Update on the genetic characterization of West Coast populations of *Halyomorpha halys* and adventive populations of its egg parasitoid *Trissolcus japonicus*

Marie Claude BON and Vincent LESIEUR

Corresponding author: mcbon@ars-ebcl.org

Brown Marmorated Stink Bug (BMSB) BioControl Identification Workshop

BMSB Integrated Pest Management (IPM) Working Group Meeting

Update on the genetic characterization of West Coast populations of *Halyomorpha halys* and adventive populations of its egg parasitoid *Trissolcus japonicus*

Active collaboration

- Elijah Talamas
- Matt Buffington
- Kim Hoelmer
- Christine Dieckhoff
- Kathleen Tatman

AND many others…

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- Phylogeography of *Halyomorpha halis* in the U.S.
- Genetic diversity of *Trissolcus japonicus*
- Phylogeny of the Asian *Trissolcus*

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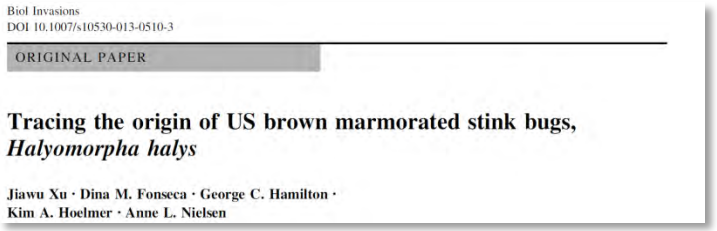
Phylogeography of BMSB in Eastern America was already depicted by Xu et al. (2013)



Biol Invasions
DOI 10.1007/s10530-013-0510-3

ORIGINAL PAPER

Tracing the origin of US brown marmorated stink bugs, *Halyomorpha halys*

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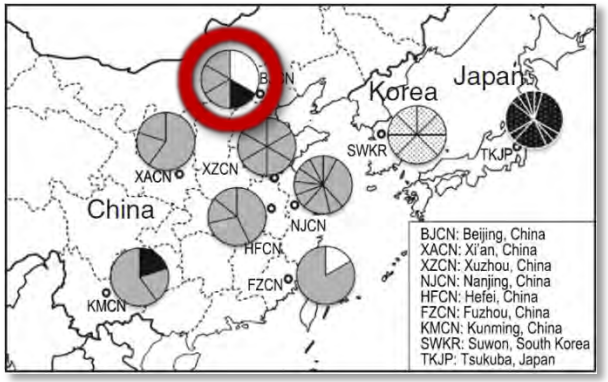

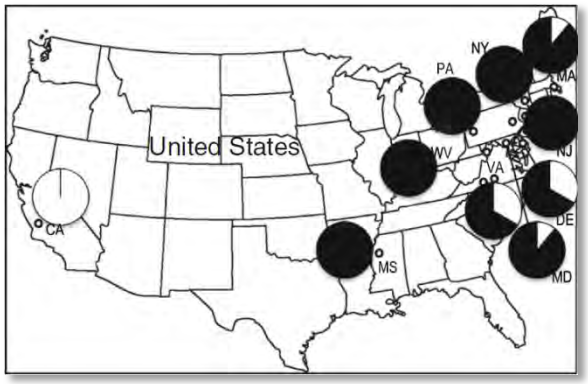

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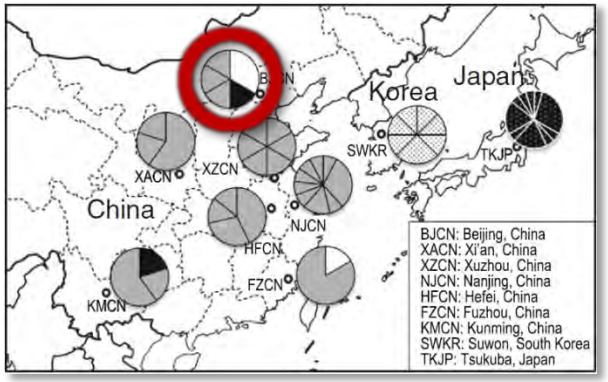
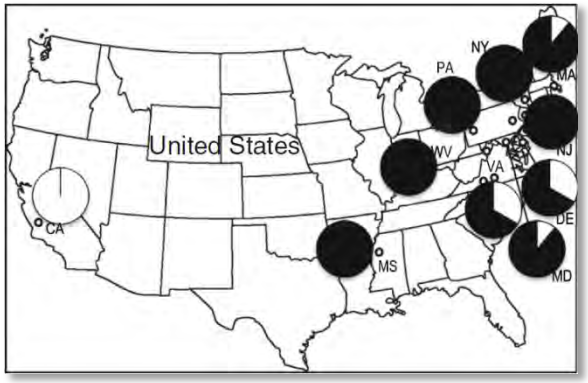
J Pest Sci (2014) 87:17–28
DOI 10.1007/s10340-013-0529-3

ORIGINAL PAPER

Occurrence, genetic diversity, and potential pathways of entry of *Halyomorpha halys* in newly invaded areas of Canada and Switzerland

T. D. Gariepy · T. Haye · H. Fraser · J. Zhang





- A single introduction of small propagules size matches the invasion history in Eastern US
- Bejing area in China being the likely source of introduction
- Similar scenario Gariepy et al (2014)

➞ US populations acted as bridgeheads for Canadian populations

What about Western US?

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Phylogeographic approach

Same methods as Xu et al. (2013)

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3 mtDNA markers

- **COII**
- **12S rRNA**
- **12S CR**

What about Western US?

Phylogeographic approach

Same methods as Xu et al. (2013)

3 mtDNA markers

- COII
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Sampling

- 119 specimens sampled
- 18 sampling locations
  - ➞ Oregon, Washington and California

Genetic diversity measures of BMSB

Geographic area	*n*	Number of haplotypes	Unique haplotype	Haplotype diversity	Nucleotide diversity
Western US (CA, OR, WA)	119	6	1	0.72	0.00168
Eastern US	55	2	0	0 - 0.67	0 – 0.0006
China	18	26 (2-5 per location)	24	0.81-1	0.0011- 0.0038
Korea (1 site)	8	7	7	0.96	0.0023
Japan (1 site)	21	10	10	0.87	0.0027

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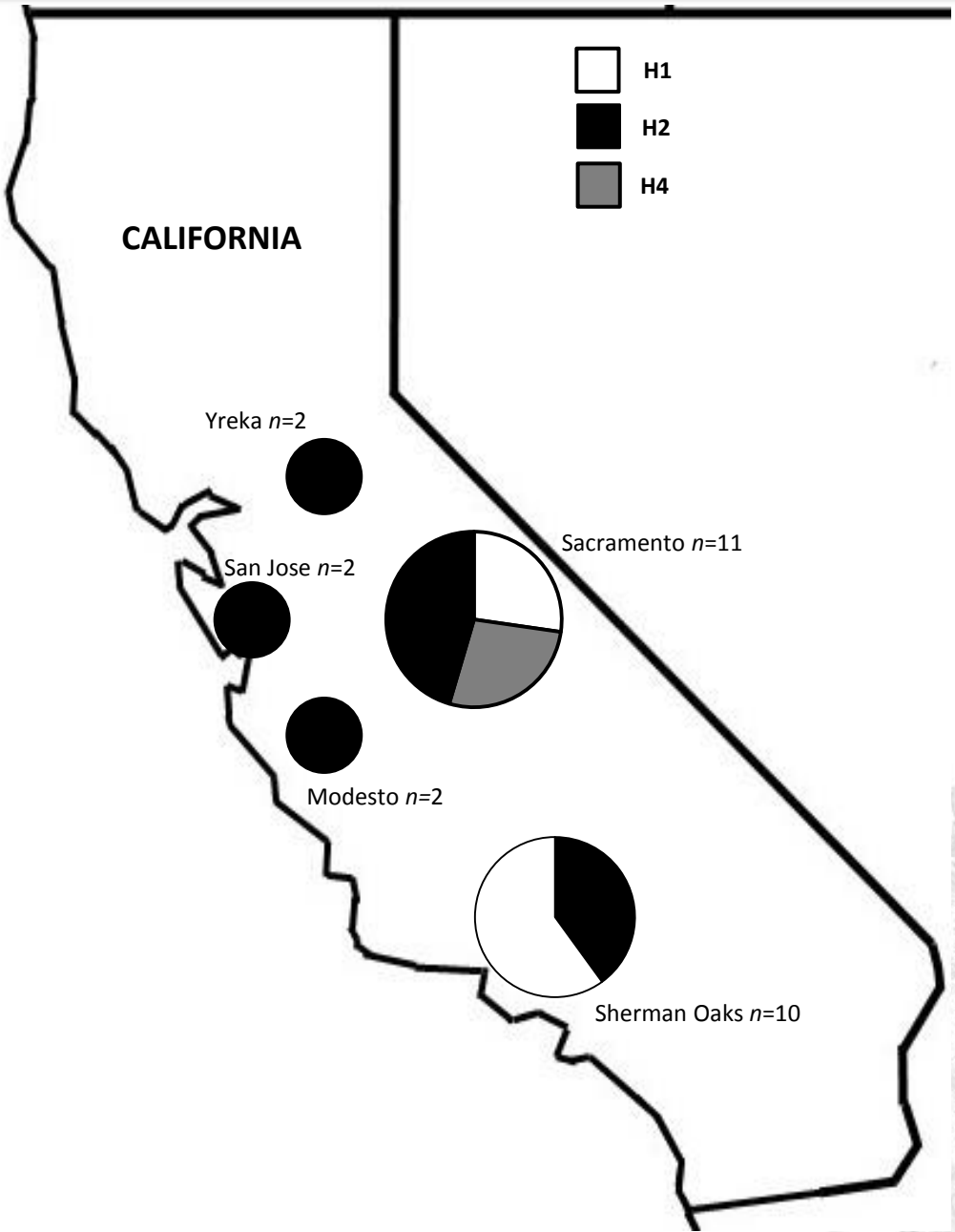
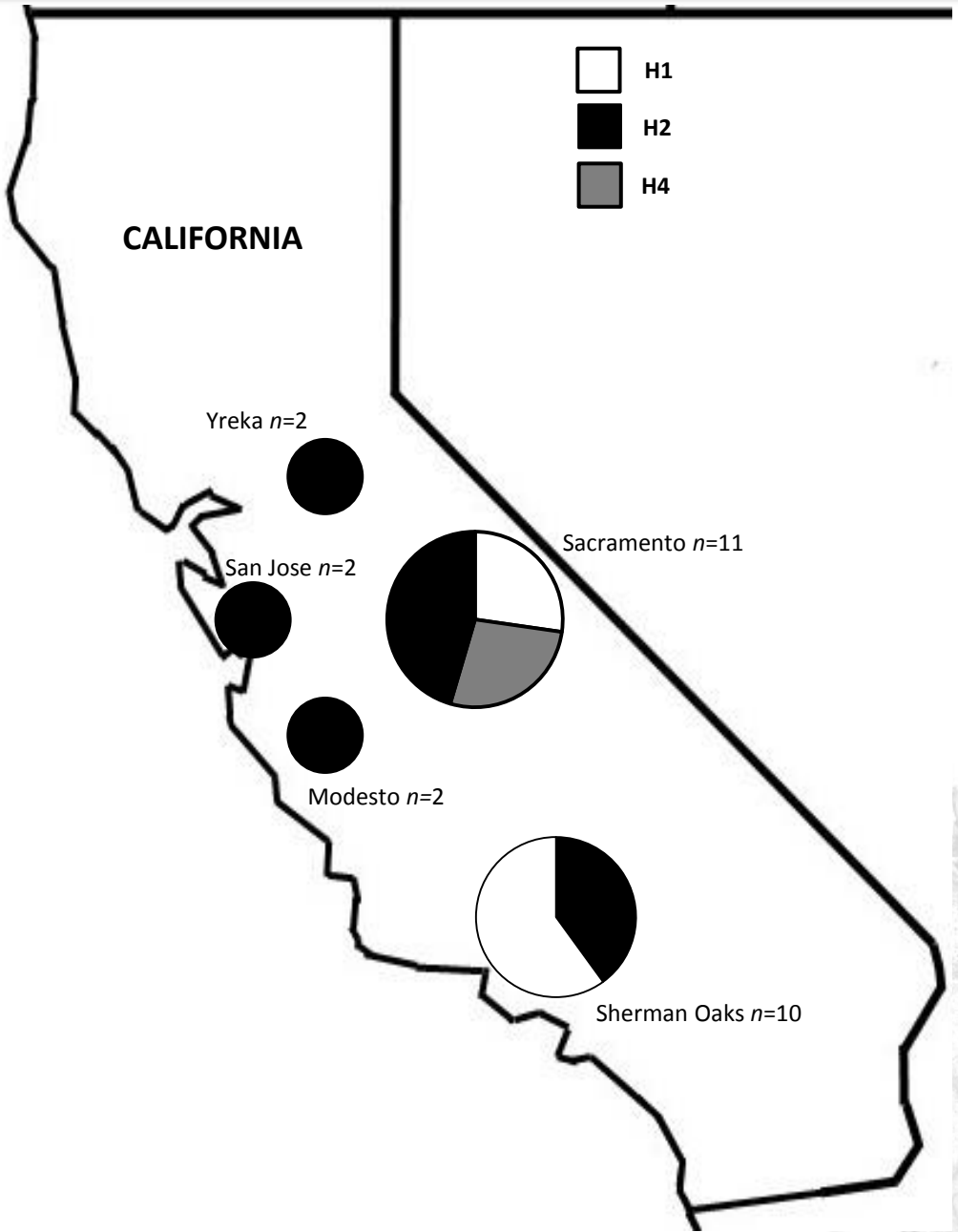
California


H1
H2
H4
CALIFORNIA
Yreka *n*=2
San Jose *n*=2
Sacramento *n*=11
Modesto *n*=2
Sherman Oaks *n*=10

California

- 3 mt DNA haplotypes (combined dataset) recovered from 31 specimens
- Prevalence of H1 and H2 (similarly to the pattern observed in East Coast)



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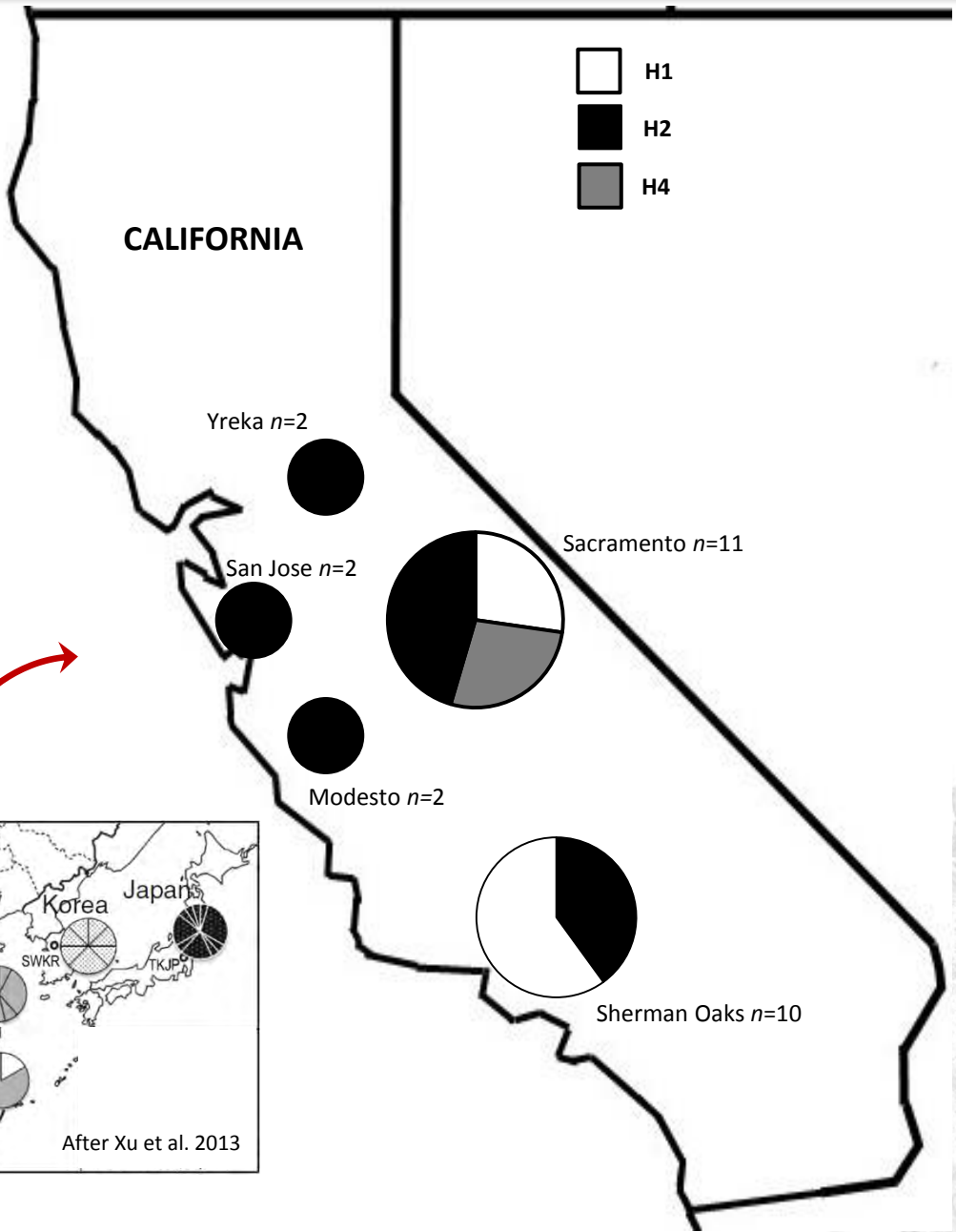
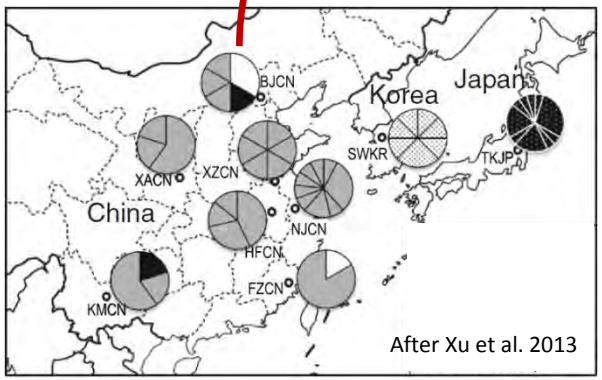
Beijing area in China:
the likely source region for California





Washington and Oregon

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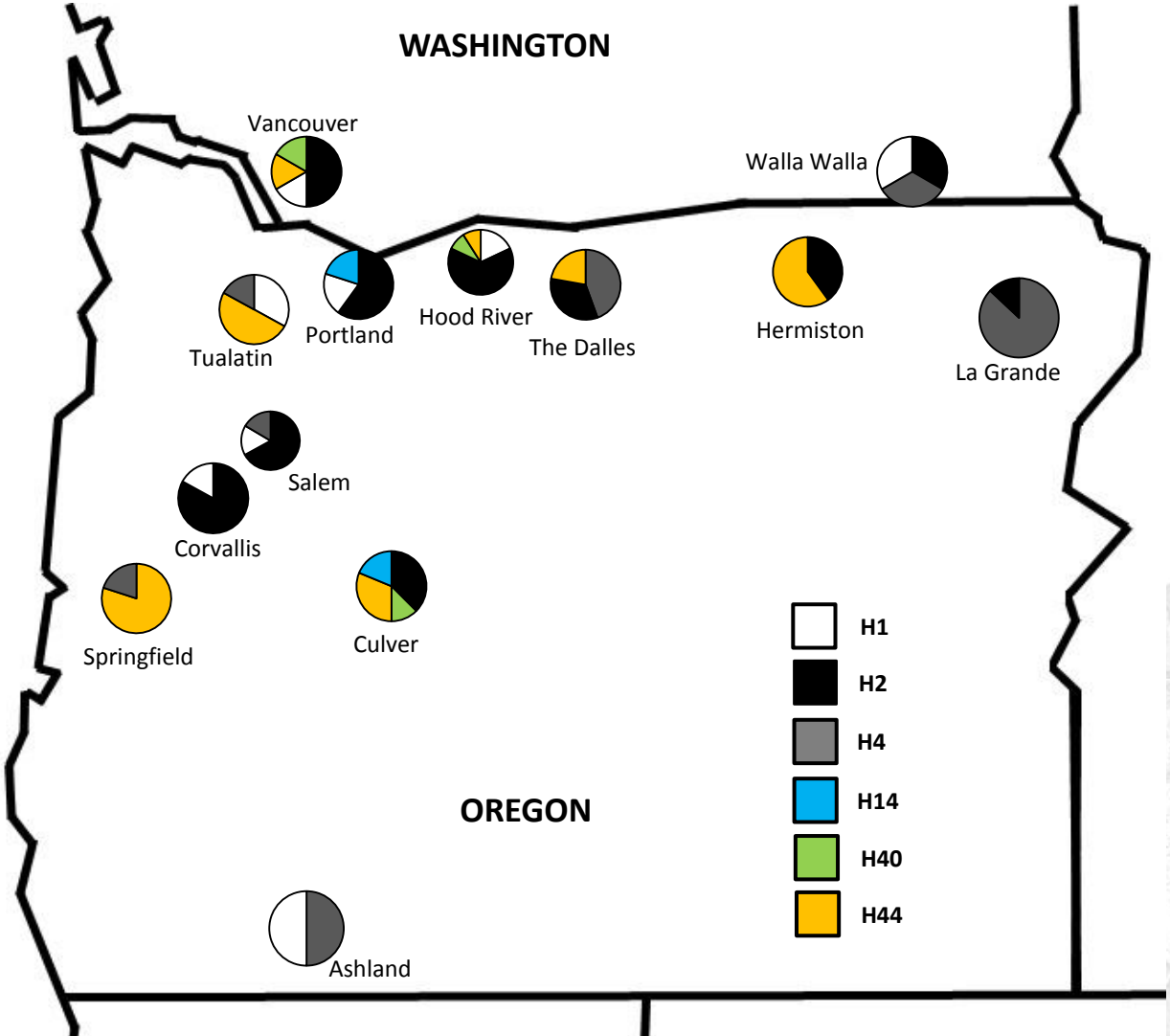
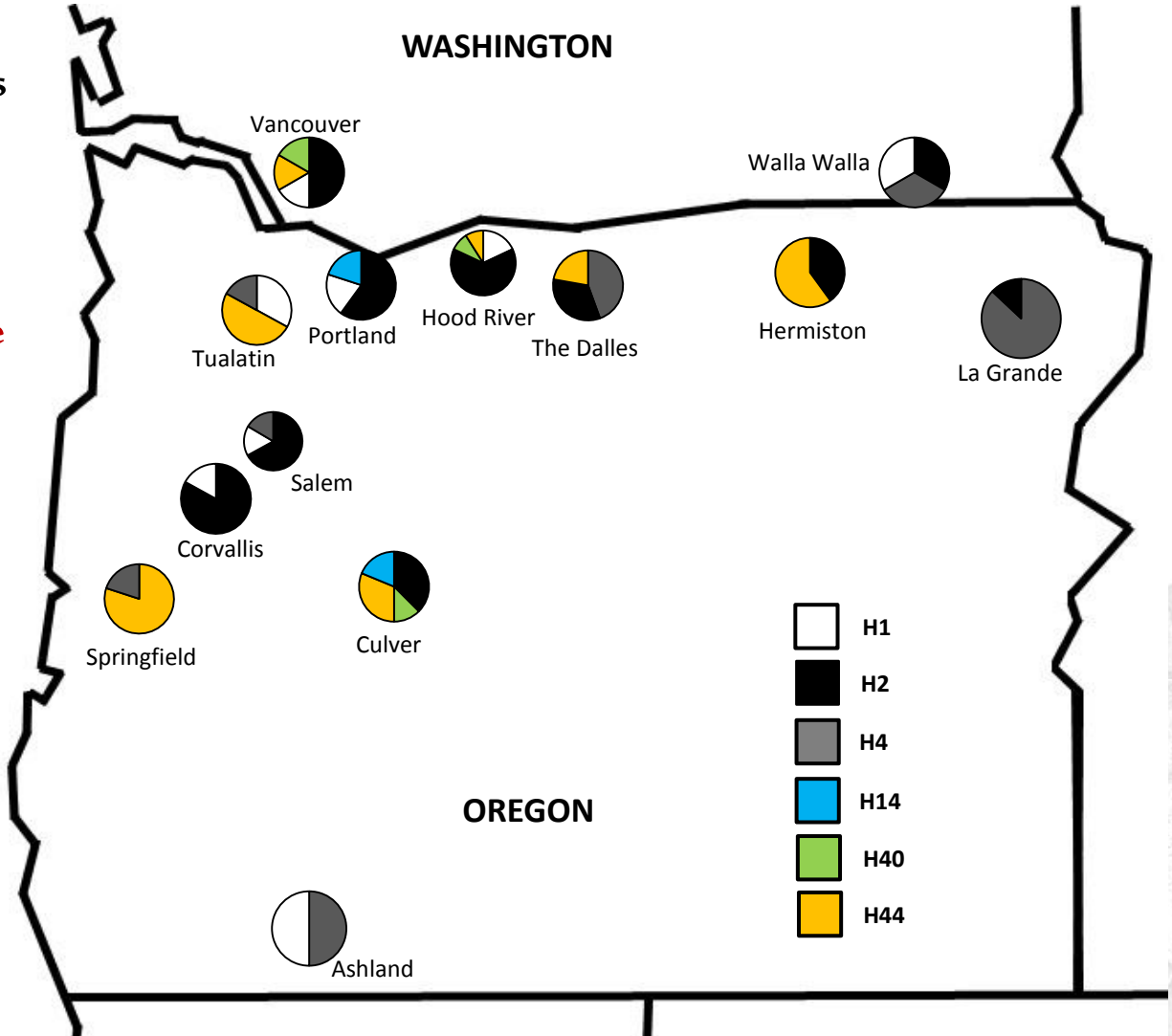
- 6 haplotypes recovered from 88 specimens



Washington and Oregon

- 6 haplotypes recovered from 88 specimens
- Prevalence of H2 (39%) and to a less extent H44 (21%)

H44 is evidenced for the first time



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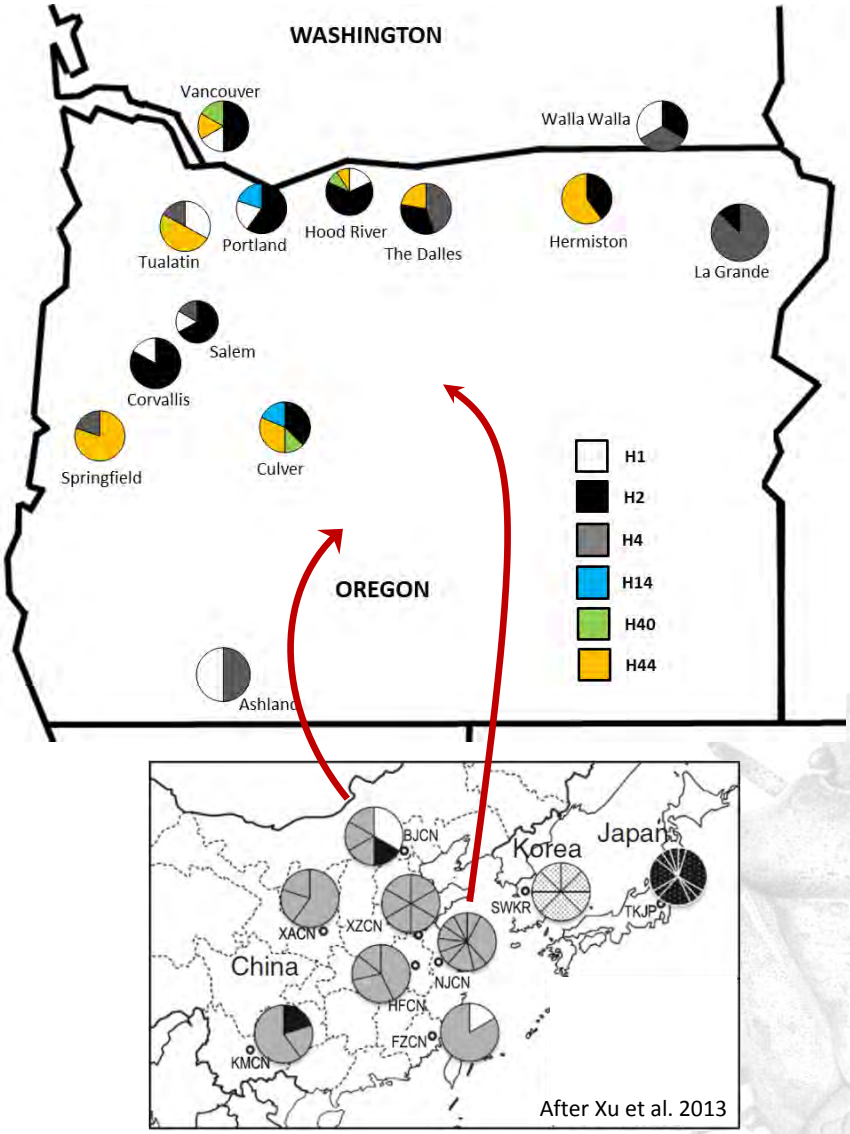
H1, H2, H4 and H14

Beijing and Nanjing areas in China: the likely source regions for WA and OR



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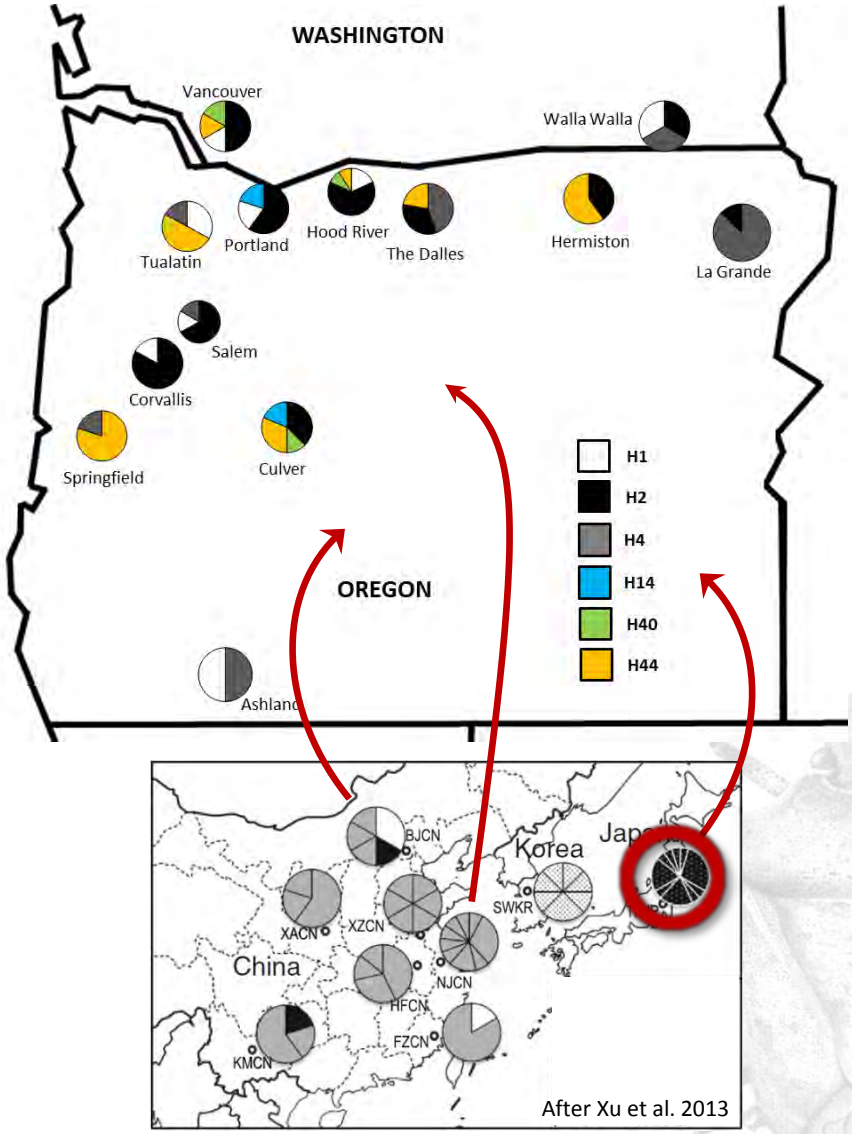
But not only… Japan

Historical interception from Japan in British Columbia

After Cariepy et al. 2014



Most likely scenario evidenced by the molecular data

- **Invasion in Western US resulting from multiple sources in Asia**

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Manuscript in preparation

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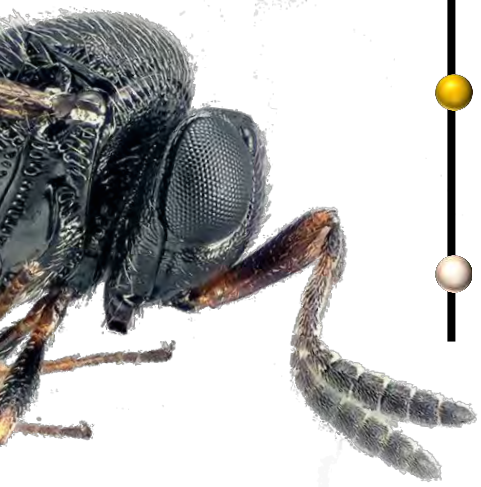

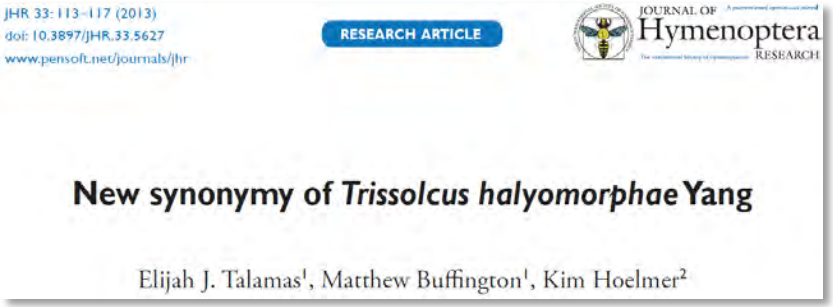
JHR 33: 113–117 (2013)
doi: 10.3897/JHR.33.5627
www.pensoft.net/journals/jhr
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JOURNAL OF Hymenoptera RESEARCH

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Elijah J. Talamas[1], Matthew Buffington[1], Kim Hoelmer[2]

Talamas et al. 2015

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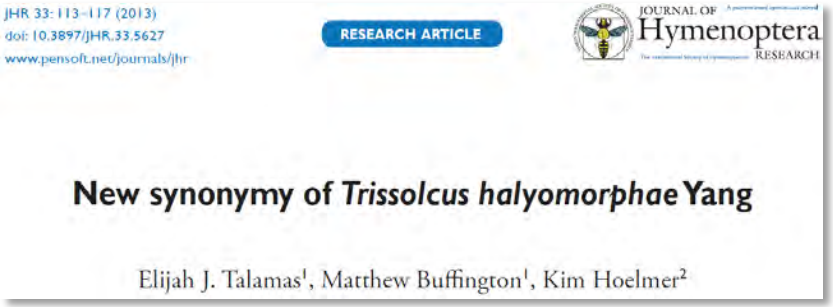
- **Different strains from China, Japan and South Korea in culture**

➞ **Evaluation of its host range and efficacy**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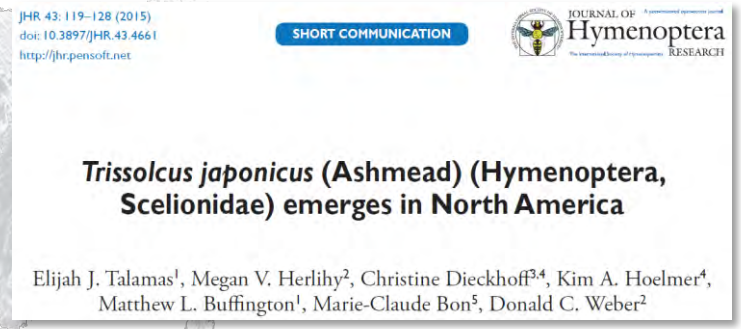

JHR 43: 119–128 (2015)
doi: 10.3897/JHR.43.4661
http://jhr.pensoft.net
SHORT COMMUNICATION
JOURNAL OF Hymenoptera RESEARCH

***Trissolcus japonicus* (Ashmead) (Hymenoptera, Scelionidae) emerges in North America**

Elijah J. Talamas[1], Megan V. Herlihy[2], Christine Dieckhoff[3,4], Kim A. Hoelmer[4], Matthew L. Buffington[1], Marie-Claude Bon[5], Donald C. Weber[2]

A survey conducted in 2014 revealed that *T. japonicus* was already present in the wild

- **How did *Trissolcus japonicus* come to this site?**
- **Accidental quarantine escape?**

Comparison of the genetic diversity

« Recovered population » **Quarantine colonies**

Comparison of the genetic diversity

« Recovered population » **Quarantine colonies**

Development of microsatellites

Population genetics requires successful development of microsatellites

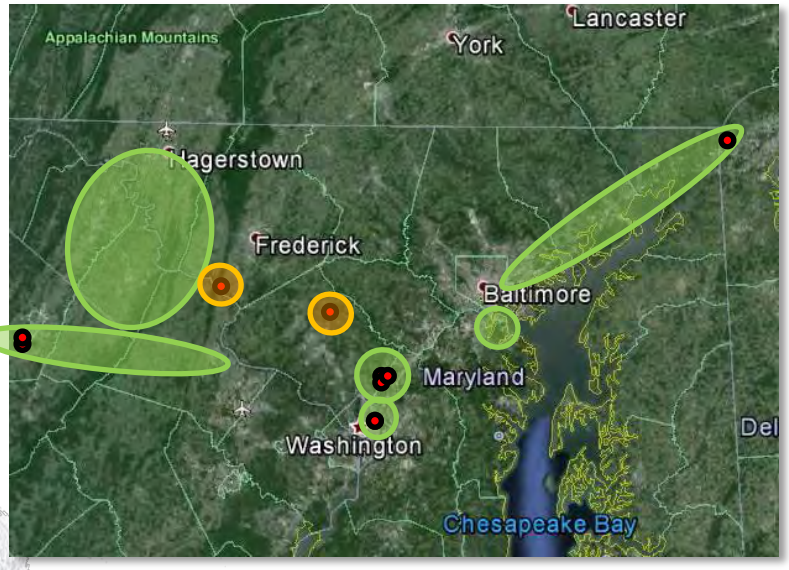
➞ These tools have been developed at the onset of the BMSB project

24 microsatellites

Sentinel egg masses → **67 individuals**

- **2014** ***n*** **= 38**
- **2015** ***n*** **= 29**

East Coast Survey



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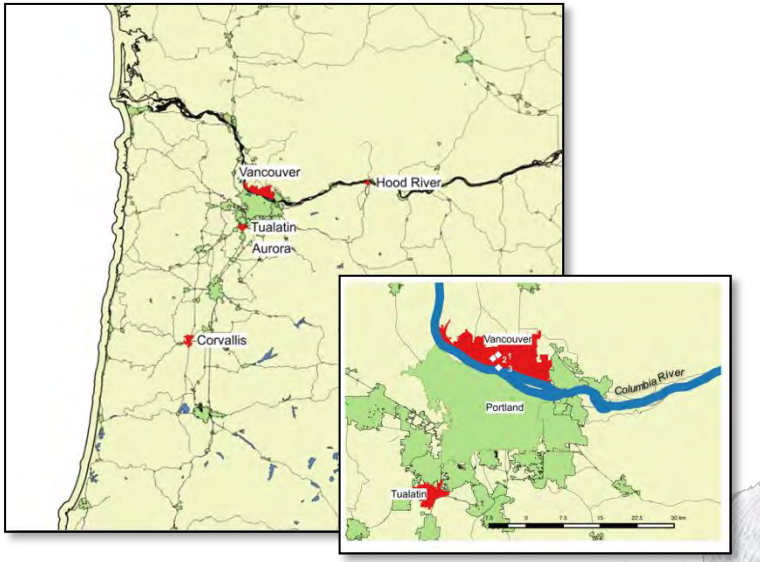

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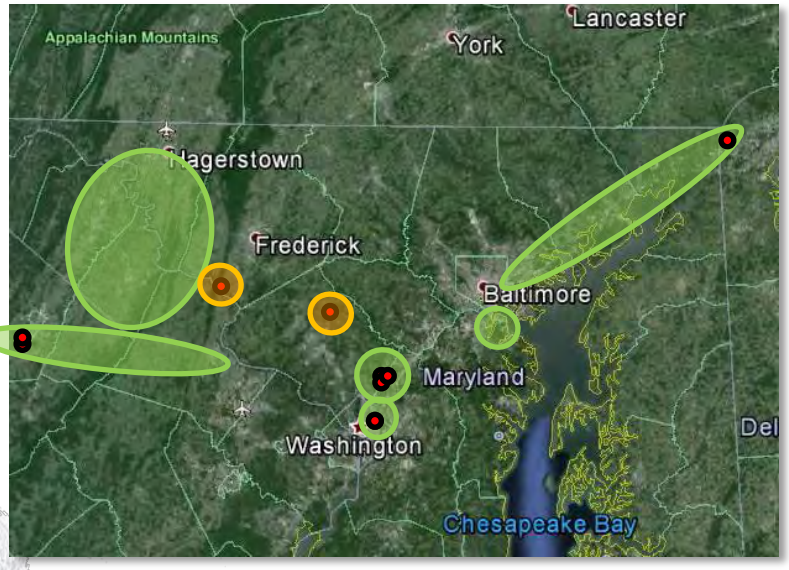

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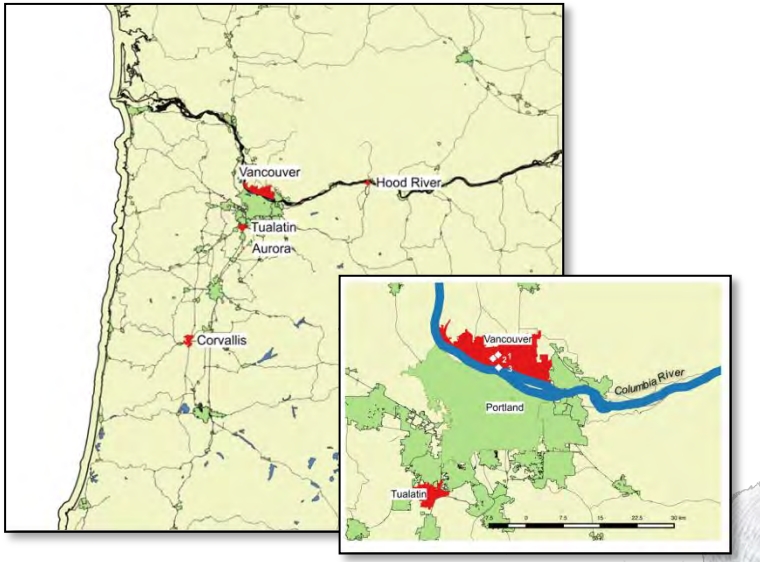

- **Taxonomy**
- **Multilocus genotyping approach (24 microsatellites)**
- **Barcode sequencing (COI)**

→ **Wild specimens can be identified**

Genus	Species		EBCL id	Sex	Cuid	Label1
Trissolcus	euschistii	fragment	Tj221	?	destructive method	dissected from wild Euschistus EM# O-15-15
Trissolcus	japonicus	diss out	Tj222	f?	destructive method	BIIR sentinel ex Thyanta on Ash# NS-13-15
Trissolcus	japonicus		Tj223	m	destructive method	BIIR ex P. mac sentinel EM on ash tree August 2015 possible sentinel lab contaminant
Trissolcus	japonicus		Tj224	m	destructive method	BIIR ex P. mac sentinel EM on ash tree August 2015 possible sentinel lab contaminant
Trissolcus	japonicus		Tj225	m	destructive method	BIIR ex P. mac sentinel EM on ash tree August 2015 possible sentinel lab contaminant
Trissolcus	japonicus		Tj226	m	destructive method	BIIR ex P. mac sentinel EM on ash tree August 2015 possible sentinel lab contaminant
Trissolcus	japonicus		Tj227	m	destructive method	BIIR ex P. mac sentinel EM on ash tree August 2015 possible sentinel lab contaminant
Trissolcus	japonicus		Tj228	m	destructive method	BIIR ex P. mac sentinel EM on ash tree August 2015 possible sentinel lab contaminant
Trissolcus	japonicus		Tj229	m	destructive method	BIIR ex P. mac sentinel EM on ash tree August 2015 possible sentinel lab contaminant
Trissolcus	japonicus	partial	Tj230	?	destructive method	WC 17 83 BARC, M. Herlihy
Trissolcus	japonicus	partial	Tj231	?	destructive method	WC 11810 BARC, M. Herlihy
Trissolcus	japonicus	partial	Tj232	?	destructive method	DE? 7 83 BARC, M. Herlihy
Trissolcus	japonicus	partial	Tj233	?	destructive method	WC10 720 BARC, M. Herlihy
Trissolcus	japonicus	partial	Tj234	?	destructive method	WC11 (1) 8 BARC, M. Herlihy
Trissolcus	japonicus	partial	Tj235	?	destructive method	WC 3831
Trissolcus	japonicus	partial	Tj236	?	destructive method	OE283
Trissolcus	japonicus	partial	Tj237	?	destructive method	ON5817
Trissolcus	japonicus	partial	Tj238	?	destructive method	WC10810
Trissolcus	japonicus		Tj239	f	non invasive method	WC10727 BARC, M. Herlihy
Trissolcus	japonicus		Tj240	f	non invasive method	OE5720 BARC, M. Herlihy
Trissolcus	japonicus		Tj241	f	non invasive method	WC 9810 BARC, M. Herlihy
Trissolcus	japonicus		Tj242	f	non invasive method	WC 18831
Trissolcus	japonicus		Tj243	f	non invasive method	WC 12831
Trissolcus	japonicus		Tj244	f	non invasive method	WC 11831
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Trissolcus	japonicus		Tj246	f	non invasive method	ON 8 817
Trissolcus	japonicus		Tj247	m	non invasive method	BARC 15 Aug 2015
Trissolcus	japonicus		Tj248	m	non invasive method	BARC 15 Aug 2015
Trissolcus	sp closely related		Tj249	f	non invasive method	BARC 15 Aug 2015
Trissolcus	sp closely related		Tj250	f	non invasive method	BARC 15 Aug 2015
Trissolcus	japonicus		Tj251	f	non invasive method	BARC EM#6, 17 June 2015
Trissolcus	japonicus		Tj252	f	non invasive method	BARC EM#6, 17 June 2015
Trissolcus	japonicus		Tj253	f	non invasive method	BARC EM#6, 17 June 2015
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Trissolcus	japonicus		Tj256	f	non invasive method	BARC EM#6, 17 June 2015
Trissolcus	japonicus		Tj257	m	non invasive method	BARC 18 Aug 2015
Trissolcus	japonicus		Tj258	m	non invasive method	BARC 18 Aug 2015
Trissolcus	japonicus		Tj259	m	non invasive method	BARC 18 Aug 2015
Trissolcus	japonicus		Tj260	m	non invasive method	BARC 18 Aug 2015

- **Prevalence of *T. japonicus* in the populations recovered**

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Trissolcus	japonicus		Tj248	m	non invasive method	BARC 15 Aug 2015
Trissolcus	sp closely related		Tj249	f	non invasive method	BARC 15 Aug 2015
Trissolcus	sp closely related		Tj250	f	non invasive method	BARC 15 Aug 2015
Trissolcus	japonicus		Tj251	f	non invasive method	BARC EM#6, 17 June 2015
Trissolcus	japonicus		Tj252	f	non invasive method	BARC EM#6, 17 June 2015
Trissolcus	japonicus		Tj253	f	non invasive method	BARC EM#6, 17 June 2015
Trissolcus	japonicus		Tj254	f	non invasive method	BARC EM#6, 17 June 2015
Trissolcus	japonicus		Tj255	f	non invasive method	BARC EM#6, 17 June 2015
Trissolcus	japonicus		Tj256	f	non invasive method	BARC EM#6, 17 June 2015
Trissolcus	japonicus		Tj257	m	non invasive method	BARC 18 Aug 2015
Trissolcus	japonicus		Tj258	m	non invasive method	BARC 18 Aug 2015
Trissolcus	japonicus		Tj259	m	non invasive method	BARC 18 Aug 2015
Trissolcus	japonicus		Tj260	m	non invasive method	BARC 18 Aug 2015

- **Prevalence of *T. japonicus* in the populations recovered**
- ***Trissolcus euschitii* = North American species**

Genus	Species		EBCL id	Sex	Cuid	Label1
Trissolcus	euschistii	fragment	Tj221	?	destructive method	dissected from wild Euschistus EM# O-15-15
Trissolcus	japonicus	diss out	Tj222	f?	destructive method	BIIR sentinel ex Thyanta on Ash# NS-13-15
Trissolcus	japonicus		Tj223	m	destructive method	BIIR ex P. mac sentinel EM on ash tree August 2015 possible sentinel lab contaminant
Trissolcus	japonicus		Tj224	m	destructive method	BIIR ex P. mac sentinel EM on ash tree August 2015 possible sentinel lab contaminant
Trissolcus	japonicus		Tj225	m	destructive method	BIIR ex P. mac sentinel EM on ash tree August 2015 possible sentinel lab contaminant
Trissolcus	japonicus		Tj226	m	destructive method	BIIR ex P. mac sentinel EM on ash tree August 2015 possible sentinel lab contaminant
Trissolcus	japonicus		Tj227	m	destructive method	BIIR ex P. mac sentinel EM on ash tree August 2015 possible sentinel lab contaminant
Trissolcus	japonicus		Tj228	m	destructive method	BIIR ex P. mac sentinel EM on ash tree August 2015 possible sentinel lab contaminant
Trissolcus	japonicus		Tj229	m	destructive method	BIIR ex P. mac sentinel EM on ash tree August 2015 possible sentinel lab contaminant
Trissolcus	japonicus	partial	Tj230	?	destructive method	WC 17 83 BARC, M. Herlihy
Trissolcus	japonicus	partial	Tj231	?	destructive method	WC 11810 BARC, M. Herlihy
Trissolcus	japonicus	partial	Tj232	?	destructive method	DE? 7 83 BARC, M. Herlihy
Trissolcus	japonicus	partial	Tj233	?	destructive method	WC10 720 BARC, M. Herlihy
Trissolcus	japonicus	partial	Tj234	?	destructive method	WC11 (1) 8 BARC, M. Herlihy
Trissolcus	japonicus	partial	Tj235	?	destructive method	WC 3831
Trissolcus	japonicus	partial	Tj236	?	destructive method	OE283
Trissolcus	japonicus	partial	Tj237	?	destructive method	ON5817
Trissolcus	japonicus	partial	Tj238	?	destructive method	WC10810
Trissolcus	japonicus		Tj239	f	non invasive method	WC10727 BARC, M. Herlihy
Trissolcus	japonicus		Tj240	f	non invasive method	OE5720 BARC, M. Herlihy
Trissolcus	japonicus		Tj241	f	non invasive method	WC 9810 BARC, M. Herlihy
Trissolcus	japonicus		Tj242	f	non invasive method	WC 18831
Trissolcus	japonicus		Tj243	f	non invasive method	WC 12831
Trissolcus	japonicus		Tj244	f	non invasive method	WC 11831
Trissolcus	japonicus		Tj245	f	non invasive method	WC 4831
Trissolcus	japonicus		Tj246	f	non invasive method	ON 8 817
Trissolcus	japonicus		Tj247	m	non invasive method	BARC 15 Aug 2015
Trissolcus	japonicus		Tj248	m	non invasive method	BARC 15 Aug 2015
Trissolcus	sp closely related		Tj249	f	non invasive method	BARC 15 Aug 2015
Trissolcus	sp closely related		Tj250	f	non invasive method	BARC 15 Aug 2015
Trissolcus	japonicus		Tj251	f	non invasive method	BARC EM#6, 17 June 2015
Trissolcus	japonicus		Tj252	f	non invasive method	BARC EM#6, 17 June 2015
Trissolcus	japonicus		Tj253	f	non invasive method	BARC EM#6, 17 June 2015
Trissolcus	japonicus		Tj254	f	non invasive method	BARC EM#6, 17 June 2015
Trissolcus	japonicus		Tj255	f	non invasive method	BARC EM#6, 17 June 2015
Trissolcus	japonicus		Tj256	f	non invasive method	BARC EM#6, 17 June 2015
Trissolcus	japonicus		Tj257	m	non invasive method	BARC 18 Aug 2015
Trissolcus	japonicus		Tj258	m	non invasive method	BARC 18 Aug 2015
Trissolcus	japonicus		Tj259	m	non invasive method	BARC 18 Aug 2015
Trissolcus	japonicus		Tj260	m	non invasive method	BARC 18 Aug 2015

- **Prevalence of *T. japonicus* in the populations recovered**
- ***Trissolcus euschitii* = North American species**

Genus	Species		EBCL id	Sex	Cuid	Label1
Trissolcus	euschistii	fragment	Tj221	?	destructive method	dissected from wild Euschistus EM# O-15-15
Trissolcus	japonicus	diss out	Tj222	f?	destructive method	BIIR sentinel ex Thyanta on Ash# NS-13-15
Trissolcus	japonicus		Tj223	m	destructive method	BIIR ex P. mac sentinel EM on ash tree August 2015 possible sentinel lab contaminant
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Trissolcus	japonicus		Tj226	m	destructive method	BIIR ex P. mac sentinel EM on ash tree August 2015 possible sentinel lab contaminant
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Trissolcus	japonicus		Tj228	m	destructive method	BIIR ex P. mac sentinel EM on ash tree August 2015 possible sentinel lab contaminant
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Trissolcus	japonicus	partial	Tj231	?	destructive method	WC 11810 BARC, M. Herlihy
Trissolcus	japonicus	partial	Tj232	?	destructive method	DE? 7 83 BARC, M. Herlihy
Trissolcus	japonicus	partial	Tj233	?	destructive method	WC10 720 BARC, M. Herlihy
Trissolcus	japonicus	partial	Tj234	?	destructive method	WC11 (1) 8 BARC, M. Herlihy
Trissolcus	japonicus	partial	Tj235	?	destructive method	WC 3831
Trissolcus	japonicus	partial	Tj236	?	destructive method	OE283
Trissolcus	japonicus	partial	Tj237	?	destructive method	ON5817
Trissolcus	japonicus	partial	Tj238	?	destructive method	WC10810
Trissolcus	japonicus		Tj239	f	non invasive method	WC10727 BARC, M. Herlihy
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Trissolcus	japonicus		Tj242	f	non invasive method	WC 18831
Trissolcus	japonicus		Tj243	f	non invasive method	WC 12831
Trissolcus	japonicus		Tj244	f	non invasive method	WC 11831
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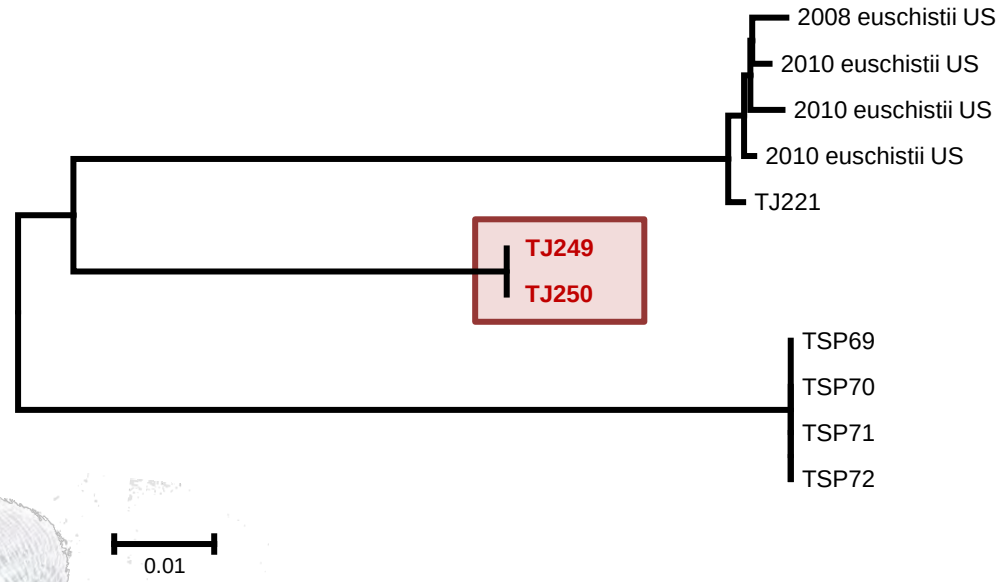
Trissolcus	japonicus		Tj254	f	non invasive method	BARC EM#6, 17 June 2015
Trissolcus	japonicus		Tj255	f	non invasive method	BARC EM#6, 17 June 2015
Trissolcus	japonicus		Tj256	f	non invasive method	BARC EM#6, 17 June 2015
Trissolcus	japonicus		Tj257	m	non invasive method	BARC 18 Aug 2015
Trissolcus	japonicus		Tj258	m	non invasive method	BARC 18 Aug 2015
Trissolcus	japonicus		Tj259	m	non invasive method	BARC 18 Aug 2015
Trissolcus	japonicus		Tj260	m	non invasive method	BARC 18 Aug 2015

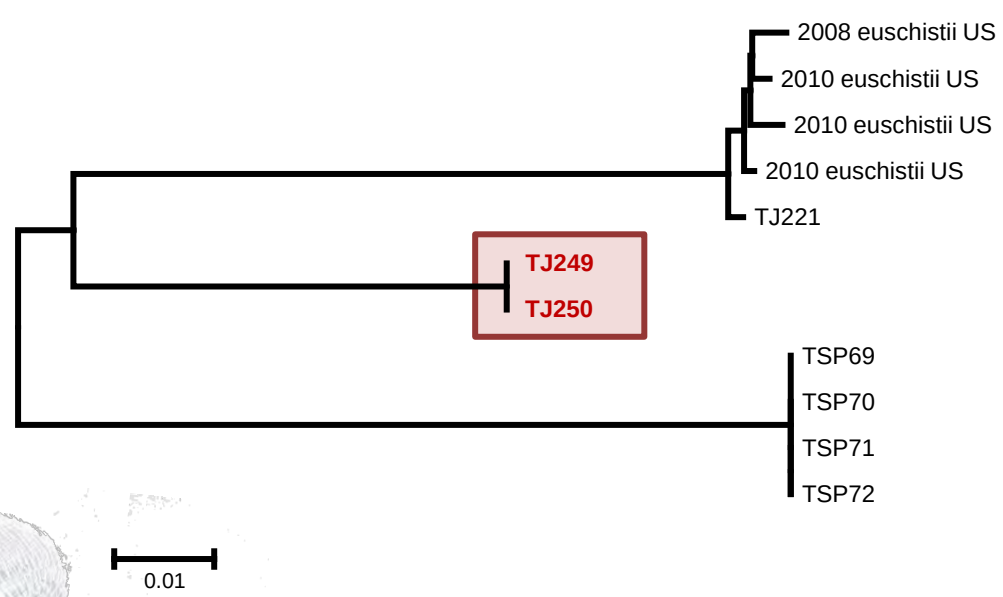
- **Evidence of a second lineage of egg parasitoid ?**

2 individuals

COI (barcode) results



COI (barcode) results



Trissolcus euschistii

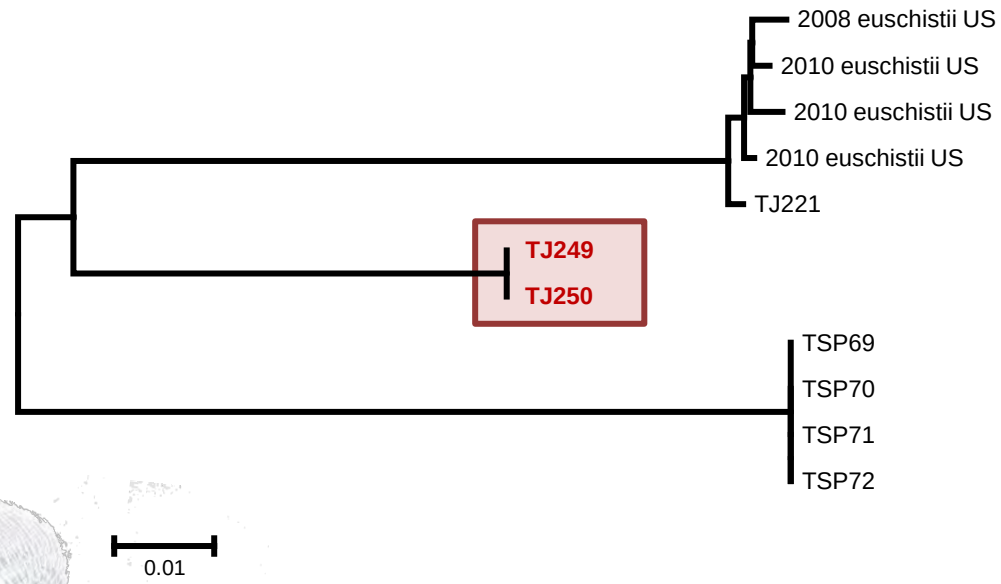
Trissolcus sp.

Trissolcus corai

The closest taxa → *Trissolcus euschistii*

The closest taxa → *Trissolcus corai*

COI (barcode) results



Trissolcus euschistii

p distance = 11.5 %

Trissolcus sp.

p distance = 12.6 %

Trissolcus corai

The closest taxa → *Trissolcus euschistii*, *Trissolcus corai* → Morphology *T. euschistii*

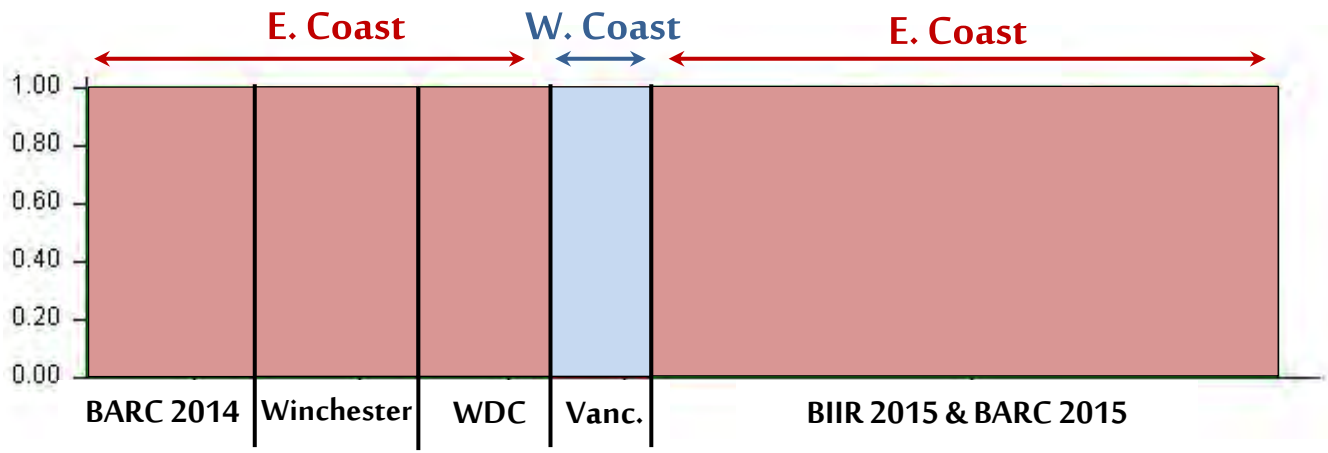
More genes !

Bayesian clustering approach

Structure software 

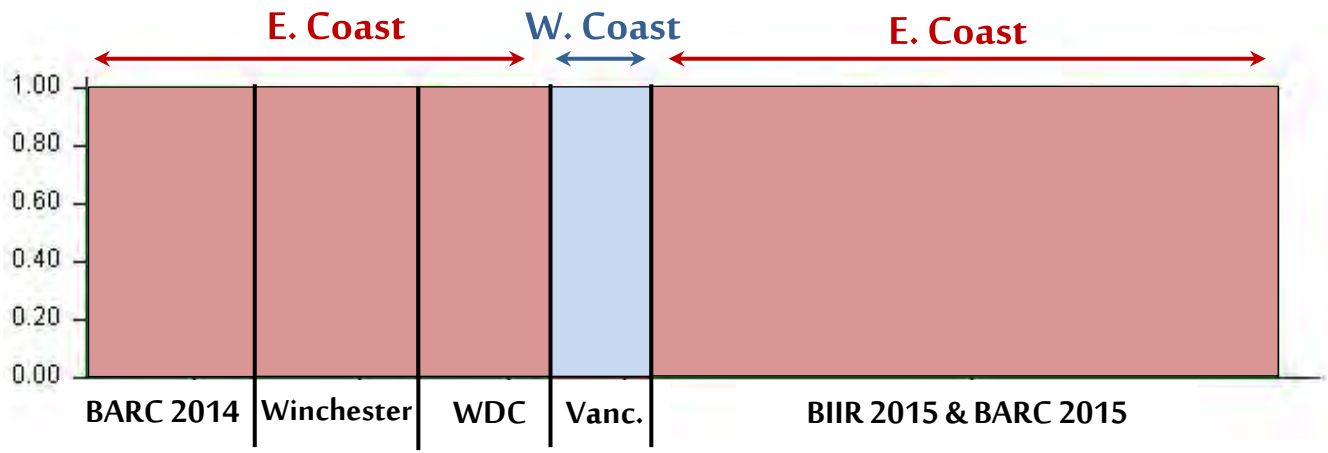
Bayesian clustering approach

Structure software



Bayesian clustering approach

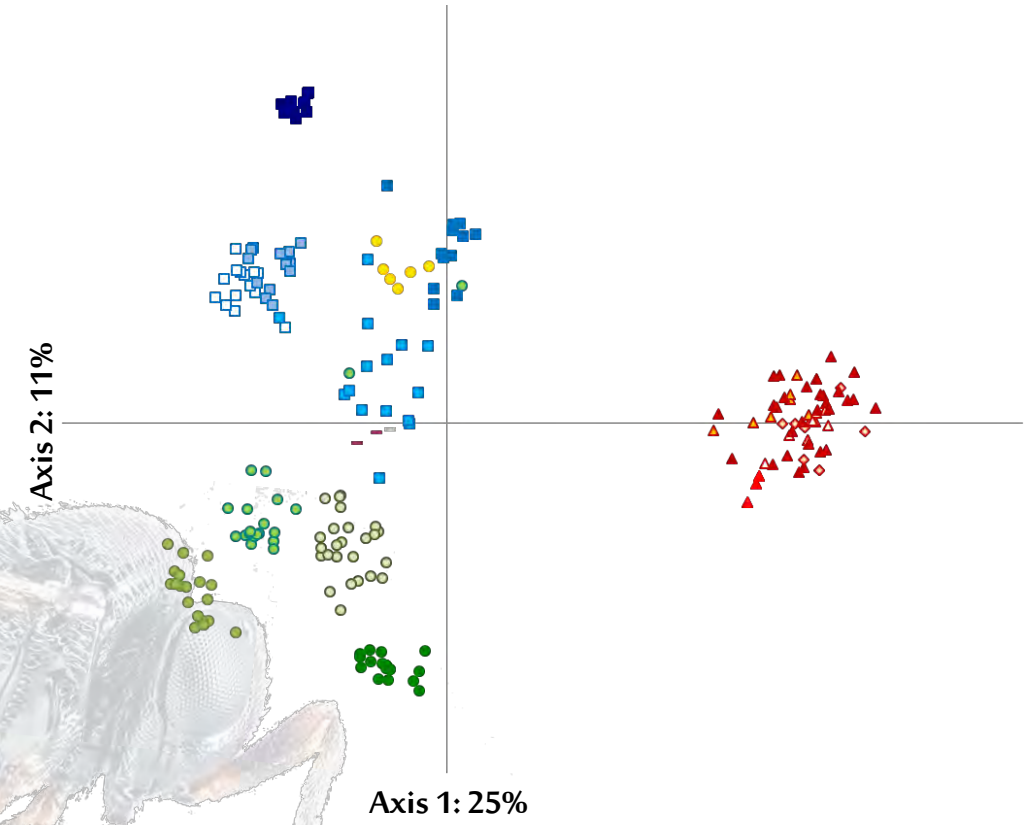
Structure software



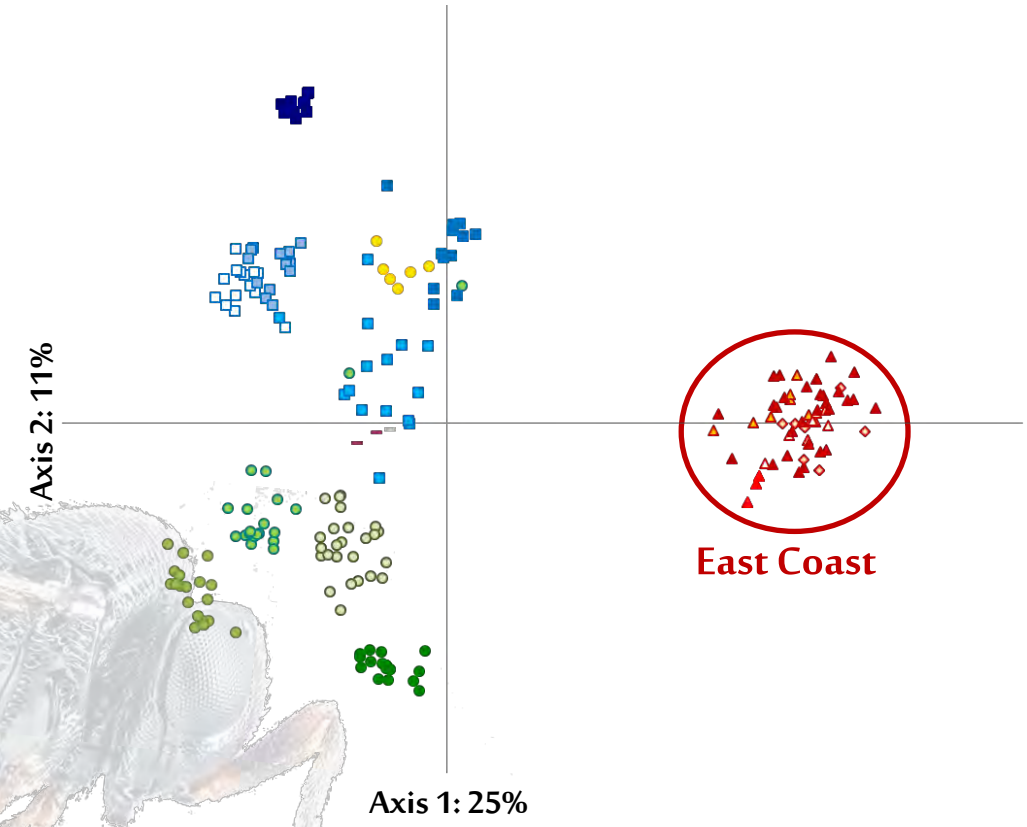
- The most likely value: K = 2
- Different clustering pattern ➞ West Coast population *versus* East Coast populations

➞ Different sources for East Coast and West Coast populations ?

Principale Coordinate Analysis (PCoA)



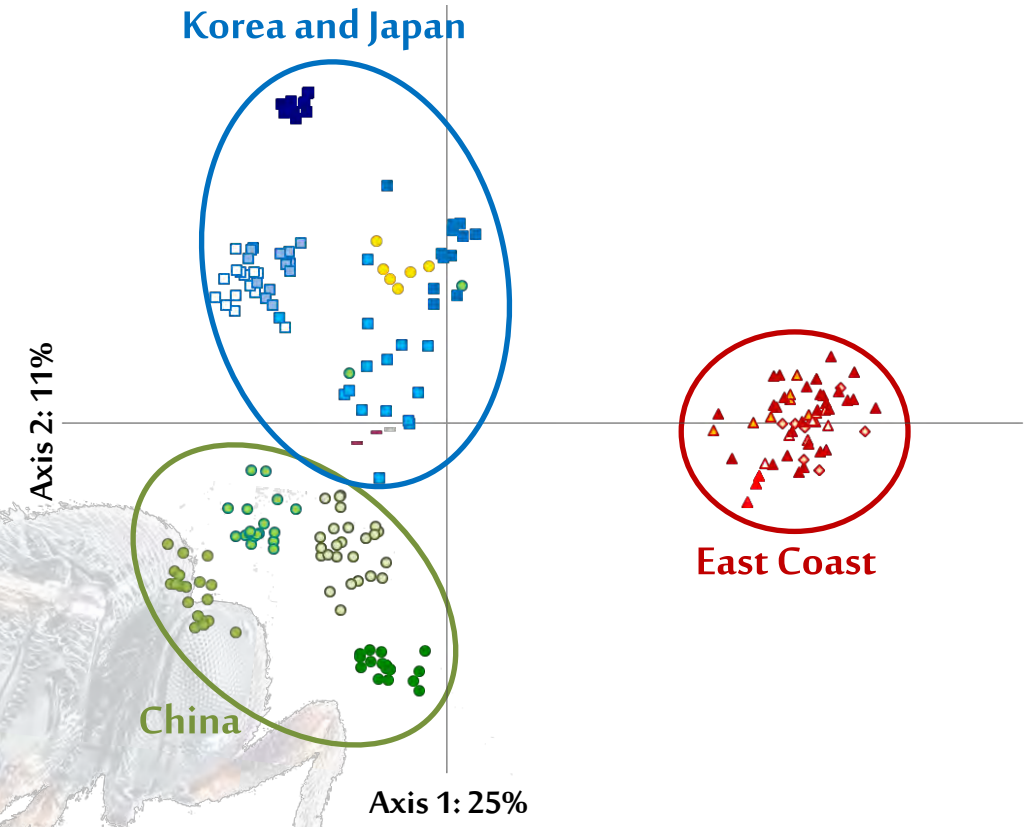
Principale Coordinate Analysis (PCoA)



- Axis 1: Split between East Coast populations and all other populations

Populations recovered in East Coast do not correspond to an accidental quarantine escape

Principale Coordinate Analysis (PCoA)



- Axis 1: Split between East Coast populations and all other populations

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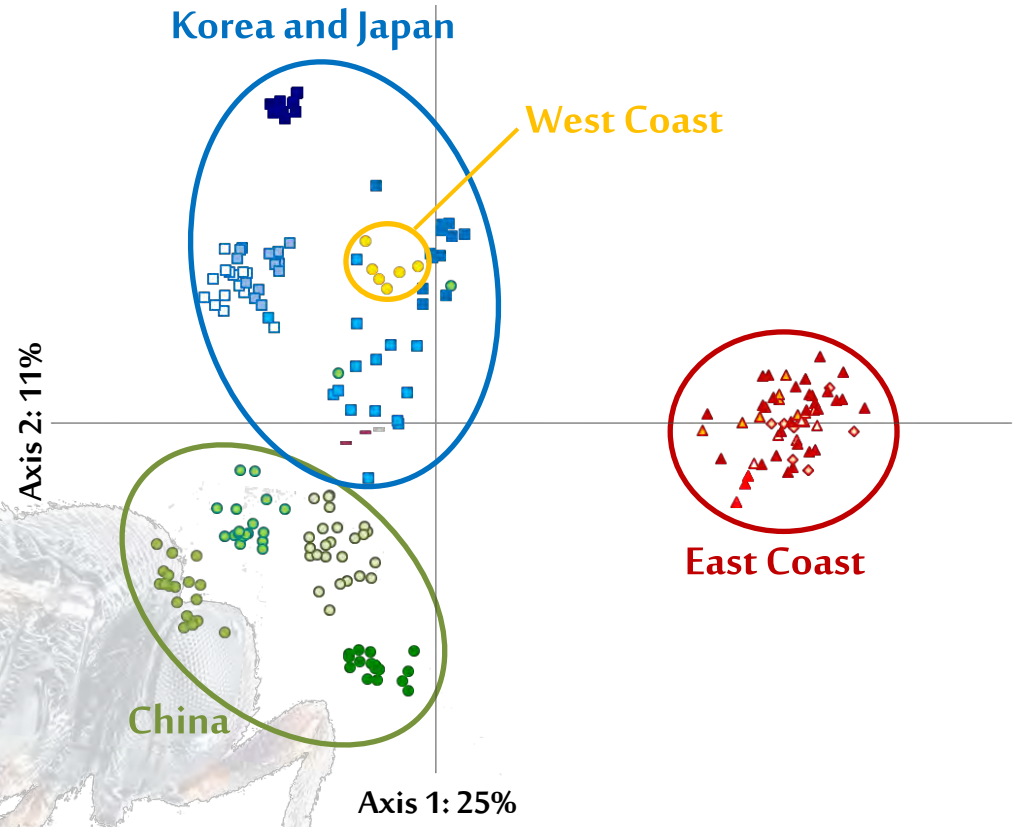
Principale Coordinate Analysis (PCoA)



- Axis 1: Split between East Coast populations and all other populations

Populations recovered in East Coast do not correspond to an accidental quarantine escape

- West Coast individuals
  - ➞ Cluster with Korean group

No reason that those individuals correspond to an accidental quarantine escape

Korean origin following an introduction of parasitized eggs or hitchiking adults

What's next?

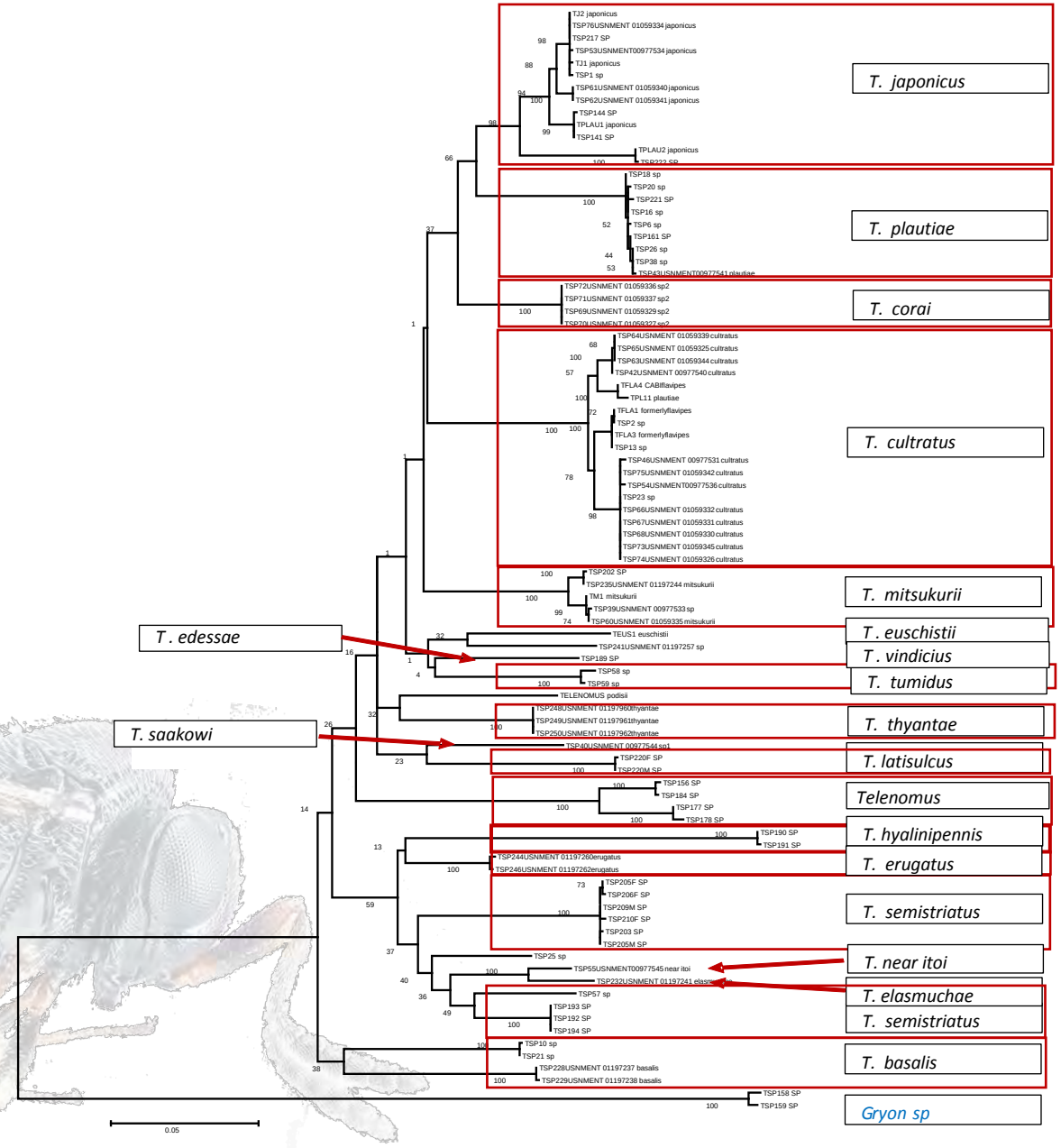
- Manuscript in preparation

- A master degree student

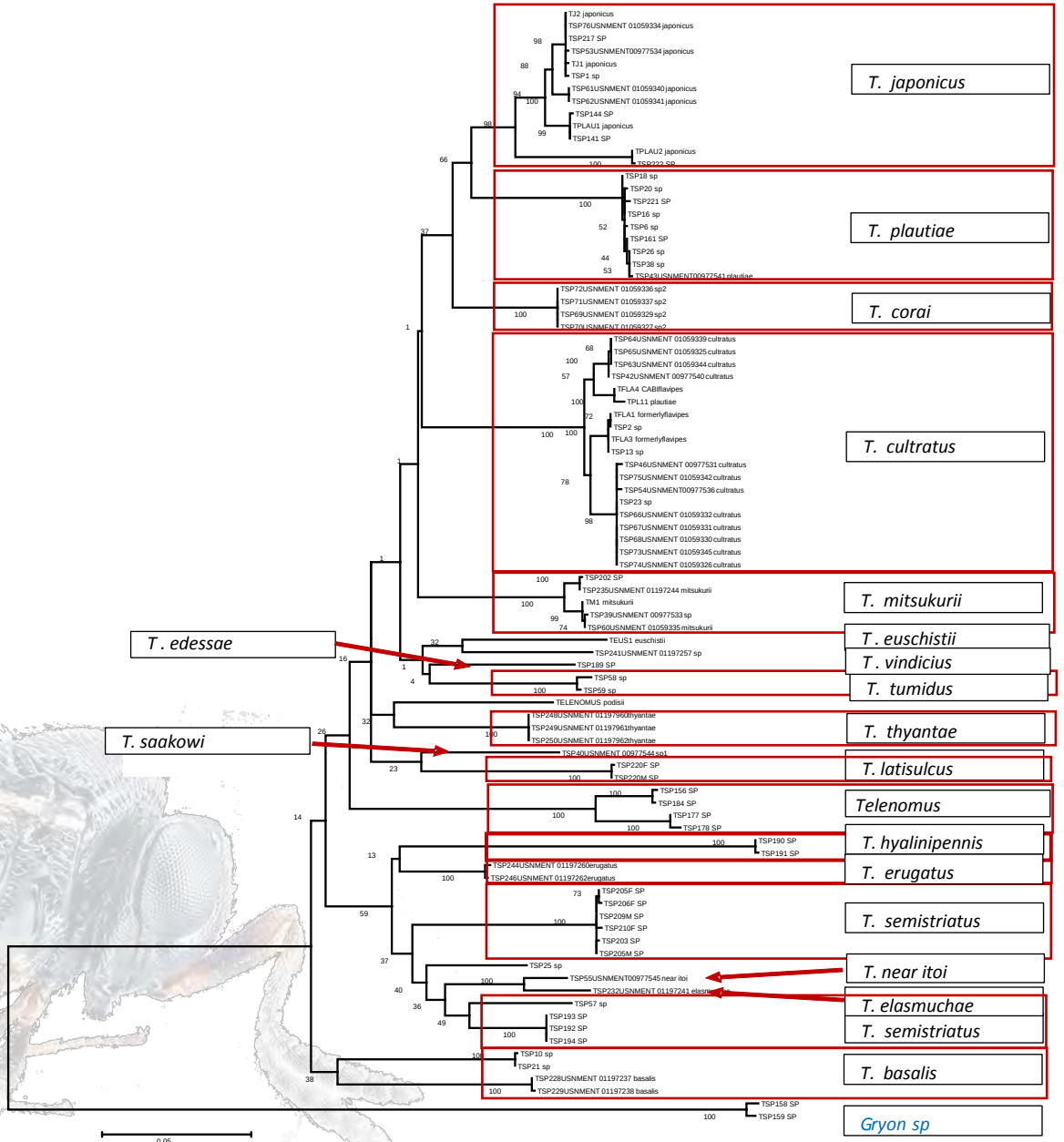
  ➞ To type all potential endosymbionts present in the quarantine colonies (using a multilocus approach)

Update on the genetic characterization of West Coast populations of *Halyomorpha halys* and adventive populations of its egg parasitoid *Trissolcus japonicus*

- Phylogeography of *Halyomorpha halis* in the U.S.
- Genetic diversity of *Trissolcus japonicus*
- **Phylogeny of the Asian *Trissolcus***



- Comprehensive Phylogenetic reconstruction based on concatenated analysis (1616bp) of mitochondrial and nuclear genes
- 289 individuals used



- Comprehensive Phylogenetic reconstruction based on concatenated analysis (1616bp) of mitochondrial and nuclear genes
- 289 individuals used

Congruence between molecular delineation and morphological characters for most lineages

Evidence of 19 *Trissolcus* species

Some are new to science

Real tool for diagnostic

What's next?

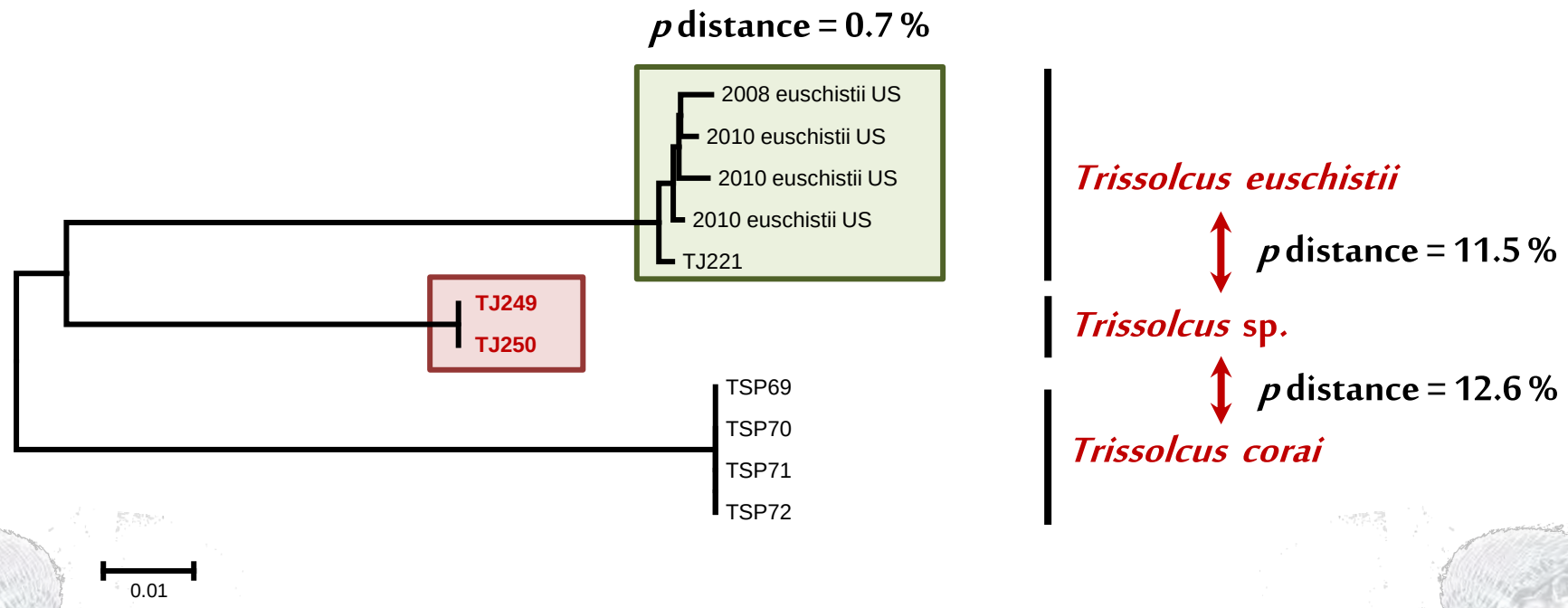
- 2 more genes are sequenced to get a robust phylogeny
- Final results for datation are expected before ICE2016
- Preliminary results and the philosophy of the project

➞ Poster and oral presentation of the project

Thank you for your attention....

Thank you for your attention....

COI (barcode) results



The closest taxa → *Trissolcus euschistii*, *Trissolcus corai* → New species ?