Journal Academica Vol. 4(4), pp. 110-120, December 31 2014 - Microbiology - ISSN 2161-3338
online edition www.journalacademica.org © 2014 Journal Academica Foundation

Full Length Research Paper

Morphological and Molecular Identification of the Fungal Population at the *Alder Ain Khiar* (*El Kala* National Park, North Eastern Algeria)

Radia Djelloul [a]*, Karima Mokrani [a], Amel Lazli [a], Abdelhak Darbouche [b]
[a] Functional and Evolutionary Ecology Laboratory, University El Tarf (Algeria)
[b] Institute of Biology, University Khenchela (Algeria)

Received November 1, 2014; Accepted December 30 2014

ABSTRACT

The National Park of El Kala, covers and preserves around 70% Alder surfaces, counted in Northern Africa. Those wetlands have been described within "Ramsar" international program, for hosting a natural heritage of major concern; or moreover, a hotspot for both Alder diversity, and their associated mycetes. Among the so-identified Alders, we have chosen Ain-El-Khiar area, in order to realize our investigation project. Our attempt to describe the ectomycorrhizal fungi specific diversity is basically related to the humidity gradient effect on ectomycorhizic communities, as well as the identification of potentially adapted ones toward xero-resistance. On the other hand, we aim to compare this diversity to refugia and non-refugia, already studied in Europe. Fungal genetics will be characterized by standard molecular typing of fruiting bodies and mycorrhizae.

Keywords: Alder Ain Khiar, ectomycorrhizae, molecular typing, ITS sequencing, fruiting bodies

1. INTRODUCTION

Alder trees are known to host a little diverse community of specific ectomycorhizic mycetes. In addition, gene-mapping studies have highlighted areas of glacial refugia (Carpathians, Caucasus, Corsica, Algeria) for the Alders, described as areas where these species have been present for a long term, so stable even in ice age (King & Ferris 1998). In the National Park of El Kala, glutinous Alder (*Alnus glutinosa*, Betulaceae) colonize permanent or intermittent wetlands, structuring an ecosystem of high ecological, biological and paleoecological interest. They develop around the lakes, on landing surfaces, wadis and in the dune/lake contact areas, or at the upwelling of groundwater accumulated in reservoirs formed by the dunes. Taken together, Alder National Park is a natural hymidity gradient for associated microbial communities.

Furthermore, if the National Park of El Kala is a glacial refuge for Alders, it should be expected that their genetic diversity is strongly compared to re-colonized areas such as southern France, the ectomycorhizic communities are consequently supposed to be highly diversified. The study of genetic diversity inside ice refugia has already been conducted with mycetes like truffle (Murat et al. 2004), but never been applied on community scale, nor in thermophilic refugia similar to Algeria.

*Corresponding author: radia_d@yahoo.fr

The comparison of various refugia leads to valorize Alders particularities in Algeria.
We use conventional methods of fruiting bodies molecular typing and mycorrhizae identification, in comparison to data already acquired on Alder communities in Europe (especially France) (Roy et al. 2013). Sampling of mycorrhizae and fruiting bodies is made at Ain-El-Khiar Alders first. Then it will be spread to other sites from the National Park of El Kala context.
Soil samples are taken for pedological analysis (pH, C / N, N, P, K, Ca, Na) and physicochemical analysis of water. In order to contribute establishing mycological inventory of the National Park, observed fruiting bodies will be described on each visited parcel.

2. MATERIALS AND METHODS

Face to the obvious signs of Ain-El-Khiar Alders decline, maintaining the forest has become an important heritage issue. National Park of El-Kala in collaboration with "*Evolutionary and Functional Ecology*" laboratory (University of El Tarf), suggests an innovative and interesting study, trying to determine the health status of our study site: Ain-El-Khiar Alders.
Our main topics for this investigation are:

- To identify the forest mycological richness (fruiting bodies and Ectomycorrhizae) using the molecular technology.
- To study the decline of Alder using fungal bio-indication.

Criteria of the Study-Site Choice

We chose Ain-El-Khiar Alders station (National Park of El Kala) to achieve our mycological surveys. As there is almost no mycological data available at the El Kala National Park, fruiting bodies and ectomycorrhizal collection was performed at the scale of an experimental device, which gather crops on a small surface area, to avoid long and tiring prospecting time by one side, and not to underestimate different species relative importance.

Choice of sampling protocol

We chose a sampling mode that can best meet our objectives. The sample units were selected, according (Arnolds 1981), by vegetation units ranging from 100 to 5000m^2; depending on the environmental description on the site, from a minimum area of 100m^2, we have divided our sampling site into 5 plots with an area of 100m^2 (Fig. 1).

Figure 1: Ain Khiar

The protocol chosen for our study is based on random transects (Favre 1948), allowing a complete inventory covering the species hosted in the chosen site various plots. Transect was arranged on the decline in Alders presenting an interesting humidity gradient.

Fruiting bodies collecting approach

In order to achieve an exhaustive inventory, regular visits scheduled at about one prospecting weekly, throughout the experimental device during the fall season, starting from October 2012 until October 2013. We have focused surveys on the fall because

of fungal ecology, as fruiting bodies materialize mostly in autumn. Surveys more spaced in time (one visit per month) have still been conducted during other seasons in the year. Therefore, these visits allow to dwell on more hidden and tardily fruiting species, mycelia under bark or fallen to the ground dead stumps, or arboreal species fruiting at the time.
Species determination was performed requiring microscopic screening compared to consulted literature. Fruiting bodies of most fungi encountered were kept as *exsiccata* (personal collection of R. DJELLOUL), along with descriptive notes and *in situ* photographies. Regarding typing sporophores on the 72 species identified, 18 were subjected to molecular analysis.

Laboratory morphotyping of fruiting bodies

A fragment of the harvested fruiting bodies is placed in an Eppendorf tube filled with 150µl of CTAB higher for DNA extraction. The tube was kept in a freezer at a temperature of +4°C.

Ectomycorrhizal Harvesting

As part of our study, the molecular analysis of ectomycorrhizal although more destructive, is a method that has the advantage of more accurately describe the composition of fungal communities.

Harvesting Roots

Within the limit of allotted time to the study, two samplings of Alder roots campaigns were conducted: the first on December 24th, 2013 at the vegetative recovery, and the second on June 4th, 2013.
Roots were harvested below the organic layer of the soil to a depth of 15 to 20cm. The removal of mycorrhizae at 5 stations (ST1, ST2, ST3, ST4 and ST5) was performed according to protocol (Gardes 2002).

During the sampling campaigns, 168 mycorrhizae were taken from the roots of Alder (*Alnus glutinosa*), as following: 90 December and 78 June; among them, only 76 were typed: 39 from December sampling, and 37 from June, whereas, the typing reached a success rate of 45.20%.

Laboratory Ectomycorrhizal Morphotyping

Back at the laboratory, roots are thoroughly washed in running water to remove residual soil to allow better observation of ectomycorrhizae under the binocular loupe. Then, ectomycorrhizae are separately removed using forceps under a dissecting microscope and stored individually in a microtube containing 100µl of CTAB Extraction Solution [Nucleic Lysis Solution (Promega)]. Each ectomycorrhizae labeled (site number and mycorrhiza number) and typed according to morphological criteria (color, dichotomous aspect, simple or branched, turgid, downy... presence/absence of mycelium contact). The choice of mycorrhizae is random, endeavoring to draw 2-3 mycorrhizae similar by root fragment. Broken mycorrhizae, wilted in damaged apex are avoided. 16 per tree mycorrhizae (minimum required by the sampling plan) are taken. Once collected, ectomycorrhizae are then stored in the laboratory at +4°C.

Molecular characterization of collected ectomycorrhizae and fruiting bodies

Molecular analysis of ectomycorrhizae and fruiting bodies to classify the collected individuals use modern molecular techniques. Such a specific characterization is carried out by studying the polymorphism of the ITS region (Internal Transcribed Spacer) from nuclear ribosomal DNA using Nested PCR-RFLP (Polymerase Chain Reaction-

Restriction Fragment Length Polymorphism) and ITS sequencing.
This part of research work was performed at the "Evolution and Biodiversity laboratory", Paul Sabatier University, Toulouse, France.

• DNA extraction
We adopted of Gardes (2002) technique to extract DNA from both ectomycorhizae and fruiting bodies.

• Amplification
ITS region of the nuclear ribosomal DNA was amplified by nested PCR corresponding to two successive PCRs, using two sets of primers: NS5 ITS4 and ITS4, ITS1-F.
The first PCR with universal primers and NS5 ITS4 (White 1990), allows the ribosomal DNA amplification for plants and the fungi; the second one intends to amplify fungal nuclear sequence using the primer ITS4 and the primer specific ITS1-F fungi (Gardes & Bruns 1996).
The amplification reaction is performed by adding 2µl of DNA diluted to 1 / 10th in a 23µl reaction mixture containing 5µ 5x buffer 0,5µl 10 mM dNTP, 50 mM ITS4 and NS5 (or ITS1-F) 50 µm, 0,2µL GoTaq of 5µ / µl and 16.5µl of sterile water.
The amplifications are carried out in a thermocycler Eppendorf Master Cycler, according to the following programs:

PCR 1: DNA undergoes an initial denaturation of 3 minutes at 95°C; followed by 5 cycles of three steps (30 s of denaturation at 95°C, primers binding for 30s at 52°C, DNA elongation during 1:30 minute at 72°C); 30 times at 51°C to fix the primers. A final elongation of 10 minutes at 72°C ends in the synthetic route chains. The samples are kept at +4°C afterwards.

PCR 2: an initial denaturation of 3 minutes at 95°C is followed by 5 cycles of three steps (30 s denaturation at 95°C, primers binding for 30s at 56°C, DNA elongation for 1 minute 30 s at 72°C); the same cycle is repeated 25 times at 55°C for the primers fixation, followed by 10-minute final elongation at 72°C. the samples are then kept at +4°C.
Amplification quality and quantity are checked by electrophoresis of a loading buffer aliquot + 2µl of 1µl agarose gel prepared with 1.5% of 0.5X TAE buffer (0.02 M Tris-acetate, 0.05mM EDTA) containing 0.10 ug/ml ethidium bromide.
Electrophoresis is conducted in 0.5X TAE buffer (0.02 M Tris-acetate, 0.5mM EDTA) at a potential of 100V. To estimate the amplified fragments size, a molecular weight marker (100pbladder, Promega) is used to migrate in parallel to the amplification products. The DNA fragments are visualized under UV and gel screening is captured by CCD camera controlled by Bio-vision software.
The ITS amplified taxa (fruiting bodies and mycorrhizae) are sequenced by the company Cogenics (Meylan).
The sequences are compared with sequences deposited in international databases using BLAST procedure on NCBI website www.ncbi.nlm.nih.gov .
Molecular analysis of Alder ectomycorrhizae leads to generate of an inventory at time zero of fungal populations experimental set.

3. RESULTS AND DISCUISSION

Molecular analysis of fruit bodies

18 selected samples on alnicole ectomycorrhizae groups are already well documented in databases (2 *Alnicola* / *Paralnicola*, 2 *Cortinarius*, 4 *Lactarius*, 1 *Russula*, 1 *Paxillus*), as well as certain worthy interesting decomposers found in Alders (1 *Gymnopilus*, 1 *Callistosporium*, 1 *Psilocybe*). It is noteworthy that we got two negative amplifications; therefore, the number of samples which have undergone sequencing is only 16.

• ALNICOLE MYCORHIZIENS

- ***Alnicola/Paralnicola***

Sample 1: *Alnicola umbrina* (Fig. 2) "pale": 100% conform to European crops. However we know that ITS does not completely solve the systematic of this complex. Notice that this taxon has been described by (Maire 1928) in the neighborhood of Algiers.

Sample 2: *Paralnicola alnetorum,* it is not about *P. inculta* ss.str. (Mostly known in Europe), but it looks like *P. alnetorum*, which was known only in the Grand-Lemps and the Pyrenees. It means that *P. inculta* has a wider distribution (at least from Estonia to Brittany and Corsica), but *P. alnetorum* is more "Mediterranean". There is a dig on this Algerian refuge and especially for Ain-El-Khiar Alders, which probably was a refuge for *P. alnetorum* while *P. inculta* took refuge elsewhere.

- ***Cortinarius:***

Sample 3: *Cortinarius croceocrystallinus* var. *alneti* small cosmopolitan *Myxacium* identical to all crops and European collections.

Sample 4: *Cortinarius sp.*: is as provided in the "blackish mess" of C. *americanus-badiovestitus*, in which we know that ITS will not provide an explanation. However, morphologically, these crops from Ain-El-Khiar Alders are singular; we therefore, expect that the TEF1, LSU or RPB2 gives better results. However, this species has been described in Sardinia, collected at a coastal Alder Cagliari to as *Cortinarius atropusillus* var. alni-*glutinosae* and published by (Contu 1994); So apparently distribution Algeria-Sardinia (TBC). We propose to call this sample: *Cortinarius alni-glutinosae* (Contu) comb.nov (Fig. 3).

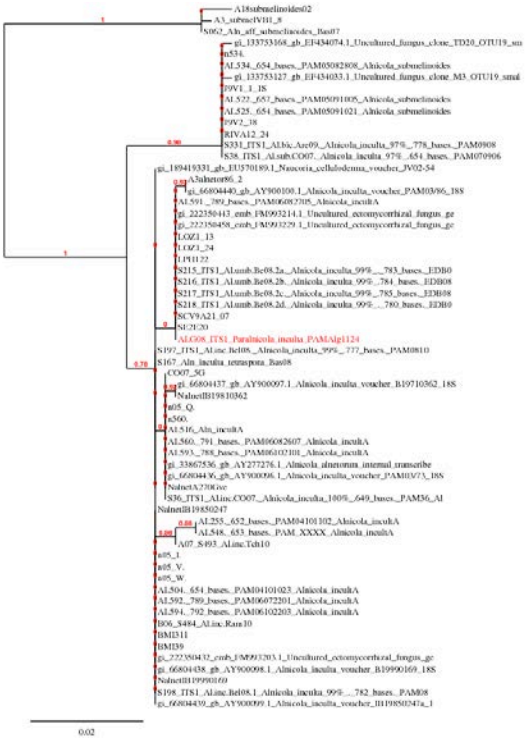

Figure 2: Clade *Alnicola/Paralnicola*

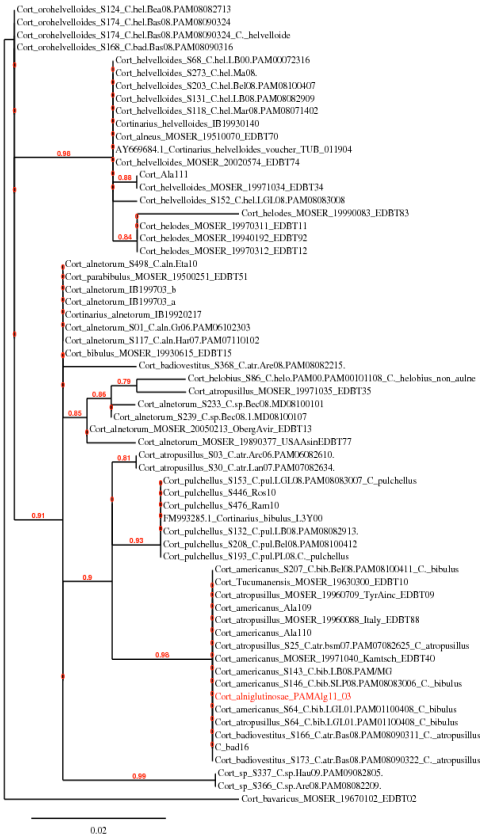

Figure 3: Cladogramme *Cortinarius alni-glutinosae*

- ***Lactarius:***

Sample 5: *Lactarius cyathuliformis*
Sample 6: *Lactarius cyathuliformis*
Sample 7: *Lactarius cyathuliformis*

These three sequences match to *Lactarius cyathuliformis* (Fig. 4) (almost indefinite *Lactarius obscuratus* on the ground). It's interesting to find only *Lactarius cyathuliformis* in this refuge, but not L. *obscuratus*! Both species are as frequent as each other, in continental Europe, but they were able to differentiate in allopatric way, before re-colonize Europe.

Sample 8: *Lactarius lilacinus*: unsurprisingly sequence is identical to the European *lilacinus*, despite a slight difference in shape (more slender and lighter).

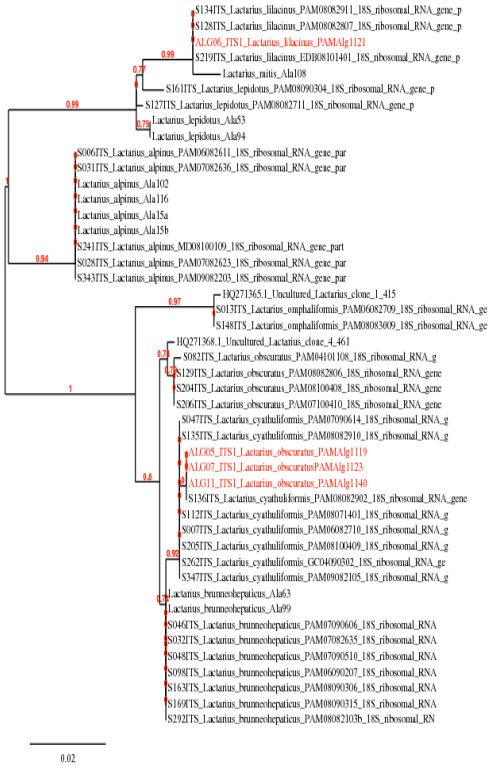

Figure 4: Clade *Lactarius*

- ***Paxillus:***

Sample 9: *Paxillus filamentosus*
Sample 10: *Paxillus rubicundulus*

On the ground we have been confused between two species that molecular biology has proved to be distinct, namely *Paxillus rubicundulus* rather southern and *Paxillus filamentosus* to wider distribution. In Corsica there that *Paxillus rubicundulus*; France (Toulouse) both species are found in combination; but in the North, *Paxillus filamentosus* was not harvested. Although not more significantly slender than average, Ain-El-Khiar harvest matches (without biogeographical surprise) to *Paxillus rubicundulus*. We assume that the sample as labeled "sample10", although without satisfactory results, is also *Paxillus rubicundulus* because it was morphology, more "typical" (Fig. 5).

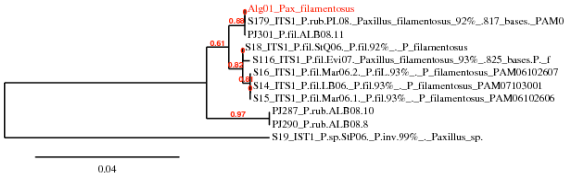

Figure 5: Clade *Paxillus*.

- ***Russula*:**

Sample 11: *Russula sp.* the only russula found in glutinous Alder that is not *Russula pumila*. This is a *Viridantinae* (the group of *Russula xerampelina*), which is located in a hygrophilous species clade related to willow alpine-arctic zone (*R. chamitae, R. pascua*) or plain (*R. subrubens*). It is morphologically distinct (ribbed margin, low browning and smell...) and the ITS is also a bit different. There was no willow near the place of harvest, we assume that there was a piece of willow "slippage" with Alder , or a species related to sustainable Alder but in any case very localized. It has no relationship to the unknown russula mycorrhizae found in Corsica or in the Alps or with the Alaskan species. This should be a new species to be described (Fig. 6).

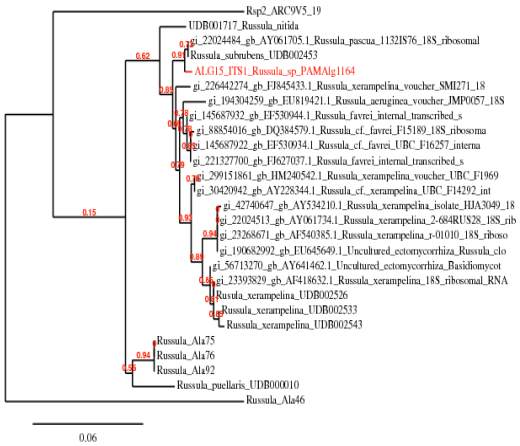

Figure 6: Clade *Russula*, illustrating the species position among the Viridantes group.

• SAPROTROPHES:

- ***Gymnopilus*:**

Sample 12: *Gymnopilus sp*. (Fig. 7) gymnopile, an apparently common on Alder trunks, and morphologically resembles *Gymnopilus suberis* (Northern African species supposedly subservient to Oak). ITS is identical to a *Gymnopilus suberis* in Genbank, and also some *Gymnopilus luteofolius* determined crops. Notice that *Gymnopilus luteofolius* is a common name given to Mediterranean or tropical gymnopiles in poor condition. It is interesting that this decomposer occurs in Algerian Alders, mainly Ain-El-Khiar ones, not by particular affinity with Alders, but because they are included in its geographical area. This is perhaps a preferential host, as it is quite rare on Oak.

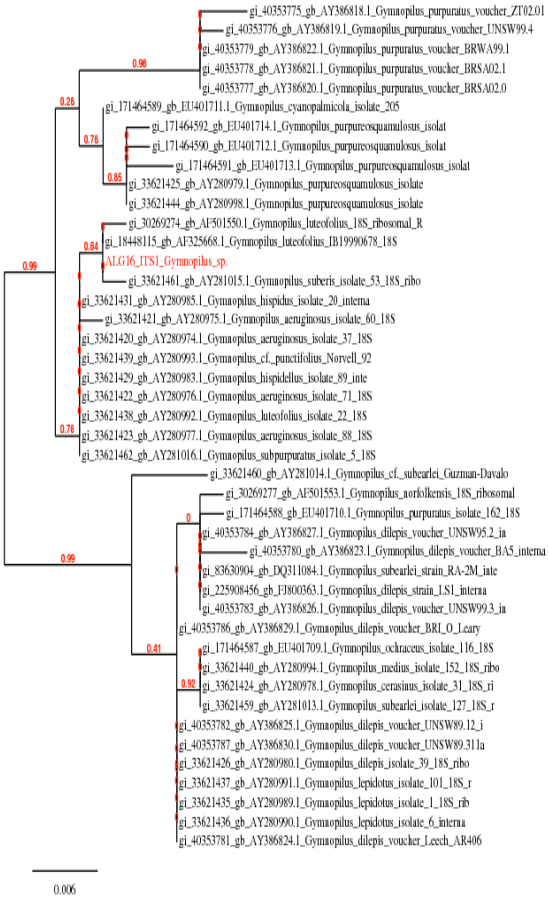

Figure 7: Clade *Gymnopilus* sect. p*urpurati*

- ***Callistosporium:***

Sample 13: *Callistosporium sp*. (Fig. 8): ITS does not fully explain the spectacular morphological and microscopic differences compared to *Callistosporium xanthophyllum*. Notice that all *Callostosporium* GenBank have the same ITS, and thaughts are on the fact that this little genus supposedly finds differences elsewhere. In any case the species can be considered to be new, because it largely differs from all described *Callistosporium*.

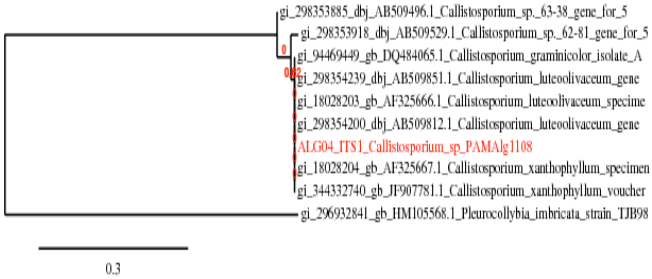

Figure 8: Clade *Callistosporium*

- ***Psilocybe:***

Sample 14: *Psilocybe romagnesii*: a species whose name was never validly published, but is fairly common in the peaty or muddy areas, ponds edges etc. The question was whether to classify this species in *Hypholoma* (from macroscopic characters) or the *Psilocybe* (after the coating structure hat devoid of hypodermis). We now compare it with *Hypholoma subericaeum*, which is very similar, to see if it wills to be described. However, there is no STC *Hypholoma subericaeum* Genbank sequence.
Through the results of fruiting bodies molecular analysis, we identified four (04) mycetes; those macroscopic and microscopic descriptions were not fully able to solve the inventory work conducted at the National Park of El Kala (Djelloul 2014):

- Two cortinaries exclusive for the Alders *Cortinarius croceocrystallinus* and *Cortinarius alni-glutinosae*, which correspond to *Cortinarius sp.* and *Cortinarius sp1 in* the list of PNEK fungi, mainly Ain-El-Khiar Alders.
- A *Psilocybe*: *Psilocybe romagnesii*.
- *Callistosporium* gender description.

Thus, of the 16 typed samples, we define 3 new species and one new genus. However, we have not been able to identify two species from russula and gymnopile in the "Genbank" database.
The mycorrhizal group includes complex species growing in mixture, in France. As decomposers, they are rather endemic Mediterranean that find refuge in the woodlands rich in Deadwood, without any underlined specificity. This is an argument to defend these Alders areas, not as closed and exclusively specific islands, but as refugia for a broader and potentially threatened diversity.

Molecular analysis results of mycorhizae

Harvested mycorrhizae were all treated (DNA extraction, amplification ITS1f + 4B or ITS3 + 4B). PCR results were rather disappointing, so that only 76 amplifications / 168 (45.20%) were positive. from these 76 PCR products, only 66 gave correct sequences (Fig. 09), as following:

- 52 could be attributed to known Alnicole species (*Alnicola citrinella*, *Alnicola umbrina, Lactarius cyathuliformis, Lactarius omphaliformis, Paxillus rubicundulus* sensui Orton*, Tomentella*). Tomentellas include *Tomentella* cf *sublilacina*, *Tomentella* aff. *stuposa*, *Tomentella* cf. *elisii*.
- Seven (07) of the sequences have been attributed to *Alnicola*, without species characterization, which is due to their bad quality.
- Five (05) sequences from *Trechisporales* close to each other but different from the one found in Czech republic (Richards & *al.* 2005).
- Two (02) sequences belong to *Ceratobasidium.*

Throughout our results obtained by molecular analysis of ectomycorhizae, we could add 07 new mycetes to our identified species list:

- Three (03) Tomentelles exclusive for Alders, *Tomentella* cf *sublilacina*, *Tomentella* aff *stuposa* et *Tomentella* cf *elisii*.
- One (01) lactarial: *Lactarius cyathuliformis*.
- One (01) paxillus: *Paxillus rubicundulus*.
- *Trechisporales* gender definition.
- *Ceratobasidium* gender definition.

Thus, from the sixty-six (66) samples typed, we have five (05) new species and two new genera.

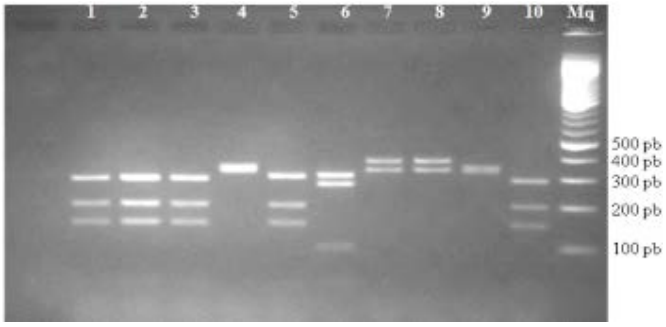

Figure 9: PCR results of ectomycorhizal DNA.

Comparison of fruiting bodies with ectomycorhizal molecular analysis

Molecular analysis of ectomycorrhizae has detected seven (07) taxa that were not identified in the fruiting bodies collection.

From the ten (10) new taxa detected by molecular methods (fruiting bodies and ectompycorhizae) six (06) are not mycorrhizal fungi and 08 are new to the forest of Ain Khiar Alders (not described in (Djelloul 2010). these are saprophytes, the mycelium was probably to be in contact either with mycorrhiza tissues or a dead mycorrhizae.

In general, new species of humid forests are mainly basidiomycetes belonging to the family of *Tomentellaceae* and *Cortinariaceae* (family consisting of many abundant mycorrhizal taxa in the forest ecosystems). Hence, mycological diversity identified in the forest of Ain-el-Khiar numbers 80 Eumycota. The list obtained by molecular technique is very different from that established from mycological campaigns, except some taxa common to both methods of analysis (*Alnicola umbrina, Paxillus filamentosus, Lactarius omphaliformis*) (Richards & *al.* 2005).

The detection of isolates by a single molecular method does not lead to the biological spectrum calculation, because it normally checks if that organizations can achieve symbiosis. Therefore, it does not identify the saprophytic fungi; and even some parasite taxa of this status were identified by this method.

To estimate the actual biological spectrum of the transect, the whole fungal community of the soil should be fully identified: spores, sclerotia, mycelium, different types of mycorrhizae. In other words, saprophytic fungi, parasites, and mycorrhizal have to be identified. This method would need to take samples of a certain volume of soil on the transect and then make determinations and/or molecular analyzes on all the fungal bodies detected in different periods.

By contrast, the molecular analysis method has got the advantage of identifying additional taxa that were not described by the mycological campaigns method, may be because they have not fructified this year, as we did just not observed at the time of the surveys. This method is also being informative and important for the detection of mycorrhizae, evolving naturally with stand age (Horton & Bruns 2001).

4. CONCLUSION

The molecular study conducted over our current work, has helped to set up the fungal community state regarding the common Alders. Although sampling underestimates the ectomycorrhizal fungi diversity (for one year sampling), the highlighted fungal procession brings very interesting information that to conclude requires further study. Indeed, mycorrhizal communities change with stand age (Durieu 1993). In addition, this association is very sensitive to environmental conditions (soil compaction, eutrophication, use of organic and mineral fertilizers, nitrogen) and forest health; in our case Alders forest.

5. REFERENCES

Andrew King R., Ferris C. [1998]: Chloroplast DNA phylogeography of Alnus glutinosa (L.) Gaertn. Molecular Ecology 7: 1151-1161

Murat C., Diez J., Luis P., Delaruelle C., Dupre C., Chevalier G., Bonfante P., Martin F. [2004]: Polymorphism at the ribosomal DNA ITS and its relation to postglacial re-colonization routes of the Perigord truffle *Tuber melanosporum*. *New Phytologist* 164: 401–411

Roy M., Rochet J., Manzi S., Jargeat P., Gryta H., Moreau P-A. & Gardes M. [2013]: What determines Alnus-associated ectomycorrhizal community diversity and specificity? A comparison of host and habitat effects at a regional scale. New Phytologist 198: 1228–1238

Arnolds E. [1981]: Ecology and Coenology of Macrofungi in Grasslinds and Moist Heathlands in Drenthe, The Netherlands, Part 1: Introduction and Synecology. Ed. Cramer Vaduz, 407 p.

Favre J. [1948]: Les Associations Fongiques des Hauts-Marais Jurassiens. Beiträge zur Kryptogamenflora der Schweiz, 10: 1–228

Gardes M. [2002]: An orchid-fungus marriage - physical promiscuity, conflict and cheating. New Phytologist 154(1): 4-6

White T.J., Bruns T.D., Lee S., Taylor J. [1990]: Amplification and direct sequencing of fungal ribosomal RNA genes for polygenetics. In: Innis M.A., Gelfand D.H., Sinsky J.J., Wthite T.J. (eEds.): *PCR Protocols: a Guide to Methods and Amplifications*. New York, USA: Academic Press: 315-322

Gardes M., Bruns TD. [1996]: Community structure of ectomycorrhizal fungi in a *Pinus muricata* forest: above- and below-ground views. *Can. J. Botany,* 74: 1572-1583

Maire R. [1928]: Diagnose de champignons inédits de l'Afrique du Nord. Bulletin Trimestriel de la Société Mycologique de France XLIV: 37-56

Contu M. [1994]: Stato attuale delle conoscenze sulla flora micologica delle dune sabbiose della Sardegna. Micol. Ital. 23(2): 109–118

Djelloul R. [2014]: Cartographie des champignons au niveau du Parc National d'El Kala, Nord Est Algérien. Thèse de doctorat Es-Sciences, Univ. Annaba

Richards F., Millot S., Gardes M., Selosse M.A., [2005]: Diversity and specificity of ectomycorhizal fungi retrieved from an oldgrowth Mediterranean forest dominated by *Quercus ilex*. New Phytologist 166: 1011-1023

Djelloul R. [2010]: Inventory and distribution of higher fungi (macrofungi) at the bog Ain Khiar. Scholars Research Library. Annals of Biological Research, 1(4): 95-105

Horton T.R., Bruns T.D. [2001]: The Molecular revolution in ectomycorrhizal ecology: peeking into the blackbox. New Phytologist 139: 331-339

Durieu G. [1993]: Ecologie des champignons. Masson, Paris, 207 p.

Paxillus filamentosus

Callistosporium sp

Cortinarius croceocrystallinus* var. *alneti

Lactarius lilacinus