Quick identification of commonly intercepted Tephritidae in Europe:

How does molecular identification help the morphology?

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Introduction

Before

Entomological identification = morphological methods

but no keys or incomplet keys => it's a challenge

Now !

Molecular analysis provides a complementary approach

1 – Statute of Tephritid

- Family of Diptera named « Fruit fly »
- All species phytophagous
- Important worldwide pest

⇒ **All non-European Tephritid on the EU quarantine list Annex IA1 and IA2**

2 – Biology

Bactrocera sp.

ovipositor

Adult female

Egg

Larva
3 instars
named L1, L2 and L3

Pupa

3 – Detection and usual species found in import

Host plants *:*

Mangifera indica
Annona
Psidium guajava
Syzydium
Ziziphus
Capsicum
Momordica
Citrus …

- Genus : *Anastrepha sp.*
- Species : *Bactrocera dorsalis*, *Bactrocera correcta*, *Bactrocera cucurbitae*, *Bactrocera latifrons*, *Bactrocera zonata*, *Ceratitis capitata*, *Ceratitis cosyra*, *Ceratitis rosa*, *Dacus ciliatus*

10 / 2.500 wordwide species of Tephritidae

4 – Distortion of sampling and rearing

A - Larvae ⟶ **rearing** ⟶ **adults**

- failure of emergence
- duration

B - 2 Larvae ⟶ **1 for morphology**

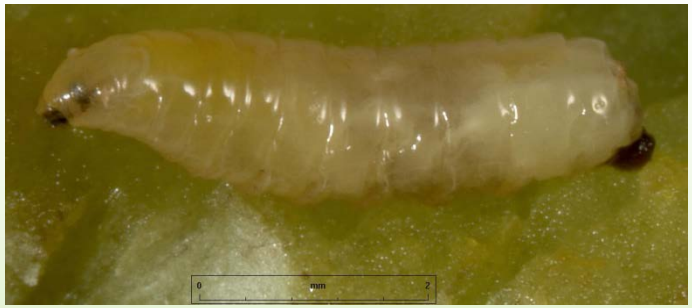
B - 2 Larvae ⟶ **1 for molecular**

If two or more species?

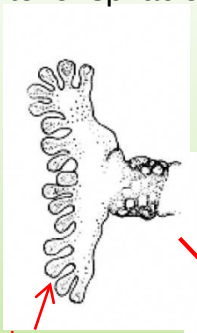
C - 1 larva ⟶ **½ for morphology**

C - 1 larva ⟶ **½ for molecular**

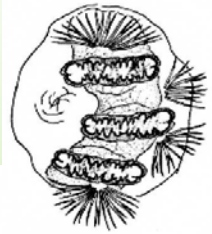
5 – Morphology of larvae



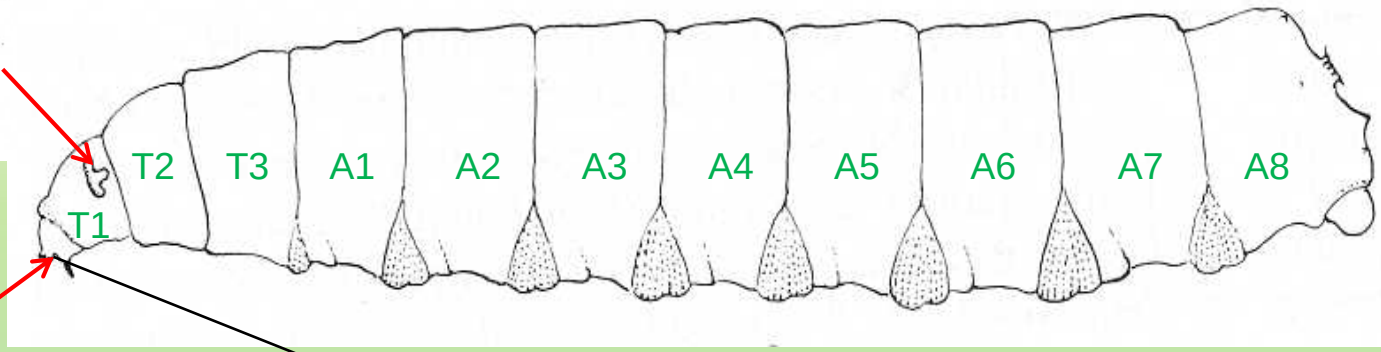
anterior spiracle



tubules

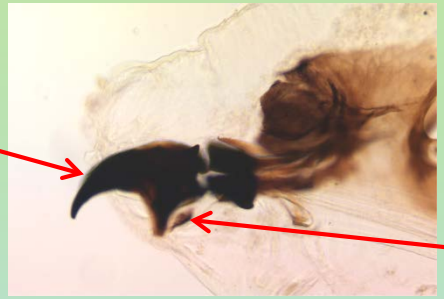


posterior spiracle



Head

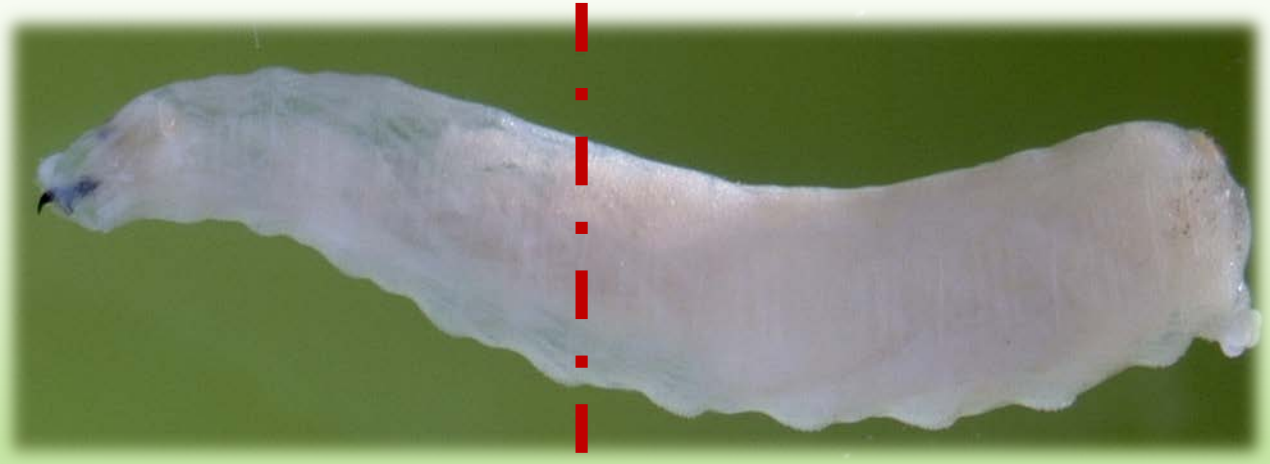
mouthhook

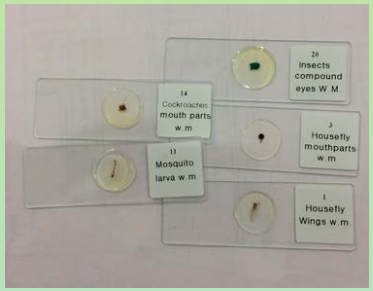


dental sclerite

Part of cephalopharyngeal skeleton

6 – Preparing and mounting larva for analysis

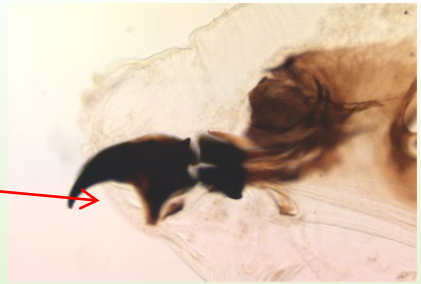


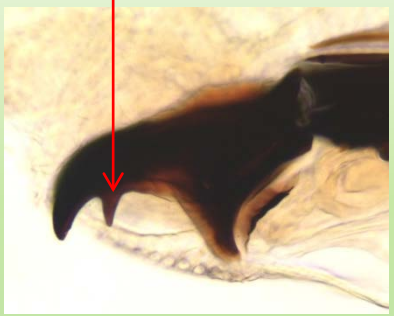


7 – Morphological analysis

Mouth hook

presence or absence of preapical tooth





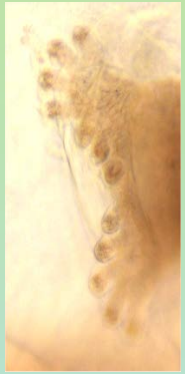
size of this tooth

Anterior spiracle

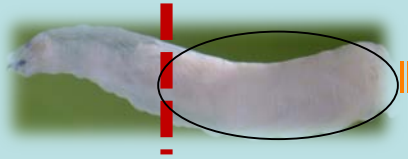
shape

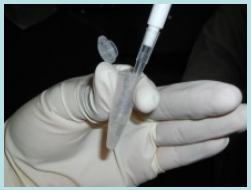
+

number of tubules



8 – Molecular analysis

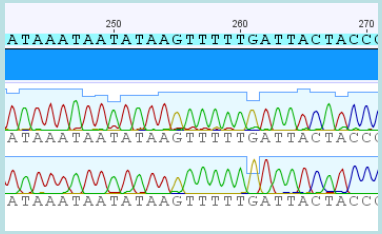




DNA extraction

PCR amplification COI

Sanger sequencing



Sequence editing

Blast

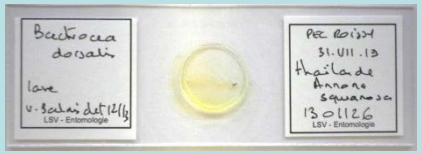
Bactrocera dorsalis
Ceratitis capitata
Ceratitis cosyra
Ceratitis rosa
Bactrocera correcta
Bactrocera cucurbitae
Bactrocera latifrons
Bactrocera zonata
Dacus ciliatus
…

Molecular ID

Workflow for molecular identification

9 – Results

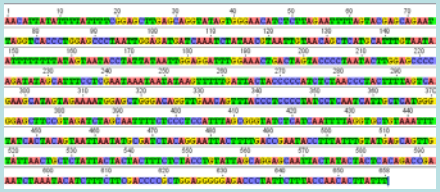
- 180 specimen analysed so far
- For each specimen, we obtain:



1 Slide

1 Morphological ID

&



1 COI sequence

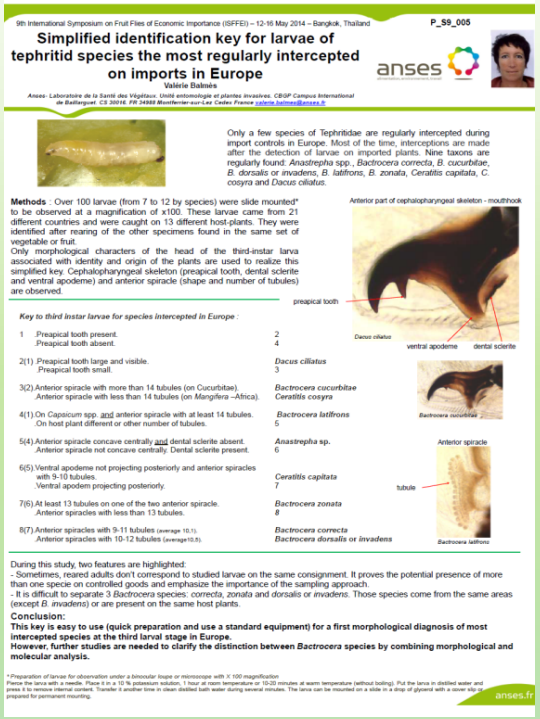
1 Molecular ID

- Possibility to assess performance criteria of the key by comparing the morpholocial ID with the molecular ID
- Work under way…

10 - Presentation of the key

Key to third instar larvae for species intercepted in Europe *:*

1	.Preapical tooth present.	2
	.Preapical tooth absent.	4
2(1)	.Preapical tooth large and visible.	***Dacus ciliatus***
	.Preapical tooth small.	3
3(2).	Anterior spiracle with more than 14 tubules (on Cucurbitae).	***Bactrocera cucurbitae***
	.Anterior spiracle with less than 14 tubules (on *Mangifera* –Africa).	***Ceratitis cosyra*** ***or Ceratitis rosa***
4(1).	On *Capsicum* spp. and anterior spiracle with at least 14 tubules.	***Bactrocera latifrons***
	.On host plant different or other number of tubules.	5
5(4).	Anterior spiracle concave centrally and dental sclerite absent.	***Anastrepha* sp.**
	.Anterior spiracle not concave centrally. Dental sclerite present.	6
6(5).	Ventral apodeme not projecting posteriorly and anterior spiracles with 9-10 tubules.	***Ceratitis capitata***
	.Ventral apodem projecting posteriorly.	7
7(6).	At least 13 tubules on one of the two anterior spiracle.	***Bactrocera zonata***
	.Anterior spiracles with less than 13 tubules.	***8***
8(7).	Anterior spiracles with 9-11 tubules (average 10,1).	***Bactrocera correcta***
	.Anterior spiracles with 10-12 tubules (average10,5).	***Bactrocera dorsalis* or *invadens***


9th International Symposium on Fruit Flies of Economic Importance (ISFFEI) – 12-16 May 2014 – Bangkok, Thailand

P_S9_005

Simplified identification key for larvae of tephritid species the most regularly intercepted on imports in Europe

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Only a few species of Tephritidae are regularly intercepted during import controls in Europe. Most of the time, interceptions are made after the detection of larvae on imported plants. Nine taxons are regularly found: *Anastrepha* spp., *Bactrocera correcta*, *B. cucurbitae*, *B. dorsalis* or *invadens*, *B. latifrons*, *B. zonata*, *Ceratitis capitata*, *C. cosyra* and *Dacus ciliatus*.

Methods : Over 100 larvae (from 7 to 12 by species) were slide mounted* to be observed at a magnification of x100. These larvae came from 21 different countries and were caught on 13 different host-plants. They were identified after rearing of the other specimens found in the same set of vegetable or fruit.
Only morphological characters of the head of the third-instar larva associated with identity and origin of the plants are used to realize this simplified key. Cephalopharyngeal skeleton (preapical tooth, dental sclerite and ventral apodeme) and anterior spiracle (shape and number of tubules) are observed.

Anterior part of cephalopharyngeal skeleton - mouthhook

preapical tooth

Dacus ciliatus

ventral apodeme

dental sclerite

Bactrocera cucurbitae

Anterior spiracle

tubule

Bactrocera latifrons

Key to third instar larvae for species intercepted in Europe *:*

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During this study, two features are highlighted:
- Sometimes, reared adults don't correspond to studied larvae on the same consignment. It proves the potential presence of more than one specie on controlled goods and emphasize the importance of the sampling approach.
- It is difficult to separate 3 *Bactrocera* species: *correcta*, *zonata* and *dorsalis* or *invadens*. Those species come from the same areas (except *B. invadens*) or are present on the same host plants.

Conclusion:
This key is easy to use (quick preparation and use a standard equipment) for a first morphological diagnosis of most intercepted species at the third larval stage in Europe.
However, further studies are needed to clarify the distinction between *Bactrocera* species by combining morphological and molecular analysis.

* *Preparation of larvae for observation under a binocular loupe or microscope with X 100 magnification*
Pierce the larva with a needle. Place it in a 10 % potassium solution, 1 hour at room temperature or 10-20 minutes at warm temperature (without boiling). Put the larva in distilled water and press it to remove internal content. Transfer it another time in clean distilled bath water during several minutes. The larva can be mounted on a slide in a drop of glycerol with a cover slip or prepared for permanent mounting.

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Important : knowing pathway of import and pests situation

11 – Strengths and limitations of the 2 methods :

Morphological :

+ :
- low cost
- rapidity
- few materials
- + or – « fresh » larva
- « field » use

- :
- only with L3
- poor reliability/ some species
- invalid / new species
- invalid /morphological anomalies
- knowledges in entomology

Molecular :

+ :
- all instar
- high reliability
- valid / new species
- valid / non-normal form
- no specific knowledges

- :
- high cost
- duration
- expensive materials
- need « good » DNA
- requiers specific laboratory

12 – Use and other applications

- Imported control or control in focus area
- IPM Integrate pest management

Application to other larvae of invasive or reguled pests :

ex : Diptera, Drosophilidae (*Zaprionus indianus/Drosophila suzukii etc…)*

ex: Lepidoptera, Tortricidae (*Thaumatotibia leucotreta/other Tortricidae)*

Thank you