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# Culturable Foliar Fungal Endophytes of Mangrove Species in Bicol Region, Philippines

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**Identification of fungi in the mangrove ecosystem is warranted because of the need to document species richness in unique ecosystems, amidst the continuous anthropogenic and climatic threats to mangrove forests and the potentials for biotechnological applications. This study aimed to identify endophytic fungi in association with mangrove species. Leaves – devoid of discoloration, wound, physical deformation, or necrosis – of 21 mangrove species in the Bicol region, Philippines were collected. Circular discs from each leaf were surface sterilized, plated on potato dextrose agar (PDA), and incubated for 7–14 d at room temperature. Growing fungi were transferred individually into sterile PDA slants for identification. A total of 53 endophytic fungi belonging to 15 orders and 19 families were isolated – 75.47% ascomycetes, 20.75% basidiomycetes, and 3.77% zygomycetes. *Trametes cubensis* (Mont.) Sacc. and *Pestalotiopsis cocculi* (Guba) were the most distributed among the mangrove hosts. The mangroves *Rhizophora mucronata* Lam. and *Lumnitzera racemosa* Willd. hosted the most number of fungal endophytes with 15 and 12, respectively.**

Key words: Bicol, fungal endophytes, *Lumnitzera*, mangroves, *Rhizophora*, *Trametes cubensis*

## INTRODUCTION

It is reported that fungal species inhabiting the mangrove ecosystem account for the second largest group of marine fungi (Cheng *et al.*

2008). The distinctiveness of the mangrove ecosystem and the mangroves themselves provide an exceptional niche for fungi to colonize. Mangroves must adapt to changing salinity, high temperature, anaerobic soils, and faunal competition (Debbab *et al.* 2013). Moreover, the rise and fall of the tides present a dynamic ecotone between terrestrial and marine habitats (Cheng *et al.* 2009). These environmental factors create unique pressures on fungal diversity that may allow them to differ from their terrestrial counterparts.

The need to research on fungal diversity in mangrove areas stems from a two-fold challenge. First, because mangroves provide goods and services to communities – as well as serving nursery to a number of fish species, crustaceans, and mollusks – they are deemed economically and ecologically important. Woods coming from mangroves are harvested to produce wood chip and charcoal for commercial purposes. They are developed as areas for ecotourism. Consequently, the mangrove forests are among the most threatened ecosystems in the world. In the Philippines, coastal communities over-harvest mangrove for firewood and construction of houses. Many areas are polluted by effluents from industries and households near them and are almost always subject to garbage disposal. It is also evident that some are converted into fishponds and resorts, relegating the ecological importance of mangroves as secondary. Losing these salt-tolerant species of plants equates to a loss of their corresponding associated fungi.

Secondly, because of the different environmental conditions experienced by fungi within the mangrove forests, the probability of finding novel compounds

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may be high. Fungal diversity corresponds to a gamut of biological applications that can be harnessed from their bioactive compounds (Strobel 2003). Antifungal, antimicrobial, anticancer, and antimalarial are among the most common lines of research (Yu *et al.* 2010). For instance, a species of *Alternaria* isolated from *Sonneratia alba* Sm. was shown to have potential antibacterial activity against multidrug-resistant *Staphylococcus aureus* Rosenbach (Kjer *et al.* 2009). Similarly, a species of *Talaromyces* isolated from *Kandelia candel* (L.) Druce showed antimicrobial activity and cytotoxic activities (Liu *et al.* 2010).

This wide spectrum of natural functionality that fungal endophytes provide underscore the need to conduct diversity studies to properly account them along with the hosts they inhabit. Strobel and Daisy (2003) said that areas with the greatest biodiversity – including tropical and temperate forests – also have the most diverse microorganisms, yet they are also the most threatened. Thus – in this study – foliar fungal endophytes of mangrove species in the Bicol region, Philippines are presented.

## MATERIALS AND METHODS

### Sampling Site

A mangrove forest from each of the six provinces in the Bicol Region, Philippines was selected as sampling site. These were the following: Cagmanaba Mangrove Area (province of Albay), Batalay Mangrove Area (province of Catanduanes), Mangrove Reforestation Area (province of Sorsogon), Pawa Mangrove Park (province of Masbate), Calabanga Mangrove Area (province of Camarines Sur), and Bagasbas Mangrove Area (province of Camarines Norte). The selected mangrove areas are protected by local ordinances and are characterized by their proximity to household and coastal communities. The study was conducted from Feb 2016 to Feb 2017. In each of the mangrove forest, species of mangroves were located and sampled randomly.

### Sampling

Ten mature, clean, and asymptomatic leaves from each mangrove species within the laid transect were collected randomly. These were placed in resealable and sterile bags and transported to the laboratory for immediate plating. Only one tree per species of mangrove was sampled per area.

### Isolation and Incubation of Leaf Discs

Two leaf discs of approximately 0.64 cm in diameter were punched out from each of the collected leaves. Leaf discs were surface sterilized following the methods of Torres and dela Cruz (2015) with modifications. Explants were soaked in 95% ethanol for 1 min, and then transferred to 0.5% NaOH solution for 2 min. Leaf discs were then washed twice in sterile distilled water and blot dried in sterile tissue paper. Sterilized leaf discs were then plated onto potato dextrose agar (PDA), prepared following manufacturer's specifications, amended with streptomycin (300 mg/L). Plates were incubated for 7–14 d at room temperature, with observation done every 2 d for fungal growth. Every new growth was transferred to new PDA plates and slants.

Prior to plating, each of the sterile leaf discs were touched on the surface of a sterile PDA, stored at room temperature for 5–7 days, and checked for fungal growth. When fungal growth was observed, the corresponding plated leaf discs were not considered valid. This is to avoid epiphytic fungi to be included in the isolates.

### Identification of Fungal Isolates

Initial identification was based on morphological and cultural characteristics. Isolates with similar colonial and morphological characteristics were assigned to a single morphospecies. Each morphospecies were sent to Macrogen Korea for DNA extraction, ITS amplification via PCR, and capillary sequencing. Identity of the isolates were determined by homology search from NCBI database using BLAST. Percent similarity of 97% or higher were accepted.

The primers ITS 1 5' (TCC GTA GAA CCT GCG G) 3' and ITS 4 5' (TCC TCC GCT TAT TGA TAT GC) 3' were used for the PCR. The PCR reaction was performed with 20 ng of genomic DNA as the template in a 30 uL reaction mixture using a EF-Taq (SolGent, Korea) as follows: activation of Taq polymerase at 95 °C for 2 min, 35 cycles of 95 °C for 1 min, 55 °C, and 72 °C for 1 min each, finishing with a 10-min step at 72 °C. The amplification products were purified with a multiscreen filter plate (Millipore Corp., Bedford, MA, USA). Sequencing reaction was performed using a PRISM BigDye Terminator v3.1 cycle sequencing kit. The DNA samples containing the extension products were added to Hi-Di formamide (Applied Biosystems, Foster City, CA). The mixture was incubated at 95 °C for 5 min, followed by 5 min on ice, and then analyzed by ABI Prism 3730XL DNA analyzer (Biosystems, Foster City, CA).

### Data Analysis

Occurrence of each species of fungi was calculated through the given equation:

$$Percent\ Occurence = \frac{occurence\ of\ species\ A}{total\ number\ of\ isolates} x\ 100 \quad (1)$$

Density of colonization (%) of a single fungal species was calculated following the equation presented by Suryanarayanan and co-workers (1998):

$$Percent\ Colonization = \frac{number\ of\ colonized\ leaf\ discs}{total\ number\ of\ leaf\ discs\ plated} x\ 100 \quad (2)$$

Isolation rate in each mangrove host is calculated through the given equation:

$$Isolate\ Rate = \frac{total\ number\ of\ isolates\ of\ mangrove\ A}{total\ number\ of\ leaf\ discs\ plated\ of\ species\ A} x\ 100 \quad (3)$$

## RESULTS

A total of 21 mangrove species were sampled in this research yielding 172 isolates from 53 fungal morphospecies (Table 1). Ten mangrove species have an isolate-to-species ratio of 1.00, namely: *Bruguiera gymnorhiza* (L.) Savigny, *B. parviflora* (Roxb.), *Ceriops decandra* (Griff.), *C. zippeliana* Blume*, C. tagal* (Perr.) C.B. Rob., *Sonneratia caseolaris* (L.) Engl., *Pemphis acidula* J.R. Forst & G. Forst, *Scyphiphora hydrophylacea* C.F. Gaertn., *Excoecaria agallocha* L., and *Lumnitzera littorea* (Jack) Voigt. A species-to-isolate ratio of 1.00 suggests that no single fungal species dominate the leaves as endophytes. However – for the mangroves *Ceriops zippeliana* Blume, *Ceriops tagal* (Pers.) C.B. Rob., *Pemphis acidula*, and *Lumnitzera littorea* – the value was only 1.00 since there was a single fungal endophyte isolated from their leaves, namely: *Rhizopus microsporus* Tiegh., *Nigrospora oryzae* H.J. Huds, *Nigrospora sphaerica* Mason, and *Lasiodiplodia theobromae* (Pat.) Griffon & Maubl., respectively. *Bruguiera sexangula* (Lour.) Poir. (*Rhizophoraceae*) had the least species-to-isolate ratio of 0.36. Overall, there was low species-to-isolate ratio of 0.31.

Fifty-three (53) species of fungi belonged to three phyla (Figure 1) – with 75.47% from Ascomycota, 20.75% from Basidiomycota, and 3.77% from Zygomycota – and to 15 orders and 20 families. Among these, *Trametes cubensis* was isolated the most with 18.02% occurrence. *Trametes cubensis* and *Pestalotiopsis cocculi* were the most distributed among the mangrove hosts, infecting nine and eight mangroves, respectively.

Of the 172 isolates (Table 2), 24.12% were from the genus *Aspergillus* – a known cosmopolitan group of fungi that shifts from endophytic to epiphytic nature of association. The presence of these *Aspergillus* species is not new, as many studies have already accounted them as endophytes

**Table 1.** Mangrove species and their corresponding number of isolates and fungal morphospecies.

| Mangroves | Mangrove species Code | Number of Isolates | Fungal Morphospecies | Species to Isolate Ratio |
|---|---|---|---|---|
| **Family: Rhizophoraceae** | | | | |
| *Rhizophora stylosa* | RS | 12 | 8 | 0.67 |
| *Rhizophora apiculata* | RA | 15 | 7 | 0.47 |
| *Rhizophora mucronata* | RM | 28 | 15 | 0.54 |
| *Bruguiera cylindrica* | BC | 12 | 6 | 0.50 |
| *Bruguiera gymnorhiza* | BG | 3 | 3 | 1.00 |
| *Bruguiera sexangula* | BS | 15 | 5 | 0.33 |
| *Bruguiera parviflora* | BP | 4 | 4 | 1.00 |
| *Ceriops decandra* | CD | 4 | 4 | 1.00 |
| *Ceriops zippeliana* | CZ | 1 | 1 | 1.00 |
| *Ceriops tagal* | CT | 1 | 1 | 1.00 |
| **Family: Acanthaceae** | | | | |
| *Avicennia marina* | AM | 9 | 5 | 0.56 |
| *Avicennia rumphiana* | AR | 9 | 8 | 0.89 |
| *Avicennia alba* | AA | 4 | 3 | 0.75 |
| **Family: Lythraceae** | | | | |
| *Sonneratia alba* | SA | 16 | 11 | 0.69 |
| *Sonneratia ovalis* Korth. | SO | 8 | 6 | 0.75 |
| *Sonneratia caseolaris* | SC | 7 | 7 | 1.00 |
| *Pemphis acidula* | PA | 1 | 1 | 1.00 |
| **Family: Rubiaceae** | | | | |
| *Scyphiphora hydrophylacea* | SH | 4 | 4 | 1.00 |
| **Family: Euphorbiaceae** | | | | |
| *Excoecaria agallocha* | EA | 3 | 3 | 1.00 |
| **Family: Combretaceae** | | | | |
| *Lumnitzera racemosa* | LR | 15 | 12 | 0.80 |
| *Lumnitzera littorea* | LL | 1 | 1 | 1.00 |
| **Total** | 21 | 172 | 53 | 0.31 |

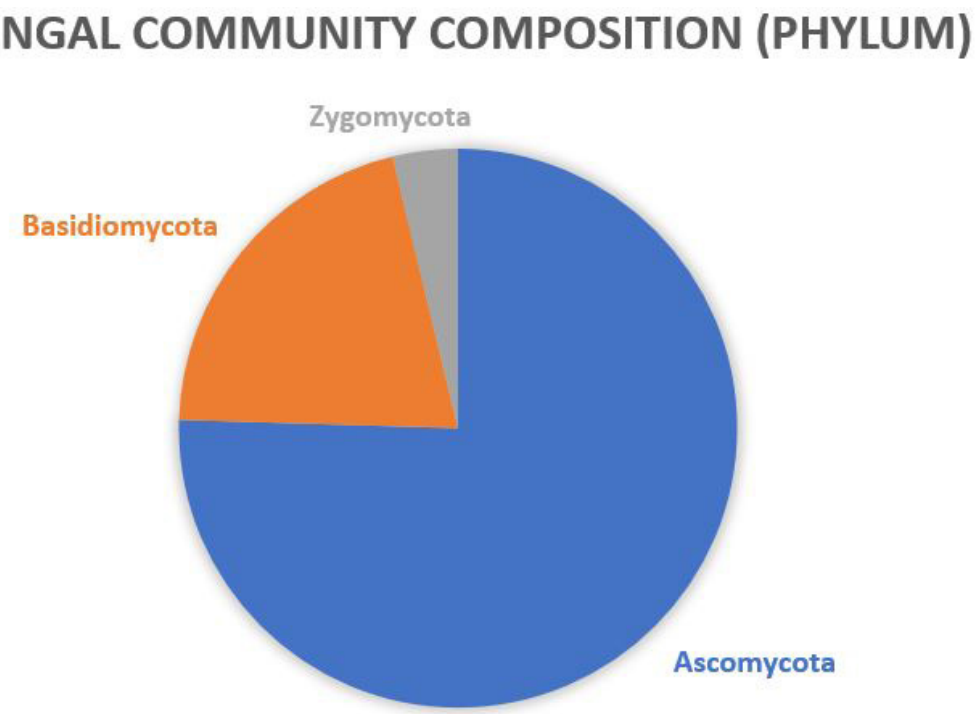


**Figure 1.** Fungal community in the mangroves sampled indicate majority of species belonging to Ascomycota (74.47%), while a small fraction belongs to Basidiomycota (20.75%) and Zygomycota (3.77%).

**Table 2.** Foliar fungal endophytes isolated from leaves of mangrove species in the Bicol region, Philippines.

| Fungal Isolates | Phylum[1] | Order | Family | Occurence | (%)[2] | Total Mangrove Species Infected[3] |
|---|---|---|---|---|---|---|
| *Alternaria* sp. | A | Pleosporales | Pleosporaceae | 1 | 0.58 | 1 |
| *Aspergillus aculeatus* | A | Eurotiales | Trichocomaceae | 9 | 5.23 | 5 |
| *Aspergillus flavus* | A | Eurotiales | Trichocomaceae | 12 | 6.98 | 6 |
| *Aspergillus fumigatus* | A | Eurotiales | Trichocomaceae | 2 | 1.16 | 2 |
| *Aspergillus japonicus* | A | Eurotiales | Trichocomaceae | 1 | 0.58 | 1 |
| *Aspergillus minisclerotigenes* | A | Eurotiales | Trichocomaceae | 9 | 5.23 | 7 |
| *Aspergillus niger* van Tieghem | A | Eurotiales | Trichocomaceae | 2 | 1.16 | 2 |
| *Aspergillus* sp. | A | Eurotiales | Trichocomaceae | 5 | 2.91 | 4 |
| *Aspergillus versicolor* (Vuillemin) Tiraboschi | A | Eurotiales | Trichocomaceae | 1 | 0.58 | 1 |
| *Botryosphaeria rhodina* (Berk. & M.A. Curtis) | A | Botryosphaeriales | Botryosphaeriaceae | 1 | 0.58 | 1 |
| *Ceriporia lacerata* | B | Polyporales | Phanerochaetaceae | 13 | 7.56 | 7 |
| *Chaetomium globosum* | A | Sordariales | Chaetomiaceae | 1 | 0.58 | 1 |
| *Colletotrichum coffeanum* | A | Glomerellales | Glomerellaceae | 1 | 0.58 | 1 |
| *Colletotrichum gloeosporioides* | A | Glomerellales | Glomerellaceae | 2 | 1.16 | 1 |
| *Colletotrichum kahawae* | A | Glomerellales | Glomerellaceae | 1 | 0.58 | 1 |
| *Colletotrichum* sp. | A | Glomerellales | Glomerellaceae | 1 | 0.58 | 1 |
| *Curvularia verrusculosa* | A | Pleosporales | Pleosporaceae | 1 | 0.58 | 1 |

Table 2 continuation next page

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| | | | | | | |
|---|---|---|---|---|---|---|
| *Daldinia eschscholtzii* (Ehrenb.) Rehm. | A | Xylariales | Xylariaceae | 1 | 0.58 | 1 |
| *Diaporthe longicolla* | A | Diaporthales | Diaporthaceae | 1 | 0.58 | 1 |
| *Fomes* sp. | B | Polyporales | Polyporaceae | 8 | 4.65 | 7 |
| *Fusarium solani* | A | Hypocreales | Nectriaceae | 1 | 0.58 | 1 |
| *Glomerella cingulata* | A | Glomerellales | Glomerellaceae | 1 | 0.58 | 1 |
| *Glomerella* sp. | A | Glomerellales | Glomerellaceae | 1 | 0.58 | 1 |
| *Hyalotiella rubi* Senan, Camporesi & K.D. Hyde | A | Amphisphaeriales | Bartaliniaceae | 4 | 2.32 | 4 |
| *Lasiodiplodia pseudotheobromae* | A | Botryosphaeriales | Botryosphaeriaceae | 1 | 0.58 | 1 |
| *Lasiodiplodia theobromae* | A | Botryosphaeriales | Botryosphaeriaceae | 1 | 0.58 | 1 |
| *Lentinus squarrosulus* | A | Polyporales | Polyporaceae | 1 | 0.58 | 1 |
| *Lichtheimia ramosa* | Z | Mucorales | Mucoraceae | 2 | 1.16 | 2 |
| *Microascus cinereus* | A | Microascales | Microascaceae | 1 | 0.58 | 1 |
| *Nigrospora oryzae* | A | Trichosphaeriales | Apiosporaceae | 4 | 2.32 | 3 |
| *Nigrospora sphaerica* | A | Trichosphaeriales | Apiosporaceae | 4 | 2.32 | 2 |
| *Nigrospora* sp. | A | Trichosphaeriales | Apiosporaceae | 1 | 0.58 | 1 |
| *Paecilomyces formosus* | A | Eurotiales | Trichocomaceae | 1 | 0.58 | 1 |
| *Paecilomyces variotii* Bainier | A | Eurotiales | Trichocomaceae | 1 | 0.58 | 1 |
| *Penicillium citrinum* | A | Eurotiales | Trichocomaceae | 2 | 1.16 | 2 |
| *Penicillium griseofulvum* | A | Eurotiales | Trichocomaceae | 1 | 0.58 | 1 |
| *Peniophora* sp. | B | Russulales | Peniophoraceae | 1 | 0.58 | 1 |
| *Pestalotiopsis mangiferae* Steyaert | A | Xylariales | Amphishpaeriaceae | 1 | 0.58 | 1 |
| *Pestalotiopsis cocculi* | A | Xylariales | Amphishpaeriaceae | 11 | 6.40 | 8 |
| *Pestalotiopsis microspora* (speg.) G.C. Zhao & N. Li | A | Xylariales | Amphishpaeriaceae | 2 | 1.16 | 1 |
| *Pestalotiopsis uvicola* | A | Xylariales | Amphishpaeriaceae | 9 | 5.23 | 9 |
| *Pestalotiopsis* sp. | A | Xylariales | Amphishpaeriaceae | 1 | 0.58 | 1 |
| *Phlebiopsis crassa* (Lev.) Floudas & Hibbett | B | Polyporales | Phanerochaetaceae | 1 | 0.58 | 1 |
| *Phomopsis longicolla* | A | Diaporthales | Diaporthaceae | 1 | 0.58 | 1 |
| *Rhizopus microsporus* | Z | Mucorales | Mucoraceae | 3 | 1.74 | 3 |
| *Rigidoporus vinctus* (Berk.) Ryvarden | B | Polyporales | Meripilaceae | 4 | 2.33 | 3 |
| *Schizophyllum commune* | B | Agaricales | Schizophyllaceae | 1 | 0.58 | 1 |
| *Trametes cubensis* | B | Polyporales | Polyporaceae | 31 | 18.02 | 9 |
| *Trametes elegans* | B | Polyporales | Polyporaceae | 1 | 0.58 | 1 |
| *Trametes ljubarskyi* Pilat | B | Polyporales | Polyporaceae | 2 | 1.16 | 2 |
| *Trametes maxima* | B | Polyporales | Polyporaceae | 1 | 0.58 | 1 |
| *Valsa euginiae* Nutman & F.M. Roberts | A | Diaporthales | Valsaceae | 1 | 0.58 | 1 |
| *Xylaria feejeensis* | A | Xylariales | Xylariaceae | 1 | 0.58 | 1 |
| **Total** | | **15** | **19** | **172** | **100.00** | |

[1]Phylum: A – Ascomycota; B – Basidiomycota; Z – Zygomycota
[2](%) = (occurrence of species/ total occurrence of all species) x 100
[3]Refers to the total number of unique mangrove species from where a particular fungal species was isolated

of leaves of mangroves (Suryanarayanan *et al.* 1998). The closely related genus *Penicillium* accounted only for two species (*P. citrinum* Thom. and *P. griseofulvum* Dierckx, R.P.). However, the importance of *Penicillium* species cannot be overemphasized as they opened the era of antimicrobials and the search for novel biological compounds. In line with this, Wang and co-workers (2009) reported new compounds from *P. griseofulvum* isolated from the mangrove *Lumnitzera racemose*, which has potential activities against human cancer cells. Isolates of *P. citrinum* from the mangrove *Bruguiera sexangula* var. *rhynchopetala* (Zheng *et al.* 2016) and from *Bruguiera gymnorhiza* (Wu *et al.* 2015) likewise were earlier reported to have antibacterial and neuroprotective metabolites, respectively. *Penicillium citrinum* in this research was isolated from *Bruguiera sexangula*.

Four species of *Colletotrichum* were isolated in this study. This genus is known for its ability to cause disease among several plants such as mango. However, it is also reported to be an endophyte of several terrestrial plants such as *Artemisia monglolica* H. Lev (Zou *et al.* 2000) and the medicinal plant *Justicia gendarussa* Burm. f (Gangadevi & Muthumary 2008). Hyde and co-workers (2009) discussed extensively the lifestyle of *Colletotrichum*, which can occur as endophyte, epiphyte, saprobe, or pathogen at different conditions. In mangroves, *Colletotrichum* is a common endophyte in many studies (Costa *et al.* 2012).

*Pestalotiopsis cocculi* was among the most ubiquitous, having been isolated in eight mangrove hosts. *Pestalotiopsis* species are common endophytes isolated in the subtropical and tropical regions (Wei *et al.* 2007) and are some of the dominant species in mangroves, such as *Rhizophora apiculata* Blume (Suryanarayanan & Thennarasan 2004). Studies such as that of Rönsberg and co-workers (2013) dwell on biological compounds produced by *Pestalotiopsis* species isolated from mangroves. The other hypomycetes, including *Fusarium* and *Alternaria*, are common endophytes already reported among mangrove leaves.

Among the 11 species of basidiomycetes, nine were from the order *Polyporales* known for their polypores or corticoid appearance, as well as some agarics such as the genus *Lentinus* – also isolated in this study. The polypores observed in this study are endophytes, as compared to their usual role as wood decaying agents. The other two basidiomycetes were a species of *Peniophora* (*Russusales*) – characterized by its hymenial cystidia – and *Schizophyllum commune* Fries, which is an agaric characterized by its wavy and lobed morphology. It was also noted that although they contributed less to total species richness, basidiomycetes were relatively widespread among the mangrove species. *Trametes cubensis* was isolated from nine mangroves – *Fomes* from seven and *Ceriporia lacerata* N. Maek, Suhara & R. Kondo from another seven species of mangroves. They had also high percent occurrence compared to the other fungal groups. A study by Gilbert and Sousa (2002) suggested that basidiomycetes have preferential hosts, which was not observed in this study.

*Rhizopus microsporus* and *Lichtheimia ramosa* (Zopf) Vuill. are the only Zygomycetes isolated in this study. Both are fast growers and are the primary colonizers of decaying organic matter.

*Rhizophora mucronata* Lam. hosted fifteen fungal species with an isolation rate of 23.33%, followed by *Lumnitzera racemosa* with 12 species and *Sonneratia alba* with eleven (Table 3). The genus *Rhizophora* hosted a total of 23 out of the 53 fungal species isolated. By extension, the family *Rhizophoraceae* which includes the genera *Rhizophora*, *Bruguiera*, and *Ceriops* host a total of 33 fungal endophytes. This, however, cannot be generalized because there were more *Rhizophoraceae* species to sample from in this study than the rest of the mangrove families.

Other mangrove families had some unique fungal endophytes. The species *Glomerella* sp., *Lentinus squarrosulus* Mont., *Peniophora* sp., and *Xylaria feejeensis* (Berk.) Fr. were only isolated from members of the *Acanthaceae* family (genus *Avicennia*); *Trametes elegans* (Spreng.) Fr., *Phomopsis longicolla* Hobbs., *Paecilomyces formosus* (Sakag., ay. Inoue & Tada) Houbraken & Samson, *Microascus cinereus* Curzi, *Lasiodiplodia pseudotheobromae* A.J.L. Phillips, A. Alves & Crous, *Glomerella cingulata* (Stoneman) Spauld. & H. Schrenk, *Fusarium solani* (Mart.) Sacc., and *Colletotrichum coffeanum* F. Noack were only isolated from *Lythraceae* family (genera *Sonneratia* and *Pemphis*); *Diaporthe longicolla* (Hobbs) J.M. Santos, Vrandecic & A.J.L. Phillips was only isolated from *Scyphyphora hydrophyllacea* C.F. Gaertn. of the *Rubiaceae* family; *Chaetomium globosum* Kunze and *Curvularia verrusculosa* Tandon & Bilgrami were only isolated from *Excoecaria agallocha* of the *Euphorbiaceae* family; and *Aspergillus japonicus* Saito, *Colletotrichum kahawae* J.M. Waller & Bridge, *Lasiodiplodia theobromae*, and *Trametes maxima* (Mont.) A. David & Rajchenb were unique to the *Combretaceae* family (genus *Lumnitzera*). It can be seen here that preference of fungal species to a family of mangrove host do not necessarily apply to the entire fungal genus. For example, *Colletotrichum coffeanum* and *Colletotrichum kahawae* are unique to two different families of mangroves, as are *Trametes elegans* and *Trametes maxima*.

Four species of mangroves hosted only one fungal species each – *Lumnitzera littorea*, *Pemphis acidula*, *Ceriops*

**Table 3**. Density of colonization of endophytic fungi in mangrove leaves.

| Endophyte | Density of Colonization[1] | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | RS[2] | RA[3] | RM[4] | BC[5] | BG[6] | BS[7] | BP[8] | CD[9] | CZ[10] | CT[11] | AM[12] | AR[13] | AA[14] | SA[15] | SO[16] | SC[17] | PA[18] | SH[19] | EA[20] | LR[21] | LL[22] |
| *Alternaria* sp. | - | - | 0.83 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| *Aspergillus aculeatus* | - | 0.83 | - | 1.67 | - | - | - | - | - | - | 0.83 | - | - | 3.33 | - | - | - | - | - | 0.83 | - |
| *Aspergillus flavus* | 0.83 | - | 3.33 | 0.83 | - | 2.50 | 0.83 | - | - | - | - | - | - | 1.67 | - | - | - | - | - | - | - |
| *Aspergillus fumigatus* | - | 0.83 | - | - | - | 0.83 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| *Aspergillus japonicus* | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | 0.83 | - |
| *Aspergillus minisclerotigenes* | 0.83 | 2.50 | - | - | - | - | 0.83 | - | - | - | - | 0.83 | - | 0.83 | 0.83 | - | - | - | - | 0.83 | - |
| *Aspergillus niger* | - | - | - | 0.83 | - | - | - | - | - | - | - | 0.83 | - | - | - | - | - | - | - | - | - |
| *Aspergillus* sp. | - | - | - | - | - | - | - | 0.83 | - | - | - | 0.83 | 1.67 | - | - | - | - | - | - | 0.83 | - |
| *Aspergillus versicolor* | 0.83 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| *Botryosphaeria rhodina* | - | - | - | 0.83 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| *Ceriporia lacerata* | 1.67 | - | 1.67 | 3.33 | - | - | 0.83 | - | - | - | - | 0.83 | - | - | - | - | - | - | 0.83 | 1.67 | - |
| *Chaetomium globosum* | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | 0.83 | - | - |
| *Colletotrichum coffeanum* | - | - | - | - | - | - | - | - | - | - | - | - | - | 0.83 | - | - | - | - | - | - | - |
| *Colletotrichum gloeosporioides* | - | 1.67 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| *Colletotrichum kahawae* | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | 0.83 | - |
| *Colletotrichum* sp. | - | - | - | - | - | - | 0.83 | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| *Curvularia verrusculosa* | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | 0.83 | - | - |
| *Daldinia eschscholtzii* | - | - | - | - | 0.83 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| *Diaporthe longicolla* | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | 0.83 | - | - | - |
| *Fomes* sp. | - | - | - | - | - | - | - | 0.83 | - | - | - | 1.67 | 0.83 | 0.83 | 0.83 | - | - | 0.83 | - | 0.83 | - |
| *Fusarium solani* | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | 0.83 | - | - | - | - | - |
| *Glomerella cingulata* | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | 0.83 | - | - | - | - | - |
| *Glomerella* sp. | - | - | - | - | - | - | - | - | - | - | 0.83 | - | - | - | - | - | - | - | - | - | - |
| *Hyalotiella rubi* | - | - | 0.83 | - | - | - | - | - | - | - | - | 0.83 | 0.83 | - | - | - | - | 0.83 | - | - | - |
| *Lasiodiplodia pseudotheobromae* | - | - | - | - | - | - | - | - | - | - | - | - | - | 0.83 | - | - | - | - | - | - | - |
| *Lasiodiplodia theobromae* | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | 0.83 |
| *Lentinus squarrosulus* | - | - | - | - | - | - | - | - | - | - | 0.83 | - | - | - | - | - | - | - | - | - | - |
| *Lichtheimia ramosa* | - | - | 0.83 | - | 0.83 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| *Microascus cinereus* | - | - | - | - | - | - | - | - | - | - | - | - | - | 0.83 | - | - | - | - | - | - | - |
| *Nigrospora oryzae* | - | - | 1.67 | - | - | - | - | - | - | 0.83 | - | - | - | - | - | - | - | - | - | 0.83 | - |
| *Nigrospora sphaerica* | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | 0.83 | - | - | 2.50 | - |

Table 3 continuation next page

Table 3 continuation . . .

| | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *Nigrospora* sp. | - | - | 0.83 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| *Paecilomyces formosus* | - | - | - | - | - | - | - | - | - | - | - | - | - | 0.83 | - | - | - | - | - | - | - | - |
| *Paecilomyces variotii* | - | - | 0.83 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| *Penicillium citrinum* | - | - | - | - | - | 0.83 | - | - | - | - | - | - | - | - | - | 0.83 | - | - | - | - | - | - |
| *Penicillium griseofulvum* | - | - | 0.83 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| *Peniophora* sp. | - | - | - | - | - | - | - | - | - | - | - | 0.83 | - | - | - | - | - | - | - | - | - | - |
| *Pestalotiopsis mangiferae* | - | - | 0.83 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| *Pestalotiopsis cocculi* | 0.83 | - | 1.67 | - | 0.83 | - | - | - | - | - | 1.67 | - | - | 0.83 | 1.67 | 0.83 | - | - | - | 0.83 | - | - |
| *Pestalotiopsis microspora* | - | - | 1.67 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| *Pestalotiopsis uvicola* | 2.50 | 1.67 | - | - | - | 3.33 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| *Pestalotiopsis* sp. | - | 0.83 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| *Phlebiopsis crassa* | - | - | 0.83 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| *Phomopsis longicolla* | - | - | - | - | - | - | - | - | - | - | - | - | - | - | 0.83 | - | - | - | - | - | - | - |
| *Rhizopus microsporus* | - | - | - | - | - | - | - | - | 0.83 | - | - | - | - | - | - | 0.83 | - | 0.83 | - | - | - | - |
| *Rigidoporus vinctus* | - | - | - | - | - | - | - | 0.83 | - | - | - | - | - | - | 1.67 | - | - | - | - | 0.83 | - | - |
| *Schizophyllum commune* | - | - | 0.83 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| *Trametes cubensis* | 1.67 | 4.17 | 5.83 | 2.50 | - | 5.00 | - | - | - | - | 3.33 | - | - | 1.67 | 0.83 | 0.83 | - | - | - | - | - | - |
| *Trametes elegans* | - | - | - | - | - | - | - | - | - | - | - | - | - | 0.83 | - | - | - | - | - | - | - | - |
| *Trametes ljabarskyi* | - | - | - | - | - | - | - | 0.83 | - | - | - | - | - | - | - | 0.83 | - | - | - | - | - | - |
| *Trametes maxima* | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | 0.83 | - | - |
| *Valsa euginiae* | 0.83 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| *Xylaria feejeensis* | - | - | - | - | - | - | - | - | - | - | - | 0.83 | - | - | - | - | - | - | - | - | - | - |
| **Total Number of Isolates** | 12 | 15 | 28 | 12 | 3 | 15 | 4 | 4 | 1 | 1 | 9 | 9 | 4 | 16 | 8 | 7 | 1 | 4 | 3 | 15 | 1 | |
| **Isolation Rate (%)** | 10.00 | 12.50 | 23.33 | 10.00 | 2.50 | 12.50 | 3.33 | 3.33 | 0.83 | 0.83 | 7.50 | 7.50 | 3.33 | 13.33 | 6.67 | 5.83 | 0.83 | 3.33 | 2.50 | 12.50 | 0.83 | |
| **Total Fungal Species Isolated** | 8 | 7 | 15 | 6 | 3 | 5 | 4 | 4 | 1 | 1 | 5 | 8 | 3 | 11 | 6 | 7 | 1 | 4 | 3 | 12 | 1 | |

N= 120 leaf discs; [1]Density of Colonization = (number of isolate/ total number of leaf discs plated) x 100; [2]*Rhizophora styolosa;* [3]*Rhizophora apiculata;* [4]*Rhizophora mucronata;* [5]*Bruguiera cylindrica;* [6]*Bruguiera gymnorhiza;* [7]*Bruguiera sexangula;* [8]*Bruguiera parviflora;* [9]*Ceriops decandra;* [10]*Ceriops zippeliana*; [11]*Ceriops tagal;* [12]*Avicennia marina;* [13]*Avicennia rumphiana;* [14]*Avicennia alba;* [15]*Sonneratia alba;* [16]*Sonneratia ovalis;* [17]*Sonneratia caseolaris;* [18]*Pemphis acidula;* [19]*Scyphiphora; hydrophylacea;* [20]*Excoecaria agallocha;* [21]*Lumnitzera racemosa;* [22]*Lumnitzera littorea*

*zippeliana*, and *Ceriops tagal* – resulting to the isolate-to-species ratio of 1.00 (Table 1). This may suggest that their endophytes are dominant in the mangrove leaves. However, this warrants verification because the research is culture-based and that no other trees were sampled to act as replicates. The commonality in the growth pattern of the fungi *Nigrospora oryzae, Nigrospora sphaerica* E.W. Mason, *Lasiodiplodia theobromae*, and *Rhizopus microsporus* – obtained from the four previously mentioned mangrove hosts – is their fast growth and development that may have prevented slow growers to spread out into the culture media.

Moreover, although *Ceriops tagal* only hosted one fungus – *Nigrospora oryzae* – this fungus was also isolated from other species of mangroves. In fact, it shares *Rhizophora mucronata* and *Lumnitzera racemosa* as host to 14 and

11 other fungal species, respectively. The same is true for *Pemphis acidula* and *Ceriops zippeliana*. Only the mangrove *Lumnitzera littorea* hosted a species of fungus (*Lasiodiplodia theobromae*) that was not isolated from any other mangrove species.

Finally, the richness of fungal taxa isolated was high but very few showed appreciable degree of colonization [2% or more according to Suryanarayanan and co-workers (1998)]. *Aspergillus aculeatus* Iizuka; *Aspergillus flavus* Link.; *Aspergillus minisclerotigenes* Vaamonde, Frisvad & Samson, *Ceriporia lacerata* N. Maek, Suhara & R. Kondo, *Nigrospora sphaerica*, *Pestalotiopsis uvicola* (Speg.) Bissett, and *Trametes cubensis* were only those which reached the 2% limit.

## DISCUSSION

### Culture-dependent Endophytic Research

There are many studies on mangrove fungal endophytes, but there are few published researches concerning such in the Philippines. These include the works of Leanio (2001), Besitulo and co-workers (2010), and Sadaba and Sarinas (2010). These researches, however, did not dwell on endophytic fungi but only on fungal occurrence on drift woods and fallen leaves. The similarity of this research with most of the literature is the utilization of culture-dependent approach on fungal isolation supplemented by molecular approach to aid in identification of species. It is fairly established that only few fungal species relative to the diversity of this kingdom can grow well in synthetic culture media. This may present a major disadvantage, especially in diversity studies when one needs to provide an estimate of species richness. Those that cannot grow in culture media cannot be accounted for and are thus omitted in the overall diversity picture. Likewise, fast growers can over-propagate in the media and prevent slow-growing species from establishing colonies. Because a culture-dependent approach is still the quickest and most available for low-funded laboratories, it becomes the primary source of data on which molecular diversity mapping can anchor on. Moreover, the immensity of endophytic studies – whether on mangroves or terrestrial plants – root from culture-dependent procedures than on recent molecular approaches. The merger between culture-dependent and independent methodologies can be seen, therefore, as complementary rather than one being better than the other.

### The Plant-Fungus Association

The definition of endophytes will matter because it will be the basis on which fungi to classify as endophytes and those which are strictly pathogens. Most literature defines an endophyte as one that inhabits a plant organ at some point in their life, which can colonize internal plant tissues without causing any apparent harm to their host. Saikkonen and co-workers (1998) agreed with this working definition, but noted that this definition results to some pathogens and saprobes being regarded as endophyte because of the considerable lengthy latency periods prior to the appearance of disease symptoms. They noted further that interactions between fungi and host plants are often variable among and within population and communities because distinction between a strict pathogen and a mutualist is not always clear. The present research agrees with this fluidity in the endophyte classification. Some fungi isolated in this study are known pathogens of terrestrial plants but were found to be asymptomatically living within the mangrove leaves. These include the genera *Alternaria*, some species of *Aspergillus*, *Colletotrichum, Curvularia, Diaporthe, Fusarium, Lasiodiplodia, Pestalotiopsis, Phomopsis*, and *Rhizopus*. This study, however, does not categorize the fungal isolates as pathogens or saprophytes of mangroves and thus warrants further study.

Carroll (1998) described fungal endophytes to be closely related to virulent pathogens but with limited, if any, pathogenic characteristic themselves. Arnold (2007) highlighted that although there is a rich literature on endophytism, there is also a parallel lack of clarity on the evolutionary and ecological relationships among endophytes, pathogens, and saprophytes – referring to them as communities of uncertain overlap. Hyde and Soytong (2008) listed alternative definitions of endophytes through time and likewise underscore the idea that fungal endophytes can become primary saprobe decomposers, mutualists, and latent pathogens. Studies have shown that – under conditions of stress – endophytes may trigger symptom formation, and that some abiotic factors such as light may favor either endosymbiotic or pathogenic development (Alvarez-Loayza *et al.* 2011). This may plausibly explain why endophytes isolates in this study are, in fact, saprophytes and pathogens of some terrestrial plants, or that these endophytes may be saprophytes and pathogens waiting for ecological and physiological imbalances for them to infect their hosts.

### Mangrove Endophytic Association with Fungi

Arnold and Lutzoni (2007) have cited that leaves of tropical trees represent hotspots for fungal endophytes. These foliar endophytes are distinct because of the dynamic interactions of the leaves with the environment (Arnold 2007). True enough, the leaves of mangrove species are unique because of the many environmental factors they encounter and the anatomy of the leaf itself.

Leaf structures share similarities even if the mangroves belong to different families (Poompozhil & Kumarasamy 2014). These include a relatively thicker lamina, palisade, and spongy layers – as well as features that relate to their functionalities. For instance, epidermal characteristics relate to transpirational control, salt glands, and mesophyll to the maintenance of salt balance and the lack of extensive sclerenchymatous structural support to high turgor. The mangroves represented in this study exhibited these characteristics.

Of important discussion is the salinity that mangroves need to tolerate and how these may affect fungal assemblage. *Avicennia* species excrete salts through salt glands in the leaves, while *Rhizophora* species exclude them in the roots. Consequently, *Rhizophora* species accumulate less salt in the leaves than the *Avicennia* species. Reports show salt excretion may be a mechanism to regulate fungal colonization (Gilbert *et al.* 2002). In the present study, there were relatively higher number of fungal isolates from *Rhizophora* species than *Avicennia* species. Some fungi, however, may tolerate high levels of salt such as *Pestalotiopsis* species. A study by Arfi and co-workers (2013) extensively discussed the salt-adapted secreted lignocellullytic enzymes, highlighting the salt tolerance of this group of fungi.

Although leaf age is not within the scope of the study, it can also be a significant factor in isolation of endophytes. Among terrestrial flora, it was already shown that leaf age can affect the fungal colony of plants in which more endophytes can be isolated among old/ mature leaves than the younger ones (Osono 2008), even suggesting that it is independent of the age of the tree (Taylor *et al.* 1999). Age of mangrove leaves may be one of the variables for research in the future.

Host preference, although not within the scope of this study, may account for the varying richness of fungi for each mangrove. Gilbert and Sousa (2002) have shown that at least three polypore basidiomycetes were almost exclusively found in a particular mangrove species. The polypore fungi *Datronia caperata* (Berk.) Ryvarden, *Phellinus swieteniae* (Murrill) S. Herrera & Bondartseva, and *Trichaptum biforme* (Fr.) Ryvarden showed strong host preference to *Avicennia*, *Rhizophora*, and *Laguncularia*, respectively. The polypores in this study – namely *Trametes cubensis* – did not show preference because it was isolated from 9 mangrove hosts, while other *Trametes* species were isolated once in a particular mangrove host. Likewise, tissue type may affect endophytic community. This has been shown in terrestrial plants, as detailed in the study by Sun and co-workers (2012) on woody plants in mixed forests.

**Conservation of Mangroves Means Conservation of Mycobiota**

As terrestrial forests are the main source of ecological services, so too are mangrove forests as the critical drivers of ecosystem functioning in the coastal environments. Mangroves provide goods and services economically and ecologically (Ewel *et al.* 1998) and improve our understanding of ecosystem resilience and overall impact of global climate change (Alongi 2008). Uncontrolled and undocumented mangrove exploitation, however, threaten not only the mangrove themselves but the many species dependent on them. The rich fauna and flora and the richer microbiota become prone whenever mangroves are pushed towards degradation. Fungal species that are well adapted to the environmental conditions of a mangrove forest will suffer the consequences of losing its host.

Likewise, the concept of bioprospecting or the search for new biological compounds sits well with mangrove fungal endophytes and further justifies the need to continuously document fungal diversity in unique environments, such as the mangrove ecosystem. Fungal endophytes of higher plants and mangroves are considered new sources of biologically active products, and that research in this area has gained traction through the years (Aly *et al.* 2010). With prior knowledge that fungal characteristics may be affected by host plants and environmental factors, bioprospecting research can narrow down the search to unique ecosystems or specific host plants.

## CONCLUSION

The study concludes with 53 fungal species found in 21 mangrove hosts. Identification of endophytic fungi, especially among mangroves, has implications to conservation and genetic diversity; likewise, it can significantly impact modern science, agriculture, and medicine. Mangroves are among the most vulnerable species, exponentially exposed to anthropogenic and climatic pressures. Limiting mangrove growth consequently limits the unique features on which endophytic fungi and the rest of mangalicolous microflora may thrive on. The increasing reports on natural products derived from fungal endophytes are testament to the mangrove ecosystem as an arsenal of biological agents for the present and future. All these are thus anchored on extensive identification of endophytes, the subsequent characterization of their biological properties, and the continuous protection of the mangroves.

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## REFERENCES

ALONGI DM. 2008. Mangrove forests: Resilience, protection from tsunamis, and responses to global climate change. Estuar Coast Shelf Sci 76(1): 1–13.

ALVAREZ-LOAYZA P, WHITE JR JF, TORRES MS, BALSLEV H, KRISTIANSEN T, SVENNING JC, GIL N. 2011. Light converts endosymbiotic fungus to pathogen, influencing seedling survival and niche-space filling of a common tropical tree, *Iriartea deltoidea*. PloS one 6(1): e16386.

ALY AH, DEBBAB A, KJER J, PROKSCH P. 2010. Fungal endophytes from higher plants: A prolific source of phytochemicals and other bioactive natural products. Fungal Divers 41(1): 1–16.

ARFI Y, CHEVRET D, HENRISSAT B, BERRIN JG, LEVASSEUR A, RECORD E. 2013. Characterization of salt adapted secreted lignocellulolytic enzymes from the mangrove fungus *Pestalotiopsis* sp. Nat Commun 4: 1810.

ARNOLD AE. 2007. Understanding the diversity of foliar endophytic fungi: Progress, challenges, and frontiers. Fungal Biol Rev 21(2): 51–66.

ARNOLD AE, LUTZONI F. 2007. Diversity and host range of foliar fungal endophytes: Are tropical leaves biodiversity hotspots? Ecology 88(3): 541–549.

BESITULO A, MOSLEM MA, HYDE KD. 2010. Occurrence and distribution of fungi in a mangrove forest on Siargao Island, Philippines. Bot Mar 53(6): 535–543.

CARROLL G. 1998. Fungal endophytes in stems and leaves: From latent pathogen to mutualistic symbiont. Ecology 69(1): 2–9.

CHENG ZS, TANG WC, SU ZJ, CAI Y, SUN SF, CHEN QJ, WANG FH, LIN YC, SHE G, VRIJMOED LLP. 2008. Identification of mangrove endophytic fungus 1403 (*Fusarium proliferatum*) based on morphological and molecular evidence. J Forest Res 19(3): 219–224.

CHENG ZS, PAN JH, TANG WC, CHEN QJ, LIN, YC. 2009. Biodiversity and biotechnological potential of mangrove-associated fungi. J Forest Res 20(1): 63–72.

COSTA IP, MAIA LC, CAVALCANTI MA. 2012. Diversity of leaf endophytic fungi in mangrove plants of Northeast Brazil. Braz J Microbiol 43(3): 1165–73.

DEBBAB A, ALY AH, PROKSCH P. 2013. Mangrove derived fungal endophytes – A chemical and biological perception. Fungal Divers 61(1): 1–27.

EWEL K, TWILLEY R, ONG J. 1998. Different kinds of mangrove forests provide different goods and services. Global Ecol Biogeog Lett 7(1): 83–94.

GANGADEVI V, MUTHUMARY J. 2008. Isolation of *Colletotrichum gloeosporioides*, a novel endophytic taxol-producing fungus from the leaves of a medicinal plant, *Justicia gendarussa*. Mycologia Balcanica 5: 1–4.

GILBERT GS, MEJÍA-CHANG M, ROJAS E. 2002. Fungal diversity and plant disease in mangrove forests: Salt excretion as a possible defense mechanism. Oecologia 132(2): 278–285.

GILBERT GS, SOUSA WP. 2002. Host specialization among wood-decay polypore fungi in a Caribbean mangrove forest. Biotropica 34(3): 396–404.

HYDE KD, CAI L, MCKENZIE EHC, YANG YL, ZHANG JZ, PRIHASTUTI H. 2009. *Colletotrichum*: A catalogue of confusion. Fungal Divers 39(2): 1–17.

HYDE KD, SOYTONG K. 2008. The fungal endophyte dilemma. Fungal Divers 33(163): 73.

KJER J, WRAY V, EDRADA-EBEL R, EBEL R, PRETSCH A, LIN W, PROKSCH P. 2009. Xanalteric acids I and II and related phenolic compounds from an endophytic *Alternaria* sp. isolated from the mangrove plant *Sonneratia alba*. J Nat Prod Resour 72(11): 2053–57.

LEANIO EM. 2001. Straminipilous organisms from fallen mangrove leaves from Panay Island, Philippines. Fungal Divers 6: 75–81.

LIU F, CAI XL, YANG H, XIA XK, GUO ZY, YUAN J, LI MF, SHE ZG, LIN YC. 2010. The bioactive metabolites of the mangrove endophytic fungus *Talaromyces* sp. ZH-154 isolated from *Kandelia candel* (L.) Druce. Planta Medica 76(02): 185–189.

OSONO T. 2008. Endophytic and epiphytic phyllosphere fungi of *Camellia japonica*: Seasonal and leaf age dependent variations. Mycologia 100(3): 387–391.

POOMPOZHIL S, KUMARASAMY D. 2014. Leaf

Anatomical Studies on Some Mangrove Plants. J Acad Indust Res 2(10): 583.

RÖNSBERG D, DEBBAB A, MÁNDI A, WRAY V, DAI H, KURTÁN T, PROKSCH P, ALY AH. 2013. Secondary metabolites from the endophytic fungus *Pestalotiopsis virgatula* isolated from the mangrove plant *Sonneratia caseolaris*. Tetrahedron Lett 54(25): 3256–59.

SADABA RB, SARINAS BG. 2010. Fungal communities in bunker C oil-impacted sites off southern Guimaras, Philippines: A post-spill assessment of Solar 1 oil spill. Bot Mar 53(6): 565–575.

SAIKKONEN K, FAETH SH, HELANDER M, SULLIVAN TJ. 1998. Fungal endophytes: A continuum of interactions with host plants. Annu Rev Ecol Evol Syst 29(1): 319–343.

STROBEL GA. 2003. Endophytes as sources of bioactive products. Microb Infect 5(6): 535–544.

STROBEL GA, DAISY B. 2003. Bioprospecting for microbial endophytes and their natural products. Microbiol Mol Biol Rev 67(4): 491–502.

SUN X, DING Q, HYDE KD, GUO LD. 2012. Community structure and preference of endophytic fungi of three woody plants in a mixed forest. Fungal Ecol 5(5): 624–632.

SURYANARAYANAN TS, KUMARESAN V, JOHNSON JA. 1998. Foliar fungal endophytes from two species of the mangrove *Rhizophora*. Can J Microbiol 44(10): 1003–06.

SURYANARAYANAN TS, THENNARASAN S. 2004. Temporal variation in endophyte assemblages of *Plumeria rubra* leaves. Fungal Divers 15: 197–204.

TAYLOR JE, HYDE KD, JONES EBG. 1999. Endophytic fungi associated with the temperate palm, *Trachycarpus fortunei*, within and outside its natural geographic range. New Phytol 142(2): 335–346.

TORRES JMO, DELA CRUZ TEE. 2015. Antibacterial activities of fungal endophytes associated with the Philippine endemic tree, *Canarium ovatum*. Mycosphere 6: 266–273.

YU H, ZHANG L, LI L, ZHENG C, GUO L, LI W, SUN P, QIN L. 2010. Recent developments and future prospects of antimicrobial metabolites produced by endophytes. Microbiol Res 165(6): 437–449.

WANG YN, TIAN L, HUA HM, LU X, SUN S, WU HH, PEI YH. 2009. Two new compounds from the broth of the marine fungus *Penicillium griseofulvum* Y19-07. J Asian Nat Prod Res 11(11): 912–917.

WEI JG, XU T, GUO LD, LIU AR, ZHANG Y, PAN XH. 2007. Endophytic *Pestalotiopsis* species associated with plants of *Podocarpaceae*, *Theaceae* and *Taxaceae* in southern China. Fungal Divers 24(1): 55–74.

WU YZ, QIAO F, XU GW, ZHAO J, TENG JF, LI C, DENG WJ. 2015. Neuroprotective metabolites from the endophytic fungus *Penicillium citrinum* of the mangrove *Bruguiera gymnorrhiza*. Phytochem Lett 12: 148–152.

ZHENG CJ, HUANG GL, XU Y, SONG XM, YAO J, LIU H, WANG RP, SUN XP. 2016. A new benzopyrans derivatives from a mangrove-derived fungus *Penicillium citrinum* from the South China Sea. Nat Prod Res 30(7): 821–825.

ZOU WX, MENG JC, LU H, CHEN GX, SHI GX, ZHANG TY, TAN RX. 2000. Metabolites of *Colletotrichum gloeosporioides*, an endophytic fungus in *Artemisia mongolica*. J Nat Prod 63(11): 1529–30.