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Occurrence of *Xyleborus monographus* (Fabricius) and *Xyleborinus saxesenii* (Ratzeburg) Together with *Platypus cylindrus*, in Cork Oak Trees in Tunisia

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Abstract: Cork oak (*Quercus suber*) is found in south-western Europe (Portugal, Spain, France and Italy) and northern Africa (Morocco, Algeria and Tunisia). Different pests and diseases affect this tree, with some important regional differences. For instance, the pinhole borer *Platypus cylindrus* is a major threat in the Iberian Peninsula, but it is not a relevant pest in Tunisia. While studying the infestation of pinhole borer in cork oak forests of Tunisia, insects of the *Xyleborus* and *Xyleborinus* genus were captured. Sampling was carried out in cork oak stands in North-western Tunisia, in 2012 and in 2018. One symptomatic tree from each stand was selected, and the logs were set up in the laboratory. Emerging Scolytinae insects were identified by morphological characters and molecular analysis. Using the mitochondrial gene cytochrome oxidase I (COI) sequences the multiple alignment analysis of partial COI sequences allowed the identification of *Xyleborus monographus* and *Xyleborinus sexesenii*. These ambrosia beetles had previously

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been reported in Tunisia but not associated with cork oak. According to CABI, *Q. suber* is not listed as a host plant for *X. saxesenii* that is cited as native and non-invasive in Tunisia. Further studies on the bioecology of *X. monographus* and *X. sexesenii* are needed as they may vector pathogenic fungi. Though the insects are known to attack only weakened trees, climatic changes may increase their potential to cause economic damage.
Key words: Ambrosia beetles; *Quercus suber*; Cytochrome oxidase I; Tunisia; Xyleborini.

Ocorrência de *Xyleborus mongraphus* (Fabricius) e *Xyleborinus saxesenii* (Ratzeburg) juntamente *com Platypus cylindrus*, em Árvores de Carvalho de Cortiça na Tunísia

Sumário: O sobreiro (*Quercus suber*) encontra-se distribuído pelo sudoeste da Europa (Portugal, Espanha, França e Itália) e no norte de África (Marrocos, Argélia e Tunísia). Diferentes pragas e doenças afetam esta árvore, com algumas diferenças regionais importantes. Por exemplo, o plátipo (*P. cylindrus*) é uma grande ameaça na Península Ibérica, mas não é uma praga relevante na Tunísia. Ao estudar a infestação do plátipo nos montados da Tunísia, foram capturados insetos dos géneros *Xyleborus* e *Xyleborinus*. A amostragem foi realizada em povoamentos de sobreiros no noroeste da Tunísia, em 2012 e em 2018. Foi selecionada uma árvore sintomática em cada povoamento, e os troncos foram colocados no laboratório. Os insetos Scolytinae emergentes desses troncos foram identificados pelos caracteres morfológicos e análise molecular. Utilizando as sequências parciais do gene mitocondrial Citocromo Oxidase I (COI) foi possível a identificação das espécies *Xyleborus monographus* e *Xyleborinus sexesenii*. Estes insetos-ambrosia já tinham sido descritos para a Tunísia, mas não associados ao sobreiro. Segundo o CABI, *Q. suber* não está listado como planta hospedeira de *X. saxesenii*, que é considerada uma espécie nativa da Tunísia. São necessários mais estudos sobre a bioecologia de *X. monographus* e *X. sexesenii*, uma vez que podem ser vetores de fungos patogénicos para o sobreiro. Embora se saiba que estes insetos atacam apenas árvores enfraquecidas, as alterações climáticas podem aumentar o seu potencial de causar danos económicos.
Palavras-chave: Insetos-ambrosia; *Quercus suber*; Cytochrome oxidase I; Tunísia; Xyleborini

Présence de *Xyleborus monographus* (Fabricius) et de *Xyleborinus saxesenii* (Ratzeburg) avec *Platypus cylindrus*, dans les Chênes-Lièges en Tunisie

Résumé: Le chêne-liège (*Quercus suber*) est présent dans le sud-ouest de l'Europe (Portugal, Espagne, France et Italie) et en Afrique du Nord (Maroc, Algérie et Tunisie). Différents ravageurs et maladies affectent cet arbre, avec quelques différences régionales importantes. Par exemple, le platype *Platypus cylindrus* est une menace majeure dans la péninsule ibérique, mais il n'est pas un ravageur important en Tunisie. Lors de l'étude de l'infestation de *P. cylindrus* dans les forêts de chêne-liège de Tunisie, des insectes du genre *Xyleborus* et *Xyleborinus* ont été capturés. L'échantillonnage a été effectué dans des

peuplements de chênes-lièges du nord-ouest de la Tunisie, en 2012 et en 2018. Un arbre symptomatique de chaque peuplement a été sélectionné, et les tronçons ont été mis en place au laboratoire. Les insectes Scolytinae émergents ont été identifiés par des caractères morphologiques et des analyses moléculaires. En utilisant les séquences de la cytochrome oxydase I (COI) du gène mitochondrial, l'analyse de l'alignement multiple des séquences partielles de la COI a permis l'identification de *Xyleborus monographus* et de *Xyleborinus sexesenii*. Ces coléoptères ambrosia avaient déjà été signalés en Tunisie mais n'étaient pas associés au chêne-liège. Selon CABI, *Q. suber* n'est pas répertorié comme une plante hôte pour *X. saxesenii* qui est citée comme indigène et non envahissante en Tunisie. Des études supplémentaires sur la bioécologie de *X. monographus* et *X. sexesenii* sont nécessaires car ils peuvent être des vecteurs de champignons pathogènes. Bien que l'on sache que ces insectes n'attaquent que les arbres affaiblis, les changements climatiques pourraient accroître leur potentiel de dommages économiques.
Mots-clés: Insectes ambrosia; *Quercus suber*; Cytochrome oxydase I; Tunisie; Xyleborini

Introduction

Cork oak (*Quercus suber*) is a common evergreen forest species of the Western Mediterranean region. The largest expanses of cork oak in the world are present in the Iberian Peninsula (BUGALHO *et al.*, 2011). Cork exploitation makes cork oak ecosystems economically beneficial. Cork oak also provides environmental and social benefits to local communities in the form of hunting, honey and mushroom collection, and livestock farming (MENDES and GRAÇA, 2009). However, at all stages of their lives, cork oak trees are vulnerable to several biotic agents mainly insects and microbial pathogens (ERIKSSON *et al.*, 2017). Amongst the most important pests, the ambrosia beetle *Platypus cylindrus* F. (the oak pinhole borer) plays a key role in the decline of cork oak in many countries, including Portugal (FERREIRA and FERREIRA, 1989; INÁCIO *et al.*, 2012a), Morocco (VILLEMANT and FRAVAL, 1993; SOUSA *et al.*, 2005), and Algeria (BELHOUCINE *et al.*, 2011). In Tunisia, this beetle remains to be a secondary pest and only attacks dead or already weakened trees (BELLAHIRECH *et al.*, 2015, 2016).

As all ambrosia beetles, *P. cylindrus*, establishes symbiotic relationships with fungi. Under favourable conditions, adult insects disperse fungi from one tree to another in spore-carrying organs called mycangia. These fungi provide nutrients for the insect offspring, simultaneously, causing decay of the wood tissues (BAKER, 1963; BEAVER, 1977; BIEDERMANN and TABORSKY, 2011). Fungi in the order Ophiostomatales are among the most common fungal associates of bark and ambrosia beetles. In the particular case of *P. cylindrus*, *Ophiostoma* sp. *sensu lato* were found to be pathogenic towards cork oak plants in Portugal (INÁCIO *et al.*, 2012b).

In the ambrosia beetles, members of the tribe Xyleborini (*Xyleborinus* plus related genera) have the greatest potential to cause economic damage. This is due to inbreeding: males mate with their sisters within the parental gallery system before females dispersal. Thus, the introduction of only a few mated females may lead to the establishment of an active population if suitable host plants can be found and environmental conditions are satisfactory (KÜHNHOLZ *et al.*, 2003).

The relationships between cork oak, *P. cylindrus* and associated mycobiota that may contribute to the decline of local cork oak forests, were studied by BELLAHIRECH (2016). Thus, this work aimed to study the diversity and the abundance of ambrosia beetles, namelly two Xyleborini species found

associated with *P. cylindrus* in Tunisian cork oak forests for the first time and their possible role in tree decline is discussed

Materials and methods

Study sites

Investigations were carried out in cork-oak forests in North-western Tunisia. The survey occurred between November 2011 and June 2012 in four sites: Ain Beya, Ain Sarouia, Mzara and Bellif and between December 2017 and August 2018 in two sites: Ain Drahem and Homran. Characteristics of each site are resumed in Table 1. Selected forests present diversity of understory and shrubs species namely *Pistacia lentiscus*, *Quercus coccifera*, *Erica arborea*, *Myrtus communis*, *Smilax aspera*, *Phillyrea media*, *Arbutus unedo*, *Scilla maritima*, *Brisa maxima* and *Lavandula stoechas* (DGF, 2006). Shrub density was notably high in the majority of the sites, including Mzara and Homran. However, it was low and considerably degraded in Ain Drahem.

Table 1 - Cork oak studied sites in northwest of Tunisia, where insects were collected

Site	Longitude (E)	Latitude (N)	Altitude (m)
Ain Beya	8.93855	36.65177	375
Ain Sarouia	8.49912	36.73101	298
Ain Drahem	9.050	36.990	728
Bellif	9.01397	37.04737	276
Homran	8.824	36.783	609
Mzara	8.72043	36.76946	653

Sampling

In each site, one symptomatic *Q. suber* tree was randomly selected and part of the debarked trunk was cut and sectioned in 10 cm logs which were installed in the nursery of the National Research Institute of Rural Engineering, Water and Forests in Tunis (INRGREF, Tunisia), enclosed in fabric tissue covers at environmental temperature. A total of six trees were monitored and emerged insects were collected and prepared for identification.

Morphological characterization

A total of 462 Xyleborini insects was examined. Morphological identification was made under a Leica magnifying glass using keys of BALACHOWSKY (1949), WOOD and BRIGHT (1992). The Xyleborini identification key of HULCR and SMITH (2010) was also used.

Molecular characterization:
To confirm morphological identification of very damaged Xyleborini specimens, mitochondrial cytochrome oxidase subunit I gene (COI) was used. PCR amplifications were performed with the primers LCO 1490 and HCO 2198 (FOLMER *et al.*, 1994). COI sequences were used to construct the phylogenetic tree using the Maximum Likelihood based on the Hasegawa-Kishino-Yano model and 1000 replicates of bootstrapping, in the MEGA6 program (TAMURA *et al.*, 2013)

Statistical analysis

To compare sites and seasonal occurrence, the multivariate statistical analysis (ANOVA) was applied to the numbers of insects collected from the trees sampled at Ain Drahen and Homran sites, using STATISTICA software package version 6.0 (StatSoft, Inc., Tulsa, OK, USA).

Results and discussion

Insects identification

Morphological identification of the Xyleborini tribe allowed differentiation between *Xyleborus* sp., *Xyleborinus* sp. and *Platypus cylindrus* (Figure 1).

Xyleborus sp. and *Xyleborinus* sp. specimens pursued for species identification through molecular analyses. Partial COI sequences were compared with the sequences registered in the GenBank and BOLD Systems databases, revealing the species *Xyleborus monographus* (Fabricius) and *Xyleborinus saxesenii* (Ratzeburg) (Figure 2).

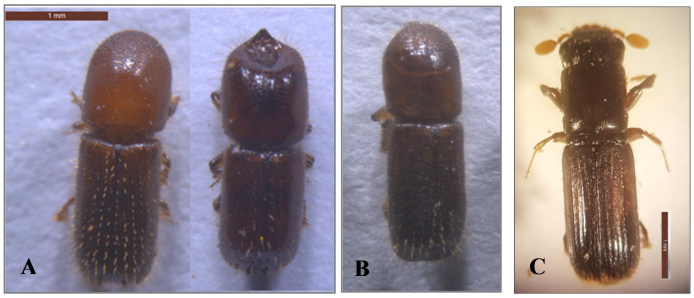

Figure 1 - A. Adults of *Xyleborus monographus* (left: female; right: male); B. *Xyleborinus saxesenii:* C. *Platypus cylindrus*

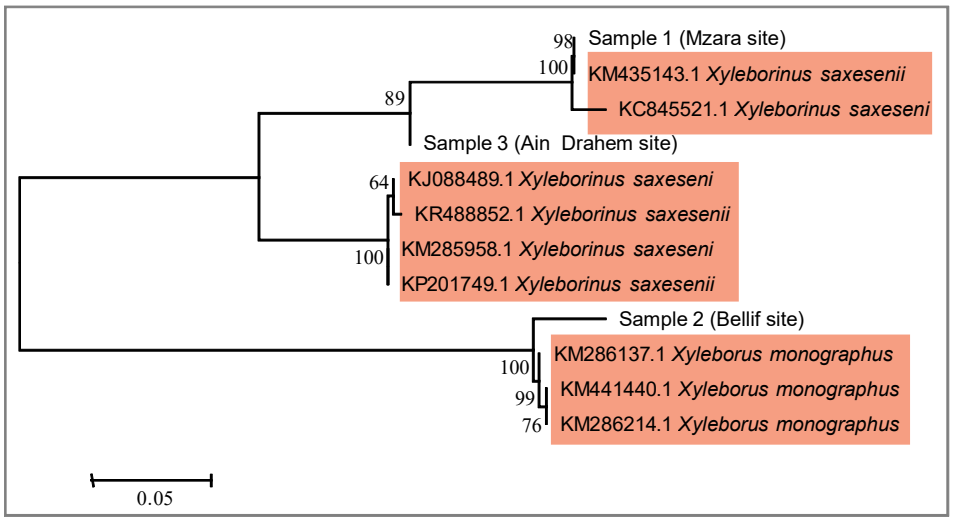

Figure 2 - Phylogenetic tree based on COI sequences of species from Xyleborinae sub-family generated using the Maximum Likelihood method based on the Hasegawa-Kishino-Yano model with 1000 bootstrap replication. Bootstrap values are indicated at the nodes. The length of each pair of branches represents the distance between sequence pairs. The sequence information at the tips of the branches includes an accession numbers of the sequences. Evolutionary analyses were conducted in MEGA6

Insects collection

A total of 376 insects were collected from the logs of the surveyed sites, during 2011/2012, and 864 specimens, during 2017/2018 survey.

Both Xyleborini species were collected from all tree sections with higher abundance in the lower trunk sections.

The pinhole borer *P. cylindrus* was the dominant species present in the cork-oak wood, being the most collected from all studied sites and with a mean of 34 insects per tree (max=436 and min= 28) ($F_{(2,252)}$= 9,8337; p= 0,0001*). Their emergences occurred from February to June on both years (2012 and 2018).

There were also significant differences in the catches between sites ($F_{(5,252)}$= 16,8319; p< 0,0001*) for all three species, where the site with most specimens collected was Ain Drahem during 2018, and Ain Beya during 2012, however the last site more relevant only for *P. cylindrus*. In fact, if we consider only the Xyleborini species, no differences are detected between the two species ($F_{(1,168)}$= 2,4786; p= 0,1173 NS) and the effect of the sites is barelly significant ($F_{(5,168)}$= 2,5111; p= 0,0320*) (Table 2).

Table 2 - Number of collected insects from logs representative of studied sites

Year	Site	*Xyleborus monographus*	*Xyleborinus saxesenii*	*Platypus cylindrus*
2012	Ain Beya	22	25	155
	Ain Sarouia	1	0	47
	Bellif	2	7	28
	Mzara	6	10	73
2018	Ain Drahem	255	113	436
	Homran	17	4	39

In these analyses we are not able to clearly evaluate the influence of the year of survey in the observed differences in the catches, since the survey wasn't repeated in same sites.

So for 2018 survey the insect populations in Ain Drahem are considerably larger than in Homran, which is directly related to the phytosanitary situation of each forests mainly with the regards to following parameters proportion of trees with trunk cavities, proportion of trees with crown yellowing, proportion of trees with trunk diseases signs, proportion of trees with crown defoliation , mean number of trunk scars per tree, proportion of trees with holes of ambrosia beetles (BELLAHIRECH *et al.*, 2019).

For surveyed sites during 2012, Ain Beya has larger insects' samples than the other three sites, also justified by the sanitary condition of cork oak trees, proved to be healthier than Ain Beya (BELLAHIRECH *et al.*, 2019).

The more reduced *P. cylindrus* populations in Homran, Ain Sarouia, Bellif and Mzara can also be due to dendrometric characteristics (Diameter at breast height and tree crown length and diameter), better phytosanitary conditions and management factors such as less trunk injuries due to cork oak removal (BELLAHIRECH *et al.*, 2019).

The numbers of captured insects of both species in Ain Drahem allowed us to establish emergence curves, revealing overlapping peak emergences within a 2-months period from April 12th to May 19th, when the majority of specimens were obtained (83% and 87% for *X. monographus* and *X. saxesenii*, respectively). Both species exhibited statistically significant variability in seasonal occurrence during the survey period ($F_{(1,12)}$= 3,260; p= 0,0220), as is clearly illustrated in Figure 3.

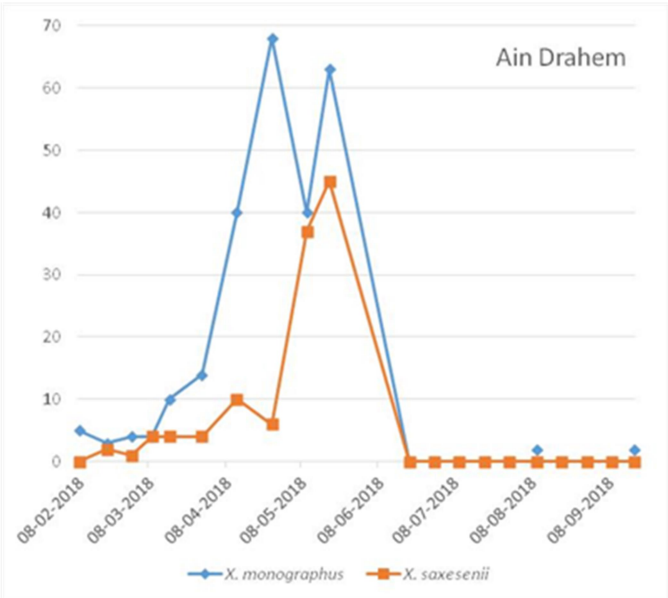

Figure 3 - Number of emerged *Xyleborus monographus* and *Xyleborinus saxesenii* in laboratorial conditions during 2018, from infested cork oak logs collected in Ain Drahem

Both species had been previously recorded in Northern Africa (WOOD and BRIGHT, 1992). *Xyleborus monographus* was reported for Algeria and Morocco on *Quercus* spp. by WOOD and BRIGHT (1992) and in Algeria on *Q. suber* (BOUCHAOUR-DJABEUR, 2013; BELHOUCINE and BOUHRAOUA, 2015), while *X. saxesenii* was reported for Tunisia in 1960 on *Ulmus* spp. and *Eucalyptus* sp. but not on *Quercus suber* (WOOD, 1960). In addition, CABI website lists only white oak (*Quercus alba*) and common oak (*Quercus robur*) as host plants for *X. saxesenii*, with no record of cork oak (CABI, 2019).

The ambrosia beetles *X. monographus* and *X. saxesenii* are reported here for the first time in Tunisia in association with cork oak trees. The two species were found in few locations and in low numbers, colonizing trees already affected by other decline agents, including the much more abundant and widespread *P. cylindrus* (Table 2).

Being ambrosia beetles, species of the *Xyleborus* genus such as *X. glabratus* (Eichhoff) have been found to carry pathgenic fungi namely *Raffaelea lauricola* in the southeastern USA, causing lethal vascular wilt disease on Lauraceae plant members (ESKALEN and MCDONALD, 2011, HARRINGTON and FRAEDRICH, 2011; FRAEDRICH *et al.*, 2015).

Furthermore, *R. montetyi* and *R. canadensis* were also identified as primary ambrosia fungi of *X. monographus* and *X. dryographus* (GEBHARDT *et al.*, 2004). In previous studies conducted in Tunisian and Portuguese cork oak forests by INÁCIO *et al.*, (2012a); BELLAHIRECH *et al.*, (2014), fungi of the *Raffaelea* and *Ophiostoma* genera were found associated with *P. cylindrus*, implying that different ambrosia beetles can transport similar pathogenic fungi which can debilitate or kill the host tree, benefiting all the insect species evolved in the process.

Taking into account that ambrosia beetles are the most representative group among invasive species, this study warrants a particular attention. In fact, recently, *X. grabratus* has been reported as an exotic invasive species in Mexico representing a major threat for native and cultivated forest ecosystems (LIRA-NORIEGA *et al.*, 2018).

Our observations suggest that Xyleborini beetles are secondary pests of cork oak trees in Tunisia, similarly to the findings reported in Morocco (CHADIGAN *et al.*, 1991). These species should be carefully monitored, as they can be associated with pathogenic fungi and therefore contribute, with other biotic and abiotic factors, to cork oak decline. Actually, in the context of climate change and commercial international trade, both of which enable the dispersal of

nonnative, disease-spreading invasive species (RASSATI *et al.*, 2018), monitoring bark and ambrosia beetles has become a priority.

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