

Ecology of vertical life in harsh environments: The case of mycorrhizal symbiosis with secular cliff climbing trees (Juniperus phoenicea L.)

Hervé Sanguin, Coralie Mathaux, Frédéric Guibal, Yves Prin, Jean-Paul Mandin, Thierry Gauquelin, Robin Duponnois

► To cite this version:

Hervé Sanguin, Coralie Mathaux, Frédéric Guibal, Yves Prin, Jean-Paul Mandin, et al.. Ecology of vertical life in harsh environments: The case of mycorrhizal symbiosis with secular cliff climbing trees (Juniperus phoenicea L.). Journal of Arid Environments, 2016, 134, pp.132–135. 10.1016/j.jaridenv.2016.07.008 hal-01444638

HAL Id: hal-01444638 https://hal.science/hal-01444638

Submitted on 15 Feb 2022

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Ecology of vertical life in harsh environments: The case of mycorrhizal symbiosis with secular cliff climbing trees (*Juniperus phoenicea* L.)

Hervé Sanguin ^{a, *}, Coralie Mathaux ^b, Frédéric Guibal ^b, Yves Prin ^a, Jean-Paul Mandin ^c, Thierry Gauquelin ^b, Robin Duponnois ^d

^a CIRAD, UMR LSTM, F-34398, Montpellier, France

^b Institut Méditerranéen de Biodiversité et d'Ecologie marine et continentale, Aix Marseille Université, CNRS, IRD, Avignon Université, UMR 7263, F-13397, Marseille, France

^c Société botanique de l'Ardèche, Réserve Naturelle Nationale des Gorges de l'Ardèche, F-30760, Saint Julien-de-Peyrolas, France

^d IRD, UMR LSTM, F-34398, Montpellier, France

ARTICLE INFO

Article history: Received 12 February 2016 Received in revised form 27 May 2016 Accepted 27 July 2016

Keywords: 454-Pyrosequencing Juniperus phoenicea Mycorrhizal community Secular tree Harsh environment

ABSTRACT

The Phoenician juniper tree (*Juniperus phoenicea* L.) is emblematic of Mediterranean ecosystems. Secular trees from a relict population are present in the cliffs of the Ardèche gorges (protected natural reserve). This population shows a remarkable adaptability to major physical, nutrient and water availability constraints. The basis of its adaptation to hostile environmental conditions is poorly understood. The aim of the study is to decipher the diversity and structure of the Phoenician juniper mycorrhizal community in order to highlight mycorrhizal characteristics related to the particular ecology of this relict population. We hypothesized that a long-term co-evolution between arbuscular mycorrhizal (AM) fungi and the plant partner, emphasized by a geographic isolation, may have selected a specific mycorrhizal community from Phoenician juniper secular trees revealed a complex community, notably composed of *Rhizophagus* and poorly-affiliated *Glomeraceae* clades. The AM fungal community characterized was mainly related to those found in arid and semi-arid habitats, strengthening the ecological specificity of the Phoenician juniper AM fungal communities to harsh environmental conditions.

1. Short note

Juniperus phoenicea L. (family Cupressaceae) is a small (8–12 m tall) gynodioic tree native to the Mediterranean basin, mainly distributed in its western part. It is a light-demanding tree resisting to dry climates and characterized by high pioneer properties (Quézel and Médail, 2003). Populations of *J. phoenicea* subsp. turbinata are characteristic of the coastal areas while *J. phoenicea* subsp. phoenicea are growing up to 2000 m (e.g. on the Atlas mountain slopes). The subsp. phoenicea shows a low level of geographic differentiation and a conservation of local characters by isolation from other subspecies (Boratynski et al., 2009).

In France, populations of *J. phoenicea* subsp. *phoenicea* stretch to the nature reserve of the Ardèche gorges (Mathaux et al., 2016), in the south of Rhône-Alpes. This reserve is characterized by a 200-m

* Corresponding author. E-mail address: herve.sanguin@cirad.fr (H. Sanguin). deep canyon composed of urgonian limestones and an extensive plateau mainly covered by typical Mediterranean forest. Old Phoenician juniper trees found on the large walls of the limestone cliffs have adapted their growth to the topological and physicochemical constraints (Mathaux et al., 2016). This ecosystem, functioning for millennium - trees have been dated to be thousandyear-old (Mathaux et al., 2016) - is probably among the last native ones in France, representing a possible relict population (geographic and physical isolation, confined distribution). Its natural regeneration is reaching a critical threshold due to erosion and to the attested warming in the Rhône-Alpes region (Météo-France Centre-Est, 2011). Phoenician juniper trees establish a symbiosis with arbuscular mycorrhizal (AM) fungi (Boullard, 1986), a key parameter in ecosystem functioning (Smith and Read, 2008). Characterizing Phoenician juniper mycorrhizal diversity may represent a major insight to better understand the biological functioning of this particular ecosystem.

Three secular Phoenician trees (J1, J2, and J3; Fig. 1) located on the reserve canyon cliffs (Gaud, Saint-Remèze, France; Fig. A1) were



Fig. 1. Photos of selected *J. phoenicea* trees in the protected nature reserve of the Ardèche gorges (Gaud, Saint-Remèze, France). Phoenician juniper trees (A) J1, (C) J2 and (E) J3 are indicated with red arrows. Red circles indicate root sampling area for each tree, visualized more precisely in (B), (D), (F) for J1, J2 and J3, respectively. (G) Schema of the general morphology of J3 growing downwards on the cliff. The trees J1 ($44^{\circ}21'57''N - 4^{\circ}27'06''E$, 268 m altitude) and J2 (J2, $44^{\circ}21'56''N 4^{\circ}27'08''E$, 268 m altitude) are distant from 25 m and are both located on a 2-m wide rocky shelf (A and C) used as pathway by feral goats. The tree J3 ($44^{\circ}21'44''N$, $4^{\circ}26'58''E$, 190 m altitude) presents an original morphology since it grows down directly along the cliff (G). The juniper roots were shown to penetrate in rock crevices partially filled by soil deposits (B, D and E). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

reached using rock-climbing equipment. The selected trees are hundred-years-old (141, 128 and 216 years old for tree J1, J2, and J3, respectively), and are characterized by their extremely slow radial growth rates (0.37, 0.21 and 0.25 mm per year, respectively) (Appendix). Only three trees could be sampled because of the very low accessibility of root systems (rock crevices) and tree instability.

A 18S-based 454-pyrosequencing approach was applied to characterize the Phoenician juniper AM fungal community. The 18S rRNA gene as well as the primer set were selected because of their reliability to assess AM fungal diversity in environmental samples (Öpik et al., 2013; Van Geel et al., 2014). 454 data-processing (for more details, see Appendix file) lead to 4647 high quality sequences from approximately 10,000 raw sequences. All the analyses were performed both with and without singletons because it has been shown that half of the singletons may represent true target taxa (Brown et al., 2015). Finally, the sequence number between environmental samples was rarefied to improve statistical robustness.

The AM fungal community was composed of 77 molecular operational taxonomic units (MOTUs) (42 without singletons), based on a 97% sequence similarity. A high diversity coverage (>97%) was reached, with less than 10 MOTUs not retrieved (Boneh estimates) (Table 1), demonstrating a suitable sequencing effort for

Table 1	
Richness and diversity inc	lexes

comparison of community richness, diversity and composition between environmental samples (Lemos et al., 2011). The AM fungal diversity and evenness among trees tended to be different, contrary to community richness (Table 1).

Approximately 80% of sequences were shared between at least two trees. Differences in the AM fungal community were only due to low abundant MOTUs membership (Fig. A2). On the other hand, the genetic structure of the AM fungal community was significantly different among trees (P < 0.001; Unifrac tests on genetic distances; Table A1). Phylogeny-based approaches are demonstrated as powerful approaches compared to similarity-based approaches to reliably analyse microbial community in environment (Senés-Guerrero and Schüßler, 2016). The differences observed in the AM fungal community structures among the juniper trees might be related to abiotic factors. The soil deposits surrounding the juniper roots showed variations for several chemical parameters (CaCO₃ content, SOC and SOM; Table A2) between [3 and the two other trees. Heterogeneity of physical and nutritive constraints and water availability are known as potential drivers of mycorrhizal communities (Tedersoo et al., 2003; Johnson et al., 2010; Jumpponen et al., 2010). The variation in abiotic factors were postulated as the main factor driving both plant and AM fungal communities in

Juniper tree ^a	Number of MOTUs	Chao (lci; hci) ^b	Shannon (lci; hci) ^b	Inverse simpson (lci; hci) ^b	Simpson evenness	Shannon evenness	Coverage (%) ^c	Boneh estimation
J1	42	70	1.55	3.10	0.07	0.41	97.69	7.58
		(52; 121)	(1.46; 1.64)	(2.93; 3.28)				
J1(- s)	21	21	1.41	2.96	0.14	0.46	99.79	3.29
		(21; 24)	(1.34; 1.49)	(2.82; 3.13)				
J2	26	61	0.96	1.59	0.06	0.30	98.49	4.37
		(36; 152)	(0.87; 1.06)	(1.50; 1.69)				
J2(- s)	21	21	0.90	1.56	0.07	0.29	99.95	0
		(21; 21)	(0.85; 0.96)	(1.50; 1.63)				
J3	20	48	0.51	1.22	0.06	0.17	98.89	3.26
		(27; 133)	(0.43; 0.60)	(1.18; 1.27)				
J3(- s)	13	14	0.45	1.20	0.09	0.18	99.75	1.34
		(13; 24)	(0.39; 0.52)	(1.16; 1.24)				

^a J1, J2, J3 indicate the juniper trees 1, 2 and 3, respectively. (- s) indicates the data without the singletons. All data correspond to normalized data based on random subsamplings.

^b lci and hci indicate the lower and higher 95% confidence intervals, respectively.

^c Good's coverage: sum of probabilities of observed classes calculated as (1 - (n/N)), where n is the number of singleton sequences and N is the total number of sequences.

successionally stable plant populations (Habitat hypothesis; Zobel and Öpik, 2014), which is the case of the Phoenician juniper population investigated. However, the impact of abiotic factors cannot be robustly evaluated in the current study due to the limited sampling and 454-data treatment intrinsic biases (Gihring et al., 2012).

The phylogenetic assignment of MOTUs (Fig. 2) revealed a predominance of *Glomeraceae* (*Rhizophagus*, 47% of sequences; *Sclerocystis*, 0.03%; *Septoglomus*, 0.27%), in accordance with previous results on *Juniperus procera* (Wubet et al., 2006). The majority of MOTUs (52% of sequences) had uncertain affiliation regarding reference strains but constituted nine well-resolved clades (Glo-a to Glo-i; Table A3) related to known virtual taxa (Öpik et al., 2014). The most abundant MOTU (1382 sequences), shared between all trees (Fig. A1) was affiliated to a cluster composed of *Rhizophagus intraradices/Rhizophagus proliferus*, distinct from the cluster defined by well-known *Rhizophagus irregularis*. The worldwide distribution of *Rhizophagus* suggests a lack of specialization. However, some species present a rapidly evolving genotypic diversity (Angelard et al., 2014) having to differential effects on plant growth (Koch et al., 2006).

The need to develop AM fungal diversity analyses at the community and population levels appears crucial to reliably evaluate AM fungal ecology and adaptation to environmental changes. The originality of the AM fungal community associated to the Phoenician juniper population was assessed using an ecosystem-related approach crossing taxonomic assignment, genetic structure and host origin data, as described in Appendix. Forty four percent of MOTUs (44% of sequences) were closely related to AM fungal sequences from desert and xeric shrublands (Biome) (Blast analysis;

Table 2

Comparison of AMF genetic structures from the relict Phoenician juniper population and different shrubland habitats corresponding to different biomes.

Comparison with limestone cliffs ^a	UWscore ^b	<i>P</i> -value ^c				
Tropical dry broadleaf forest biome-related habitats						
Dry afromontane forest	0.91,667	0.0170,093				
Seasonal tropical forest	0.97,744	0.00100,921				
Tropical shrubland biome-related habitat						
Arid montane shrubland	0.913,445	0.0650,088				
Subtropical dry broadleaf forest biome-related habitat						
Dry eucalyptus forest	0.923,016	0.00400,924				
Subtropical shrubland biome-related habitats						
Fynbos	0.931,909	0.105,009				
Semi-arid mediterranean shrubland	0.931,909	0.0660,085				
Desert and xeric shrubland biome-related habitats						
Desert	0.918,605	0.044,092				
Dolomitic vegetation	0.880,386	0.358,008				
Gypsophilous vegetation	0.914,079	0.0790,092				

^a "Limestone cliffs" represents the habitat of AM fungal MOTUs from Phoenician juniper trees J1, J2, J3. The biome and habitat references were defined in MAARJAM database.

^b "UWscore" indicate the score obtained with the unweighted Unifrac tests.

^c Statistical tests were done at the habitat level (P < 0.05).

Table A4). The genetic structure of AM fungal community from the Ardèche Gorges limestone cliffs showed significant similarity with AM fungal communities from arid and semi-arid habitats characterized by low water availability (unweighted unifrac test; Table 2 and Table A5). For example, the similarity with the dolomitic habitat may reflect the comparable abiotic and physical constraints between both habitats (drought due to rainwater percolation and high level of calcium carbonate). Only habitats in Tropical and



Fig. 2. Network phylogenetic analysis of AM fungal 18S sequences from the Phoenician juniper trees (J1, J2, J3). All sequences were clustered in MOTUs (97% similarity). 18S sequences from public database used as references are from Krüger et al. (2012) and MAARJAM database (Öpik et al., 2013). The circles indicate the presence of MOTUs and the size is indicative of the sequence number in MOTUs (see scale at the top-right corner). Blue color corresponds to sequences from J1, red from J2 and green from J3. Glo-a to Glo-i correspond to *Clomeraceae* clades with uncertain position in *Clomus* « sensu lato » closely related to one or several fungal virtual taxa (MAARJAM database) (see Table A3 for details). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

subtropical dry broadleaf forest biomes showed significant differences. Overall, the results highlighted the specific status of the Phoenician juniper AM fungal community regarding harsh environmental conditions, and support the habitat preference of AM fungal taxa detailed in Helgason and Fitter (2009), rather than the plant host preference.

The current study constitutes a preliminary exploration of AM fungal community associated to a relict Phoenician juniper population, growing in extreme conditions on limestone cliffs. Unraveling the complexity of mycorrhizal symbiosis associated to relict plant populations represents a major insight towards a better understanding of microbial life in harsh ecosystems, and the impacts of long-term environmental changes on plant-fungal interactions (Woolbright et al., 2014). Climate-induced die off in juniper woodlands has been shown to strongly affect soil microbial functioning (Lloret et al., 2015), but many gaps still exist in our knowledge of plant-fungal symbiosis responses to climate changes (Johnson et al., 2013; Kivlin et al., 2013). A more extensive exploration of Phoenician juniper tree ecotypes and other juniper relict populations (El-Bana et al., 2015) may allow the determination of an "island" effect (Peay et al., 2007) of the relict Phoenician juniper on AM fungal community and improve our knowledge about the laws that rule mycorrhizal symbiosis during long-term coevolution.

Acknowledgements

This work was supported by the Office de Génie Ecologique et l'Agence de l'Arbre (O.G.E.). We are grateful to the Comité consultatif de la Réserve des Gorges de l'Ardèche and the Syndicat de gestion des Gorges de l'Ardèche for authorization and helping fieldwork. We thank C. Lecareux (UMR IMBE, France) for soil chemical analyses, M. Öpik (University of Tartu, Estonia) and M. Héry (UMR Hydrosciences, France) for scientific discussion and technical advises.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.jaridenv.2016.07.008.

References

- Angelard, C., Tanner, C.J., Fontanillas, P., Niculita-Hirzel, H., Masclaux, F., Sanders, I.R., 2014. Rapid genotypic change and plasticity in arbuscular mycorrhizal fungi is caused by a host shift and enhanced by segregation. ISME J. 8, 284–294.
- Boratynski, A., Lewandowski, A., Boratynska, K., Montserrat, J.M., Romo, A., 2009. High level of genetic differentiation of *Juniperus phoenicea* (Cupressaceae) in the Mediterranean region: geographic implications. New. Phytol. 277, 163–172.
- Boullard, B., 1986. Les mycorhizes des *Juniperus* de la flore française. Bull. Soc. Mycol. Fr. 102, 1–18.
- Brown, S.P., Veach, A.M., Rigdon-Huss, A.R., Grond, K., Lickteig, S.K., Lothamer, K., Oliver, A.K., Jumpponen, A., 2015. Scraping the bottom of the barrel: are rare high throughput sequences artifacts? Fungal Ecol. 13, 221–225.
- El-Bana, M., Shaltout, K., Khalafallah, A., Hosni, M., 2015. Ecological status of the

Mediterranean Juniperus phoenicea L. relicts in the desert mountains of Nort Sinai. Egypt. Flora 205, 171–178.

- Gihring, T.M., Green, S.J., Schadt, C.W., 2012. Massively parallel rRNA gene sequencing exacerbates the potential for biased community diversity comparisons due to variable library sizes. Environ. Microbiol. 14, 285–290.
- Helgason, T., Fitter, A.H., 2009. Natural selection and the evolutionary ecology of the arbuscular mycorrhizal fungi (Phylum Glomeromycota). J. Exp. Bot. 60, 2465–2480.
- Johnson, N.C., Wilson, G.W.T., Bowker, M.A., Wilson, J.A., Miller, R.M., 2010. Resource limitation is a driver of local adaptation in mycorrhizal symbioses. Proc. Natl. Acad. Sci. U. S. A. 107, 2093–2098.
- Johnson, N.C., Angelard, C., Sanders, I.R., Kiers, E.T., 2013. Predicting community and ecosystem outcome of mycorrhizal responses to global change. Ecol. Lett. 16, 140–153.
- Jumpponen, A., Jones, K.L., Mattox, D., Yaege, C., 2010. Massively parallel 454sequencing of fungal communities in *Quercus* spp. ectomycorrhizas indicates seasonal dynamics in urban and rural sites. Mol. Ecol. 19, 41–53.
- Kivlin, S.N., Emery, S.M., Rudgers, J.A., 2013. Fungal symbionts alter plant responses to global change. Am. J. Bot. 100, 1445–1457.
- Koch, A.M., Croll, D., Sanders, I.R., 2006. Genetic variability in a population of arbuscular mycorrhizal fungi causes variation in plant growth. Ecol. Lett. 9, 103–110.
- Krüger, M., Krüger, C., Walker, C., Stockinger, H., Schüssler, A., 2012. Phylogenetic reference data for systematics and phylotaxonomy of arbuscular mycorrhizal fungi from phylum to species level. New phytol. 193, 970–984.
- Lemos, L.N., Fulthorpe, R.R., Triplett, E.W., Roesh, L.F.W., 2011. Rethinking microbial diversity analysis in the high throughput sequencing era. J. Microbiol. Meth. 86, 42-51.
- Lloret, F., Mattana, S., Curiel Yuste, J., 2015. Climate-induced die-off affects plantsoil-microbe ecological relationship and functioning. FEMS Microbiol. Ecol. 91, 1–12.
- Mathaux, C., Mandin, J.P., Oberlin, C., Edouard, J.-L., Gauquelin, T., Guibal, F., 2016. Ancient Juniper trees growing on cliffs: towards a long Mediterranean tree-ring chronology. Dendrochronologia 37, 79–88.
- Météo-France Centre-Est, 2011. Étude du changement climatique pour le SRCAE Rhône-Alpes. Étude réalisée Météo-France Centre-Est Div. Développements-Études-Climatologie la DREAL Rhône-Alpes, janvier 2011, 73.
- Öpik, M., Davison, J., Moora, M., Zobel, M., 2014. DNA-based detection and identification of *Glomeromycota*: the virtual taxonomy of environmental sequences. Botanique 92, 135–147.
- Öpik, M., Zobel, M., Cantero, J.J., Davison, J., Facelli, J.M., Hiiesalu, I., et al., 2013. Global sampling of plant roots expands the described molecular diversity of arbuscular mycorrhizal fungi. Mycorrhiza 23, 411–430.
- Peay, K.G., Bruns, T.D., Kennedy, P.G., Bergemann, S.E., Garbelotto, M., 2007. A strong species-area relationship for eukaryotic soil microbes: island size matters for ectomycorrhizal fungi. Ecol. Lett. 10, 470–480.
- Quézel, P., Médail, F., 2003. Ecologie et biogéographie des forêts du bassin méditerranéen. Elsevier, Paris, p. 572.
- Senés-Guerrero, C., Schüßler, A., 2016. A conserved arbuscular mycorrhizal fungal core-species community colonizes potato roots in the Andes. Fungal Divers. 77, 317–333.
- Smith, S.E., Read, D.J., 2008. Mycorrhizal Symbiosis, 3nd edn. Academic press, San Diego, CA, USA.
- Tedersoo, L, Kõljalg, U, Hallenberg, N., Larsson, K.-H., 2003. Fine scale distribution of ectomycorrhizal fungi and roots across substrate layers including coarse woody debris in a mixed forest. New Phytol. 159, 153–165.
- Van Geel, M., Busschaert, P., Honnay, O., Lievens, B., 2014. Evaluation of six primer pairs targeting the nuclear rRNA operon for characterization of arbuscular mycorrhizal fungal (AMF) communities using 454 pyrosequencing. J. Microbiol. Meth. 106, 93–100.
- Woolbright, S.A., Whitham, T.G., Gehring, C.A., Allan, G.J., Bailey, J.K., 2014. Climate relicts and their associated communities as natural ecology and evolution laboratories. Trends Ecol. Evol. 29, 406–416.
- Wubet, T., Weiss, M., Kottke, I., Teketay, D., Oberwinkler, F., 2006. Phylogenetic analysis of nuclear small subunit rDNA sequences suggests that the endangered African Pencil Cedar, *Juniperus procera*, is associated with distinct members of *Glomeraceae*. Mycol. Res. 110, 1059–1069.
- Zobel, M., Öpik, M., 2014. Plant and arbuscular mycorrhizal fungal (AMF) communities – which drives which? J. Veg. Sci. 25, 1133–1140.