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ORIGINAL PAPER

Mycorrhizal characterization of the boojum tree, *Fouquieria columnaris*, an endemic ancient tree from the Baja California Peninsula, Mexico

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Abstract The mycorrhizal association with the boojum tree, *Fouquieria columnaris* (=*Idria columnaris*), was studied. This unusual tree is almost exclusively endemic to granite and volcanic soils in highly arid areas of the Baja California Peninsula of Mexico. Soil and root samples from ten sites, covering the extent of geographic distribution of the tree on the peninsula, were analyzed. The roots of the boojum tree contained all structures of an arbuscular mycorrhizal (AM) association. Morphologically different species, 23 in number, were identified in close vicinity to the boojum tree indicating that *F. columnaris* is associated with a high number of AM species of several AM genera and families.

Keywords Arbuscular mycorrhizal fungi · Baja California Peninsula · Boojum tree · *Fouquieria columnaris*

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Introduction

Scarce information is available about the boojum tree (Fig. 1), *Fouquieria columnaris* (Kell) Kell. ex Curran (= *Idria columnaris* Kell, which was previously assigned as a single species to the genus *Idria*). The boojum tree, known in Spanish as ''cirio'' is found in the Baja California Peninsula of Mexico in a restricted portion of the main peninsular mountain crest (Valle de los Cirios, ranging from 27.5 to 30.5°N), with isolated populations on Isla Angel de la Guarda in the Gulf of California and a small colony in Puerto Libertad in the state of Sonora in northern Mexico (Turner et al. 1995) (Fig. 2). Humphery (1974) postulated that the genus is descended from a relict derived from a tropical antecedent that had become adapted to drier climates about 200 million years ago.

Boojum trees thrive in deep volcanic soils of some interior valleys and shallow soils of the rocky hills of the interior of the central peninsula where rainfall is low, averaging 120 mm a year (51-year record). Summer temperatures are very high, rarely dropping below 35–40°C on clear days and frequently exceeding 45°C in exposed areas (Bashan et al. 2003, 2006).

Boojums commonly grow in loose associations with other plants, endemic to that part of the Sonoran Desert, such as the elephant tree (Bashan et al. 2006), datillilo (a

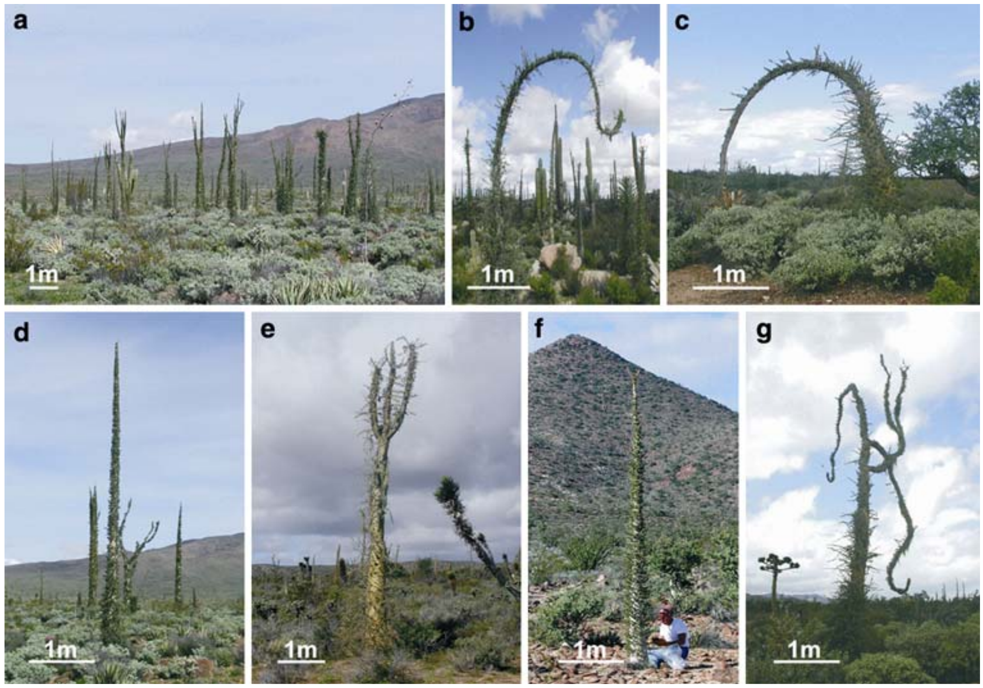

Fig. 1 The boojum tree (*Fouquieria columnaris*) and its habitat in the desert preserve of Valle de los Cirios in the state of Baja California, Mexico. **a** A small ''forest'' of boojum (cirio) trees; **b** the boojum trees can form loops, **c** boojum arcs and **g** strange formations during its very long life span. **d** Common formation of the tree, looking like an upside-down carrot, **e** chandelier structure. **f** Typical young boojum tree where sampling was done

tree-like yucca) and giant cardon cactus (Humphery 2001; León de la Luz et al. 1995). The boojum grows very slowly, about 3-4 cm in a good rainy year. It may take 50–100 years from germination until the plant produces its first flowers (Bashan et al. 2003; Humphery 1974). Boojums that are older than 500 years are common and even trees over 700 years can be found (Humphrey 2001). Although there is some information on the above-ground part of the boojum, virtually nothing is known about the roots and the root-associated microflora (Humphery 1974, 2001).

Arbuscular mycorrhizal fungi (AMF) are obligate mutualistic fungi, which form a symbiotic association with the roots of a majority of land plants. The importance of this 400-million-year-old association for plant nutrition is well documented (Smith and Read 1997). Moreover, AMF improve the water status of the host plant (Augé 2001; Smith and Read 1997), making this symbiosis of particular interest in regions that suffer drought most of the year. A number of reports have postulated that AMF are essential components of desert plant–soil systems (Allen 1991; Bethlenfalvay et al. 1984; Jacobson et al. 1993; Requena et al. 1996; Uhlmann et al. 2006). Few data are available on the mycorrhizal status of plants growing in highly arid Baja California (Carillo-Garcia et al. 1999; Rose 1981). We studied the AM status of *F. columnaris* and identified the AMF spores associated with *F. columnaris* at ten sites covering the full geographic distribution of the boojum tree in Baja California.

Material and methods

Sampling

Samples were collected at the end of February and the beginning of March 2005 at sites with dense populations of boojum trees (Fig. 1). From ten sites, samples of roots and soil (about 300 g) were collected (Fig. 2). The sites were located at: #1 (28°39′N, 114°02′W), #2 (28°46′N, 114°06′W), #3 (28°51′N, 114°07′W), #4 (29°04′N, 114°04′W), #5 (29°46′N, 114°45′W), #6 (29°47′N, 114°47′W), #7 (29°45′N, 114°41′W), #8 (29°41′N, 114°39′W), #9 (29°29′N, 114°28′W), #10 (29°12′N, 114°09′W). Root and soil samples from the base area of four trees (samples a–d) were taken at each site (total number of samples; $n = 40$ trees). Boojum trees were selected where no other plants grew within a radius of at least 50 cm around it. Root and soil samples were collected at a depth of 5–15 cm and as close as possible to the trunk of the boojum (0–15 cm from each trunk) after the topsoil was removed. Soil samples were dried at 40°C in an oven and stored until spores were isolated. Root samples (10–15 pieces per plant, 1–2 cm average length) were stored in 70% ethanol.

Determination of mycorrhization

To determine fungal colonization of roots that were stored in ethanol (70%), samples were cleared (6 min boiling in 10% KOH solution) and then stained by boiling for 4 min

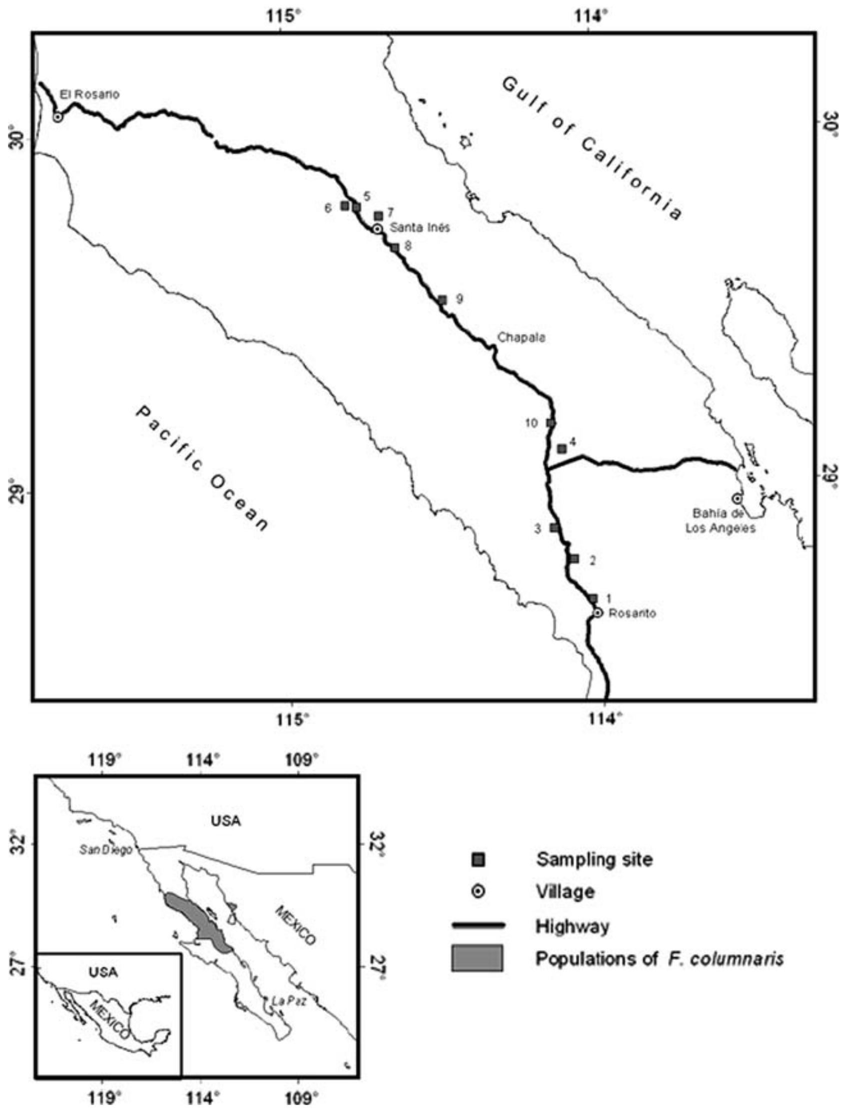

Fig. 2 Distribution of *Fouquieria columnaris* and location of the sampling sites

in a 5% jet-black commercial ink (Schaeffer)—5% commercial household vinegar solution (Vierheilig et al. 1998, 2005). Stained roots were observed under a light microscope to determine the percentage of root colonization (mean ± SE) with the gridline intercept method (Giovannetti and Mosse 1980).

Isolation and identification of AMF spores

Arbuscular mycorrhizal fungi spores occurring in the rhizosphere samples were extracted from the soil by wet sieving and using a sucrose density gradient centrifugation method described by Oehl et al. (2003). Briefly, 25 g oven-dried (40°C) soil samples were sieved (1,000, 125 and 32 μm mesh). The spores were mounted on slides with polyvinyl–lactic acid–glycerine (PLVG)(Koske and Tessier 1983) or a mixture (1:1; v/v) of PLVG with Melzer's reagent (Brundrett et al. 1994) and viewed under a dissecting microscope at up to 90× magnification. Subsequently, they were examined under a compound microscope at up to 400× magnification. Identification, as far as possible, was based on current species descriptions and identification manuals (Schenck and Pérez 1990; International Culture Collection of Arbuscular and Vesicular–Arbuscular Endomycorrhizal Fungi at http://www.invam.caf.wvu.edu/Myc_Info/Taxonomy/species.htm.).

Results

Dense population of boojum trees occurred at all sites (Fig. 1). In boojum roots at all sites, only AMF could be detected and no other fungi. All AMF structures, such as intraradical hyphae, arbuscules and vesicles were found

(Figs. 3a–d). AMF root colonization averaged 14 ± 9% at all sites, with the highest root colonization at #8 (21.5 ± 12.1%) and the lowest at #3 (7.5 ± 4.2%) (Fig. 4). Only two boojum, out of 40 evaluated (#2d and #6a), showed no sign of root colonization by AMF.

In the soil samples, 2–7 spores per gram of soil were found. We identified 23 morphologically distinct AMF species at the ten sites (Table 1). Over half (12) belonged to the genus *Glomus* Tul and C. Tul in the Glomeraceae. Three species belonged to the genus *Scutellospora* C. Walker and F.E. Sanders in the Gigasporaceae, while three species were from *Appendicispora* Spain, Oehl and Sieverding, one from *Archaeospora* J.B Morton and D. Redecker and one from *Intraspora* (Sieverding and S. Toro) Oehl and Sieverding of the Archaeosporaceae (Morton and Redecker 2001; Spain et al. 2006; Sieverding and Oehl 2006). Furthermore, one each species was from *Pacispora* Oehl and Sieverding (2004), from *Entrophospora* (Sieverding and Oehl 2006) and from *Paraglomus* (Morton and Redecker 2001) (Table 1). Remarkably, no species of *Acaulospora* Gerd. and Trappe and *Gigaspora* Gerd. and Trappe were recovered. Five AMF species could not or not clearly be attributed to AMF species described so far (*Glomus* sp. MX1, *Scutellospora* sp. MX2, *Pacispora* sp. MX3 and *Appendicispora* spp. MX4 and MX5; Table 1).

The most frequently detected species were *G. mosseae*, *G. etunicatum*, *G. intraradices* and *G. macrocarpum*. They were found at all sites and in at least 75% of all samples (Table 1). Spores of *G. microcarpum*, *G. constrictum* and *Glomus* sp. MX1 were abundant, but not at all sites. Only a few spores of *G. coronatum*, *Scutellospora* sp. MX3, *Pacispora* sp. MX4, *E. infrequens*, *Archaeospora gerdemannii* and *Intraspora schenckii* were found.

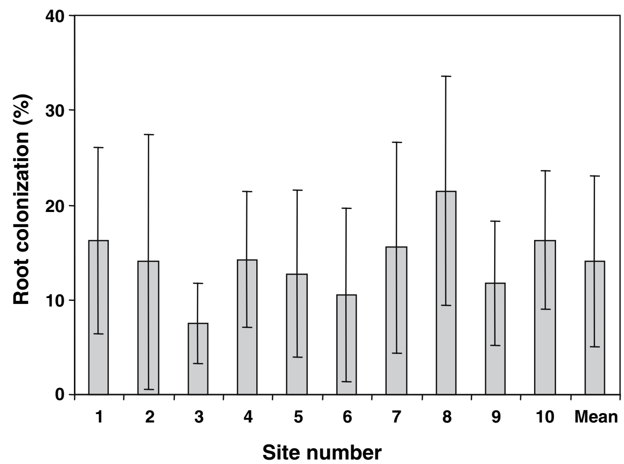

Fig. 4 Degree of root colonization by AMF of *Fouquieria columnaris* at different sites. Samples were taken from four plants at each site (mean ± SE)

Discussion

In this study, we showed that the boojum tree *F. columnaris* maintains a typical arbuscular mycorrhizal fungi–plant symbiosis. We observed intraradical hyphae, vesicles, arbuscules and spores. The arbuscules filled whole root cells of *F. columnaris*, indicating that they were highly branched and functional. The spore density around roots of boojum

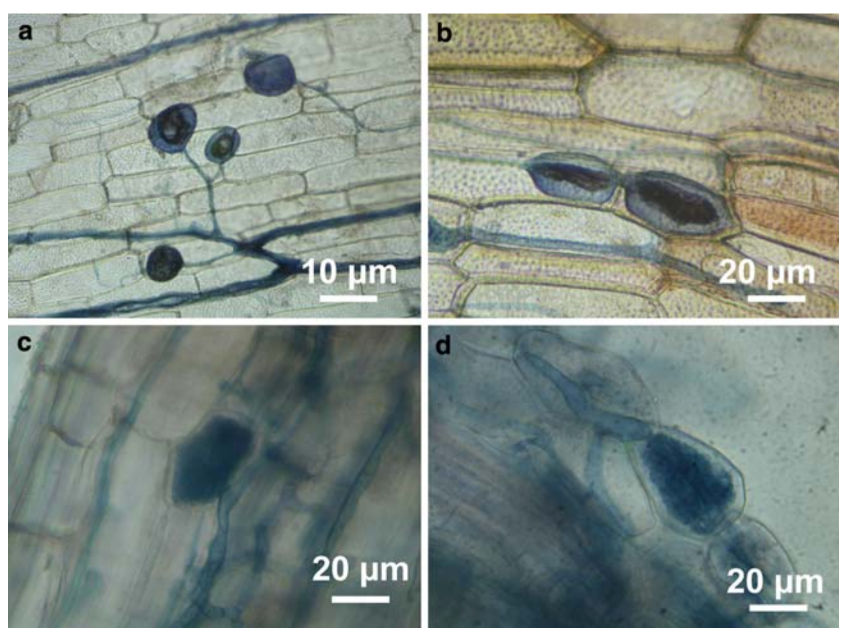

Fig. 3 Arbuscular mycorrhizal root colonization in roots of *Fouquieria columnaris*; **a** hyphae and spores on root surface; **b** hyphae and vesicles; **c**, **d** intraradical hyphae and arbuscules

Table 1 AMF species detected in rhizosphere soils of boojum tree (*Fouquieria columnaris*)

AMF species	Site										Frequency[b] (%)
	1	2	3	4	5	6	7	8	9	10	
Glomeraceae											
Glomus etunicatum	a, b, c, d	a, b, c, d	b, c, d	b, c, d	a, b, c, d	a, b, d	b, c, d	a, b, c, d	a, b, c, d	d	82.5
G. mosseae	a, b, d	a, b, c, d	a, b, c	c, d	b, c	a, b, c, d	a, b, d	a, b, c, d	a, b, c, d	b, c, d	80
G. intraradices	a, b, c, d	a, b, d	a, b, c, d	a, b, c, d	a, b, c, d	a, d	a, b, c, d	a, b	a, b, c	b, c	80
G. macrocarpum	a, c	a, b, c	a, b, c, d	a, b, c	d	a, c, d	a, b, c, d	a, b, d	a, b, c, d	a, b, d	75
Glomus sp. MX1[c]	a		a		b	c		a, b, c	a, c	a, c, d	30
G. microcarpum	a, d		d		b		a				12.5
G. constrictum								c, d	b, c	a	12.5
G. coronatum			c				b			d	7.5
G. claroideum				a, b			a				7.5
G. versiforme			b					d			5
G. fasciculatum							a, c				5
G. microaggregatum	a										2.5
Gigasporaceae											
Scutellospora fulgida				d		a, d					7.5
S. pellucida	b, c, d										7.5
Scutellospora sp. MX2			a								2.5
Entrophosporaceae											
Entrophospora infrequens	a										2.5
Pacisporaceae											
Pacispora sp. MX3										a	2.5
Paraglomeraceae											
Paraglomus occultum			b			d					5
Archaeosporaceae[d]											
Archaeospora trappei		b						d			5
Appendicispora sp. MX4					b	b, c			b, d		12.5
Appendicispora sp. MX5					c	a, b, c					10
Ap. gerdemannii				d							2.5
Intraspora schenckii							d				2.5
Number of AMF species at site	9	5	10	7	8	9	9	8	7	8	

[a] Ten sites (1–10) with four trees (a–d) per site

[b] Percentage of presence of each species in a total of 40 samples (=trees) investigated

[c] Resembling *Glomus rubiforme*

[d] The genera *Appendicispora* and *Intraspora* were recently described (Spain et al. 2006; Sieverding and Oehl 2006)

trees was highly variable, ranging from 2 to 7 spores per gram soil. These values are much higher than spore densities reported from the rhizosphere of other plants growing in arid and semiarid environment under similar harsh arid environmental conditions (Carillo-Garcia et al. 1999; Jacobson 1997; Uhlmann et al. 2004).

Interestingly, looking at mycorrhization densities in other plants growing in arid environments, such as *Welwitschia mirabilis* or members of the Poaceae in the Namib Desert (Jacobson et al. 1993; Uhlmann et al. 2004) and plants of the desert in the Baja California Peninsula (Carrillo-Garcia et al. 1999), root colonization in *F. columnaris* was relatively low. Lacking or even negative correlation between spore density and mycorrhization densities has already been reported (Mathur and Vyas 1994; López-Sánchez and Honrubia 1992; Oehl et al. 2003; Uhlmann et al. 2004).

The highest mycorrhization found in boojum roots reached 32%; however, in two plants, we did not detect AMF colonization. The absence of AMF seems to be contradictory to studies of other desert plant–soil systems, where AMF root colonization was considered to be an essential component of plant systems in arid environments (Bashan et al. 2000; Bethlenfalvay et al. 1984; Carrillo-Garcia et al. 1999; Allen 1991; Jacobson et al. 1993; Requena et al. 1996; Uhlmann et al. 2006). However, since the number of root samples were relatively small and sampling was performed in a restricted zone around the trunk of the boojum, we cannot conclude that the entire root system of these two trees was nonmycorrhizal. A complete absence of AMF in these two cases is questionable because we found spores belonging to three AMF species at site 2d and six AMF species at site 6a under these two trees.

The number of 23 AMF species we found was surprisingly high, considering the extremely arid conditions compared with results from other arid regions, where up to 14 species have been reported (Uhlmann et al. 2006; Stutz et al. 2000; Jacobson et al. 1993). Uhlmann et al. (2004) reported 44 AMF species in semi-arid areas in Namibia. These data seem to support the hypothesis that diversity may decrease with increasing aridity (Dhillion and Zak 1993; Jacobson 1997; Uhlmann et al. 2006). Nevertheless, the climatic condition is only one factor that affects the diversity of AMF. The diversity of AMF can be linked to soil characteristics (Coughlan et al. 2000), land use (Oehl et al. 2003), diversity of plant species (Helgason et al. 2002) and depth of soil (Oehl et al. 2005).

Four AMF species (*G. etunicatum, G. mosseae, G. intraradices* and *G. macrocarpum*) were detected at all sites and we concluded that they are always associated with boojum trees. It would be interesting to learn whether these AMF, individually or in combination, are essential for the growth of boojum trees. Other species were detected as spores only at particular sites, suggesting that although they can form AMF–boojum interactions, their role is secondary.

In summary, our results show that the boojum tree is an AMF host plant associated with a high number of AMF species ranging over several AMF genera and families. The importance of the arbuscular mycorrhizal symbiosis for the growth of *F. columnaris* under the extremely harsh climatic conditions needs further studies.

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