# Dominant fungi from Australian coral reefs

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This report describes 617 fungi isolated from coral reefs in tropical Australian marine environments. Host substrates include 62 sediments, algae (8 Rhodophyta, 9 Chlorophyta, 3 Phaeophyta) and vertebrates/invertebrates (16 Bryozoa, 21 Chordata, 16 Cnidaria, 70 Porifera). Results indicate that some reef dwellers may provide a natural reservoir for fungal genera normally associated with other organisms. Taxa such as *Aspergillus* and *Penicillium*, commonly thought to originate from terrestrial run-off, were frequently isolated from offshore hosts. One hundred and twenty one isolates (19.6% of the total) sporulated, but could not be identified using the available taxonomic keys, while 99 isolates (16%) did not sporulate, and thus were classified as sterile mycelium. Some isolates, such as *Cochliobolus* spp., have not previously been described from marine sources, and could represent novel taxa. Slow growing marine ascomycetes were not isolated, probably because they were outgrown by faster growing taxa.

Key words: coral reef, filamentous, fungi, marine.

## Introduction

Ecosystem fertility is dependent upon microbial activity (Moore and de Ruiter, 1991), and therefore filamentous fungi are likely to play a role in the heterotrophic conversion of reef biomass to nutrients. Only 800-1000 taxa of marine fungi have been identified (Hawksworth *et al.*, 1995; Jones, 1995), compared with the thousands of species of terrestrial fungi described. The majority of these taxa have been identified from mangroves, beach sand, driftwood, shell matter or as pathogens of marine organisms of economic value (Hyde, 1996). Marine fungi are generally placed into one of the following groups: manglicolous - those found in the mangrove environment; arenicolous - fungi associated with sand grains; lignicolous - wood inhabiting fungi; caulicolous - growing on or in herbaceous stems; foliicolous - fungi growing on leaves and algicolous - inhabiting marine algae (Hughes, 1975; Hyde, 1989).

Research into the fungi associated with coral reef ecosystems has predominantly been concerned with pathogenic disease symptoms in various

host organisms, usually in the form of tissue necrosis or biomineralisation (Rand, 2000). The majority of marine mycological research has focused on organisms of economic value, particularly those of farmed crustaceans (Alderman, 1982, 1984; Hose *et al.*, 1984; Chen *et al.*, 1992; Huang *et al.*, 1996a) fish (Alderman, 1982; Rand, 2000), and to a lesser extent algae (Muehlstein, 1992). In total only 22 species of ascomycetes and mitosporic fungi have been identified from carbonaceous sources in the tropics, in association with crustose sponges, coralline algae, coral slabs, forams, molluscs and barnacles (Kohlmeyer and Volkmann-Kohlmeyer, 1992).

Reports of fungal taxa in the ecological context of marine environments are scarce. Littler and Littler (1998) describe an unidentified fungal pathogen of reef-forming crustose coralline algae in American Samoa, found predominantly in shallow (<20 m) waters. Fungi have also been observed inhabiting the skeleton of *Porites lobata*, causing local decomposition of dense skeletal material (Le Campion-Alsumard *et al.*, 1995), with gorgonians in the Carribean (Smith *et al.*, 1997; Geiser *et al.*, 1998), and associated with sea cucumbers (Afiyatullov *et al.*, 2000; Pivkin, 2000). Surveys of the biodiversity of marine fungi in Australian marine environments, and studies of fungal interactions with reef-specific organisms are even more limited, with a majority of research taking place prior to 1970 (Cribb and Herbert, 1954; Cribb and Cribb, 1955, 1956, 1960, 1969; Kohlmeyer and Volkmann-Kohlmeyer, 1991, 1992; Hyde, 1996).

This paper explores the diversity and ecology of natural populations of filamentous fungi isolated from the Australian marine environment, with an emphasis on host organisms sourced from the general reef community.

## Materials and Methods

### *Sample collection*

A wide range of sediments and marine organisms were targeted for collection in an attempt to maximise the diversity of fungal isolates obtained from marine environments around Australia.

Marine organisms were collected via reef walking, snorkelling, SCUBA, and, in a few instances, trawling. Each sample was placed wet into a sterile plastic bag and maintained at the seawater temperature for transport to the laboratory. A 1 $cm^3$ section was excised from each invertebrate or algal sample, and placed into a sterile 0.06% sodium hypochlorite solution, gently shaken and left for one minute, followed successively by two 1 minute rinses in 10 mL of sterile ocean nature artificial seawater (sASW) to ensure surface contaminants were removed prior to plating. The shaking ensures that sponge

vacuoles and pores were also cleaned. The sample block was removed and cut into small sections (<1 $mm^3$) with a sterile scalpel. Cut sections (5 per plate) were placed onto the following selective media: filter paper (sterile filter paper on a Petri-dish, moistened with sASW)); modified malt extract agar (MEA; 10g Difco malt extract, 1g yeast extract, 1g Difco bacteriological peptone, 15g of Bacto agar, 1L sASW); yeast peptone agar (YPA; 1g Difco yeast extract, 1g Difco bacteriological peptone, sASW); potato carrot agar (PCA; made in 1L sASW, 15g Bacto agar). Streptomycin sulphate (10 mg/L) was incorporated into each of the agar media to minimise bacterial contamination.

Sediments were collected by divers (snorkel or using SCUBA directly from the sea floor), or with the use of Smith-MacIntyre (>50 m depth) or hand-held scissor grabs (<50 m depth). Each sediment sample (3 $cm^3$) was collected in a pre-sterilised sawn-off syringe, and were plated onto the isolation media within two hours of collection: Sediment (2 $cm^3$) was collected *in situ* or from the centre of the resultant grab sample, to minimise extraneous contamination. The sample was added to 8 mL sASW and vortexed at high speed for 10 minutes. Two hundred µl of the resultant suspension was plated onto MEA, PCA and YPA plates. Small amounts of sediment were also dropped onto individual plates of MEA, YPA, PCA and filter paper with sterile forceps.

***Isolation***

All inoculations (sediments, algae and animals) were completed within two hours of collection. Inoculated plates were sealed with parafilm and incubated at 27 C. Samples were checked daily for the emergence of fungal hyphae, which were transferred to a fresh agar plate and purified. Only hyphae emerging from the source material was sub-cultured onto media for purification. Hyphae originating from samples on the filter paper media were subcultured onto MEA.

Six-hundred and seventeen fungal isolates collected between August 1995 and August 1997 were chosen for taxonomic identification. Microscopic slides were prepared from plate cultures using both the sticky tape method and teasing hyphae in lactophenol cotton blue on a glass slide (Kohlmeyer, 1979). Glass slides were covered with coverslips and left 24-48 hours before sealing with clear nail varnish. To document each isolate digital photographic images are curated within the marine bioproducts database (Australian Institute of Marine Science). Fungi were identified to genera whenever possible, using a range of taxonomic keys (Kohlmeyer, 1979; Barron, 1983; Domsch *et al.*, 1993; Alexopolous, 1996). NID (not identified) was used to indicate that the fungi could not be taxonomically assigned using the keys available, while SM (sterile mycelium) was used to indicate no spores (or spore bearing structures) as viewed under the microscope.

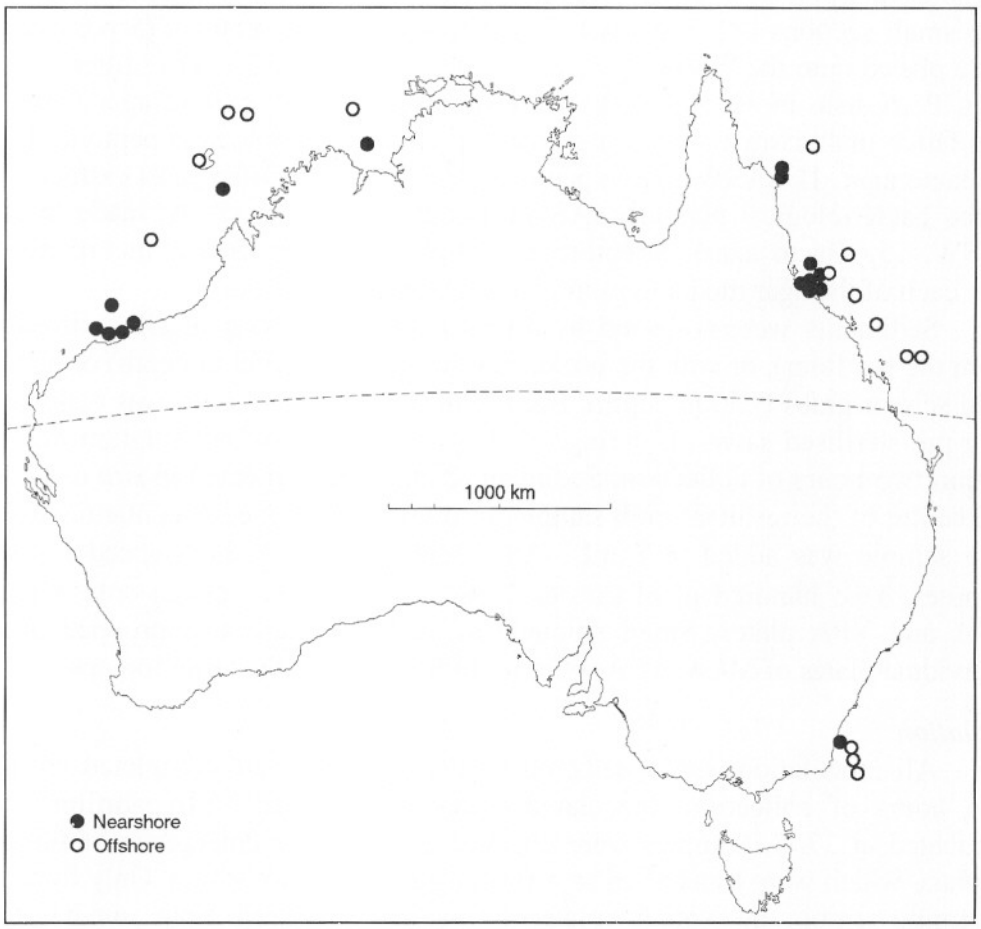


**Fig. 1.** Map of Australian collection sites for source material collected during 80 separate collection trips between 1995 and 1998. 1403 fungi were isolated from source materials collected at near-shore and offshore locations.

All of the fungi collected were cryopreserved in 30% glycerol/tryptic soy broth at -80 C and -130 C, and are curated in the marine microbial collection at the Australian Institute of Marine Science.

## Results

One-hundred and sixty sediment samples and representatives of the following marine phylum were collected: 3 Annelida; 20 Bryozoa; 53 Chordata; 61 Cnidaria; 4 Crustacea; 8 Echinodermata; 7 Mollusca; 166 Porifera; 20 Chlorophyta; 18 Rhodophyta; 11 Phaeophyta. Source substrates were collected between June 1995 and September 1998, from a number of locations around Australia (Fig. 1), and resulted in the isolation of 1403

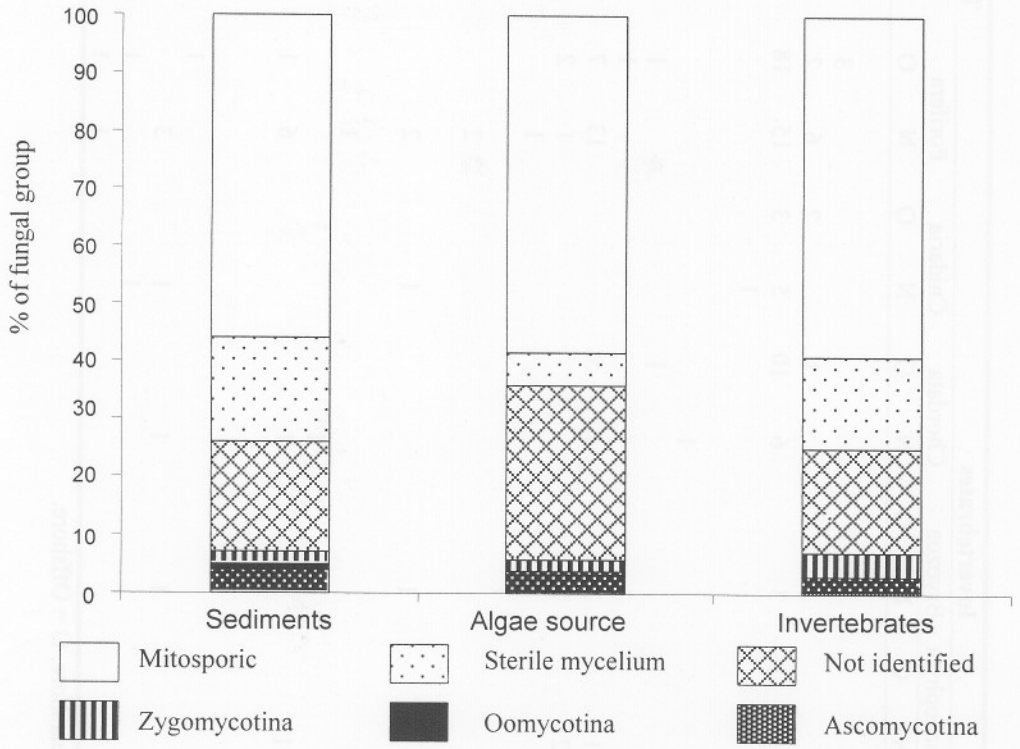


**Fig. 2**. The distribution of fungal classes within sediments, algae and marine invertebrates/ chordates.

filamentous fungi. Collection sites were split into near-shore or offshore locations (Fig. 1), based on distance to shoreline, biodiversity and tidal influences, as inferred by the Great Barrier Reef Marine Park Authority.

Of the 1403 fungi collected, 617 were studied morphologically for taxonomic identification (Table 1), resulting in the identification of 54 distinct taxa, and sterile mycelia. The majority, 41 taxa, were mitosporic fungi. Nine taxa were ascomycetes, one genus (*Pythium* sp.) an oomycete and three taxa, zygomycetes. Many (19.6%) of the isolates could not be identified, while 16% of the isolates were sterile mycelium. Twenty taxa have previously been recognised as true marine isolates (Kohlmeyer, 1979), and contributed to 17.5% (108 individuals) of the identified collection (marked with "*" in Table 1).

Algae supported a high proportion of mitosporic taxa when compared to other fungal groups. Combined algae (Chlorophyta, Rhodophyta and Phaeophyta) hosted 58% mitosporic fungi, 4% ascomycetes, 2% zygomycetes, 30% NID and 6% SM (Fig. 2). The ascomycetes isolated from algae were identified as *Cochliobolus* spp., a teleomorph of the mitosporic *Curvularia*

**Table 1.** Taxonomic characterisation of 617 fungi collected from Australian nearshore and offshore marine sites.

| | **Sediment** | | **Algae** | | | | | | **Invertebrates** | | | | | | | | **Total** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Sediment | | Chlorophyta | | Rhodophyta | | Phaeophyta | | Bryozoa | | Chordata | | Cnidaria | | Porifera | | |
| | N** | O | N | O | N | O | N | O | N | O | N | O | N | O | N | O | |
| **Mitosporic fungi** | | | | | | | | | | | | | | | | | |
| *Acremonium* spp. | 3 | | | | | | | | | | | | | | | 3 | 6 |
| *Alternaria** spp. | 7 | 4 | 1 | | | 1 | | | 1 | | | 1 | | 2 | 6 | 2 | 25 |
| *Aspergillus* spp. | 6 | 16 | | 2 | 1 | 4 | | 1 | 3 | | 6 | 10 | 5 | 3 | 15 | 18 | 90 |
| *Beauveria* spp. | | 2 | | | | | | | | | | | 1 | | | | 3 |
| *Blodgettia** sp. | | 2 | | | | | | | | | | | | | | | 2 |
| *Camarosporium** sp. | | | | | | | | | | | 1 | | | | | | 1 |
| *Chrysosporium* sp. | | | | | | | | | | | | 1 | | | | 1 | 2 |
| *Cirrenalia** sp. | | | | | | | | | 1 | | | | | | | 1 | 2 |
| *Cladosporium** spp. | 4 | 2 | | 1 | 1 | | 1 | | 2 | | 1 | 5 | | | 13 | 7 | 37 |
| *Curvularia* spp. | 1 | 1 | 2 | | 2 | | 2 | | 2 | | | | | | 1 | 2 | 13 |
| *Cylindrocarpon** sp. | | | | | | | | | | | | | | | 1 | | 1 |
| *Dactylella* sp. | | | | | | | | | 1 | | | | | | | | 1 |
| *Dactlyosporium** sp. | | | | | | | | | | | | | | | 2 | | 2 |
| *Diheterospora** sp. | 1 | | | | | | | | | | | | | | | | 1 |
| *Dreschlera** spp. | | | | | | | | | 1 | | | | 1 | | 2 | | 4 |
| *Echinobotryum* sp. | | 1 | | | | | | | | | | | | | | | 1 |
| *Epicoccum** sp. | | | | | | | | | | | | | | | 1 | | 1 |
| *Exserohilum** spp. | 1 | | 1 | | | | | | | | 1 | | | | | | 3 |
| *Fusarium* spp. | 2 | 5 | 1 | | | | 1 | | 3 | | 1 | | | | 6 | 1 | 20 |
| *Gilmaniella* sp. | | | 1 | | | | | | | | | | | | | | 1 |
| *Gliomastix* spp. | 1 | 2 | | | | | | | | | | | | | | | 3 |
| *Gonatobotryum* sp. | | | | | | | | | | | | | | | | 1 | 1 |
| *Humicola** spp. | 4 | 1 | 2 | 1 | | | | | 1 | | 1 | | 1 | | 3 | | 14 |
| *Monilia* sp. | | | | | | | | | | | | | 1 | | | 1 | 2 |
| *Nigrospora* sp. | | 2 | | | | | | | | | | | | | 1 | 1 | 4 |

* indicate taxa previously recognised as marine isolates; ** N = Nearshore, O = Offshore.

**Table 1.** (continued).

| | **Sediment** | | **Algae** | | | | | | **Invertebrates** | | | | | | | | **Total** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Sediment | | Chlorophyta | | Rhodophyta | | Phaeophyta | | Bryozoa | | Chordata | | Cnidaria | | Porifera | | |
| | N** | O | N | O | N | O | N | O | N | O | N | O | N | O | N | O | |
| *Oidiodendron* spp. | 1 | | | | | | | | 1 | | | 1 | 1 | 1 | | | 5 |
| *Papulaspora** sp. | 1 | | | | | | | | | | | | | | | | 1 |
| *Penicillium* spp. | 19 | 15 | 1 | 1 | | 2 | | | 3 | | 2 | 4 | 2 | 8 | 7 | 16 | 80 |
| *Periconia** sp. | | | | | | | | | | | 1 | | | | | | 1 |
| *Phialophora* sp. | | | | | | | | | | | | 1 | | | | | 1 |
| *Phoma** sp. | | | | | | | | | | | | 1 | | | | | 1 |
| *Scolecobasidum* sp. | 2 | | | | | | | | | | | | | | | | 2 |
| *Stachybotrys* sp. | 1 | | | | | | | | | | | | | | | | 1 |
| *Thysanophora* sp. | 1 | | | | | | | | | | | | | | | | 1 |
| *Torulomyces* spp. | | 2 | | | | | | | 2 | | | 1 | | | 2 | | 7 |
| *Trichothecium* sp. | 1 | 1 | | | | | | | | | | | | | | | 2 |
| *Tritirachium* sp. | | | | | | | | | | | | | | | 1 | | 1 |
| *Verticillium* sp. | | | | | | | | | | | 1 | | | | | 1 | 2 |
| *Wardomyces* sp. | | | | | | | | | | | | | | | 1 | | 1 |
| *Zalerion** spp. | | | | | | | | | | | 1 | | | | 2 | | 3 |
| *Zygosporium* spp. | 1 | | | | | 1 | | | 1 | | | | | | 1 | 1 | 5 |
| **Ascomycotina** | | | | | | | | | | | | | | | | | |
| *Aniptodera** sp. | | | | | | | | | 1 | | | | | | | | 1 |
| *Carbosphaerella** sp. | 1 | | | | | | | | | | | | | | | | 1 |
| *Cochliobolus* spp. | 1 | 1 | 1 | | 1 | | | | | | 1 | | | 1 | | | 6 |
| *Coronopapilla** sp. | | | | | | | | | | | | | | | 1 | | 1 |
| *Gaeumannomyces* sp. | | 1 | | | | | | | | | | | | | | | 1 |
| *Haloguignardia** sp. | | | | | | | | | | | 1 | | | | | | 1 |
| *Pseudeurotium* sp. | | 2 | | | | | | | | | | | | | | | 2 |
| *Sporormiella* sp. | 1 | | | | | | | | | | | | | | | | 1 |
| *Pestalotiopsis*-like* sp. | 2 | | | | | | | | | | | 1 | 1 | 1 | 2 | | 7 |

**Table 1.** (continued).

| | **Sediment** | | **Algae** | | | | | | **Invertebrates** | | | | | | | | **Total** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Sediment | | Chlorophyta | | Rhodophyta | | Phaeophyta | | Bryozoa | | Chordata | | Cnidaria | | Porifera | | |
| | N** | O | N | O | N | O | N | O | N | O | N | O | N | O | N | O | |
| **Oomycotina** | | | | | | | | | | | | | | | | | |
| *Pythium** sp. | 2 | | | | | | | | | | | | | | | | 2 |
| **Zygomycotina** | | | | | | | | | | | | | | | | | |
| *Absidia* spp. | 2 | | 1 | | | | | | | | 1 | | 1 | | 3 | 3 | 11 |
| *Mucor* spp. | | 1 | | | | | | | 1 | | 1 | | | | | 2 | 5 |
| *Rhizopus* spp. | 4 | | | | | | | | | | | | | | | | 4 |
| Not identified | 21 | 19 | 4 | 4 | 5 | 1 | 2 | | 10 | | 5 | 4 | 5 | 1 | 22 | 18 | 121 |
| Sterile mycelia | 20 | 18 | 1 | 1 | 1 | | | | 9 | | 10 | 3 | 1 | | 29 | 6 | 99 |
| Sub-total | 111 | | 16 | | 11 | | 6 | | 43 | | 35 | | 20 | | 122 | | 364 |
| Total offshore | | 98 | | 10 | | 9 | | 1 | | 0 | | 32 | | 17 | | 86 | 253 |
| Grand total | | | | | | | | | | | | | | | | | **617** |
| Number of sources sampled | 26 | 36 | 6 | 3 | 4 | 4 | 1 | 2 | 14 | 2 | 8 | 13 | 6 | 10 | 29 | 41 | 205 |

spp., which was isolated from all three near shore algae. A similar trend is seen in the invertebrate/ chordate group, with taxa identified as 59% mitosporic, 3% ascomycetes, 4% zygomycetes, 18% NID and 16% SM.

Sediment samples showed the greatest fungal diversity supporting 35 of the 56 identified taxonomic groups, with the following composition: 56% mitosporic, 4% ascomycetes, 1% oomycetes, 2% zygomycetes, 19% not identified and 18% sterile mycelia (Fig. 2).

The taxa most commonly observed (appearing from 6 or more sources) were identified as *Alternaria* spp., *Aspergillus* spp., *Cladosporium* spp., *Cochliobolus* spp., *Curvularia* spp., *Fusarium* spp., *Humicola* spp. and *Penicillium* spp., which in combination represent 46% of all taxa identified.

The data collected was difficult to analyse statistically due to the inherent nature of variables in each data set, but some general trends within these taxa are apparent. One such trend is finding *Cladosporium* spp. in similar proportions to *Aspergillus* spp. and *Penicillium* spp. from many of the Porifera (sponge) samples. Twenty *Cladosporium* spp. were identified from 70 poriferan sources (28% of all poriferans collected). Similarly, *Aspergillus* spp. were collected in higher proportions from algae and chordates, with relatively low proportions occurring in sediments. Indeed only 44% (16/36) of offshore sediments yielded *Aspergillus* spp., compared to 77% (10/13) associated with off shore chordates. This trend is also seen in the nearshore collections, with 23% (6/26) originating from sediments and 75% (6/8) from chordates. *Penicillium* spp. were isolated consistently in higher proportions from offshore samples, excepting sediments and bryozoans.

Taxa identified as *Fusarium* spp. appeared more commonly in near shore source materials, with 14 of the 20 identified originating from near-shore sites. Twenty-one percent of near shore bryozoans and poriferans yielded *Fusarium* spp., with the offshore representatives originating only from sediments (14% of off shore sediments) and sponges (2% of off shore Porifera).

*Acremonium* spp. are common to soil, therefore it was not surprising to isolate three specimens from near shore sediment samples. Their presence associated with Porifera from offshore sites was surprising (7% of poriferans sampled), since none were recorded from the 29 near shore Porifera. Database examination revealed that all three were found in Axinellid sponges; two were identified as *Carteriospongia* spp., and one from an unidentified sponge.

*Alternaria* spp. were associated with each substrate group type: sediment, alga and invertebrates, showing no preference for a specific source. The *Pestalotiopsis*-like sp. in this collection were mostly isolated from marine animals, and showed no preference for collection site. Cnidarians were the only source that consistently harboured this fungus in both near shore (17%) and

offshore (10%) locations.

Forty-six of the taxa identified were found in low numbers and isolated from less than six source materials (Table 1). Many of these exhibited slow growth characteristics in comparison to some of the more dominant genera, and could have been present in higher numbers, but were overgrown or growth inhibited by other competing, fast growing fungal genera present. Isolates that produced spores but could not be identified (121), or those that produced sterile mycelium (99) were common to each host group, and in combination represented 35% of the 617 fungi examined. Sediments, chlorophytes, rhodophytes, chordates and poriferans all had greater than 30% of the total fungi identified falling into these groups, with Phaeophyta, Cnidaria and Bryozoa, hosting 15%, 18% and 22% (respectively) of these unidentified taxa.

Differences between genera collected from near shore and offshore sites were compared using a student t-Test (two sample assuming unequal variances). Significantly more genera ($t = 4.97$, $DF = 142$, $P=<0.001$) were collected from near shore sites than from offshore sites. Pooling of the sources into six groups, near shore and offshore sediments, algae and animals, allowed further statistical analysis of the data. An F-test comparing the six groups indicates that the differences in the mean values among the groups is greater then would be expected by chance ($F = 30.257$, $DF = 5$, $P = <0.001$). Tukeys test (pair wise multiple comparison procedures) on these data sets, indicates a greater diversity of fungi collected from near shore sediments compared to offshore algae and offshore invertebrates ($Q = 2$, $DF = 6$, $P = <0.05$). Near shore invertebrates also showed higher diversity in the numbers of genera than offshore invertebrates ($Q = 1.8$, $DF = 6$, $P = <0.05$) or algae ($Q = 2.3$, $DF = 6$, $P = <0.05$).

**Discussion**

The most widely accepted definition of a marine fungus is one "...capable of producing successive generations by sexual and asexual means in natural oceanic waters and oceans diluted by freshwater or on intertidal substrates" (Hughes, 1975). Many fungi that are isolated from marine environments have been classified as geofungi (a fungus from terrestrial origins that has adapted to life in marine environments). Common genera identified in this study, and in a comparative study from Singapore (Huang *et al.*, 1996b) show that these geofungi are frequently isolated from a wide range of marine hosts (Table 1) occurring both at near shore and offshore locations.

Previous research into fungi in the marine environment has focussed on the isolation of obligate marine fungi, finding these species extensively in sea foam and seawater, marine plants and occasionally marine animals (Vrijmoed,

2000). Thus it would be expected that these fungi should also have been present in sediments, and the guts of filter feeders, such as poriferans and chordates. While some obligate marine fungi were isolated from these sources (Table 1), the majority of taxa isolated fell into those described as geofungi, most of which grow at a faster rate than the obligate marine taxa. Also the methods followed were adaptations of those used to isolate terrestrial taxa, hence it is not surprising that these form the majority of taxa isolated.

The greater diversity of fungi isolated from near shore collection sites ($p = <0.01$) is influenced by the near shore environment. Mangroves, beach sand, rivers and estuaries, and to some extent land use, all support fungi specific to these diverse micro-niches. Environmental influences (flooding, winds, run-off) often present terrestrial fungi to the marine environments, where they must either adapt or die. Hence fungi routinely isolated from marine hosts often display identical morphological characteristics to terrestrial taxa (Geiser, 1998; Laurence, 2000; Pivkin, 2000). Past research into the mycostatic effect of seawater on these geofungi (Kirk, 1980) found that spore germination of some taxa (*Penicillia* and *Aspergilli*) was inhibited by seawater, however some "marine" taxa were also inhibited to the same degree. It was concluded that the mycostatic factor was not a useful criterion to differentiate fungal origins. Thus, although it is likely that some fungi isolated from near shore sites may have a terrestrial origin, it is equally possible that they are also suitably adapted to life in the marine environment. To fully understand the true ancestry of these genera, genetic comparisons in conjunction with enzymatic studies should be performed between morphologically identical isolates obtained from marine and terrestrial environments.

One such geofungus is *Acremonium*, which has previously been described from soils and plants (Donnison *et al.*, 2000; Kelemu *et al.*, 2001) and one report of an association with sea cucumbers (Afiyatullov *et al.*, 2000). This research isolated *Acremonium* from near shore sediments and three offshore Axinellidae sponges. *Carteriospongia* spp. are common to slopes and reef edges, particularly those sites having moderate currents (Berquist *et al.*, 1988). It is possible that some of the fungal taxa described in Table 1 occurring in offshore sponges, such as *Acremonium* spp., had travelled by currents from near shore origins. However, if this was the case the fungus should also have been isolated from organisms. One explanation of this observation could be due to differences in the natural toxins produced as secondary metabolites by Porifera, as a chemical defence to invaders (Sennet *et al.*, 1990). Genera of Porifera common to near shore areas may produce protectants cytotoxic to some *Acremonium* spp. Nevertheless, 27 of the 55 taxa were isolated from poriferan sources. Evidence of fungal isolations from diseased sponge tissues

(Galstoff, 1942; Vacelet *et al.*, 1994) implies that the fungi are opportunistic, acting as secondary colonisers to other infections or stresses, enhancing the decay process of affected hosts. Spores may sit dormant for some time in sponges (or other hosts) until tissues are challenged, compromised or immunosuppressed before actively growing. It remains to be seen if the three *Acremonium* spp. identified from poriferan sources are novel taxa commensal to Axinellid sponges.

*Aspergillus* spp. are also common to tropical soils and are most frequently reported from sediments or vegetation (Polishook *et al.*, 2000). It is reasonable to assume that the presence of this genus in marine environments would also be greatest on algae and in sediments (as a wash effect), when in fact the results in Table 1 show greater numbers from algae and chordates, with relatively low occurrence in the sediments. Recent research has implicated *Aspergillus sydowii* as the infectious agent causing mass mortalities of sea fans in the Carribean (Smith *et al.*, 1997; Geiser *et al.*, 1998). Similar results are reported from GBR studies (Morrison-Gardiner, 2001), where *Penicillium* spp. have been routinely isolated from patches of necrotic gorgonian tissues collected throughout the Great Barrier Reef. *Penicillium* spp. are widely recognised terrestrial fungi, but are also isolated frequently from marine sources. *Penicillium* spp. appear to occur over a wide range of hosts, and have been isolated too frequently from offshore marine invertebrates and chordates to be a result of terrestrial wash-in (Huang *et al.*, 1996b).

*Cladosporium* spp. are also ubiquitous in terrestrial, freshwater and marine environments (Renault *et al.*, 1993; Majer *et al.*, 1996; Pelaez *et al.*, 1998; Zvereva, 1998; Laurence *et al.*, 2000), and have been recognised in pathogenic conjunction with aquacultured fish and crustaceans (Owens and Hall-Mendelin, 1990; McClelland, 1997). *Cladosporium algarum* is a recognised marine fungus that infects decaying alga and plant materials (Kohlmeyer, 1979). The ecological role of *Cladosporium* spp. in marine environments appears to be broader than only algal affiliations, with the discovery of proteolytic producing *Cladosporium* spp. enteric in sea-cucumber digestive tracts (Pivkin, 2000). Pivkin (2000) hypothesised that the *Cladosporium* sp. was a possible pathogen, but taking into account the role of proteases in digestion, it could be that the fungi are acting as symbionts, aiding the production of digestive enzymes within the holothurian. Protease production from invertebrate derived *Cladosporium* spp. has also been recognised in a separate study where eight marine derived *Cladosporium* spp. actively produced extracellular protease (Morrison-Gardiner, 2001). That *Cladosporium* spp. are active in marine invertebrates is further highlighted by the discovery of a compound highly active against *Cladosporium*

*cucumerinium* from the poriferan *Xestospongia* sp. The compound is not active to Gram-positive bacteria, indicating that the sponge is selectively producing a fungal inhibitor (Edrada *et al.*, 1996). The results of this study have shown *Cladosporium* spp. are frequently collected from marine invertebrates, chordates, as well as algae.

*Fusarium* spp. occurred in most source types, showing a preference for near shore habitats. This was not surprising as most *Fusarium* spp. are soil inhabitants, or associated with cellulosic plant substrates (Badran and Abdel-Rehiem, 1996; Rodriguez *et al.*, 1996). The high proportion collected from offshore sediments is possibly due to settlement of transported spores, but this does not explain the relatively high numbers collected from near shore bryozoans and poriferans. Again, further studies are necessary to determine the ecological range of Fusarium spp. in the marine environment.

*Alternaria* spp. (attributed as plant parasites) have been isolated from a wide range of marine hosts, including seawater, mangroves and algae (Domsch *et al.*, 1993). The *Alternaria* spp. in this collection were associated with each source group type: sediment, alga and animals, showing no preference for a specific substrate.

Other terrestrially common taxa isolated included *Cochliobolus* spp. (teleomorphic state of *Curvularia* and *Dreschlera* spp.), which are found in soils and plants of the tropics (Domsch *et al.*, 1993; Berbee *et al.*, 1999; Zhong and Steffenson, 2001). All three states of the taxa were isolated from sediments, algae and invertebrates, with offshore algae supporting more *Curvularia* and *Cochliobolus* spp. then other source groups. Since *Cochliobolus* spp. have only previously been described from terrestrial plants (Domsch *et al.*, 1993; Berbee *et al.*, 1999), it is possible that these species represent new taxa that are adapted to (or have originated from) life in the marine environment and are utilising algae as a host substrate.

*Camarosporium* is a recognised conidial state of marine fungi of *Leptosphaeria*. *Leptosphaeria australiensis* has previously been reported from coastal and mangrove trees, and intertidal wood in Queensland. The isolate in this collection was found on a near-shore chordate (ascidian). It is unknown whether the chordate is a true host of the fungus.

Although much data is presented in Table 1, statistical analysis was difficult due to the variable nature of the dataset (collection site and source) between taxa. However, a difference was apparent between the number of genera collected from near shore and offshore sites, with nearshore sites supporting a wider variety of culturable taxa. A significant difference ($P = <0.01$) was also apparent when the six groups were compared (near shore and offshore, sediments, algae and invertebrates), with Tukeys test results also

supporting a greater diversity of near shore taxa, particularly from sediments and invertebrates.

The research presented is intended to highlight the wide occurrence of filamentous fungi in coral reef ecosystems. It was beyond the scope of this project to make any further evaluations on the role these taxa play within this environment, or to make identifications to species. However the consistent isolation of many taxa previously not recognised from marine sources suggests that these taxa may have a primary role within tropical coral reefs. Further taxonomic identification of the isolates presented within this paper, and of the remaining 786 undescribed fungal isolates, may uncover many new fungal taxa. All of the isolates presented in this study are available for research purposes by contacting the author, or http:/ www.aims.gov.au.

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