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

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Running title: Host determine AMF community in facultative epiphytic

ABSTRACT

The epiphytic vascular flora is scarce and facultative in semiarid Mediterranean ecosystems, thus covering diverse taxonomic groups. However, differently to terrestrial 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 in 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 roots were examined by molecular and phylogenetic analysis of AMF community. The plant affiliation to species strongly influenced the AMF assemblages. Plant life-form and plant life-cycle also shaped AMF interactions. The AMF community differed between annual and perennial species and higher AMF richness was detected in perennial plants. The indicator species analysis revealed three Operational Taxonomic Units belonging to the *Glomeraceae*, associated with annual species. However, the epiphytic plants associated with AMF irrespective of whether they were native or not, probably because here no functional differences derive from plant origin.

IMPORTANCE

Arbuscular mycorrhizal (AM) symbiosis has a decisive role in plant nutrient and water uptake by plants, with particular importance in stressful environments. Under semiarid conditions, the facultative epiphytic flora should cope with harsh conditions. While numerous studies have been conducted on factors driving terrestrial AM assemblages,

the epiphytic environment remains unexplored. We offer new insights into composition of AM communities as shaped by epiphytic plant host identity and functional traits.

Keywords: Facultative epiphytes**;** arbuscular mycorrhizal fungi; diversity; SSU rDNA; semiarid ecosystems;

INTRODUCTION

Epiphytic habitats are considered as extreme plant environments due to the large fluxes of temperature and low water and nutrient availability they are subjected to. 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 (38). 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 semiarid Mediterranean ecosystems the epiphytic vascular flora 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 (44,52).

Since most vascular epiphytic plants are ferns and their relatives or monocots (11), the mycorrhizal condition, specificity, and dependence have therefore mainly been studied

in these taxonomic groups. Most studies concerning epiphytism-mycorrhizas relationships have been conducted in humid tropical habitats and involve orchids, which form mycorrhizas with Basidiomycetes (23,30,31), or other species that form arbuscular mycorrhizas, like those of the Araceae, Clusiaceae, Bromeliaceae, or Begoniaceae (20,33,36). However, very little is known about the facultative epiphytic plant species, belonging to diverse phylogenetic groups, which grow under semiarid Mediterranean conditions. Only the widespread *Sonchus tenerrimus* has been reported to establish a symbiotic relationship with arbuscular mycorrhizal fungi (AMF), belonging to the *Glomeromycota* (44).

But more interesting than checking the mycorrhizal status of epiphytic plants is to understand the factors driving symbiotic communities in epiphytic habitats and consequently their role in the tree canopy ecosystems, which still has been investigated less than in their terrestrial counterparts.

There is evidence that AMF communities in terrestrial habitats depend on host-plant preference (1,22,42), but the specificity in biotic interactions has been suggested to be mediated by plant functional traits rather than phylogeny (19,37). Plant life-cycle, for instance, may explain differences in AMF communities (2,41). 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, phylogeny, 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 to 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 (4,5,13,26). Moreover, functional traits are often conserved during evolution, resulting in closely related species that tend to

interact with similar species in such a way that significant correlations have been observed between the phylogenetic composition of plants and AMF assemblages (24). But, as previous reports show, the AMF communities on epiphytic plants are different from those of the surrounding soil habitats (23,44). These results suggest that facultative epiphytic plants from semiarid sites might support distinctive AMF communities shaped by particular plant traits.

In this study we investigated facultative epiphytic plant species occurring in the trunks of *Phoenix dactylifera* trees cultivated in orchards under Mediterranean semiarid conditions and we assessed the arbuscular mycorrhizal communities they harbored. We wanted to ascertain 1) if there are AMF in plants growing epiphytically and 2) if 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.

RESULTS

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. 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 AM fungal 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 and are shown in bold in Fig. S1.

Phylogenetic analysis of AMF groups

After phylogenetic analyses of the sequences, 23 AM fungal OTUs were detected in this study (Fig. S1; Table S1). 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 not related to any sequences of AMF in the database.

Effect of plant origin, life-cycle, life-form and plant species on AM fungal community composition

In order to determine whether the number of clones sequenced was sufficient to represent the AM fungal diversity in all the facultative epiphytic species, rarefaction curves were constructed (Fig. 1). With the clones sequenced for each root sample, we covered perfectly the diversity of the AM fungal 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 (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 AMF communities composition and structure (F=14.45, P=1e-04). Also, the life-cycle and life-form significantly influenced the distribution of the AMF (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).

DISCUSSION

In this study we found a high number of facultative epiphytic plant species growing on *Phoenix dactylifera* palm trees being colonized by arbuscular mycorrhizal fungi under semiarid Mediterranean conditions (Table 1). There is only a recent study carried out with one plant species, *Sonchus tenerrimus* L. growing as facultative epiphyte in *P. dactylifera* in semiarid conditions (44), the rest of studies concerning the

174 occurrence of AM symbiosis in epiphytic vascular plants have been conducted in
175 temperate and tropical ecosystems with plant species such as ferns and lycophytes
176 (15,27,32,50).

177 In our study the AMF richness was covered in the thirty one epiphytic plants
178 studied with the method used, since all accumulation curves reached a well-defined
179 asymptote. Consistent results for the fungal community structure and relative
180 abundances of fungal taxa can be obtained whatever the sequencing approach used; i.e.,
181 NGS vs cloning-Sanger sequencing (29).

182 The species indicator analysis revealed three OTUs (Glo1, Glo2 and Fu)
183 belonging to the *Glomeraceae* and associated with annual species, Glo2 was also an
184 indicator taxon for native species. One OTU (Pa7) belonging to the *Paraglomeraceae*
185 was an indicator for both woody and exotic species (Table 3). These OTUs showed
186 similarity to database sequences reported previously from a wide range of
187 environmental conditions in terrestrial habitat, varying from stressful to optimal
188 conditions (1,14,18,42,48,49). This shows the high plasticity of these AMF taxa.

189 Epiphytic habitats are characterized by nutritional insufficiency (3), since the
190 substrate on which epiphytic plants grow is accumulate organic matter and rainwater. In
191 our case, the different facultative epiphytic plants species grew in small ledges of cut
192 leaves in the trunks of date palms (*P. dactylifera*) with high organic carbon content
193 (36%) and subjected to large periods of low water availability. Under these conditions,
194 it has been suggested a set of morphological and physiological adaptations in epiphytic
195 taxa to cope water and nutrient shortage (25,36), being reported only the association
196 with arbuscular mycorrhizal fungi in Mediterranean semiarid ecosystems (44).

197 Our results suggest that the AMF community composition is plant host identity-
198 dependent. Plant affiliation to a species strongly influenced the AMF assemblages in the

199 epiphytic environment (Table 3). This agrees with the hypothesis put forward to explain
200 the phylogenetic distribution of mycorrhizas in land plants (46) and suggests that plant
201 phylogeny might influence the AMF community - as proposed by Montesinos-Navarro,
202 et al. (24), who demonstrated that changes in the phylogenetic composition of plant and
203 AMF assemblages do not occur independently in a patchy environment. The differences
204 in dispersal processes between AMF and plants might explain which group drives the
205 other. According to this hypothesis, in primary successions plants arrive before AMF
206 and then act as a potential filter for the latter, as seems to be the case in the AMF
207 community in palm trunks.

208 Functional traits such as plant life-form and plant cycle also shaped the AMF
209 interactions. The AMF community differed between the annual and perennial epiphytic
210 species ($F=2.4082$; $P=0.0365$) and between herbaceous and woody plant species
211 ($F=3.0367$; $P=0.0128$). The dependence of the composition of the AMF community on
212 the host-plant has been previously reported in different habitats around the world
213 (1,2,6,17,39,42), with different plant ecological groups (e.g. habitat generalists vs
214 specialists) (7,43) or ecosystems (45). In a study in the same semiarid Mediterranean
215 conditions, Alguacil et al. (2) found a different AMF community composition and
216 greater diversity in perennial plant species than in annuals, in accordance with our
217 results (where we detected greater AMF richness in perennial (21 OTUs) than in annual
218 plants (15 OTUs)). These authors explained these differences as being due to the fact
219 that the former are in the soil for longer, giving more opportunities for mycorrhization
220 establishment. Similarly, the continuity of the roots of an individual with time is critical
221 in an epiphytic environment, taking into account the strong limitations to AMF
222 dispersal.

Several authors have pointed out the constraints to AMF dispersal in epiphytic habitats, as propagules are mainly dispersed by biotic vectors (10,21,44). 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.

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. (4), who compared 67 publications with divergent hypotheses about the enhancement or loss of AMF following invasions by exotic plants and concluded that other factors - such as plant functional groups - better predict AM variations in fungal associations (not only in terms of AM status but also fungal colonization or growth response), as we also found for non-invasive exotic species. Unlike invasive plants - which have specific functional traits that confer the ability to colonize new habitats, usually displacing native species - no invasive ability was observed in the exotic plant species growing in the epiphytic environment provided by the palm orchards.

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

MATERIAL AND METHODS

Study area and sampling

The study area was located at the Historic Palm Grove of Elche (Alicante), southern Spain (38º15’ 51, 28’’N, 0º 41’ 51, 23’’ W, 82 m.a.s.l). It covers 550 Ha and contains about 180.000 adult, *Phoenix dactylifera* date palms aged 40-60, planted in one or two

rows around cultivated, rectangular-shaped orchard (35). The climate is Mediterranean semiarid, with a mean annual temperature of 17.7^{o}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 1.5-2 m height on date palm trunks were sampled at the spring growing season (three individuals of each species, one per plot). Plant species were classified according to their origin into native or exotic (authoctonous or aloctonous), according to their life cycle into annual or perennial and according to life form into herbaceous or wood. Canopy or bare-limb epiphytes were not found. Roots systems were collected, fine foots were separated, briefly rinsed, quickly dried on paper and used for molecular analysis.

Roots DNA extraction and PCR

For each sample, 0.2 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 -20ºC.

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 (47). PCR was carried out in a final volume of 25 µl using PuReTaq^{TM} Ready-To-Go PCR beads (Amershan Pharmacia Biotech), 0.2µM 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, 2µl from the first PCR were used as template DNA in a second PCR reaction performed using the specific primers AML1 and AML2 (16). PCR reactions were carried out in a final volume of 25 µl using the PuReTaq™ Ready-To-Go PCR beads (Amershan Pharmacia Biotech), 0.2 µM dNTPs and 0.5 µlM 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. Reactions yields were estimated by using a 1.2% agarose gel containing *GelRed*™ (Biotium).

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 unit of RedTaq DNA polymerase (Sigma) and the supplied reaction buffer to a final volume of 25µ and a 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).

Phylogenetical analysis

298 Sequence editing was done using the program FinchTV 1.4.0 (Geospiza, Inc.; Seattle,
299 WA, USA; http://www.geospiza.com). A search for similar sequences to the ones from
300 this study was conducted with the BLAST tool (51) provided by GenBank.
301 Phylogenetic analysis was carried out on the sequences obtained in this study and those
302 corresponding to the closest matches from GenBank as well as sequences from cultured
303 AMF taxa including representatives of the major taxonomical groups described by (34).
304 All the sequences were aligned, using the multiple sequence comparison program,
305 MAFFT, version 7.0 (available at http://align.bmr.kyushu-u.ac.jp/mafft/software) and
306 the alignment was adjusted manually in BioEdit software version 7.2.5. (12). The
307 program CHIMERA_CHECK 2.7 (Ribosomal Database Project II;
308 http://rdp.cme.msu.edu) was used to check for chimeric artifacts among the 18S rDNA
309 sequences.
310 Maximum likelihood (ML) phylogenetic tree inference was performed with MEGA
311 software (version 5.05) (40). Nucleotide data files were first tested to find the best DNA
312 evolution model. The general time reversible model with a discrete gamma distribution
313 showed the lowest Bayesian information criterion (BIC) scores and was deemed to best
314 describe the nucleotide substitution pattern. Initial trees for the heuristic search were
315 obtained by applying the neighbor-joining method to a matrix of pairwise distances
316 estimated using the maximum composite likelihood (MCL) approach. The robustness of
317 all trees obtained was evaluated by 1000 bootstrap replications. *Endogone pisiformis*
318 Link and *Mortierella polycephala* Coem, were used as the out-groups.
319 Different AMF sequence types or OTUs (operational taxonomic units), were defined as
320 groups of closely related sequences, with a high level of bootstrap support in the
321 phylogenetic analyses (higher than 80%) and sequence similarity ≥ 97%.
322

323 **Statistical analysis**

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

329 In order to ascertain whether the AMF communities composition and structure were
330 significantly affected by the experimental factors (plant origin, life form, life cycle and
331 plant species) a permutational multivariate analysis of variance (perMANOVA) was
332 performed with the adonis function in vegan (28) using the Jaccard distance matrix and
333 999 permutations.

334 Three different indicator species analyses (ISA) were conducted on the OTUs
335 presence/absence dataset using the "indicspecies" package implemented in R (8). This
336 analysis allows us identifying species which are associated to a classifier factor by
337 calculating an Indicator Value (IndVal) (9). We considered as classifier factors the plant
338 origin, life form and life cycle. The statistical significance of the indicator values was
339 tested using a permutation test with 999 permutations.

340 The AMF OTUs richness was subjected to ANOVA to test for significant differences
341 between different factors (plant origin, life cycle, life form and plant species**)**. For the
342 comparisons among means the Duncan's test at $P<0.05$ was used. All the statistical
343 procedures were carried out with the software package IBM SPSS Statistic 24.0 for
344 Windows.

345 **Nucleotide sequence accession numbers**. A total of 109 representative sequences of
346 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.

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Figure 1. 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 europea;* 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 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 europea 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

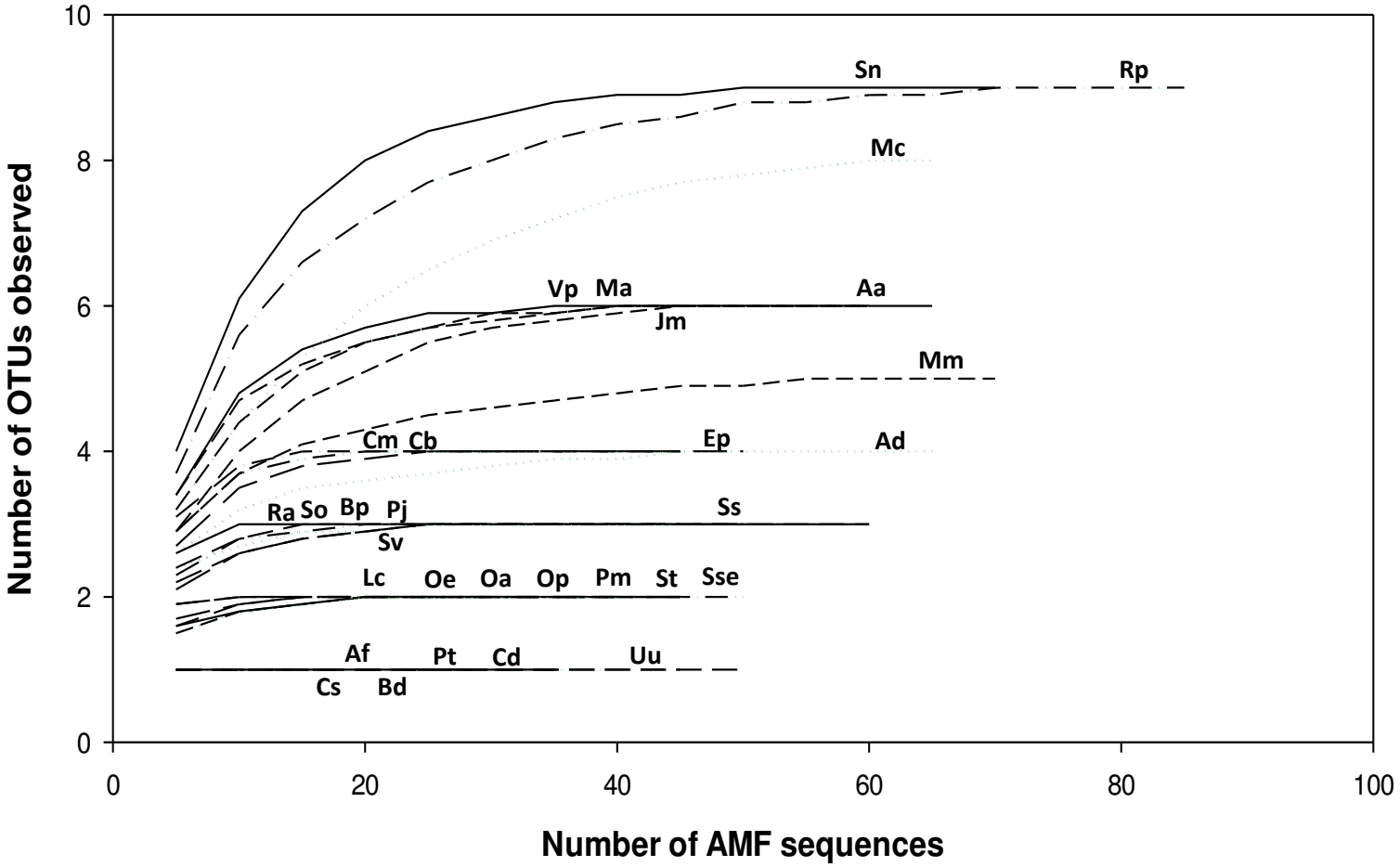
Table 2. Indicator species analyses.

OTUs associated to plant origin				
Group Exotic			Indicator Value	
#sps. 1	A	B	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 to plant life cycle				
Group Annual				
#sps. 4				
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 to plant life form				
Group Woody				
#sps. 1				
Pa7	1.0000	0.1000	0.316	0.0323

Table 3. PERMANOVA analysis of the effect of plant origin, life cycle, life form and plant species on the distribution of AMF OTUs in plant species growing as facultative epiphytes on date palm trees (*Phoenix dactylifera*) under semiarid 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***
Life form	1	0.750	0.750	3.037	0.032	**0.013***
Plant species	30	20.75	0.692	14.46	0.874	**1e-04*****

Df, degrees of freedom; SS, sum of squares; MS, mean squares; Pr value by permutation
*In bold, statistically significant relationships ($P \leq 0.05$)


Number of OTUs observed
Number of AMF sequences
0
2
4
6
8
10
0
20
40
60
80
100
Sn
Rp
Mc
Vp
Ma
Aa
Jm
Mm
Cm
Cb
Ep
Ad
Ra
So
Bp
Pj
Ss
Sv
Lc
Oe
Oa
Op
Pm
St
Sse
Af
Pt
Cd
Uu
Cs
Bd