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Molecular Identification of Filamentous Fungi Diversity in North Coast Beaches Sands of Puerto Rico

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Abstract:

The Northern region has a great variety of beaches with diverse microbial characteristics. Beach sands receive direct contamination from the garbage generated by people, which serves as nutrient for fungi growth. The objectives of this investigation were to assess the filamentous fungi diversity of four popular beaches; identify the genus and species; and identify the taxonomic relationship between the most abundant fungi. The beaches studied are located in the towns of Vega Baja, Manatí, Barceloneta and Arecibo. One sample of dry sand per month from three equidistant points was acquired every month for a year in each beach. The samples were homogenized according to dry (December-April) and humid (May-November) seasons, for a total of four composite samples per season. The DNA of each sample was isolated and quantified; and, upon sequencing, evaluated by metagenomics analysis with MG-RAST. There were 104 fungi species identified by DNA sequencing analysis. The most abundant were: *Aspergillus penicillioides*, *Aspergillus terreus*, *Microascus sp.*, *Arthrographis kalrae*, *Paramicrosporidium sp.*, *Dokmaia sp.*, *Glomastix polychroma* and *Aspergillus sp.* The taxonomic analysis demonstrated that there is no relationship in the genus of the most abundant species. As significant finding, 66 species of new registries were identified, including *Malassezia restricta*, *Arthrographis eremomyces*, and *Cephalophora tropica*. Not only were many of the species pathogenic, several genera of filamentous fungi have been previously isolated from patients in nasal culture, and can cause eye, respiratory and skin disease. The majority of these fungi use direct contact and air transport as transmission vehicle to the host.

Keywords: PCR, DNA, pathogen, beach, sand, filamentous fungi, soil fungi.

SUPPLEMENTARY MATERIALS

Table S1. Total species by samples of each season by beach.

Simple count of species								
Species	Caza Pesca (w)	Caza pesca (d)	Marbella (w)	Marbella (d)	Criolla (d)	Criolla (w)	Tubos (d)	Tubos (w)
<i>Cephalophora tropica</i>	0	0	0	0	387	0	0	0
<i>Lecanicillium saksenae</i>	0	0	0	0	0	0	0	598
<i>Microascus trigonosporus</i>	0	138	0	0	0	0	0	0
<i>Penicillium citrinum</i>	0	22	12	0	236	0	0	0
<i>Microdochium sp.</i>	0	191	0	78	0	0	0	0
<i>Aspergillus nidulans</i>	0	0	68	0	0	0	0	0
<i>Auricularia polytricha</i>	0	438	0	0	0	0	0	0
<i>Cladosporium cladosporioides</i>	0	569	9	0	0	0	0	0
<i>Oedogoniomyces sp.</i>	0	11	0	0	0	0	0	0
<i>Dactylellina lobata</i>	0	0	0	149	0	0	0	0
<i>Lulwoana sp.</i>	0	0	0	28	0	0	0	0
<i>Paraphaeosphaeria sp.</i>	0	274	0	0	0	0	0	0
<i>Stachybotrys sp.</i>	0	0	0	0	487	0	0	0
<i>Phlyctochytrium sp.</i>	0	1202	0	0	0	0	0	0
<i>Rhizocarpon disporum</i>	0	0	0	41	0	0	0	0
<i>Aspergillus penicillioides</i>	3675	533	23	1	229	2	24	1
<i>Strobilomyces luteolus</i>	0	0	0	0	66	0	0	0
<i>Lentinula edodes</i>	0	0	4	0	0	0	0	0
<i>Pyrenochaeta sp.</i>	0	0	0	0	0	0	0	627
<i>Aspergillus oryzae</i>	0	0	0	0	269	0	0	0
<i>Teratosphaeria sp.</i>	0	105	0	0	0	0	0	0
<i>Teratosphaeria toledana</i>	0	0	0	81	0	0	0	0
<i>Acremonium psammosporum</i>	0	0	0	0	315	0	0	0
<i>Magnaporthe grisea</i>	0	0	0	0	23	0	0	0
<i>Nigrospora sp.</i>	0	0	0	0	0	0	0	555
<i>Catenaria sp.</i>	0	0	0	0	14	0	0	0
<i>Chaetomium sp.</i>	0	0	0	0	780	0	0	0
<i>Magnaporthe salvinii</i>	0	0	0	0	205	0	0	0
<i>Pestalotiopsis sinensis</i>	0	0	0	0	421	0	0	0
<i>Coprinopsis sp.</i>	0	593	0	0	0	0	0	0
<i>Gibellulopsis nigrescens</i>	0	0	32	0	0	0	0	0
<i>Lecanicillium psalliotae</i>	1648	0	0	0	0	0	0	0
<i>Aspergillus niger</i>	0	0	26	0	88	0	0	0
<i>Mycocleptodiscus sp.</i>	0	0	0	0	0	0	0	643

<i>Sarocladium strictum</i>	0	345	0	0	0	0	0	0
<i>Aspergillus sp.</i>	0	152	0	0	0	0	0	1043
<i>Myrothecium roridum</i>	0	0	0	0	80	0	0	0
<i>Phoma sp.</i>	0	56	0	0	37	0	0	0
<i>Hygrocybe splendidissima</i>	0	72	0	0	0	0	0	0
<i>Malassezia restricta</i>	0	0	0	16	0	0	104	167
<i>Phaeosphaeria eustoma</i>	0	0	33	0	0	0	0	0
<i>Gliomastix polychroma</i>	0	0	0	0	0	0	3301	0
<i>Hortaea sp.</i>	0	0	31	0	0	0	0	0
<i>Graphium dubautiae</i>	0	177	0	0	0	0	0	0
<i>Arthrographis kalrae</i>	0	0	0	10581	0	0	0	0
<i>Geosmithia pallida</i>	0	0	0	0	0	0	196	0
<i>Sordaria fimicola</i>	0	0	0	0	411	0	0	0
<i>Aspergillus aculeatus</i>	0	315	0	0	0	0	0	0
<i>Wickerhamomyces sp.</i>	0	0	0	0	0	0	88	0
<i>Penicillium sp.</i>	0	377	0	0	0	0	0	158
<i>Tripacticalcar sp.</i>	0	0	0	9	0	0	0	0
<i>Faurelina indica</i>	0	0	0	29	0	0	0	0
<i>Sterigmatomyces elviae</i>	0	53	0	0	0	0	0	0
<i>Boletus floridanus</i>	0	0	0	0	152	0	0	0
<i>Curvularia lunata</i>	1765	0	0	0	0	0	0	0
<i>Corollospora gracilis</i>	0	0	78	0	0	0	0	0
<i>Dokmaia monthadangii</i>	0	0	0	0	0	118	0	0
<i>Dokmaia sp.</i>	0	0	0	0	0	2512	0	0
<i>Sarocladium glaucum</i>	0	0	0	0	0	0	91	0
<i>Clavulina sp.</i>	86	0	0	0	0	0	0	0
<i>Stromatonectria caraganae</i>	0	3	0	0	0	0	0	0
<i>Euoidium mutisiae</i>	8	0	0	0	0	0	0	0
<i>Paramicrosporidium fungal sp.</i>	0	0	0	0	1249	0	0	0
<i>Aspergillus terreus</i>	0	1355	46	0	469	0	0	0
<i>Chaetomium aureum</i>	0	0	0	0	21	0	0	0
<i>Periconia sp.</i>	0	0	0	0	0	0	0	92
<i>Phaeosphaeria halima</i>	0	22	0	0	28	0	0	0
<i>Aspergillus parasiticus</i>	0	0	0	0	3	0	0	0
<i>Mycogone perniciosa</i>	0	15	0	0	0	0	0	0
<i>Agaricostilbum hyphaenes</i>	0	7	0	0	0	0	0	0
<i>Stachybotrys bisbyi</i>	0	11	0	0	0	0	0	0
<i>Coniosporium sp.</i>	0	0	0	18	0	0	0	0
<i>Mycosphaerella sp.</i>	0	0	0	3	0	0	0	0
<i>Corollospora portsaidica</i>	0	0	850	45	0	0	34	0

<i>Metarhizium anisopliae</i>	0	0	0	16	0	0	0	0
<i>Hypomyces cervinigenus</i>	0	1207	0	0	0	0	0	0
<i>Corollospora maritima</i>	0	0	142	5	0	0	0	0
<i>Paxillus involutus</i>	0	0	0	0	57	0	0	0
<i>Passalora sp.</i>	0	149	0	0	0	0	0	0
<i>Chytridium lagenaria</i>	0	3	0	0	0	0	0	0
<i>Galactomyces geotrichum</i>	0	3	0	0	0	0	0	0
<i>Cladosporium sp.</i>	173	0	0	0	0	0	0	0
<i>Devries lagerstroemiae</i>	0	912	0	0	0	0	0	0
<i>Epichloe festucae</i>	0	0	0	0	352	0	0	0
<i>Cordyceps memorabilis</i>	695	0	0	0	0	0	2154	0
<i>Phaeosphaeria typharum</i>	0	189	0	0	159	0	0	0
<i>Acremonium sp.</i>	0	0	73	0	374	0	0	0
<i>Cephalosporium curtipes</i>	0	0	0	0	0	0	0	841
<i>Malassezia globosa</i>	53	0	0	0	0	0	0	0
<i>Lasiodiplodia pseudotheobromae</i>	0	0	29	0	65	0	0	0
<i>Scolecobasidium dendroides</i>	0	0	56	0	0	0	0	0
<i>Verticillium sp.</i>	0	0	0	0	786	0	0	0
<i>Sigmoidea parvula</i>	0	0	5	0	0	0	0	0
<i>Calcarisporiella thermophila</i>	0	0	0	0	37	0	0	0
<i>Laetisaria sp.</i>	0	0	0	0	12	0	0	0
<i>Stachybotrys zeae</i>	0	0	0	0	310	0	0	0
<i>Metarhizium flavoviride</i>	0	0	0	0	0	0	0	130
<i>Engyodontium album</i>	0	0	0	0	0	0	0	819
<i>Microascus sp.</i>	0	0	7101	2	0	0	0	0
<i>Strumella coryneoidea</i>	0	0	8	854	0	0	0	0
<i>Aspergillus tamarii</i>	0	0	20	0	510	0	0	0
<i>Myrothecium atroviride</i>	0	0	126	0	0	0	0	0
<i>Eremomyces langeronii</i>	0	0	0	730	0	0	0	0
(d) dry season (w) wet season								

Table S2. Summary of some pathogenic species. (Echevarría 2017).

Species	Disease
<i>Geotrichum galactomyces</i>	Geotrichosis (ingestion or inhalation) bronchial and pulmonary, buccal, vaginal and skin infections.
<i>Cladosporiodes</i>	Seasonal allergic diseases.
<i>Aspergillus terreus</i>	Pulmonary aspergillosis
<i>Cladosporium</i>	Infections in the skin and nails
<i>Acremonium</i>	Mycetoma, Onychomycosis, Hyalomycosis, keratitis.
<i>Penicillium</i>	Mycotoxins (food)
<i>Chaetomium</i>	Brain infection.
<i>Periconia</i>	Mycotic keratitis.
<i>Coniosporium</i>	Cutaneous infections.
<i>Aspergillus tamaritii</i>	Keratitis
<i>Penicillium citrina</i>	Mycotoxins Pneumonia.
<i>Microascus trigonosporus</i>	Pulmonary infections.
<i>Curvularia lunata</i>	Nails and skin.

Table S3. Summary of some species a new record and diseases (Echevarría 2017).

Species	Taxonomy ID (NCBI)	Disease
<i>Cephalophora tropica</i>	88273	It causes keratitis.
<i>Lecanicillium saksenae</i>	468837	Fungicola causes dry bubble disease in commercially cultivated mushroom.
<i>Microdochium sp</i>	1789825	Diseases in plants.
<i>Oedogoniomyces sp</i>	252682	Epiphytic algae colonize submersed and emergent plants. These are the most widely studied group of benthic algae in lakes, perhaps because of their obvious accumulation in the littoral zone.
<i>Dactylellina lobato</i>	1796066	Nematophagous fungus.
<i>Lulwoana sp</i>	575476	A dark septate endophyte in roots.
<i>Paraphaeosphaeria sp</i>	321238	The fungus is considered halotolerant above the rhizome, and is thus regularly exposed to salt spray. It is found on the Atlantic Coast of the United States.
<i>Phlyctochytrium sp</i>	355342	Marine microalgae.
<i>Rhizocarpon disporum</i>	168923	Lichenized fungus.
<i>Aspergillus penicilloides</i>	41959	Allergic rinitis.
<i>Strobilomyces luteolus</i>	1611890	Cause diseases of plants.
<i>Teratosphaeria sp</i>	488699	A serious leaf disease pathogen.
<i>Teratosphaeria toledana</i>	306885	A serious leaf disease pathogen.
<i>Acremonium psammosporum</i>	745571	Cause human superficial infections including mycetoma.
<i>Catenaria sp</i>	287	Is a widespread endoparasite, but a weak parasite.
<i>Pestalotiopsis sinensis</i>	1912845	Is second most important fungal pathogen causing grey blight disease in tea plant.
<i>Gibellulopsis nigrescens</i>	796325	Endophytic fungi.
<i>Lecanicillium psalliotae</i>	73499	Entomopathogenic fungus
<i>Mycocleptodiscus sp</i>	582818	Skin excoriations (dogs)
<i>Sarocladium strictum</i>	582818	Hialohifomycosis
<i>Hygrocybe splendissima</i>	557327	Mushroom
<i>Malassezai restricta</i>	395497	Superficial micosis.
<i>Phaeosphaeria eustoma</i>	85909	Species causing diseases of grain crops.
<i>Gliomastix polychroma</i>	1036751	It can cause opportunistic infections in humans and

		animals.
<i>Arthrographis kalrae</i>	241728	Fungus responsible for human nail infections.
<i>Geosmithia pallida</i>	1450457	Causing Foamy Bark Canker.
<i>Aspergillus aculeatus</i>	5053	Is considered to be a ubiquitous species that could be usually isolated from rotting fruits and soil.
<i>Wickerhamomyces sp</i>	1123656	Diversity of ascomycetous yeasts associated with migratory birds.
<i>Triparticalcar sp</i>	693134	Is endogenous, isolated usually from the soil. Parasite of a number of organisms and plants.
<i>Faurelina indica</i>	796324	Causing blue stain of lumber, Dutch elm disease.
<i>Sterigmatomyces elviae</i>	5616	It has worldwide distribution and is common. It has been isolated from air, soil, plants, marine and plants. Yeast species.
<i>Corollospora gracilis</i>	470010	Marine fungus.
<i>Dokmaia monthadangii</i>	91347	Phytopathogenic fungus.
<i>Dokmaia sp.,</i>	397757	Fungus endophytes.
<i>Sarocladium glaucum</i>	706495	Soil fungus.
<i>Stromatonectria caraganae</i>	4565	Pea root diseases caused by soil-borne fungal pathogens.
<i>Euoidium mutisiae</i>	366515	Pathogenic fungus in plants.
<i>Paramicrosporidium fungal sp.</i>	1493516	Infectious spores (respiratory system)
<i>Chaetomium aureum</i>	155868	Fungus endophyte.
<i>Phaeosphaeria halima,</i>	113242	Endophytic fungus and saprophyte.
<i>Mycogone pernicioso,</i>	494059	Disease known as moisture bubbles in commercial mushroom crops.
<i>Agaricostilbum hyphaenes</i>	44565	Mycoparasite fungus that has been found in the palm litter.
<i>Stachybotrys bisbyi</i>	80385	Mycotoxins Inflammation of the skin, skin reactions, pharyngitis and cough. Estaquibotriotoxicosis (horses)
<i>Corollospora portsaidica</i>	470013	Mycoviral infection to mushrooms
<i>Hypomyces cervinigenus</i>	100997	Plant pathogens.
<i>Corollospora maritima</i>	45810	Sand fungi
<i>Paxillus involutus</i>	71150	Ectomycorrhic symbiotic fungi.
<i>Chytridium lagenaria,</i>	64509	Parasite in filamentous green algae cells and plants.
<i>Devries lagerstroemia</i>	141186	Endophyte, saprophyte and pathogen.
<i>Epichloe festucae</i>	73839	Endophytic fungus.
<i>Phaeosphaeria typharum</i>	113244	Disease on leaves and stems.
<i>Malassezia globosa</i>	425265	It is found in the skin. Dandruff, <i>Pitiriasis versicolor</i> .
<i>Lasiodiplodia pseudotheobromae</i>	466954	Disease on leaves and stems.
<i>Scolecobasidium dendroides</i>	644593	Root disease.
<i>Sigmoidea parvula</i>	297362	The aquatic hyphomycete genus <i>Sigmoidea</i> , with freshwater and marine representatives.
<i>Calcarisporiella thermophila</i>	911321	Basal fungi.
<i>Laetisaria sp</i>	1008976	Plant pathogen.
<i>Stachybotrys zeae</i>	1304211	Asthma (respiratory diseases)
<i>Metarhizium flavoviride</i>	92630	Entomopathogenic fungus.
<i>Engyodontium album</i>	37998	Keratitis and endocarditis.
<i>Strumella coryneoidea</i>	201881	Plant Pathogen.
<i>Myrothecium atroviride</i>	145970	Diseases respiratory system.
<i>Eremomyces langeronii</i>	1537659	Chronic skin infections, keratitis, sinusitis and meningitis.

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