

First Report of three Tortricidae species on *Quercus suber* Forest in Northwestern Tunisia

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ABSTRACT

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The Tunisian fauna of Tortricidae has been poorly investigated despite the great economic importance of this family. Sampling of Tortricidae insects was carried out in spring and summer 2010 in two cork oak (*Quercus suber*) forests in northwestern Tunisia. Three species are reported for the first time: *Archips xylosteana*, *Pammene splendidulana*, *Pammene giganteana*. Their identification was achieved using DNA barcodes.

Keywords: *Archips xylosteana*, DNA barcoding, *Pammene splendidulana*, *Pammene giganteana*, *Quercus suber*, Tortricidae, Tunisia

The Tortricidae family is one of the most diverse families in the so-called "Microlepidoptera" with more than 10,300 species described (Gilligan et al. 2012). The common name "leafrollers" refers to the behavior of their caterpillars that have the habit of winding or twisting the leaves of the plants on which they feed, using silk to fix the leaf pockets (Harrachi 1994). However, the larvae of those tortricids cover a wide range of feeding strategies, sometimes fairly divergent from the typical leaf-rolling habit (Horak and Brown 1991; Powell et al. 1998). There are

gall-makers, root-borers, fruit-borers, seed-predators, flower-feeders, and tip-tiers (Brown et al. 2008).

The Tortricidae family is of great economic importance; the larvae of many species cause major economic damage in agriculture, horticulture and forestry on a wide variety of crops including pome and stone fruits, citrus fruits, grapes, ornamental crops, tea, coffee, cereals and cotton. In forestry, many species of both coniferous and deciduous trees are attacked by the pest species belonging to this family (Meijerman and Ulenberg 2000). Mannai (2017) identified 82 species of Lepidoptera defoliators of *Quercus* species, containing eight species of the Tortricidae family. This work

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presents the first report of three Tortricidae species on *Q. suber* forests in northwestern Tunisia.

MATERIALS AND METHODS

Sampling of larvae.

Larvae of Tortricidae insects were collected on *Q. suber* from the cork oak forests Bellif (Long. 9°538'; Lat. 37°152'; alt. 70 m) and El Jouza (Long. 9°015'; Lat. 36°513'; alt. 550 m) in 2010 and were conserved into ethanol (96%) for the DNA analysis.

Molecular Identification.

DNA analysis of larvae was performed to identify the species. PCR amplification and DNA sequencing were conducted at the CCDB (Canadian Centre for DNA Barcoding, Guelph, Canada) following standard high-throughput protocols (Ivanova et al. 2006) that can be accessed under the website <<http://www.dnabarcoding.ca/pa/ge/research/protocols>>. PCR amplification with a single pair of primers consistently recovered a 658 bp region near the 5' terminus of the mitochondrial Cytochrome c Oxidase I (COI) gene that included the standard 658 bp barcode region for the animal kingdom (Hebert et al. 2003). DNA extracts are stored at the CCDB. All sequences are deposited also in GenBank according to the iBOL (international BARCODE OF LIFE) data release policy. Species identification and delineation were based on the system of Barcode Index Numbers (BINs: Hausmann et al. 2013; Ratnasingham and Hebert 2013).

RESULTS

Using PCR techniques, three Tortricidae species were identified: *Archips xylosteana*, and *Pammene splendidulana*, *Pammene giganteana* (Figs. 1-3).

Archips xylosteana.

BIN: BOLD: AAC0366; five DNA barcode sequences (INRGREF 0138; 0261; 0264; 0283; 0293; all with full length of 658 bp) of *A. xylosteana* were obtained from Tunisia; sequences and metadata are free accessible under the public dataset DS-TORTRITU on BOLD. Compared to 44 other DNA barcodes of *A. xylosteana* on BOLD from nine countries in Europe and north-eastern Canada, 99.1-99.7% similarity was found; all specimens are BIN-sharing.

Pammene splendidulana.

BIN: BOLD: ACS5540; one DNA barcode sequence (INRGREF 0073; with full length of 658 bp) of *P. splendidulana* was obtained from Tunisia; sequence and metadata are free accessible under the public dataset DS-TORTRITU on BOLD. Compared to seven other DNA barcodes of *P. splendidulana* on BOLD Bin-sharing and high similarity, 99.1-99.2% was found with two samples from Austria and Slovenia, but with other five samples from Germany and Finland belonging to another BIN (AAU2599), similarity was 97.7-98%. Additional taxonomic work is required to address potential cryptic diversity.

Pammene giganteana.

BIN: BOLD: AAI2598; one DNA barcode sequence (INRGREF 0065; with full length of 658 bp) of *P. splendidulana* was obtained from Tunisia, sequence and metadata are free accessible under the public dataset DS-TORTRITU on BOLD. Compared to twelve other DNA barcodes of *P. giganteana* on BOLD from eight European countries, 99.1-99.5% similarity was found; all specimens are BIN-sharing.



Fig. 1. Larva of *Archips xylosteana*

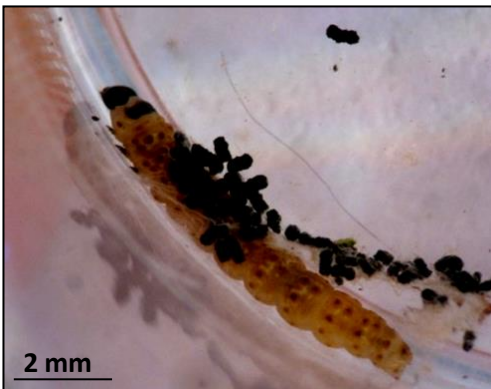


Fig. 2. Larva of *Pammene splendidulana*



Fig. 3. Larva of *Pammene giganteana*

DISCUSSION

Leaf-rollers.

A. xylosteana is a Palearctic species. In Europe, it has been observed in most European countries (Karsholt and Razowski 1996; Zhang 1994). In North Africa, this insect was reported in Algeria (Leraut et Luquet 1995). In Asia, *A. xylosteana* was detected in Korea (Razowski 1977), China, Japan and Asia Minor (Bradley et al. 1973; Hwang 1974;

Konno 2005; Zhang 1994). Schall (2006) reported the species in America, confirmed by a Canadian DNA barcode on BOLD.

Larvae of *A. xylosteana* are polyphagous (Dickler 1991) and may cause significant defoliation by feeding on new foliage and buds of ornamental trees and shrubs (Spears 2006). The larvae feed on the foliage of numerous trees and woody plants such as *Q. ilex*, *Q.*

pyrenaica, *Q. robur* (CAB 2006; Toimil 1987), *Acer* sp., *Corylus* sp., *Fraxinus* sp., (Bradley et al. 1973) and *Prunus* sp. (Luciano and Roversi 2001). *A. xylosteana* is univoltine (Dickler 1991). Adults are active from late June or early July to mid-August in Europe and Japan (Razowski 1977). The insect overwinters in the egg stage (Razowski 1977). Larval development requires 30-40 days. The pupal stage lasts 9-12 days (Razowski 1977). Adult moths rest in foliage during daytime and fly at night or when disturbed (Bradley et al. 1973).

The Genus *Pammene* is one of the genera related to *Grapholita* and belongs to the tribe Grapholitini of the subfamily Olethreutinae (Bae and Park 1998). *P. splendidulana* is widespread in the Western Palearctic zone. It is present in most European countries including the east to the Caucasus and Asia Minor (Karsholt and Razowski 1996). Their larvae feed on the underside of the leaves of *Quercus*, developing inside a small silky tube

interwoven between the leaves, within which they accumulate their own excrement (Ylla Ullastre and Marcia Vila 2005). This species shows two generations in the year in April and June (Razowski 2003). Larvae of *P. splendidulana* were observed on *Q. suber*.

Gall-makers.

Besides the leaf-roller *P. splendidulana*, the gall-maker *P. giganteana* was observed on *Quercus* species. *P. giganteana* is present in many countries of Europe such as Bulgaria (Toshova et al. 2016), Italy (Pinzari et al. 2013) and Hungary (Csóka and Szabóky 2005) and many others (Karsholt and Razowski 1996 under the synonymous name "*P. inquilana*"). This species is univoltine. Adults are active in spring (Kimber 2018). Their larvae feed on gall tissues inside the galls formed by gall wasps belonging to the hymenopteran family Cynipidae (Csóka and Szabóky 2005).

RESUME

Mannai Y., Ezzine O., Hausmann A., et Ben Jamâa M.L. 2018. Premier rapport de trois espèces de Tortricidae sur la forêt de *Quercus suber* dans le nord-ouest de la Tunisie. *Tunisian Journal of Plant Protection* 13 (2): 275-280.

La faune tunisienne des Tortricidae a été mal étudiée malgré la grande importance économique de cette famille. Un échantillonnage d'insectes *Tortricidae* a été effectué au printemps et en été 2010 dans deux forêts de chêne-liège (*Quercus suber*) du nord-ouest de la Tunisie. Trois espèces ont été signalées pour la première fois: *Archips xylosteana*, *Pammene splendidulana*, *Pammene giganteana*. Leurs identifications ont été réalisées moyennant l'ADN barcoding.

Mots clés : *Archips xylosteana*, ADN barcoding, *Pammene splendidulana*, *Pammene giganteana*, *Quercus suber*, Tortricidae, Tunisie

ملخص

مناعي، يسرى وألفة الزين وأكسال هوزمان ومحمد لحبيب بن جامع. 2018. أول تسجيل لثلاث أنواع من الفراشيات الفتالة في غابات بلوط الفلين (*Quercus suber*) في شمال غرب تونس. *Tunisian Journal of Plant Protection* 13 (2): 275-280.

إن الفراشيات الفتالة ليست مدروسة جيداً في تونس رغم أهميتها الاقتصادية الكبيرة. تم جمع عينات في ربيع وصيف 2010 من الفراشيات الفتالة في غابات بلوط الفلين (*Quercus suber*) في شمال غرب تونس. سجلت ثلاثة أنواع لأول مرة:

Pammene giganteana و *Pammene splendidulana* و *Archips xylosteana*. تم تشخيص أنواع الحشرات باعتماد ترميز الخطوط للحمض النووي ADN.

كلمات مفتاحية: ترميز الخطوط للحمض النووي ADN، تونس، فراشيات قتالة، *Pammene*، *Archips xylosteana*، *Quercus suber*، *Pammene giganteana*، *splendidulana*

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