Ophiostomatoid Fungi Associated with the Ambrosia Beetle *Platypus cylindrus* in Cork Oak Forests in Tunisia

Amani Bellahirech, Laboratoire de Gestion et Valorisation des Ressources Forestières, INRGREF, Université de Carthage, Rue Hédi Karray, BP 10, 2080 Ariana, Tunisia; INAT, Université de Carthage, 43, Avenue Charles Nicolle, 1082 Tunis, Cité Mahrajène, Tunisia, **Maria Lurdes Inácio**, INIAV-IP, Quinta do Marquês, 2780-159 Oeiras, Portugal, **Mohamed Lahbib Ben Jamâa**, Laboratoire de Gestion et Valorisation des Ressources Forestières, INRGREF, Université de Carthage, Rue Hédi Karray, BP 10, 2080 Ariana, Tunisia, **and Filomena Nóbrega,** INIAV-IP, Quinta do Marquês, 2780-159 Oeiras, Portugal

ABSTRACT

Bellahirech, A., Inácio, M.L., Ben Jamâa, M.L., and Nóbrega, F. 2018. Ophiostomatoid fungi associated with the ambrosia beetle *Platypus cylindrus* in cork oak forests in Tunisia. Tunisian Journal of Plant Protection 13 (si): 61-75.

Cork oak (*Quercus suber*) is a unique species of the Western Mediterranean region and over the last decades it has been threatened by several pests and diseases. Amongst the main dangerous pests, the ambrosia beetle *Platypus cylindrus* (the oak pinhole borer) has a key role on the process of cork oak decline namely in Portugal, Morocco, and Algeria. However, in Tunisia, where cork oak forests cover around 90.000 ha of the territory, this insect continues to have a secondary pest status. As all ambrosia insects, *P. cylindrus* is able to establish symbiotic relationships with fungi and it is known as the vector of ophiostomatoid fungi, a group including primary tree pathogens. The aim of this study was to identify these beetle-associated fungi in Tunisian forests and to understand the contribution of this association in cork oak decline by comparing with the results from other countries. The present study was conducted in 2012 in ten cork oak forests in the western-north of Tunisia and focused on ophiostomatoid fungi associated with the cork oak pinhole borer. Twenty four isolates were grouped based on morphological identification, and five representative isolates were included in phylogenetic analyses based on sequence data of ITS and β-tubulin loci. The fungi were assigned to five species namely *Raffaelea montetyi*, *R. canadensis*, *Ophiostoma* sp., *O. tsotsi* and *O. quercus*, some of them were already reported in Portugal and Algeria to be associated with cork oak decline. All these species were identified and reported for the first time in Tunisia to be associated with *P. cylindrus* in cork oak trees and their role in the cork oak loss of vitality needs to be investigated.

Keywords: Ambrosia fungi, oak pinhole borer, Ophiostomatales, *Quercus suber*.

Platypus cylindrus, the cork oak pinhole borer, is an ambrosia beetle infesting cork oaks (*Quercus suber*) that has assumed increasing importance in the Iberian Peninsula, specifically Portugal and in Algeria where it is directly associated with cork oak decline (Belhoucine et al. 2011; Inácio et al. 2011, 2012; Sousa and Debouzie 1999). Nevertheless, in some countries of the North African region, the insect is not a relevant problem, and in Tunisia it is considered as a secondary pest, attacking

Corresponding author: Amani Bellahirech
Email: amany21@hotmail.fr

Accepted for publication 08 April 2018

mainly weakened or dead trees (Bellahirech et al. 2015; Ben Jamâa et al. 2010). As an ambrosia beetle, it carries and inoculates fungal inoculum, in specialized organs called mycangia (Beaver1989).Several Ophiostomatales were reported to be associated with *P. cylindrus* in Portugal, Algeria and France, unlike Tunisia where no information has been previously reported until the beginning of new work in 2010 (Bellahirech et al. 2012).

Ambrosia beetles are associated with mutualistic fungi that serve as a nutrition source for larvae and adult beetles (Hofstetter et al. 2015), such as species of the Ophiostomatales (De Beer et al. 2013; Zipfel et al. 2006).This order includesthe genera *Ophiostoma*, *Ceratocystiopsis*, *Leptographium*, *Sporothrix*, *Raffaelea*, and *Graphilbum* (De Beer and Wingfield 2013; De Beer et al. 2014, 2016). More specifically, wood-boring ambrosia beetles are commonly associated with fungi of the genera *Ophiostoma* sensu stricto, and specific fungi, known as "ambrosia fungi", of the genera *Ambrosiella*, *Raffaelea*, and *Phialophoropsis* (Gebhardt et al. 2004; Harrington et al. 2010; Kowalski 1991; Massoumi Alamouti et al. 2009;Mayers et al. 2015).Due to their morphological and ecological similarities, Ascomycota fungi from the orders Ophiostomatales and Microascales are often designated as ophiostomatoid fungi (De Beer et al. 2013; Spatafora and Blackwell 1994). The ophiostomatoid fungi is a convenient term for a group of species that produce either ascospores or conidia, or both spore types, in sticky drops on elevated ascomatal necks or conidiophores (De Beer et al. 2013). The two main ophiostomatoid genera are *Ceratocystis* and *Ophiostoma*, and have been taxonomically confused for many years. Presently, they are placed indifferent orders with Ophiostomatales, including the genus *Ophiostoma*, and Microascales, comprising the genus *Ceratocystis* (De Beer et al. 2013, 2014).

Many of these fungi are serious tree pathogens (Wingfield et al. 2013) and some considered as agents of sapstain(Seifert 1993). For instance, in Japan, *Raffaelea quercivora*is responsible for the mortality of oaks infested by *Platypus quercivorus* since the 1980s (Kubono and Ito 2002; Matsuda et al. 2010; Murata et al. 2005). In addition, *R. lauricola* transported by *Xyleborus glabratus* to the southern United States is the causal agent of the lethal vascular wilt on *Lauraceae* (Fraedrich et al. 2008; Harrington et al. 2010).

Platypus cylindrus carries and inoculates mycelium that develops along the galleries serving both as offspring source of food and to weaken the host tree (Batra 1963). Several ophiostomatoid fungi were reported to be associated with *P. cylindrus* in France (Morelet 1998), Algeria (Belhoucine et al. 2011) and Portugal (Inácio et al. 2012). Based on this relationship developed between insects and associated fungi, the present study was elaborated. The main goals of this work were to (i): identify ambrosia fungi transported by the pinhole borer in Tunisian cork oak stands and; (ii): compare ongoing studies in Tunisia with previous reports in the Mediterranean cork oak region namely in Portugal, where it has been observed that the combined action of *P. cylindrus* massive attacks and extensive boring with the inoculation of ambrosia fungi, leads to an increase of tree mortality. The increase of cork oaks mortality in these past two decades can thus be in part attributed to these new associations between the insect and more aggressive wilt causing fungi (Inácio 2011). Thus, it is of paramount importance to prevent the spread and

establishment of these more aggressive ambrosial fungi in Tunisian cork oak forests where *P. cylindrus* is already infesting trees. The first step is the complete characterization of the insect associated mycoflora and the investigation of the pathogenic potential of each fungal species towards the cork oak trees.

MATERIALS AND METHODS

Study area and sampling.

The present study was conducted between January and July 2012 inselected trees of tencork oak forests (Ain Beya, Ain Drahem, Ain Sarouia, Babouch, Bellif, El Jouza, Mzara, Oued Zen, Sejnène and Tabouba) located in the western-north of Tunisia, the main cork oak region (Fig. 1).

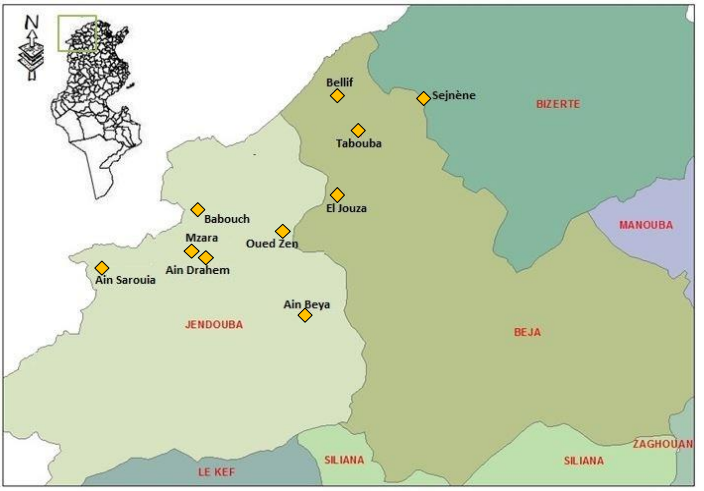

Fig. 1. Localization of studied sites (cork oak forests are indicated with yellow diamond).

One infested cork oak tree from each site was allowed to be cut and sectioned into three logs of 50 cm length. The logs were installed in the laboratory and the insects were captured while emerging in fabric traps attached to the entrance/exit hole (Sousa and Inácio 2005).

Insects were aseptically dissected and their mycangia were delicately detached under a stereo binocular microscope following the procedures of Sousa et al. (1997) and Inácio et al. (2008). After insects' emergence, cross-sections of the logs were sawn and the tunnel system of the beetles in the sapwood was open. Three galleries from each tree were taken and sectioned into small pieces.

Fungi isolation.

Both mycangia and pieces of wood from the trees' galleries were surface sterilized with sodium hypochlorite solution (1%) for one minute and rinsed with sterilized distillated water (Cassieret al. 1996). The material was plated in Malt Extract Agar medium (MEA, Difco) added with streptomycin (500 mg/l) and cycloheximide (500 mg/l). The latter is an antibiotic used to isolate only fungi of the genus *Ophiostoma* (Harrington 1981;

Harrington et al. 2010). Plates were incubated at 25°C in darkness for 15 days.

Morphological identification.

Morphological observations were made on 3 to 10-day-old cultures and after 15 days, slide cultures of each isolate (Riddell 1950) were mounted in lactophenol and examined with light microscopy (Olympus BX-41 with Olympus DP11, Hamburg, Germany).Identification at genus level was based on cultural and morphological features according to Ellis (1971, 1976), Kiffer and Morelet (1997) and Barnett and Hunter (1998).

DNA extraction and PCR amplification.

DNA of the five representative isolates was extracted from fresh myceliumscraped from the surface of the pure cultures growing on agar plates with a sterile scalpel. Mycelium was transferred to a pre-cooled and sterile mortar and pestle, frozen with liquid nitrogen and ground to a fine powder. Total DNA was isolated from approximately 100 mg of the mycelia powder using the DNeasy Plant Mini kit (Qiagen, Germany) following the manufacturer's instructions. DNA concentration and purity were checked using a NanoDrop 2000 UV-Vis Spectrophotometer (Thermo Fisher Scientific, Massachusetts, USA). The 5,8S nuclear ribosomal RNA gene and the flanking internal transcribed spacers (ITS1 and ITS2) were amplified using primers ITS1F and ITS4 (White et al. 1990) and a fragment of the 5' end of the β-tubulin gene was amplified with primers T1 (O'Donnell and Cigelnik 1997) and Bt2b (Glass and Donaldson 1995).

PCR reactions were carried out using the Dream Taq PCR Master Mix (2X) (QIAGEN, Germany) in a Biometra TGradient thermocycler (Biometra, GmbH). Each reaction mixture was performed in a final reaction volume of 25 µl containing 1 µl (50-150 ng) of template DNA, 1 µl of each primer (10 µM stock), 12.5 µl of PCR Master Mix buffer (2X), which included 1.5 mM $MgCl_2$ and 0.2 mM of each dNTP. The thermal cycling conditions were initially denatured at 95°C for 4 min, followed by 35 cycles of 1 min at 94°C, annealing at 50°C for 1.5 min and extension at 72°C for 1 min, followed by a final elongation for 10 min at 72ºC.

The amplified products were loaded into a 1.5 % agarose gel containing 0.5 µg/ml ethidium bromide and 0.5× Tris-borate-EDTA (TBE) running buffer and electrophoresed at 5 V/cm. Amplifications were visualized using the Versa Doc Gel Imaging System (Bio-Rad, USA). PCR products were cleaned using the GeneJET PCR Purification Kit (Fermentas, Germany) according to the manufacturer's protocol.

Sequencing of PCR amplicons and bioinformatics analysis.

Amplicons were sequenced in forward direction at STABVida Sequencing Laboratory (Lisbon, Portugal) on a DNA analyzer ABI PRISM 3730xl (Applied Biosystems). The nucleotide sequences were edited and analyzed using BioEdit v7.2.0 program (Hall 2007). The nucleotide sequences were compared with those of reference available at NCBI (National Center for Biotechnology Information) GenBank database using the BLAST (Basic Local Alignment Search Tool) sequence analysis tool (http://www.ncbi.nlm.nih.gov/BLAST/). Phylogenetic trees were generated from the aligned sequences in

MEGA version 6 program (Tamura et al. 2013) using a Neighbour-joining analysis method. The evolutionary distances were computed using Maximum Likelihood methodology. Percentage reliability values at each internal node of the trees were obtained by performing 1000 bootstrap analyses.

Outgroup sequences were selected based on their genetic distance to Ophiostomatales used in the phylogenetic analyses Matsuda et al. (2010). Sequence from *Taphrina virginica* was used which is a frequent used outgroup in Ophiostomatales phylogenetic studies. The homologous sequences were retrieved from the GenBank database and their accession numbers were plotted in phylogenetic trees.

RESULTS

Morphological and molecular characterization of isolates.

A total of 200 isolates were obtained. Several of them were cosmopolitan fungi belonging to the genera *Acremonium*, *Aspergillus*, *Fusarium*, *Gliocladium*, *Penicillium*, and *Trichoderma*and are not part of the present work. We could observe five different morphological groups among twenty four ophiotomatales (Fig. 2) and coded respectively as TN11.001, TN12.002, TN12.003, TN12.004 and TN12.005 (Table1). Microscopic observations of conidia and conidiophores are presented in Fig. 3.

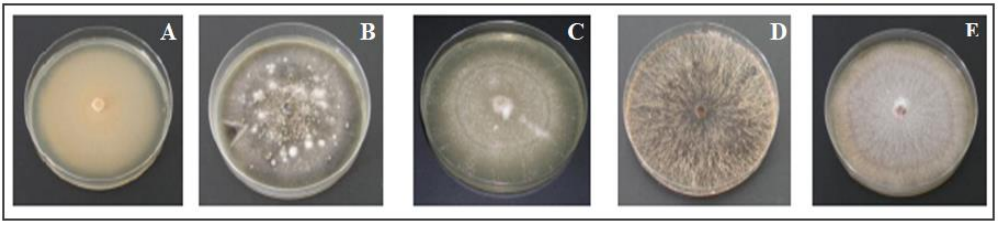

Fig. 2. Ten days old cultures of five representative species of Ophiostomatales:**A)***Ophiostoma* sp1, **B)**. *Ophiostoma* sp2, **C)***Ophiostoma* sp3, **D)***Raffaelea* sp1, **E)***Raffaelea* sp2.

Table 1. Ten days old cultural description of five putative Ophiostomatales species

Isolate	Cultural aspect	Density	Color	Shape
TN11.001	Appearance type yeast to flour with long filaments, vigorous and aerial mycelium	Medium	Fulgurant, light yellowish	Absent
TN12.002	Yeast-like to farinaceous with long filaments, vigorous and aerial mycelium with a central colony	Medium	Fulgurant, olive green	Clear
TN12.003	Yeast-like to farinaceous with long filaments, vigorous and aerial mycelium with a central colony	Not very dense	Light green olive	Clear
TN12.004	Appearance like yeast flour with long filaments, vigorous and aerial mycelium	Not very dense	Light brown	Absent
TN12.005	Yeast-like to farinaceous with long filaments, mycelium and aerial, with a central colony	Medium	Yellowish with a whitish central colony	Absent

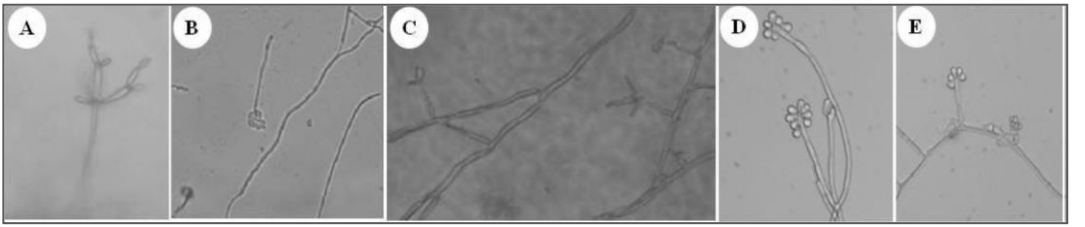

Fig. 3. Conidia and conidiophores of Ophiostomatales isolated from *Playpus cylindrus* and its galleries after 5 days incubation in darkness: **A**) *Ophiostoma* sp.1 (x400), **B)** *Ophiostoma* sp. 2 (x200), **C)** *Ophiostoma* sp. 1 (x400), **D)** *Raffaelea* sp. 2 (x400), **E)** *Raffaelea* sp. 3 (x400).

Phylogenetic analysis.

PCR analysis using ITS1F/ITS4 and T1/BT2b primer pairs yielded specific products of approximately 650 bp (ITS-PCR products) and 1000 bp (β-tubulin), respectively. For each pair of primers used in the PCR amplifications, no secondary band was obtained except for the specific products. In addition, no PCR products were obtained for the controls.

Comparison of the sequences with GenBank nucleotide sequences showed that the similarity of some sequences is not very high, but some of them can be assigned to a particular species.

Comparison of the sequences obtained with the *Raffaelea* nucleotide sequences in the GenBank sequence database for both ITS and β-tubulin regions, confirmed high degree of sequence identity of two *Raffaelea* species with *R. canadensis* and *R. montetyi*. The position of the two *Raffaelea* species was consistent, as they were placed in the Ophiostomatales clade (Harrington et al. 2010; Massoumi Alamouti et al. 2009; Matsuda et al. 2010). On the other hand, a similarity of 100% of the TN12.002 isolate with *Ophiostoma tsotsi* and 96% of the TN12.003 isolate with *O. quercus* was noted for the partial β-tubulin gene.

The phylogenetic analysis was carried out separately for both regions (ITS and β-tubulin) and the sequences of *Taphrina pruni* and *T. wiesneri* were used as outgroup, according to Inácio (2011).This phylogenetic tree underlined a separation of isolates into two different groups. A phylogenetic group including isolates TN12.002 and TN12.003, closely related to each other and to *Ophiostoma* strains, whereas isolates TN11.001, TN12.004 and TN12.005 were more similar to strains close to *Raffaelea* (Fig. 4).

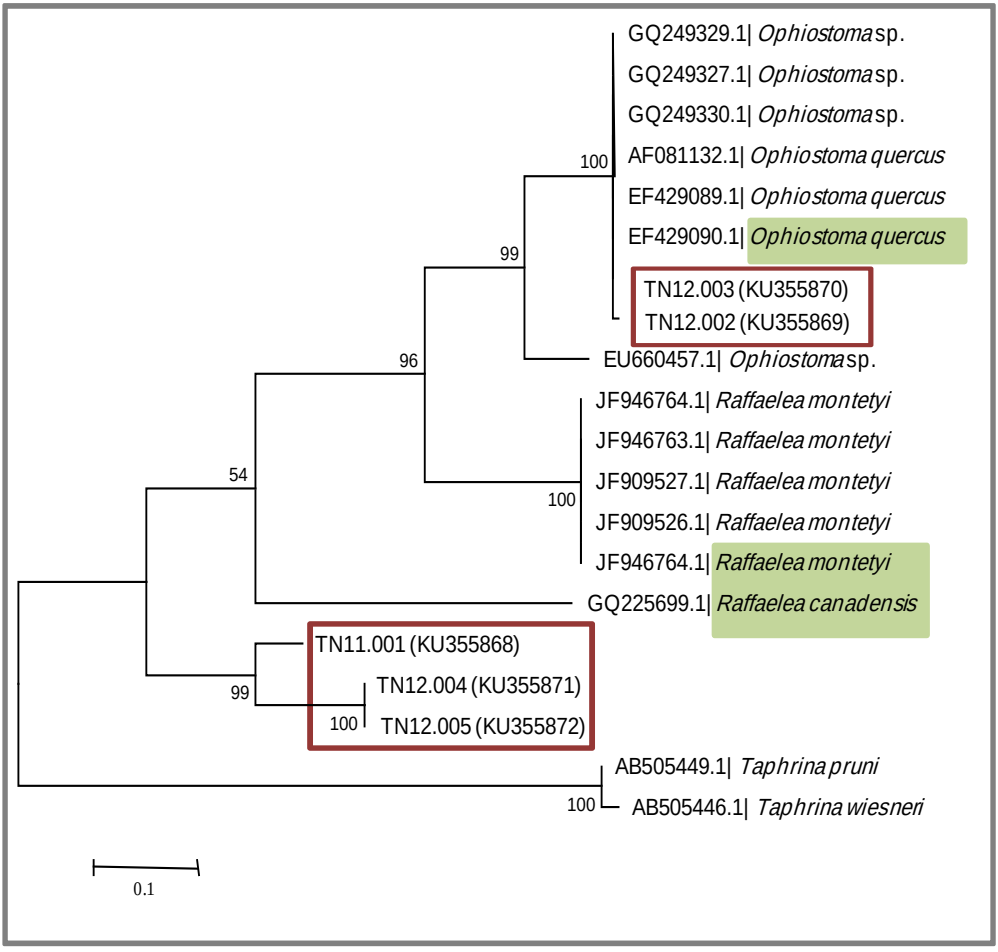

Fig. 4. Molecular phylogenetic analysis of 20 nucleotide sequences of rDNA (5.8S-ITS2-28S) of Ophiostomatales isolated from *Platypus cylindrus* on cork oak stands in Tunisia by Maximum Likelihood (ML) method based on Tamura 3-parameter model (Tamura and Nei1993) and supported by 1000 replicates of bootstraps. Evolutionary analyses were conducted with the MEGA 6 software (Tamura et al. 2013).

The comparison of the partial β-tubulin sequences with sequences already present in the database using the BLAST search of the *Ophiostoma* sp., *Raffaeleamontetyi* and *R. canadensis* sequences yielded the phylogenetic tree below (Fig. 5). A sequence from *Taphrina virginica* was included as outgroup. Homologous sequences were retrieved from GenBank and accession numbers included in the phylogenetic tree.

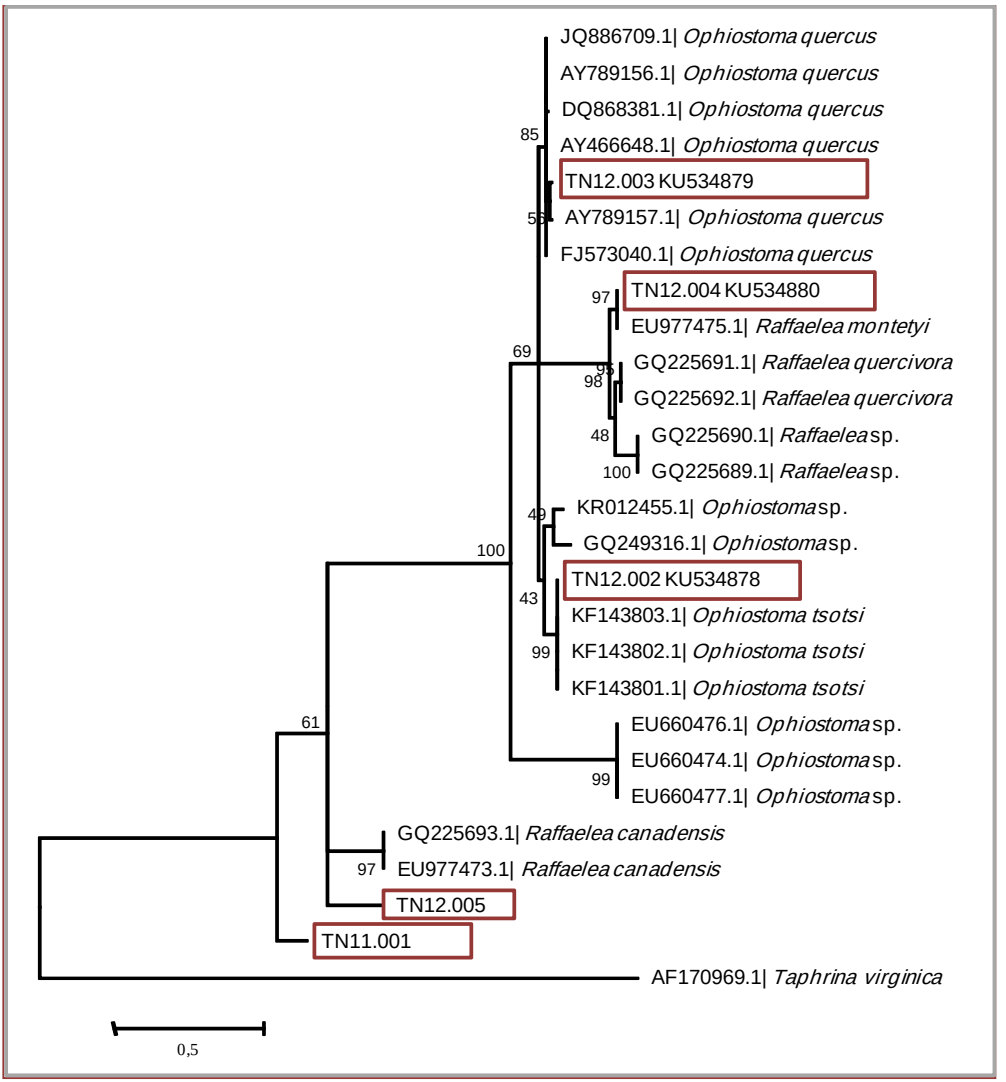

Fig. 5. Molecular phylogenetic analysis of 27 nucleotide sequences encoding the β-tubulin protein gene of Ophiostomatales isolated from *Platypus cylindrus* on cork oak stands using the Maximum Likelihood method (ML) based on the Tamura 3-parameter model. Evolutionary analyses were conducted using the MEGA v.6.0 software (Tamura et al. 2013).

The phylogenetic tree showed that the TN12.005 isolate is directly related to *R. canadensis* whereas TN12.004 is closer to *R. montetyi*. The other *Ophiostoma* isolates belonged to three different species, two of which could be identified, and a third one preliminary called *Ophiostoma* sp. The isolate

TN12.003 has more similarity with *O. quercus* while TN12.002 has more homology with *O. tsotsi*. TN11.001 isolate is *Ophiostoma* sp. since its position on the phylogenetic tree did not allow to assign it to any known species.

The sequences of the isolates obtained by each pair of primers were deposited to GenBank database (NCBI) (http://www.ncbi.nlm.nih.gov/genbank) under the accession numbers from KU534878 to KU534880 for β-tubulin and from KU355868 to KU355872 for the ITS region (Fig. 4 and Fig. 5).

Based on the sequences for both regions of the obtained ophiostomatales associated with *P. cylindrus*, five different species were identified: *Ophiostoma* sp. (KU355868), *O. tsotsi* (KU534878; KU355869), *O. quercus* (KU534879; KU355870), *Raffaelea montetyi* (KU534880; KU355871), and *R. canadensis* (KU355872).

The distribution of the fungal species in the sampled forests revealed some differences between sites (Table 2).

Table 2. Presence of the fungal species in the Ophiostomatales order associated with *Platypus cylindrus* in Tunisian cork oak forests (in grey)

Fungi	Wed Zen	Ain Sarouia	Ain Beya	Babouch	Mzara	Ain Drahem	El Jouza	Tabouba	Bellif	Sejnène
Raffaelea montetyi										
R. canadensis										
Ophiostoma quercus										
O. tsotsi										
***Ophiostoma* sp.**										

R. montetyi is present in seven forests and absent in Babouch and Mzara while *R. canadensis* occurs only in three forests namely Ain Beya, Bellif and Sejnène. The genus *Ophiostoma* represented by three species is less present. *O. quercus* was isolated from trees of the forests of Babouch and Mzara, which in addition to Ain Sarouia forest reveals the presence of *O. tsotsi*. As for the unidentified *Ophiostoma* species, it has been isolated from the forests of Wed Zen and Ain Beya.

DISCUSSION

Morphological and molecular identification of Ophiostomatales associated with *P. cylindrus* and its galleries revealed the presence of the fungi *O. quercus*, *O. tsotsi*, *R. montetyi* and *R. canadensis* and a third species of *Ophiostoma* not yet fully characterized, *Ophiostoma* sp.

Taking into account previous studies on *P. cylindrus* in Portugal (Henriques 2007; Inácio et al. 2011,2012) and in Algeria (Belhoucine et al. 2011), the presence of *R. montetyi* in Tunisia confirms this symbiotic relationship with the insect that appears to be an ecological adaptation aiming at colonizing the host in the fastest and most efficient way.

R. montetyi was detected in the forests of Ain Beya, Ain Drahem, Ain Sarouia, El Jouza, Tabouba and Wed Zen which are characterized by a humid bioclimate with mild and temperate winters and by the presence of dense and abundant shrubs (Bellahirech 2016). These conditions seem to enhance the presence of this fungal species. Among *Raffaelea* genus, several species were related to the massive mortality of oaks in Japan (Kubono and Ito 2002; Matsuda et al. 2010) and of laurel wilt (Fraedrich et al. 2008) and avocado (*Persea americana*) in the United States (Eskalen and McDonald 2011). In Portugal, *R. montetyi* was shown to be pathogenic towards cork oak (Inácio et al. 2012), therefore playing an important role in the cork oak decline. In Portugal, a *Raffaelea* species closely related to *R. canadensis* was noticed for the first time and in association with the ambrosia beetle (Inácio et al. 2012). In Tunisia, *R. canadensis* is reported for the first time in North Africa, and it has been detected only in Ain Beya forest where insect infestation did not exceed 10% (Bellahirech 2016).

Regarding molecular studies, the ITS region was widely used for fungi diagnosis and proposed as a universal marker for fungal DNA barcodes (Schoch et al. 2012). Unfortunately, the locus is particularly difficult to use for *Raffaelea* fungi (Harrington et al. 2011; Jeyaprakash et al. 2014). Thus, the differentiation between *Raffaelea* species was achieved only through the analysis of both ß-tubulin and ITS regions.In the present work, we identified two species of the genus *Ophiostoma* namely *O. quercus* and *O. tsotsi*. *O. quercus* was isolated for the first time in Yugoslavia from *Q. pedunculata* (Georgevitch 1927). This fungus was associated with the decline of various oaks in central Europe (Cech et al. 1990). In Spain, Luque et al. (2000) isolated *O. quercus* from cork oak, and was considered to be the agent of blue stain and xylem discoloration of many other trees (De Beer et al. 2003; Geldenhuis et al. 2004; Nkuekam et al. 2008). *O. quercus* is associated with many insects, particularly bark beetles (Kirisits 2007; Zhou et al. 2006). In Norway, *O. quercus*was associated with *Scolytusrafzeburgi* in *Betula* sp. (Linnakoski et al. 2009). It was also found in Finland and Russia (Linnakoski et al. 2008).

O. quercus was reported in Algeria on cork oak associated with *P. cylindrus*, (Behoucine et al. 2011) and in Tunisia,as shown in the present work, in Babouch and Mzara forests with presence average of 16.6 and 8.3%, respectively. These forests are characterized by similar climatic, edaphic and phytosanitary conditions and dense vegetation cover. Phylogenetic β-tubulin analyzes confirmed that the TN12.002 isolate associated with *P. cylindrus* and its galleries was 100% similar to the *O. tsotsi* species. This group of fungi is closely related to *O. quercus* and to the Dutch disease pathogens, *O. ulmi* and *O. novo-ulmi*, in the clade of the O. *piceae* complex (Grobbelaar et al. 2010). This is coherent with our results which show, through the phylogenetic tree of the ITS region, an assembly of these two species under a monophylogenic line (*O. quercus*). The differentiation between these species was only possible through

the β-tubulin analyzes alone, due to a better resolution.

It is noteworthy that *O. tsotsi* is frequently found in association with *O. quercus* and it is not surprising that it remained hidden from recognition as a discrete entity. The available knowledge of this fungus is limited, but it appears that its distribution and host range are confounded with those of *O. quercus* (De Beer et al. 2003; Grobbelaar et al. 2010; Harrington et al. 2001).*O. tsotsi* was found on hardwood trees in South Africa (Grobbelaar et al. 2010) and it is considered a native species in the northern hemisphere (Brasier 1990). Its presence in Tunisia suggests that it was introduced from this region which also represents all the industrialized countries (Europe, Asia, and North America). This could easily occur with the commercial trade of timber and its products from the northern hemisphere to Africa (Grobbelaar et al. 2010) including Tunisia. On the other hand, this fungus was identified on *Eucalyptus* in China (Grobbelaar et al. 2011) and Australia (Nkuekam et al. 2011). It is important to verify that *O. tsotsi* was always isolated in association with *O. quercus* (Grobbelaar et al. 2010, 2011; Nkuekam et al. 2011). This observation corroborates our study where the two species were isolated in the same forest (Mzara) where *P. cylindrus* infestation is about13% and the global phytosanitary situation of trees is good (Bellahirech 2016).

In Tunisia, these species are newly reported in symbiotic relationship with the cork oak pinhole borer. *Ophiostoma* and *Raffaelea* fungi were obtained from both insect mycangia and galleries. Without *P. cylindrus* as vector, it would not be possible for these fungal species to infect new hosts because Ophiostomatales require pre-existing wounds to infect their hosts (Nkuekam et al. 2012).On the other hand, without these fungal associates, the insects would have enormous difficulty in colonizing new trees and to continue their life cycle. Hence, there is thus an obligatory relationship of symbiosis as defended by Lieutier (2007). In addition, several studies showed that only one or a few species of fungi are associated with a particular ambrosia insect (Batra 1963; Funk 1970). However, more recent studies have pointed out that symbiotic relationships are more diverse,more promiscuousand more competitive than assumed and that *Raffaelea* species compete with each other for growth into mycangia (Harrington et al. 2011; Inácio et al. 2012).

Understanding the ecology and population dynamics of *P. cylindrus*-associated fungi is important for the surveillance and management of the beetle-fungal complex, and could improve prediction and modeling. Biological control of the pathogens may prove possible through manipulation of the mycangial mycoflora (Inácio 2011).

Finally, it is important to point out that the research described herein contributed to clarify aspects of putative reasons to trigger the decline of trees in Tunisian cork oak forests. This investigation should continue, namely through the pathogenicity assessment of the identified Ophiostomatales, considering however that it is rather difficult to perform such studies in adult trees. The clarification of these fungal symbionts role and the prevention of their spread will help to preserve the natural patrimony in Tunisia and cultural heritage of the unique cork oak stands in Mediterranean Basin.

RESUME

Bellahirech A., Inácio M.L., Ben Jamâa M.L. et Nóbrega F. 2018. Champignons ophiostomatoïdes associés à l'insecte ambrosia *Platypus cylindrus* dans les forêts de chênes-lièges en Tunisie. Tunisian Journal of Plant Protection 13 (si): 61-75.

Le chêne-liège (Quercus suber) est une espèce unique de la région méditerranéenne occidentale et, au cours des dernières décennies, il a été menacé par plusieurs ravageurs et maladies. Parmi les principaux ravageurs dangereux, l'insecte ambroisia *Platypus cylindrus* (le platype du chêne) qui joue un rôle clé dans le processus de déclin du chêne-liège, notamment au Portugal, au Maroc et en Algérie. Cependant, en Tunisie, où les forêts de chênes-lièges couvrent environ 90 000 ha, cet insecte continue d'avoir un statut de ravageur secondaire. Comme tous les insectes ambrosia, *P. cylindrus* est capable d'établir des relations symbiotiques avec les champignons et il est connu comme étant le vecteur des champignons ophiostomatoïdes, un groupe comprenant les pathogènes primaires des arbres. Le but de cette étude était d'identifier ces champignons associés au coléoptère dans les forêts tunisiennes et de comprendre la contribution de cette association dans le déclin du chêne-liège en comparant avec les résultats des autres pays. La présente étude a été réalisée en 2012 dans d'importantes forêts de chênes-lièges de l'ouest-nord de la Tunisie et s'est concentrée sur les champignons ophiostomatoïdes associés au platype de chêne-liège. Vingt-quatre isolats ont été regroupés en fonction de l'identification morphologique et cinq isolats représentatifs ont été inclus dans les analyses phylogénétiques basées sur les données de séquençage des locus ITS et β-tubuline. Les champignons ont été attribués à cinq espèces: *Raffaelea montetyi*, *R. canadensis*, *Ophiostoma* sp. *O. tsotsi* et *O. quercus*, certaines d'entre elles ont déjà été signalées au Portugal et en Algérie en relation avec le déclin du chêne-liège. Toutes ces espèces ont été identifiées et signalées pour la première fois en Tunisie en association avec *P. cylindrus* sur chêne-liège et leur rôle dans la perte de vitalité du chêne-liège devra être étudié.

Mots clés: Champignons ambrosia, Ophiostomatales, platype, *Quercus suber*

ملخص

بلاحيرش، أماني وماريا لوردس أناسيو ومحمد الحبيب بن جامع وفلومينا نبرجا .2015. مساهمة الفطريات ophiostomatoids المرتبطة بالحشرة *Platypus cylindrus* في غابات بلوط الفلين بتونس.

Tunisian Journal of Plant Protection 13 (si): 61-75.

بلوط الفلين/الفرنان (*Quercus suber*) هو جنس فريد من نوعه في منطقة غرب البحر الأبيض المتوسط، وخلال العقود الماضية، يتعرض باستمرار لخطر مختلف الآفات والأمراض. من بين الآفات الخطيرة الرئيسية، هناك الحشرة الأمبروزية *Platypus cylindrus* (ناخرة الخشب) التي تتواجد في دول أخرى ولها دور رئيسي في عملية تدهور بلوط الفلين، خاصة في البرتغال والمغرب والجزائر. رغم ذلك، في تونس حيث تغطي أشجار بلوط الفلين حوالي 90.000 هكتار، تظل هذه الحشرة آفة ثانوية. مثل جميع الحشرات الأمبروزية، الحشرة *P. cylindrus* قادرة على إقامة علاقات تكافلية مع الفطريات، وتعمل كناقلة للفطريات ophiostomatoids، وهي مجموعة من الفطريات التي تضم مسببات الأمراض الأولية للشجرة. كان الغرض من هذه الدراسة هو تشخيص هذه الفطريات ذات الصلة بالحشرات لفهم مساهمة هذه العلاقة في تدهور اشجار بلوط الفلين. أجريت هذه الدراسة في عام 2012 في عشر غابات في شمال غرب تونس وتركزت على رتبة الفطريات Ophiostomatales المرتبطة بالحشرات الناخرة في غابات بلوط الفلين التونسية. تم توصيف أربع وعشرون عزلة بناءً على التشخيص المورفولوجي، وتمت دراسة خمس عزلات في تحليل جيني استنادا إلى تتابع منطقتيْ ITS وβ-tubulin. تم ربط الفطريات إلى خمسة أنواع هي *Raffaelea montetyi* و *R. canadensis* و sp. *Ophiostoma*. و *O. quercus* و *O. tsotsi*. تمت الإشارة إلى البعض منها في البرتغال والجزائر لارتباطها بتدهور بلوط الفلين. وقد تم تشخيص جميع هذه الأنواع وإرادها لأول مرة في تونس مع ارتباطها بـ *P. cylindrus* في أشجار بلوط الفلين ويبقى دورها في فقدان الفرنان لحيويته بحاجة إلى بحث.

كلمات مفتاحية: بلوط الفلين، ناخرة الخشب، فطريات أمبروزية، Ophiostomatales

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