REPORT ON FUNGI ASSOCIATED WITH CORAL SKELETON FROM SAINT MARTIN'S ISLAND, BANGLADESH

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ABSTRACT

A total of 9 fungal species belonging to four genera were found to be associated with coral skeleton collected from Saint Martin's Island, Bangladesh. The isolated fungi were *Arthrinium* Kunze, *Aspergillus flavus* Link, *Fusarium nivale* (Fr.) Sorauer, *F. oxysporum* Schltdl., *F. sambucinum* Fuckel, *F. semitectum* Berk. & Ravenel, *F. stoveri* Booth, *F. trichothecioides* Wollenw and *Penicillium* Link. The genus *Fusarium* was predominant among the associated fungi. This is the first report of fungal association with solid calcium carbonate substrate like coral skeleton from Bangladesh. *Fusarium sambucinum* and *F. stoveri* are first time recorded from Bangladesh.

KEYWORDS: Coral skeleton; Fungal ecology; Morphology; Identification; Saint Martin's Island.

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Introduction

Bangladesh is blessed with Saint Martin's Island which is the only island in the northeastern part of the Bay of Bengal where corals are grown exclusively. Coral reefs are the world's most spectacular and biologically diversified ecosystem in the marine realm providing a plethora of micro-habitats to support enormous biodiversity (Buhl and Mortensen, 2004; Thrush and Dayton, 2002; Tsounis *et al.*, 2006). Fungal association and their unraveling diversity in corals have received a lot of attention from research community nowadays. Many fungi that are found in the sea are also found in terrestrial environments, indicating the remarkably effective adaptive capabilities within the fungal kingdom (Amend *et al.*, 2019). Several researchers have reported number of fungi to be associated with corals including *Aspergillus sydowii*, *A. restrictus*, *Penicillium* sp., *Trichoderma* sp., *Xylaria* sp., *Chaetomium* sp., *Acremonium* sp., *Cladosporium sphaerospermum*, *Bipolaris rostrata*, *Fusarium* sp., *Labyrinthula* sp., *Alternaria* sp., *Aureobasidium pullulans*, *Phoma* sp. and so on (Kendrick *et al.*, 1982; Smith *et al.*, 1996; Koh *et al.*, 2000; Ravindran *et al.*, 2001; Yarden *et al.*, 2007; Soler-Hurtado *et al.*, 2016; Barrero-Canosa *et al.*, 2013). Some of these fungi were pathogenic causing severe diseases to corals including aspergillosis (Smith *et al.* 1996; Soler-Hurtado *et al.*, 2016), while most of them were found to be associated with corals (Kendrick *et al.*, 1982; Koh *et al.*, 2000). Presence of several fungi have been conformed in bleached and diseased corals having possible function in degradation or decomposition (Ravindran *et al.*, 2001; Yarden *et al.*, 2007). Apart from those reports, there might be a good number of fungi present in corals without being undetected. Being the most abundant and diverse eukaryotic microorganisms on earth, a substantial part of the fungal species diversity, habitat and ecological role remain to be discovered and characterized. Fungi of different types and habitat were studied in different times but fungal association particularly with carbonate structures like coral skeleton has not investigated yet from Bangladesh. Hence, the present study was undertaken to characterize mycoflora that are associated with dead coral skeleton collected from Saint Martin's Island, Bangladesh.

Materials and Methods

The sample was collected from Saint Martin's island in February 2017 (Fig. 1A-B) and stored in 4 °C refrigerator for further analysis. Fungi associated with the different grooves of the coral skeleton were isolated following "Tissue planting method" on potato dextrose agar (PDA) medium (CAB, 1968). The microscopic structural characters of the isolated fungi were recorded with a digital camera (Nikon optiphot- 2 trinocular microscope, Japan). Micro-morphological structures of the fungi were drawn in detail with the help of Camera Lucida. Detailed morphological and micro-morphological studies of the isolated fungi were done in order to determine their identity. Identities of the fungi were determined following standard literatures (Thom and Raper, 1945; Booth, 1971; Barnett and Hunter, 1972; Ellis, 1971, 1976). All specimens included in the present study were preserved in Mycology and Plant Pathology laboratory, Department of Botany, University of Dhaka, Bangladesh.

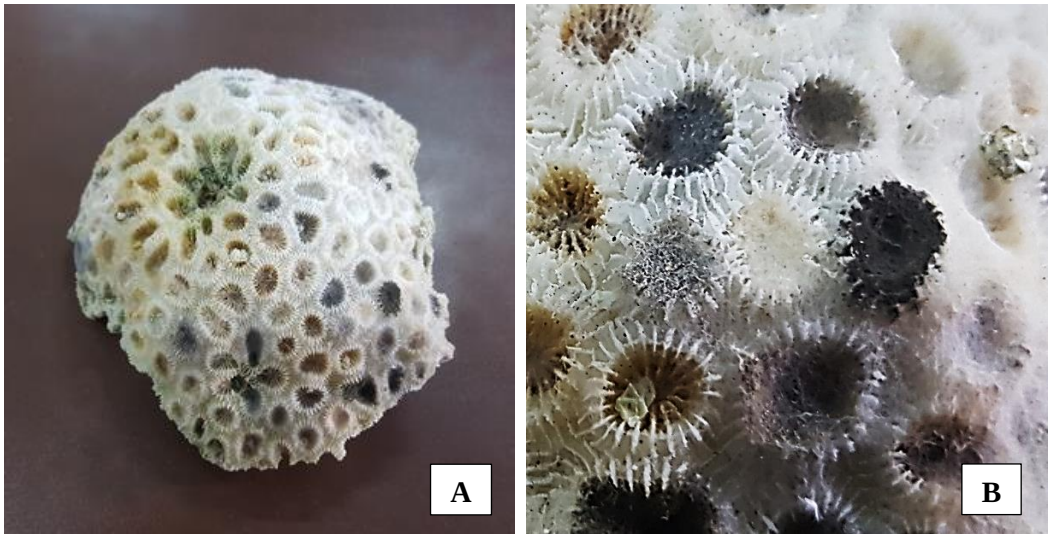

Figure 1. A-B. Coral skeleton showing colonization of different fungi in different grooves.

Results and Discussion

A total of 9 fungal species representing four different genera were identified from the studied coral skeleton. Associated fungal taxa were *Arthrinium* Kunze, *Aspergillus flavus* Link, *Fusarium nivale* (Fr.) Sorauer, *F. oxysporum* Schltdl., *F. sambucinum* Fuckel, *F. semitectum* Berk. & Ravenel, *F. stoveri* Booth, *F. trichothecioides* Wollenw and *Penicillium* Link. Abundance of the genus *Fusarium* was high among the identified fungi.

Taxonomic descriptions of fungal taxa associated with coral skeleton are as follows:

Arthrinium Kunze, Mykol. Hefte **1**: 9 (1817) (**Fig. 2A**)
Colonies rapidly growing, dark brown to black, cottony, reverse blackish. Hyphae were hyaline and septate and bear pale, short or elongated conidiogenous cells. Setae and hyphopodia absent. Conidia formed in clusters, dark brown, lentil shaped, aseptate, 3–4 µm in diameter.
Specimen examined: Isolated from dead coral skeleton collected from Saint Martin's island, MAA Noman 02, 24 July 2017.

Aspergillus flavus Link, Magazin der Gesellschaft Naturf. Freunde Berlin **3**(1): 16 (1809) (**Fig. 2B**)
Colonies dark yellow green. Conidiophores hyaline, coarsely roughened, up to 1–2 mm in length. Vesicles globose to subglobose, 20–35 µm in diameter. Conidia globose to sub-globose, pale green, 3–4 µm in diameter.
Specimen examined: Isolated from dead coral skeleton collected from Saint Martin's Island, MAA Noman 03, 24 July 2017.

Fusarium nivale (Fr.) Sorauer, Zeitschrift für Pflanzenkrankheiten **11**: 220 (1901) (**Fig. 2C**)
Colonies white to pale peach apricot, reverse light pink. Mycelium sparse to densely floccose or felted. Chlamydospores absent. Conidia hyaline, curved a bit, broadly falcate with a pointed apex and flattened, 1–3 septate, 16–20 × 3–6 µm.
Specimen examined: Isolated from dead coral skeleton collected from Saint Martin's Island, S Hosen 29, 24 July 2017.

Fusarium oxysporum Schltdl.: Fr., emend. Snyder & Hansen, Am. J. Bot. **27**: 6067 (1940) (**Fig. 2D**)
Aerial mycelium white, reverse hyaline to purple, rapidly growing. Macroconidia borne on more elaborately branched conidiophores. Chlamydospores absent. Conidia fusiform, thin walled, slightly curved, 3-5 septate, pointed at the tip, 27–45 × 3-5 µm.
Specimen examined: Isolated from dead coral skeleton collected from Saint Martin's Island, S Hosen 30, 24 July 2017.

Fusarium sambucinum Fuckel, Fungi Rhenani Exsiccati Fasc. **3**: 211 (1863) (**Fig. 2E**)
Aerial mycelium white, floccose and tinged with rose. Ascospores fusiform to ellipsoid, sometimes curved, slightly constricted at the three transverse septa, 22–29 × 6–7 µm. Chlamydospores formed as single globose cell or in chain, 10–12 µm in diameter.
Specimen examined: Isolated from dead coral skeleton collected from Saint Martin's Island, S Shamsi 3095, 24 July 2017.

Fusarium semitectum Berk. & Ravenel, Grevillea **3:** 98 (1875) (**Fig. 2F**)
Cultures initially white but gradually became brownish. Aerial mycelium floccose. Macroconidia formed in the aerial mycelium from loosely branched conidiophores, 0–5 septate, curved, pointed at the tip, 15–22 × 3–6 µm. Chlamydospores not seen.
Specimen examined: Isolated from dead coral skeleton collected from Saint Martin's Island, S Hosen 31, 24 July 2017.
Fusarium stoveri C. Booth, The Genus Fusarium: 37 (1971) **(Fig. 2G)**

Colony surface initially white but gradually turned orange. No true microconidia developed, macroconidia not seen, numerous ascospores formed. Ascospores hyaline, clavate to ellipsoid with 3 transverse septa and measure 20–35 × 6–8 µm.

Specimen examined: Isolated from dead coral skeleton collected from Saint Martin's Island, S Shamsi 3096, 24 July 2017.

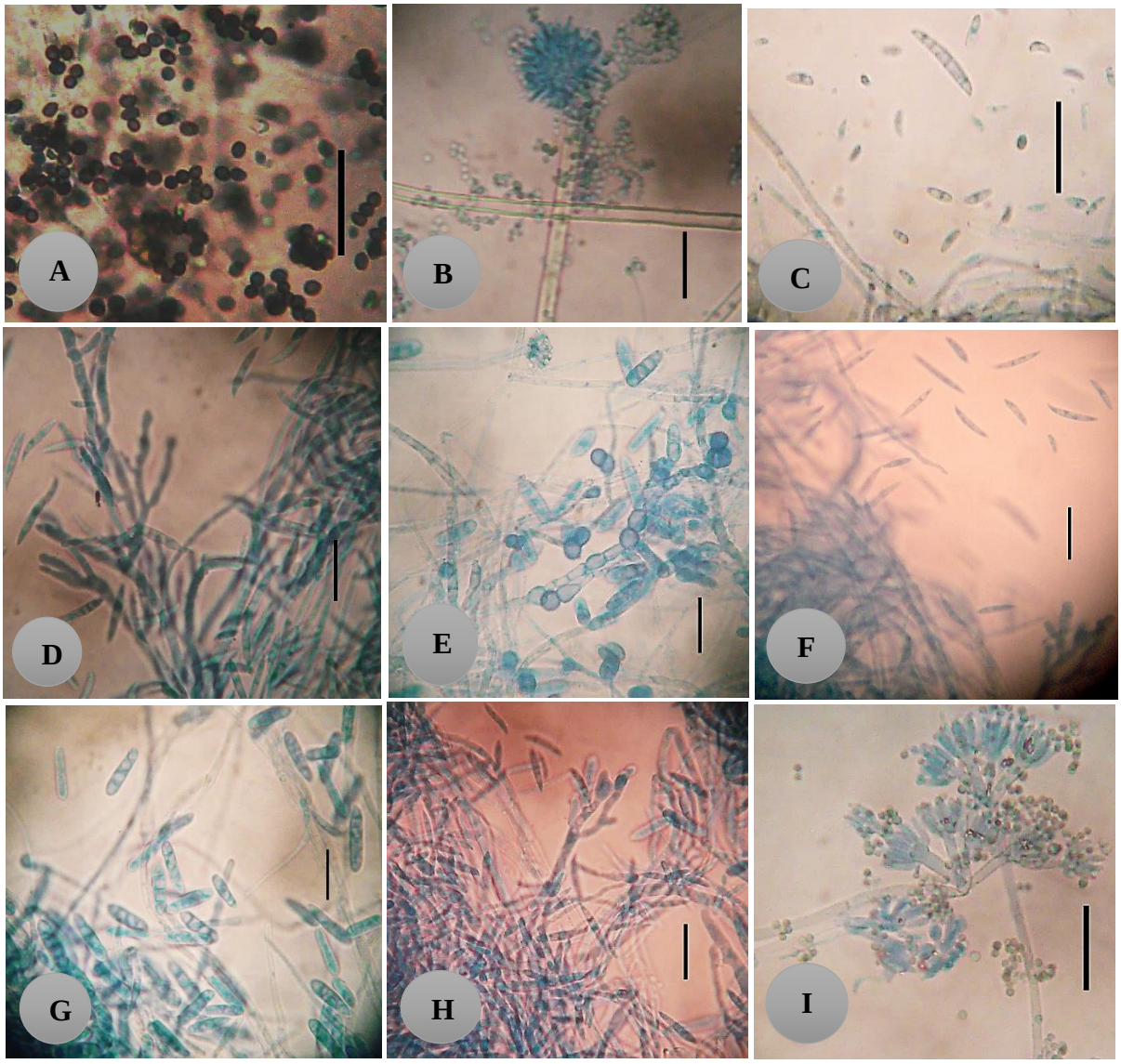

Figure 2. Microphotographs of the fungi associated with coral skeleton. A. Mycelia and conidia of *Arthrinium* sp., B. Conidiophore, vesicle and conidia of *Aspergillus flavus*, C. Mycelia with micro and macroconidia of *F. nivale*, D. Mycelia, phialides and conidia of *Fusarium oxysporum*, E. Mycelia, chlamydospores and conidia of *F. sambucinum* , F. Mycelia, phialides and conidia of *F. semitectum*, G. Mycelia and ascospores of *F. stoveri*, H. Mycelia, phialides and conidia of *F. trichothecioides* and I. Conidiophores, sterigmata and conidia of *Penicillium* sp. (Bar = 50 µm).

Fusarium trichothecioides Wollenw, J. Washington Aca. Sci. **2**: 147 (1912) (**Fig. 2H**)

Aerial mycelium floccose, white to pale pinkish. Microconidia were not found. Macroconidia formed sparsely from lateral conidiophores on aerial hyphae, 3–5 septate, 21– 26 × 3–4 µm. *Specimen examined:* Isolated from dead coral skeleton collected from Saint Martin's Island, S Shamsi 3097, 24 July 2017.

Penicillium Link, Magazin der Gesell. Naturf. Freunde Berlin **3**(1): 16 (1809) (**Fig. 2I**)

Colony small, greenish with white margin, reverse creamy. Hyphae hyaline, septate. Conidiophores hyaline, branched. Phialides formed brush-like clusters at the ends of the conidiophores. Conidia unicellular, greenish, smooth walled, globose to sub-globose, 3–4 µm in diameter.

Specimen examined: Isolated from dead coral skeleton collected from Saint Martin's island, MAA Noman 04, 24 July 2017.

Till now, a diverse array of fungi was reported to be associated with corals among which some were pathogenic while most of which were saprotrophic (Kendrick *et al.*, 1982; Koh *et al.*, 2000; Yarden *et al.*, 2007; Barrero-Canosa *et al.*, 2013). Soler-Hurtado *et al.* (2016) reported 17 fungal species that are potentially pathogenic to corals including *Aspergillus sydowii* which is responsible for aspergillosis in corals. Barrero-Canosa *et al.* (2013) identified 13 fungal genera including *Aspergillus* sp., *Penicillium* sp., *Xylaria* sp. *and Chaetomium* sp. while studying gorgonian corals. Kendrick *et al.* (1982) isolated several bioeroding fungal species *viz.*, *Acremonium* sp., *Aspergillus* spp., *Penicillium* spp., *Cladosporium* sp. and some common terrestrial fungi including *Aspergillus sydowii*, *Aspergillus versicolor*, *Cladosporium* sp., *Bipolaris rostrata* and *Penicillium restrictum* from live corals. Ravindran *et al.* (2001) also isolated common terrestrial fungi including *Acremonium* sp., *Fusarium* sp., *Aspergillus* sp., *Cladosporium* sp., *Chaetomium* sp. and *Aureobasidium* sp. from healthy, bleached and dead corals. Moreover, several ascomycetous fungi including *Fusarium* sp., *Phoma* sp., *Alternaria* sp., *Cladosporium* sp. and *Penicillium citrinum* were reported from healthy and diseased branched corals by Yarden *et al.* (2007). Paulino *et al.* (2019) reported 50 fungal isolates from four different corals (*viz.*, *Favia gravida*, *Palythoa caribaeorum*, *Palythoa variabilis* and *Zoanthus sociatus*) of which *Aspergillus*, *Penicillium*, *Trichoderma* and *Cladosporium* were most frequently isolated fungal genus from northeast part of Brazil. From the previous reports, it is evident that common terrestrial fungi have found their home in corals.

In the present study, nine fungal species belonging to four different genera were identified all of which are predominantly terrestrial fungi. Whether these associated fungi have any role in coral ecosystem or in nutrient cycling might be an area for further investigation.

A comprehensive overview of literature expressed that *F. sambucinum* and *F. stoveri* has not been reported in any relevant literature of Bangladesh (Talukder 1974, Siddiqui *et al.*, 2007; Kibria *et al.*, 2016; Shamsi *et al.*, 2017, 2018, 2019). Hence, *F. sambucinum* and *F. stoveri* are reported here as a new fungal record for Bangladesh.

In conclusion, this study investigated fungal association with coral skeleton and tried to bring forth fungal taxa that can grow on solid calcium carbonate substrate. The outcome of this study will provide additional knowledge on fungal ecology and their habitat.

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