Distribution of Marine Fungi and Fungus-Like Organisms in the South China Sea and Their Potential Use in Industry and Pharmaceutical Application

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ABSTRACT In this article we document the distribution and ecology of marine fungi in the South China Sea. They have been widely studied in this area, resulting in approximately a third of all described marine fungi (560 species) reported from the South China Sea, and especially from Hong Kong, Malaysia, Thailand and Taiwan. Sixty-nine marine fungi have been described from countries bordering the South China Sea, mainly ascomycetes species, with many from Malaysia (25 species). Many of the fungi documented have been found on mangrove substrates, including attached and drift mangrove wood. This has resulted in detailed studies of their vertical and horizontal distribution in mangroves, and sequence of colonisation on submerged test blocks. Many marine fungi isolated from the South China Sea have been screened for new chemical structures with bioactivity. Foremost has been Professor Y. C. Lin and his colleagues at Guangzhou University who have described a wide range of chemical structures including 42 new or novel compounds and 35 known compounds from 25 fungal strains (comprising endophytes, obligate and marine-derived fungi). Although filamentous fungi have been widely collected in the South China Sea, information on chytrids, mucoraceous fungi and fungal-like organisms is meagre and warrants further investigation.

(Keywords: distribution, ecology marine fungi, South China Sea, omega-3-fatty acids, bioactive compounds, industrial application).

INTRODUCTION

In comparison to other marine organisms, fungi and fungus-like organisms are not as numerous, but yet play a vital role in the recycling of organic matter in marine environments [46][61][76][83]. The Ascomycota, Basidiomycota and asexual morphs are the best known groups of marine fungi as testified by the number of books published [30][41][38]. Few marine members of the Chytridiomycota have been described and are rarely included in general books or reviews, while marine yeasts are generally documented in reviews on yeasts [15][43]. However, the book "Marine Fungi and Fungal-like Organisms" [35] offers a broad coverage of all taxonomic groups, their phylogeny, ecology and industrial application.

In this article we wish to consider four topics:

- the organisms,
- their occurrence and distribution of marine fungi in the South China Sea,
- chemical structures from marine fungi,
- production of omega-3-fatty acids, and,
- conclusions.

MARINE FUNGI AND FUNGUS-LIKE ORGANISMS

Various estimates have been made of the number of marine fungi and much depends on the criteria used to characterise these organisms. Many mycologists no longer use the straight jacket terms obligate and facultative marine organisms, but rather accept a broad ecological definition of all that are repeatedly collected or isolated from marine habitats [33]. Early studies tend to consider only fungi sporulating on the substrate, ignoring those present in the water columns as plankton or single cells, e.g. marine yeasts [16]. The current number of described marine fungi stands as 530 species in the Ascomycota, Basidiomycota and asexual

morphs [38]. However this figure does not include the Chytridiomycota and yeasts. Estimates of the number of marine fungi and fungus-like organisms are presented in Tables 1 and 2, respectively.

So where are the missing fungi to be found? Substrates most intensively studied for the occurrence of marine fungi have been driftwood, intertidal attached wood, and trapped wood in rocks, mangrove wood, saltmarsh grasses and on seaweeds. Even so these collections are restricted to locations where marine mycologists work: Asia, Europe, Americas, while others have hardly been sampled for marine fungi, e.g. Africa, Antarctic waters.

OCCURRENCE AND DISTRIBUTION OF MARINE FUNGI IN THE SOUTH CHINA SEA

From a geographical point of view, marine fungi in Asia have been well served from the extensive work by many strong research teams in the countries bordering the South China Sea: Hong Kong [86][5][37], Brunei, Indonesia [22], Malaysia [1][2][26], Philippines [17] [40][5], Singapore [79][51], Taiwan [66] and Thailand [23][24][70][78](Figure 1). Coastal waters of mainland China remain largely unexplored with only reports of marine fungi from the islands Macau and Hainan [87] [88]. These studies resulted in the description of a wide range of new marine fungi: *Calathella mangrovei* [34], *Caryospora mangrovei* [23], *Diaporthe salsuginosa and Aniptodera haispora* [87], *Phragmitensis marina* [89], and *Sedecimiella taiwanensis* [65], to mention but a few.

Sixty-nine marine fungi have been described from countries bordering the South China Sea, mainly ascomycetes species (Table 3). No fewer than 25 new species were described from Malaysia and 18 from Brunei, with only four from Singapore. This reflects the intense studies of marine and mangrove fungi by Kevin Hyde in Brunei, Hong Kong and Thailand.

The number of marine fungi recorded for the South China Sea varies from country to country, depending on the intensity of collecting, with only five for Vietnam (E.B.G. Jones unpublished data) to over 139 for Malaysia (Table 4).

Many of the marine fungi documented for the South China Sea are mangrove species occurring on a wide range of mangrove trees, especially those on *Avicennia*, *Bruguiera*, *Kandelia* and *Rhizophora* species [75]. These biodiversity studies have enabled the characterization of a core group of mangrove fungi, although differences occur between individual mangroves [1][75] (Table 4). Two monographs have been published for the mangrove fungi of Malaysia [1] and Taiwan [66], illustrating 18 and 54 species, respectively.

Studies of marine fungi in the South China Sea have covered a wide range of topics: horizontal [2] and vertical distribution in mangroves [24][2], sequence of colonization of exposed timber [79][51], decomposition of lignocellulose and patterns of decay [68][56], endophytes of mangrove trees [31][62][7][71], and which have contributed immensely to our understanding of the biology, ecology and distribution of mangrove fungi. As a result of these investigations, marine fungi have been isolated into axenic culture and used for screening of bioactive compounds (see section 3). They have also enabled phylogenetic studies of selected taxa leading to the discovery of a number of new lineages of marine fungi [71].

Fungus-like organisms in the South China Sea have fared less well when it comes to their study with published data from Hong Kong [49][50][48][12][14], Philippines [44][45] and Thailand [82]. These studies have focused on their biodiversity in coastal mangroves [50][12][28], zoospore chemotaxis [14], utilization of food processing waste [11], tolerance to heavy metals [54], source of animal feed in marine aquaculture [29], and their growth and polyunsaturated fatty acid production (PUFA), in particular docosapentaneoic acid (DHA) [11][82] (see section 4).

Other fungus-like organisms include *Halophytophthora*, *Haliphthoros* and *Sirolpidium* species, with some causing diseases of marine animals such as abalone, lobster, and prawns, generally of eggs or larvae [18]. Hatai et al. (1980) described *Haliphthoros philippinensis* as a parasite of cultivated larvae of jumbo tiger prawn (*Penaeus japonicus*), and subsequently parasitic on eggs of captive mud crab (*Scylla serrata*) by Leano (2002). *Halophytophthora* species have been widely collected on senescent mangrove leaves in the intertidal zone with new species described from Taiwan [21][20], and Thailand [59]. An up-to-date list of fungus-like organisms reported from the South China Sea can be found in Marano et al. (2012).

CHEMICAL STRUCTURES FROM MARINE FUNGI

Marine fungi have attracted much attention in the search for new sources of biodiversity for screening

for new chemical structures with antimicrobial activity. The first antibiotic documented from a marine fungus was siccayne [42] from the marine basidiomycetes *Halocyphina villosa*, a compound also known from a terrestrial fungus *Helminthosporium siccans*. Ebel (2012) estimates that some 1,100 new compounds are known from marine fungi, with over 100 published each year from 2007-2010. All major groups of chemical structures are documented for marine derived-fungi: polyketides (41%), alkaloids (20%), peptides (12 %), terpenoids (14%), prenylatedpolyketides (8 %), shikimates 2%) and lipids (1%) [69].

Many university departments and research organisations have investigated the potential of marine fungi from the South China Sea to produce new or novel chemical structures. Foremost have been Professor Y. C. Lin and his colleagues at Guangzhou University who have described a wide range of chemical structures from fungi in the South China Sea. Pan et al. (2008) documented the bioactive compounds obtained from 25 fungal strains (comprising endophytes, obligate and marine-derived fungi) with 42 new or novel compounds and 35 known compounds. Some of the new compounds they described include: xyloketals A-H from a *Xylaria* sp. found growing on seeds in Mai Po Mangrove, Hong Kong [55], eutypoid A, a new lactone from *Eutypa* sp., and seven anthroquinones from a strain of the ascomycete *Halorosellinia* [90].

Chinese scientists have also described new compounds from fungi, plants and animals collected from the tropical island of Hainan, China [58][73]. An unidentified endophytic fungus isolated from the mangrove plant *Scyphiphora hydrophyllavea* yielded four new meroterpenes (guignardones 1-4) and two known guignardones A and B [58], with guignardone 1 showing modest activity against *Staphylococcus aureus* and MRSA.

Many new compounds have been described by scientists at BIOTEC, Thailand: five aigialomycins A-E which are 14-membered resorcyclic macrolides and one hypothemycin [27], and aigialone A and aigialospirol A (a hypothemycin related compound) two structurally unique compounds [84], all from the mangrove ascomycete *Aigialus parvus*. *Halorosellinia oceanica* is a prolific producer of novel compounds, including cytochalasin Q, 5-carboxymellein and halorosellinic acid (an ophiobolane sesterterpene) reported from a Thai strain (Chinworrungsee et al. 2001). Another Thai group working on natural products research is from Prince of Songkla University under the leadership of Professor Vatcharin Rukachaisirikul that screens marine-derived fungi from the sea fan *Annella* sp. and endophytes of mangrove trees. Many new chemical structures have been reported, such as, epxydons and pyrones from the marine-derived *Nigrospora* sp. [80] and anthraquinone, cyclopentanone and napththoquinone derivatives from a *Fusarium* sp. isolated from the sea fan *Annella* [81].

Debbab et al. (2012) opine that marine fungal endophytes remain an underexplored group when compared with their bacterial counterparts and fungi from terrestrial habitats, a feature also commented on by Jones (2008, 2011a). In this respect endophytes of seaweeds are a group worthy of much greater investigation [31][72] [33]. Some preliminary observations on marine-derived fungi from sea grasses [71] and mangrove trees have been reported [62][7]. The fungi reported are primarily asexual morphs, similar to terrestrial counterparts: *Aspergillus*, *Penicillium* and *Pestalotiopsis* spp., and since few of these are fully identified, their marine origin remains to be resolved [33].

Although most natural products studies have focused on antimicrobials, others have looked for antifouling activities and the production of unique enzymes [31] [73].

PRODUCTION OF OMEGA-3-FATTY ACIDS

The ability of fungus-like organisms, such as thraustochytrids, to produce the polyunsaturated fatty acid docosahexaenoic acid (DHA, c22:6n3) has been known for many years [4][74][60][52]. DHA for commercial use has been extracted from trash fish, but only a few microorganisms can synthesise it as most organisms do not possess the synthetic pathway for its production. Therefore the ability of organisms, such as, *Cryptothoconidium cohnii*, *Thraustochytrium*, *Schizochytrium* and *Ulkenia* species to produce Poly unsaturated Fatty Acids (PUFA) have attracted considerable interest over the past 20 years. Most research has been undertaken in Hong Kong (Fan et al. 2001), Japan [91][92][53][6].

Studies have focused on strains that produce high yields of PUFA's, especially *Schizochytrium* spp., and to determine the optimum conditions for DHA production. Surprisingly this is a topic that has not been widely researched in countries bordering the South China Sea. In Hong Kong studies have focused on the physiology of thraustochytrid strains [12][14], and

their use to enhance the value of waste products such as Okara [11][13]. In Thailand, Unagul et al. (2005) have concentrated on enhancing the growth media for the growth of *Schizochytrium mangrovei*, especially the effects of glucose, temperature and salinity. The highest yield of DHA reported by Unagul et al. (2005) was 65 mg l^{-1} h^{-1} (biomass 22.5 g l^{-1}) and is comparable to those reported by Yokochi et al. (1998) (35 mg l^{-1} h^{-1}) and Fan et al. (2001) (52 mg l^{-1} h^{-1}). Other studies in Thailand examined the enhanced growth of *Artemia* larvae when fed on different concentrations of thraustochytrid biomass [29], and in particular their potential use in aquaculture [28]. Preliminary studies by Leano et al. (2003) explored the growth and fatty acid production of thraustochytrids isolated in Philippine mangroves.

CONCLUSIONS

Jones and Vrijmoed in 1993 gave a presentation on the biodiversity marine fungi in South China Sea at a conference on "Perspective on marine environment change in Hong Kong and Southern China 1977-2001". Since then considerable progress has been made to document the fungal biodiversity of this region, as documented above. Marine fungi can be grouped according to their geographical distribution temperate, tropical/subtropical and Arctic/Antarctic, while some are cosmopolitan. Some 50 % of the described marine fungi are tropical and most of these have been recorded from the South China Sea, with 69 new fungi described from this region. New fungi continue to be described from the South China Sea, such as *Kitesporella keelungensis* and *Pileomyces formosanus* (64) (63).

Over the past 20 years marine fungi have been screened for a variety of products, in particular compounds with antimicrobial activity from filamentous species, PUFA production from thraustochytrids, and enzymes. Filamentous marine fungi have been shown to produce a diverse range of enzymes as they grow under extreme conditions: high salt tolerance, deep waters, alkaline pH and low temperatures. Many industries require enzymes that are alkaline tolerant and with optimal activity at low temperatures. Despite this biochemical and physiological diversity, few have been commercially successful.

Compounds produced by marine fungi which have been commercially developed include: cephalosporin C isolated from seawater near a sewage outlet, off the Sardinian coast, phomactins, initially isolated from a *Phoma* sp. isolated from crab shell and plinabulin, a diketopiperazine halimide, which is in advanced clinical trials for anticancer therapy. What are the challenges for the future? Many new marine fungi remain to be discovered, especially the marine derived taxa (e.g. *Aspergillus*, *Penicillium* spp.), which need to be fully described and confirmed by molecular studies to show how they differ from their terrestrial counterparts. Marine yeasts and chytrids have hardly been looked for in the South China Sea, and many substrata need to be examined for fungi: diseases of mangrove plants, corals, mollusc shells, deep-sea water samples and endophytes of seaweeds. Our knowledge on fungus-like organisms is limited to studies of thraustochytrids, and greater effort is required in their study. Biochemical and physiological studies of fungi from the South China Sea have been few and warrants greater attention.

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Table 1. Estimated number of marine fungi (Jones 2011b).

Group	Number
Marine fungi	560
Facultative marine fungi	100
Marine yeasts	1500
Misidentified fungi	100
Marine-derived	1500
Deep-sea fungi	300
Planktonic fungi	500
Endophytes, algicolous and cryptic species	7 500
Total	12 060

Table 2. Estimates of marine fungal-like organisms (Jones and Pang 2012).

Group	Number
Cryptomycota	5?
Mesomycetozoea	5?
Oomycota	74
Hyphochytriomycota	7
Labyrinthulomycota	66
Phytomyxea	9
Total	166

Table 3. New marine fungi described from countries boarding the South China Sea.

Ascomycota		
Acrocordiopsis sphaerica	Mangrove wood	Philippines
Aniptodera haispora	Mangrove wood	Macau
Aniptodera intermedia	*Nypa fruticans*	Malaysia
Aniptodera longispora	Mangrove wood	Brunei
Aniptodera nypae	*Nypa fruticans*	Malaysia
Anthostomella nypae	*Nypa fruticans*	Malaysia
Anthostomella nypensis	*Nypa fruticans*	Malaysia
Anthostomella nypicola	*Nypa fruticans*	Malaysia
Apioclypea nypicola	*Nypa fruticans*	Malaysia
Arecophila nypae	*Nypa fruticans*	Malaysia
Astrocystis nypae	*Nypa fruticans*	Malaysia
Astrocystis selangorensis	*Nypa fruticans*	Malaysia
Crinispora nypae	*Nypa fruticans*	Brunei
Capillataspora corticola	*Rhizophora* prop roots	Brunei
Caryospora mangrovei	Mangrove wood	Thailand
Corollospora besarispora	Mangrove wood	Malaysia
Cryptovalsa mangrovei	*Kandelia obovata*	Hong Kong
Dactylospora mangrovei	Mangrove wood	Hong Kong, Malaysia, Taiwan, Thailand
Fasciatispora lignicola	*Mangrove wood*	Malaysia
Fasciatispora nypae	*Nypa fruticans*	Brunei
Halosarpheia kandeliae	*Kandelia obovata*	Hong Kong
Halosarpheia minuta	Mangrove wood	Singapore
Helicascus nypae	*Nypa fruticans*	Brunei
Herpotrichia nypicola	*Nypa fruticans*	Malaysia
Hypophloeda rhizophora	*Nypa fruticans*	Brunei
Frondicola tunitricuspis	Mangrove wood	Brunei
Kitesporella keelungensis	Mangrove wood	Taiwan

Lautospora gigantea	Mangrove wood	Brunei
Leprosphaerulia mangrovei	*Acanthus ilicifolius*	Hong Kong
Leptosphaeria nypicola	*Nypa fruticans*	Malaysia
Ligninicola nypae	*Nypa fruticans*	Malaysia
Linocarpon nypae	*Nypa fruticans*	Malaysia
Marinosphaera mangrovei	Mangrove wood	Brunei
Massarina acrostichi	Fern *Acrostichum* sp.	Brunei
Melaspilea mangrovei	Mangrove wood	Hong Kong
Nemania maritimqa	Mangrove wood	Taiwan
Neolinocarpon globosicarpum	*Nypa fruticans*	Brunei
Neolinocarpon nypicola	*Nypa fruticans*	Malaysia
Nipicola selangorensis	*Nypa fruticans*	Malaysia
Nypaella frondicola	*Nypa fruticans*	Brunei
Oxydothis nypae	*Nypa fruticans*	Brunei
Payosphaeria minuta	Mangrove wood	Singapore
Pedumispora rhizophorae	Mangrove wood	Brunei
Phomatospora kandelae	Mangrove wood	Thailand
Phomatospora nypae	*Nypa fruticans*	Malaysia
Phomatospora nypicola	*Nypa fruticans*	Malaysia
Phragmitensis marina	*Phragmites australis*	Hong Kong
Pileomyces formosanus	Driftwood	Taiwan
Remispora minuta	Driftwood	Singapore
Sablecola chinensis	Driftwood	Singapore
Salsuginea ramicola	Mangrove wood	Brunei
Saccardoella mangrovei	Mangrove wood	Malaysia
Saccardoella marinospora	Mangrove wood	Brunei
Saccardoella rhizophorae	Mangrove wood	Thailand
Savoryella longispora	Mangrove wood	Thailand
Sedecimiella taiwanensis	Mangrove wood	Taiwan
Thalespora appendiculata	Mangrove wood	Thailand
Tirispora unicaudata	Mangrove wood	Hong Kong
Tirisporella beccariana	*Nypa fruticans*	Malaysia
Trematosphaeria malaysiana	Mangrove driftwood	Malaysia
Vibrissea nypicola	*Nypa fruticans*	Malaysia
Asexual morphs		
Arthrobotrys mangrovispora	Mangrove wood	Hong Kong
Phomopsis mangrovei	*Rhizophora* prop roots	Thailand
Plectophomella nypae	*Nypa fruticans*	Brunei
Pleurophomopsis nypae	*Nypa fruticans*	Brunei
Trichocladium nypae	*Nypa fruticans*	Malaysia

Basidiomycota		
Calathella mangrovei	*Bruguiera* sp.	Malaysia
Physalacria maipoensis	Mangrove wood	Hong Kong

Table 4. Number of marine fungi recorded for the South China Sea.

Country	Number
Thailand	154
Malaysia	139
Hong Kong	117
Taiwan	107
Philippines	80
Brunei	68
Indonesia	48
Singapore	41
Vietnam	5

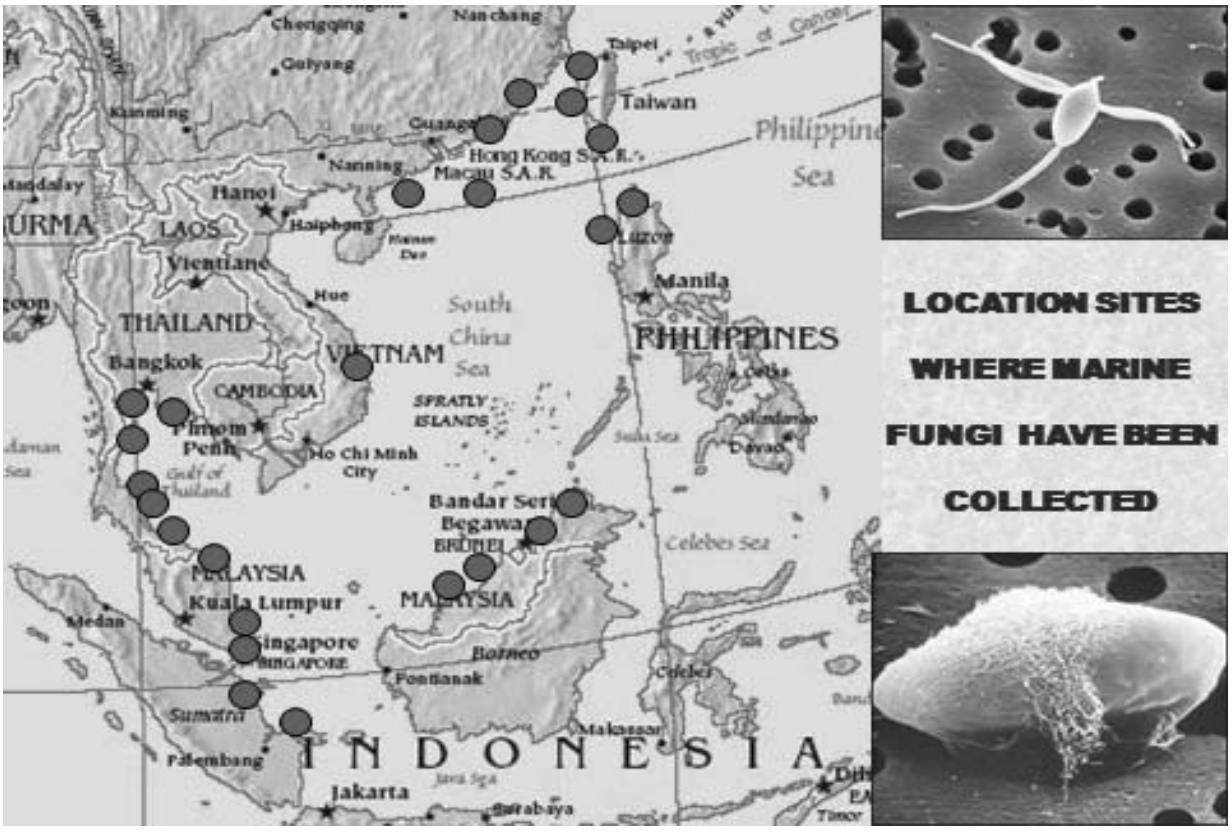

Figure 1. Distribution of sites surveyed for marine fungi (Jones, unpublished data)