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Cover image: The mudskipper *Periophthalmus argentilineatus* on the mangrove of Ulo Beach, Cabo Delgado, North Mozambique. © José Paula, 2006

Diversity of mangrove fungal endophytes from selected mangrove species of coastal Kenya

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Endophytes are bacteria or fungi living asymptomatically in the internal tissues of plants. They are symbiotic in nature and can be exploited for novel bioactive metabolites with applications in agriculture, medicine and industry. Mangrove fungal endophytes from the marine environment are abundant and have been recognized as sources of bioactive natural products. The study was designed to isolate, purify and identify mangrove fungal endophytes from selected common mangrove species of Gazi Bay, Tudor and Mida creek on the Kenya coast. The colonization rate and isolation rate of the mangrove fungal endophytes were determined. The studied mangrove species were *Rhizophora mucronata* (red mangrove), *Sonneratia alba* (mangrove apple), *Avicennia marina* (grey or white mangrove), and *Ceriops tagal* (spurred mangrove). Samples from twigs of these mangrove species were collected and analyzed using standard methods. Isolation of pure cultures of the endophytes was performed using Potato Dextrose Agar (PDA) incubated at 28 ± 1°C for 5 days. The fungal isolates were identified under a light microscope based on colony morphology characteristics, type and presentation of conidiophores and conidia. A total of 18 different mangrove fungal endophytes were identified and these belonged to 5 genera. These were *Aspergillus*, *Penicillium*, *Fusarium*, *Cephalosporium* and *Blastomyces*, with *Aspergillus* being the most dominant genus. Tudor Creek recorded the highest fungal community diversity (H' = 1.35) and Gazi Bay had the lowest diversity (H' = 0.45). Fungal community similarity based on the identified genera was highest between Gazi Bay and Mida Creek (0.80) and lowest between Tudor Creek and Mida Creek (0.57). The selected mangrove species recorded a colonization rate of endophytic fungi of between 38.9 – 94.4 % with the highest habitation being associated with *S. alba* and *C. tagal*. There were differences and similarities in the colonization rate within mangrove species across study sites. Findings of this study have confirmed that the selected mangrove species exhibit high diversity of fungal endophytes with host recurrence and spatial heterogeneity.

Keywords: diversity, mangroves, fungal endophytes, colonization, Kenya coast

Introduction

Mangroves differ from other plants in morphology, anatomy and reproduction due to the influence of several factors such as salinity, pH and soil condition (Thorati *et al*., 2016). Mangrove plants harbor different species of endophytes which are a valuable source of useful metabolites gaining increasing importance in the pharmaceutical industry. The Kenyan mangrove forests are part of the Western Indian Ocean (WIO) region which covers 1 million ha; about 5 % of the global mangrove cover (UNEP-Nairobi Convention/USAID/WIOMSA, 2020). Two of the selected mangrove species in this study, *R. mucronata* (Lamk) and *C. tagal* (Perr.) belong to the largest mangrove family Rhizophoraceae while *A. marina* (Forsk.) is of the family Acanthaceae, and *S. alba* (Sm) from the Lythraceae family (Kairo, 2001). Mangroves have been used in traditional medicines and extracts of some species have been known to exhibit activity against human, animal and plant pathogens (Hamzah *et al*., 2018).

Endophytic fungi are eukaryotic microorganisms that colonize living internal tissues of plants asymptomatically and are under-explored to meet the various

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needs of mankind, including medicine (Dar *et al*., 2015). These microorganisms represent an important and quantifiable component of fungal biodiversity, affecting plant community biodiversity and structure (Dar *et al*., 2015). Studies have shown that these fungi have been found in every plant examined to date and are a potential source of natural products for exploration in medicine, agriculture and industry (Thorati *et al*., 2016). Recent studies have shown the uniqueness of endophytic fungi with about 1 million species inhabiting plant tissues (Huang *et al*., 2008).

The discovery of penicillin by Alexander Fleming in 1928 from the fungus *Penicillium* sp., coupled with re-isolation and clinical studies by Chain, Florey, and co-workers in the early 1940s, and commercialization of synthetic penicillin, revolutionized drug discovery research to a large extent (Sonia, 2006). This enormous success prompted drug companies and research groups to assemble collections of microorganisms in order to uncover new antibiotics. This led to the discovery of streptomycin, chloramphenicol, chlortetracycline, erythromycin, vancomycin and cephalosporin C. The antibiotic cephalosporin C was extracted from a fungus *Cephalosporium acremonium* and was found to show activity against *Staphylococcus aureus*, *Salmonella typhii* and *Escherichia coli.* All of the compounds stated, or their derivatives, are still in use as drugs to date (Sonia, 2006; Ling *et al*., 2010).

Interest in endophytic fungi has further grown since the discovery of an endophytic fungus, *Taxus brevifolia,* which led to the production of the billion-dollar anti-cancer drug, taxol (Saryono *et al*., 2015). Taxol or paclitaxel was first isolated from an endophytic fungus, *Taxomyces adreanae* by Stierle and colleagues in 1993 (Ling *et al*., 2010). This fungus inhabited the bark of a yew tree (*Taxus brevifolia),* an evergreen shrub. Taxol generated more attention and interest than any other new drug since its discovery, possibly due to its unique mode of action compared to other anticancer agents found to interfere with the multiplication of cancer (Sonia, 2006).

The relationship of endophytic fungi with single or multiple plant hosts can be described in terms of host specificity, host-recurrence, host-selectivity or host-preference (Huang *et al*., 2008). Host-specificity refers to a relationship between a living host and a fungus. This is restricted to a single host or a group of related species but does not occur in other unrelated plants in the same habitat (Nogueira-Melo *et al*., 2017). Frequent or predominant occurrence of an endophytic fungus on a particular host or a group of plant hosts is refered to as host-recurrence while the fungus can still occur in other plant hosts in the habitat (Huang *et al*., 2008). Host-selectivity is a phenomenon whereby an endophytic fungal species forms relationships with two related plant species but demonstrates preference to one of the plant hosts. Endophytic fungi may exhibit host specificity for a particular plant species; the distribution of which may then be influenced by environmental conditions, leading to spatial heterogeneity (Huang *et al*., 2008).

Endophytes in mangrove species along the Kenya coast have scarcely been investigated. The microbial resources of the Kenya coast, particularly the endophytic populations in the mangrove plants, are still under-explored. Jenoh *et al*., (2019) studied the infestation mechanisms of two woodborer species in the mangrove *S. alba* in Kenya and the co-occurrence of endophytic fungi. These authors established the occurrence of secondary infestation by endophytic fungi in the infested branches of *S. alba*. Endophytic fungal isolation was achieved using standard procedures by Barnett (1998) resulting in 15 species distributed in 6 fungal genera namely *Aspergillus*, *Penicillium*, *Trichoderma*, *Giberella*, *Talaromyces*, and *Cladosporium*. Hamzah *et al*., (2018) isolated and identified a total of 78 fungal isolates from the leaves of *R. mucronata* from Matang Mangrove Forest Reserve (MMFR) in Malaysia.

This study therefore aimed at isolating and identifying fungal endophytes from selected mangrove species from samples of twigs collected from Gazi Bay, Tudor Creek and Mida Creek along the Kenya coast. Colonization rate (CR) or the percentage of colonized segments, isolation rate (IR) which is a measure of fungal richness in a sample of plant tissue, relative frequency (RF) (Huang *et al*., 2008), and fungal community diversity were determined. The findings obtained from this study provide preliminary data on the diversity of mangrove endophytic fungi from the Kenya coast for future investigations on marine bioactive molecules.

Materials and methods

Study area

This study was conducted in three selected mangrove ecosystems along the Kenya coast (Fig. 1). These included Gazi Bay on the south coast of Kenya located at 4°25'S, 39°50'E in Kwale County, about 50 km south of Mombasa City. The bay comprises

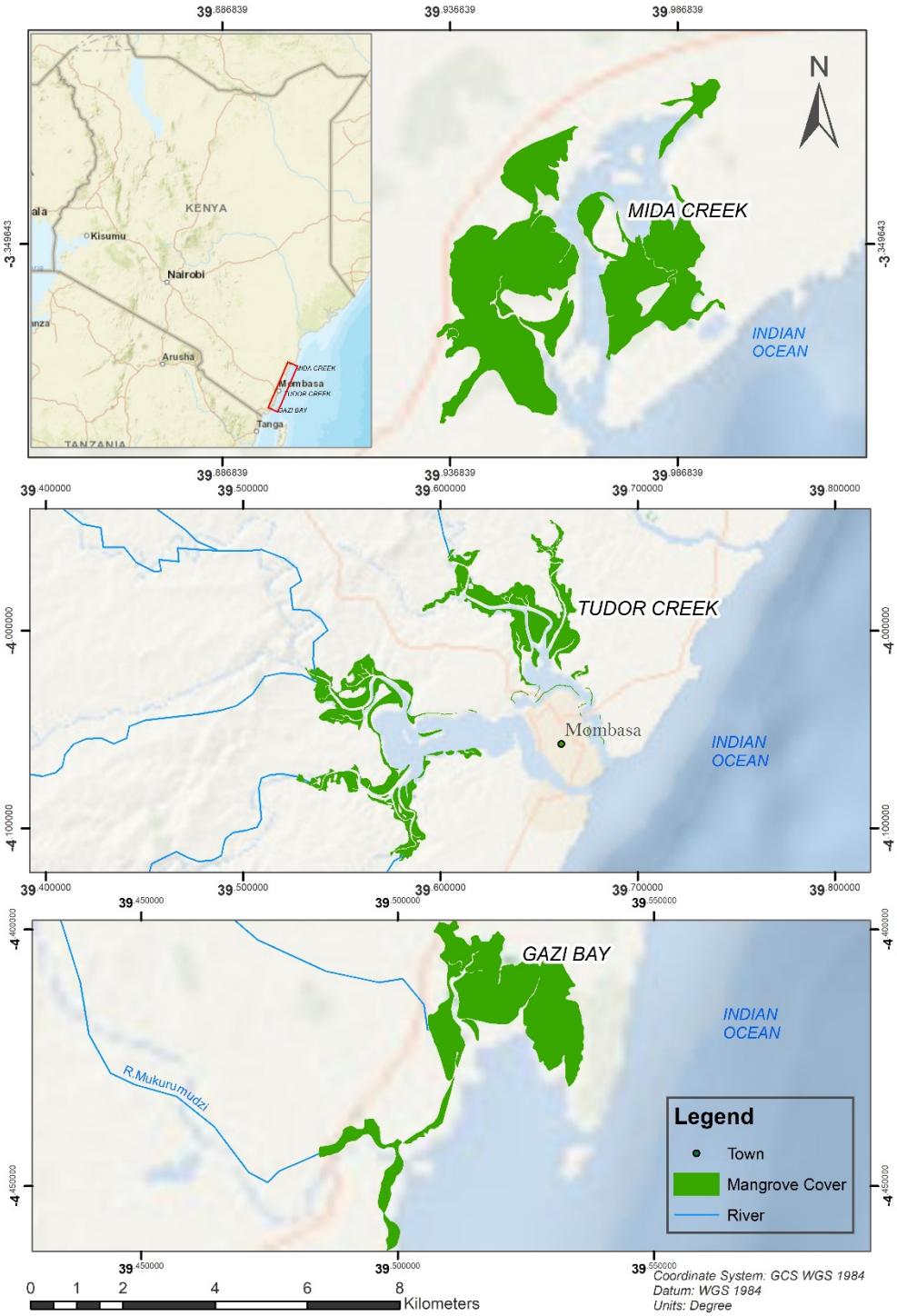

Figure 1. A map of the Kenyan coast showing the mangrove forests of Gazi Bay, Tudor Creek and Mida Creek where samples of mangrove twigs were collected for the study.

a shallow channel approximately 4 km long fringed by mangrove forest dominated by the species *S. alba* and *R. mucronata* (Bosire *et al*., 2003). Tudor Creek (4°2'S, 39°40'E) which is located at the northwest of Mombasa Island, extends some 10 – 15 km inland with two main seasonal rivers, Kombeni and Tsalu, draining over 45,000 and 10,000 ha, respectively (Bosire *et al*., 2014). Within the creek is a mangrove forest extending over an area of 1,641 ha, principally composed of *R. mucronata*, *S. alba*, and *A. marina*. The forest does not display distinct mangrove species zonation along the tidal gradient and is covered by sediments that are predominantly made up of mud, and sand in some parts (Bosire *et al*., 2014). Mida

Creek (03°21'S, 39°59'E) is located in Kilifi County, 88 km north of Mombasa and is approximately 25 km south of Malindi Town (Dahdouh-Guebas *et al*., 2000). Mangrove forest is the most dominant habitat in Mida Creek and occupies approximately 1,746 ha supporting 7 of the 9 mangrove species found in Kenya (Kairo, 2001; Owuor *et al*., 2017).

Collection of samples

Identification and sampling of the selected common mangrove species was conducted in each study site. Subjective or non-probability sampling was employed. From the landward side, samples of twigs of *A. marina* were collected. Between the landward and seaward side, samples of twigs of *C. tagal* and *R. mucronata* were collected, and to the seaward side, samples of twigs of *S. alba* were collected. A total of 3 twig cuttings from 2 mangrove trees of each species in the 3 study sites were randomly sampled using a sharp laboratory knife; [3 x 2 x 4] x 3. This gave an overall total of 72 twig cuttings comprising of 18 segments from each mangrove species.

Isolation and purification of fungal endophytes

All 72 twig cuttings were taken to the laboratory where tissue segments were washed in running water to remove soil and debris. Mangrove fungal endophytes were isolated according to Arnold (2001) with a few modifications. The twig cuttings were surface sterilized with 1 % sodium hypochlorite for 30 seconds instead of 0.525 % for 2 minutes. Traces of sodium hypochlorite were removed and then rinsed in sterile distilled water before treatment with 90 % for 30 seconds instead of 70 % ethanol for 2 minutes according to Arnold (2001). The outer tissues from the twigs were removed after drying the plant tissues under sterile laminar air flow and passing through a flame for 30 seconds. The internal tissues were then cut into smaller pieces of between 0.5 and 1 cm and inoculated on Potato Dextrose Agar (PDA) plates to capture a broad spectrum of the fungal community. The plates were later incubated at 28 ± 1°C for 5 days (Saunders, 2010; Bijaya, 2015; Prabukumar, 2015). Hyphal tips of fungi, emerging out of the mangrove fungal endophytes were then isolated and sub-cultured on PDA at an optimum temperature of 28 ± 1°C and a pure culture of each isolate obtained (Xing *et al*., 2011).

Morphological and microscopic identification of the fungal isolates

Identification of the mangrove fungal endophytes was based on macroscopic morphological features such as fungal colony characteristics, fungal growth, colony color (front and reverse), characteristics of the spores and discernible vegetative features on the PDA plate. Microscopic slides of each endophyte were prepared by placing a drop of lacto phenol- cotton-blue on a clean slide. A small tuft with spores and spore bearing structures of the fungus was picked using a sterilized inoculation needle and placed onto the drop. The stain was mixed with the mold structures and a cover glass was placed over the preparation. The slides were observed under a light microscope (Primostar) at x40 and x100 with oil emulsion. Microscopic features of the endophytes were photographed and processed by an Axiocam ER 5s camera linked to a Zen blue 71 software. Features included conidial development, shape of conidia and conidial head, size of and attachment of conidia (Barnett and Hunter, 1998; Huang *et al*., 2008; Xing *et al*., 2011).

Determination of colonization rate, isolation rate and relative frequency

Colonization rate (CR) was calculated as the total number of segments colonized by endophytic fungi divided by the total number of segments incubated for a given plant sample and expressed in percentage. This compares the degree of infection by endophytic fungi between plant tissues. Isolation rate (IR) is a measure of fungal richness in a sample of plant tissue and the incidence of plant infections per segment. It was calculated as the total number of segments incubated from the twig cuttings of a given mangrove species divided by the endophytic fungi isolated from the segments. This gave an indication of the fungal richness per sample of plant material. Fungal density or relative frequency (RF) was calculated as the number of isolates of one fungal genus divided by the total number of isolates and expressed in percentage (Huang *et al*., 2008; Liu *et al*., 2019).

Data analysis

Determination of fungal isolate diversity

Diversity of fungal isolates (taxa richness) contained in each selected mangrove species and the relative abundance of the fungal isolates were determined. The Shannon Wiener diversity index (*H'*) was used as a measure reflecting the number of different isolates and how evenly the individuals are distributed among the species of the selected mangroves (Pielou, 1977). The degree of community similarity of endophytic fungi between the individual selected mangrove species was determined by employing Sorenson's Coefficient (CC) (Magurran, 2004).

Results

Isolation of fungal endophytes

Cultural identification of endophytic fungi isolates resulted in a total of 50 mangrove endophytic fungal isolates from the 72 twig cuttings of *R. mucronata*, *S. alba, A. marina*, and *C. tagal*. Due to the large number of the isolated fungi, selection was made based on good growth characteristics, color and margin characteristics. The resulting fungal endophytes were classified into 4 groups according to their host mangrove species (Fig. 2). The isolated fungal endophytes demonstrated a brown color with a white margin and different shades of brown, green and white.

Morphological characterization of mangrove fungal endophytes

A total of 19 endophytic fungal isolates were selected for further morphological characterization based on their colony morphology, microscopic and macroscopic features (Table 1) (Fig. 6-10). Sporulated cultures were examined for colony color, conidial size, shape and development. A total of 18 isolates were successfully identified morphologically into 5 mangrove endophytic fungal genera namely; *Aspergillus*, *Penicillium*, *Fusarium*, *Cephalosporium* and *Blastomyces* (Fig. 4). One of the isolates was not fully characterized because its conidia did not grow in PDA media. The dominant fungal genus identified in this study was *Aspergillus* (55 %). This was followed by *Penicillium* (22 %) and *Fusarium* at 11 %. The least dominant were *Cephalosporium* and *Blastomyces* each recording an abundance of 5.5 %. *Aspergillus* was found in all the 3 study sites of Gazi Bay, Tudor and Mida creek and in all the selected mangrove species of *A. marina, C. tagal, R. mucronata* and *S. alba.* The genus *Penicillium*, the second most abundant, was distributed in *A. marina* from the 3 study sites and in *S. alba* from Tudor Creek. The 2 isolates of *Fusarium* were both found in Tudor Creek in *S. alba* and *C. tagal,* respectively. *Cephalosporium* isolate 1 and *Blastomyces* isolate 1 were found in *S. alba* as well as in *R. mucronata* of Tudor Creek. A total of 4 out of 5 fungal genera were found in Tudor Creek alone, namely *Aspergillus*, *Penicillium*, *Fusarium* and *Cephalosporium*. Gazi recorded 2 fungal genera, *Aspergillus* and

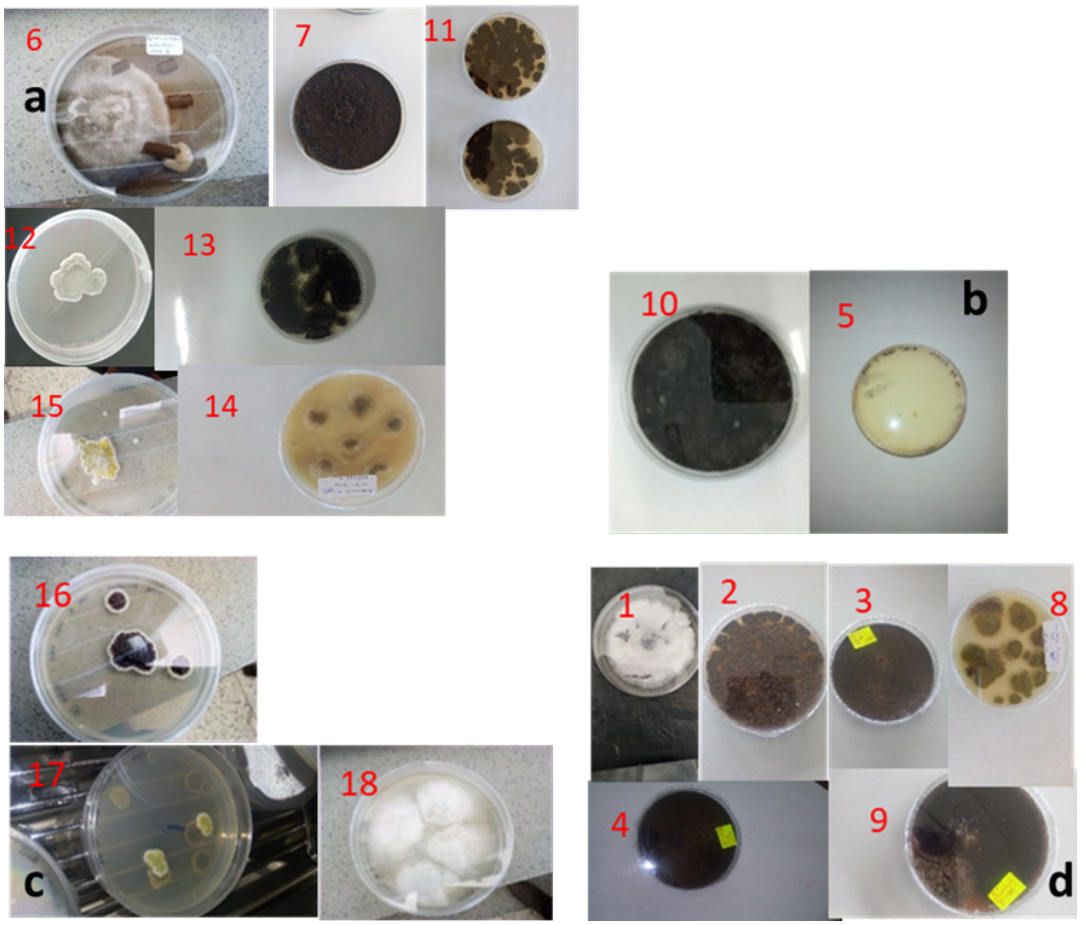

Figure 2. A section of the culturally identified mangrove fungal endophytes from the twig cuttings of selected mangrove species of coastal Kenya. **(a)** *Avicenia marina*, **(b)** *Ceriops tagal*, **(c)** *Rhizophora mucronata*, **(d)** *Sonneratia alba*.

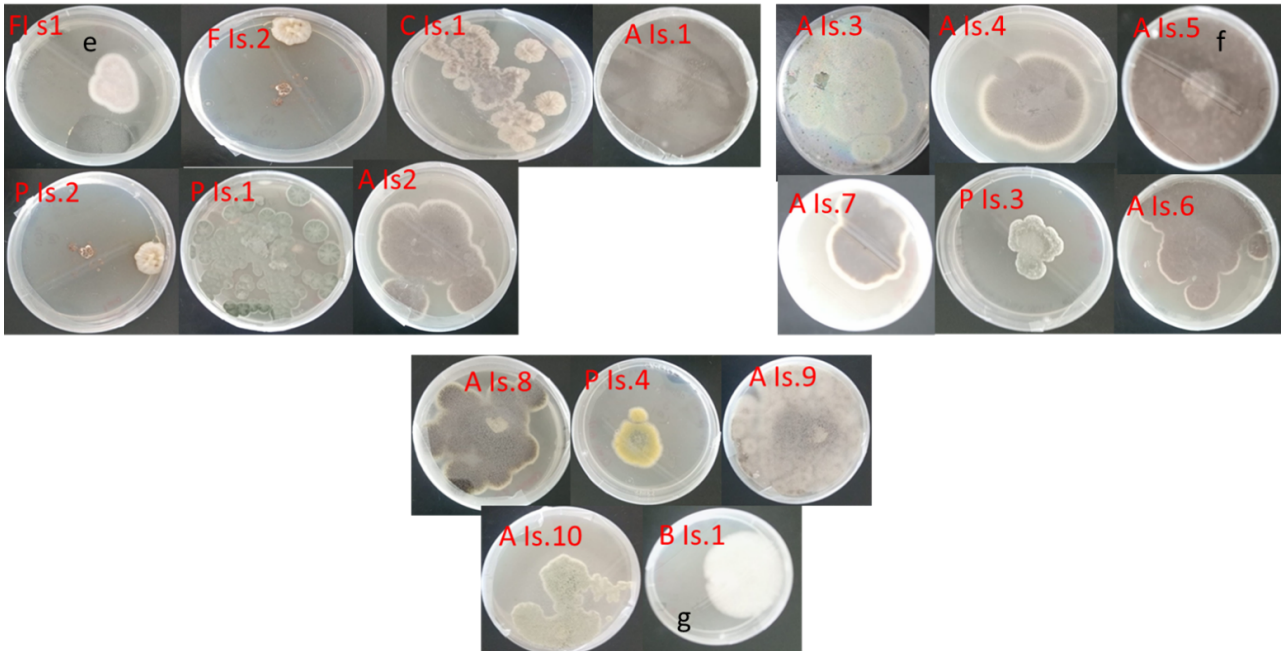

Figure 3. Spatial distribution of mangrove endophytic fungal genera in (**e**) Tudor Creek, (**f**) Gazi Bay, and (**g**) Mida Creek.

Table 1. Morphological characteristics of fungal mangrove endophytes isolated from selected study sites in coastal Kenya.

S/No	Fungal genera	Macroscopic features	Microscopic features	Reverse side
1	*Aspergillus*	Fast growing brown colony with a white margin a powdery texture	Have regular mycelium and hyphae that are septate, Conidiophores are erect and terminate in a vesicle. The vesicle and conidia form the conidial head	Cream
2	*Penicillium*	Grow moderately fast, colony green and velvet like	Hyphae are septate, hyaline simple conidiophores. Phialides are branched in brush like clusters at the tips of conidiophores. Conidia are round and unicellular	Yellow
3	*Fusarium*	White colony filled the plate with aerial mycelia	Hyaline, septate hyphae leading to branched conidiaphore with both macro and microconidia. Microconidia are sickle-shaped and produced from phialides of branched conidiophores	White
4	*Cephalosporium*	Dark grey in color and cream in reverse. Initially glabrous and shortly became felt like	Well-developed hyaline slender hyphae with unbranched conidiophores. Conidia hyaline and unicellular. Phialides are erect, unbranched, tapering and are form directly on narrow hyphae	Cream
5	*Blastomyces*	The texture is membranous and downy to woolly. Produced aerial mycelium	They have septate hyaline hyphae and unbranched short conidiophores. Conidia are hyaline and unicellular. Conidia are round and are produced perpendicular to the hyphal axis on short, thin conidiophores.	White

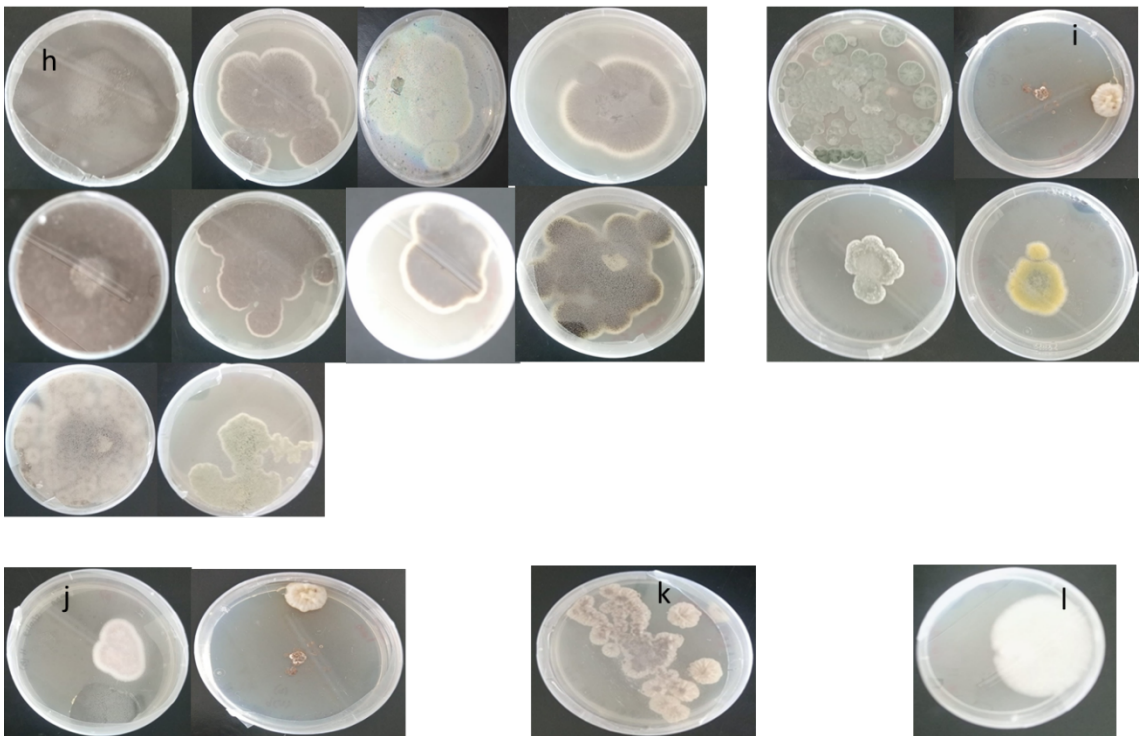

Figure 4. Morphologically identified mangrove endophytic fungal genera from selected mangrove species of coastal Kenya. (**h**) *Aspergillus*, (**i**) *Penicillium*, (**j**) *Fusarium*, (**k**) *Cephalosporium*, (**l**) *Blastomyces*.

Penicillium while Mida Creek recorded 3 fungal genera, *Aspergillus*, *Penicillium* and *Blastomyces*. The mangrove species *A. marina* hosted the largest number (7) of mangrove endophytic fungal genera while *S. alba* hosted 6 isolates, *R. mucronata* 3 isolates, and 2 isolates were found in the mangrove species *C. tagal* (Fig. 4).

Colonization and isolation rates

Endophytic CR ranged between 38.9 – 98 % for the mangrove species in this study. The highest CR was 94.4 % associated with the mangrove species *S. alba* and *C. tagal* in Tudor Creek. This was followed by *R. mucronata* (88.9 %) in Tudor Creek, *R. mucronata* (83.3 %) in Mida Creek, and *A. marina* with 72.2 % in Tudor Creek, *C. tagal* and *A. marina*, 66.7 % in Mida Creek and Gazi Bay, respectively (Fig. 5). Endophytic IR was highest in *A. marina* at 0.30 followed by *C. tagal* and *R. mucronata* each at 0.24, and lowest for *S. alba* at 0.14. Fungal diversity varied across the study sites. The highest fungal community diversity (H' = 1.35) was found in Tudor Creek followed by Mida Creek (H' = 0.95) and Gazi Bay was the least diverse (H' = 0.45).

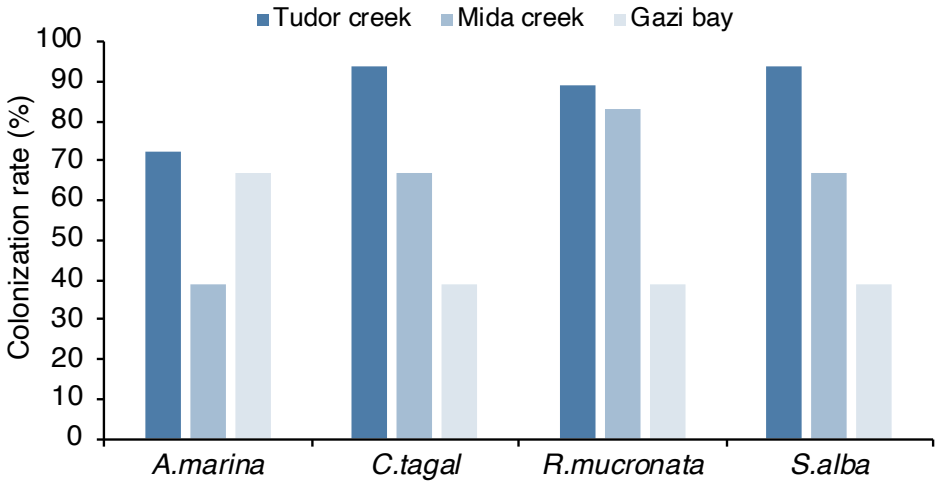

Figure 5. The colonization rates of mangrove fungal endophytes across the study sites in coastal Kenya.

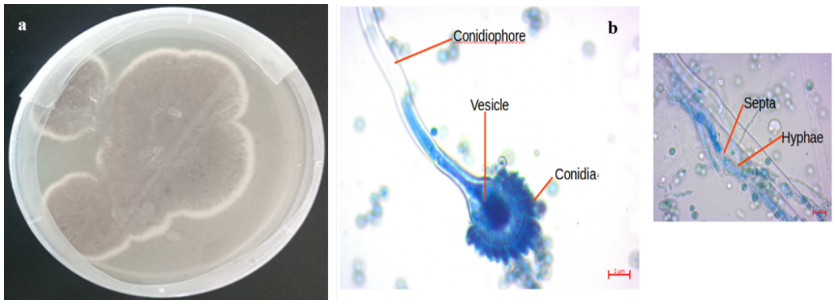

Figure 6. Colony morphology of *Aspergillus*: a) front view; and b) microscopic features of *Aspergillus* (x 100).

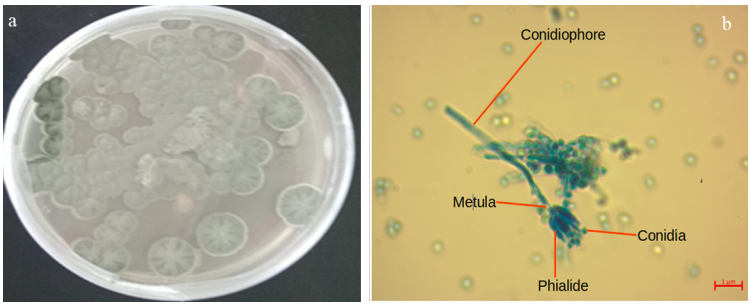

Figure 7. a) Colony morphology of *Penicillium*; b) microscopic features of *Penicillium* (x 100).

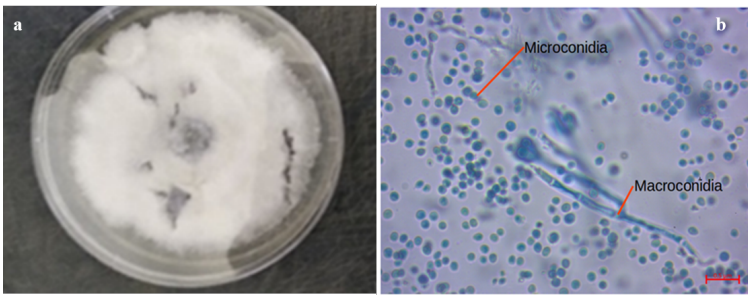

Figure 8. Colony morphology of *Fusarium*: a) front view; b) microscopic features of *Fusarium* (x 100).

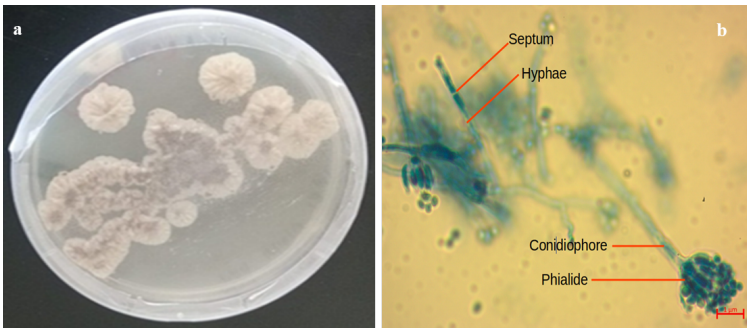

Figure 9. Colony morphology of *Cephalosporium*: a) front view; and b) microscopic features of *Cephalosporium* (x 100).

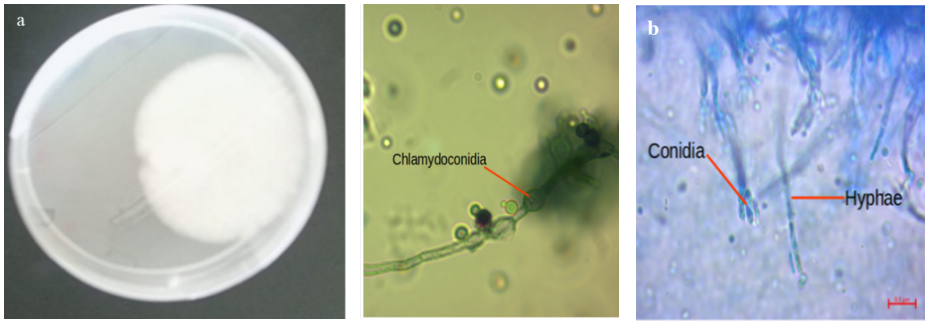

Figure 10. Colony morphology of *Blastomyces*: a) front view; and b) microscopic features of *Blastomyces* (x 100).

Fungal community similarity based on the identified genera was highest between Gazi Bay and Mida Creek (CC = 0.80) and lowest between Tudor Creek and Mida Creek (CC = 0.57).

Mangrove endophytic fungal genera found in Tudor Creek were *Aspergillus* isolate 1 and 2, *Penicillium* isolate 1 and 2, *Fusarium* isolate 1 and 2 and *Cephalosporium* isolate 1. Gazi recorded a total of 6 mangrove endophytic fungal isolates; *Aspergillus* isolate 3, 4, 5, 6 and 7 and *Penicillium* isolate 4. A total of three out of ten isolates of *Aspergillus* (8, 9 and 10), and *Blastomyces* isolate 1 were recorded in Mida Creek (Fig. 3). The isolates were clustered in 5 groups (h-l) according to color of their colonies and other morphological characteristics. Group h represents the genus *Aspergillus* sp. ranging from different forms of green, whitish brown, dark and various forms of brown with different colony morphology. Group i represents *Penicillium* genera, j *Fusarium* genera, k *Cephalosporium* genera and l *Blastomyces* genera (Fig. 4).

A summary of morpho taxonomic characteristics of fungal endophytes by study site and mangrove species showed that the brown fungal endophyte recorded the highest relative frequency (RF) of 16 % followed by green (12 %), and white and black with 10 % each. The brown fungal endophyte is found in *R. mucronata* and *S. Alba* whereas the green fungal endophyte appears more in *A. marina* of Gazi Bay and Mida Creek. A total of 2 isolates (greenish white and the yellowish green) of the green fungal endophyte was observed in *R. mucronata* of Mida Creek only. The black and the grey fungal endophytes occurred in *C. tagal* more than in any other mangrove species. Generally endophytic fungi were recorded in more than one location except the stranded brown and the yellowish green fungal endophyte that was recorded only in Tudor and Mida Creeks, respectively (Fig. 3).

Discussion

Diversity of mangrove fungal endophytes

Morphological characterization resulted in a total of 5 fungal genera, namely *Aspergillus*, *Penicillium*, *Fusarium*, *Cephalosporium* and *Blastomyces* (Fig. 4). The most abundant endophytic fungal genus was *Aspergillus*; an observation that has been commonly encountered in other endophytic studies (Prihanto *et al*., 2011; Rossiana, *et al*., 2016; Jenoh *et al*., 2019). Thorati *et al*. (2016), recorded the prominence of *Aspergillus* in a study of isolation and identification of endophytic fungi from mangrove roots of *R. apiculata, R. mucronata* and *Bruguiera gymnorrhiza* along the coast of South Andaman sea, Andaman and Nicobar Islands, India. The genus *Penicillium* (Fig. 6) was moderately abundant and had been identified previously along the Kenyan coast in the infested *S. alba* of Gazi Bay and Mida Creek (Jenoh *et al*., 2019). *Fusarium* (Fig. 7) was the third most abundant mangrove fungal endophyte genus in this study and was also observed in other endophytic fungal studies (Prihanto *et al*., 2011; Hamzah *et al*., 2018). Hamzah *et al*., (2018) identified the genus *Fusarium* in the leaves of *R. mucronata* growing in the Malaysian mangrove forest. The 2 isolates of *Fusarium* in this study were isolated from the twigs of *S. alba* and *R. mucronata* in Tudor Creek. The least encountered endophytic fungal genera were *Cephalosporium* isolate 1 and *Blastomyces* isolate 1. *Cephalosporium*, only found in *R. mucronata* of Mida Creek, is listed among fungal endophytes with the potential for producing bioactive compounds (Kumar, 2020).

Tudor Creek recorded the highest diversity of endophytic fungi and these were mostly present in *A. marina* located on the landward side of the creek. On the other hand, Mida Creek recorded the least diversity and the associated fungi came mostly from the mangrove species *C. tagal* that was located between the landward side and the seaward zone of the mangrove forest (Fig. 3). The highest number of fungal isolates from Tudor Creek was attributed to differences in environmental conditions where samples of twigs were collected towards the landward side of the creek that was more exposed to air, land and water pollution from land-based sources. Such differences in environmental conditions may include elevated levels of pollution along Tudor Creek arising from both domestic and industrial sources (Mohamed *et al*., 2008). Endophytic fungi in plant tissues are known to help the host plant adapt to both biotic and abiotic stress factors (Wang *et al*., 2014). Therefore, Tudor Creek being a highly polluted environment is the likely cause of the presence of the highest number of endophytic fungal genera. This has been cited as an adaptation strategy for marine plants in surviving stressful environments as documented by Thatoi *et al*. (2013).

Most of the mangrove fungal isolates showed colony pigmentation attributed to the production of pigments such as carotenoids, melanins, flavins, phenazins and quinones which are associated with crucial antifungal, antibacterial and herbicidal activities. Previous studies on endophytic fungi have also shown that fungal isolates produce pigmentation in response

to adverse conditions such as low moisture, pH and UV light (Hamzah *et al*., 2018). A summary of morpho-taxonomic characteristics revealed that the most frequently encountered fungal endophyte was the brown isolate identified as *Aspergillus* (Fig. 6) (Barnett and Hunter, 1998). The least common fungal endophytes were stranded brown and the yellowish green fungal isolate from Mida Creek, each with a relative frequency of 2 %. These results corroborate with findings from a study conducted in Hong Kong Island by Huang *et al*. (2008). Tudor Creek recorded the highest fungal community diversity whereas the lowest diversity was encountered at Gazi Bay. This is indicative of variations in environmental conditions in the respective study sites. Similarity in fungal community based on the identified genera was highest between Gazi Bay and Mida Creek and lowest between Tudor Creek and Mida Creek. This may be attributed to the fact that Mida Creek is located in a less polluted environment and has similarities to Gazi Bay which is a natural harbor pushing deep into the mainland coast.

Host recurrence of mangrove endophytic fungi

A phenomenon whereby an endophytic fungus frequently occurs or dominates a particular plant host or a group of plant hosts is termed 'host-recurrence'. However, the endophytic fungi can still colonize other plant hosts in the same habitat. Host selectivity occurs when endophytic fungi form relationships with two related plant species but demonstrates preference to one plant host (Huang *et al*., 2008; Nogueira-Melo *et al*., 2017). All the selected mangrove species under study were found to harbor different types of endophytic fungi, an observation that was also made on endophytic fungi from 29 traditional Chinese medicinal plants (Huang *et al*., 2008). There were variations in the kind of endophytic fungi isolated from different mangrove species. Endophytic fungi colonized the same mangrove species from different locations. The brown fungal endophyte for instance occurs in many forms and was isolated mainly from *R. mucronata* and *S. alba* and was identified as belonging to the genus *Aspergillus* (Barnett and Hunter, 1998). *Aspergillus* is a common genus and has been reported in many endophytic fungal studies (Barathidasan and Panneerselvam, 2015). The black and grey fungal endophytes identified as *Aspergillus* isolate 1, 5, 6 and 7 and isolated from *C. tagal* exhibit host-recurrence as described by Nogueira-Melo *et al*., (2017) since they are rarely observed in other mangrove species. These 4 dominant fungal isolates (brown, white, black and green) showed preference for specific mangrove species. The brown fungal isolate was mainly isolated from *A. marina* of Gazi Bay and Mida Creek whereas the white fungal endophytes were consistently common to *R. mucronata.* The white mangrove fungal isolates identified as *Blastomyces* (Figure 11 a and b) was found in *R. mucronata* of Mida Creek while 2 other white endophytic fungal isolates identified as *Fusarium* isolate 1 and 2 were isolated from *S. alba* and *C. tagal* of Tudor Creek, but are also more rarely observed in *R. mucronata* and *A. marina,* thereby exhibiting host-specificity and host-recurrence according to Huang *et al*., (2008). The other 3 endophytic fungi (stranded brown, pinkish fluffy and yellowish green) were only isolated from *S. Alba, C. tagal* and *R. mucronata* of Tudor and Mida Creeks respectively, thus exhibiting host selectivity (Nogueira-Melo *et al*., 2017).

Spatial heterogeneity of mangrove endophytic fungi

Mangrove twig cuttings were obtained from different sites, however the overall CR and IR differed slightly such that they ranged from between 38.9 % to 94.4 % and 0.14 to 0.30, respectively. This observation corroborates the study by Huang *et al*. (2008) where CR values of endophytic fungi of medicinal plants ranged from 58.3 % to 83.3 %. Differences in CR may be due to differences in structure and nutrient content of the mangrove species (Liu *et al*., 2019). Different genera of mangrove fungal endophytes showed different relative frequencies in different plant species (Huang, 2008). The fact that *S. alba* was most colonized is significant in that endophytic fungi are known to be associated with plant hosts for shelter and in turn protect the host plant against invasion by pathogens. Protection against invasion by pathogens is enhanced through the synthesis of biologically active secondary metabolites. Huang *et al*. (2008) suggested that differences in endophytic assemblages from different hosts may be related to chemical differences of the plant. The fact that *S. alba* is the most colonized may be due to its location on the seaward side where it is submerged in water during high tides. However, though the CR of *S. alba* was high, its IR was much lower because it was mainly colonized by *Aspergillus*. According to Kairo (2001) *S. alba* is found on the seaward side because it cannot tolerate wide salinity fluctuations. *A. marina* which enjoys double zonation and tolerates high salinity levels on the landward side of the intertidal zones (Kairo, 2001) had the highest number of fungal genera. A relationship exists between extreme environments and physiological adaptations of mangroves; some of which may be the colonization of

fungal endophytes which in turn produces secondary metabolites that aid host plants fight against invasion by pathogens (Jose and Christy, 2013; Ling *et al*., 2016).

Conclusion

Mangrove fungal endophytes of the Kenyan coast are diverse, depicting host recurrence and spatial heterogeneity. The most abundant and widely distributed fungal genus was *Aspergillus* with 10 isolates representing 55 % of the total fungal genera identified morphologically. Most of the fungal genera were found to be host specific despite differences in location and hence exhibiting host-specificity. The dominance of endophytic fungal genera, diversity and similarity coefficients of mangrove fungal endophytes differed across the study sites. The colonization of Kenyan mangrove species by fungal endophytes coupled with the high diversity indicate that these endophytes can further be explored as sources of novel bioactive molecules contributing to the blue economy. This further emphasizes the need for continued conservation of mangroves along the Kenya coast.

Most fungal endophytes identified in this study grew well in PDA and poorly in PDB, therefore posing a challenge in the examination of their crude extracts since the study was method specific. It is recommended that other types of media and broths be explored to overcome this challenge leading to further investigation of a wider range of mangrove fungal endophytes in coastal Kenya which are largely underexplored. It is proposed that confirmation of the 18 fungal isolates be subjected subjected to DNA extraction, amplification, sequencing and analysis for complete identification of the fungal isolates to species level. Antimicrobial properties of the fungal isolates should be determined and bioactive compounds responsible for the different activities of isolated compounds purified, and structures elucidated by appropriate techniques.

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References

Arnold AE, Maynard Z, Gilbert GS (2001) Fungal endophytes in dicotyledonous neotropical trees: Patterns of abundance and diversity. Mycological Research 150 (12): 1502-1507

Barathidasan R, Panneerselvam A (2015) Antibacterial activity of endophytic fungi extracts from the mangrove plant *Avicennia marina* (Forsk) Vierh. International Journal of Advanced Research in Biological Sciences 2 (9): 145-148

Barnett HL, Hunter BB (1998) Illustrated genera of imperfect fungi, 4th ed. APS Press, St. Paul, Minnesota. pp 56-60

Bijaya KN (2015) Studies on endophytic fungal diversity from different leaf samples of *Pongamia pinnata*. International Journal of MediPharm Research 1 (2):134-138

Bosire JO, Dahdouh-Guebas F, Kairo JG, Koedam N (2003) Colonization of non-planted mangrove species into restored mangrove stands in Gazi Bay, Kenya. Aquatic Botany 76 (4): 267-279

Bosire JO, Kaino JJ, Olagoke AO, Mwihaki LM, Ogendi GM, Kairo JG, Berger U, Macharia D (2014) Mangroves in peril: Unprecedented degradation rates of Peri-urban mangroves in Kenya. Biogeosciences 11: 2623-2634

Dahdouh-Guebas F, Mathenge C, Kairo JG, Koedam N (2000) Utilization of mangrove wood products around Mida Creek (Kenya) among subsistence and commercial users. Economic Botany 54 (4): 513-527

Dar FA, Rather SA, Mushtaq S, Qazi PH (2015) Purification and characterization of endophytic fungal strains from four different high value medicinal plants of Kashmir valley. International Journal of Photochemistry 5 (1): 8-11

Hamzah TNT, Lee SY, Hadiyat A, Terhem A, Hanum IF, Mohamed I (2018) Diversity and characterization of endophytic fungi isolated from the tropical mangrove species, *Rhizophora mucronata,* and identification of potential antagonists against the soil-borne fungus, *Fusarium solani*. Frontiers in Microbiology 9 (1707): 1-17

Huang WY, Cai YZ, Hyde KD, Corke H, Sun M (2008) Biodiversity of endophytic fungi associated with 29 Chinese medicinal plants. Fungal diversity 33: 61-75

Jenoh EM, de Villiers EP, de Villiers SM, Okoth S, Jefwa J, Kioko E (2019). Infestation mechanisms of two woodborer species in the mangrove *Sonneratia alba* J Smith, in Kenya and co-occurring endophytic fungi. PLoS ONE 14 (10): 1-20

Jose AC, Christy PH (2013) Assessment of antimicrobial potential of endophytic bacteria isolated from *Rhizophora mucronata*. International Journal of Current Microbiology and Applied Science 2 (10): 88-194

Kairo JG, Dahdouh-Guebas F, Bosire J, Koedam N (2001) Restoration and management of mangrove systems: a lesson for and from the East African region. South African Journal of Botany 67: 383–389

Kumar R (2020) Microbial endophytes: Endophytic fungi from medicinal plants; Biodiversity and biotechnological applications, Woodhead Publishers. Elsevier. pp 1-122

Ling M, Onn Lim, Teen P, Aazani M, Peter P, Moritz M (2016) Initial screening of mangrove endophytic fungi for antimicrobial compounds and heavy metal bio-sorption potential. Sains Malaysiana 45 (7): 1063-1071

Liu ZZ, Chen Y, Lian B, Zhang Z, Zhao YY, Ji ZH, Lv YN, Li HG (2019) Comparative study of population ecological distribution and extracellular enzyme activities of endophytic fungi in *Artemisia annua*. Journal of Biosciences and Medicines 7: 94-105

Magurran AE (2004) Measuring biological diversity. Wiley-Blackwell, Oxford (UK). pp 19-20

Mohamed MOS, Neukerman G, Kairo GM, Dahdouh-Guebas F, Koedam N (2008) Mangrove forests in a peri urban setting: the case of Mombasa, Kenya. Wetlands Ecology Management (2009) 17: 243-255

Nogueira-Melo GS, Santos PJP, Gibertoni TB (2017) Host-exclusivity and host-recurrence of wood fungi (Basidiomycota-Agaricomycetes) in Brazilian mangroves. Acta Botanica Brasilica (31) 4: 566-570

Owuor MA, Icely J, Newton A, Nyunja J, Otieno P, Tuda AO, Oduor N (2017) Mapping of ecosystem services flow in Mida Creek, Kenya. Ocean & Coastal Management 140: 11-21

Pielou E (1977) Ecological diversity. John Wiley & Sons. New York. viii + 165 pp

Prabukumar S, Rajkuberan C, Ravindran K, Sivaramakrishna S (2015) Isolation and characterization of endophytic fungi from medicinal plant *Crescentia cujete* L. and their antibacterial, antioxidant and anticancer properties. International Journal of Pharmacy and Pharmaceutical Sciences 7 (11): 316-331

Prihanto AA, Firdaus M, Nurdiani R (2011) Endophytic fungi isolated from mangrove (*Rhizophora mucronata*) and its antibacterial activity on *Staphylococcus aureus* and *Escherichia coli*. Journal of Food Science and Engineering 1: 237-243

Rossiana N, Miranti M, Rahmawati R (2016) Antibacterial activities from endophytic fungi from mangrove plants *Rhizophora apiculata* L. and *Bruguiera Gymnorrhiza* (L.) Lamk. on *Salmonella typhii* AIP Conference Proceedings1729: 020074

Saunders M (2010) Biotic filters in fungal endophyte community assembly, a thesis. Graduate Department of Ecology and Evolutionary Biology, University of Toronto. pp 19-23

Saryono, Hendris S, Fitriyah D, Jose C, Titania TN, Ardhi A (2015) Antimicrobial activity and molecular characterization of endophytic fungi strain isolated from *Dahlia variabilis*. Journal of Chemical and Pharmaceutical Research 7 (9S): 201-208

Sonia v der S (2006) Studies in the chemistry of fungal natural products. PhD Thesis, University of Canterbury. pp 27-30

Thorati M, Mishra JK, Kumar S (2015) Isolation, identification of endophytic fungi from mangrove roots along the coast of South Andaman Sea, Andaman and Nicobar Islands, India. Journal of Marine Biology and Oceanography 5 (2): 1-5

Thatoi H, Behera BC, Mishra RR (2013) Ecological role and biotechnological potential of mangrove fungi: a review. Mycology 4 (1): 54-71

UNEP-Nairobi Convention/USAID/WIOMSA (2020) Guidelines on mangrove ecosystem restoration for the Western Indian Ocean region. UNEP, Nairobi. 71 pp

Wang J, Weijia D, Ruimin W, Yipeng D, Huanliang L, Xuehua K, Chunyuan L (2014) Identification and bioactivity of compounds from the mangrove endophytic fungus *Alternaria* sp. Marine Drugs 13: 4492-4504

Xing XK, Chen J, Xu MJ, Lin WH, Guo SX (2011) Fungal endophytes associated with *Sonneratia* (Sonneratiaceae) mangrove plants on the south coast of China. Plant Pathology 41: 334-340