbiont and the *Symbiodinium* was acquired from the corals on which the worms live. However, they specifically mention that there is no evidence that the worms fed on any of the host corals (Trench and Winsor 1987).

In order to study the type of physical interaction between corals and worms we used two approaches. The first one checked whether the worms damage the coral and compared the surface area of corals that were infected with worms to those that were devoid of worms. The second one studied the identity of *Symbiodinium* sp. symbionts in both the corals and the worms and examined the genotype of *Symbiodinium* sp. in the two partners using PCR-DGGE (LaJeunesse 2001). The SEM images of the surface area of the coral *Stereonephthya cundabiluensis* with and without worms (Figs. 4, 5) suggest that the worms may be removing the surface mucus layer, using their dense cilia cover to brush off the mucus. Possible nutritional value of the mucus (see Wild et al. 2004) for the worms awaits further examination. Although these SEM images indicated that the tissue of worm-infected colonies did not contain any lesions or injuries, there is a possibility that the worms interfere with certain biochemical processes of the coral, such as photosynthesis by causing a "shading" effect when present in high numbers on its surface. Furthermore, a recent review by Brown and Bythell (2005) highlights a variety of functions of the coral mucus-layer mostly as a defense agent against pathogens, space invasion by other corals, UVR damage, desiccation, smothering by sediment, and pollutants. Considering these data, the removal of the mucus by the worms might make the coral more susceptible to various biotic and a-biotic disturbances.

Most acoel worms found on corals do not appear to obtain *Symbiodinium* sp. from the coral tissues with which they are in contact. The *Symbiodinium* sp. symbionts in the worms differed in most cases to those in the "host" coral colony. This marked difference in specificity implies that the worm acquires its symbionts via another means. To this end we have determined that these worms vertically transmit both *Amphidinium* sp. and *Symbiodinium* sp. to the oocytes during gametogenesis (Barneah et al. in press). We realize symbiont populations inherited by the juvenile from the parent may not remain the same at reproductive maturity, especially while under constant exposure to environmental pools of viable symbionts. Several nudibranch snails such as *Spurilla neapolitana* temporarily incorporate zooxanthellae into their tissues while ingesting cnidarian prey (Marin and Ros 1991). These "second hand" zooxanthellae are acquired via feeding and translocated to vacuoles in the cytoplasm of endothelial cells of the digestive gland (Kempf 1991; Marin and Ros 1991). This does not appear to be the case in the three-way relationship under study. Based on consistent differences in symbiont identity between the worms and their coral hosts as was shown in the

DGGE analysis, and the lack of any noticeable tissue loss on "host" corals, it is concluded that the worms do not prey on their coral hosts, nor do they obtain their algal symbionts directly from them.

The PCR-DGGE fingerprints of algal symbionts derived from six colonies of *Turbinaria* sp. corals and their worms revealed high consistency in identity of symbionts found in both the worms and their coral hosts (Fig. 7). However, there were two groups of worms collected from *Turbinaria* sp. and *Stylophora pistillata* that did show heterogeneity in algal symbionts content. In these cases certain individuals possessed symbiont species belonging to two different clades. Additional work is required to determine whether different species of *Waminoa* contain different *Symbiodinium* spp. or that variation in these symbioses occurs among individuals of the same species. There is also the possibility that individual worms simultaneously contain two different species of *Symbiodinium*. Because DNA extractions were performed on large pools of worms, these distinctions cannot be made at present.

The occurrence of acoelomorph worms on many coral hosts in Eilat's reefs may be a unique phenomenon. The extent and prevalence of epizoic symbiotic acoelomorph worms on corals in other regions around the Indo-Pacific is virtually unknown. This three-party symbiotic relationship, comprised of two invertebrate hosts harboring algal symbionts and living together, exemplifies the complexity and interdependence of life on a coral reef. Determining the relative energetic contribution each of these associations provides, would further elucidate their importance.

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