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Tracking origins of invasive leaf-mining moths using herbaria and archival DNA

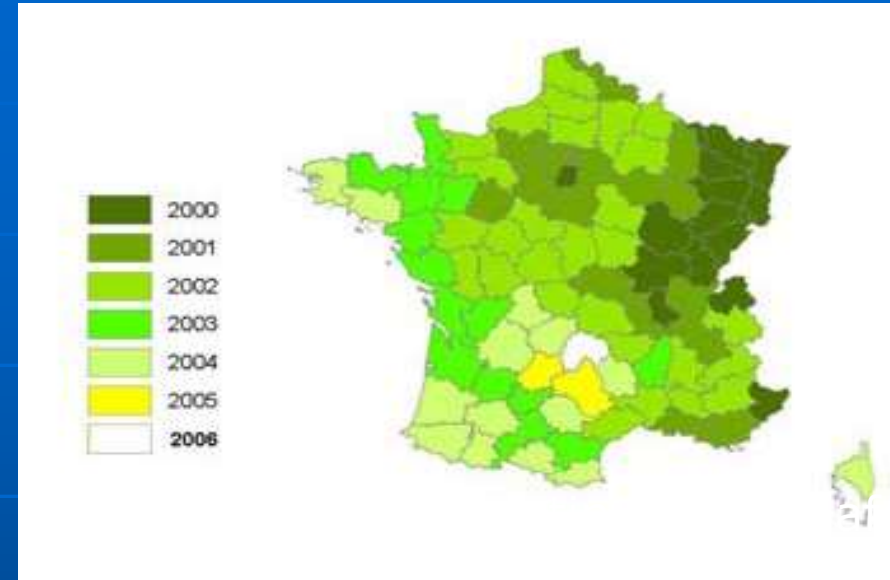
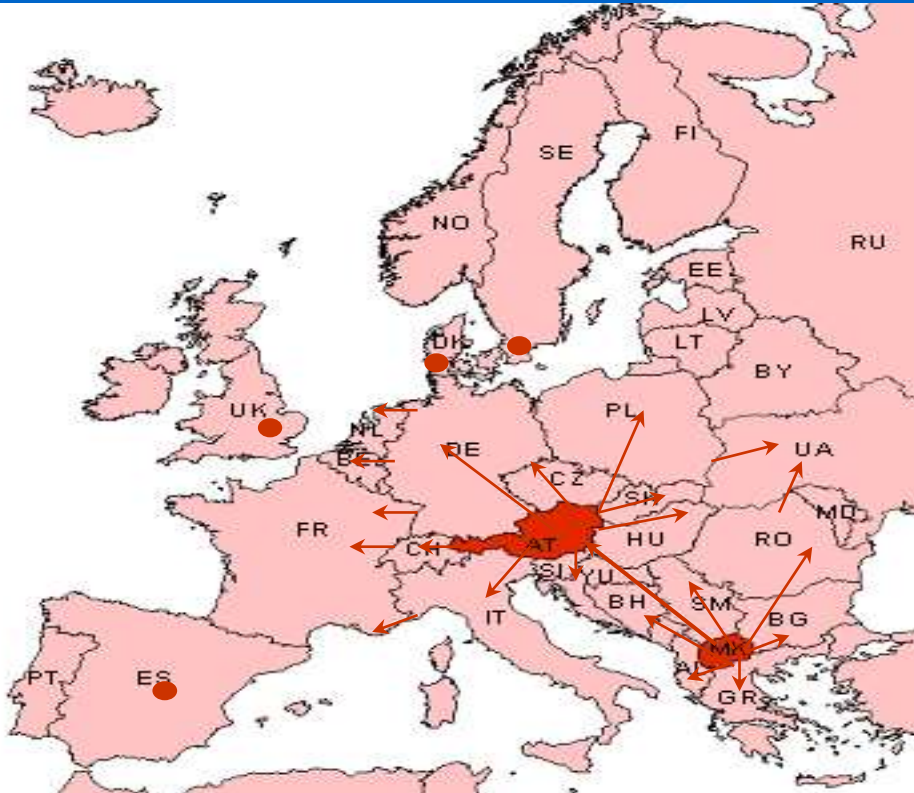
Carlos Lopez-Vaamonde

INRA Orleans, France

Evolutionary Ecology of Invasion

- Invasive species: Rapid expansion in a new environment, preadaptation, phenotypic plasticity.
- To understand the success of invasive species and establish biocontrol:
 - Determine the number of introductions
 - Level of genetic diversity. Bottleneck?
 - Invasion pathways
 - Sources (populations/regions) of invasion

Horse-chestnut leafminer, *Cameraria ohridella*



- First discovered in Macedonia in 1984.
- Invaded Europe in 20 years and France in just five years!
- It has become one of the best known micro-moth species (80 papers published)



by David Lees

Host Plants

- Horse-chestnut, ornamental tree present throughout Europe
- Extreme browning of leaves
- Aesthetic impact
- *Acer pseudoplatanus* and *A. platanoides*



Natural Forests: Tertiary relict of Balkan origin
Scattered populations in deep river gorges in Albania, Macedonia and Greece.

Possible areas of Origin of *Cameraria ohridella*:

- **Balkans** - Origin of the host tree
 - First found in this region

BUT

- Why spreading only in the 80s?
- Lepidopteran fauna well known in Europe
- No other *Cameraria* in Europe
- Only polyphagous parasitoids reared
- Still at outbreak density after 20 years

- sudden host plant shift to horse-chestnut, probably from maple or sycamore (*Acer* spp.)?
- originated in North America (52 out of 74 described *Cameraria* species)?

Tracking down *Cameraria ohridella* origin: looking for a needle in a haystack

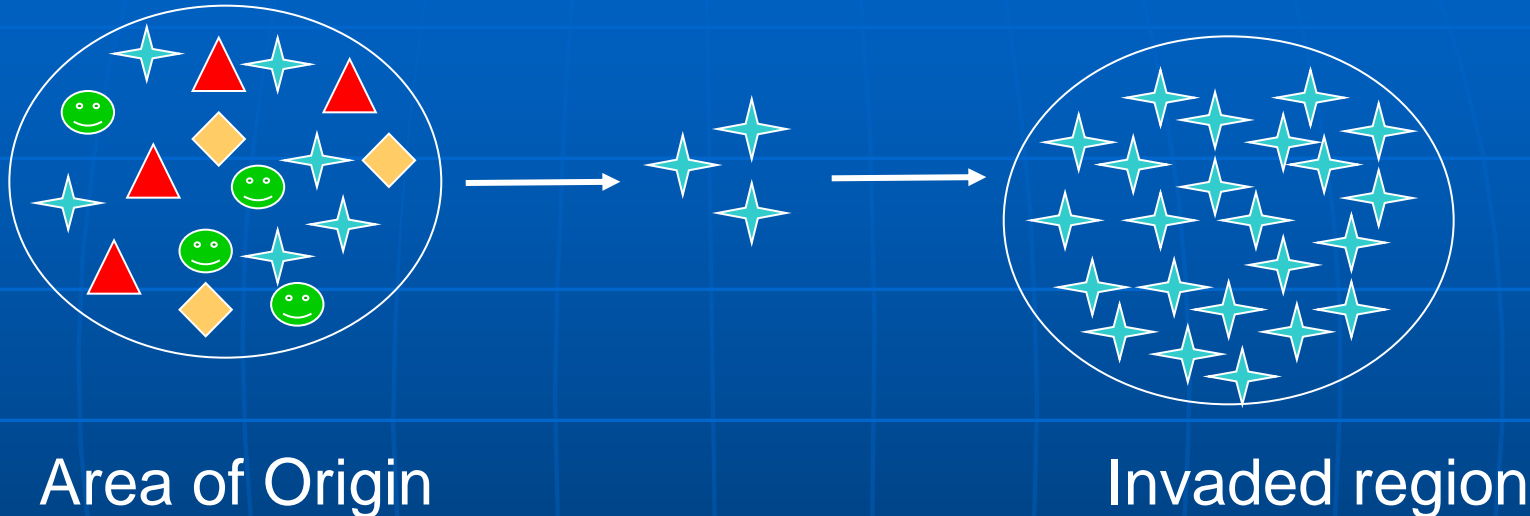


EU project CONTROCAM
(2001-2006):

- Expeditions to China, northamerica, Japan to look for *Cameraria ohridella* without succes

Genetics of Invasion

Introduction = loss of genetic diversity (bottlenecks)



Hypothesis (Balkan origin):

Genetic diversity should be higher in the Balkans than in central Europe (invaded area)

Strategy

- Mitochondrial DNA
- Microsatellites
- Herbaria and archival DNA
 - Minibarcoding & microsatellites

Is genetic diversity higher in the Balkans? Mitochondrial DNA

→633-bp fragment of the mitochondrial COI (barcode fragment)

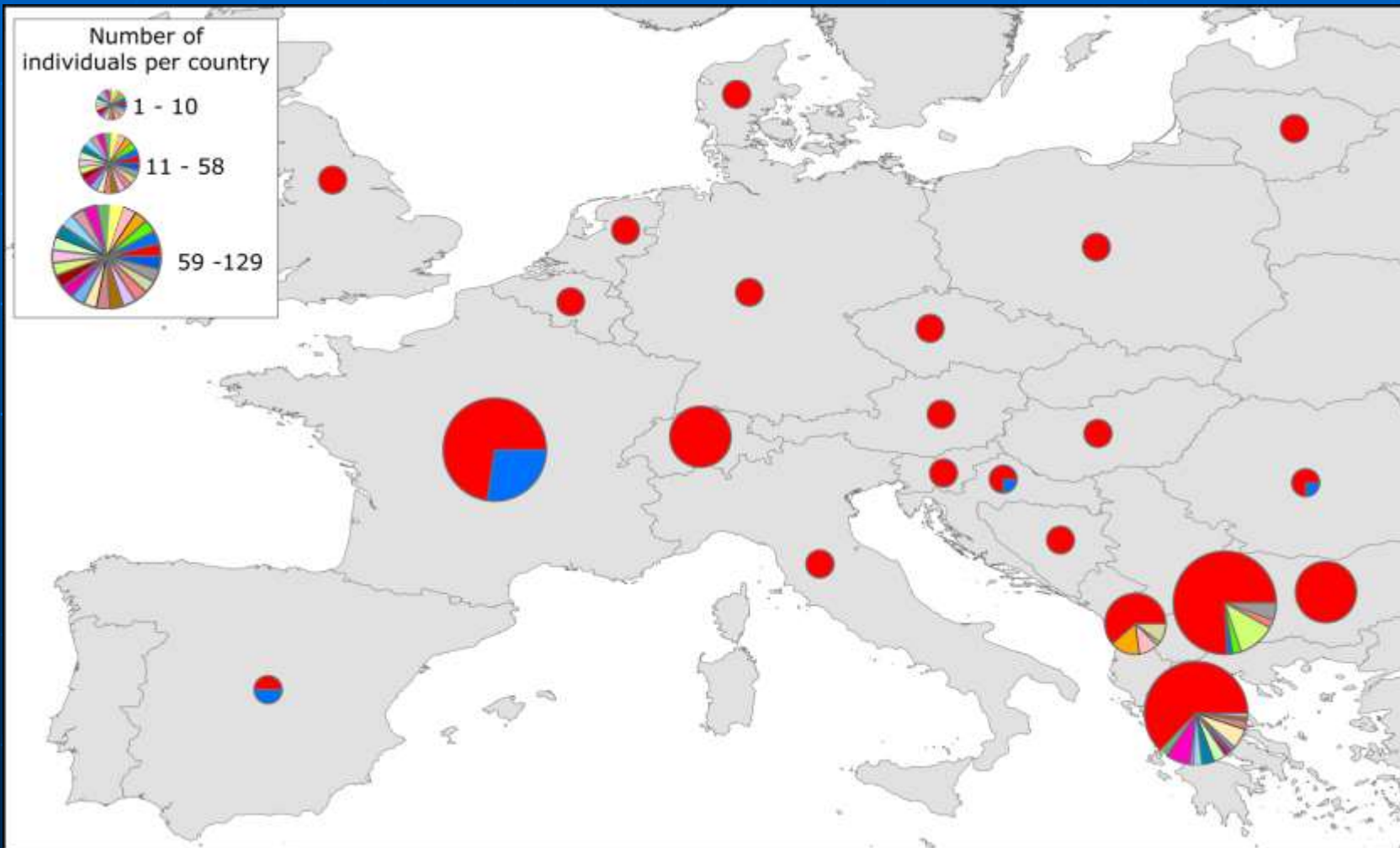
→486 *C. ohridella*: 351 individuals from 78 parks and 135 individuals from 9 natural stands

→88 localities from 22 countries



Haplotype diversity in Europe

-25 haplotypes Only haplotype “A” is dominant (at a frequency of 67–100%), not only throughout its expanding range in Europe but, intriguingly, also in about 90% of Balkan relict horse-chestnut sites



Romain Valade MSc

Valade et al (2009)
Molecular Ecology

Haplotype diversity higher in natural stands

Median number of haplotypes per locality :

Parks: 1 (0.0001) (n = 78 localities)

Natural stands: 2 (3.25) (n = 9 localities)

Haplotype diversity (\pm S.D.):

Parks: 0.280 ± 0.029 (n = 8 haplotypes)

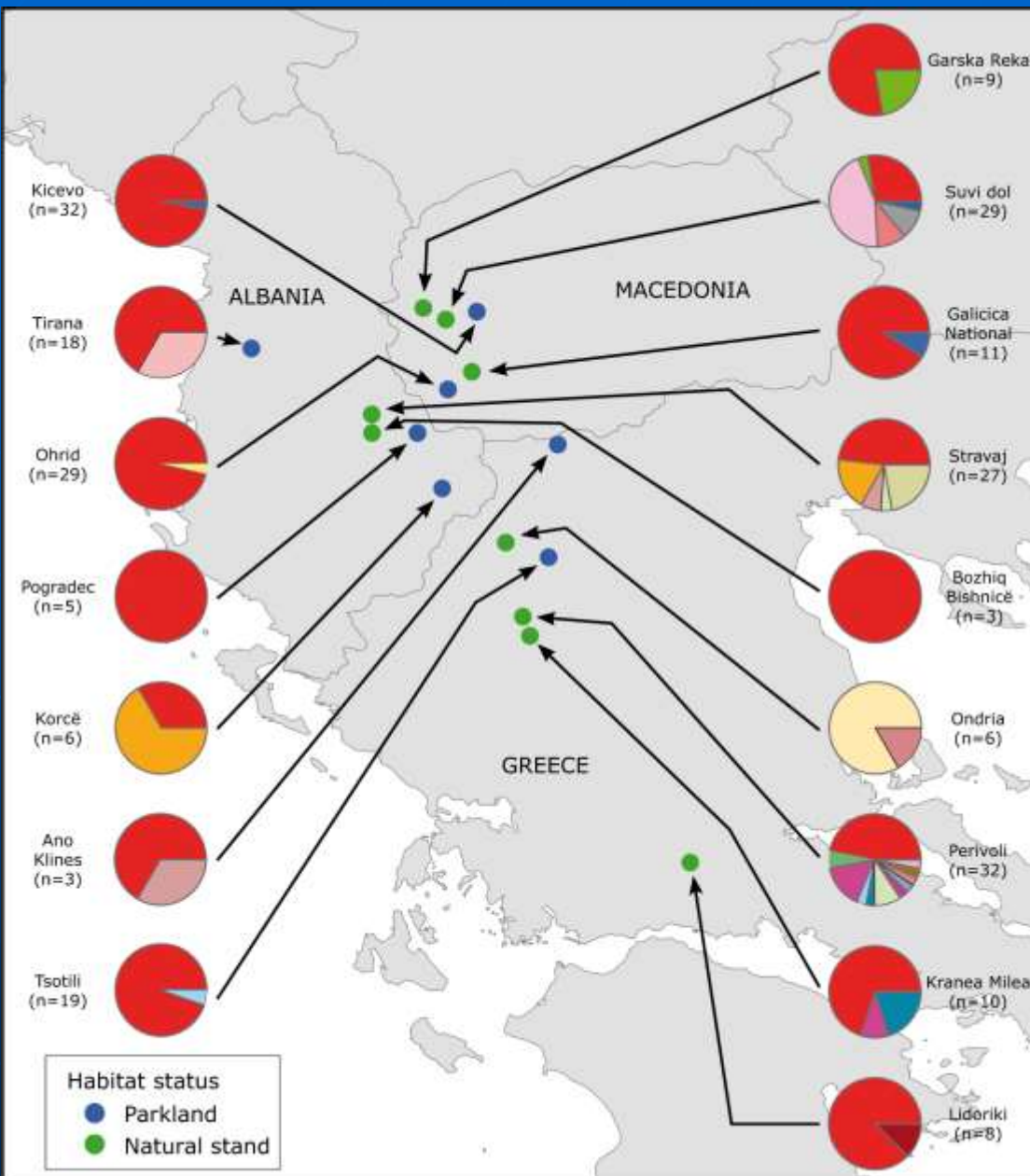
Natural stands: 0.717 ± 0.041 (n = 23 haplotypes)

Nucleotide diversity (\pm S.D.):

Parks: 0.00087 ± 0.0001 (n = 351 sequences)

Natural stands: 0.00265 ± 0.00025 (n = 135 sequences)

Haplotype diversity in Balkans



Parks: 112 individuals

Natural stands: 135 individuals

Parks: 0.232 ± 0.053 (n=7 haplotypes)

Natural stands: 0.717 ± 0.041 (n = 23 haplotypes)

11 haplotypes in Perivoli!!!

One major invasive haplotype

Most COI haplotypes of *C. ohridella* are either endemic (14) or very narrowly distributed (three) among natural horse-chestnut populations



Twenty-five mitochondrial cytochrome c oxidase sub-unit 1 [COI] haplotypes – each differing by at least one single nucleotide mutation

Microsatellites

Six Microsatellite loci

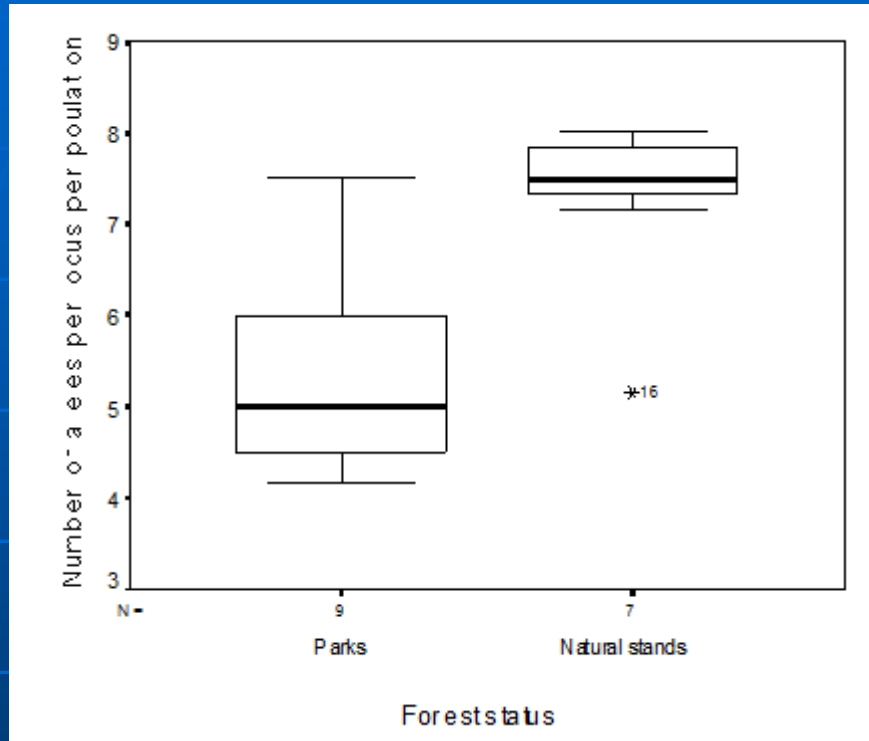
- 480 individuals genotyped from 16 populations:
 - 280 individuals from 9 parks (Albania, Austria, France, Germany, Greece, UK)
 - 200 individuals from 7 natural stands (Albania, Greece, Macedonia)



Neus Mari MSc

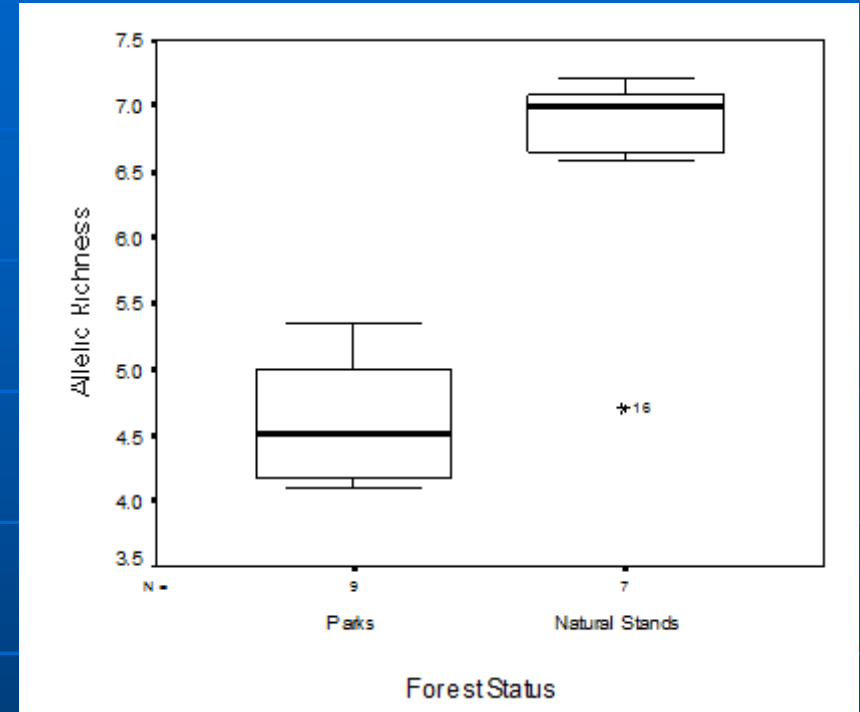


Higher genetic diversity in natural stands



Mean number of alleles per locus per population

Parks, NA= 5 (n = 54 loci)
Natural stands, NA= 7.5 (n = 42 loci)
(Kruskal-Wallis test: $P = 0.007$)



Allelic Richness per locus per population

Parks, AR= 4.51
Natural stands, AR= 7
($P = 0.004$)

Conclusions: molecular markers

- Both mitochondrial and nuclear data show a decrease in diversity away from the Balkans and in artificial plantations.
- Also consistent with a Balkan origin, a higher frequency of rare or “private” alleles was found in natural stands
- DNA Barcodes are an effective tool to trace the origin and spread of an invasive insect

Why the invasion was delayed until two decades ago?

- (H_0) haplotype "A" was historically widespread and abundant among natural sites, and thus is most likely to have spread to artificial plantings;
- (H_1) haplotype "A" was, like other haplotypes, rare and very localised in the Balkans but has only recently become highly invasive – even (re)-invading natural horse-chestnut stands.

HERBARIA: a historical approach

No pre-1984 *C. ohridella* specimens conserved in entomological

Given a quarter-century-long record of outbreaks, a historical trace of *C. ohridella* should exist in herbarium collections of *A. hippocastanum*.

David Lees (Studium fellow)

Walter Lack (Botanical Museum, Freie
Universität Berlin)



- Visit six historic herbaria (Kew, London, Berlin, Vienna, Paris, Jena)
- 71 archival sheets representing 17 different localities (natural stands)
- Temporal series: Karitsa, 1936, 1974, 1981, 2008

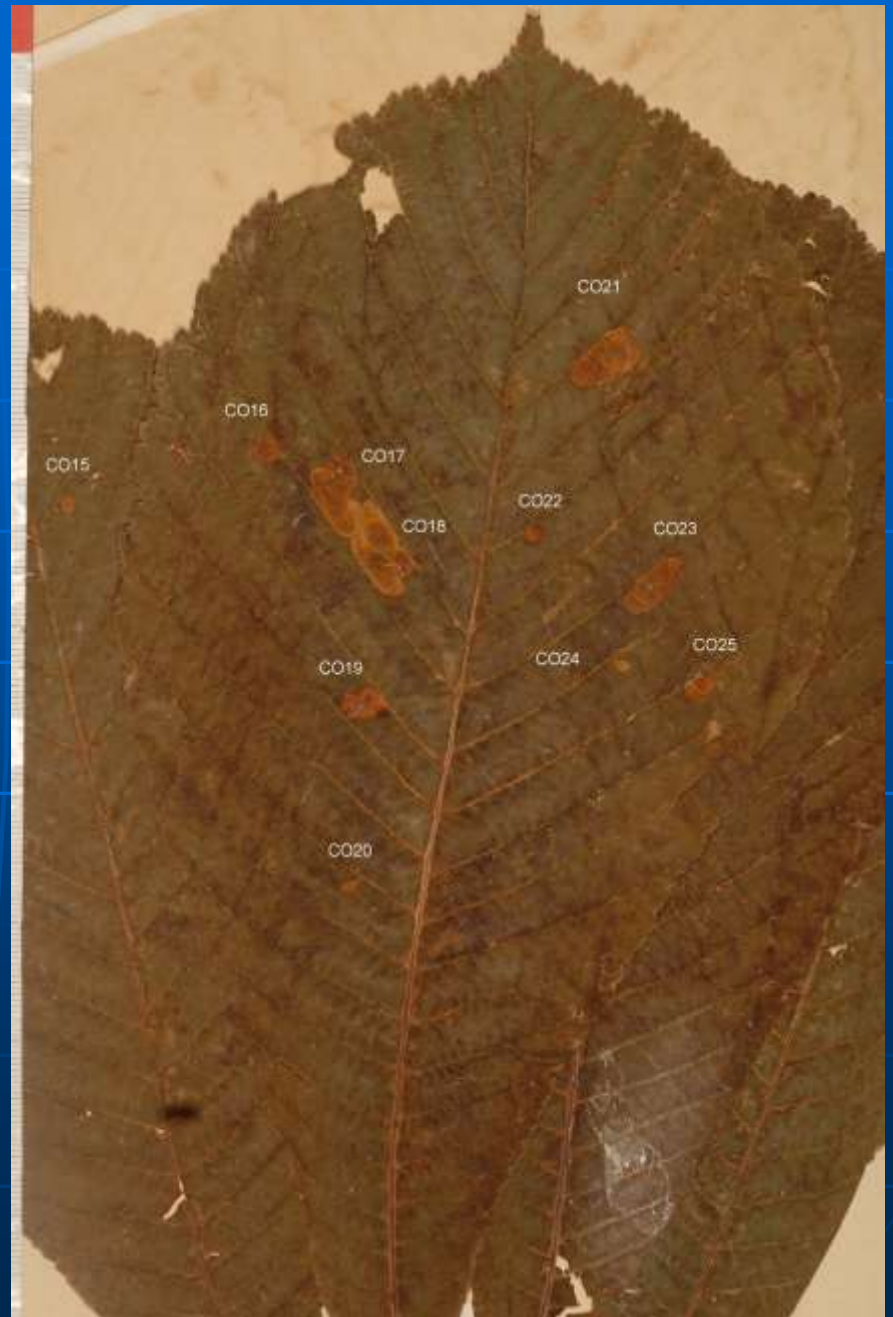
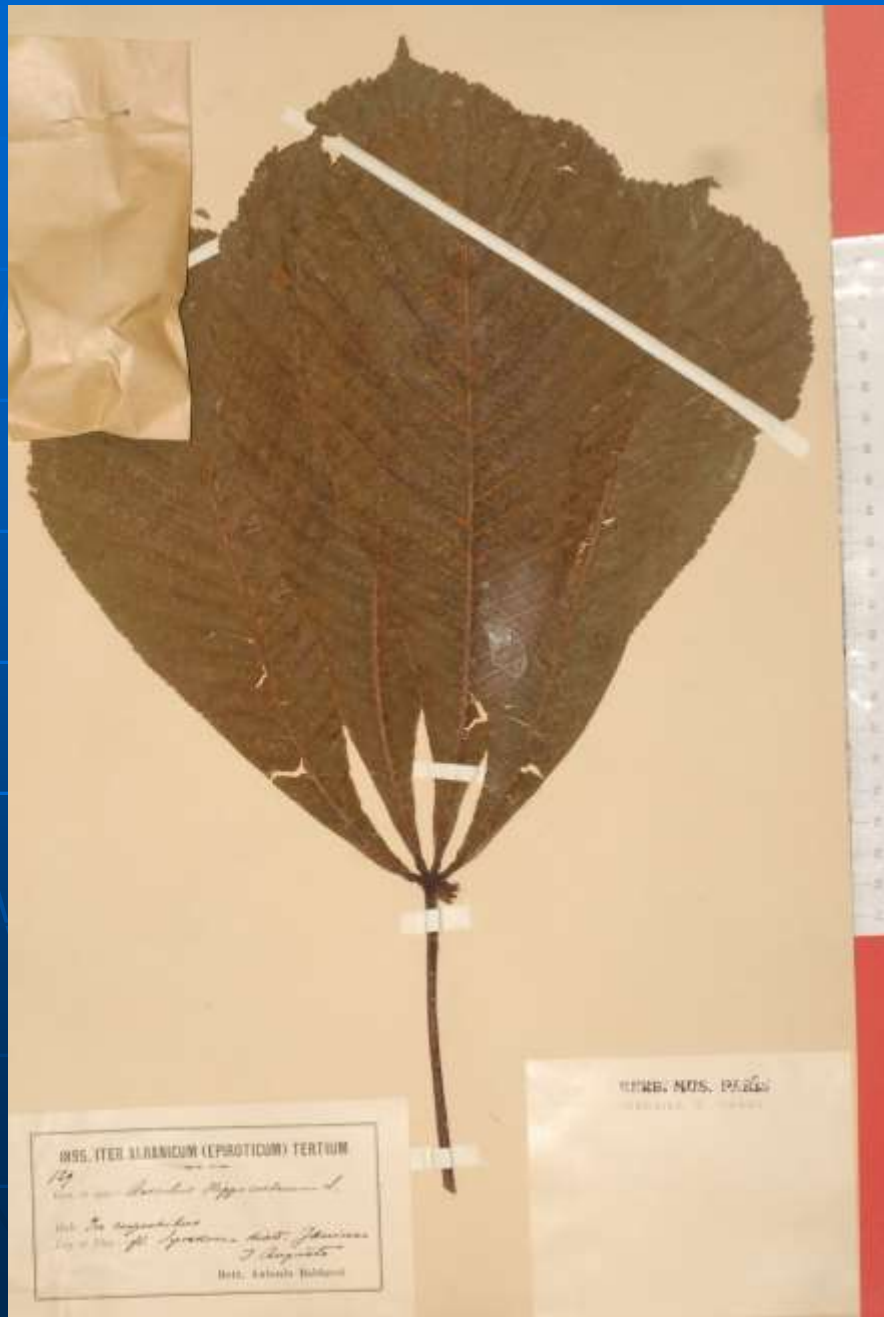


Historic collections with mines

- 32 sheets out of 71 had leaf mines with larvae/pupae inside (natural stands in Albania & Greece) date from 1981 back to 1879
- No mine in herbaria from artificial *Aesculus* collections outside Balkans (back 1737)

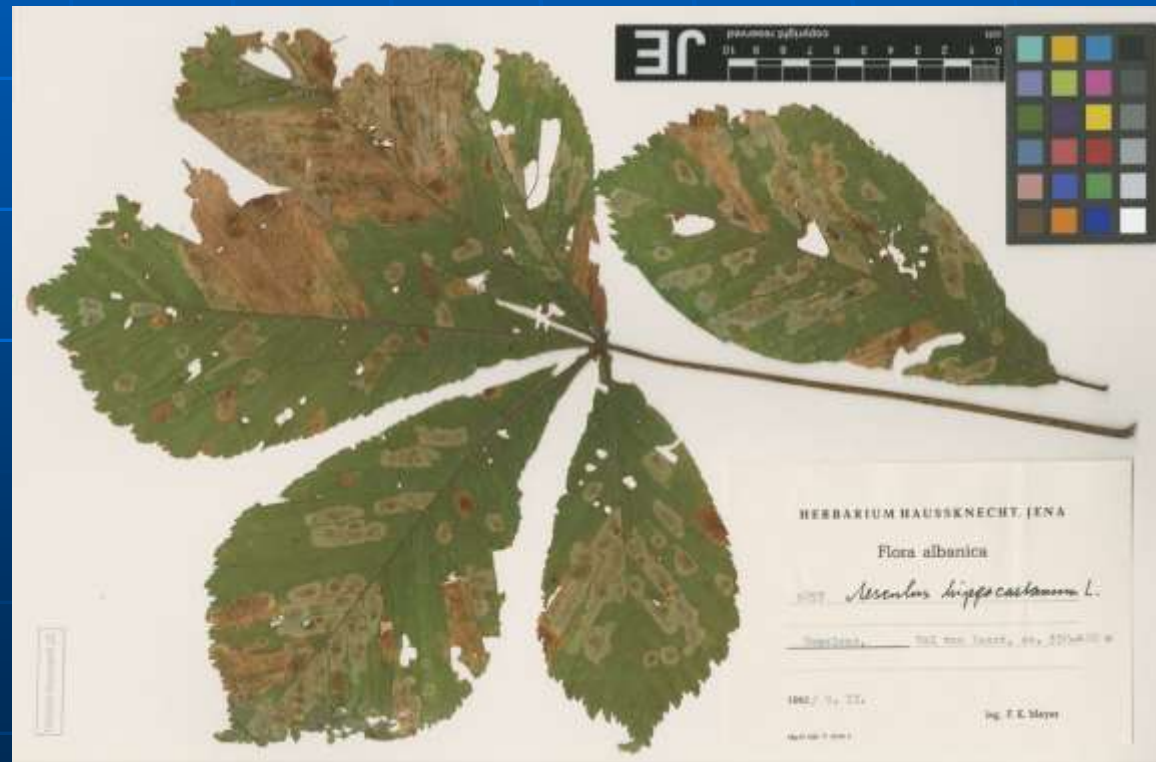


Botanists prefer to display “perfect” specimens of leaves



Leaf-mine density data

- Most archival collections, mine densities were low but increase June-september (season of collection)
- But up to 32 mines per leaflet in 1961 (5-7 leaflets per leaf) similar to late-summer damage in 21st century European parks



Parasitoid: Pnigalio (from 1964)



- 54 archival DNA extractions
- Five COI minibarcode primer pairs specially designed: 136-182 bp long fragments



1a



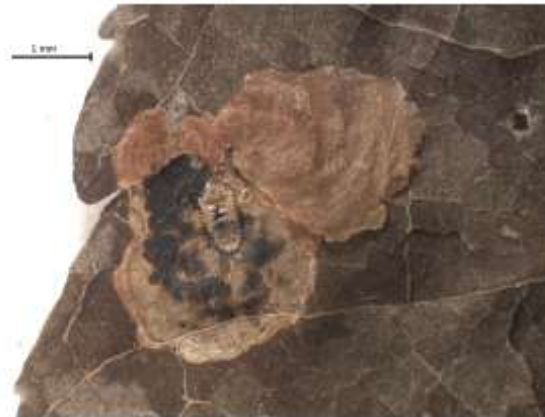
1b



2a



2b



3a



3b

Minibarcodes & Microsatellites

- COI: 10 sequences obtained from 1936 to 1981.
 - Three unique haplotypes
 - Microsatellites: Out of 6 pair primers 2 amplified:
 - 30 out of 54 extractions amplified (back to 1879)
- Private alleles.

Conclusions: Historical Herbaria

- Archival sequences confirm identification of *C.ohridella*, setting its history in Europe back over a century, revealing previously undocumented alleles
- Herbaria show outbreaks occurred in the early 1960's long before the discovery of the species: natural phenomenon?
- Even a pest may have unique, isolated and endangered populations in the area of origin worthy of conservation
- Major oversight of herbaria by entomologists working for over two decades to crack the origin of *C. ohridella*

Looking for natural enemies in the Balkans: *Pediobius saulius* as potential biocontrol agent?



Antonio Hernandez Lopez (Postdoc EFPA INRA)

Pediobius saulius (Walker, 1839)

- Pupal generalist parasitoid:

76 espèces Lepidoptera: Gracillariidae (59 espèces)

20 espèces de Hymenoptera et 11 Coleoptera

Principal parasitoid of *C. ohridella* in the Balkans

Host Race specific to *C. ohridella* in the Balkans?



Phyllonorycter spp.

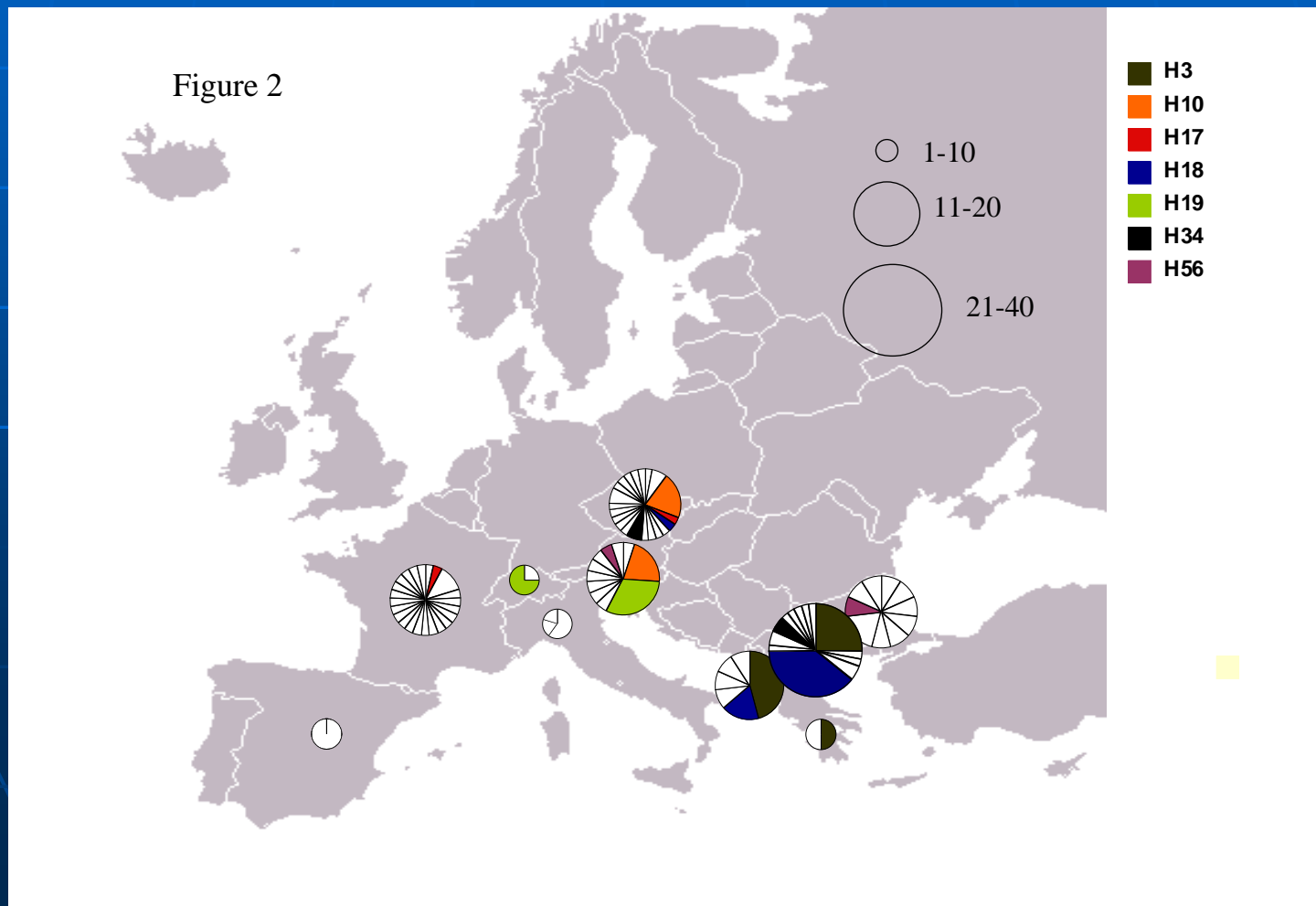


Orchestes spp.



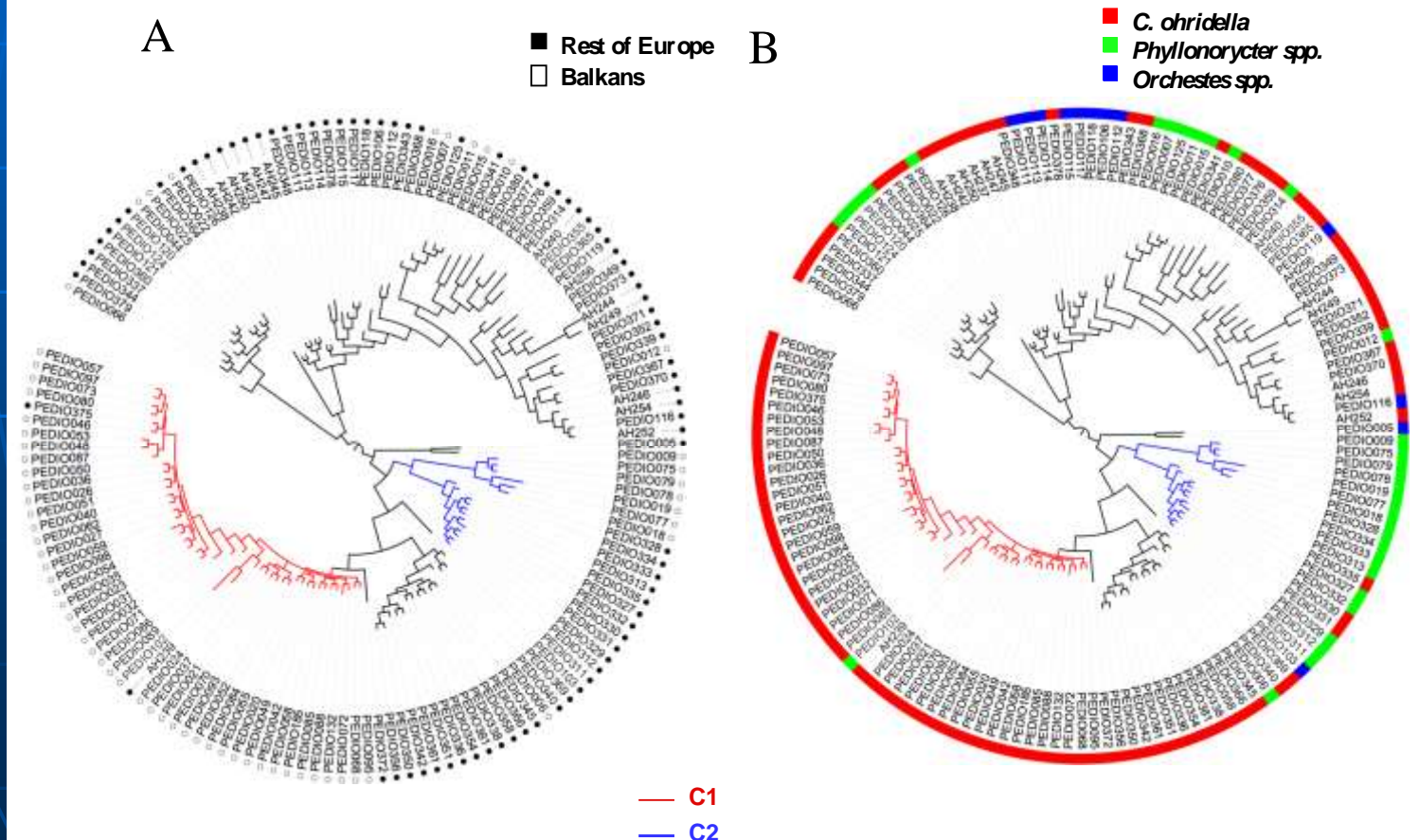
C. ohridella

- 83 haplotypes (146 individuals, 38 localities, 10 countries)
- A major haplotype (H18)
- only seven haplotypes are present in more than one country
- Divide between Balkan and European haplotypes, as only three haplotypes (H18, H34, H56) are present in both geographic regions



- Most of the Balkan individuals (45 out of 63) are grouped in a monophyletic clade (C1)
- All individuals in C1 apart from one were reared from *C. ohridella*

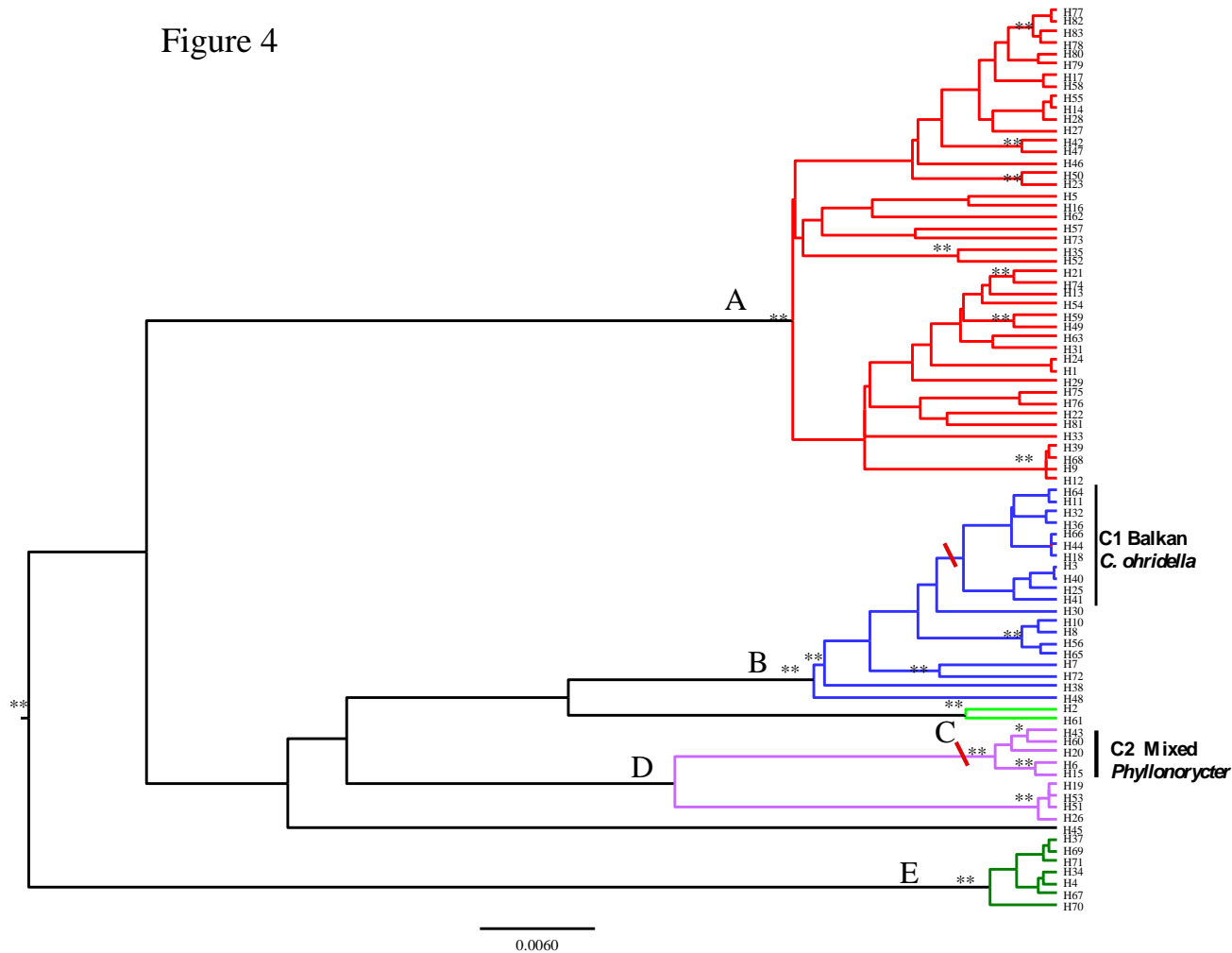
Figure 3



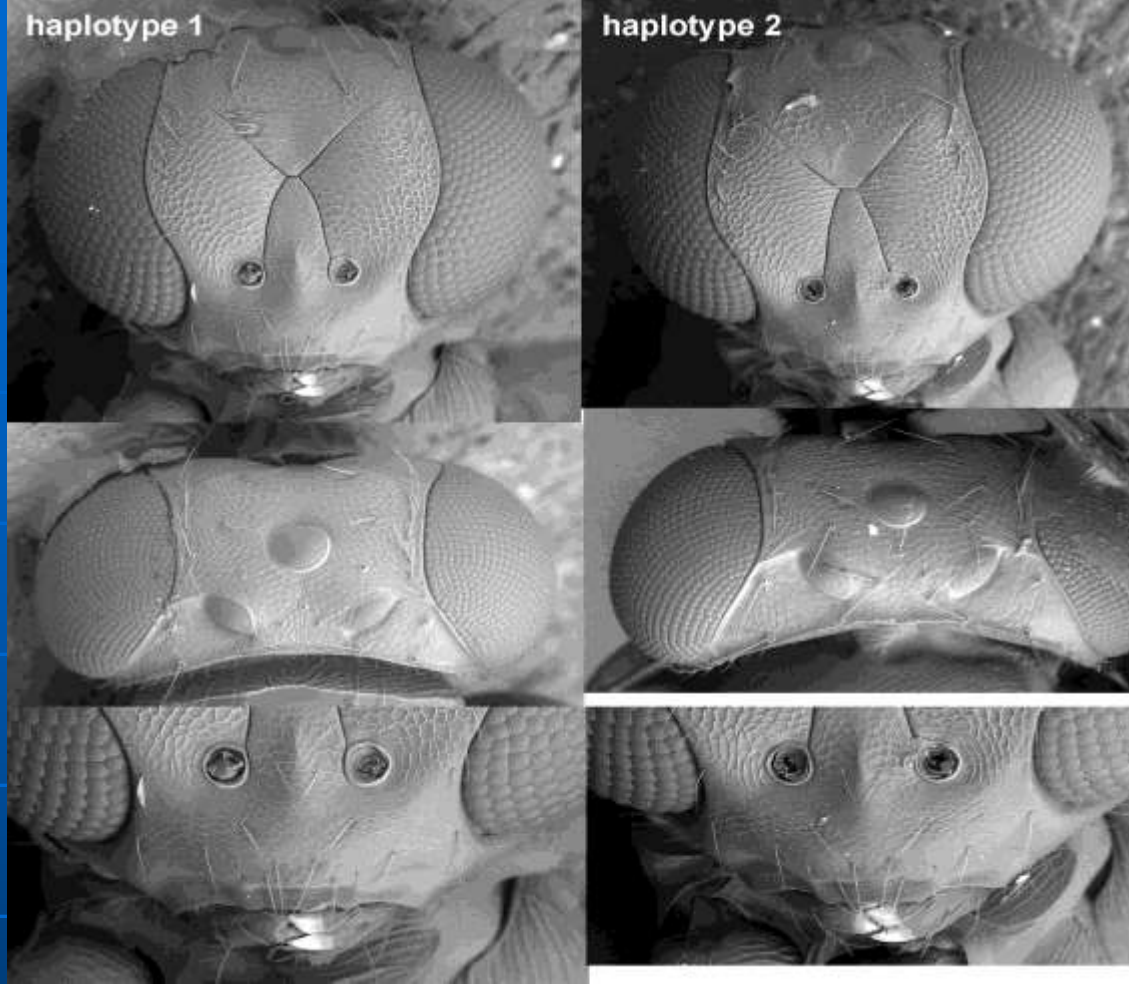
High mitochondrial divergence (up to 7.6%)

GMYC model revealed five independent coalescent ML clusters

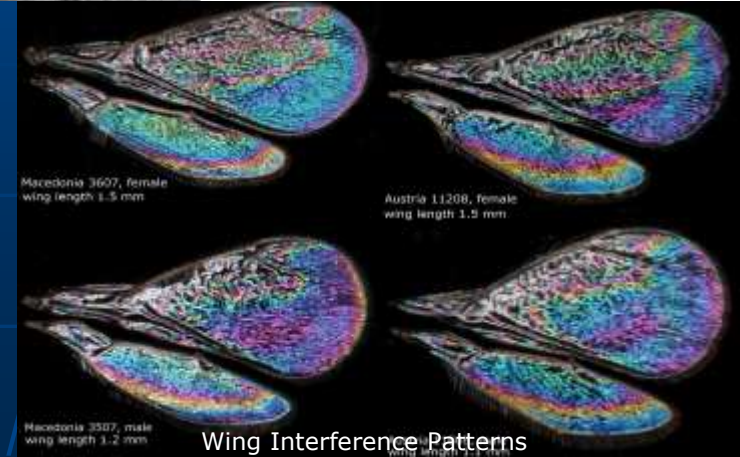
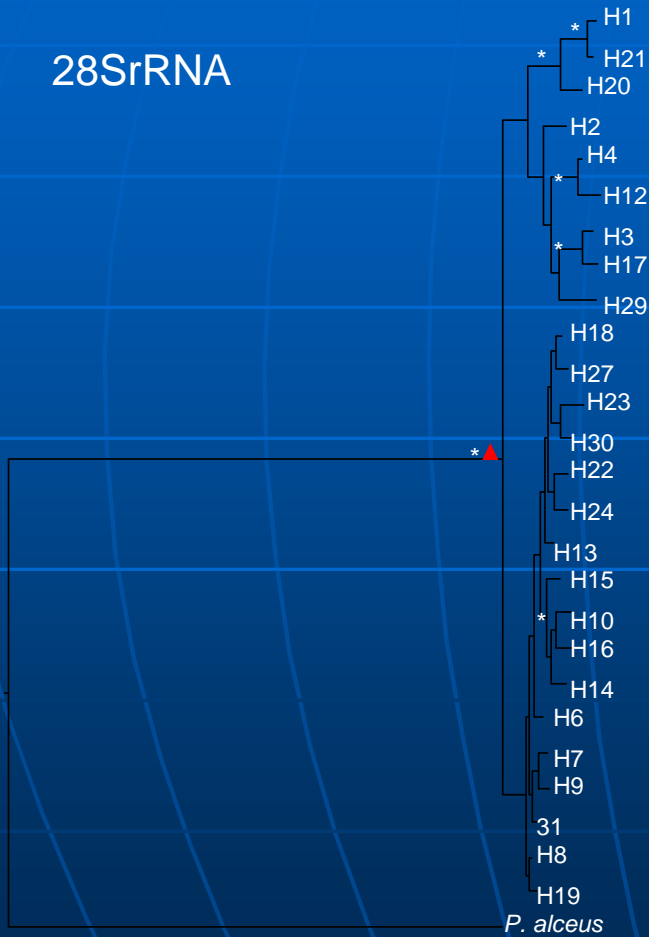
Figure 4



But neither nuclear (28S, ITS2) nor morphological differentiation



28SrRNA



Conclusions: natural enemies

- Much higher intraspecific genetic diversity than typical values obtained for other Hymenoptera
- High divergence among haplogroups yet no differentiation for nuclear DNA (28SrRNA, ITS2) and morphology
- Haplogroup (C1) associated mainly to *C. ohridella* in the Balkans: potential biocontrol candidate?

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