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Host identity and functional traits determine the community composition of the arbuscular mycorrhizal fungi in facultative epiphytic plant species

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ABSTRACT

The epiphytic vascular mycobiota is scarce and facultative in semi-arid Mediterranean ecosystems. However, unlike in soil conditions, little is known about the factors driving mycorrhizal communities in epiphytic environments. Here, we investigated the arbuscular mycorrhizal fungi (AMF) harboured by 31 plant species occurring on the trunks of *Phoenix dactylifera*. We wanted to ascertain if host identity and plant functional traits shape mycorrhizal communities. Specifically, we tested the plant life-cycle (perennial versus annual), the plant life-form (herbaceous versus woody), the plant origin (exotic versus native) and the plant species. The plant affiliation to species strongly influenced the AMF community composition. Plant life-form and plant life-cycle also shaped indicator taxa. The AMF structure differed between annual and perennial species and higher AMF richness was detected in perennial plants. The epiphytic plants associated with AMF irrespective of whether they were native or not, probably because here no functional differences derive from plant origin.

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1. Introduction

Epiphytic habitats are considered as extreme plant environments due to the large fluxes of temperature and low water and nutrient availability to which they are subjected. In these conditions, symbiotic associations such as mycorrhizal symbiosis could be crucial because of their widely demonstrated role in plant nutrient and water uptake in terrestrial habits, with particular importance in stressful environments (Smith and Read, 2008). Furthermore, the availability of compatible and suitable mycorrhizal fungi might be a key factor constraining the development and distribution of epiphytic plants and *vice versa*.

In semi-arid Mediterranean ecosystems the epiphytic vascular mycobiota is scarce and facultative or accidental, mainly occurring on the trunks of certain palm species. Here, the microhabitat conditions that originate in the cut leaves formed by the pruning of dead or old leaves allow water and debris accumulation, which enables occasional plant establishment (Torrecillas et al., 2013; Zotz, 2013).

Since most vascular epiphytic plants are ferns and their relatives or monocots (Freitas et al., 2016), the mycorrhizal condition, specificity and dependence have, therefore, mainly been studied in these taxonomic groups. Most studies concerning epiphyte-mycorrhiza relationships have been conducted in humid tropical habitats and involve orchids, which form mycorrhizas with basidiomycetes (Otero et al., 2004, 2007; Martos et al., 2012), or other species that form arbuscular mycorrhizas, like those of the Araceae, Clusiaceae, Bromeliaceae, or Begoniaceae (Maffia et al., 2000; Rains et al., 2003; Rowe and Pringle, 2005). However, very little is known about the facultative epiphytic plant species which grow under semi-arid Mediterranean conditions. Only the widespread *Sonchus tenerrimus* has been reported to establish a symbiotic relationship with arbuscular mycorrhizal fungi (AMF), belonging to the Glomeromycota (Torrecillas et al., 2013).

The mycorrhizal status of epiphytic plants and the factors

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driving symbiotic communities in epiphytic habitats is a topic of interest, which has not been investigated in depth.

There is evidence that AMF communities in terrestrial habitats depend on host-plant preference (Alguacil et al., 2011; Martínez-García and Pugnaire, 2011; Torrecillas et al., 2012b), but the specificity in biotic interactions has been suggested to be mediated by plant functional traits rather than plant identity (Scheublin et al., 2004; Lopez-García et al., 2017). Plant life-cycle, for instance, may explain differences in AMF communities (Alguacil et al., 2012; Torrecillas et al., 2012a). It is likely that differences in AMF communities are due not only to plant life-cycle but also to a combination of factors such as life-form, physiology or host origin (native vs exotic). It is expected that native plant species have co-evolved together with local AMF and thus their mycorrhizal communities would be different and more diverse than those of exotic plants in a particular environment. Some studies have demonstrated that the AMF associated with exotic plant invaders shift in both abundance and community composition, compared with native species (Mummey et al., 2005; Hawkes et al., 2006; Busby et al., 2012; Bunn et al., 2015), but, as previous reports show, the AMF communities of epiphytic plants are different from those of the surrounding soil habitats (Martos et al., 2012; Torrecillas et al., 2013). These results suggest that facultative epiphytic plants from semi-arid sites might support distinctive AMF communities shaped by particular plant traits.

In this study we investigated facultative epiphytic plant species occurring on the trunks of *Phoenix dactylifera* trees cultivated in orchards under Mediterranean semi-arid conditions, and we assessed the arbuscular mycorrhizal communities they harbored. We wanted to ascertain if: (1) there are AMF in plants growing epiphytically; and (2) AMF host identity and plant functional traits shape these mycorrhizal communities. Specifically, we tested the plant life-cycle (perennial versus annual), the plant life-form (herbaceous versus woody), the plant origin (exotic versus native) and the plant species as driving factors.

2. Material and methods

2.1. Study area and sampling

The study area was located at the Historic Palm Grove of Elche (Alicante), southern Spain (38º15′ 51, 28″N, 0° 41′ 51, 23″ W, 82 m.a.s.l). It covers 550 ha and contains about 180,000 adult, *P. dactylifera* date palms aged 40–60 y, planted in one or two rows in cultivated, rectangular-shaped orchards (Rivera et al., 2014). The climate is Mediterranean semi-arid, with a mean annual temperature of 17.7 °C and a mean annual rainfall of 266 mm (www.aemet.es). Three sampling plots separated at least 300 m from each other, and each consisting of two palm orchards (2.000 m^2 area, 250 palm trees ha^{-1}), were selected. A total of 31 plant species growing epiphytically between 1.5 and 2 m height on date palm trunks were sampled at the spring growing season (three individuals of each plant species, one per plot, total 93 individuals). Plant species were classified according to their origin into native or exotic (autochthonous or allochthonous), according to their life cycle into annual or perennial and according to life-form into herbaceous or woody. Canopy or bare-limb epiphytes were not found. Root systems were collected, fine foots were separated, briefly rinsed, quickly dried on paper and used for molecular analysis.

2.2. AMF colonization

To assess AM colonization, roots were cleared with 10% KOH and stained with 0.05% trypan blue (Phillips and Hayman, 1970). The percentage of root length colonized by AM fungi was calculated by the gridline intersect method (Giovanetti and Mosse, 1980). Positive counts for AM colonization included the presence of vesicles, arbuscules or mycelium within the roots.

2.3. Roots DNA extraction and PCR

DNA extractions from 93 samples (one root sample per replicate for each epiphytic plant species) were carried out. For each sample, 0.1 g fresh root material was frozen with liquid nitrogen, placed into a 2 ml screw-cap propylene tube together with two tungsten carbide balls (3 mm) and ground (3 min, 13000 r.p.m.) using a mixer mill (MM 400, Retsch, Haan, Germany). Total DNA was extracted using a DNeasy Plant Mini Kit following the manufacturer's recommendations (Qiagen). The extracted DNA was resuspended in 20 μl of water and stored at −20 °C.

Several dilutions of extracted DNA (1/10, 1/50, 1/100) were prepared and 2 μl were used as template. Partial small subunit (SSU) ribosomal RNA gene fragments were amplified using nested PCR with the universal eukaryotic primers NS1 and NS4 (White et al., 1990). PCR was carried out in a final volume of 25 μl using PuReTaq™ Ready-To-Go PCR beads (Amersham Pharmacia Biotech), 0.2 μM dNTPs and 0.5 μM of each primer (PCR conditions: 94 °C for 3 min, then 30 cycles at 94 °C for 30 s, 40 °C for 1 min, 72 °C for 1 min, followed by a final extension period at 72 °C for 10 min).

Then, 2 μl from the first PCR were used as template DNA in a second PCR reaction performed using the specific primers AML1 and AML2 (Lee et al., 2008). PCR reactions were carried out in a final volume of 25 μl using the PuReTaq™ Ready-To-Go PCR beads (Amersham Pharmacia Biotech), 0.2 μM dNTPs and 0.5 μl M of each primer (PCR conditions: 94 °C for 3 min, then 30 cycles of 1 min denaturation at 94 °C, 1 min primer annealing at 50 °C and 1 min extension at 72 °C, followed by a final extension period of 10 min at 72 °C). Positive and negative controls using PCR positive products and sterile water respectively were also included in all amplifications. All the PCR reactions were run on a Perkin-Elmer Cetus DNA Thermal Cycler. Reaction yields were estimated by using a 1.2% agarose gel containing GelRed™ (Biotium).

2.4. Cloning and sequencing

The PCR products of the expected band length, approximately 795 bp were purified using a Gel extraction Kit (Qiagen) cloned into pGEM-T Easy vector (Promega) and transformed into *Escherichia coli* (Xl1 blue). Putative positive transformants were screened in each resulting SSU rRNA gene library, using 0.7 units of RedTaq DNA polymerase (Sigma) and the supplied reaction buffer in a final volume of 25 μl and re-amplification with AML1 and AML2 primers with the same cycling conditions described above. Product quality and size were checked in agarose gels as described above. All clones having inserts of the correct size (795 bp) in each library were sequenced using the universal primers SP6 and T7 by Laboratory of Sistemas Genómicos (Valencia, Spain).

2.5. Phylogenetical analysis

Sequence editing was done using the program FinchTV 1.4.0 (Geospiza, Inc.; Seattle, WA, USA; http://www.geospiza.com). A search for similar sequences to the ones from this study was conducted with the BLAST tool (Zhang et al., 2000) provided by GenBank. Phylogenetic analysis was carried out on the sequences obtained in this study and those corresponding to the closest matches from GenBank and referenced to the MaarjAM database

(Öpik et al., 2010) as well as sequences from cultured AMF taxa including representatives of the major taxonomical groups described by Redecker et al. (2013).

All the sequences were aligned, using the multiple sequence comparison program, MAFFT, version 7.0 (available at http://align.bmr.kyushu-u.ac.jp/mafft/software) and the alignment was adjusted manually in BioEdit software version 7.2.5. (Hall, 1999). The program CHIMERA_CHECK 2.7 (Ribosomal Database Project II; http://rdp.cme.msu.edu) was used to check for chimeric artifacts among the 18S rDNA sequences.

Maximum likelihood (ML) phylogenetic tree inference was performed with MEGA software (version 5.05) (Tamura et al., 2011). Nucleotide data files were first tested to find the best DNA evolution model. The general time reversible model with a discrete gamma distribution showed the lowest Bayesian information criterion (BIC) scores and was deemed to best describe the nucleotide substitution pattern. Initial trees for the heuristic search were obtained by applying the neighbour-joining method to a matrix of pairwise distances estimated using the maximum composite likelihood (MCL) approach. The robustness of all trees obtained was evaluated by 1000 bootstrap replications. *Endogone pisiformis* and *Mortierella polycephala* were used as the out-groups.

Different AMF sequence types or OTUs (operational taxonomic units), were defined as groups of closely related sequences, with a high level of bootstrap support in the phylogenetic analyses (higher than 80%) and sequence similarity $\geq$ 97%.

2.6. Statistical analysis

The number of clones for each AMF OTU in each plant species was used to calculate the rarefaction curves. The rarefaction curves were produced by plotting the number of OTUs observed against the number of sequences obtained using the freely available Analytic Rarefaction software (version 1.3) (http://www.uga.edu/~strata/software/anRareReadme.html).

To ascertain whether the composition and structure of AMF communities were significantly affected by the experimental factors (plant origin, life-form, life cycle and plant species) a permutational multivariate analysis of variance (perMANOVA) was performed with the adonis function in vegan (Oksanen et al., 2016) using the Jaccard distance matrix and 999 permutations.

Three different indicator species analyses (ISA) were conducted on the OTUs presence/absence dataset using the “indicspecies” package implemented in R (De Cáceres and Legendre, 2009). This analysis allows identification of species which are associated with a classifier factor by calculating an Indicator Value (IndVal) (Dufrêne and Legendre, 1997). We considered the plant origin, life-form and life cycle as classifier factors. The statistical significance of the indicator values was tested using a permutation test with 999 permutations.

The number of clones detected for each OTU (Table S1) was used to calculate the Shannon-Weaver diversity index (H’). The influence of the plant origin, life cycle and life-form on S and H′ was determined by a two-way ANOVA analysis and a *post hoc* Tukey-HSD test. To avoid bias in analysis due to differences in sequencing effort between samples, the number of sequences per sample was rarefied to the lowest one (37). This procedure was performed with the vegan package for R (R.Core Team, 2016).

Nucleotide sequence accession numbers. A total of 109 representative sequences of OTUs from root samples generated in this study have been deposited at the National Centre for Biotechnology Information (NCBI) GenBank (http://www.ncbi.nlm.nih.gov) under the accession numbers MG835459-MG835567.

3. Results

3.1. Morphological analysis

Microscopic examination of stained root samples from all epiphytic plants was performed and mycorrhizal fungal structures (vesicles and/or arbuscles) were detected in all cases, colonization ranging from 10 to 25% (data not shown).

3.2. PCR and sequence analysis

Arbuscular mycorrhizal fungal DNA was successfully amplified from 93 root samples (corresponding to 31 facultative epiphytic species) (Table 1) by nested PCR with the primer combination AML1/AML2, and generated PCR products of the expected band of approximately 795 bps, which were used for cloning and creating the clone libraries. This implies that 100% of samples contained amplifiable AMF DNA. Ninety-three clone libraries were created. We screened 2790 clones in total (30 clones were analyzed per library); out of these, 1992 clones contained an SSU rDNA fragment and, subsequently, were sequenced. The BLAST search revealed that 1694 sequences had a high degree of similarity (97–100% identity) to sequences from AMF taxa and belonged to members of the phylum Glomeromycota. The rest of the sequences showed BLAST similarity to plants.

Representative sequences of OTUs from root samples of epiphytic species were submitted to the GenBank database (Fig. 1).

3.3. Phylogenetic analysis of AMF groups

After phylogenetic analyses of the sequences, 23 AMF OTUs were detected (Fig. 1; Tables S1 and S2.). Sequences of the families Glomeraceae (6 OTUs), Diversisporaceae (3 OTUs), Gigasporaceae (1 OTU), Claroideoglomeraceae (5 OTUs), Paraglomeraceae (7 OTUs), and Archaeosporaceae (1 OTU) were obtained. Eleven groups of AMF sequences or OTUs - namely *Glomus macrocarpum* (Glo1), *Septoglomus constrictum* (Sep), *Funneliformis mosseae-fragilistratum-caledonium-geosporum-coronatum* group (Fu), *Sclerocystis sinuosa* (Sc), *Rhizophagus intraradices-irregularis-fasciculatus* group (Rh), *Diversispora spurca-aurantia-eburnea* group (Div1), *Redeckera fulvum* (Red), *Scutellospora aurigloba-callospora* group (Scut), *Claroideoglomus luteum-claroideum-lamellosum-etunicatum* group (Cl2), *Paraglomus laccatum-occultum-brasilianum* group (Pa3), and *Archaeospora schenckii-trappei* group (Arch) - clustered with previously identified AMF sequences. Seven OTUs (Glo2, Div2, Cl3, Cl4, Cl5, Pa5, Pa7) did not cluster with any known Glomeromycota sequence. The remaining five OTUs (Cl1, Pa1, Pa2, Pa4, Pa6) were Glomeromycota not related to any sequences in the database.

3.4. Effect of plant origin, life-cycle, life-form and plant species on the composition and diversity of AMF communities

To determine whether the number of clones sequenced was sufficient to represent the AMF diversity in all the facultative epiphytic species, rarefaction curves were constructed (Fig. 2). With the clones sequenced for each root sample, we covered perfectly the diversity of the AMF communities in all the plant species studied, since there was a well-defined leveling-off of all curves, and it is highly unlikely that the sequencing of more clones would have revealed more OTUs.

An indicator species analysis (ISA) was conducted to find specific OTUs associated with plant origin, life-cycle, and life-form (Table 2). When the ISA was performed considering the plant origin as the grouping factor, two OTUs were associated with the exotic group

Table 1
Plant species growing as facultative epiphytes on date palm trees (*Phoenix dactylifera*) at the Historic Palm Grove of Elche (Alicante, Spain).

Species	Abb	Life Form	Life Cycle	Origin
Asparagus acutifolius L.	Aa	Woody	Perennial	Native
Asparagus densiflorus (Kunth)	Ad	Woody	Perennial	Exotic
Asphodelus fistulosus L.	Af	Herbaceous	Perennial	Native
Brachychiton populneus (Schott & Endl.) R. Br.	Bp	Woody	Perennial	Exotic
Brachypodium distachyon (L.) P. Beauv.	Bd	Herbaceous	Perennial	Native
Centaurium spicatum (L.) Fristch.	Cs	Herbaceous	Annual	Native
Chenopodium murale L.	Cm	Herbaceous	Annual	Native
Conyza bonariensis (L.) Cronquist	Cb	Herbaceous	Annual	Exotic
Cynodon dactylon (L.) Pers.	Cd	Herbaceous	Perennial	Native
Euphorbia peplus L.	Ep	Herbaceous	Annual	Native
Jacaranda mimosifolia D. Don	Jm	Woody	Perennial	Exotic
Lantana camara L.	Lc	Woody	Perennial	Exotic
Medicago minima (L.) L.	Mm	Herbaceous	Annual	Native
Melia azedarach L.	Ma	Woody	Perennial	Exotic
Myrtus communis L.	Mc	Woody	Perennial	Native
Olea europaea L.	Oe	Woody	Perennial	Native
Oxalis acetosella L.	Oa	Herbaceous	Perennial	Exotic
Oxalis pes-caprae L.	Op	Herbaceous	Perennial	Exotic
Parietaria judaica L.	Pj	Herbaceous	Annual	Native
Piptatherum miliaceum (L.) Coss.	Pm	Herbaceous	Perennial	Native
Pittosporum tobira (Thunb.) W.T. Aiton	Pt	Woody	Perennial	Exotic
Rhamnus alaternus L.	Ra	Woody	Perennial	Native
Rubia peregrina L.	Rp	Herbaceous	Perennial	Native
Sedum sediforme (Jacq.) Grulich.	Sse	Herbaceous	Perennial	Native
Setaria viridis (L).P. Beauv.	Sv	Herbaceous	Annual	Native
Solanum nigrum L.	Sn	Herbaceous	Annual	Exotic
Sonchus oleraceus L.	So	Herbaceous	Annual	Native
Sonchus tenerrimus L.	St	Herbaceous	Annual	Native
Stenotaphrum secundatum (Walter) Kuntze	Ss	Herbaceous	Perennial	Exotic
Urtica urens L.	Uu	Herbaceous	Annual	Native
Veronica persica Poir.	Vp	Herbaceous	Annual	Native

(Cl4 and Pa7) and one OTU with the native group (Glo2). Three OTUs were found to be specific for the roots of annual plants when the plant life-cycle factor was considered (Glo1, Fu, Glo2). With respect to plant life-form, one OTU was specific for woody species (Pa7).

As shown by the perMANOVA, the plant species factor had highly significant effects on the composition of AMF communities and structure ($F = 14.45$, $P = 1e\text{-}04$). Also, the life-cycle and life-form significantly influenced the structure of the AMF community ($F = 2.4082$, $P = 0.0365$ and $F = 3.0367$, $P = 0.0128$, respectively), whereas the plant origin did not have a significant effect ($F = 1.6434$, $P = 0.1250$) (Table 3).

The two-way ANOVA showed that the AMF richness and Shannon diversity index differed significantly between plants with different life cycles (annual/perennial) and life-forms (herbaceous/woody) ($P < 0.05$) (Table 4). The perennial plants showed a mean richness and diversity index of 2.9 and 0.72 respectively whereas annual plants had 3.3 and 0.92 (Table S3). With respect to life-forms, woody plants showed a mean richness of 3.5 and diversity index of 0.94 and herbaceous plants 2.8 and 0.73 respectively.

4. Discussion

In this study we found a high number of facultative epiphytic plant species (31) growing on *P. dactylifera* palm trees and being colonized by arbuscular mycorrhizal fungi under semi-arid Mediterranean conditions (Table 1). Other studies concerning the occurrence of AM symbiosis in epiphytic vascular plants have been conducted in temperate and tropical ecosystems with plant species such as ferns and lycophytes (Zhang et al., 2004; Prashar et al., 2005; Muthukumar and Prabha, 2013; Lara-Perez et al., 2015) or in Mediterranean conditions focusing in one plant species, *S. tenerrimus* growing as a facultative epiphyte on *P. dactylifera* (Torrecillas et al., 2013). The presence of AMF in plants generally regarded as non-mycorrhizal such as *Chenopodium* or *Urtica* is also a remarkable finding. Nevertheless, the mycorrhizal status of these plants is still controversial and they have been documented both as mycorrhizal and non-mycorrhizal (Wang and Qiu, 2006; Hempel et al., 2013). As Hempel et al. (2013) proposed, the benefit from forming mycorrhiza and consequently the ability to establish the symbiosis could vary depending on the context and this might explain the presence of mycorrhiza in the epiphytic environment and its absence in others.

Although the study was done with cloning and sequencing (not NGS), it was very thoroughly sampled and species accumulation curves had reached asymptotes. Thus, the method was robust and using a different technology probably would not change our conclusions. The species indicator analysis revealed three OTUs (Glo1, Glo2 and Fu) belonging to the Glomeraceae and associated with annual species, Glo2 was also an indicator taxon for native species. One OTU (Pa7) belonging to the Paraglomeraceae was an indicator for both woody and exotic species (Table 2). These OTUs showed similarity to database sequences reported previously from a wide range of environmental conditions in terrestrial habitat, varying from stressful to optimal conditions (Wubet et al., 2006; Kottke et al., 2008; Long et al., 2010; Alguacil et al., 2011; Yamato et al., 2011; Torrecillas et al., 2012b). This shows the high plasticity of these AMF taxa. Seven OTUs from our study (Glo1, Sep, Rh, Div2, Cl2, Cl3 and Cl5) matched with sequences of AMF colonizing annual and perennial herbaceous plant species in terrestrial habitat in the same ecosystem (semi-arid Mediterranean areas) (Torrecillas et al., 2012a). However, 12 out of 23 OTUs detected were distinct from any previously published sequence and may be either known, yet unsequenced, or still unknown taxa. This suggests that the facultative epiphytes on palm trunks may be a unique source of AMF diversity, with fungi which are members of the Glomeraceae, Diversisporaceae, Gigasporaceae, Claroideoglomeraceae and Paraglomeraceae (Fig. 1) and novel. These novel OTUs likely possess

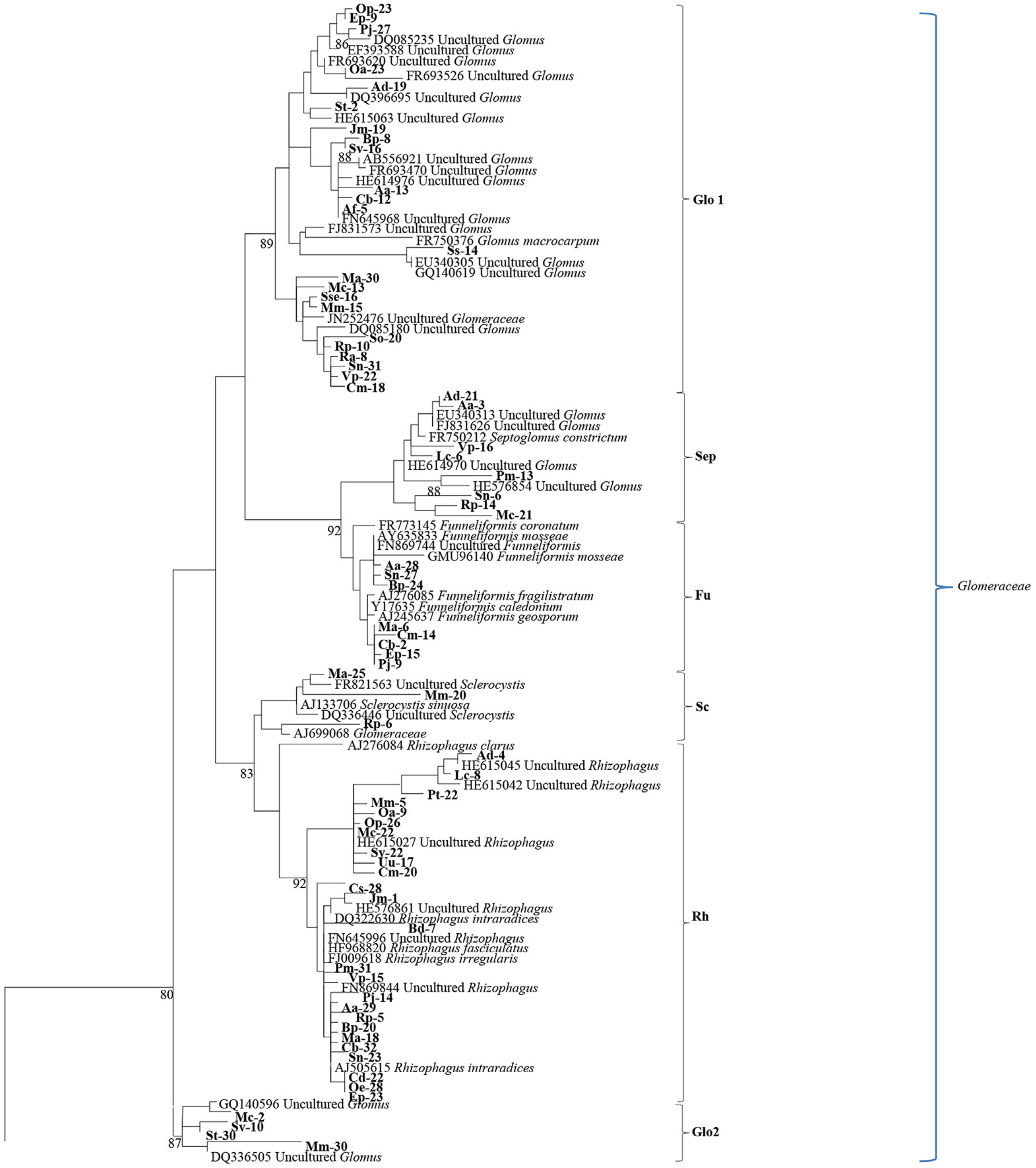

Fig. 1. Neighbour-Joining (NJ) phylogenetic tree showing AM fungal sequences isolated from thirty-one plants species growing as facultative epiphytes on date palm trees (*Phoenix dactylifera*) and reference sequences from GenBank. All bootstrap values > 80% are shown (1000 replicates). Numbers above branches indicate the bootstrap values of the maximum likelihood analysis. Sequences obtained in the present study are shown in bold type. They are labelled with the plant species abbreviation (Aa, *Asparagus acutifolius*; Ad, *Asparagus densiflorus*; Af, *Asphodelus fistulosus*; Bp, *Brachychiton populneus*; Bd, *Brachypodium distachyon*; Cs, *Centaurium spicatum*; Cm, *Chenopodium murale*; Cb, *Conyza bonariensis*; Cd, *Cynodon dactylon*; Ep, *Euphorbia peplus*; Jm, *Jacaranda mimosifolia*; Lc, *Lantana camara*; Ma, *Melia azedarach*; Mc, *Myrtus communis*; Mm, *Medicago minima*; Oa, *Oxalis acetosella*; Oe, *Olea europaea*; Op, *Oxalis pes-caprae*; Pj, *Parietaria judaica*; Pm, *Piptatherum miliaceum*; Pt, *Pittosporum tobira*; Ra, *Rhamnus alaternus*; Rp, *Rubia peregrina*; Sn, *Solanum nigrum*; So, *Sonchus oleraceus*; Sse, *Sedum sediforme*; Ss, *Stenotaphrum secundatum*; St, *Sonchus tenerrimus*; Sv, *Setaria viridis*; Uu, *Urtica urens*; Vp, *Veronica persica*). *Endogone pisiformis* and *Mortierella polycephala* were used as out-groups.

some functional traits which provide them with the capacity to disperse and proliferate under the epiphytic conditions. We also observed that Glo1 and Rh had the highest number of clones in our research, being found in almost all plant species (Table S1). Both OTUs are considered very invasive AMF taxa with a generalist lifestyle that produces a large quantity of extra-radical mycelium and small spores (Öpik et al., 2006; Helgason et al., 2007), which could be easily dispersed. Moreover, it has been suggested that those AMF seem to be better adapted to stressful environments, since their high sporulation rates can enable them to recover more readily (Oehl et al., 2003). This unique and distinctive AMF community inhabiting the epiphytic environment might be shaped by its particular conditions. For instance, epiphytic habitats are characterized by nutritional insufficiency (Benzing, 1990), since the substrate on which epiphytic plants grow is just accumulated organic matter originating from the decomposition of dead or old leaves. In our case, the facultative epiphytic plants species grew in small ledges of cut leaves in the trunks of date palms (*P. dactylifera*)

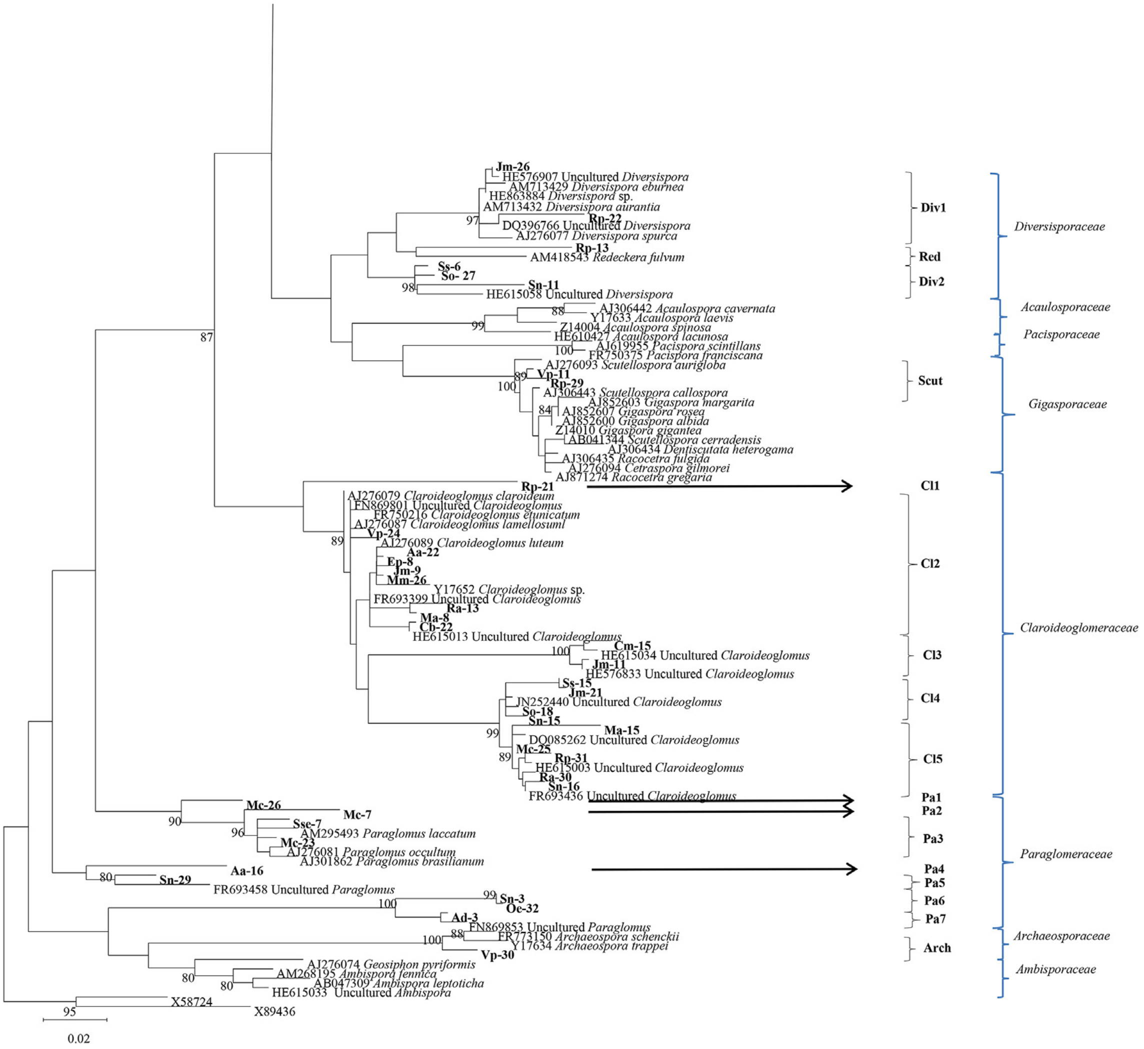

Fig. 1. (*continued*).

and are subjected to large periods of low water availability. Under these conditions, it has been suggested that symbiosis with arbuscular mycorrhizal fungi can ameliorate such unusual stressful conditions (Torrecillas et al., 2013).

Our results suggest that the AMF community composition is plant host identity-dependent. Plant affiliation to a species strongly influenced the AMF assemblages in the epiphytic environment (Table 3), as occur in others ecosystems (Wang and Qiu, 2006; Sýkorová et al., 2007; Li et al., 2010; Martínez-García and Pugnaire, 2011; Alguacil et al., 2011, 2012; Torrecillas et al., 2012b; Montesinos-Navarro et al., 2015; Ciccolini et al., 2016). According to Horn et al. (2017) plants can either drive or be driven by AMF but which drives which might depend on successional stage, which is related to the differences in dispersal processes between AMF and plants. The epiphytic environment on palm trunks can be considered, to some extent, the first stage of plant succession and, as Zobel and Öpik (2014) proposed in primary successions, plants arrive before AMF and then act as a potential filter for the latter. Therefore it can be hypothesized that host plant identity shaped AMF structure in this habitat.

Functional traits such as plant life-form and plant cycle also shaped the AMF interactions. The AMF community differed between the annual and perennial epiphytic species ($F = 2.4082$; $P = 0.0365$) and between herbaceous and woody plant species ($F = 3.0367$; $P = 0.0128$). The dependence of the composition of the AMF community on host plant traits has been previously reported with different plant ecological groups (e.g. habitat generalists vs specialists) (Davison et al., 2011; Torrecillas et al., 2014) or ecosystems (Veresoglou and Rillig, 2014). In a study in the same semi-arid Mediterranean conditions, Alguacil et al. (2012) found a different AMF community composition and greater diversity in perennial plant species than in annuals, in accordance with our results (where we detected greater AMF richness in perennial (21 OTUs) than in annual plants (15 OTUs) $P < 0.05$ (Table 4)). These authors explained these differences as being due to the fact that the former are in the soil for longer, giving more opportunities for mycorrhization establishment. Similarly, the continuity of the roots of an individual with time is critical in an epiphytic environment, taking into account the strong limitations to AMF dispersal.

Several authors have pointed out the constraints to AMF dispersal in epiphytic habitats, as propagules are mainly dispersed by biotic vectors (Mangan and Adler, 2000; Fernández et al., 2010; Torrecillas et al., 2013). Moreover, in this particular habitat, plants generally grow as individuals inside the cut leaves without any connections among root systems, thus preventing AMF spread. Under these circumstances spatial and temporal coincidence of the plants and fungi, which is higher in perennials plants than in annuals, is crucial for mycorrhiza development.

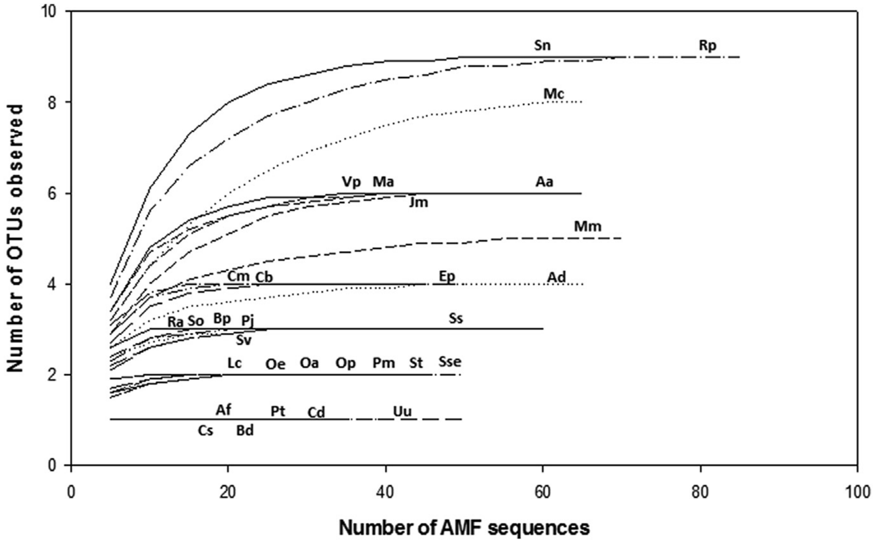

Fig. 2. Sampling effort curves for the AM fungal community in the plants species growing as facultative epiphytes on date palm trees (*Phoenix dactylifera*). Aa, *Asparagus acutifolius*; Ad, *Asparagus densiflorus*; Af, *Asphodelus fistulosus*; Bp, *Brachychiton populneus*; Bd, *Brachypodium distachyon*; Cs, *Centaurium spicatum*; Cm, *Chenopodium murale*; Cb, *Conyza bonariensis*; Cd, *Cynodon dactylon*; Ep, *Euphorbia peplus*; Jm, *Jacaranda mimosifolia*; Lc, *Lantana camara*; Ma, *Melia azedarach*; Mc, *Myrtus communis*; Mm, *Medicago minima*; Oa, *Oxalis acetosella*; Oe, *Olea europaea*; Op, *Oxalis pes-caprae*; Pj, *Parietaria judaica*; Pm, *Piptatherum miliaceum*; Pt, *Pittosporum tobira*; Ra, *Rhamnus alaternus*; Rp, *Rubia peregrina*; Sn, *Solanum nigrum*; So, *Sonchus oleraceus*; Sse, *Sedum sediforme*; Ss, *Stenotaphrum secundatum*; St, *Sonchus tenerrimus*; Sv, *Setaria viridis*; Uu, *Urtica urens*; Vp, *Veronica persica.*

Table 2
Indicator species analyses. OTUs associated with plant origin.

	Probability[a]			
Group Exotic #sps. 2	A	B	Indicator Value Index	p-value
Cl4	0.8791	0.2424	0.462	0.0035
Pa7	1.0000	0.0909	0.302	0.0473
Group Native #sps. 1				
Glo2	1.0000	0.1667	0.408	0.0307
OTUs associated with plant life cycle Group Annual				
#sps. 3				
Glo1	0.5746	0.8056	0.680	0.0297
Fu	0.7037	0.3333	0.484	0.0275
Glo2	0.8636	0.2222	0.438	0.0063
OTUS associated with plant life form Group Woody				
#sps. 1				
Pa7	1.0000	0.1000	0.316	0.0323

[a] Probability A, is the probability that the surveyed site belongs to the target site group given the fact that the species has been found; probability B, is the probability of finding the species in sites belonging to the site group.

Table 3
PerMANOVA analysis of the effect of plant origin, life cycle, life-form, plant species and plant family on the distribution of AMF OTUs in plant species growing as facultative epiphytes on date palm trees (*Phoenix dactylifera*) under semi-arid Mediterranean conditions.

	Df	SS	MS	F. Model	R^2	Pr (>F)
Origin	1	0.406	0.406	1.643	0.017	0.125
Life cycle	1	0.594	0.595	2.408	0.025	**0.036**[a]
Life-form	1	0.750	0.750	3.037	0.032	**0.013**[a]
Plant species	30	20.75	0.692	14.46	0.874	**1e-04**[a]

Df, degrees of freedom; SS, sum of squares; MS, mean squares; Pr value by permutation.

[a] In bold, statistically significant relationships ($P \leq 0.05$).

Table 4
ANOVA analysis of the effect of plant origin, life cycle and life-form, on the AMF richness and Shannon diversity index detected in roots of 31 plant species growing as facultative epiphytes on date palm trees (*Phoenix dactylifera*) under semi-arid Mediterranean conditions. F-values (P-values) are shown.

	Richness (S)	Shannon diversity index (H′)
Plant origin	2.206 (0.1411)	2.989 (0.0874)
Life cycle	3.527 (0.0497)*	5.877 (0.0174)*
Life-form	4.158 (0.0445)*	6.099 (0.0155)*

*, significant at $P < 0.05$.

In this work the epiphytic plants associated with AMF irrespective of whether they are native or not. This is in accordance with Bunn et al. (2015), who studied the enhancement or loss of AMF following invasions by exotic plants and concluded that plant functional traits better predict AM variations in fungal associations than the invasive character.

In conclusion, a surprisingly high diversity of facultative epiphytic plant species are colonized by AMF in the semi-arid Mediterranean conditions studied. Plant identity and the functional plant life-cycle and life-form features determine the AMF assemblages.

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Supplementary data

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