

## Diversity and distribution patterns of endophytic mycoflora of Atlas cedar, *Cedrus atlantica* (Endl) G. Manetti ex Carrière, needles in Belezma biosphere reserve (Batna, Algeria)

Imen Cherak<sup>1,2\*</sup>, Abdelkrim Si Bachir<sup>2</sup>, Lakhdar Cherak<sup>2</sup>, Cherif Ghazi<sup>3</sup>, Lotfi Loucif<sup>2</sup> & Mahdi Sellami<sup>1</sup>

<sup>1</sup>National School of Agronomy, El Harrach, Algiers, 16200 Algeria

<sup>2</sup>Faculty of Nature and Life Sciences, University of Batna 2, Batna, 05110 Algeria

<sup>3</sup>Department of Biology, Faculty of Nature and Life Sciences, University of Ghardaia, 47000 Algeria

\*Corresponding author, e-mail: i.cherak@univ-batna2.dz

### ABSTRACT

This study aims to assess the diversity and distribution of fungal mycoflora developing on *Cedrus atlantica* (Endl) G. Manetti ex Carrière needles in three sites in the Belezma National Park (Biosphere Reserve, Northeast - Algeria). Three sites were sampled according to a cedar decline gradient, these are the massifs of: Telmet (healthy site), Boumerzoug (moderately depressed) and Tougurt (decayed site). Polymerase chain reaction (PCR) molecular analysis, allows identifying 19 endophytic mycotaxa. All the identified species have a weak occurrence frequency (less than 25%). In terms of specific richness, the moderately depressed site (Boumerzoug) homes the largest number of taxa (S = 17), followed by healthy site of Telmet (12 taxa), while the depressed site of Tougurt was the least populated (8 taxa). The hierarchical classification analysis (HCA) showed that the taxonomic composition of endophyte associations differs clearly from one site to another according to the cedar decline. The clustering representing healthy massif brings 2 species which are demanding phytoparasitic endophytes (*Fusarium* sp. and *Xylaria* sp.). The group associated to moderately depressed site hosts 7 taxa with a wide ecological valence, such as: *Canariomyces notabilis*, *Canariomyces vonarxii*, *Chaetomium aegilopsis*, *Coniolaria hispanica* and *Penicillium kubanicum*. Then, mycoflora group noted in the decayed cedar includes 10 taxa, in particular, saprophytic mycotaxa relatively less demanding with a high ecological valence like: *Biscogniauxia mediterranea*, *Alternaria arborescens*, *A. tenuissima* and three species of *Chaetomium* genus. The mycotaxa distribution is related to the specific conditions of colonized trees. Taxa specific to healthy and decayed massifs would represent bio indicators of the phytosanitary and ecological conditions of colonized cedars.

### KEY WORDS

Algeria; Atlas cedar; Belezma National Park; Decline; Endophytic; mycotaxa.

Received 18.03.2021; accepted 02.06.2021; published online 24.07.2021

### INTRODUCTION

Atlas cedar, *Cedrus atlantica* (Endl) G. Manetti ex Carrière, is an endemic species of North Africa. It is an essence that has always aroused a significant interest because of its numerous forestry qualities such as the maintenance of a biological balance, its low flammability, production of a good quality wood (M'hirit, 1982). Genetic ana-

lyses indicated that there are two major subpopulations: one in the Rif and Middle Atlas Mountains in Morocco and the other in the Algerian Tell Atlas and the Aures Mountains (Linares et al., 2011).

In recent years, this species is threatened by the decline in its optimal development area. The factors can be multiple and the risks seem to be assigned to the climate (decrease of the water balance, increase in temperature, drought, etc.) (Sli-

mani et al., 2014; Mouhli et al., 2018). Grazing, illegal logging and pollution also influence the sustainability of Atlas cedar (Taleb et al., 2016). Other factors that trigger and aggravate this decline are exclusively biotic, especially insect pests and phytopathogenic fungi (Kherchouche et al., 2013). The most affected region in Algeria is the Belezma Forest (Aures, Batna), where the situation is accentuated in the last years (Bentouati, 2008).

The Atlas cedar needles can be colonized by endophytic, saprophytic and pathogenic fungi. Endophytic fungi are all organisms inhabiting plant organs that at some time in their life can colonize internal plant tissues without causing apparent harm to the host (Petrini, 1991). In fact, many endophytic mycotaxa are asymptomatic, while others can change their trophic behavior, in particular to a saprophyte with the natural evolution related for example to the physiological seasonality of their host plants (senescence, end of biological and organic residues composition); but they can also be in phytopathogenic state, thus generating symptoms and/or signs (externalization by sporulation) and in this case, they are also quiescent or latent pathogens (Romero et al., 2001; Schulz & Boyle 2005).

Otherwise, endophytic fungi are taxonomically and ecologically heterogeneous organisms. The number of taxa isolated from a host species is usually large; they are specific for the host species, but species composition and frequencies are significantly affected by site-specific conditions (exposure, soil type, etc.). In addition, the relative importance and number of endophytic species vary among individuals in the sites (Petrini et al., 1992).

Several studies have been devoted to endophytic fungi on the Pinaceae (Petrini et al., 1992; Sieber et al., 1999; Ganley & Newcombe, 2006; Ladjal et al., 2013), but few studies have been dedicated to the mycoflora associated with *C. atlantica* (Harzallah et al., 2009; Bensaci et al., 2015).

This study aims to describe the different endophytic fungal species associated with Atlas cedar needles in the forest massifs of the Belezma National Park (Biosphere Reserve, Batna North-eastern of Algeria) using Polymerase chain reaction (PCR) molecular analysis. We also plan to highlight the assembly of endophytes groups developing on the Atlas cedar, depending on decline degree of subjects. This will deduct the relationship between fungal diversity and the health of *C. atlantica*.

## MATERIAL AND METHODS

### *Study area and sampling sites*

Three cedar forests of *C. atlantica* at the Belezma National Park, which is located in the semi-arid bioclimatic stage with cold winters and altitude variations, were sampled. The three explored sites contain relatively the same floristic composition; *C. atlantica*, *Quercus ilex* and *Juniperus oxycedrus*: (i) a cedar in the Telmet Massif (relatively healthy site: 35° 35' 21" N, 6° 02' 06" E, altitude 1740 m); (ii) a cedar in the Boumerzoug Massif (moderately depressed site: 35° 35' 32" N, 6° 05' 11" E, altitude 1510 m) and (iii) a cedar forest in the Tougurt massif (decayed site: 35° 34' 38" N, 6° 03' 24" E, altitude 1480 m) (Figure 1).

The degree of decline in the three sampled sites was defined with naked eye taking into account the number of Atlas cedar decayed per site: the cedar is considered healthy when more than 80% of the trees are healthy; it is moderately depressed when the rate of decayed trees is between 50% and 80% and is considered to be decayed when more than 80% of the cedars are depressed.

### *Sampling and treatment of Atlas cedar needles*

Needles of different ages of *C. atlantica* were sampled from 15 trees in April 2013 and from 27 trees in March 2015. Guo et al. (2008) demonstrated that the extent of endophytic leaf colonization for some Pinaceae follows a seasonal pattern; infection is most significant during the spring and fall season.

A total of 10 needles were selected from each sampled tree and randomly collected at the height of the person (1.5 to 2.0 m) on trees and are placed in sterile paper bags. A total of 420 needles were collected, 140 needles in each site (150 in 2013 and 270 in 2015). All these sampled needles were stored in the laboratory at a temperature of 4 °C, waiting to be examined within 24 hours (Khan et al., 2007). The surface was sterilized according to the method of Helander et al. (1994) modified by Bensaci et al. (2015); that showed its effectiveness in disinfecting the needles surface. Briefly, the needles were first treated with ethanol (95%) for 2 minutes, then with sodium hypochlorite (NaOCl, 3%) for 3 minutes and finally with ethanol (95%) for 30 seconds. Each treatment was rinsed with distilled water. After drying them with sterile blotting paper, needles were

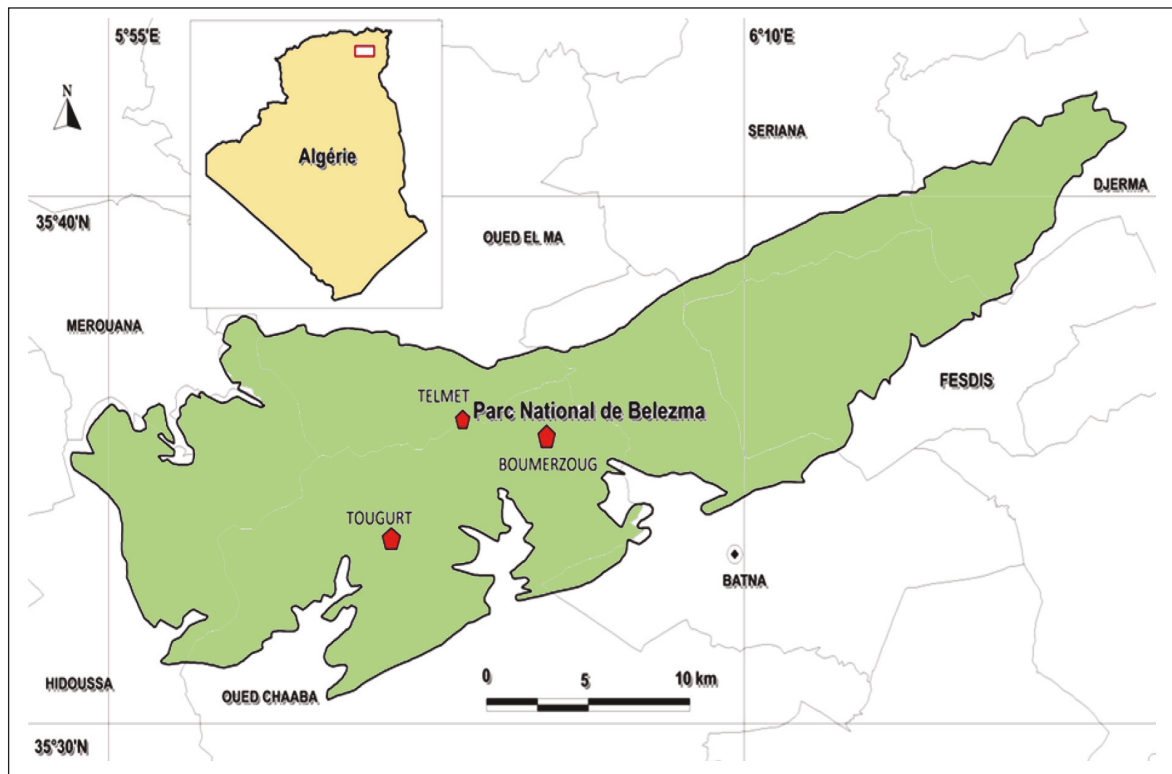


Figure 1. Localization of the 3 sampling sites in the Belezma National Park (north-east Algeria).

cut into 2 to 4 mm and were placed in Petri dishes containing Sabouraud medium supplemented with two antibiotics (Gentamycin and Chloramphenicol) to inhibit the bacterial growth. Five to six segments were deposited per dish; then they were incubated at 28 °C (Pimentel et al., 2006; Khan et al., 2010).

Petri dishes were incubated at 28 °C in the dark and were examined every day during the colony development (Pimentel et al., 2006). Pure cultures were prepared from the original mycelia which grew from the needle segments by transferring them to Petri dishes with Sabouraud or Malt medium without antibiotics. Then they were conserved to be identified.

#### ***Polymerase chain reaction (PCR) molecular analysis***

The fungal isolates were grown in 30 ml of Potato Dextrose broth (Sigma-Aldrich) in 100 ml conical flasks statically at 25 °C. Using a small sieve, the fungal mycelia were harvested and then rinsed with sterile water, carefully blotted and dried with filter paper and finally frozen at -20 °C. For the DNA extraction, the frozen fungal mycelia were

snap frozen in liquid nitrogen and ground using mortar and pestles. DNA was isolated from the ground fungal mycelia with DNeasy Plant Mini Kit (Qiagen). The Internal Transcribed Spacer (ITS) region was amplified using general primers ITS1 and ITS4 (White et al., 1990). Each reaction contained 1 x PCR Buffer (containing 1.5 mM of MgCl<sub>2</sub>), 0.2 mM each dNTP, 0.2 μM of each primer, 1 U of DNA Taq polymerase (Promega, UK) and 2 μl of template DNA. The PCR mix was adjusted to a final volume of 25 μl with water. The program used for amplification of the ITS region was: 95 °C for 3 min; 35 cycles of 94 °C for 30 s, 55 °C for 30 s, 72 °C for 1 min. The final extension was carried out at 72 °C for 10 min. PCR products were visualized by electrophoresis on 1.5 % agarose gel. Amplified samples were purified with Qiaquick PCR Purification Kit (Qiagen) and quantified using a Nanodrop ND-100 spectrophotometer (Nanodrop Technologies, Wilmington, DE). PCR products were adjusted to the required concentration and sequenced by Macrogen Europe with the ITS1 primer. Sequencing results were compared with databases in GenBank using the BLAST tool with the algorithm 'blastn'.

### Data analysis

The diversity of endophytic communities recorded was expressed by the total species richness "S", estimated by the total number of species/taxa identified in each studied site (Magurran, 2004).

The occurrence frequency was calculated for each species/taxa by the percentage of the number of records containing the species/taxa (i) in the total number of records (N) (Magurran, 2004). Four occurrence groups were defined according to the occurrence frequency (Bigot & Bodot, 1973): very accidental group (VA) where occurrence frequency is less than 12.5%, accidental group (AC) where occurrence ranges between 12.5 and 25%; the common group (CM) is present in more than 25 to 50% of the carried surveys and the constant group (CN) is present in more than 50% of the samples.

In order to describe the different endophytes assemblages and their relationship with the decline state of cedar trees, a hierarchical classification analysis (HCA) with complete linkage (hierarchical clustering) was used considering the presence/absence of identified species/taxa per needle and per cedar decline scale. Starting by considering each species/taxa as a class, we try to merge two or more appropriate classes (depending on the similarity) to form a new class. The process is iterated until all species/taxa are in the same class. This classification generates a tree that can be cut at different levels to obtain a greater or lesser number of classes (Lebart et al., 1997). The statistical test was performed by the software XLSTAT (2010).

## RESULTS

Based on the phylogenetic analysis, we found 19 species/taxa on 34 strains of fungal endophytes isolated from *C. atlantica* needles (Table 1). These taxa are distributed over 5 families, 5 orders and 3 different classes. The Sordariomycetes class is the most represented taxa with 13 species/taxa (Table 2).

According to the presence/absence of taxa in each studied site, Boumerzoug site (moderately depressed) records the highest species richness with 17 taxa; Telmet site (healthy) recorded 12 taxa and 8 taxa were identified in Tougurt cedar forest (depressed site) (Table 2).

Among the nineteen fungal taxa inventoried, the endophytic mycoflora is represented by 7 taxa re-

corded at the three studied sites. These taxa are: *Alternaria arborescens* (Simmons E.G. 1999), *Alternaria* sp., *Chaetomium grande* (Kunze, 1817), *C. senegalense* (Ames L.M., 1963) (= *Ovastospora senegalense*), *Chaetomium* sp. (Kunze, 1817), *Pleosporales* sp. and *Biscogniauxia mediterranea* (Kuntze, 1891). Among the 420 examined needles, most taxa are with very accidental to accidental occurrence. Only *Alternaria arborescens* species was noted with constant frequency of 26.72 % and considered of common occurrence (Table 2).

Clustering dendrogram established by the hierarchical classification analysis shows differences in the fungal microflora distribution on *C. atlantica* needles. Indeed, the HCA results reveals three clusters grouped according to their presence on the cedar needles and its decline degree (Fig. 2).

The cluster representing the endophytes group associated to the decayed site contains the largest number of taxa (10 taxa), such as: *Biscogniauxia mediterranea*, *Pleosporales* sp., *Alternaria arborescens*, *A. tenuissima* (Wiltshire S.P., 1933), *Alternaria* sp., *Chaetomium grande*, *C. senegalense* (*Ovastospora senegalense*), *C. strumarium* (Rai J.N., 1964), *Chaetomium* sp. and *Stolonocarpus gigasporus* (Wang et al., 2019). The moderately depressed site records a cluster which regrouped 7 taxa: *Byssoschlamys verrucosa* (Samson & Tansey, 1975), *Canariomyces notabilis* (Arx, 1984), *Canariomyces vonarxii*, *Conioliariella hispanica* (Checa J., 2008), *Chaetomium aegilopsis*, *Rosellinia australis* (Saccardo, P.A., 1882) and *Penicillium kabunicum* (Baghdadi, 1968). The healthy site hosts the less diversified mycoflora group including only 2 taxa: *Fusarium* sp. and *Xylaria* sp. (Fig. 2).

## DISCUSSION

The Atlas cedar of Belezma National Park hosts a fairly diverse range of fungal endophytes developing on its needles. The taxa identification was carried out at the species level as much as possible. In fact, among the 19 inventoried taxa, only 14 endophytes were determined up to the species. In the same studied area, Bensaci et al. (2015) have identified only 6 species out of 17 taxa. Harzallah et al. (2009) determined only 3 species out of 20 taxa. However, the inventory of our identified species presents several differences compared to the systematic lists established by the authors cited above.



Strain	Closely related species/genus	Query Cover	Per. Ident	Accession
1	<i>Alternaria arborescens</i>	100%	100%	KC464334.1
2	<i>Coniolaria hispanica</i>	93%	97%	FJ172294.1
3	<i>Rosellinia australis</i>	90%	98%	AY908997.1
4	<i>Chaetomium</i> sp.	100%	99%	GU207839.1
5	<i>Alternaria</i> sp.	100%	100%	KC623557.1
6	<i>Alternaria arborescens</i>	100%	99%	KC464334.1
7	<i>Alternaria</i> sp.	100%	99%	KC623557.1
8	<i>Pleosporales</i> sp.	100%	100%	JX179239.1
9	<i>Alternaria arborescens</i>	100%	100%	KC464334.1
10	<i>Chaetomium strumarium</i>	100%	99%	JQ796877.1
11	<i>Pleosporales</i> sp.	100%	100%	JX179239.1
12	<i>Alternaria</i> sp.	100%	100%	KC623557.1
13	<i>Alternaria arborescens</i>	100%	100%	KC464334.1
14	<i>Alternaria tenuissima</i>	100%	100%	KC818614.1
15	<i>Alternaria</i> sp.	95%	100%	GU584946.1
>160325-01_O24_184FZAA050.ab1	<i>Ovatospora senegalensis</i>	90%	99.28%	MH860871.1
>160325-01_K20_184FZAA051.ab1	<i>Ovatospora senegalensis</i>	90%	99.28%	MH860871.1
>160325-01_E22_184FZAA052.ab1	<i>Biscogniauxia</i> sp.	90%	98.42%	KF367566.1
>160325-01_O22_184FZAA053.ab1	<i>Chaetomium</i> sp. R01	47%	98.53%	GU207839.1
>160325-01_G22_184FZAA054.ab1	<i>Ovatospora senegalensis</i>	49%	99.64%	MH860871.1
>160325-01_M22_184FZAA056.ab1	<i>Ovatospora senegalensis</i>	54%	99.82%	MH860871.1
>160325-01_A24_84FZAA058.ab1	<i>Achaetomium aegilopsis</i>	48%	99.42%	MT568841.1
>160325-01_O20_184FZAA059.ab1	<i>Penicillium kabunicum</i>	39%	99.63%	MH862240.1
>160325-01_G20_184FZAA060.ab1	<i>Canariomyces vonarxii</i>	59%	99.44%	MK926806.1
>160325-01_G24_184FZAA062.ab1	<i>Ovatospora senegalensis</i>	48%	99.64%	MH860871.1
>160325-01_K22_184FZAA063.ab1	<i>Stolonocarpus gigasporus</i>	89%	91.87%	NR_165592.1
>160325-01_M20_184FZAA064.ab1	<i>Chaetomium</i> sp. EF7	66%	99.62%	GQ176270.1
>160325-01_I24_184FZAA065.ab1	<i>Biscogniauxia mediterranea</i>	90%	99.09%	KM216754.1
>160325-01_I20_184FZAA066.ab1	<i>Ovatospora senegalensis</i>	53%	99.82%	MH860871.1
>160325-01_K24_184FZAA067.ab1	<i>Canariomyces notabilis</i>	87%	98.32%	MK926803.1
>160325-01_C24_184FZAA068.ab1	<i>Biscogniauxia</i> sp. BRO-2013	51%	99.28%	KF367566.1
>160325-01_E20_184FZAA069.ab1	<i>Achaetomium strumarium</i>	46%	97.80%	NR_144811.1
>160325-01_M24_184FZAA070.ab1	<i>Penicillium kabunicum</i>	33%	99.46%	MH862240.1
>160325-01_E24_184FZAA071.ab1	<i>Biscogniauxia</i> sp. BRO-2013	82%	98.74%	KF367566.1

Table 1. Molecular identification of fungal endophyte recovered from needles of *Cedrus atlantica* based on ITS sequences.

Indeed, our observations led to the identification of 14 species not mentioned by these authors. However, they did also mention 28 taxa not identified in our study: 16 mycotaxa by Harzallah et al. (2009) and 12 by Bensaci et al. (2015). This would be related to the fact that the method used here (PCR) is more precise than that used in old studies which are only based on the microscopic observations of

the hyphae morphological characters (partitioning, coloring) and the reproductive forms (fructifications, forms and spore colors) with reference to Larnier et al. (1978) identification keys.

This study showed that most of the isolated endophytes belong to the class Dothideomycetes and Sordariomycetes which constitutes the major part of mycoendophytes associated with woody plants.

Class	Order	Family	Species	Telmet (healthy site)	Boumerzoug (moderately depressed)	Tougurt (depressed)	C%	Occurrence scale
Dothideomycetes	Pleosporales	Pleosporaceae	<i>Alternaria arborescens</i>	+	+	+	26.72	CM
			<i>Alternaria tenuissima</i>	+	+	-	3.05	VA
			<i>Alternaria</i> sp.	+	+	+	22.14	AC
			<i>Pleosporales</i> sp.	+	+	+	17.56	AC
Sordariomycetes	Sordariales	Chaetomiaceae	<i>Chaetomium aegilopis</i>	-	+	-	0.76	VA
			<i>Chaetomium grande</i>	+	+	+	21.37	AC
			<i>Chaetomium senegalense</i> ( <i>Ovatospora senegalensis</i> )	+	+	+	20.61	AC
			<i>Chaetomium strumarium</i>	+	+	-	3.82	VA
			<i>Chaetomium</i> sp. <i>Stolonocarpus gigasporus</i>	+	+	+	16.79	AC
			<i>Canariomyces notabilis</i>	-	+	-	1.53	VA
			<i>Canariomyces subthermophilus</i>	-	+	-	3.05	VA
			<i>Xylaria</i> sp.	+	-	-	2.29	VA
	Xylariales	Xylariaceae	<i>Coniolaria hispanica</i>	-	+	-	2.29	VA
			<i>Rosellinia australis</i>	-	+	-	2.29	VA
			<i>Biscogniauxia mediterranea</i>	+	+	+	11.45	VA
			Hypocreales	Nictiaceae	<i>Fusarium</i> sp.	+	-	-
	Eurotiomycetes	Eurotiales	Trichocomaceae	<i>Penicillium kabunicum</i>	-	+	-	1.53
<i>Byssochlamys verrucosa</i>				-	+	+	2.29	VA
Total specific richness (S)			19	12	17	8		

Table 2. Systematic list, occurrence rate and constancy scales (C %) of most closely species of fungal endophytes associated with Atlas cedar identified in the Belezma National Park (Batna, Northeast Algeria).

Constancy scale: CM (common species), AC (accidental), VA (very accidental).

These same classes represent more than 75% of isolated endophytes going from arctic regions to tropical area (Arnold & Lutzoni, 2007).

The fungal endophytes colonization marked differences between sites; this may be attributed to several factors which are in the majority of the site origins such as relief, altitude of site, hygrometry, as well as edaphic condition (Harzallah et al., 2009). Similar results were obtained for other Pinaceae trees (Sieber et al., 1999).

Spring period reflects a determining physiological status for Atlas cedar, according to Helander et

al. (1993), marked by defoliation and the budding of new needle rosettes. Infection is high during this growing season for Atlas cedar, which is thus more susceptible to infection.

In terms of mycotaxa diversity the Boumerzoug site (moderately depressed) shelters the largest taxa number (17) than in healthy and depressed sites (12 in Telmet and 8 taxa in Tougurt). In fact, the moderately depressed cedar massif represents a mixture of healthy and decayed trees where fungi find large and favorable conditions for their development. The tree population density may be implicated in needles

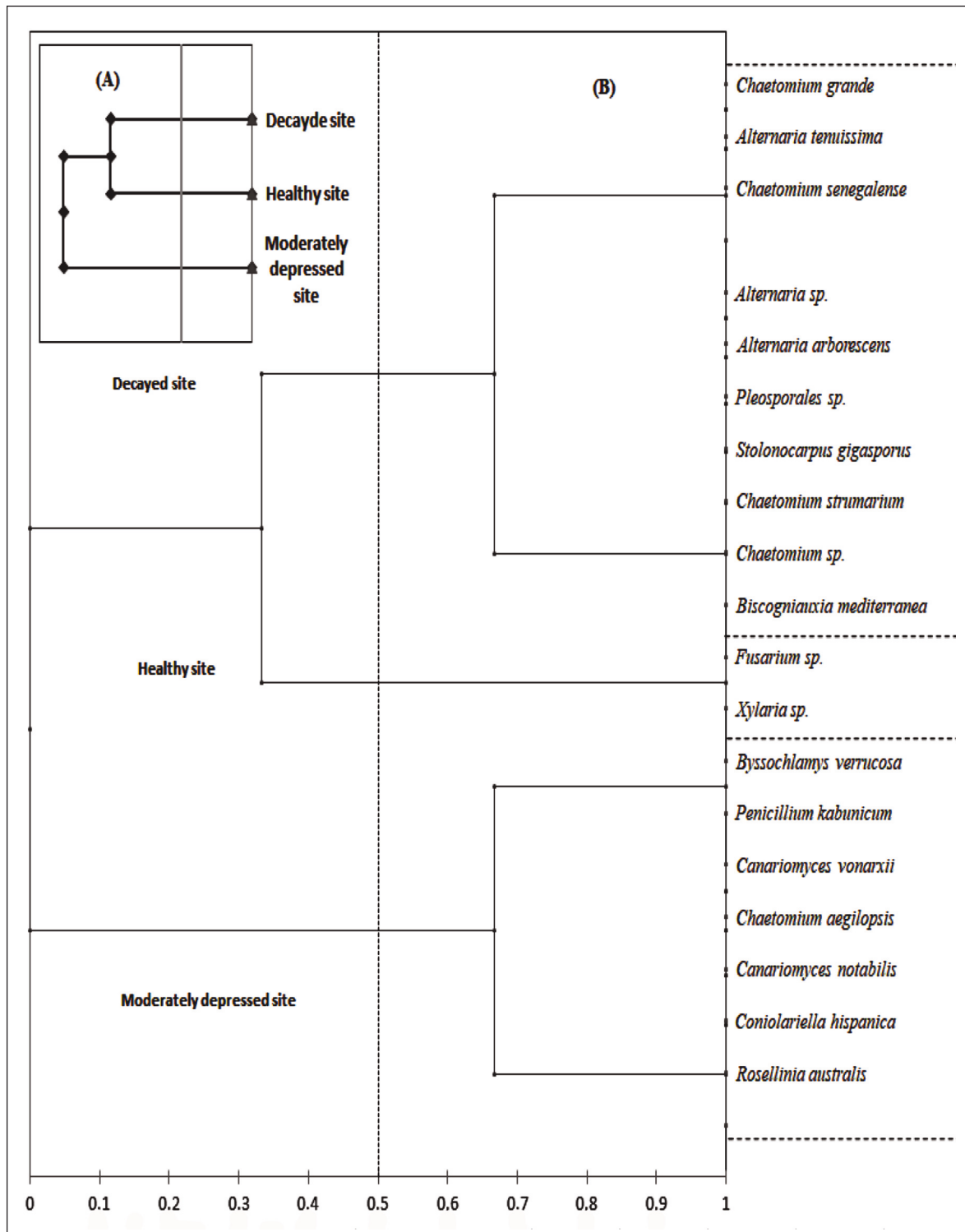


Figure 2. Hierarchical classification analysis HCA representing fungal endophytes associations in three *Cedrus atlantica* sites of Belezma National Park (Algeria), according to the decline degree.

infection variations. Boumerzoug site recorded an important population density of *C. atlantica* with a large area; on the other hand, the Tougurt station is characterized by sparse vegetation which was confirmed by Harzallah et al. (2009).

According to Deckert (2000), needle age is a determinant factor of endophyte infection. Adult needles are more infected by fungi. The influence of the age of tissues on frequency and species composition of endophyte assemblages has been reported in several studies (Harzallah et al., 2009). This parameter was not taken into consideration during our sampling.

Otherwise, the endophytes distribution in the cedar forests analyzed by the HCA allowed identifying three groups each associated with one of the three decline levels in studied cedar forests. Indeed, the first cluster representing Telmet site, where the trees are healthy, is the shelter of specific taxa with restricted ecological valence reflecting the good health of trees. This cluster is particularly composed of phytoparasitic taxa, such as *Xylaria* and *Fusarium*.

*Xylaria* endophytes were isolated from leaves and seeds of two trees species in Puerto Rico, *Casuarina equisetifolia* (Australian pine) and *Manilkara bidentata* (Ausubo) (Bayman et al., 1998), and from Eastern white pine (*Pinus strobus*) needles (Deckert & Peterson, 2000; Richardson et al., 2014). Also, within these endophyte taxa associated with healthy trees is known to be weak to strong parasite on woody plants (Ramesh et al., 2012).

The endophytic form of *Fusarium* was also isolated from the roots and foliage of herbaceous plants belonging to the Fabaceae and Poaceae families (Boyle et al., 2001), but also from the needles and scales of *Pinus strobus* (Deckert & Peterson, 2000). Although, the colonization of the leaf tissues is more discreet and highly localized. Many *Fusarium* species are associated with wilt diseases and canker (Stone et al., 2004) and also known as phytopathogens (Smahi, 2008).

Regarding the second cluster grouping together the taxa (7) associated with moderately depressed site (Boumerzoug), all the listed taxa have a wide ecological valence supporting the ecological variability conditions (generalist species, not indicators). We can cite *Penicillium* fungi which are common in the environment and may be responsible for great degradation (Samson et al., 2004); they have been isolated from the needles and root parts of *Picea abies* (Holdendrieder & Sieber, 1992) and of *Pseu-*

*dotsuga menziesii*, *Pinus ponderosa* and *P. monticola* (Ridout et al., 2017). Also, *Coniolaria hispanica* was isolated as an endophyte from leaves of *Eryngium campestre* on the Iberian Peninsula (Checa et al., 2008). The species of *Canariomyces* such as *Canariomyces notabilis* were isolated from litter Spain *Phoenix canariensis* (Spain) in the saprobe form, and *Canariomyces vonarxii* from dried flower of *Hibiscus* (Sudan) (Mehrabi et al., 2020). Also, we have determined in this group *Byssochlamys* species, capable of producing mycotoxins (Beuchat & Rice, 1979).

However, the third cluster representing Tougurt, marking a massive degradation of the Atlas cedar landscap, is the richest site hosting saprophytic mycotaxons (10 taxa) with wide ecological valence and opportunistically taking advantage of cedar weak state (good indicators of cedar advanced decline state). Including *Alternaria* sp. cited as an endophytic fungal by Elgorban et al. (2019). Our results corroborate with those of Messiaen et al. (1991), who showed that *Alternaria* could lead a saprophytic existence for long or short periods. Some species such as *A. chartarum*, *A. consortiale* and *A. tenuis* have mostly saprophytic habitat and are commonly found on organic debris or dead vegetation. Other endophytic fungi belonging to the order Pleosporales may be epiphytes, endophytes or parasites on leaves and live stems (Zhang et al., 2012). Most species are saprophytes on decaying plant matter (Shearer et al., 2009).

Although, *Biscogniauxia mediterranea* is well known as the causal agent of charcoal canker in Cork oak (*Quercus suber* L.) (Santos, 2003), this fungus can live as an endophyte in all the aerial organs of the oak plants and can act as an opportunistic pathogen when the hosts suffer prolonged periods of stress. In those conditions, *B. mediterranea* can rapidly colonize the xylem and bark tissues, induce necrosis and canker formation, accelerate tree decline and eventually death (Linaldeddu et al., 2011). This explains the presence of this species in the decaying cedar, where it finds a favorable environment, tree decline (weakness), allowing this type of fungus to spread easily.

*Chaetomium* species are found worldwide in soil, dung, or decaying plants and known as wood rot agent (Samson et al., 1984). Most known species of *Chaetomium* taxa are prolific producers of cellulase, an enzyme that degrades cellulose and shows a strong ability to destroy materials (Miller & McMullin 2014).



This study highlighted the qualitative and quantitative composition of mycoflora associated to Atlas cedar phyllosphere and the distribution patterns of endophytes taxa considering a decline gradient. Atlas cedar is a reservoir of a relatively wide range of fungal, pathogenic or saprophytic endophytes. Species groups tend to prefer one sanitary state of the cedar forests over another. These endophytes groups can be considered as bioindicators of cedar forests health. We propose that future research's concentrate on finding the mechanism of fungal infection and ecophysiological responses of Atlas cedar according to decline degree and tissue age that would find biocontrol agents.

## ACKNOWLEDGEMENTS

We would like to thank Dr. Steve Woodward, Dr. Hedda Weitz, Eleni Siasou and Alexandra Puértolas Cerdà (Department of Plant and Soil Sciences, University of Aberdeen - Scotland, UK) for their valuable assistance. The authors thank the staff of the National Park of Belezma, in particular the Hamla sector, we also thank Yamina Oussaid and Khalida Bouakziz (INRF, Algeria) for their valuable help.

## REFERENCES

- Arnold A.E. & Lutzoni F., 2007. Diversity and host range of foliar fungal endophytes: are tropical leaves biodiversity hotspots? *Ecology*, 88: 541–549. <https://doi.org/10.1890/05-1459>
- Bayman P., Angulo-Sandoval P., Báez-ortiz Z. & Lodge D., 1998. Distribution and dispersal of *Xylaria* endophytes in two tree species in Puerto Rico. *Mycological Research*, 102: 944–948. <https://doi.org/10.1017/S095375629700590X>
- Bensaci O., Harzallah D. & Gouaref K., 2015. Endophytic mycoflora of *Cedrus atlantica*: diversity patterns and determinism of the phytosanitary situation of Atlas cedar forests in Belezma massif (Algeria). *Forest Science and Technology*, 11: 36–43. <https://doi.org/10.1080/21580103.2014.957352>
- Bentouati A., 2008. La situation du Cèdre de l'Atlas dans les Aurès (Algérie), Témoignages d'ailleurs. *Forêt méditerranéenne*, 24: 203–208. <http://www.forêt-méditerranéenne.org/fr/catalogue/id-1108-la-situation-du-cedre-de-l-atlas-en-algerie>
- Beuchat L.R. & Rice S.L., 1979. *Byssochlamys* spp. and processed fruits. *Advances in Food Research*, 25: 237–288. [https://doi.org/10.1016/S0065-2628\(08\)60239-6](https://doi.org/10.1016/S0065-2628(08)60239-6)
- Bigot L. & Bodot P., 1973. Contribution à l'étude biocénologique de la garrigue à *Quercus coccifera*-composition biotique du peuplement des invertébrés. *Life and environment* 23: 229–249.
- Boyle C., Götz M., Dammann-Tugend U. & Schulz B., 2001. Endophyte-host interactions III. Local vs. systemic colonization. *Symbiosis*, 31: 259–281. <http://pascal-francis.inist.fr/vibad/index.php?action=getRecordDetail&idt=14189860>
- Checa J., Blanco N., Arenal F. & Rogers J.D., 2008. *Coniolarrella hispanica* sp nov and other additions to *Coniolarrella*. *Mycological Research*, 112: 795–801. <https://doi.org/10.1016/j.mycres.2008.01.014>
- Deckert R.J., 2000. Structural and ecological aspects of the relationship of phyllosphere fungi with their host *Pinus strobus*. PhD thesis, University of Guelph, Canada, 172 pp.
- Deckert R.J. & Peterson R.L., 2000. Distribution of foliar fungal endophytes of *Pinus strobus* between and within host trees. *Canadian Journal of Forest Research*, 30: 1436–1442. <https://doi.org/10.1139/x00-078>
- Elgorban A.M., Bahkali A.H., Al Farraj D.A. & Abdel-Wahab M.A., 2019. Natural products of *Alternaria* sp., an endophytic fungus isolated from *Salvadora persica* from Saudi Arabia. *Saudi Journal of Biological Sciences*, 26: 1068–1077. <https://doi.org/10.1016/j.sjbs.2018.04.010>
- Ganley R.J. & Newcombe G., 2006. Fungal endophytes in seeds and needles of *Pinus monticola*. *Mycological Research*, 110 : 318–327. <https://doi.org/10.1016/j.mycres.2005.10.005>
- Guo L.D., Huang G.R. & Wang Y., 2008. Seasonal and tissue age influences on endophytic fungi of *Pinus tabulaeformis* (Pinaceae) in the Dongling Mountains, Beijing. *Journal of Integrative Plant Biology*, 50: 997–1003. <https://doi.org/10.1111/j.1744-7909.2008.00394.x>
- Harzallah D., Bensaci O.A. & Bouharati S., 2009. Endophytic mycoflora of Atlas cedar (*Cedrus atlantica*. Man.) in Belezma massif (Aures, Algeria). Actes 9<sup>ème</sup> conférence internationale sur les maladies des plantes, Tours, France: 159–167. <https://www.researchgate.net/publication/296195809>
- Helander M.L., Neuvonen S., Sieber T.N. & Petrini O., 1993. Stimulated acid rain affects birch leaf endophyte population. *Microbial Ecology*, 26: 227–234. <https://doi.org/10.1007/BF00176955>
- Helander M.L., Sieber T.N., Petrini O. & Neuvonen S. 1994. Endophytic fungi in Scot's Pine needles: spatial variation and consequences of simulated acid rain. *Canadian Journal of Botany*, 72: 1108–1113. <https://doi.org/10.1139/b94-135>
- Holdendrieder O. & Sieber T.N., 1992. Fungal associations of serially washed healthy non-mycorrhizal roots of

- Picea abies*. Mycological Research, 96: 151–156.  
[https://doi.org/10.1016/s0953-7562\(09\)80932-5](https://doi.org/10.1016/s0953-7562(09)80932-5)
- Khan R., Shahzad S., Choudhary I.M., Khan S.A. & Ahmed A., 2007. Biodiversity of the endophytic fungi isolated from *Calotropis procera* (Ait.). Pakistan Journal of Botany, 39: 2233–2239.  
<http://agris.fao.org/agris-search/search.do?recordID=PK2008000956>
- Khan R., Shahzad S., Choudhary M.I., Khan S.A. & Ahmad A., 2010. Communities of endophytic fungi in medicinal plant *Withania somnifera*. Pakistan Journal of Botany, 42: 1281–1287.  
<https://pdfs.semanticscholar.org/c068/eb4bc03436de9f8fd9aecfaa6eca9372a7e2.pdf>
- Kherchouche D., Kalla M., Gutierrez E., Briki A. & Hamchi A., 2013. La sécheresse et le dépérissement du cèdre de l'Atlas (*Cedrus atlantica* Manetti) dans le massif du Belezma (Algérie). Science et changements planétaires/Sécheresse, 24: 129–137.  
<https://doi.org/10.1684/sec.2013.0384>
- Ladjal S., Harzallah D., Dahamna S., Bouamra D., Bouharati S. & Khenouf S., 2013. Endophytic fungi isolated from *Pinus halepensis* needles in M'sila (Algeria) region and their bioactivities. Communications in Agricultural and Applied Biological Sciences, 78: 625–631.
- Lanier L., Joly P., Bondoux P. & Bellemere A., 1978. Mycologie et Pathologie Forestière, Volume I : Mycologie forestière. Edition Masson, 487 pp.
- Lebart L., Morineau A. & Piron M., 1997. Statistiques exploratoires multidimensionnelles, 2ème édition, Dunod.
- Linaldeddu B.T., Sirca C., Spano D. & Franceschini A., 2011. Variation of endophytic cork oak-associated fungal communities in relation to plant health and water stress. Forest Pathology, 41: 193–201.  
<https://doi.org/10.1111/j.1439-0329.2010.00652.x>
- Linares J.C., Taiqui L. & Camarero J.J., 2011. Increasing drought sensitivity and decline of Atlas cedar (*Cedrus atlantica*) in the Moroccan Middle Atlas Forests. Forests, 2: 777–796. <https://doi.org/10.3390/f2030777>
- Magurran A.E., 2004. Measuring biological diversity. Wiley-Blackwell, New York. 71 pp.
- Messiaen C.M., Blancard D., Rouxel F. & Lafon R., 1991. Les maladies des plantes maraichères. INRA Paris, 552 pp.
- Mehrabi M., Asgari B. & Zare R., 2020. Description of *Allocanariomyces* and *Parachaetomium*, two new genera, and *Achaetomium aegilopsis* sp. nov. in the Chaetomiaceae. Mycological Progress, 19:1415–1427.  
<https://doi.org/10.1007/s11557-020-01636-x>
- M'hirit O., 1982. Etude écologique et forestière des cédraies du Rif Marocain: Essai sur une approche multidimensionnelle de la phytoécologie et de la production du Cèdre de l'Atlas. Annales de la Recherche Forestière au Maroc, 2 : 1–499.
- Miller J.D. & McMullin D.R., 2014. Fungal secondary metabolites as harmful indoor air contaminants: 10 years on. Applied Microbiology & Biotechnology, 98: 9953–9966.
- Mouhli N., Nedjahi A., Laouer H. & Riahi W., 2018. Meteorological drought in Batna region and its consequence on biodiversity (case of *Cedrus atlantica* Manetti). International Journal of Scientific Engineering and Research, 9: 581–586.  
<http://dx.doi.org/10.14299/ijser.2018.02.003>
- Petrini O., 1991. Fungal Endophytes of Tree Leaves. Microbial Ecology of Leaves. Springer-Verlag, New York Inc, pp. 179–187.
- Petrini O., Sieber T.N., Toti L. & Viret O., 1992. Ecology, metabolite production, and substrate utilization in endophytic fungi. Natural Toxins, 1: 185–196.  
<https://doi.org/10.1002/nt.2620010306>
- Pimentel I.C., Glienke-Blanco C., Gabardo J., Stuart R.M. & Azevedo J.L., 2006. Identification and colonization of endophytic fungi from soybean (*Glycine max* (L.) Merrill) under different environmental conditions. Brazilian Archives of Biology and Technology, 49: 705–711.  
<http://dx.doi.org/10.1590/S1516-89132006000600003>
- Ramesh V., Thalavaipandian A., Karunakaran C. & Rajendran A., 2012. Identification and comparison of *Xylaria curta* and *Xylaria* sp. from Western Ghats-Courtallum Hills, India. Mycosphere, 3: 607–615.  
<http://dx.doi.org/10.5943/mycosphere/3/5/8>
- Richardson S.N., Walker A.K., Nsiama T.K., McFarlane J., Sumarah M.W., Ibrahim A.J. & Miller J.D., 2014. Griseofulvin-producing *Xylaria* endophytes of *Pinus strobus* and *Vaccinium angustifolium*: evidence for a conifer-understory species endophyte ecology. Fungal Ecology, 11: 107–113.  
<https://doi.org/10.1016/j.funeco.2014.05.004>
- Ridout M., Houbraken J. & Newcombe G., 2017. Xerotolerance of *Penicillium* and *Phialocephala* fungi, dominant taxa of fine lateral roots of woody plants in the intermountain Pacific Northwest, USA. Rhizosphere, 4: 94–103.  
<https://doi.org/10.1016/j.rhisph.2017.09.004>
- Romero A., Carrion G. & Rico-Gray V., 2001. fungal latent pathogens and endophytes from leaves of *Parthenium hysterophorus* (Asteraceae). Fungal Diversity, 7: 81–87.
- Samson R.A., Hoekstra E.S. & Oorschot A.N., 1984. Introduction to food - borne fungi. 2nd ed. Central bureau voor Schimmelcultures. Institute of the Royal Netherlands. Academy of arts and sciences, 247 pp.
- Samson R.A., Hoekstra E.S. & Frisvad J.C., 2004. Introduction to food and airborne fungi. 7th Baarn Central albureau voor Schimmelcultures. Institute of the Royal Netherlands Academy of Arts and Sciences, 389 pp.

- Santos M.N.S., 2003. Contribuição para o conhecimento das relações *Quercus suber Biscogniauxia mediterranea* (syn. *Hypoxylon mediterraneum*). *Silva Lus*, 11: 21–29.
- Schulz B. & Boyle C., 2005. The endophytic continuum. *Mycological Research*, 109: 661–686. <https://doi.org/10.1017/S095375620500273X>
- Shearer C.A., Raja H.A., Miller A.N., Nelson P., Tanaka K., Hirayama K., Marvanová L., Hyde K.D. & Zhang Y., 2009. The molecular phylogeny of freshwater Dothideomycetes. *Studies in Mycology*, 64: 145–153. <https://doi.org/10.3114/sim.2009.64.08>
- Sieber T.N., Rys J. & Holdenrieder O., 1999. Mycobiota in symptomless needles of *Pinus mugo* ssp. *uncinata*. *Mycological Research*, 103: 306–310.
- Slimani S., Derridj A. & Gutierrez E., 2014. Ecological response of *Cedrus atlantica* to climate variability in the Massif of Guetiane (Algeria). *Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria (INIA). Forest Systems*, 23: 448–460. <http://dx.doi.org/10.5424/fs/2014233-05175>
- Smahi A., 2008. Contrôle biologique de la Fusariose vasculaire de la Tomate Causée par *Fusarium oxysporum* f. sp. *lycopersici*. Mémoire de magister, University of Oran (Algeria), 127 pp.
- Stone J.K., Polishook J.D. & White J.R.J., 2004. Endophytic fungi. In: Mueller G., Bills G.F., Foster M.S. (Eds.), *Biodiversity of fungi: Inventory and monitoring methods*. Elsevier, Burlington, pp. 241–270. <https://doi.org/10.13140/2.1.3835.2169>
- Taleb M.L., Maatoug M., Azouzi B., Zedek M. & Hellal B., 2016. Etude éco-dendrométrique du dépérissement du Cèdre de l'Atlas dans Le Parc National de Theniet El Had (Algérie). *Emerging Series Journal*, 12: 1857–7881. <http://dx.doi.org/10.19044/esj.2016.v12n29p112>
- White T.J., Bruns T.D., Lee S.B. & Taylor J.W., 1990. Amplification and direct sequencing of fungal ribosomal RNA Genes for phylogenetics. *PCR - Protocols and Applications - A Laboratory Manual*, Publisher: Academic Press, pp. 315–322.
- Zhang Y., Schoch C.L., Fournier J., Crous P.W., Gruyter J. De., Woudenberg J.H.C., Hirayama K., Tanaka K., Pointing S.B & Hyde K.D., 2009. Multi-locus phylogeny of the Pleosporales: a taxonomic, ecological and evolutionary re-evaluation. *Studies in Mycology*, 64: 85–102. <https://doi.org/10.3114/sim.2009.64.04>
- Zhang Y., Crous W.P., Schoch C.L. & Hyde K.D., 2012. Pleosporales. *Fungal Diversity*, 53: 1–221. <https://doi.org/10.1007/s13225-011-0117-x>



