

EAPR 2017  
20<sup>th</sup> TRIENNIAL CONFERENCE  
Versailles • 9-14 july 2017 

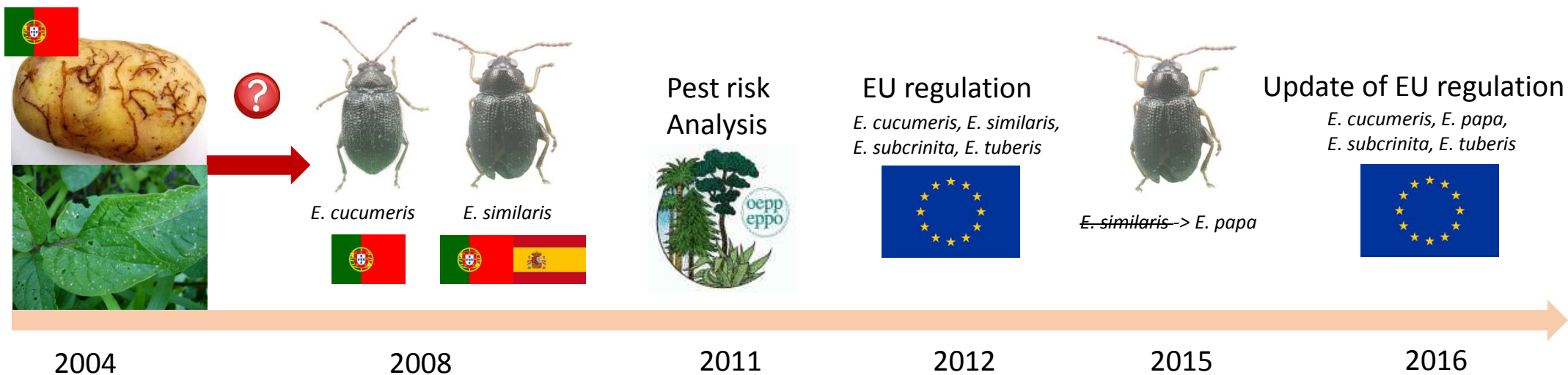
# Who's who among *Epitrix* potato flea beetles ?

R. Mouttet, J-F. Germain (Anses – Laboratoire de la Santé des Végétaux)



# Introduction

The genus *Epitrix*, a group of quarantine significance in Europe



EU emergency measures concern:

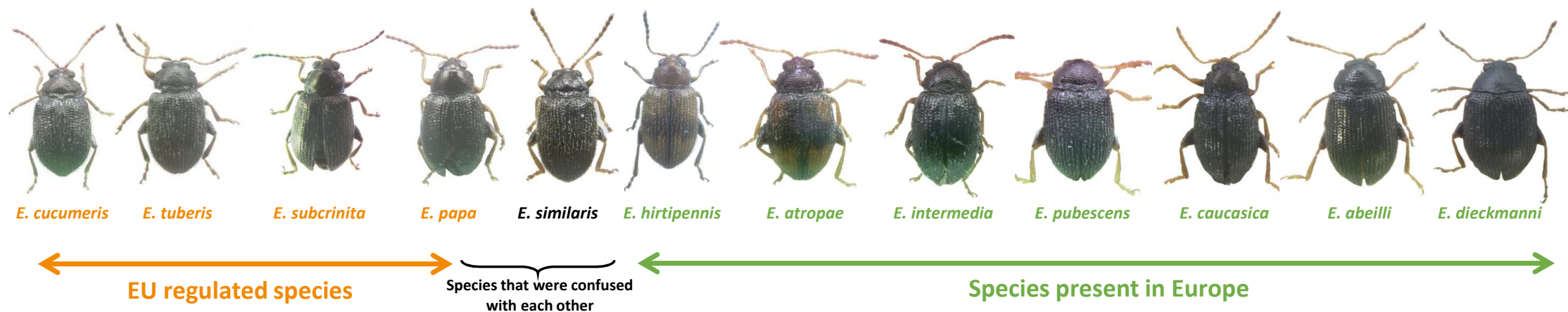
introduction of potato tubers into the Union / movement of potato tubers within the Union / surveys and notifications



# Introduction

The genus *Epitrix*, a diverse and morphologically similar group

Over 160 species worldwide, tiny pubescent beetles (1,5 – 2mm), feeding on Solanaceae





## Introduction

### Why knowing who's who is important?

the scientific name is the only universal way to refer to a species

specific identification is the first step in invasive species management



the scientific name gives access to data on biology, control...

specific identification is required for quarantine pests

-> Participation to an  **Euphresco** project to develop morphological and molecular detection methods for European *Epitrix* species and North American species developing on *Solanum tuberosum*



# Material and methods

## Sample collection

- Looking for specimens of the species of interest



- A collaborative work



- The case of *Epitrix similaris*

Only known from California

In 2015, collection of specimens from the type locality (Santa Barbara)

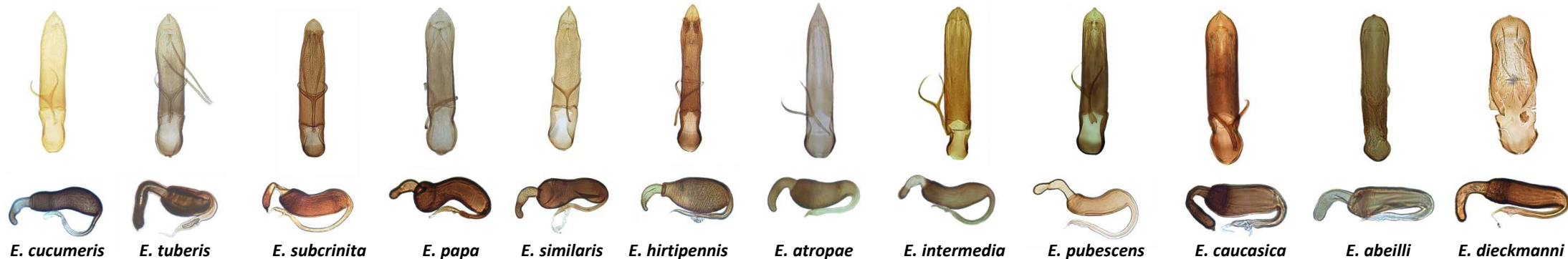
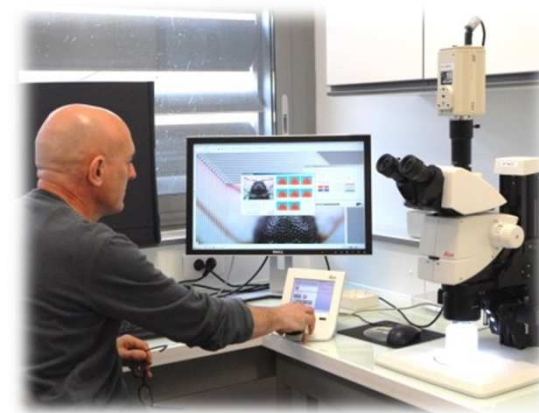
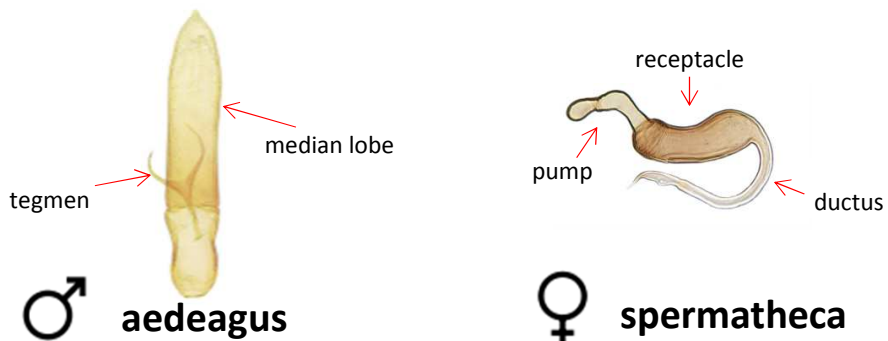




# Material and methods

## Morphological studies

- Important diagnostic features: morphology of male and female genitalia:

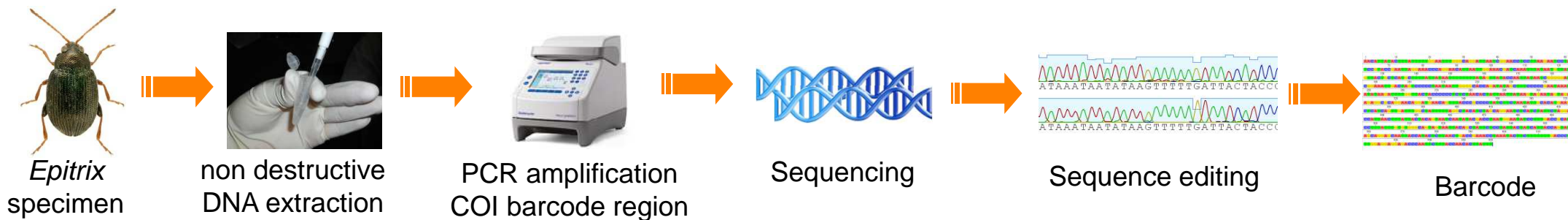




# Material and methods

## Molecular studies

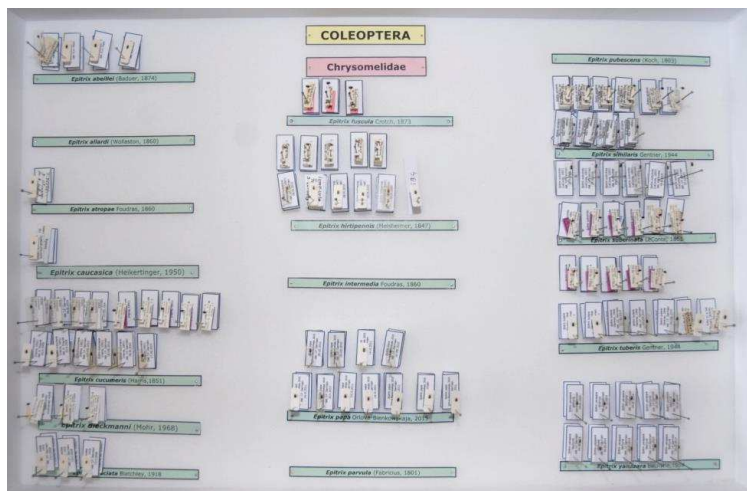
- Barcoding approach  
Use of a standardized genetic marker to aid species identification  
Identification by comparison with reference barcodes deposited in databases
- Need to produce reference barcodes when they do not exist
- A standardized protocol





# Results

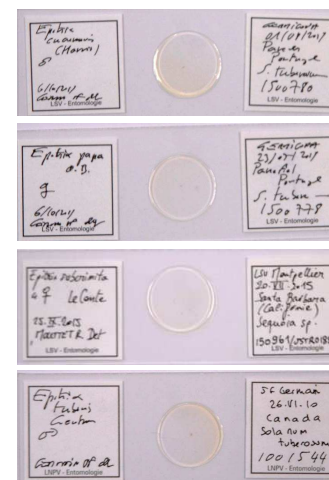
Sample collection -> A reference collection



pinned specimens



ethanol preserved specimens



slides with genitalia



DNA extracts

A collection of reference specimens, kept over the long term, available upon request for EU National Plant Protection Organizations (NPPOs)





# Results

## Morphological studies -> An identification key

Bulletin OEPP/EPPPO Bulletin (2017) 47 (1), 10-17

ISSN 0250-8052. DOI: 10.1111/epp.12382

European and Mediterranean Plant Protection Organization  
Organisation Européenne et Méditerranéenne pour la Protection des Plantes

PM 7/109 (2)

### Diagnosics Diagnostic

### PM 7/109 (2) *Epirix cucumeris*, *Epirix papa*, *Epirix subcrinita*, *Epirix tuberos*

#### Specific scope

This Standard describes a diagnostic protocol for adults of *Epirix cucumeris*, *Epirix papa*, *Epirix subcrinita* and *Epirix tuberos*.

#### Specific approval and amendment

First approved in 2011-09.  
Revision approved in 2016-01.

### 1. Introduction

*Epirix subcrinita*, *Epirix tuberos* and *Epirix cucumeris* are flea beetles belonging to the North American species of *Epirix* (developing on *Solanum tuberosum* (Solanaceae), *Epirix cucumeris* is established in the Azores Islands (Portugal) where it was collected for the first time in Faial Island around 1979 (Boavida & Germain, 2009). In mainland Portugal, *E. cucumeris* was detected with another species new to science, *Epirix papa*, in approximately 2004 when unusual damage to potato crops was observed (Oliveira *et al.*, 2008). *Epirix papa* was initially misidentified as *Epirix similaris*, another North American species (Orlova-Bienkowskaja, 2015). Both species have also been detected in Spain. To date, *E. tuberos* and *E. subcrinita* have not been detected in the EPPO region.

Nine species of *Epirix* were recorded as present in the EPPO region before these detections (Warchalowski, 2003), these are *Epirix abeillei*, *Epirix allardi*, *Epirix atropae*, *Epirix caucasica*, *Epirix dieckmanni*, *Epirix hirtipennis*, *Epirix intermedia*, *Epirix priesteri* and *Epirix pubescens*. Until recently, among the seven *Epirix* species present in Europe (Warchalowski, 2003) none was causing damage on *S. tuberosum*, although *E. hirtipennis*, known as a pest of tobacco in Italy (Santino *et al.*, 1984), is occasionally recorded on potato. This species also has a North American origin. Two other species which are present in the non-European part of the EPPO region, *Epirix allardi* and *Epirix priesteri*, are not covered in this diagnostic protocol.

Further information on the host range, geographical distribution and biology of the four species recommended for regulation can be found in the EPPO Global Database

(<https://gd.eppo.int/>). This protocol focuses on detection of *Epirix* species in potato.

### 2. Identity

**Name:** *Epirix cucumeris* (Harris)  
**Taxonomic position:** Coleoptera: Chrysomelidae: Alticinae  
**EPPO code:** EPXUCU  
**Phytosanitary categorization:** EPPO A2, EU Commission decision 2016/1359/EU

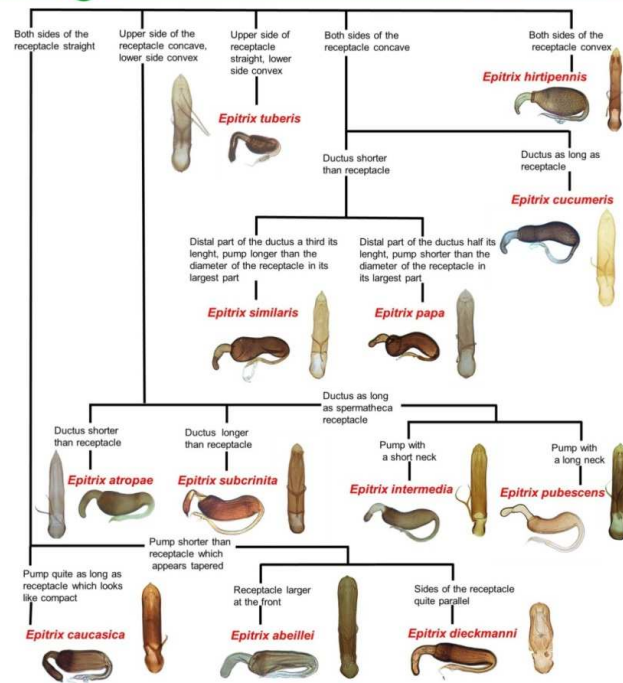
**Name:** *Epirix papa* Orlova-Bienkowskaja  
**Taxonomic position:** Coleoptera: Chrysomelidae: Alticinae  
**EPPO code:** EPXPAP  
**Phytosanitary categorization:** EPPO A2, EU Commission decision 2016/1359/EU

**Name:** *Epirix subcrinita* (LeConte)  
**Taxonomic position:** Coleoptera: Chrysomelidae: Alticinae  
**EPPO code:** EPXSSU  
**Phytosanitary categorization:** EPPO A1, EU Commission decision 2016/1359/EU

**Name:** *Epirix tuberos* Gentner  
**Taxonomic position:** Coleoptera: Chrysomelidae: Alticinae  
**EPPO code:** EPXTTU  
**Phytosanitary categorization:** EPPO A1, EU Commission decision 2016/1359/EU

### 3. Detection

**Symptoms on potato**  
Adults of *Epirix* species may be found on all above-ground parts of the plant as well as on the soil surface.



A reliable diagnostic needs the observation of the female spermatheca associated with the male aedeagus as shown in this key

anses.fr

## A published identification key to allow specific identification of *Epirix* species in the European context





# Results

## Molecular studies -> A DNA barcode library

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### Molecular identification of *Epitrix* potato flea beetles (Coleoptera: Chrysomelidae) in Europe and North America

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#### Abstract

*Epitrix* species (Coleoptera: Chrysomelidae) feed mostly on plants from the family Solanaceae and some of them are major pests of potato crops. All *Epitrix* species are morphologically highly similar, which makes them difficult to identify and limits their study and management. Identification of species is mostly based on the observation of the genitalia and requires a high level of expertise. Here, we propose a tool to reliably identify all developmental stages of the most economically important *Epitrix* species feeding on potato in Europe and North America (*Epitrix cucumeris*, *Epitrix similis*, *Epitrix tuberosa*, *Epitrix suberinata* and *Epitrix hirtipennis*). We first sequenced two DNA markers (mitochondrial cytochrome c oxidase I (COI) and nuclear internal transcribed spacer 2 (ITS2)) to test their effectiveness in differentiating among six *Epitrix* species (126 specimens). Morphospecies of *Epitrix* were well-differentiated by both DNA barcodes and no mitochondrial introgression was detected. Then, we developed an RFLP-based diagnostic method and showed that unambiguous species discrimination can be achieved by using the sole restriction enzyme *TaqI* on COI polymerase chain reaction products. The tool proposed here should improve our knowledge about *Epitrix* species biology, distribution and host range, three capacities that are particularly important in the detection and management of these pest species. Specifically, this tool should help prevent the introduction of *E. tuberosa* and *E. suberinata* in Europe and limit the spread of the recently introduced *E. cucumeris* and *E. similis*, with minimal disruption to Solanaceae trade.

**Keywords:** Alticinae, barcoding, M13-tailed primer cocktails, PCR-RFLP, QBOL, solanaceae

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COMPREHENSIVE DATABASES ON QUARANTINE PLANT PESTS AND DISEASES

Home Organisms included Methodology General Search ID References Credits Help

Pairwise sequence alignment

I have read the disclaimer and I agree with the conditions and limitations associated with the usage of the software

Pairwise sequence alignment parameters

Paste sequence to align:

```
AACTTATATTTTATTTGGGATCTGAGCAGGATTAATCGGGACCTCTTAAGAATTAATCCGTAAGTGGGGACCCCGCTCATAATGGTAATGATC
AAATTTATATGTTGTAAGTCCGCTGCTTTCATTATAATTTTATAGTAATACCGATTATAATGGGGATTGGTAAGTACTAGTCCCTCATAATCG
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GGAGCTATAATTTTACTTACTATTATAATATACACCATGGCATATCTATAGATGTACCCCTTATTGTGTGACTGTAATAATTAATCAATCTATTA
```

Start alignment

Draw Tree Expand Alignments Export data

Pairwise Results		Clustering results						
#	Reference description	Score	Probability	Similarity%	Fragments	Overlap%	Direction	Rating
1	CCOC1253_0103 CCOC1253_0103_COI Epitrix papa, nlini892: COI Arthropods	1044.49	0	100	1	100	+/+	*****
2	CCOC1252_0101 CCOC1252_0101_COI Epitrix papa, nlini892: COI Arthropods	1044.49	0	100	1	100	+/+	*****
3	CCOC1248_0101 CCOC1248_0101_COI Epitrix papa, nlini892: COI	1044.49	0	100	1	100	+/+	*****

A barcode library available online ([www.q-bank.eu](http://www.q-bank.eu)) to detect *Epitrix* quarantine species



# Conclusions

## Highlights and perspectives

*Epitrix* flea beetles in Europe, an interesting case study showing that:

- species identification is the first step in invasive pest management
- taxonomic expertise is needed to ensure that pests are adequately identified
- the development of accurate diagnostic tools is helpful for surveillance surveys

A study of a limited scope since most *Epitrix* species occur in the Neotropics:

- little is known about South American *Epitrix* species
- some of them are potato pests
- would we be able to detect one of them if it were introduced in Europe ?

-> towards a second  **Euphresco** project...



*Epitrix yanazara*



**Thanks for your attention**

