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Ectomycorrhizal fungi associated with *Coccoloba uvifera* (Polygonaceae) in coastal ecosystems of Eastern Cuba

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ABSTRACT

Introduction: *Coccoloba uvifera*, named also seagrape, establishes symbiotic relationships with many ectomycorrhizal fungi. However, in Cuba, these fungi have been little studied.

Objective: To characterize the diversity of sporocarps and ectomycorrhizae of ectomycorrhizal fungi associated with *C. uvifera* in three coastal ecosystems of Eastern Cuba.

Methods: Four samplings of sporocarps and ectomycorrhizae were carried out at three-week intervals during the rainy season, from June to September, in 2018 and 2019. Ectomycorrhizae were collected from three mature trees and 30 young individuals per tree. The samples were transferred to the Abiotic Stress Laboratory of the Center for Plant Biotechnology Studies of the University of Granma and the Laboratory of Plant Biology and Physiology of the University of French West Indies for processing and subsequent identification.

Results: Five species of ectomycorrhizal fungi were identified from sporocarps collected under *C. uvifera* in the three sampling sites (*Scleroderma bermudense*, *Russula* sp., *Cantharellus* sp., *Inocybe* sp., and *Amanita* sp.). Using internal transcribed spacer sequencing, six taxa of ectomycorrhizal fungi were identified from ectomycorrhizas of mature trees and seedlings (*S. bermudense*, two *Tuber* spp., *Tomentella* sp., *Inocybe* sp., and *Thelephora* sp.). Only *S. bermudense* coincided (similarity 99-100 %) with sporocarps. *S. bermudense* was the most frequent ectomycorrhizal fungus in the ectomycorrhizas and sporocarps collected. Mature trees and *C. uvifera* seedlings shared between 75 and 100 % of the ectomycorrhizal fungal communities, being able to form potential common ectomycorrhizal networks.

Conclusions: At the three collection sites, sporocarp weakly reflected the belowground ectomycorrhizal fungal community, ectomycorrhizal fungal diversity is quite limited, and *S. bermudense* was the only ectomycorrhizal fungus that overlapped in sporocarps and ectomycorrhizae.

Key words: seagrape; fungal diversity; fruiting body; ectomycorrhizae; ITS sequencing.

RESUMEN

Hongos ectomicorrícicos asociados con *Coccoloba uvifera* (Polygonaceae) en ecosistemas costeros del oriente cubano

Introducción: *Coccoloba uvifera,* también llamada uva de mar, establece relaciones simbióticas con varios hongos ectomicorrícicos, sin embargo, en Cuba, estos hongos han sido poco estudiados.
Objetivo: Caracterizar la diversidad de esporocarpos y ectomicorrizas de hongos ectomicorrízicos asociados a *C. uvifera* en tres ecosistemas costeros del oriente de Cuba.
Métodos: Se realizaron cuatro muestreos de esporocarpos y ectomicorrizas a intervalos de tres semanas durante la temporada de lluvias, de junio a septiembre, entre 2018 y 2019. Las ectomicorrizas se recolectaron en tres árboles maduros y en 30 individuos jóvenes por árbol. Las muestras se trasladaron al Laboratorio de Estrés Abiótico del Centro de Estudios de Biotecnología Vegetal de la Universidad de Granma y al Laboratorio de Biología y Fisiología Vegetal de la Universidad de las Antillas, para su procesamiento y posterior identificación.
Resultados: A partir de esporocarpos recolectados bajo *C. uvifera* en los tres sitios de muestreo se identificaron cinco especies de hongos ectomicorrícicos (*Scleroderma bermudense*, *Russula* sp., *Cantharellus* sp., *Inocybe* sp. y *Amanita* sp.). Utilizando la secuenciación espaciador transcrito interno se identificaron seis taxones de hongos ectomicorrícicos a partir de ectomicorrizas de árboles maduros y plántulas (*S. bermudense*, dos *Tuber* spp., *Tomentella* sp., *Inocybe* sp., y *Thelephora* sp.). Solo *S. bermudense* coincidió (similitud 99-100 %) con esporocarpos. *S. bermudense* fue el hongo ectomicorrícico más frecuente en las ectomicorrizas y los esporocarpos recolectados. Los árboles maduros y las plántulas de *C. uvifera* compartieron entre el 75 y el 100 % de las comunidades fúngicas ectomicorrícicas, pudiendo formar redes ectomicorrícicas comunes.
Conclusiones: En los tres sitios de recolección, los esporocarpos reflejaron débilmente la comunidad de hongos ectomicorrízicos subterránea, la diversidad de hongos ectomicorrízicos fue bastante limitada y *S. bermudense* fue el único hongo ectomicorrícico que se superpuso en esporocarpos y ectomicorrizas.

Palabras clave: uva de mar; diversidad fúngica; cuerpo fructífero; micorrizas; secuenciación ITS.

INTRODUCTION

Many trees form ectomycorrhizal (ECM) symbioses with diverse Basidiomycota and Ascomycota, which play a key role in many tropical forests (Bâ et al., 2012; Corrales et al., 2018), affecting tree growth and nutrient absorption as well as protection against pathogens (Smith & Read, 2008). Large areas of tropical and neotropical forests are dominated by ECM trees (Bâ et al., 2012; Corrales et al., 2018), suggesting a key role of these symbioses in the functioning of some tropical forest ecosystems. ECM surveys from tropical forests show that Africa has the highest number of confirmed ECM plant species, followed by Neotropics, including Central and South America and the Caribbean (Corrales et al., 2018). ECM host plants in the neotropical lowlands are from predominantly tropical lineages in the Fabaceae, Cistaceae, Dipterocarpaceae, Polygonaceae, and Nyctaginaceae families (Corrales et al., 2018).

In Guadeloupe and Martinique (Lesser Antilles), the ECM fungal diversity associated with the Polygonaceae *Coccoloba uvifera* L. (seagrape) was quite poor (Bâ et al., 2014; Séne et al., 2015; Séne et al., 2018) as compared with the ECM fungal diversity of the Fabaceae *Afzelia africana* Sm. from West Africa (Bâ et al., 2012). This low diversity does not result from insufficient sampling, because species accumulation curves of sporocarps and ECM fungal taxa reached an asymptote at all sites in Guadeloupe (Séne et al., 2015). However, other Caribbean and South American sites have to be investigated before this feature can be generalized to the whole distribution area of seagrape (Bâ et al., 2014; Põlme et al., 2017). There is evidence that the number of host tree species drives diversity (Ishida et al., 2007), and indeed, seagrape is the only ECM host of the investigated beach sites (Séne et al., 2015). Study of sporocarps fruiting near seagrape weakly reflected the belowground ECM fungal community, although five fruiting species were found in ECM roots. Seagrape seedlings and mature trees had very similar communities of ECM fungi and could share potential common mycorrhizal networks that could play a major role in nutrient transfers (e.g., C, P, and N) through hyphae from mature trees to seedlings of *Coccoloba* (Karst et al., 2022; Séne et al., 2015). This tree is associated with ECM fungi in the genera *Amanita*, *Inocybe*, *Cantharellus*, *Melanogaster*, *Cenococcum*, *Lactarius*, *Russula*, Thelephoraceae, *Xerocomus*, and *Scleroderma* (Álvarez, 2012; Bâ et al., 2014; Guzmán et al., 2004; Kreisel, 1971; Miller et al., 2000; Pegler, 1983; Séne et al., 2015; Séne et al., 2018). The ECM fungal species *Scleroderma bermudense* plays a major role in mitigating salt stress for seagrape (Bandou et al., 2006; Bullaín-Galardis et al., 2023; Bullaín et al., 2022).

In the flora of Cuba, 34 species of *Coccoloba* including seagrape are listed and 25 are endemic, suggesting that Cuba is an important center of specific diversity of the genus *Coccoloba* in the Greater Antilles (Castañeda, 2014). As host tree species drive diversity (Ishida et al., 2007), higher ECM fungal diversity should be expected in Cuba. However, the ECM fungi associated with seagrape in Cuba are poorly studied. Therefore, the objective of this research was to characterize the diversity of sporocarps and ectomycorrhizae (EMe) of ECM fungi associated with seagrape in three coastal ecosystems in Eastern Cuba.

MATERIALS AND METHODS

Study area: The ECM roots of seagrape and sporocarps were collected at Las Coloradas beach (19º55’31.9” N & 77º41’14.1” W), Cabo Cruz (19º50’23.5” N & 77º43’14.4” W), and Punta de Tomate (21º16’16.6” N & 76º31’ 18.5” W) in Cuba (Fig. 1).

These sites are representative of sand-growing littoral seagrape forests with abundant seedling recruitment in the crowns of the mature trees, except on Cabo Cruz beach. Due to their geographical proximity, barely 10 km, Las Coloradas and Cabo Cruz both have an average annual rainfall and temperature of 942 mm and 27.0 °C. The precipitation and average annual temperature of Punta de Tomate is

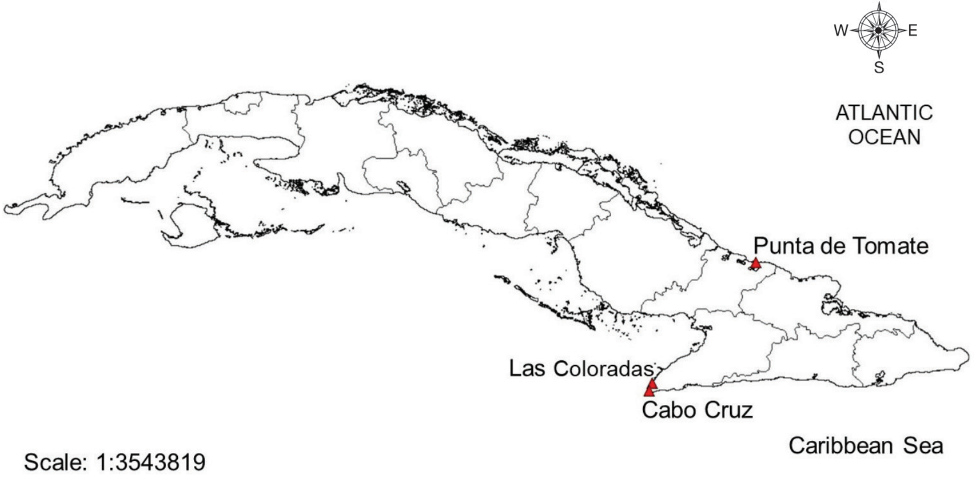

Fig. 1. Location of the three coastal collection sites (Punta de Tomate, Las Coloradas, and Cabo Cruz) in Cuba.

920 mm and 31 °C. Seagrape is the dominant tree in the three sampling sites. In Las Coloradas, the presence of trees of *Thespesia populnea* (Majaguilla), *Cocos nucifera*, *Terminalia catappa* (Indian almond tree), *Rhizophora mangle* (Red mangrove), and creeping plants such as *Ipomoea pes-caprae* (beach sweet potato) were observed. In Punta de Tomate trees such as *Casuarina equisetifolia*, *Rhizophora mangle*, and creeping plants such as *Canavalia rosea* (mate of the coast) were present. In Cabo Cruz, only creeping plants such as *Ipomoea pes-caprae*, *Canavalia rosea*, and *Sessuvium maritium* (sea purslane) shared the same habitat.

The soils of the three collection sites are formed on limestone, especially Cabo Cruz, where calcareous rocks predominate, mainly limestone. Unlike Cabo Cruz and Punta de Tomate, Las Coloradas is a beach protected from strong winds and waves due to its location in the Gulf of Guacanayabo, which has mostly muddy bottoms with little contaminated sediment (Arencibia et al., 2014).

Sampling of sporocarps and EMe: The study area was divided into two permanent plots in each forest site. The dimensions of the plots were 750 m^2 (150 x 5). The first plot extended from the shoreline to five meters and the other from five to ten meters from the shoreline, both with a length of 150 meters.

The sporocarps and ECM roots were collected from both plots. Each plot was sampled four times a year, with an interval of three weeks between each sampling during the rainy season, from June to September, in 2018 and 2019. With the help of a garden spade, the sporocarps were collected, grouped according to their morphology, photographed, and placed separately in a woven wooden basket with handle to be transported to the laboratory. A fragment of fresh tissue from each sporocarp was placed in 50 ml centrifuge tubes containing silica gel, labeled with the sample number and date and place of collection until DNA extraction. The voucher specimens were deposited in the herbarium of the Laboratory of Abiotic Stress of the Center for Plant Biotechnology Studies of the University of Granma (Cuba) and the herbarium of the Laboratory of Plant Biology and Physiology of the University of French West Indies (Guadeloupe). The identification of the sporocarps was carried out in accordance with Coker (1939), Courtecuisse (2006), Courtecuisse (2009), Guzmán et al. (2004), Miller et al. (2000), and Pegler (1983).

Natural regeneration of seedlings was observed under the crown of all mature seagrape trees except at the Cabo Cruz site. Each mature tree and its seedlings covered approximately 4 m^2 (2 × 2 m) in area and were distant by at least 2 m from the other sampled trees. Three mature trees and 30 young individuals (1-2 months old) with two cotyledons and two leaves, < 20 cm in height, and < 2 mm diameter at ground level were randomly chosen and collected during the rainy season under each mature tree, carefully avoiding mature tree roots that sometimes intermingled with seedling roots. To sample the ECM roots of mature trees, we carefully sampled ten soil cores (15 cm diameter and 20 cm depth, approximately 250 g of soil) under the crown where seedlings were absent, avoiding mixing the root sizes and reducing the damage to the seedlings. The ECM roots of mature trees and seedlings were gently washed separately with tap water to remove the excess substrate. Subsequently, they were placed in a Petri dish with water using sleeved needles and a scalpel under a stereoscopic microscope with 4X magnification. Special attention was paid to the presence of any type of swelling, color change, or defined growth pattern recognized as a fungal morphotype, based on macroscopic characteristics, such as the color and texture of the mantle, and microscopic characteristics, such as the presence or absence of hyphae, mycelial filaments, and sclerotia, according to Thoen and Bâ (1989) and Agerer (1991). The ECM colonization was confirmed by observing root apex sections of each fungal morphotype to verify the presence of the fungal mantle and the Hartig network. A fragment of each fresh ECM morphotype was placed in 50 ml centrifuge tubes containing silica gel, labeled with the sample number and date and place of collection for later identification using molecular techniques.

DNA extraction and amplification of the ITS region: DNA was extracted from sporocarps (100 mg dry weight) and from each ECM root tip using the DNeasy Plant Kit (Qiagen, USA) according to the manufacturer's instructions. Sporocarp and EMe were separately placed in extraction tubes containing 400 µL of extraction buffer (AP1) and 4 µL of RNase A. The samples were incubated for 30 min at 65 °C. Thereafter, 50 µL of buffer (AE) was added to each tube, and the extracted DNA was stored at 4 °C. The internal transcribed spacer (ITS) region of nuclear rDNA was amplified in a mix containing 10 µL of Sigma Taq 5X Ready To Go beads (buffer, dNTPs, $MgCl_2$), 1 µL of each primer ITS1-F (5'-CTTGGTCATTTAGAGGAAGTAA-3') and ITS4 (5'-TCCTCCGCTTATTGATAT GC-3') at 10 µM each, 6 µL of water, and 2 µL of the total DNA extract. The PCR conditions were programmed as follows: 1 cycle of 4 min at 95 °C followed by 35 cycles at 95 °C for 30 s, 53 °C for 30 s, 72 °C for 1 min 30 s, and extension at 72 °C for 7 min. Negative controls (no DNA template) were included to test for the presence of DNA contamination. The PCR products were separated by electrophoresis in 1 % agarose gel (Promega, Spain) in 1x TAE buffer (0.04 M Tris-acetate, 1 mM EDTA, pH 8.0) with 10 µL of GelRed Nucleic Acid Stain per 100 ml of gel. DNA bands were visualized by fluorescence under ultraviolet light. The amplification products were sent to Genoscreen (Lille, France) for sequencing.

Sequencing of the ITS region: PCR products were sequenced for both strands. Forward and reverse DNA sequences were edited and manually corrected to obtain the consensus sequence using the software BioEdit (version 7.0.8). For taxonomic affiliation of EMe and sporocarps, consensus sequences were compared with the GenBank database using the BLASTN algorithm to identify the most similar sequences. ECM fungal taxa names were defined following the BLAST score. Species were considered to have been identified when a sequence presented more than 97 % full-length similarity to sequences derived from sporocarps or from well-identified sequences in GenBank. Alternatively, sequences with less than 97 % identity were identified at the genus or family level. The phylogenetic tree was built with the MEGA 6.06 software using the maximum likelihood method. Representative sequences for each ITS OTU sequence were queried against GenBank using BLAST to define fungal taxa. The frequency of each fungal taxon was calculated as the ratio of the number of each fungal taxon over the total number of fungal taxa.

RESULTS

Poor diversity of ECM fungi: Sampling revealed limited sporocarp diversity at the three study sites (Fig. 2, Fig. 3). In all, 111 sporocarps were collected and five ECM fungal taxa were identified from sporocarps, including *Amanita* sp., *Russula* sp., *Inocybe* sp., *Scleroderma bermudense*, and *Cantharellus* sp. (Fig. 2, Fig. 3). Of these five taxa, only *S. bermudense* was subhypogeous, the remaining four were epigeous. All collected sporocarps belong to basidiomycetes. Sporocarps of the five ECM fungal species were found in Las Coloradas (Fig. 2B), two (*S. bermudense* and *Russula* sp.) were found in Punta de Tomate (Fig. 2C), and one (*S. bermudense*) in Cabo Cruz (Fig. 2D). The ECM fungus *S. bermudense* was the most common ECM fungus found in the three sites and represented 50 % of the sporocarps collected (Fig. 2A).

We morphotyped 169 ECM roots and sorted them into six EMe (Table 1, Table 2, Fig. 4). All ECM roots were sampled for subsequent DNA analysis and were identified within six fungal taxa, of which four were identified as Basidiomycota (66.7) and two as Ascomycota (33.3 %). The frequencies of ECM fungal taxa on roots were determined and *S. bermudense* appeared the most representative on root tips of seagrape (Fig. 5). Overall, *S. bermudense* was the only ECM fungus detected on sporomes and on EMe in all sites.

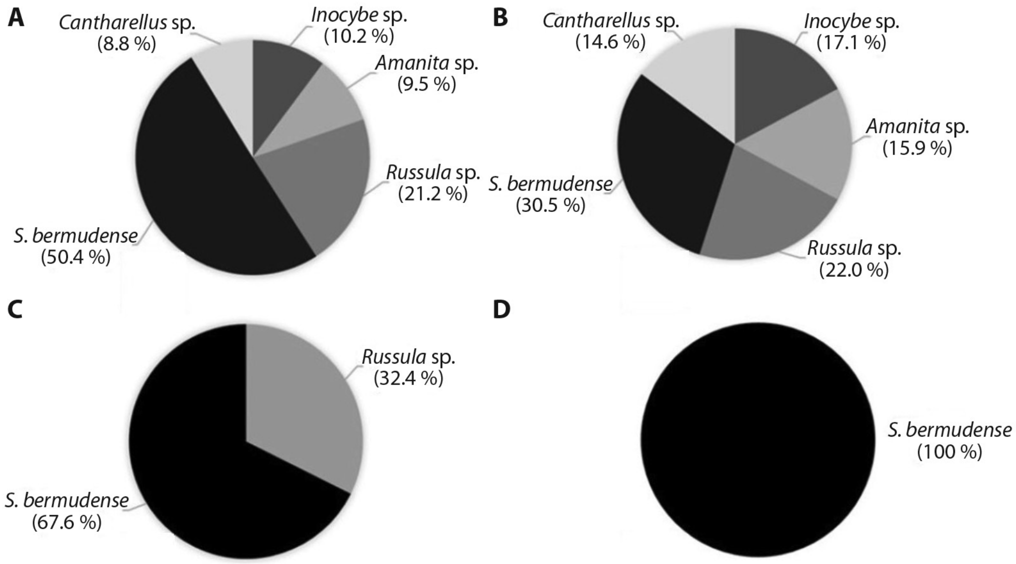

Fig. 2. Frequencies of sporocarps collected under adult seagrape trees and seedlings. **A.** In the three sites. **B.** Las Coloradas. **C.** Punta de Tomate. **D.** Cabo Cruz.

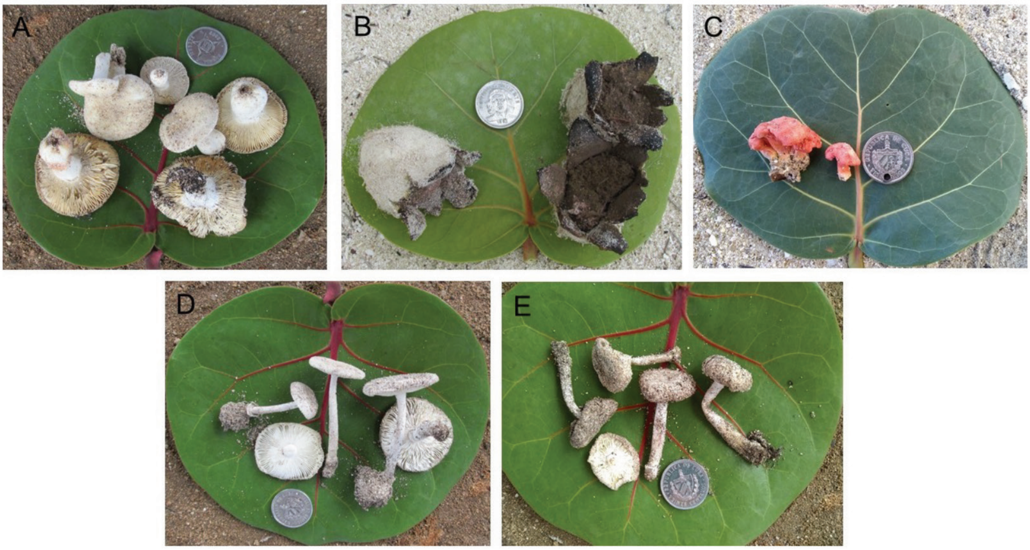

Fig. 3. Sporocarps fruiting from under seagrape. **A.** *Russula* sp. SUA06. **B.** *S. bermudense* SUA09. **C.** *Cantharellus* sp. SUA07. **D.** *Amanita* sp. SUA01. **E.** *Inocybe* sp. SUA13.

A low similarity between aboveground and belowground ECM fungi: A comparison of the aboveground and belowground ECM fungi revealed that *S. bermudense* was identified from sporocarps and ECM roots (99-100 % homology). *Cantharellus* sp., *Russula* sp., and *Amanita* sp. were not found on roots. We were not able to sequence ITS from *Inocybe* sp. Some ECM fungi found on roots, such as *Tuber* spp., *Inocybe* sp., and Thelephoraceae (*Tomentella*

Table 1
ECM fungi associated with *C. uvifera* in the three coastal zones of Cuba.

Herbarium number	Fungal taxa	Origin	Seagrape	Fungal form	Size ITS (pb)	GenBank accession numbers	Reference sequence in NCBI and origin	Similarity (%)
EUA01	*Inocybe* sp.	Cuba (Las Coloradas)	Seedling	Ectomycorrhizae	647	OQ351717	*Inocybe xerophytica* (Guadeloupe)	99
SUA13	*Inocybe* sp.	Cuba (Las Coloradas)	Seedling and mature tree	Sporocarp	nd	nd	nd	nd
EUA02	*Scleroderma bermudense*	Cuba (Las Coloradas)	Seedling and mature tree	Ectomycorrhizae	611	OQ351718	*Scleroderma bermudense* Ecu446 (Guadeloupe)	100
EUA03	*Tomentella* sp.	Cuba (Las Coloradas)	Seedling and mature tree	Ectomycorrhizae	635	OQ351719	*Tomentella* sp. Ecu210 (Guadeloupe)	95
EUA04	*Tuber* sp.	Cuba (Las Coloradas)	Seedling and mature tree	Ectomycorrhizae	564	OQ351720	*Tuber beyerlei* (Colombia)	81
SUA01	*Amanita* sp.	Cuba (Las Coloradas)	Seedling and mature tree	Sporocarp	475	OQ351727	*Amanita arenicola* BA 03.12.26 (Guadeloupe)	92
SUA02	*Russula* sp.	Cuba (Las Coloradas)	Seedling and mature tree	Sporocarp	682	OQ351728	*Russula aeruginea* (North West America)	90
SUA03	*Scleroderma bermudense*	Cuba (Las Coloradas)	Seedling and mature tree	Sporocarp	685	OQ351729	*Scleroderma bermudense* Scu14 (Guadeloupe)	99
SUA07	*Cantharellus* sp.	Cuba (Las Coloradas)	Seedling and mature tree	Sporocarp	538	OQ351733	*Cantharellus cinnabarinus* (Guadeloupe)	82
EUA05	*Scleroderma bermudense*	Cuba (Punta de Tomate)	Seedling and mature tree	Ectomycorrhizae	592	OQ351721	*Scleroderma bermudense* Scu14 (Guadeloupe)	100
EUA06	*Thelephora* sp.	Cuba (Punta de Tomate)	Seedling and mature tree	Ectomycorrhizae	625	OQ351722	*Thelephora* sp. Cu P16 (Guadeloupe)	99

Nd = not determined; NCBI = National Center for Biotechnology Information.

Table 2

Number of sampled individuals, putative EMe, and ECM fungal taxa, and percentage of ECM fungal taxa fruiting and found on roots of seagrape trees and seedlings in Las Coloradas, Punta de Tomate, and Cabo Cruz.

	Las Coloradas		Punta de Tomate		Cabo Cruz	
	Tree	Seedling	Tree	Seedling	Tree	Seedling
Number of sampled individuals	3	30	3	30	3	0
Number of putative ECM roots	36	28	54	36	15	0
Number of putative ECM sampled for DNA extraction	36	28	54	36	15	0
Number of putative ECM sampled for Sanger sequencing	26	22	31	21	7	0
Number of sequences	24	21	22	18	3	0
Number of ECM taxa	4	4	4	3	2	0
Shared ECM fungal taxa (%) (1)	75	75	100	100	0	0
Similarity (%) of ECM fungal taxa found in sporocarps and EMe (2)	11	22	16	16	33	0

(1) % of ECM fungal taxa occurring on mature trees and seedlings at each site. / (2) % of ECM fungal taxa found aboveground and belowground at each site.

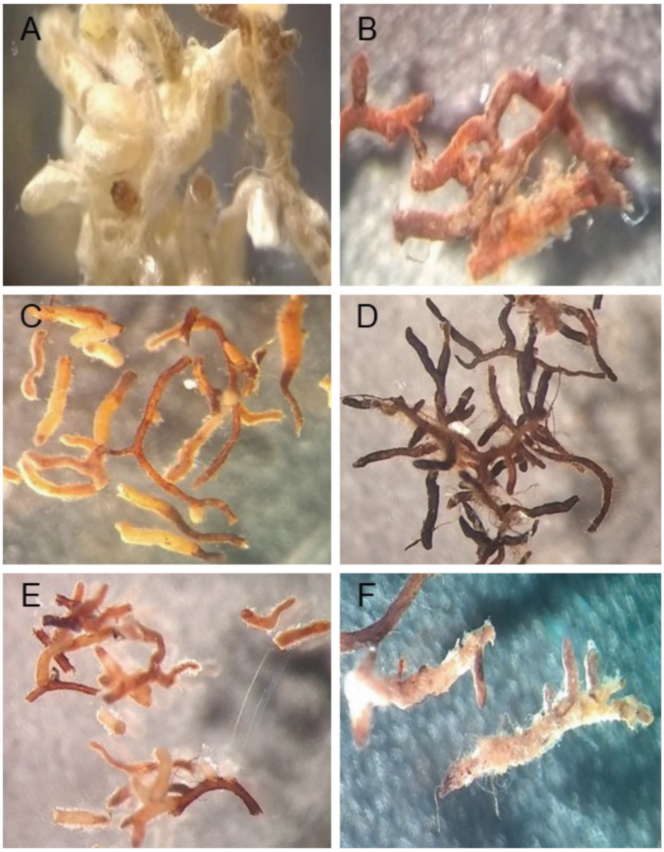

Fig. 4. EMe collected on seagrape. **A.** Bright white EUA02 of *S. bermudense.* **B.** Brown EUA01 of *Inocybe* sp. **C.** Yellow EUA04 of *Tuber* sp. **D.** Brown EUA03 of *Tomentella* sp. **E.** Brown light EUA06 of *Thelephora* sp. **F.** White EUA07 of *Tuber* sp.

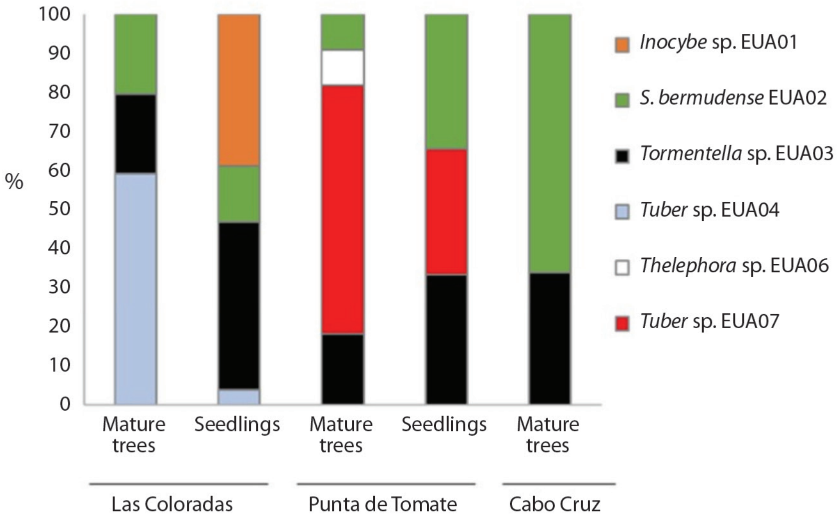

Fig. 5. Relative frequencies of ECM fungal taxa on seagrape at Las Coloradas, Punta de Tomate, and Cabo Cruz.

sp. and *Thelephora* sp.), were not found as sporocarps, but were present in the roots. In each site the ECM fungal taxa on roots that matched with sporocarps were 11 % in Las Coloradas, 16 % in Punta de Tomate and 33 % in Cabo Cruz (Table 2).

Sequence analysis and phylogenetic grouping of the ITS region was further used to identify the unknown fungal symbionts by comparing to a previously published database of sequences from that region in GenBank (Fig. 6). Sequences from sporocarps and ECM roots were compared to the GenBank database using the algorithm BLASTN to identify the most similar ITS sequences. In all cases, sequence similarity was 97-100 % between unknown EMe and their closest known genera within the family or subfamily of Basidiomycota or Ascomycota sequences in the GenBank database. Morphological identification of sporocarps were all confirmed by phylogenetic analysis except *Inocybe* sp. (Fig. 6). Two EMe were closely related to the Thelephoroid taxa (*Tomentella* and *Thelephora*), two to *Tuber*, and one to *Scleroderma* (Fig. 5). Phylogenetic analysis as displayed by the high bootstrap values shown in the neighbor-joining-based tree in Fig. 6 also demonstrates that the different species fall into the seven ECM fungal lineages proposed by Tedersoo et al., (2010), including six from Basidiomycota (/russula-lactarius-lactifluus, /amanita, /cantharellus, /thelephora-tomentella, /pisolithus-scleroderma, /inocybe) and one from Ascomycota (/tuber-helvella) (Fig. 4). Based on phylogenetic analysis and % of similarity (99-100 %), only three common ECM fungi compared to what was found using Blast, one sporocarp *S. bermudense* and two EMe of *Inocybe* sp. EUA01 and *Thelephora* sp. EUA06 of Cuba matching with *Inocybe xerophytica* and *Thelephora* sp. Cu P16 of Guadeloupe, respectively (Fig. 6).

Many ECM fungi are shared by mature seagrape trees and seedlings: Of the six ECM fungal taxa identified on roots, four were shared between mature trees and seedlings (Table 2, Fig. 5). The other ECM fungi, *Inocybe* sp. EUA01 and *Thelephora* sp. EUA06, were found only on seedlings in Las Coloradas and mature trees in Punta de Tomate, respectively. Two fungal taxa belonging to Ascomycota, *Tuber* sp. EUA04 and EUA07, were found on roots of mature trees and seedlings in Las Coloradas and Punta de Tomate, respectively (Table 2). In all, mature trees and seedlings share between 75

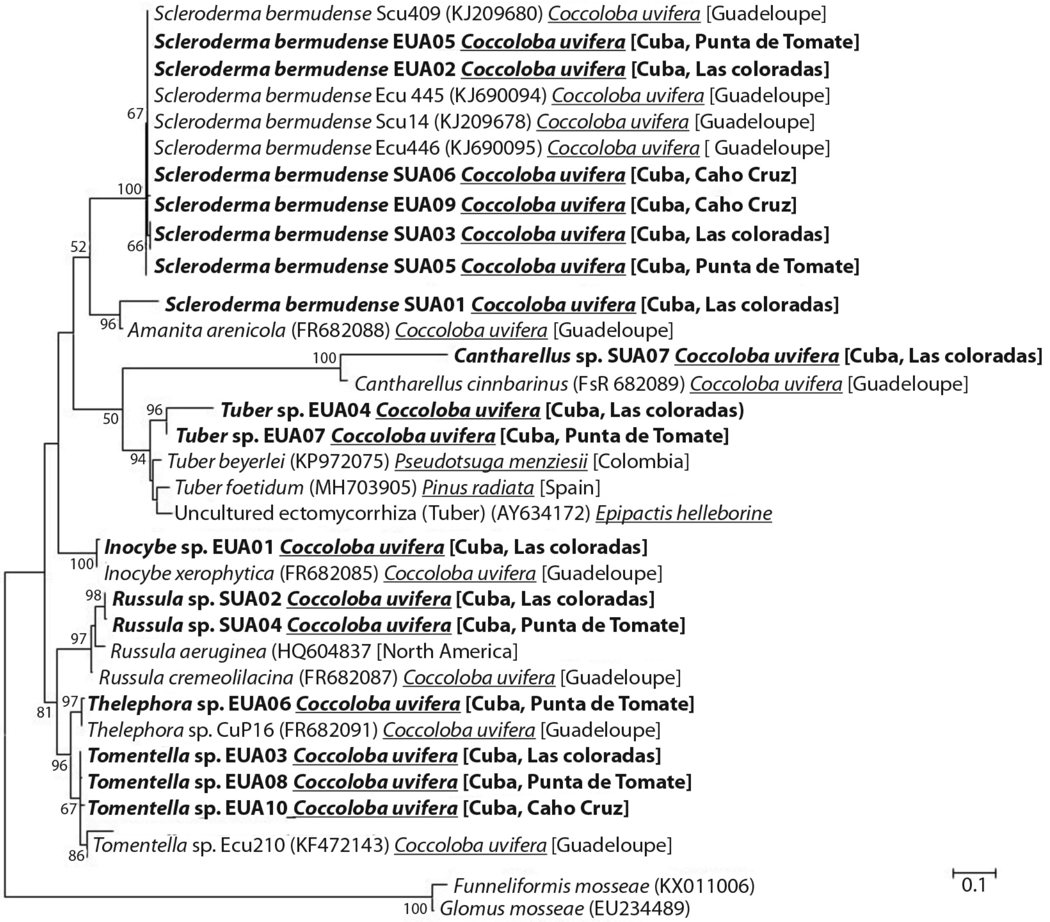

Fig. 6. Phylogenetic tree showing the relationships between ITS sequences of ECM fungi of seagrape from Cuba (in bold) compared to reference sequences in GenBank. The geographical locations of ECM fungi are indicated in square brackets. GenBank accession numbers are indicated in brackets. Host plants are underlined. The herbarium numbers “EUA” represent EMe and “SUA” sporocarps. Three common ECM fungi compared to what was found using Blast, one sporocarp *S. bermudense* and two EMe of *Inocybe* sp. EUA01 and *Thelephora* sp. EUA06 of Cuba matching with *Inocybe xerophytica* and *Thelephora* sp. Cu P16 of Guadeloupe, respectively. The phylogenetic tree was rooted with strains of *Funneliformis mosseae* and *Glomus mosseae*.

and 100 % of the fungal taxa in Las Coloradas and Punta de Tomate respectively (Table 2).

DISCUSSION

Poor diversity of ECM fungi: The limited diversity and uneven distribution and frequency of ECM fungi in the three sampling sites, based on sporocarp sampling, coincides with what was observed by Séne et al. (2015) at four sampling sites on the island of Guadeloupe (France). In that study of 546 collected sporocarps only seven species of ECM fungi were identified. The distribution of the sporocarps of the different species was different in the four sampling sites and *S. bermudense* and *R. cremeolilacina* were the most abundantly fruiting genera at the four sites. However, *S. bermudense* was the most abundantly fruiting species in the littoral forests of the Greater

Antilles (Cuba) and the Lesser Antilles (Martinique and Guadeloupe) (Bâ et al., 2014; Séne et al., 2015). This result coincides with the report that *S. bermudense* is an ECM fungus associated with seagrape and follows its distribution in the tropics (Guzmán et al., 2004).

Given the few sites investigated in Cuba, it is difficult to identify the causes of the poor diversity of ECM fungi and the differences between the three collection sites in terms of the presence of sporocarps of ECM fungi, but this behavior could be related to the properties of the soil, particularly salinity, and the average annual rainfall at the three sites. Other authors also suggest that the distribution of ECM fungi, like many vascular and nonvascular plants, is largely defined by adaptation and competition for niches related to stress tolerance (e.g., drought, soil acidity) and resource availability (especially organic N, NH_4^+, and NO_3^-) in soils (Dickie et al., 2002; Koide et al., 2005).

Precipitation could also structure the diversity, distribution, and fruiting of ECM fungal species in the littoral forest where soil water potential partly depends on the levels of annual rainfall and salinity, which may be two important factors structuring the ECM fungal community (Aučina et al., 2011; Séne et al., 2015).

Alternatively, the low diversity of ECM fungi from sporocarps and ECM roots observed in Cuba and Guadeloupe may be the result of the adverse environmental conditions in which seagrape grows, in sandy, calcareous, and rocky soils with a low percentage of organic matter and high levels of salinity (Bullaín et al., 2022; Bullaín-Galardis et al., 2023; Parrotta, 2000; Séne et al., 2015). Additionally, Bâ et al. (2014) observed that the number of mycorrhizal roots was almost three times greater in seagrape trees that grew in soils with a lower level of salinity (0-2 %) than those that grew in soils with a higher level of salinity (2-15 %). They also observed that the amount of ECM fungi collected under seagrape was lower than under mature trees of *C. swartzii* and *C. pubescens* that grew at a greater distance from the coastline with a lower level of salinity.

Another possible cause may be the fact that seagrape is the only ECM host of the investigated sites. Ishida et al. (2007) suggest there is evidence that the number of host tree species drives fungal diversity. On the other hand, it is well known that there are various levels of specificity on the fungal side of the ECM symbiosis (Borowicz & Juliano, 1991; Molina et al., 1992).

A low similarity between aboveground and belowground ECM fungi: The poor similarity between aboveground and belowground ECM fungi in this research could be the result of insufficient sampling, since Arnolds (1991) states that the type and number of ECM sporocarps reflect the belowground abundance of mycorrhizas to a large extent, but exceptions occur. Alternatively, Lang and Polle (2008) report that belowground mycorrhizal diversity drives aboveground diversity. In this sense, it is well known that the Thelephoraceae family rarely produces sporocarps but they commonly form EMe on roots of trees (Kõljalg et al., 2000). The family Thelephoraceae is very common worldwide, but rare and often inconspicuous in terms of sporocarps. Its fungi are frequent in species-poor ECM fungal communities of tropical rainforests (Bâ et al., 2012; Diédhiou et al., 2010; Furtado et al., 2023) and dominate on *Nyctaginaceae* spp. from Southern Ecuador (Henkel et al., 2005). According to Furtado et al. (2023), the members of this family establish ectomycorrhizal associations with low fungal diversity in the Neotropics, but in a study carried out by Alvarez et al. (2018), Nyctaginaceae was the family with the highest number of ECM host species. Our study has once again confirmed that belowground ECM fungal diversity from ECM roots is dissimilar from that of aboveground sporocarps (Ebenye et al., 2017; Séne et al., 2015; van der Heijden et al., 1999). Therefore, neither the diversity nor abundance of aboveground ECM fungi can be used to assess belowground ECM diversity or abundance (van der Heijden et al., 1999), underlining the importance of molecular analyses for the assessment of ECM fungal diversity. However, the absence of *Amanita* sp., *Russula* sp., *Inocybe* sp., and *Cantharellus* sp. on roots suggests that a better view of the ECM fungal diversity is achieved by combining sporocarps and ECM roots surveys.

The two ECM fungal taxa belonging to the genus *Tuber* had not previously been reported from seagrape forests in the Lesser Antilles and the Greater Antilles (Bâ et al., 2014; Séne et al., 2015). This may be related to the criterion that host diversity contributes to ECM fungal diversity (Ishida et al., 2007), as the greatest speciation and radiation of the genus *Coccoloba* in the Antilles occurred in Cuba, and Eastern Cuba, with 26 species, of which 15 are endemic, is considered the main center of diversification on the largest island of the Antilles (Castañeda, 2017).

The low number of species of ascomycetes detected is likely due to their specific ecological requirements more than a methodological issue (Tedersoo & Smith, 2013). Previous studies in tropical ecosystems also found low diversity of ECM ascomycetes (Diédhiou et al., 2010; Ebenye et al., 2017; Henry et al., 2015). The ECM fungus *Scleroderma bermudense* was the most common fungal taxa on roots of mature trees and seedlings whatever the studied site (Fig. 5). This ECM taxon may form potential common mycorrhizal networks (CMNs) between these two cohorts. Similarity of ECM fungal taxa composition between mature trees and seedlings has often been reported in temperate (Aučina et al., 2011) and tropical forests (Diédhiou et al., 2010; Ebenye et al., 2017; Séne et al., 2015). For instance, mature trees and seedlings of dominant seagrape forests shared three ECM fungal taxa representing 80% of the ECM colonization (Séne et al., 2015). In a mixed tropical rainforest in Guinea, ECM fungi shared by mature trees and seedlings represented 79 % of the ECM colonization (Diédhiou et al., 2010). The CMNs and their impact on the nutrition, growth, and fitness of regenerating seedlings should be further investigated experimentally in seagrape coastal forests.

As in Guadeloupe, sampling at the three coastal collection sites in Cuba showed that sporocarps of ECM fungi associated with seagrape only weakly reflected the belowground ECM fungal community, although some fruiting species were also found on roots. The diversity of ECM fungi associated with seagrape was rather limited, which is also true for other ECM plants of the Polygonaceae family. In seedlings and mature trees, the same representatives of the ectomycorrhizal fungal community coincide with *S. bermudense*, Thelephoraceae (*Tomentella* and *Thelephora*), and *Tuber* spp. predominating in the roots of both cohorts, which may allow seedlings to join the potential CMN existing under mature trees, where *S. bermudense* forms the main potential CMN between mature trees and seedlings. The role of CMNs in the regeneration of seagrape seedlings remains to be clarified. Given the few sites sampled and investigated in Cuba, it is difficult to make broader comments on the differences in sporocarps and EMe in the whole territory of the largest island of the Antilles. Thus, more exploration and sampling work should be carried out to contrast the results obtained.

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