

AMPHIBIOUS MICROBORERS: BIOERODING FUNGI ISOLATED FROM LIVE CORALS

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ABSTRACT

Pure cultures of bioeroding fungi have been obtained from the interior of the aragonite skeleton of living corals in the Caribbean and the South Pacific. Most isolates represent omnivorous, saprobic, dikaryomycotan anamorphs.

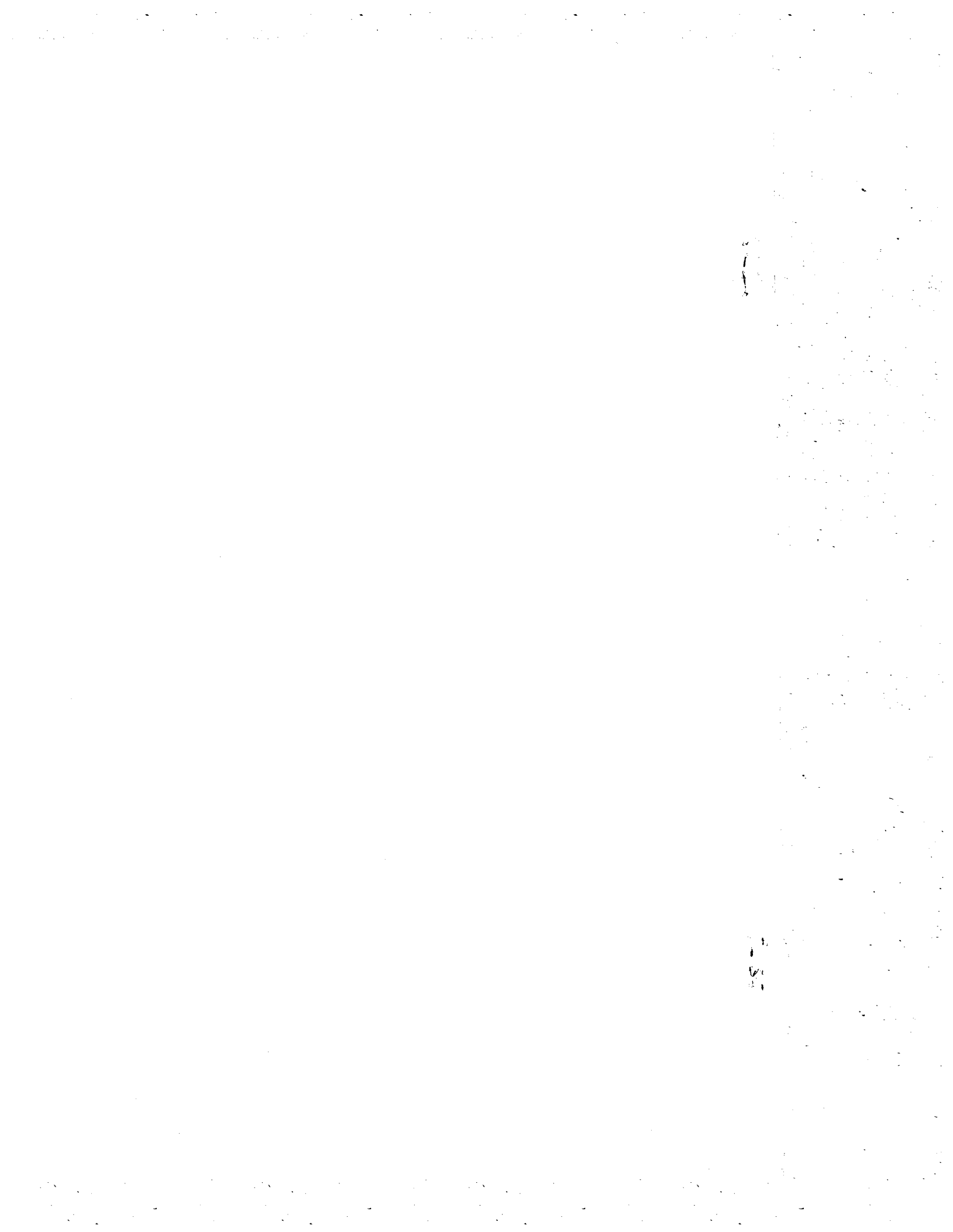
Bioerosion of calcareous substrates is of considerable ecological and economic importance. The strength of modern coral reef ramparts can be drastically reduced by cryptic organisms which may destroy existing coral skeleton from within as fast as new material is laid down at the periphery (Hein and Risk, 1975; Tunnicliffe, 1979; 1980). The porosity and permeability of carbonate reservoir rocks may have been altered by similar bioeroders.

Most published studies of bioerosion have emphasized the macroborers, such as sponges and polychaetes, which are generally believed to be the most destructive (Goreau and Hartman, 1963; Neumann, 1966; MacGeachy and Stearn, 1976). As yet we know very little about the taxonomy, distribution and mode of action of the less obvious but apparently equally ubiquitous microborers—bacteria, algae and fungi. This is especially regrettable in view of suggestions that bioerosion represents an example of succession in which one group of organisms prepares the substrate for the next (McCloskey, 1970; Risk and MacGeachy, 1978), and that there may be an anthropogenic acceleration of bioerosion in coastal waters (Risk and MacGeachy, 1978).

Chitinoclastic bacteria have been isolated from coral heads, and are believed to weaken the coral skeleton by removing the organic matrix (Di Salvo, 1969). Marine bacteria also cause *in vitro* etching of Iceland spar (Risk and MacGeachy, 1978). Boring (endolithic) algae were described early in the century (Duerden, 1902), and are capable of eroding 80% of the surface of Iceland spar crystals within 40 days (Kobluk and Risk, 1977).

The excavations made by microborers have been studied in a series of elegantly-illustrated papers employing casting techniques (Perkins and Halsey, 1971; Edwards and Perkins, 1974; Golubic et al., 1975; Le Campion-Alsumard, 1979; Zeff and Perkins, 1979), which revealed algae and fungi as pervasive penetrators (Figs. 2, 3). Nevertheless, we still knew almost nothing about the identities and activities of the fungi involved.

Fungal bioerosion is an ancient phenomenon, dating back at least to the lower Paleozoic (Kobluk and Risk, 1974). The ability of some fungi to utilize the organic matrix of carbonate skeletons in the absence of light appears to give them an advantage over the algae, and means that they may have, at any depth, the same skeleton-weakening effects attributed to the bacteria. Boring fungi are widespread in modern corals (Fig. 2). Because most fungal hyphae display considerable morphological uniformity, taxonomic and experimental approaches to this group depend on successful culturing and isolation. In this paper, we report on the first phase of cooperative research on bioeroding fungi, which has produced the first isolations of identifiable species of these fungi.





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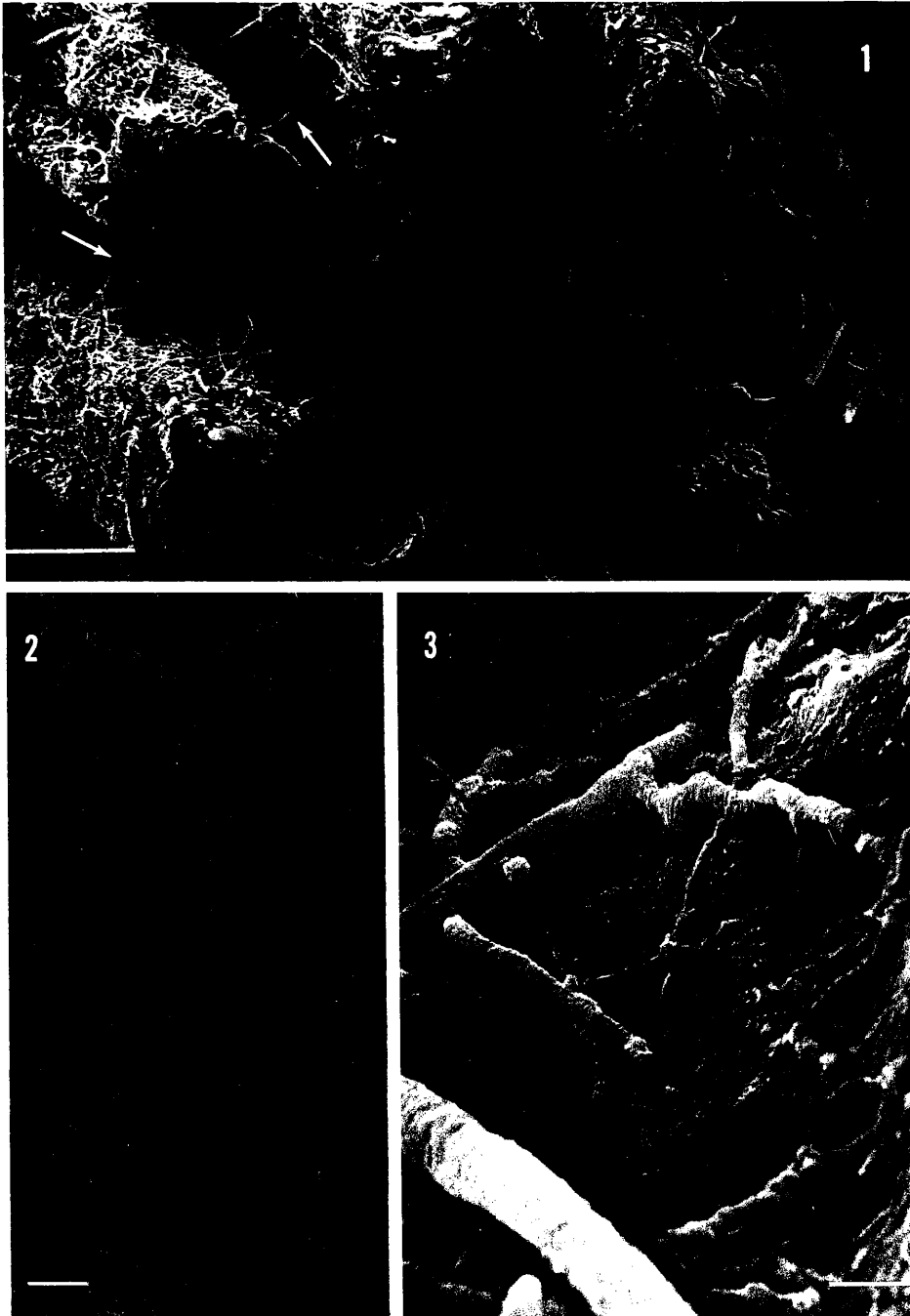


Figure 1. Scanning electron micrograph of a plastic mould of a fungus-bored corallite of *Siderastrea siderea*. Hyphae may be seen penetrating the very youngest layers, and sometimes passing right through a septum (arrows). Scale bar = 200 μm .

Figure 2. Transmitted light micrograph of horizontal section through naturally-bored aragonite skeleton of *Goniastrea australensis*. Scale bar = 40 μm .

Figure 3. Scanning electron micrograph of a plastic mould of naturally-bored skeleton of *Siderastrea siderea*. Note broad and narrow borings. Scale bar = 5 μm .

riddling modern corals belong to common terrestrial conidial (anamorphic) fungi. Many of these anamorph-species, such as those of *Aspergillus*, *Penicillium* and *Cladosporium*, are omnivorous, and have no difficulty in coping with the salinity of the habitat. Members of these genera are among the most osmotolerant organisms known, often being found in habitats of very high osmotic pressure, such as jams and jellies (Pitt, 1981), while *Wallemia ichthyophaga* is commonly recorded on salt fish (Frank and Hess, 1941). It is now accepted by a majority of mycologists that the dikaryomycotan fungi—Ascomycetes, Basidiomycetes and their anamorphs—evolved on land (Savile, 1955; 1968), but since some of them, particularly in the anamorphic phases, have become such omnipresent and adaptable saprobes, there is little reason why they should not compete successfully in the oceans with the older, more conservative, primarily aquatic protocystan fungi, such as the chytrids.

How these opportunistic fungi enter the coral skeleton, and how they spread, will be the subject of future investigations. Reproduction and dissemination which, in the air-bounded terrestrial environment, usually involve the formation and liberation of conidia, must operate differently in the sea, since many terrestrial anamorphs do not form conidia in submerged culture. Although there may well be a continuous influx of conidia from the land, these may in many, though not all, cases be prevented from germinating by a mycostatic factor or factors that may be present in seawater (Kirk, 1980). We suspect that much spread within reefs is simply by growth of hyphae through the substrate, or by the dispersal of infected shore rock, sediment, or broken coral fragments during storms.

The regularity with which such "terrestrial" fungi occur within corals from widely separated geographic areas suggests that some of these fungi are even more omnipresent and ecologically important than has been realized.

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MATERIALS AND METHODS

After a lengthy process of trial and error, we developed a simple technique for culturing fungi from inside coral skeletons with an acceptably low (or, at worst, a quantifiable) contamination rate, and a high success rate (up to 50%).

Isolation had to be attempted as soon as possible after the coral head had been removed from the sea, to avoid chemical changes inside the head (Risk and Kramer, 1981) and contamination by opportunistic airborne fungi. The coral heads were cleaved. With a flamed scalpel blade tiny samples were gouged out from the freshly exposed surface at pinpointed, predetermined levels within the head. The samples were transferred immediately to small screw-top vials (47×15 mm) containing 1–2 ml seawater-based liquid medium with a broad-spectrum, autoclavable, antibacterial antibiotic (10 g peptone, 0.1 g chloramphenicol, 1 l seawater). All resulting colonies were subcultured onto solid PDA or MEA in slants or plates for identification. Levels of atmospheric fungal contaminants were tested by opening a series of control vials to the air for 10 sec each at intervals during the isolation procedure. This step was necessitated by the general lack of sterile facilities in the vicinity of most coral reefs. Subsequent comparison of these controls with the inoculated vials helped to establish the origin of isolates developing in the latter.

Preliminary reinoculation experiments have been carried out with some of our fungal isolates. Although it is difficult to find completely unbored samples of coral (Fig. 1), the peripheral, most recently deposited, layer of the coral skeleton is often virtually free of borings. We inoculated small samples of such material, derived from *Siderastrea siderea*, with *Aspergillus versicolor* and *Penicillium stoloniferum*, two of our fungal isolates. After incubation for 28 days in seawater at room temperature, the specimens were rinsed repeatedly in sterile water then fixed, dehydrated and plastic-embedded using the following schedule: (1) Fix in 6% glutaraldehyde in 0.1 M Na cacodylate buffer (24–72 h); (2) Wash in distilled water (10–30 min); (3) Dehydrate in ethanol series: 50% EtOH (30 min), 70% EtOH (30 min—may be stored at this point for up to 1 year), 95% EtOH (30 min), 100% EtOH (30 min), 100% EtOH (30 min); (4) Embed: 100% propylene oxide (PO) (30 min), 50% PO + 50% Spurr's (30 min), 25% PO + 75% Spurr's (30 min), 100% Spurr's (overnight in vacuum oven at 65°C), 100% Spurr's (8 h at 65°C), 100% Spurr's (16 h at 65°C).

Samples were then cut to expose an aragonite surface and decalcified in 10% (v/v) HCl. The remaining plastic matrix was then gold sputter-coated and observed in the SEM.

RESULTS AND DISCUSSION

We isolated 20 fungi, representing 18 species of 12 anamorph-genera and 2 unidentified sterile mycelia, from 15 different hermatypic (reef-building) corals and one hydrozoan (*Millepora*). The reefs sampled were at Barbados, West Indies; Lizard Island and Heron Island, respectively at the north and south ends of the Great Barrier Reef, Australia; and Rarotonga in the Cook Islands. Fungi were isolated from coral samples taken at depths up to 20 m, and from all levels within the coral heads. The results of our preliminary survey are shown in Table 1.

Reinoculation produced the kind of phenomenon revealed by the scanning electron micrograph reproduced in Figure 1, which is derived from the plastic-infiltration technique discussed above. Here, the radially arranged columns represent the spaces between the septa of a corallite. The spaces in the picture represent the septa themselves. The fine filaments seen growing outside the solid columns represent plastic-infiltrated microborings produced by fungi which penetrated and grew within the aragonite, occasionally passing right through a septum (arrows).

Since our thin sections of corals regularly show fungal borings deep within the coral skeleton, as in Figure 2, and similar fungal penetration of calcareous shore rock has been reported (Le Campion-Alsumard, 1979), our experimental confirmation of the boring potential of these fungi is not unexpected.

In naturally bored corals, both light microscopy of sections (Fig. 2) and scanning electron microscopy of plastic-infiltrated borings (Fig. 3) show the presence of two major classes of microborings. These are commonly described as wide and narrow. The wide borings have generally been ascribed to algae and the

Table 1. Fungi, substrates and locations

Bioeroding Fungi	Coral Substrate	Location
<i>Acremonium</i> sp.	<i>Meandrina meandrites</i>	Barbados
<i>Aspergillus restrictus</i> Smith	<i>Diploastrea heliopora</i>	Lizard Is.
<i>A. sydowi</i> (Bain. & Sart.)	<i>Acropora palmata</i> <i>Montastrea annularis</i> <i>Porites porites</i>	Barbados
<i>A. versicolor</i> (Vuill.) Tiraboschi	<i>Acropora hyacinthus</i> <i>Acropora</i> sp. <i>Goniastrea retiformis</i> <i>Porites australensis</i>	Heron Is. Lizard Is.
<i>Asteromella</i> sp.	<i>Diploastrea heliopora</i>	Lizard Is.
<i>Bipolaris rostrata</i> (Drechs.) Shoem.	<i>Acropora palifera</i>	Heron Is.
<i>Cladosporium sphaerospermum</i> Penz.	<i>Montastrea annularis</i> <i>Porites</i> sp.	Barbados Rarotonga
<i>Hormonema dematioides</i> Lagerb. & Melin	<i>Acropora</i> sp. <i>Diploastrea heliopora</i> <i>Goniastrea retiformis</i> <i>Porites australensis</i> <i>Stylophora pistillata</i>	Lizard Is. Rarotonga
<i>Humicola alopollonella</i> Meyers & Moore	<i>Acropora hyacinthus</i>	Heron Is.
<i>Paecilomyces lilacinus</i> (Thom.) Samson	<i>Goniastrea australensis</i>	Heron Is.
<i>Penicillium avellaneum</i> Thom & Turesson	<i>Porites porites</i>	Barbados
<i>P. expansum</i> Link ex S.F. Gray	<i>Acropora palmata</i>	Barbados
<i>P. godlewskii</i> Zaleski	<i>Montastrea cavernosa</i>	Barbados
<i>P. restrictum</i> Gilm. & Abbott	<i>Goniastrea australensis</i> <i>Porites australensis</i>	Lizard Is. Heron Is.
<i>P. stoloniferum</i> Thom	<i>Acropora palmata</i> <i>Diploria labyrinthiformis</i> <i>Millepora complanata</i>	Barbados
<i>Phialophora bubaki</i> (Laxa) Schol-Schwarz	<i>Diploastrea heliopora</i> <i>Goniastrea retiformis</i> <i>Porites australensis</i>	Lizard Is.
<i>Pithomyces chartarum</i> (Berk. & Curt.) M.B. Ellis	<i>Acropora</i> sp.	Lizard Is.
<i>Wallema ichthyophaga</i> Johan-Olson	<i>Porites</i> sp.	Rarotonga
Sterile hyaline mycelium with swellings	<i>Acropora</i> sp. <i>Diploastrea heliopora</i> <i>Goniastrea australensis</i>	Lizard Is. Heron Is.
Sterile dark mycelium with swellings	<i>Acropora palifera</i> <i>Acropora</i> sp. <i>Porites</i> sp.	Lizard Is. Heron Is. Rarotonga

narrow borings to fungi; the literature has been summarized by Lukas (1973). We are currently seeking further confirmation of this using fluorescence techniques.

It is highly probable, given that our culture methods are similar to those commonly used in marine mycology, that at least some of the fungal hyphae seen