Mycorrhiza (2020) 30:269–283
https://doi.org/10.1007/s00572-020-00944-0

ORIGINAL ARTICLE

# Seasonal shifts of arbuscular mycorrhizal fungi in *Cocos nucifera* roots in Yucatan, Mexico

**Luis A. Lara-Pérez**[1,2] · **Iván Oros-Ortega**[2] · **Iván Córdova-Lara**[1] · **Héctor Estrada-Medina**[3] · **Aileen O'Connor-Sánchez**[1] · **Elsa Góngora-Castillo**[4] · **Luis Sáenz-Carbonell**[1]

Received: 30 August 2019 / Accepted: 27 February 2020

## Abstract

The diversity and community structure of arbuscular mycorrhizal fungi (AMF) associated with coconut (*Cocos nucifera*) roots was evaluated by next generation sequencing (NGS) using partial sequences of the 18S rDNA gene and by spore isolation and morphological identification from rhizosphere soil. Root samples from six different Green Dwarf coconut plantations and from one organic plantation surrounded by tropical dry forest along the coastal sand dunes in Yucatan, Mexico, were collected during the rainy and dry seasons. In total, 14 root samples were sequenced with the Illumina MiSeq platform. Additionally, soil samples from the dry season were collected to identify AMF glomerospores. Based on a 95–97% similarity, a total of 36 virtual taxa (VT) belonging to nine genera were identified including one new genus-like clade. *Glomus* was the most abundant genus, both in number of VT and sequences. The comparison of dry and rainy season samples revealed differences in the richness and composition of AMF communities colonizing coconut roots. Our study shows that the main AMF genera associated with coconut tree roots in all samples were *Glomus*, *Sclerocystis*, *Rhizophagus*, *Redeckera*, and *Diversispora.* Based on glomerospore morphology, 22 morphospecies were recorded among which 14 were identified to species. *Sclerocystis sinuosa*, *Sclerocystis rubiformis*, *Glomus microaggregatum*, and *Acaulospora scrobiculata* were dominant in field rhizosphere samples. This is the first assessment of the composition of AMF communities colonizing coconut roots in rainy and dry seasons. It is of importance for selection of AMF species to investigate for their potential application in sustainable agriculture of coconut.

**Keywords** Established fore dune · Tropical forest · Wetland plantations · Dynamic fungi community structure · Glomerospores · Illumina MiSeq

## Introduction

Coconut (*Cocos nucifera* L.) is a crop of recognized high economic importance, and its potential becomes even greater due to the growing demand for its high-value products such as packaged coconut water, coconut milk products, virgin coconut oil, and products derived from its fibers (Sáenz et al. 2018). Globally, it is estimated that 12 million hectares of *C. nucifera* are planted across 89 countries (APCC-Secretariat 2016), and Mexico is one of the most important coconut-producing countries in America with approximately 166,000–190,000 ha planted across 13 states (Faostat 2016). However, to satisfy the demand for coconut products, farmers are faced with important challenges such as a decrease in plant productivity due to the senility of an important portion of current plantations, reduction in soil fertility, natural calamities (Samosir and Adkins 2014), and pests and diseases (Gurr et al. 2016). Therefore, the International Coconut Community

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**Electronic supplementary material** The online version of this article (https://doi.org/10.1007/s00572-020-00944-0) contains supplementary material, which is available to authorized users.

✉ Luis Sáenz-Carbonell
vyca@cicy.mx

[1] Unidad de Biotecnología, Centro de Investigación Científica de Yucatán, Mérida, Yucatán, Mexico

[2] Instituto Tecnológico de la Zona Maya, Tecnológico Nacional de México, Carretera Chetumal-Escárcega km 21.5, C.P. 77965 Ejido Juan Sarabia, Quintana Roo, Mexico

[3] Facultad de Medicina Veterinaria y Zootecnia, Departamento de Manejo y Conservación de Recursos Naturales Tropicales, Campus de Ciencias Biológicas y Agropecuarias, Universidad Autónoma de Yucatán, Mérida, Yucatán, Mexico

[4] CONACYT-Unidad de Biotecnología, Centro de Investigación Científica de Yucatán, Mérida, Yucatán, Mexico

(ICC, previously Asian and Pacific Coconut Community) projects to replace 50% of the currently planted palms around the world within the next 20 years to try to meet the increasing demand set by global markets (APCC-Secretariat 2016).

Even though coconut producers in Mexico suffer similar problems to their international counterparts, Mexican plantations are affected most significantly by a disease called lethal yellowing (LY), caused by a phytoplasma (Mollicutes) of the 16SrIV group, which has already devastated Mexican coconut groves and has also heavily harmed the landscape of tourist resorts in the Yucatan Peninsula, an additional important income source for the local economy (Zizumbo-Villarreal et al. 2008). To overcome the LY infestation in Mexico, coconut producers have initiated programs to renew plantations and expand the cultivation area with varieties resistant or tolerant to the phytoplasma (Zizumbo-Villarreal et al. 2008). These new commercial coconut plantations require a high input of fertilizers (2910 g of $K_2O$, 300 g of $P_2O_5$, and 2353 g of N $palm^{-1}$ $year^{-1}$) and pesticides for optimal development and production and to reduce the dispersal rates of the disease (Reinert 1977; Pushparajah et al. 1990; Miguel et al. 2011). However, intensive use of agricultural fertilizers is an important contributor to nitrate and phosphate contamination of groundwater, turning this strategy into a potential risk for human health and raising environmental concerns about eutrophication of aquatic ecosystems (Pacheco et al. 2001; Gonzalez-Herrera et al. 2014; Rojas-Fabro et al. 2015).

Arbuscular mycorrhizal fungi (AMF) belong to the subphylum *Glomeromycotina*, in the phylum *Mucoromycota* (Spatafora et al. 2016). Species of this group are associated with the roots of over 80% of land plant species (Brundrett 2009; Van der Heijden et al. 2015; Brundrett and Tedersoo 2018) and are considered among the most ecologically important soil microorganisms due to the role they play in ecosystem functionality, increasing plant biomass and improving resistance to moderate salinity and hydric stress as well as tolerance to some pathogens (Porcel et al. 2012; van der Heijden et al. 2015). AMF also play a crucial role in nutrient cycling and can reduce the leaching of nitrogen and phosphorus from soils in which these elements are scarce (Köhl and van der Heijden 2016). However, their precise role in plant nutrition depends on the specific AMF species involved (Leigh et al. 2009; Köhl and van der Heijden 2016). For all the benefits mentioned above, AMF have gained increased attention as an asset in sustainable agriculture (Priyadharsini and Muthukumar 2015).

The beneficial effects on plant growth of the association of AMF and members of the Arecaceae family have been well reported (Ramos-Zapata et al. 2006; Sosamma et al. 1998). It has also been well documented that *C. nucifera* is consistently colonized by AMF (Wang and Qiu 2006; Muthukumar and Prakash 2009; Muthukumar and Vediyappan 2010), and that it shows high levels of arbuscule colonization (Thomas and Ghai 1987; Koske et al. 1992). Furthermore, due to the absence of root hairs from the coconut root system, it is likely that AMF play a crucial role in nutrient intake (Thomas and Ghai 1987). Mycorrhizae also promote plant growth and lead to differential effects in growth rates in coconut seedlings, as well as suppressing plague infections, depending on the AMF species (Sosamma et al. 1998).

So far, the richness of glomerospores of AMF in the rhizosphere of coconut has been studied only in India, one of the two centers of origin for coconut cultivation (Ramesh 1984; Ambili et al. 2012; Rajeshkumar et al. 2015). Surprisingly, and despite its great economic importance and wide pantropical distribution, no molecular or morphological studies on AMF community composition of coconut have been made in areas where it is considered exotic or naturalized.

Coconut is not native to Mexico, and the current genetic diversity is a result of successive introductions of genotypes from different origins for over 400 years (Zizumbo-Villarreal et al. 2008; Baudouin and Lebrun 2009). Today, this species is considered a naturalized plant and is present in 21 states, mainly because of its agronomic importance (Richardson et al. 2000). Although there are not many data about dune colonization by *C. nucifera* in Mexico, some records of natural communities at very low densities in sand dunes exist, but there are no reports of aggressive invasive coconut varieties with potential to displace native plants (Castillo and Moreno-Casasola 1996; Espejel et al. 2017).

Alien plants, such as coconut in Mexico, may influence the composition and density of resident AMF communities and either increase or reduce the mutualism between plants and AMF (Moora et al. 2011). Alien plants also have been documented to associate mostly with widespread generalist AMF taxa across a broad distribution range (Moora et al. 2011). Prior to utilizing AMF for sustainable agriculture, it is crucial to identify the richness of AMF populations and to understand their spatio-temporal dynamics with exotic or naturalized plants, grown under different cropping systems.

Even though the best environments for coconut production are coastal areas, crops can currently be found in a wide range of areas with different types of soils (from loam to clay), at altitudes ranging from sea level to 1200 m, and in areas of low rainfall with well-aerated soils (Chan and Elevitch 2006; Khan and Krishnakumar 2018; Perera et al. 2009). The coconut-growing areas in Yucatan are mainly distributed in small populations along the coastline, which has been divided into west, middle, and east based on type of climate and vegetation (Espejel 1987). More recently, the cultivation of coconut has expanded inland, into the tropical forest of Yucatan, with shallow soils and a higher content of clay, organic matter, and phosphorus when compared with the sandy soils of the coastal dunes.

The majority of the plant species analyzed along the coastline have exhibited AMF colonization, independently from the type of dunes (embryonic or stabilized) (Ramos-Zapata et al. 2011; Guadarrama et al. 2012; Carmona-Escalante et al. 2013; Polanco et al. 2013) and including wetland and coastal lagoons (Ramírez-Viga et al. 2019). Based on glomerospore morphology, the richness of AMF populations is low (from two to seven species), and this richness shows a tendency to increase when moving inland, in wetland (22 species), and tropical agroecosystems (18 species) (Guadarrama et al. 2012; Ramos-Zapata et al. 2012). In these environments, the rainy and dry seasons are well defined, and seasonal differences have been shown to influence fluctuations in root colonization, richness, and abundances of spores and sporocarps (Ramos-Zapata et al. 2011). Analyses of seasonal shifts in the composition of AMF communities mainly have been based on root colonization and spore counts, but do not provide information about the identities of the fungus species and only offer a limited understanding of the dynamics of AMF colonizing the roots of studied plant species.

The aim of this study is to describe the AMF diversity in the roots of *C. nucifera*, based on rDNA sequences from the rainy and dry seasons. To this end, seven representatives and contrasting sites of coconut growing areas along the 320 km coastline in west, middle, and east Yucatan and one site inland, in the tropical forest, were selected. Distinct AMF communities and diversities are expected to be colonizing the roots of coconut plants during the rainy and dry seasons. Because *C. nucifera* has long been a naturalized species, we anticipate that it hosts not only generalist AMF but also rare native species.

## Materials and methods

### Study sites

For the study, six representative sites of coconut-growing areas along the 320 km of coastline (divided into west, central, and east) and one site inland, in the tropical forest of Yucatan, Mexico, were selected. All selected sites present contrasting climatic types and vegetation and different cultural practices for the handling of coconut production (Table 1). The coastal areas in the region are formed by an uplifted section of the Quaternary platform, with a beach with coarse sand composed of 90% calcium carbonate ($CaCO_3$) all along the coast and with a mix of mangroves (wetland) and dune thickets, barrier beaches, and coastal lagoons (Duch-Gary 1991). Tropical forest soils are shallow and have a higher content of clay and organic matter than the sand dunes (Table 1). Dominant plant species in the coastal dunes are *Ambrosia hispida* Pursh, *Coccoloba uvifera* L., *Scaevola plumieri* L., and *Tournefortia gnaphalodes* (L.) R. Br. ex Roem. & Schul. (for further details on vegetation, see Moreno-Casasola and Espejel 1986; Espejel 1987; Torres et al. 2010). Although the tropical forest presents a high diversity of tree species, the understory is structurally poor. Some common tree species in the area are *Sabal* sp., *Piscidia piscipula* (L.), *Havardia albicans* (Kunth) Britton & Rose, *Gymnopodium floribundum* Rolfe, and *Bursera simaruba* (L.) Sarg. Coconut plantations in the wetlands are surrounded by the common mangrove species *Avicennia germinans* (L.) L. and *Rhizophora mangle* L. The weather in all sites is semi-arid, with a well-defined rainy season running from May to October with an average temperature of 23 °C, and a dry season, running from November to April, with an average temperature of 25.8–26.8 °C. The mean annual precipitation for the western and eastern sections of the coast is 687 mm and 779 mm of rain, respectively.

### Root samples

Root sampling of *C. nucifera* was carried out in April (2016) and September (2016), for dry and rainy seasons, respectively. In each of the seven sites, roots from three healthy trees, at least 50 m apart from one another, were collected (Table 1). For each tree, roots of the four cardinal points were collected, 30–50 cm away from the base of the palm and 15–20 cm in depth. Trees were georeferenced and labeled in order to select the same three healthy trees in both seasons. Each sample was immediately covered with cetyltrimethyl ammonium bromide (CTAB) buffer described by Harrison et al. (1994), 2% (CTAB), 0.1% 1-Thioglycerol, 0.5% of spermidine, 100 mM Tris-HCl pH 8, 2.0 M NaCl, and 20 mM EDTA pH 8, and transported to the laboratory where they were kept at − 90 °C until further processed. For DNA extraction, we selected fine roots (approximately 100 mg from each tree) to form one composite root sample for each site.

### 18S rRNA gene sequencing

DNA was extracted from a total of 14 root composite samples (seven from the dry season and seven from the rainy season) (Table 1). Extraction was performed within 24 h after collection using 300 mg of each composite sample of fresh coconut roots with the aid of the DNeasy Plant Mini Kit (QIAGEN, Hilden, Germany) according to the manufacturer's instructions with 50 μl of elution buffer. Quantity and quality of the DNA were estimated with a NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA). To confirm *Glomeromycotina* presence in these samples, PCR analyses were performed using specific primers according to Krüger et al. (2009). Plants were determined to be free of LY infection before submitting samples for high throughput sequencing, following the DNA-based molecular diagnostic assay established by Córdova et al. (2014). LY

**Table 1** Management characteristics of the study sites

| Site | Locality (coordinates) | Ecosystem | Management/age of establishment | Fertilizer | Climate type |
|---|---|---|---|---|---|
| 1 | Celestun (21° 51′ 43″ N; 90° 23′ 11″ W) | Wetland | Undergrowth slashing/ 10 years | None | Dry and warm |
| 2 | Celestun (20° 54′ 20″ N; 90° 23′ 04″ W) | Established foredune | None/18–20 years | None | Dry and warm |
| 3 | Chicxulub (21° 18′ 29″ N; 89° 32′ 50″ W) | Established foredune | None/18–20 years | None | Warm and semidry |
| 4 | San Crisanto (21° 21′ 5″ N; 89° 11′ 48″ W) | Established foredune | None/18–20 years | None | Warm and semi-dry |
| 5 | El Cuyo (21° 30′ 50″ N; 87° 39′ 39″ W) | Established foredune | None/18–20 years | None | Warm sub-humid |
| 6 | San Crisanto (21° 20′ 56″ N; 89° 11′ 49″ W) | Established foredune | Undergrowth slashing/ 19 years | Organic management, based on mulching the base of the palms with coconut husks and residual leaves | Warn sub-humid |
| 7 | Espita (21° 03′ 35″ N; 88° 19′ 14″ W) | Tropical deciduous forest | Undergrowth slashing/ 4 years | Organic fertilizer, enriched soil with the use of bokashi. Application of commercial *Bacillus subtilis* and *Trichoderma harsianum* | Warm sub-humid |

disease is still present in southwest Mexico and some of the symptoms are reduced levels of sugar concentration and necrotic roots of infected plants (Maust et al. 2003). LY infection can drastically change or limit AMF colonization and, although infection by phytoplasma in coconut usually exhibits visual symptoms, the disease can be asymptomatic in some plants. Extracted DNA was submitted to Research and Testing Laboratory (Lubbock, TX, USA) for Illumina MiSeq sequencing targeting a partial sequence of the small-subunit (SSU) 18S rRNA gene using Wanda F (5′-CAGCCGCGGTAATTCCAGCT-3′) and AM1 R primers (5′-GTTTCCCGTAAGGCGCCGAA-3′) (Helgason et al. 1998; Dumbrell et al. 2011). Denoising, correction of errors, and omission of chimeric sequences were performed using UCHIME (Edgar et al. 2011) according to the RTL Data Analysis Methodology document (RTL Genomics 2016). Singletons were excluded from the analysis as likely sequencing errors (Tedersoo et al. 2010). Samples with fewer than 100 sequences were omitted from the final dataset (Herrmann et al. 2016). Sequence analyses were carried out using sequences with ≥ 328 pb after removal of oligonucleotide primers and adapters. For taxonomic assignment of reads to virtual taxa (VT), representative sequences were aligned to the MaarjAM database using BLAST (Öpik et al. 2010; August 2018) with the following criteria: sequence similarity ≥ 97%, alignment length ≥ 95% of the shorter of the query, and subject sequences and BLAST e-value < 1e–50. The best hits of the BLAST were recorded for each read. Sequence alignments with a sequence similarity lower than 96% were aligned to the non-redundant International Nucleotide Sequence Database (INSD) with sequence similarity and alignment coverage equal to 90% of the shorter of the sequences. Phylogenetic analysis was conducted to putatively determine *Glomeromycotina* DNA sequences that represent novel VT. Representative sequences of each VT were submitted to the NCBI database under accession numbers MK551286-MK551328.

## Phylogenetic analysis

A phylogenetic analysis was performed to determine to which *Glomeromycotina* genera or families the obtained VT belonged. VT that did not group in a cluster corresponding to a known genus were considered new genus-like clades (Jiang et al. 2018). Reference sequences from the MaarjAM database (Öpik et al. 2010), NCBI (www.ncbi.nlm.nih.gov), and representative consensus sequences from each VT were aligned using MAFFT version 7.149bv (Katoh et al. 2002). A tree was built with MEGA X (Kumar et al. 2018) using Neighbor-Joining analysis (Saitou and Nei 1987) and the Tamura 3-parameter nucleotide substitution with bootstrap values based on 1000 runs (Jiang et al. 2018). *Lichtheimia hyalospora* (NG 064991) and *Rhizopus microsporus* var. *chinensis* (EU410422) (*Mucoromycotina*) sequences were taken from the NCBI database (www.ncbi.nlm.nih.gov) and used as outgroups (Spatafora et al. 2016). Nomenclature of AMF families and genera was assigned according to the current classification described by Schüßler and Walker (2010).

## AMF glomerospore isolation and identification

For AMF glomerospore and sporocarp isolation, three composite samples of soil for each of the seven sites were considered (21 samples in all) for only the dry season.

Approximately 200 g of soil was collected from the four cardinal points of each plant where roots were collected (with the same considerations of tree base distance and depth as root samples) and was then mixed to get one composite soil sample. Soil samples were transported to the laboratory in black polythene bags and stored at 4 °C, for no longer than 1 week. Separation of glomerospores and sporocarps from 20 g of dry season soil samples of the rhizosphere of coconut was carried out by using a series of sieves (1000 to 37 μm) (Gerdemann and Nicolson 1963), followed by sucrose centrifugation (Daniels and Skipper 1982). Only healthy and intact glomerospores were counted and grouped according to their morphological characteristics. Sporocarps were counted as one individual. Glomerospores and sporocap permanent preparations were made with at least 20 glomerospores mounted with polyvinyl alcohol in lactoglycerol (PVLG) and Melzer's reagent in PVLG 1:1 (v/v) (Morton 1988, 1993). Identifications were based on Schenck and Perez (1990), Morton and Msiska (2010), Schüßler and Walker (2010), and Redecker et al. (2013).

### Soil analyses

Chemical and physical soil analyses were performed on three technical replicates of one composite soil sample for each of the seven sites from the dry season following standard procedures: Organic matter by colorimetric determination (Nelson and Sommers 1996); total nitrogen by the Kjeldahl method (Bremner 1996); REDOX by potentiometry (Patrick et al. 1996); pH by potentiometry (Thomas 1996); phosphorus by the Olsen method (Kuo 1996); carbonates by the acetic acid method (Loeppert and Suarez 1996); electric conductivity by potentiometry and particle size analysis (Kilmer and Alexander 1949).

### Statistical analyses

Samples with fewer than 100 reads were omitted from the analyses. AMF richness was determined as the number of VT present in a sample. EstimateS software version 8.2.0 was used to produce VT accumulation curves for all sites and for rainy and dry seasons (Colwell 2009). Richness, Shannon entropy, and Gini-Simpson indexes were calculated and then transformed to an effective number of species (Hill numbers 0, 1, 2), to assess the diversity of AMF VT species for all sites (Hill 1973; Jost 2006). In order to detect differences between seasons, we compared only the similarly managed sites of established foredune, namely Celestún (site 2), Chicxulub (site 3), San Crisanto (site 4), and El Cuyo (site 5) (Table 1). The three measures of effective number of species were subjected to Student's *t* test ($P < 0.05$) to assess differences between the rainy and dry seasons. Non-metric multidimensional scaling was used to visualize differences in community composition of AMF in rainy and dry seasons based on Bray-Curtis distances. For pairwise differences between community compositions between the rainy and dry season, a Permutational Multivariate Analysis of Variance (PERMANOVA) (Anderson 2001) based on Bray-Curtis dissimilarity was performed with 999 permutations and Bonferroni correction of probabilities. Similarity Percentages (SIMPER) Analysis based on species abundance data was used to identify the contribution of each species to differences between VT obtained from the rainy and dry seasons. All analyses were performed using PAST version 2.02 software (Hammer et al. 2001), unless indicated otherwise.

## Results

### Soil characteristics

As shown in Table 2, all tested soil samples of coastal sand dunes were slightly alkaline (pH 7.5–7.6), with a low content of organic matter (1.9–3.6%), nitrogen, phosphorus, and potassium, and rich in carbonates (106–108%) and calcium (5083–9375 mg/kg). On the contrary, in Espita, the location with an organic plantation surrounded by tropical dry forest, the soil pH was slightly acid (6.9), with a high content of clay (51.4%) when compared with Celestún and San Crisanto (the lowest percentages of clay with 6.1% and 5.4%, respectively), a low content of carbonates (13.6%), and twice the amount of organic carbon (6.3%) of coastal dunes.

### Molecular diversity of AMF associated with coconut

After removal of the samples with < 100 reads (a rainy season sample from Espita and a dry season sample from Celestun wetland), chimeras, and non-Glomeromycotinan species, 12 *C. nucifera* root samples with a total of 68,147 quality sequences remained. Of these, 43,175 sequences were from the dry season and 24,972 from the rainy season (Table 3). Considering all sequences together, 36 known virtual taxa and one new genus-like clade were identified. The frequency of VT per sample is presented in Table 3.

Rarefaction curves showed that the number of sequences in the 12 samples obtained from coconut roots was sufficient to asymptote, indicating that an acceptable mycorrhizal richness was recovered (Fig. 1S; Table 3). From the rainy season samples, the highest number of reads and VT were recorded in San Crisanto (sand dune with management), with 13,919 and 16, respectively, while Chicxulub (foredune) recorded the lowest number of reads (408) and VTs (5). The highest number of sequences from the dry season samples was detected in Espita (tropical dry forest) with 14,406 and 24 VT, while the lowest number of sequences was detected again in Chicxulub (foredune) with 405 reads and 9 VT (Table 3). The mean VT

**Table 2** Mean values (± SD) of physicochemical soil properties measured in the study sites

| Parameters | Study sites | | | | | | |
|---|---|---|---|---|---|---|---|
| | Celestum wetland | Celestun foredune | Chicxulub foredune | San Crisanto foredune | El Cuyo foredune | Espita tropical forest | San Crisanto plantation |
| pH | 7.5 ± 0.2 | 7.6 ± 0.2 | 7.6 ± 0.1 | 7.4 ± 0.1 | 7.5 ± 0.5 | 6.9 ± 0.6 | 7.7 ± 0.1 |
| EC 1:5 (μS/cm) | 740.0 ± 581.5 | 663.7 ± 371.6 | 405.0 ± 15.1 | 459.7 ± 48.8 | 763.7 ± 25.6 | 422.3 ± 322.2 | 617.3 ± 425.5 |
| REDOX potencial (mV) | 60.8 ± 6.2 | 40.5 ± 4.4 | 21.7 ± 13.5 | 48.8 ± 6.3 | 42.9 ± 3.2 | 62.9 ± 22.6 | 60.6 ± 4.2 |
| Phosphorus (mg/kg) | 10.8 ± 2.1 | 10.5 ± 3.2 | 31.3 ± 12.8 | 15.1 ± 0.4 | 15.7 ± 1 | 3.9 ± 5.1 | 5.8 ± 1.7 |
| Clay (%) | 18.1 ± 4.2 | 6.1 ± 0 | 12.8 ± 6.1 | 5.4 ± 0 | 16.7 ± 1.2 | 51.4 ± 11.1 | 11.4 ± 4 |
| Silt (%) | 9.4 ± 4.6 | 2.0 ± 2 | 4.0 ± 0 | 6.7 ± 3.5 | 10.1 ± 3.1 | 28.7 ± 10.6 | 6.7 ± 2 |
| Sand (%) | 72.5 ± 1.2 | 91.9 ± 2 | 83.2 ± 6.1 | 87.9 ± 3.5 | 73.2 ± 4.2 | 19.9 ± 19.7 | 81.9 ± 3.5 |
| Organic carbon (%) | 3.0 ± 0.5 | 2.0 ± 0.4 | 3.0 ± 1.7 | 1.9 ± 0.4 | 3.6 ± 0.8 | 6.3 ± 2.1 | 2.0 ± 0.3 |
| Sodium (mg/kg) | 486.7 ± 570.6 | 440.0 ± 225.4 | 246.7 ± 91.7 | 230.0 ± 25 | 663.3 ± 20.8 | 171.7 | 476.7 ± 484 |
| Potassium (mg/kg) | 91.7 ± 16.1 | 148.3 ± 36.2 | 75.0 ± 54.1 | 108.3 ± 23.6 | 113.3 ± 2.9 | 475.0 ± 129.9 | 128.3 ± 67.5 |
| Calcium (mg/kg) | 9375 ± 700.4 | 6423.3 ± 909.9 | 5183.3 ± 851.8 | 5221.7 ± 1124.5 | 5083.3 ± 130 | 5535.0 ± 3412.3 | 8706.7 ± 737 |
| Nitrogen (%) | 0.4 ± 0.02 | 0.2 ± 0.02 | 0.3 ± 0.1 | 0.2 ± 0.1 | 0.2 ± 02 | 0.7 ± 0.3 | 0.2 ± 0.01 |
| Carbonates (%) | 104.9 ± 4 | 108.6 ± 1.7 | 109.1 ± 0.6 | 97.0 ± 19.7 | 97.6 ± 9.1 | 13.6 ± 15.6 | 106.0 ± 1.9 |

*EC*, electrical conductivity

richness per sample, Shannon entropy, and Gini-Simpson diversity did not differ between the rainy and dry seasons in sand dunes associated with coconut roots (Student's $t$ test, $P = 0.05$) (Fig. 1).

Phylogenetic analyses of representative AMF sequences from coconut roots revealed 36 groups with $\geq$97% and seven sequences with 95% of similarity forming one new genus-like clade (Fig. 2). The detected AMF sequences belong to five families: Acaulosporaceae (3), Claroideoglomeraceae (1), Diversisporaceae (2), Gigasporaceae (2), Glomeracae (28), and the new genus-like clade (Fig. 2). The family Glomeraceae, followed by Diversisporaceae dominated in the sampled coconut roots (Fig. 3a). The overall community of AMF in coconut roots was mostly represented by *Glomus* species in all seven sites, in both seasons, followed by *Sclerocystis*, *Rhizophagus*, *Redeckera*, *Diversispora*, *Septoglomus*, *Claroideoglomus*, and *Scutellospora* (Fig. 3b). The five most dominant VT in all sites were VT175 (*Glomus*), VT64 (*Septoglomus*), VT156 (*Glomus*), and VT103 (*Glomus*). However, VT185 (*Glomus*), VT103 (*Glomus*), VT100 (*Rhizophagus*), VT69 (*Sclerocystis*), and VT156 (*Glomus*) were the best represented VT in all sites, according to their frequency of occurrence (Table 3).

In coconut samples from established dunes with no management, an average of 9.25 ± 0.5 VT from the rainy and 10.75 ± 3 VT from dry season were detected in composite root samples. The highest number of reads in both seasons in established dunes was recorded in "El Cuyo" with 17,387 sequences in dry season samples and 5231 sequences from the rainy season. The lowest number of sequences was found in Chicxulub with 196 from the dry and 405 from the rainy season (Table 3).

The highest number of VT from dry season samples was found in the managed coconut plantation in the sand dune in "San Crisanto" and in the tropical forest in "Espita," with 16 and 24 VT, respectively. Similarly, the greatest number of VT from the rainy season was found in the sample corresponding to "San Crisanto," with 17 VT. The highest number of sequences was obtained from the "Espita" sample, with 14,406 during the dry season while the lowest number of sequences was found in the "Celestun" samples with 408 sequences and five VT during the rainy season (Table 3).

## Beta diversity in established dunes

Compositions of AMF communities were found to change between dry and rainy seasons in the coastal sand dune environment (PERMANOVA: $F$-value = 3.8, $P$ value = 0.02). The similarity percentage analysis based on Bray-Curtis dissimilarities showed dissimilarity for rainy and dry seasons (Fig. 2S). Virtual taxa 185, VT156, VT64, and VT167 contribute with 17.77, 14.95, 13.72, and 10.79% of dissimilarity between the rainy and dry seasons. The families Diversisporaceae, Gigasporaceae, and Glomeracae were found in both seasons but with different assemblages at the genus and species levels. Out of the 27 VT found in the rhizospheres of the established foredune ecosystem, only seven VT were shared between the rainy and dry seasons. In the dry season, 48.14% (13 VT) were exclusive species while 25.9% (7 VT) were exclusive for the rainy season. The rainy season was represented by 14 VT, and 20 VT represented the dry season. Exclusive and dominant species of the rainy season were VT156, VT159, VT199, VT126, VT53, VT214 (*Glomus*), and VT262

**Table 3** Heat map of AM VT based on a neighbor-joining phylogenetic tree, found in coconut roots in Yucatan, Mexico, during the rainy and dry seasons. Nomenclature according to the classification described at http://www.amf-phylogeny.com

| Family | *Genus* | VT | Rainy season | | | | | | Dry season | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Wetland | Coastal Sand Dune | | | | Sand dune (management) | Coastal Sand Dune | | | | Tropical forest | Sand dune (management) |
| | | | Cel | Cel | Chi | SC | EC | SC | Cel | Chi | SC | EC | Esp | SC |
| Acaulosporaceae | *Acaulospora* | VT026 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.2 | 0.0 |
| Acaulosporaceae | *Acaulospora* | VT027 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.4 | 0.0 |
| Acaulosporaceae | *Acaulospora* | VT047 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 3.4 |
| Claroideoglomeraceae | *Claroideoglomus* | VT055 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.5 | 0.0 |
| Diversisporaceae | *Redeckera* | VT262 | 0.0 | 0.0 | 16.3 | 0.3 | 2.0 | 9.1 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.1 |
| Diversisporaceae | *Diversispora* | VT061 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.4 | 0.0 | 1.3 | 0.6 | 0.0 | 23.8 |
| Gigasporaceae | *Scutellospora* | VT041 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.4 |
| Gigasporaceae | *Scutellospora* | VT049 | 2.7 | 0.2 | 0.0 | 3.0 | 0.0 | 0.7 | 0.3 | 0.0 | 0.0 | 0.0 | 2.2 | 0.6 |
| Glomeraceae | *Glomus* | VT214 | 0.0 | 0.0 | 23.0 | 2.0 | 3.3 | 10.4 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| Glomeraceae | *Glomus* | VT167 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.4 | 3.5 | 77.1 | 1.0 | 0.0 | 0.2 |
| Glomeraceae | *Glomus* | VT248 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 1.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| Glomeraceae | *Glomus* | VT159 | 0.0 | 16.7 | 14.3 | 0.0 | 9.6 | 6.7 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| Glomeraceae | *Glomus* | VT053 | 0.0 | 9.1 | 2.0 | 9.2 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| Glomeraceae | *Glomus* | VT185 | 0.0 | 0.6 | 0.0 | 9.0 | 1.3 | 3.1 | 63.1 | 70.9 | 1.4 | 4.8 | 0.3 | 0.0 |
| Glomeraceae | *Glomus* | VT126 | 31.4 | 3.9 | 0.5 | 1.2 | 7.6 | 2.8 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| Glomeraceae | *Glomus* | VT199 | 0.0 | 3.1 | 1.5 | 1.3 | 9.1 | 2.6 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| Glomeraceae | *Glomus* | VT103 | 0.7 | 0.0 | 0.0 | 24.3 | 0.0 | 4.8 | 1.1 | 4.0 | 15.4 | 0.4 | 11.6 | 1.4 |
| Glomeraceae | *Rhizophagus* | VT113 | 45.3 | 0.0 | 0.0 | 0.0 | 0.0 | 13.7 | 0.3 | 0.7 | 0.0 | 0.0 | 0.2 | 0.0 |
| Glomeraceae | *Glomus* | VT175 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.9 | 58.6 | 2.4 | 7.9 |
| Glomeraceae | *Glomus* | VT093 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 1.1 | 0.0 | 0.0 | 0.2 |
| Glomeraceae | *Septoglomus* | VT064 | 0.0 | 25.1 | 8.7 | 10.1 | 66.0 | 26.9 | 5.6 | 0.0 | 0.0 | 0.0 | 19.4 | 0.0 |
| Glomeraceae | *Glomus* | VT222 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 1.4 | 0.0 | 0.7 | 0.0 | 0.2 | 0.0 |
| Glomeraceae | *Glomus* | VT191 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.3 | 0.5 | 0.0 | 0.0 | 0.5 | 0.0 |
| Glomeraceae | *Glomus* | VT342 | 0.0 | 0.2 | 0.0 | 0.0 | 0.0 | 4.4 | 0.0 | 0.0 | 0.0 | 0.0 | 1.5 | 0.0 |
| Glomeraceae | *Glomus* | VT156 | 19.9 | 41.0 | 32.7 | 39.6 | 0.3 | 13.0 | 0.0 | 0.0 | 0.0 | 0.0 | 2.3 | 0.0 |
| Glomeraceae | *Rhizophagus* | VT100 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 3.5 | 0.0 |
| Glomeraceae | *Glomus* | VT280 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 3.5 | 0.0 |
| Glomeraceae | *Glomus* | VT418 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 4.6 | 0.0 |
| Glomeraceae | *Glomus* | VT121 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 1.1 | 2.5 | 0.0 | 0.6 | 6.0 | 2.0 |
| Glomeraceae | *Sclerocystis* | VT069 | 0.0 | 0.0 | 0.0 | 0.0 | 0.8 | 0.6 | 1.1 | 11.9 | 0.0 | 6.4 | 1.1 | 1.1 |
| Glomeraceae | *Glomus* | VT132 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 4.1 | 0.0 | 0.0 | 2.3 | 0.0 | 11.7 |
| Glomeraceae | *Glomus* | VT130 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 17.1 | 5.4 | 0.0 | 0.7 | 0.0 | 22.6 |
| Glomeraceae | *Glomus* | VT092 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 3.3 | 23.4 |
| Glomeraceae | *Glomus* | VT063 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 3.1 | 0.0 | 0.0 | 5.2 | 10.3 | 1.2 |
| Glomeraceae | *Glomus* | VT146 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 20.1 | 0.0 |
| Glomeraceae | *Glomus* | VT120 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 2.0 | 19.1 | 0.0 | 0.0 |
| Glomeraceae | *Glomeomycotina* | VT New | 0.0 | 0.0 | 1.0 | 0.0 | 0.0 | 0.2 | 0.8 | 0.7 | 0.0 | 0.0 | 5.8 | 0.0 |
| Total | | | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| Reads | | | 408 | 814 | 196 | 4404 | 5231 | 13919 | 785 | 405 | 2152 | 17387 | 14406 | 8040 |
| Richness | | | 5 | 9 | 9 | 11 | 9 | 16 | 15 | 9 | 9 | 13 | 24 | 16 |
| Shannon entropy | | | 3.24 | 4.59 | 5.51 | 5.42 | 3.38 | 9.37 | 3.78 | 2.97 | 2.26 | 3.92 | 11.66 | 6.77 |
| Gini-Simpson | | | 2.91 | 3.71 | 4.66 | 4.10 | 2.17 | 7.23 | 2.31 | 1.91 | 1.61 | 2.56 | 8.58 | 5.43 |

*Cel*, Celestún; *Chix*, Chicxulub; *SC*, San Crisanto; *EC*, El Cuyo; *Esp*, Espita

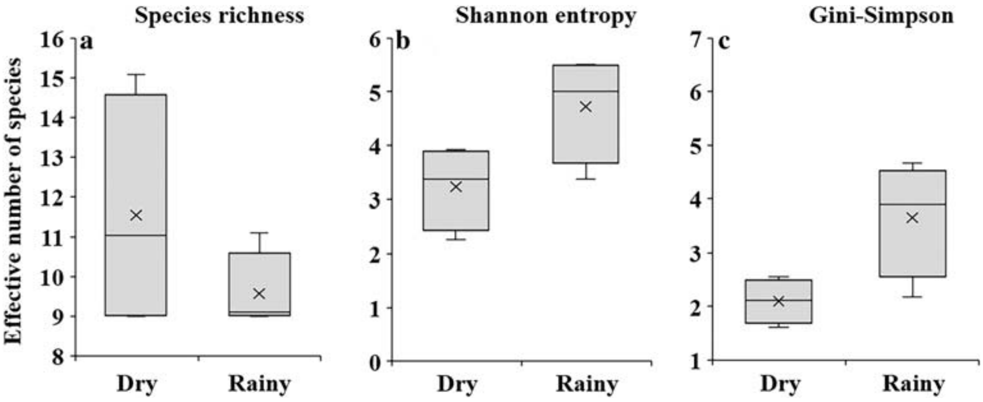


**Fig. 1** Effective number of species (true diversity) of arbuscular mycorrhizal fungi virtual taxa of coastal sand dunes in rainy and dry seasons. **a** Species richness. **b** Shannon entropy. **c** Gini-Simpson. Solid lines inside the box indicate medians, x represents the mean, and boxes and whiskers indicate quartiles and ranges, respectively

(*Redeckera*). In the dry season, exclusive and dominant species were VT175, VT120, VT167, VT63, and VT132 (*Glomus*). Virtual taxon 69 (*Sclerocystis*) is present in both seasons but it is clearly dominant in dry season samples, with more than 20 times the relative abundance when compared with the rainy season samples. Conversely, the relative abundance of VT185 (*Glomus*) is three times higher and VT64 (*Septoglomus*) is more than 10 times higher in the rainy season than in the dry season (Table 3).

### Morphological glomerospore diversity associated with coconut

A total of 1680 glomerospores were isolated from coconut tree rhizospheres from all studied sites (Table 4). We found 22 morphospecies with 9 genera of AMF: *Acaulospora* (3), *Claroideoglomus* (1), *Dominikia* (1) *Gigaspora* (2), *Glomus* (4), *Rhizophagus* (1), *Racocetra* (1), *Septoglomus* (1), and *Sclerocystis* (2) (Table 4; Fig. 3S). The highest richness in glomerospores was found in "San Crisanto" and "Espita," both with management, while the lowest richness was observed in samples from "Chicxulub" and "Celestun" in established foredunes (Table 4).

## Discussion

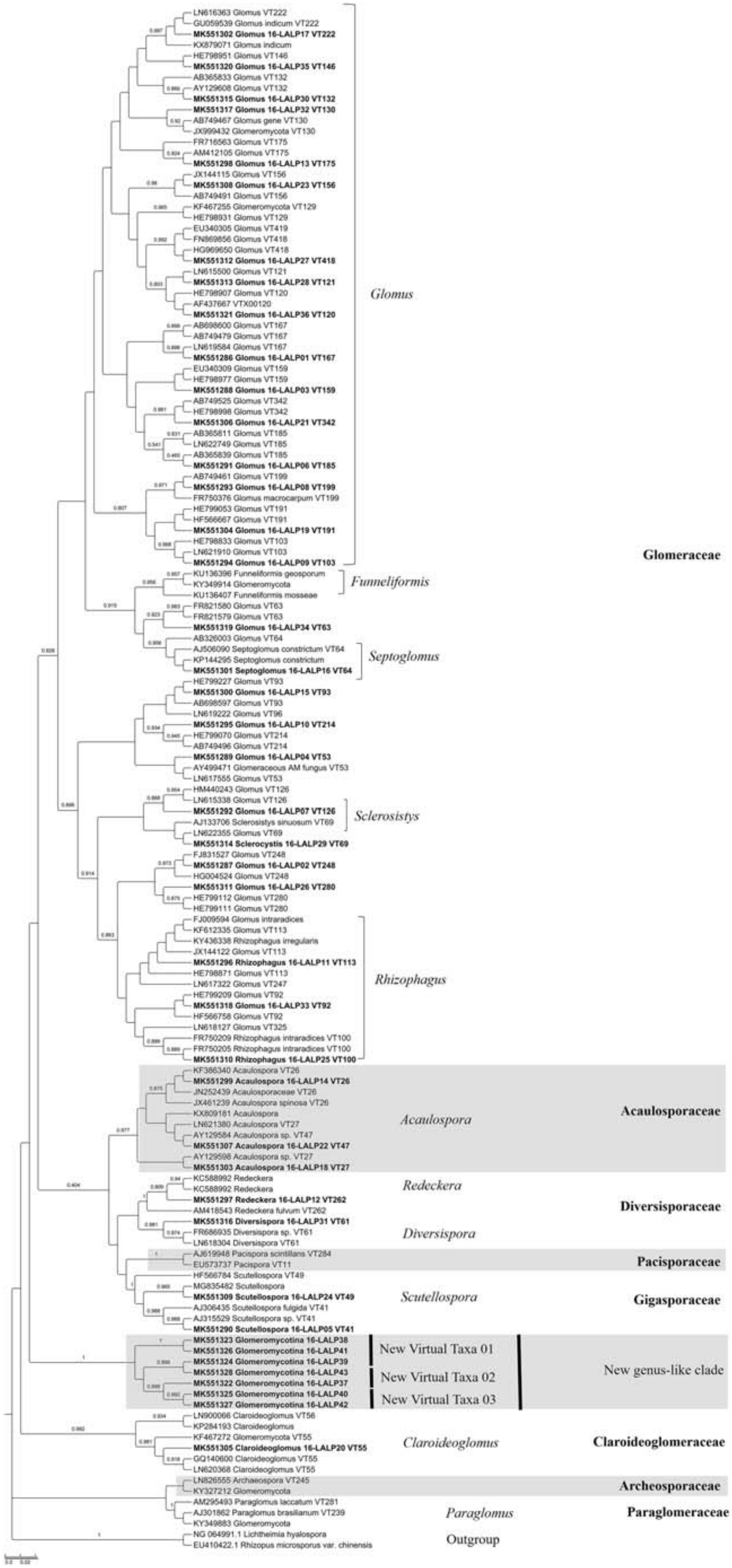
Our study reveals the existence of a high number of AMF species associated with naturalized coconut roots when compared with the richness of AMF communities recorded in sand dunes in Mexico. The results also highlight the significant interseasonal differences in the composition of AMF communities. Coconut hosts common generalist AMF species but also hosts rare native species in their growing sites in Yucatan, Mexico. Glomeraceae was the dominant family colonizing roots and in glomerospore counts, in all sites. The applied molecular and morphological approach enhanced the analyses of the diversity of AMF, including the detection of a new virtual taxa clade.

Coconut germplasm has a long history in Mexico since it was first introduced, and populations are well established in native soils of the coasts of the Pacific Ocean, the Gulf of Mexico, and the Yucatan Peninsula (Zizumbo-Villarreal et al. 2008). The success of the establishment of *C. nucifera* outside of its natural regions could have been a result of its mutualistic interaction with AMF, since this is one of the main factors influencing the outcome of a plant's naturalization (Pringle et al. 2009; David et al. 2000). The facultative mycorrhizal nature of coconut may enable it to use different ecological strategies to successfully spread, resulting in a wide distribution of the species (Menzel et al. 2017). This interaction between native AMF and newly introduced coconut plants could increase the tolerance of the plants to the typical low nutrient availability, low content of organic matter, and high salinity of tropical sand dunes (Martínez and Psuty 2004). Coconut plants growing in Yucatan can associate with a diverse number of generalist AMF and also show the required flexibility to associate with local fungi.

Rarefaction curves with the number of VT reveal that our sampling method was effective to detect the majority of AMF colonizing coconut roots in growing areas in Yucatan, but need more sampling effort during the rainy and dry seasons. The combination of the molecular and morphological approaches allowed us to find a total of 11 genera: *Acaulospora*, *Claroideoglomus*, *Diversispora*, *Glomus*, *Redeckera*, *Rhizophagus*, *Sclerocystis*, *Septoglomus*, *Gigaspora*, *Racocetra*, and *Dominikia.* The first eight genera were identified based on the DNA sequences, while the latter three were identified using glomerospore morphology, with six overlapping species: A*caulospora*, *Diversispora*, *Glomus*, *Sclerocystis*, *Septoglomus*, and *Rhizophagus*. The VT found include seven phylotypes that form a single clade within the Glomeromycotina, and can be considered a new virtual taxon (Öpik et al. 2010).

The total AMF richness (37 virtual taxa, 22 morphospecies) associated with naturalized coconut reported in the present study is high when compared with the 14 AMF species recorded for the same coastline (Ramos-Zapata et al. 2010) and with the six species identified in coastal sand dunes of

**Fig. 2** Neighbor-joining phylogenetic tree of representative sequences of arbuscular mycorrhizal fungi virtual taxa associated with coconut roots at family level (GenBank accession numbers: MK551286-MK551328) and reference sequences from MaarjAM database (Öpik et al. 2010) and International Nucleotide Sequences Database. NCBI (www.ncbi.nlm.nih.gov). Bootstrap support values > 50 (999 iterations) are shown



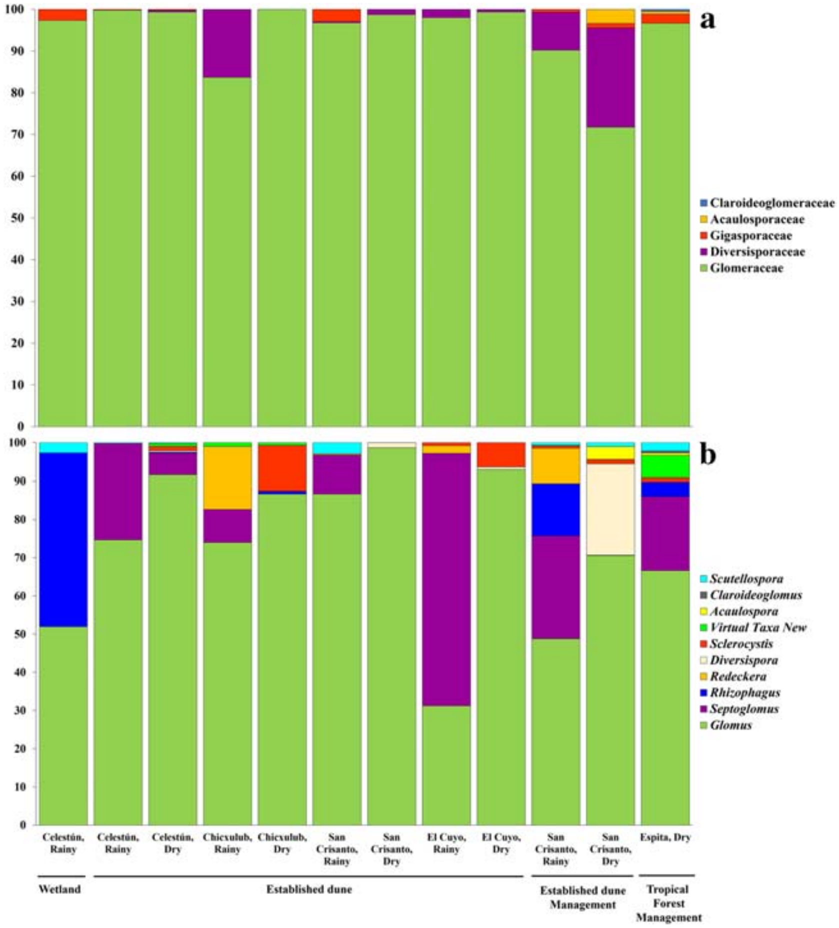


**Fig. 3** Relative abundance (%) of the community structure of the arbuscular mycorrhizal fungi virtual taxa at family (**a**) and genus level (**b**) based on a neighbor-joining phylogenetic tree and nomenclature according to the classification described at http://www.amf-phylogeny.com

Baja California (Sigüenza et al. 1996). Previous high AMF richness values in the local area have been found in mangroves of the coast, associated with *Conocarpus erectus* L. (13 species) and in coastal wetland, associated with *Acoelorraphe wrightii* (Griseb. & H. Wendl.) H. Wendl. ex Becc. with 22 AMF species (Ramírez-Viga et al. 2019). Our results using DNA sequences also were high when compared with the grass species *Spinifex sericeus* R. Br., native to coastal sand dunes in New Zealand for which 22 OTUs were recorded (Johansen et al. 2015). In Yucatan coastline, the highest AMF richness recorded in conserved sand dunes is seven species, and only two species in degraded areas (Guadarrama et al. 2012). Based on the analyses of glomerospore morphology associated with the coconut rhizosphere presented herein, the lowest AMF richness found in a single site was 11 species (Chicxulub), while the highest richness was 17 species, in San Crisanto.

*Glomus* was the dominant genus in coconut root systems in regard to both the number of VT and sequences, which is in accordance with previous field studies (Öpik et al. 2013a; Öpik et al. 2013b; Davison et al. 2015). Here, we found that the dominant AMF in coconut roots are widespread, generalist species, as also found for the alien palm *Trachycarpus fortune* (Hook.) H. Wendl. (Moora et al. 2011). Virtual taxon 64 (related to *Septoglomus constrictum*), VT185 (*Glomus*), VT132 (*Glomus*), VT156 (*Glomus*), and VT113 (related to *Rhizophagus irregularis*) were the dominant VT in *C. nucifera* roots along the coastline, wetland, and tropical forest. These VT have a wide distribution, from natural to anthropogenic ecosystems, and have been associated with a variety of host plants around the world (Öpik et al. 2010). *Rhizophagus* VT113 is known to be a generalist and a dominant species across continents and ecosystems (Moora et al. 2011). The species *S. constrictum* has been associated with coconut in India (Rajeshkumar et al. 2015), as well as in the present study. *Septoglomus constrictum* is the only known species associated with the rhizosphere of coconut in Mexico that was designated as a new species of glomerospore and was found in Veracruz, Mexico (Trappe 1977). The species with the widest distribution across all study sites were VT103 (*Glomus*), VT69 (related to *Sclerocystis sinuosa*), VT185 (*Glomus*), VT49 (related to *Gigaspora/Scutellospora*), and VT121 (*Glomus*). Our findings suggest the existence of undescribed AMF species associated with coconut roots, highlighting the importance of morphological and molecular studies in these environments, especially in poorly sampled regions in Mexico which is considered a hotspot of biodiversity (Myers et al. 2000). Overall, sand dunes have an

**Table 4** Relative glomerospore abundance (%) (mean of three field sample replicates) isolated from coconut rhizosphere soil in Yucatan, Mexico, in wetland, coastal sand dune, and tropical dry forest from the dry season

| AMF species | Wetland | Costal sand dune | | | | Sand dune (management) | Tropical dry forest |
|---|---|---|---|---|---|---|---|
| | Cel | Cel | Chix | SC | EC | SC | Esp |
| Acaulosporaceae | | | | | | | |
| *Acaulospora rehmii* Sieverd. & S. Toro 1987 | 3.5 | 1.7 | 5.8 | 6.1 | 2.7 | 11.9 | 10.9 |
| *Acaulospora spinosa* C. Walker & Trappe 1981 | 0.9 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| *Acaulospora* sp.1 | 18.4 | 18.2 | 18.4 | 0.0 | 0.0 | 0.0 | 3.6 |
| *Acaulospora* sp.2 | 0.9 | 0.0 | 0.0 | 0.3 | 0.0 | 0.0 | 2.3 |
| *Acaulospora scrobiculata* Trappe 1977 | 12.3 | 9.9 | 8.7 | 9.6 | 3.6 | 16.0 | 19.9 |
| Diversisporaceae | | | | | | | |
| *Diversispora* sp. | 2.6 | 0.4 | 4.9 | 0.0 | 0.0 | 4.9 | 0.0 |
| Gigasporaceae | | | | | | | |
| *Gigaspora* aff. *gigantea* (T.H. Nicolson & Gerd.) Gerd. & Trappe 1974 | 0.0 | 1.7 | 0.0 | 3.4 | 3.2 | 3.0 | 3.6 |
| *Racocetra sp.* | 0.0 | 0.0 | 0.0 | 0.0 | 1.4 | 2.2 | 0.0 |
| Glomeraceae | | | | | | | |
| *Dominikia aurea* (Oehl & Sieverd.) Błaszk., Chwat, G.A. Silva & Oehl | 0.0 | 0.0 | 0.0 | 0.0 | 3.6 | 0.0 | 3.6 |
| *Glomus glomerulatum* Sieverd. 1987 | 0.0 | 0.0 | 0.0 | 9.9 | 0.0 | 6.3 | 4.1 |
| *Glomus* sp. 1 | 2.6 | 2.5 | 24.8 | 7.2 | 1.4 | 2.2 | 1.8 |
| *Glomus* sp. 2 | 19.3 | 0.4 | 15.5 | 0.3 | 1.4 | 5.2 | 8.6 |
| *Glomus* sp. 3 | 10.5 | 0.0 | 0.0 | 0.3 | 18.0 | 3.4 | 4.1 |
| *Glomus* sp. 4 | 0.9 | 0.0 | 0.0 | 18.1 | 0.5 | 6.3 | 3.6 |
| *Rhizophagus intraradices* (N.C. Schenck & G.S. Sm.) C. Walker & A. Schüßler 2010 | 0.9 | 2.5 | 0.5 | 2.0 | 3.6 | 3.0 | 1.8 |
| *Rhizophagus irregularis* (Błaszk., Wubet, Renker & Buscot) C. Walker & A. Schüßler 2010 | 0.0 | 0.4 | 0.0 | 7.8 | 2.3 | 0.7 | 3.6 |
| *Glomus microaggregatum* (Koske, Gemma & P.D. Olexia) Sieverd., G.A. Silva & Oehl 2015 | 5.3 | 3.7 | 2.9 | 2.0 | 19.8 | 6.3 | 2.3 |
| *Sclerocystis rubiformis* Gerd. & Trappe 1974 | 10.5 | 27.3 | 6.3 | 17.1 | 24.8 | 1.1 | 2.7 |
| *Sclerocystis* aff. *taiwanensis* C.G. Wu & Z.C. Chen 1987 | 0.0 | 1.7 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| *Sclerocystis sinuosa* Gerd. & B.K. Bakshi 1976 | 5.3 | 14.0 | 5.8 | 15.0 | 8.6 | 0.4 | 18.1 |
| *Septoglomus constrictum* (Trappe) Sieverd., G.A. Silva & Oehl 2011 | 6.1 | 15.7 | 0.0 | 0.0 | 0.0 | 10.1 | 4.1 |
| Total | 228 | 242 | 206 | 293 | 222 | 268 | 221 |
| Richness | 15 | 14 | 11 | 15 | 15 | 17 | 18 |
| Shannon entropy (Hill 1) | 8.1 | 6.6 | 7.5 | 8.3 | 7.1 | 9.3 | 12.1 |
| Gini-Simpson (Hill 2) | 10.0 | 8.3 | 8.7 | 9.8 | 9.5 | 12.0 | 14.6 |

AMF, arbuscular mycorrhizal fungi; *Cel*, Celestún; *Chix*, Chicxulub; *SC*, San Crisanto; *EC*, El Cuyo; *Esp*, Espita

important richness of AMF (199 species), representing 44% of the 288 species described worldwide (Öpik and Davison 2016; Stürmer et al. 2018) among which more than 40% have been described from dune systems (Sridhar and Beena 2001). It is possible that new species await description in sand dunes around the world.

In the present study, AMF communities associated with coconut roots in established foredunes showed marked differences between the rainy and dry seasons, with similar richness associated with the dry season, while the rainy season was characterized by high dominance. Virtual taxa 185, VT156, VT167 (*Glomus*), and VT64 (*Septoglomus constrictum*) contribute with 54% of the interseasonal dissimilarities. These results suggest that the colonization of coconut roots is a dynamic phenomenon related to seasonality. Seasonal variation in root colonization and glomerospores in the rhizosphere have been observed to occur in coastal sand dunes in several countries around the world. For instance, a 2-year study in Spain recorded a higher glomospore frequency during the spring than in autumn, while winter and summer did not show differences (Guillén et al. 2019). In Portugal, Rodríguez-Echeverría et al. (2008) found a clear peak of sporulation during autumn associated with *Ammophila arenaria.* In Mexico, Sigüenza et al. (1996) showed seasonal shifts in colonization and spore abundances with higher percentages in summer than in spring. In Brazil, Stürmer and Bellei

(1994) found that the number of spores of *G. constrictum*, *Glomus etunicatum*, *Acaulospora* sp. associated with *Spartina ciliata* reached a maximum abundance during the winter, whereas *Gigaspora albida* did so in the spring.

In the present study, the mean density of spores in all seven growing areas ranged from 206 to 293 per 20 g of soil. Similar numbers of spores (213–298) were found in coconut plantations cultivated along with intercrops in 50 g soil samples in India (Rajeshkumar et al. 2015). In coconut cropping systems, the fungal spore density ranged from 40 to 154 spores and when in association with Areca Nut, glomerospore density ranged between 33 and 44 spores per 10 g of soil (Ambili et al. 2012). In this study, the dominant species identified from soil sieving were *Sclerocystis sinuosa*, *Sclerocystis rubiformis*, *Glomus microaggregatum*, and *Acaulospora scrobiculata*. So far, AMF richness associated with the rhizosphere of *C. nucifera* had only been studied in India based on glomerospore morphology, with 47 recorded species (Ramesh 1984; Sosamma et al. 1998; Ambili et al. 2012; Rajeshkumar et al. 2015). These studies and ours reveal a similar dominance of the Glomeraceae family in VT colonizing roots and glomerospores in rhizosphere soil. The present study provides seven new records of glomerospore species associated with coconut rhizospheres (*Acaulospora rehmii*, *Acaulospora spinosa*, *Dominikia aureum*, *Glomus glomerulatum*, *Rhizophagus intraradices*, *Rhizophagus irregularis*, *Glomus microaggregatum*) raising the number of known species in association with coconut roots to a total of 54. *Glomus indicum* (VT222) has been identified by the analysis of DNA sequences but not in the form of resting spores in the rhizosphere of coconut roots. This species constitutes a new record in Mexico (Varela et al. 2019).

Coconut root samples in this work included four sites of established sand foredunes in rainy and dry seasons. In sand dunes, the dominance of the Glomeraceae family inside and outside roots prevailed, which agrees with the richness of glomerospores observed along the same coastline (Carmona-Escalante et al. 2013). Previous research, conducted at a larger biogeographical scale, indicates that Gigasporaceae and Glomeraceae co-dominate in sand dunes worldwide (Stürmer et al. 2018). However, in America, Gigasporaceae species are thought to be dominant, based on samples taken along the Atlantic Coast of the USA and Brazil (Stürmer et al. 2018). Thus, the observed dominance of Glomeraceae colonizing coconut roots may not reflect the actual diversity in sand dunes in the Yucatan coastline. A wider richness should be expected to be retrieved from soil samples, as host plants exhibit seasonal variation (Berruti et al. 2018). In this sense, field samples and AMF bait cultures have confirmed that tropical coastlines harbor up to 54 species of AMF, based on spore morphology (da Silva et al. 2015; de Assis et al. 2016).

In conclusion, this study provides the first assessment of the AMF community colonizing coconut roots. Our results indicate that molecular and morphological approaches are complementary techniques to investigate the diversity of AMF coexisting in association with coconut plants. Our study reveals a high number of AMF species, and the dominant genera colonizing roots are *Glomus*, *Septoglomus*, and *Rhizophagus*, while the most dominant, as far as glomerospore counts, were *Sclerocystis*, *Glomus*, and *Acaulospora.* Additionally, coconut roots associate mainly with generalist AMF species but also with rare species that had previously not been recorded in natural environments. Coconut samples from sand dunes also revealed a high number of VT and exhibited seasonal differences in composition.

Further research efforts should be directed towards evaluating AMF diversity in naturalized or invasive coconut at a broader scale and in different environments, in order to better understand its distribution and shifts in AMF community composition. The results presented here are a useful baseline for the selection of AMF for investigations to find a positive effect on growth and development of coconut. This would allow the use of appropriately selected AMF in sustainable agriculture and could have a high impact in countries where the cultivation of coconut has high economic importance, such as the Philippines, Indonesia, and India.

**Acknowledgments** We are in a great debt to Juan Humberto Coronado Navarro for providing logistic support and collecting in the field.

**Author contribution** LALP and LS designed and conducted the experiments, analyzed the data, and wrote the manuscript. I O-O conducted the statistical analysis and edited the manuscript. I C-L conducted experiments, H E-M conducted the soil analysis, analyzed the data, and edited the manuscript. A O-S and E G-C analyzed the data and edited the manuscript.

**Funding information** LALP wishes to thank the Consejo Nacional de Ciencia y Tecnología (CONACYT), Mexico, for its financial support through a postdoctoral scholarship. This work was partially supported by FORDECYT 296195 and used infrastructure acquired through funding of the project CONACYT 269833.

## Compliance with ethical standards

**Conflict of interest** The authors declare that they have no conflicts of interest.

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