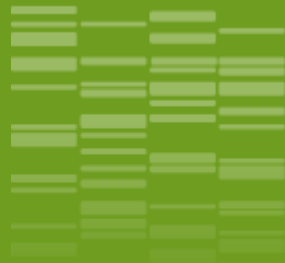




A barcode database to identify the vectors of *Xylella fastidiosa* in Europe.



By Streito Jean-Claude, Pierre Eric, Genson Guénaëlle,
Bellifa Maxime, Chartois Marguerite, †Germain Jean-François,
Astrid Cruaud & Jean-Yves Rasplus.



_01

Introduction

Why DNA barcoding of vectors of *Xf* is important?

Why barcoding?

1) Potential vectors of *Xf* are diversified

4 Hemiptera families, 120 spp. for Europe

Aphrophoridae (29 spp.)

Cercopidae (7 spp.)

Cicadellidae (9 spp.)

Cicadidae (79 spp.)

For an overview of *Xf* vectors I invite you to go and see the poster P58 by Wilson et al.



Why barcoding?

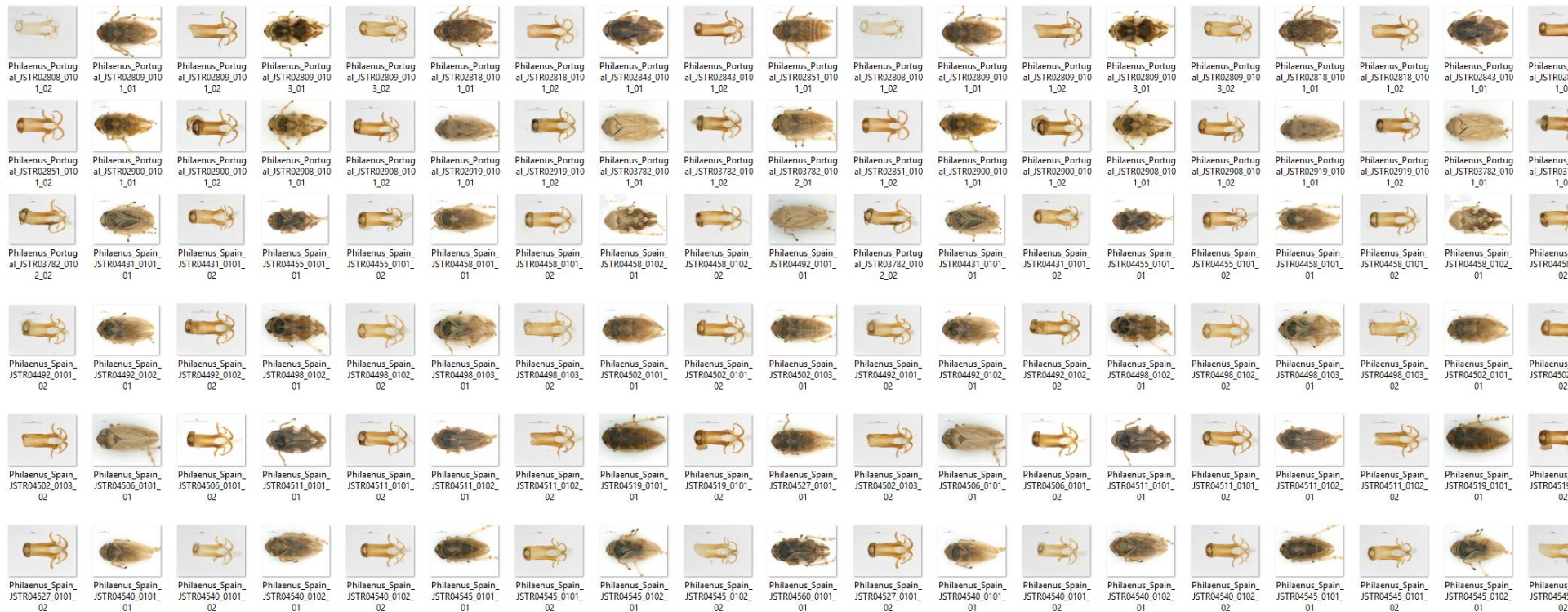
2) Main vectors must be dissected to be identified



It is possible and reliable but time-consuming and not suitable for mass screening of *Xf* in insects



Why barcoding?



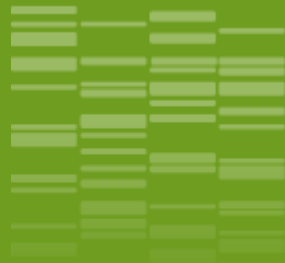
Why barcoding?

- 1) Potential vectors of *Xf* are diversified
- 2) Main vectors must be dissected to be identified
- 3) The taxonomy of *Xf*'s vectors is not 100% satisfactory

Barcoding is known to be adequate to check for taxonomical problems: cryptic species and synonymies

This is why we started to barcode the vectors of *Xf* when the bacterium was detected in Corsica in summer 2015





_02

Materials & Methods

Our main concern is to avoid misidentification and to provide reliable sequences

Barcoding methods: multiple quality controls

Species to be included

Philaenus italosignus
Philaenus lukasi
Philaenus maghresignus
Philaenus signatus
Philaenus spumarius
Philaenus tarifa
Philaenus tessellatus

Sampling conditioning storage



1) Identification by a taxonomist



Databases Web portal



2) Non destructive extraction

PCR amplification
COI

Sequencing



1st quality control: sequences

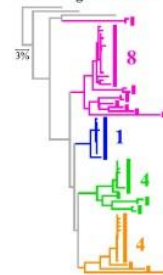
Collections

3) Vouchers



2nd quality control

Barcoding with numts



Phylogenetic analysis

Barcoding methods:

Standard Barcode: COI 658 bp (Herbert *et al.*, 2003)



First: Classical PCR and Sanger sequencing



```
@1_2213_15635_101411_1
AAGTAGAGGATGCTCAGAAGATCAGACAGACCGTGATAGATTGCTTTGAAAAGGCATCCTCTCGTGTCTAAGTGAAGAAGAGAGGAGCCAACTCT
+
EHD<<G1FE71<<DGCHIIGHE<FF<GHHHHIHHIIIGHH<GHHIHHHHIIIIIEGHFF7GCF1GHFFGEGFEGHCHIEHG0FCCGGHIEHH
@1_2213_8494_101069_1
ACCTTCGACATGTCGGCGGCAGCCTGCTTGTGTCTTCGCGACGAGCGCGTGTACCGAGCGGTCTCGGGCATTTCGAGCCAGTAGTAGGTTA
+
HHNEHHHHIIIIIGIIIIHHIIIGIHHIIIGHHIIIGHHIIIIIIIGHHIHDHIIIIHHIIHHHCHHHIIIGIHHIIIDHIIHHIHHIIII
@1_2213_10323_100879_1
ATCTCCGTCCTGGCGGATCAAGTGATCTCGTGACAGCGCGCGGACCATGATCTCGATCAACCCTGTCGAGCCGGCGACTGACAGTCTGAAAT
+
<D1<C@CFH0GH@111CFH7@11<1<D@EHHEH1DHHHHI/CCCHH7EECHFHHHHFF11<EC1FHHI10<<0CH/CEECHH/<1<1<D0<DHH
@1_2213_9533_100933_1
TCCTTGAGAAGCTTAAGGATTTGAGCTTTGTCCAATGGCGGTTTCGGGGTGGGATGCGATCGAAACCATGTGACGAATGCTCGGTGCCACCGCTCT
+
```

Now: two-step PCR and Miseq sequencing

SCIENTIFIC REPORTS

OPEN

High-throughput sequencing of multiple amplicons for barcoding and integrative taxonomy

Perrine Cruaud¹, Jean-Yves Rasplus¹, Lillian Jennifer Rodriguez^{1,2} & Astrid Cruaud¹

Barcoding methods: multiple quality controls

Species to be included

Philaenus italosignus
Philaenus lukasi
Philaenus maghresignus
Philaenus signatus
Philaenus spumarius
Philaenus tarifa
Philaenus tessellatus

Sampling conditioning storage



1) Identification by a taxonomist



Databases Web portal



2) Non destructive extraction



Collections

3) Vouchers

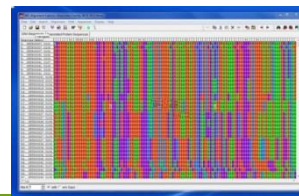
5) Consistency morpho/molecular identification

PCR amplification
COI

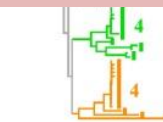
Sequencing



4) Pipeline to check sequences (non-coding, contaminants, numts...)



2nd quality control



Phylogenetic analysis

Photography of all species available

Help for morphological identification

On the CBGP collection platform:

with a Keyence VHX5000
microscope



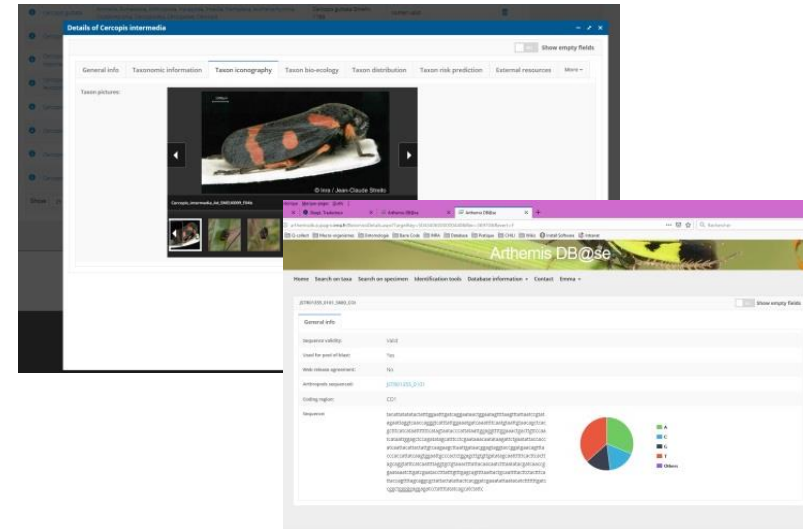
Database : validated sequences and photos

Data are stored in the Database Arthemis

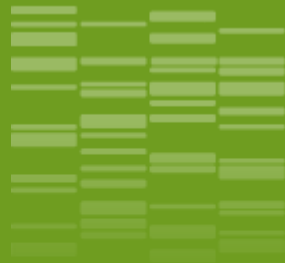
<http://arthemisdb.supagro.inra.fr/DefaultInfo.aspx?Page=Home>

Freely available in Arthemis :

- Pictures
- Sequences validated



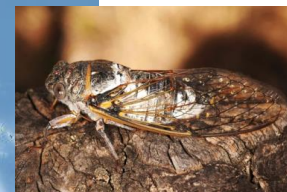
Arthemis DB@se focuses on pests and their natural enemies.
To date it contains 21 169 COI sequences of Arthropods



_03

Results

Sampling



North American vectors

European vectors

Total putative vectors : 22 849

- Aphrophoridae : 11 199
- Cercopidae : 165
- Cicadellidae : 11 364
- Cicadidae : 121

- *Xylella* samples
- QBOL samples

Number of barcodes and pictures

Number of Xf vectors included:

European vectors: **21(+16)** spp. **234 (+151)** COI

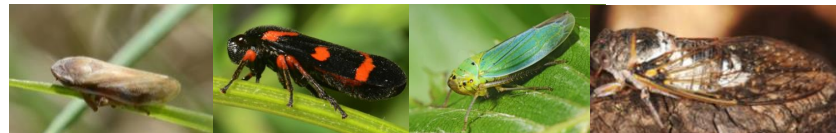
Non European vectors: **23** spp. **173** COI

Total number of spp. **44(+32)** **407(+151)** COI

Number of pictures : **271**
(24 European spp. and 5 non European spp.)



<http://arthemisdb.supagro.inra.fr/DefaultInfo.aspx?Page=Home>



Results:

Philaenus



Espèces / sous-espèces	Pictures	Number of Séquences (in process)	Bold GenBank
<i>Philaenus italosignus</i>	7	4	1
<i>Philaenus loukasi</i>	0	0	1
<i>Philaenus maghresignus</i>	9	1	2
<i>Philaenus signatus</i>	(7)	(7)	1
<i>Philaenus spumarius</i>	32	39 (11)	> 100
<i>Philaenus tarifa</i>	0	0	2
<i>Philaenus tesselatus</i>	11	29 (8)	2
<i>Philaenus arslani</i> (Liban)	0	0	1
<i>Philaenus elbursianus</i> (Iran)	0	0	0
<i>Philaenus iranicus</i> (Iran)	0	0	0

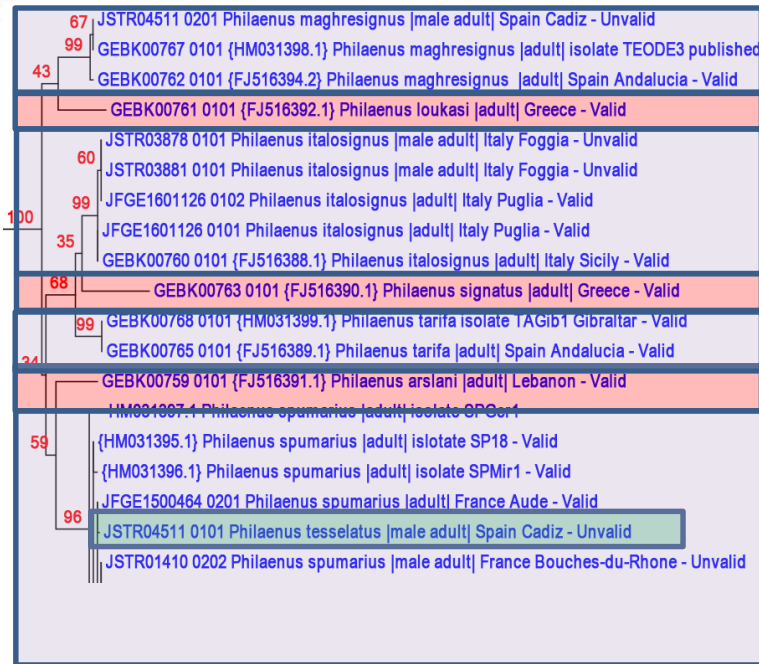
7 spp. in Europe
5 spp. sequenced and imaged

+
few sequences available in international databases

All European spp. sequenced

Results: taxonomic concerns

Philaenus



COI separates all spp.
 studied except
spumarius/tessellatus



Results: taxonomic concerns

Philaenus



Philaenus spp. :

- same variable habitus
- aedeagus diagnostic but difficult between *spumarius*/*tesselatus*



Is *P. tesselatus* a good species ?

For more details I invite you to go and see the poster P60 by Seabra et al. !!



spumarius/*tesselatus*



but



Results

Neophilaenus



Espèces / sous-espèces	Pictures	Number of Séquences (in process)	Bold GenBank
<i>Neophilaenus albipennis</i>	2	(1)	0
<i>Neophilaenus angustipennis</i>	0	0	0
<i>Neophilaenus campestris</i>	9	31 (8)	3
<i>Neophilaenus curvicaudatus</i>	0	(1)	0
<i>Neophilaenus fuscicornis</i>	0	0	0
<i>Neophilaenus glaberrimus</i>	0	0	0
<i>Neophilaenus longiceps</i>	0	0	>100
<i>Neophilaenus minor</i>	2	(2)	0
<i>Neophilaenus modestus</i>	0	0	0
<i>Neophilaenus pallidus</i>	0	0	0

A difficult genus, more barcodes are needed

11 spp. in Europe
2 spp. sequenced
5 imaged
+
Nearly no barcodes in international db

<50% European spp. sequenced

Results

Aphrophora



Espèces / sous-espèces	Pictures	Number of Séquences (in process)	Bold GenBank
<i>Aphrophora alni</i>	7	11 (6)	>100
A lot of taxonomic problems remains before reliable barcode-based identification can be performed			
<i>Aphrophora willemsi</i>	0	0	0

7 spp. in Europe
4 spp. sequenced and imaged

In international db confusion between *alni/paralella* and *salicina/pectoralis*

Results

Lepyronia and other genus



Espèces / sous-espèces	Pictures	Number of Séquences (in process)	Bold GenBank
<i>Lepyronia coleoptrata</i>	15	7 (9)	14
<i>Lepyroniella petrovi</i> (Pologne, Russie...)	0	0	0
<i>Paraphilaenus notatus</i> [F, Ukraine, Russie...]	0	0	0
<i>Peuceptyelus coriaceus</i>	0	0	0



Lepyronia coleoptrata : COI and morpho ok
Other genus are missing

Results

Cercopidae

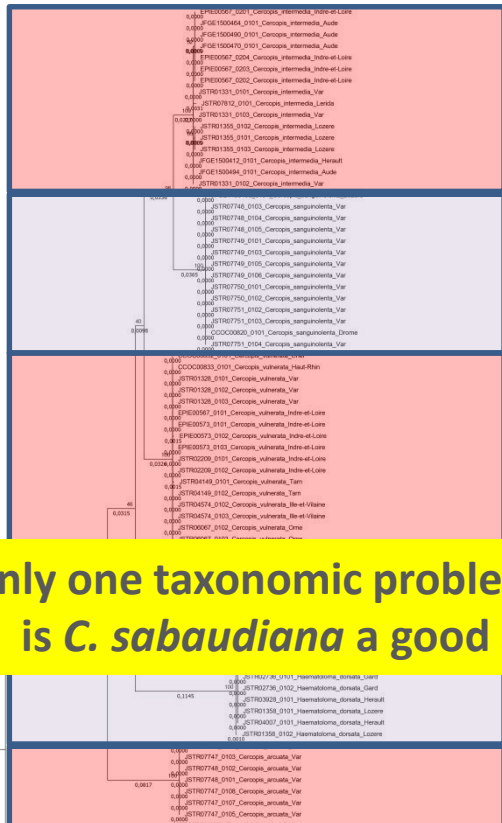


Espèces / sous-espèces	Pictures	Number of Séquences (in process)	Bold GenBank
<i>Cercopis arcuata</i>	(15)	(7)	0
<i>Cercopis intermedia</i>	7	15	0
<i>Cercopis sabaudiana</i>	0	(1?)	0
<i>Cercopis sanguinolenta</i>	7	2(12)	5
<i>Cercopis vulnerata</i>	9	19	8
<i>Haematoloma dorsata</i>	5	15 (3?)	2
<i>Triecphorella geniculata</i>	(10)	0	0

7 spp. in Europe
5 spp. sequenced
and 6 imaged
+
misidentifications in
international db

Results: taxonomic concerns

Cercopidae



C. intermedia

C. sanguinolenta

C. vulnerata

C. arcuata

Only one taxonomic problem remains :
is *C. sabaudiana* a good species ?

COI discriminates
all spp. studied

Sequences of
C. sabaudiana
and *Triecphorella*
are missing

Results

Cicadellidae putative vectors of *Xf*



Espèces / sous-espèces	Pictures	Number of Séquences (in process)	Bold GenBank
<i>Ledra aurita</i>	8	11	0
<i>Cicadella viridis</i>	13	14	81
<i>Cicadella lasiocarpae</i>	0	0	0
<i>Graphocephala fennahi</i>	1	6	21
<i>Evacanthus acuminatus</i>	2	2 (1)	10
<i>Evacanthus interruptus</i>	7	5 (4)	11
<i>Evacanthus rostagnoi</i>	0	0	0
<i>Anoterostemma ivanoffi</i>	0	0	0
<i>Errhomenus brachypterus</i>	(4)	0	0

9 spp. listed for Europe

5 spp. sequenced and 6 imaged

+

G. fennahi/*G. coccinea* confused in international db

Results

Cicadidae



We need more specimens and more sequences but we anticipate taxonomic problem and presume that COI will not be helpful enough to resolve them

Espèces / sous-espèces	Pictures	Number of Sequences (in process)	Bold GenBank
<i>Cicada orni</i>	7	4 (9)	5
<i>Lyristes plebejus</i>	5	(6)	0
<i>Cicadetta fangoana</i>	0	(6)	1
<i>Cicadetta</i> sp.	0	(3)	0
<i>Cicadatra atra</i>	0	(3)	0
<i>Tettigettna argentata</i>	0	(1)	0
<i>Tettigettna pygmaea</i>	0	(2)	0
<i>Tibicina tomentosa</i>	(1)	(4)	0
<i>Tibicina garricola</i>	0	(1)	0
<i>Tibicina haematodes</i>	0	(3)	0
Not identified	0	(8)	0

Cicadidae (1,779 spp. Europe : 1,205 spp. France)
 Aetuanella aestuans (Fabricius 1794)
 Cicada barbara ssp. barbara (Sahl, 1866)
 Cicada barbara ssp. iustitiana Bouard, 1982
 Cicada cretensis Quartau & Simbeni, 2005
 Cicada morio-geminis Bouard, 1979
 Cicada ora Linell, 1762 [C]
 Cicadatra albagos (Kolenati, 1857)
 Cicadatra atra (Olivier, 1790) [F]
 Cicadatra glycyrrhizae (Kolenati, 1857)
 Cicadatra hyalina (F., 1798)
 Cicadatra Carl Simbeni, Samborn & Quartau, 2013
 Cicadatra kargabonensis Simbeni, Samborn & Quartau, 2013
 Cicadatra persica Kirilady, 1909
 Cicadatra platyptera Fieber, 1876
 Cicadetta albigenis Fieber, 1876
 Cicadetta anapaistica ssp. anapaistica Herrsch 2011
 Cicadetta anapaistica ssp. lucana Herrsch 2015
 Cicadetta brevipennis Fieber, 1876 [F]
 Cicadetta cantabrica Guér. & Pussant, 2007 [F]
 Cicadetta caucasica Kolenati, 1857
 Cicadetta confamilienis Pussant & Bouard, 2000 [F]
 Cicadetta concinna ssp. androcopa Gogolia, Trilar & Krpal, 2014
 Cicadetta concinna ssp. concinna Germar, 1821
 Cicadetta dirrica Gogolia, Trilar & Drosopoulos, 2011
 Cicadetta fangoana Bouard, 1976 [C]
 Cicadetta hageni Fieber, 1872
 Cicadetta hanneseae Gogolia, Drosopoulos & Trilar, 2008
 Cicadetta oliginea Emejevsky, 1916
 Cicadetta hispani Gogolia, Drosopoulos & Trilar, 2009
 Cicadetta macedoniana Scholtz, 1999
 Cicadetta mediterranea Fieber, 1876
 Cicadetta montana (Scopoli, 1772) [F]
 Cicadetta olympica Gogolia, Drosopoulos & Trilar, 2009
 Cicadetta salinae Herrsch 2015
 Cicadivetta carayoni Bouard, 1982
 Cicadivetta flavida (Brullé, 1832)
 Cicadivetta graenicheria Gogolia, Drosopoulos & Trilar, 2012
 Cicadivetta tibialis (Panzer 1798) [F]
 Dimisualta dimisua (Hagen, 1856)
 Eubonina caranovagii Gogolia, Trilar & Drosopoulos, 2011
 Euryphara contentei Bouard, 1982
 Euryphara dubia (Rambur, 1840)
 Euryphara undulata (Waltl, 1837)
 Hilaphura varipes (Waltl 1837)
 Lyristes gemellus Bouard 1988
 Lyristes pliebei (Scopoli, 1763) [F]
 Melampalpa lobulata Fieber, 1876
 Melampalpa musiva (Germar, 1830)
 Pappophora annulata (Brullé, 1832)
 Pappophora aschei Karnal, 1978
 Palmocharias querula (Pallas, 1773)
 Pseudotettigetta melanophrys ssp. heuani (Bouard, 2000)
 Pseudotettigetta melanophrys ssp. melanophrys (Horváth, 1907)
 Saticula coriaria Sahl, 1866
 Tettigetta grassini (Pallas, 1773)
 Tettigettna baenai (Bouard, 2000)
 Tettigettna aneabi (Bouard, 2000)
 Tettigettna argentea (Olivier 1790) [F]
 Tettigettna armandi Pussant, 2010
 Tettigettna bouardi Pussant, 2010
 Tettigettna dehaui Pussant, 2010
 Tettigettna estrellae (Bouard, 1982)
 Tettigettna hellantheni ssp. galantei Pussant, 2010
 Tettigettna hellantheni ssp. hellantheni (Rambur, 1840)
 Tettigettna josi Bouard, 1982
 Tettigettna mariae (Quartau & Bouard, 1995)
 Tettigettna pygmaea (Olivier 1790) [F]
 Tibicina cisticola (Hagen, 1855) [C]
 Tibicina corsica ssp. corsica (Rambur, 1840) [C]
 Tibicina corsica ssp. balearica Bouard, 1980 [F]
 Tibicina garricola Bouard, 1983 [F]
 Tibicina haematodes ssp. haematodes (Scopoli, 1763) [F]
 Tibicina haematodes ssp. helveta (Germar, 1821)
 Tibicina nigronervis Fieber, 1876 [C]
 Tibicina picta (Fabricius, 1794) [F]
 Tibicina quadripunctata (Hagen, 1855) [F]
 Tibicina saxeii (Krynitz, 1837) [F]
 Tympanistalna distincta (Rambur, 1840)
 Tympanistalna gastrica (Sahl, 1864)

Conclusion

Our database contains:

- european vectors of *Xf* (21 spp. and 234 barcodes)
(+ 16 spp. and 151 barcodes in progress)
- non european vectors (23 spp. and 173 barcodes)

That is 45% of european listed species (excluded Cicadids)
(if the ongoing sequences are valid we will reach 58%)



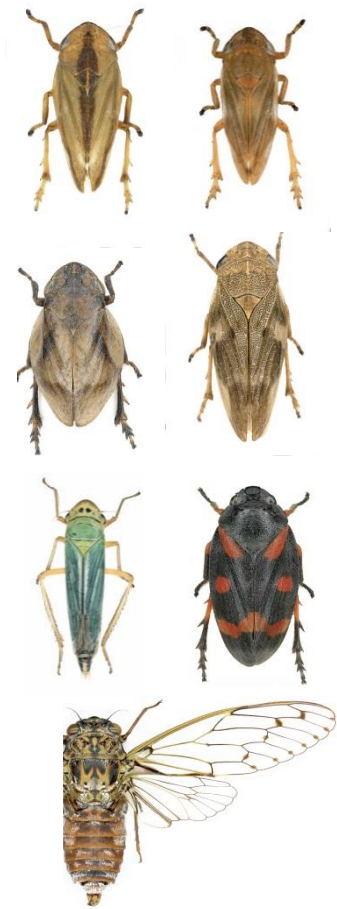
Conclusion

Standard barcode (COI) allows a reliable identification of about 80% of the species studied

All important vector species for cropping systems are sequenced.
(The remaining species are rare or localized less prevalent in crops. They are important for taxonomic issues but not in terms of disease epidemiology).

COI is a good marker and can be used for mass identification
(considering that species abundant in agrosystems were validated).

Some taxonomic problems remain
(spp. cannot be identified neither morphologically nor by barcoding).



Conclusion

The main problem of taxonomy: is *P. tessellatus* a different species from *P. spumarius*?

Question is very important because it concerns the main European vector and a large geographical area where the risk Xf is highest.

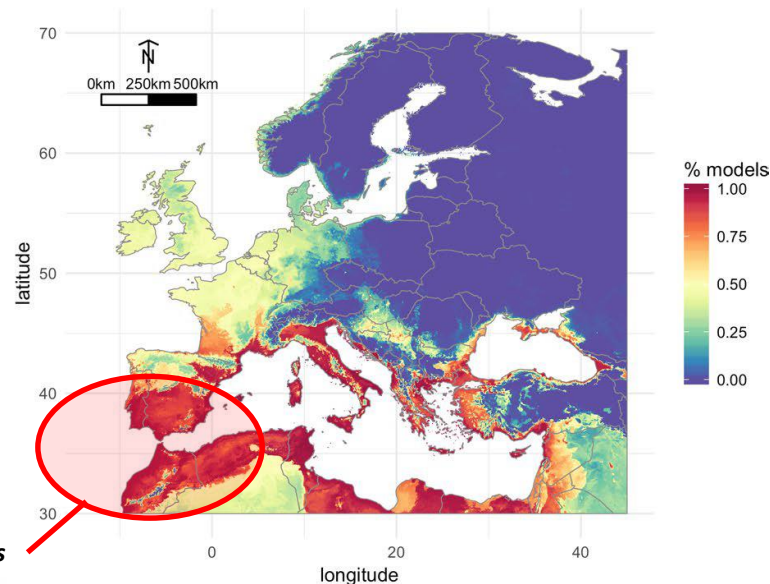
An other important question: are Cicadids vectors of Xf ?

They are abundant and feed on olive tree and other crops

Philaenus tessellatus



A *Xylella fastidiosa fastidiosa*



Godefroid et al., 2019. *Xylella fastidiosa*: climate suitability of european continent. Scientific Reports 9(1) 8844 : 1-10. <https://doi.org/10.1038/s41598-019-45365>



Thank you for your attention

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INRA, XF-actors, DGAL and
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