



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

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Evolutionary Ecology of Invasion

- Invasive species: Rapid expansion in a new environment, preadaptation, phenotypic plasticity.

- To understand the sucess of invasive species and establish biocontrol:
  - Determine the number of introductions
  - Level of genetic diversity. Bottleneck?
  - Invasion pathways
  - Sources (populations/regions) of invasion

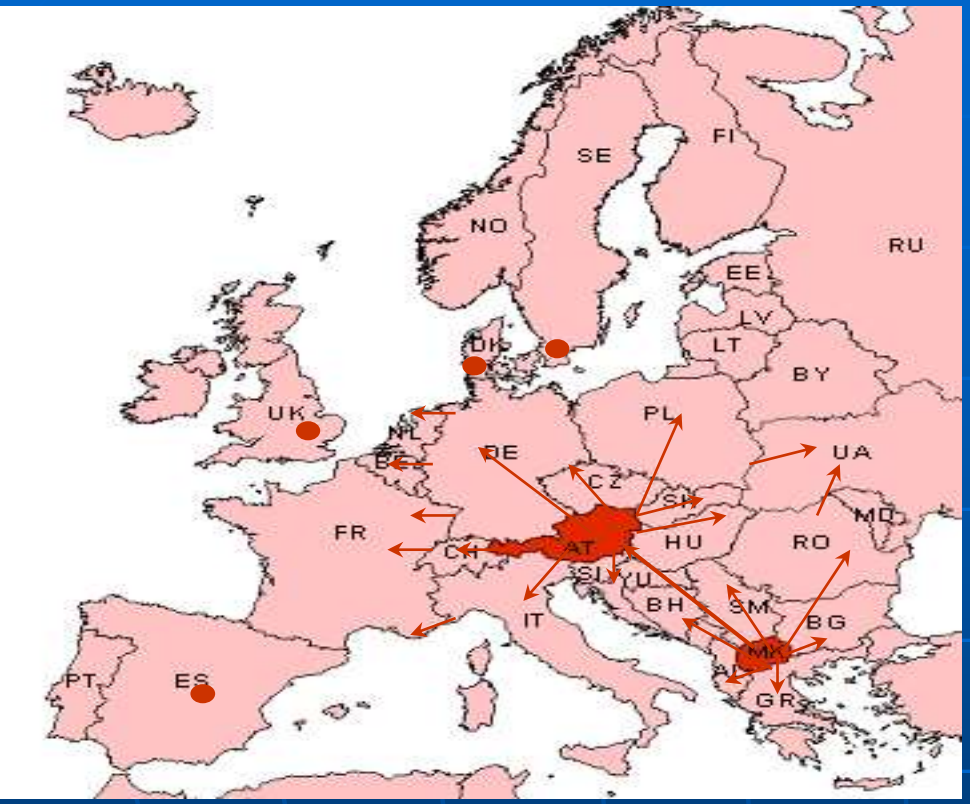
Identifying areas of origin

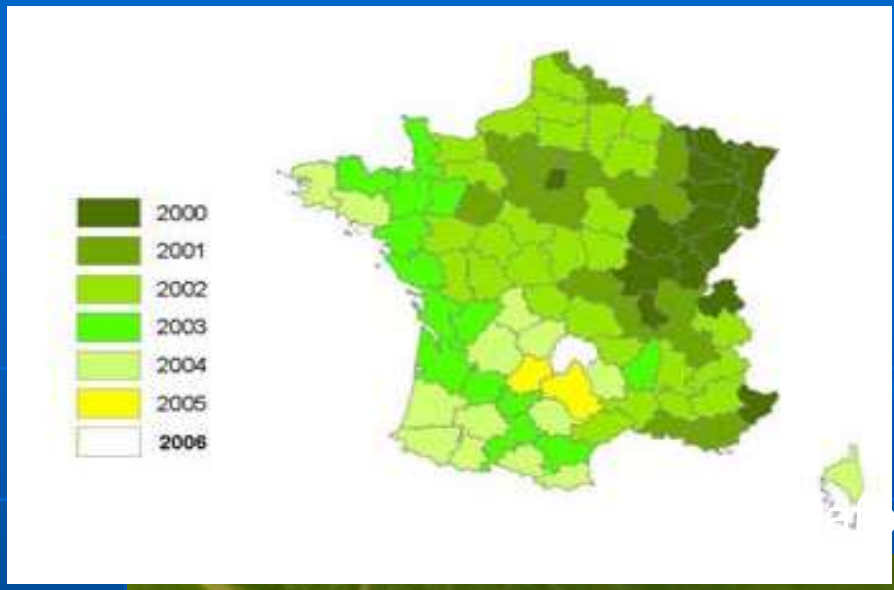
Out of 1514 exotic invertebrate species established in Europe , 221 (14.6%) have an unknown origin (Roques et al 2010)





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)



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

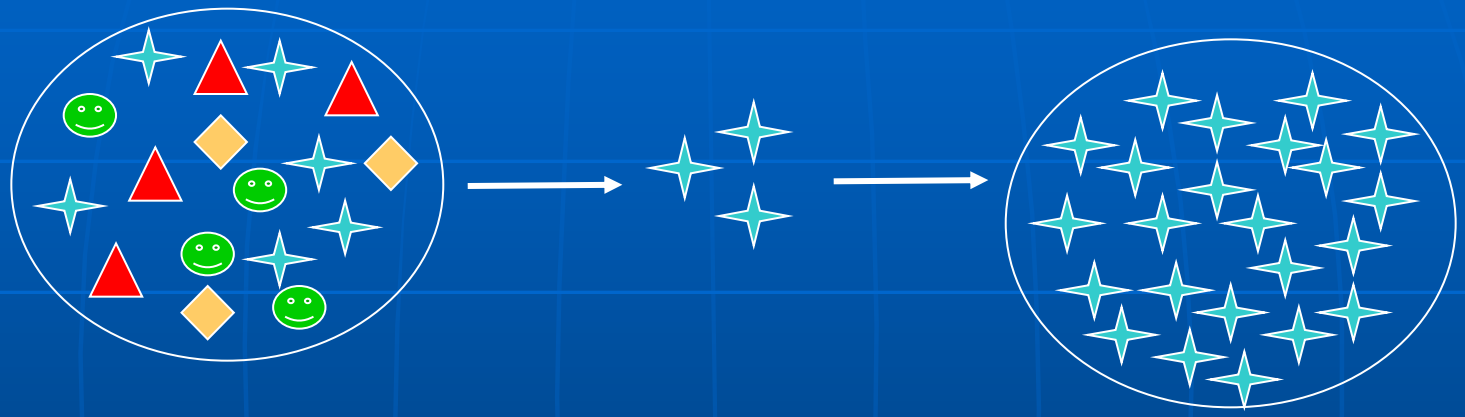


EU project CONTROCAM (2001-2006):

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

Genetics of Invasion

Introduction = loss of genetic diversity (bottlenecks)



Area of Origin

Invaded region

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)

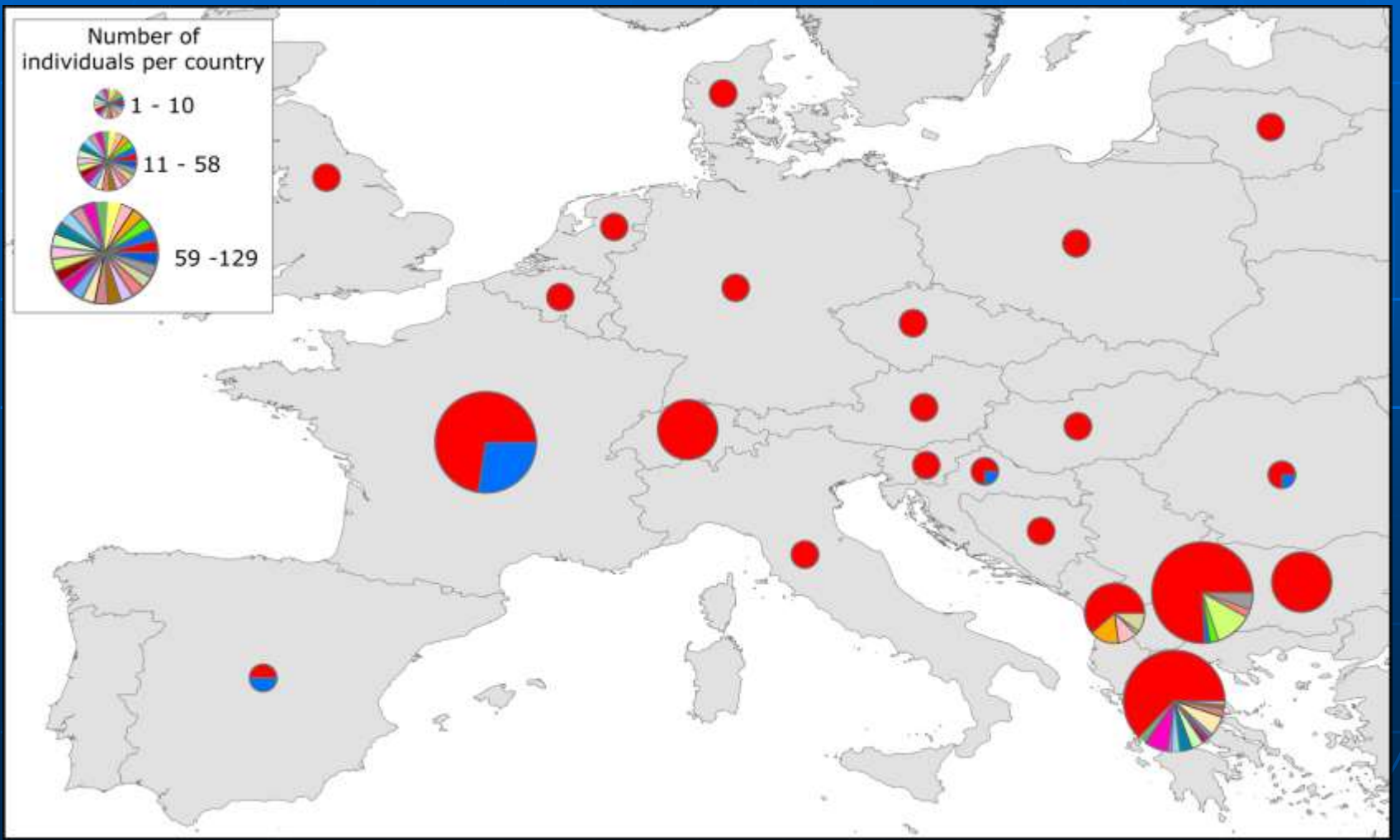
→486 *C. ohridella:* 351 individuals from 78 parks and 135 individuals from 9 natural stands

→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 (± S.D.):

Parks: 0.280 ± 0.029 (n = 8 haplotypes)

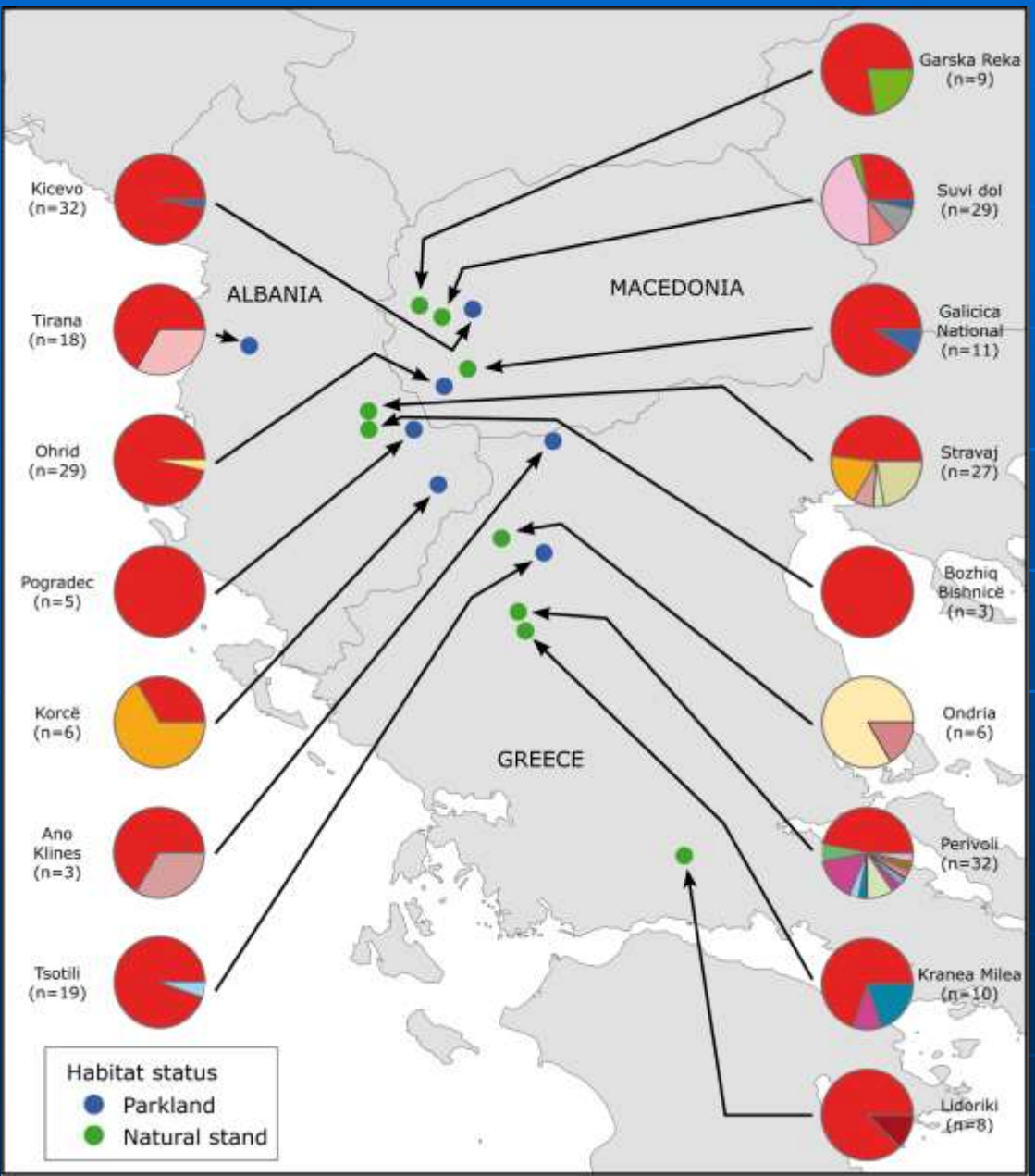
Natural stands: 0.717 ± 0.041 (n = 23 haplotypes)

Nucleotide diversity (± 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

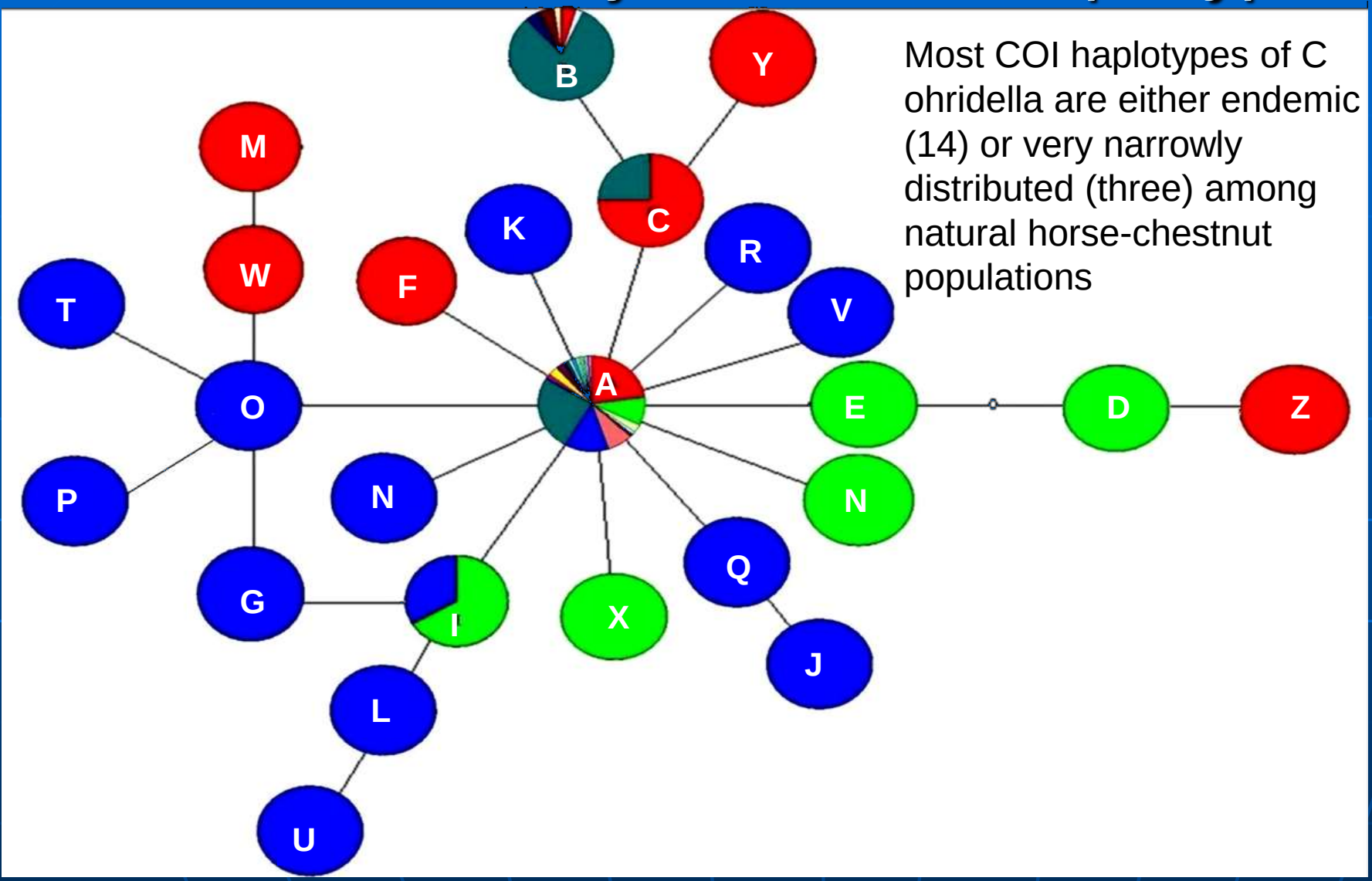
Natural stands: 135 individuals

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

Macedonia
Albania
Slovenia
Croatia
Bosnia
Bulgaria
Greece
France
Spain
Germany
Switzerland
Poland
Austria
Hungary
Czech Republic
Italia
Belgium
Netherland
Denmark
Romania
England

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

Microsatellites



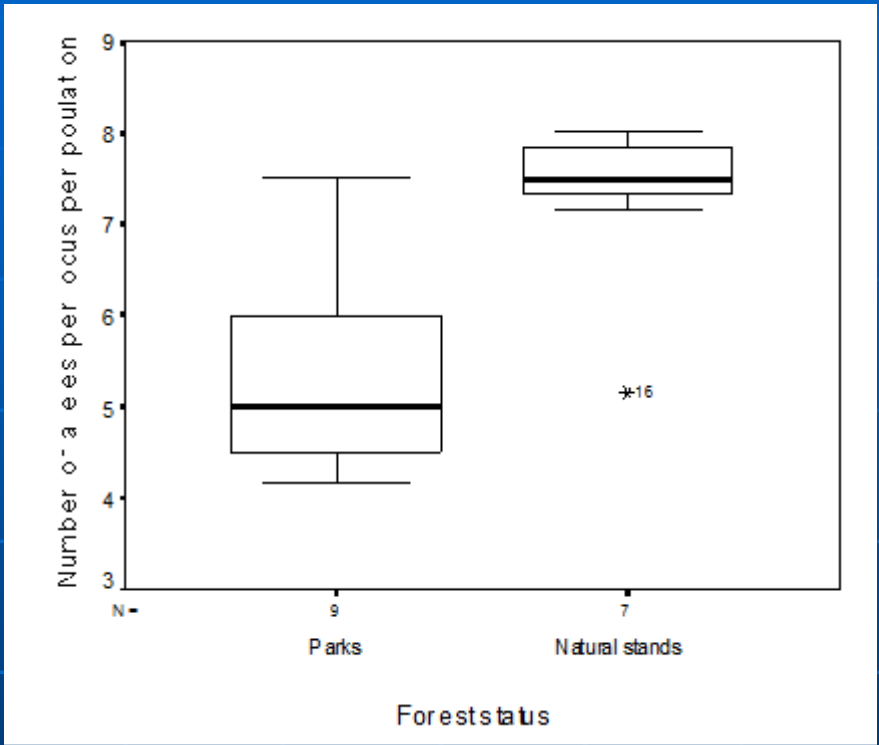
Neus Mari MSc

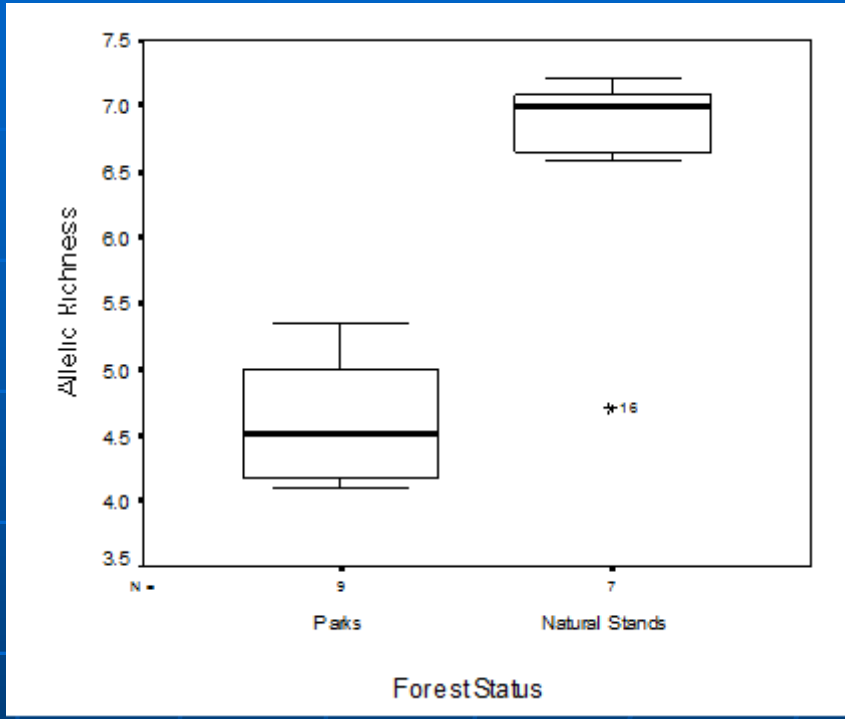
- Six Microsatellite loci
  - 480 individuals genotyped from 16 populations:
    - 280 individuals from 9 parks (Albania, Austria, France, Germany, Greece, UK)
    - 200 individuals from 7 natural stands (Albania, Greece, Macedonia)



Mari Mena et al., 2008 European Journal of Entomology

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_o) 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

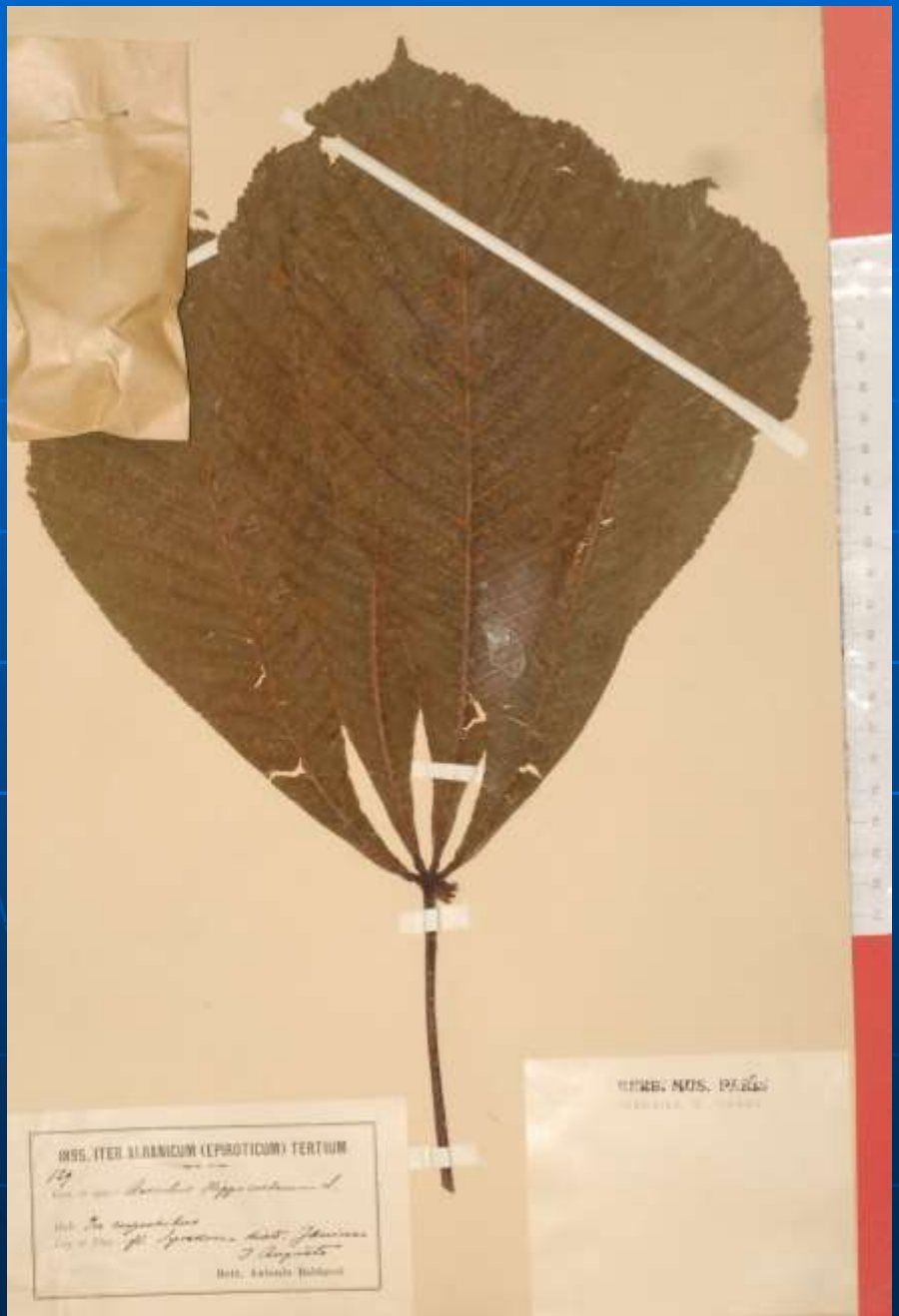
- 32 sheets out of 71 had leaf mines with larvae/pupae inside (natural stands in Albania & Greece) date from 1981 back to 1879

- No mine in herbaria from artificial *Aesculus* collections outside Balkans (back 1737)





Botanists prefer to display “perfect” specimens of leaves





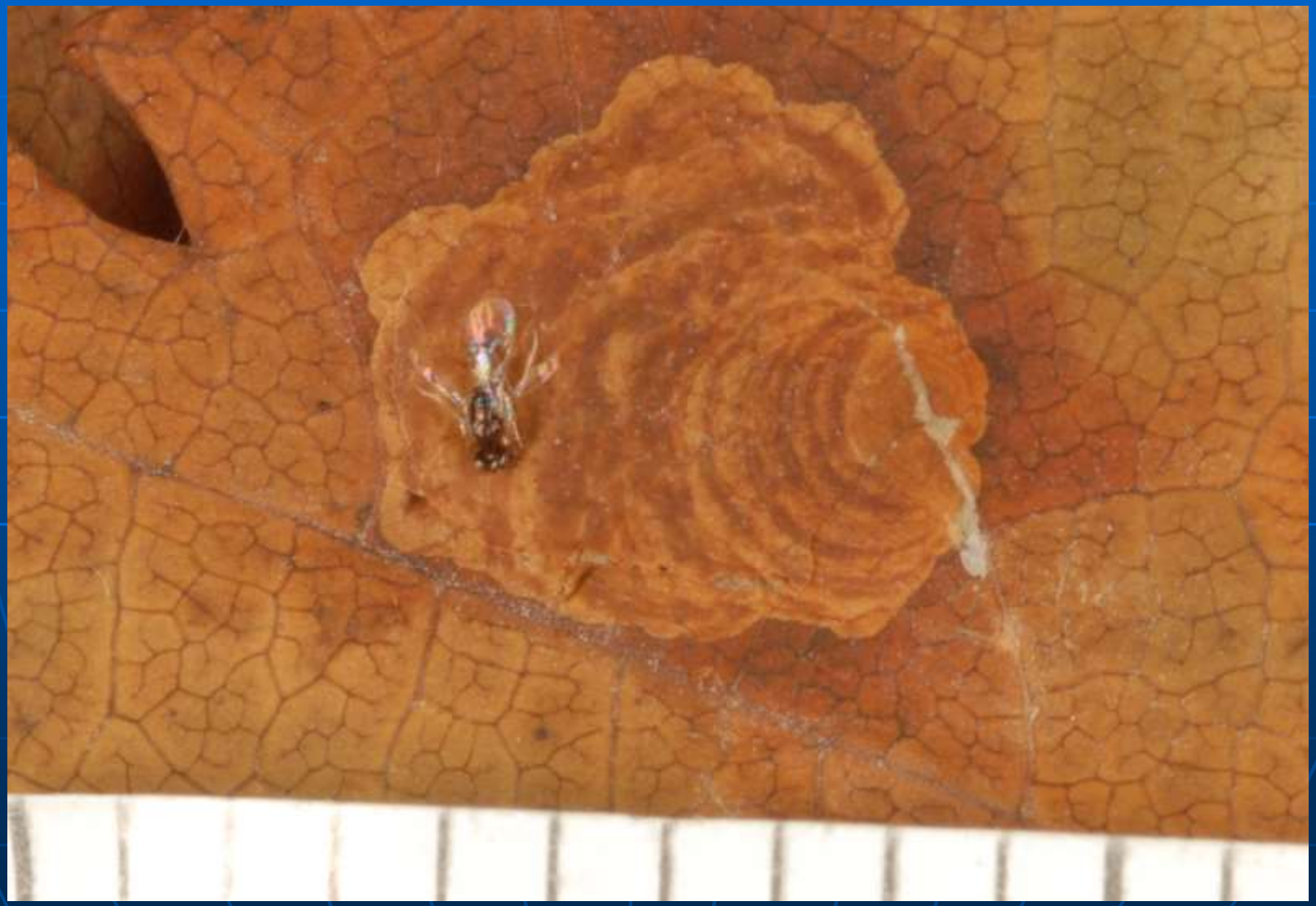
Leaf-mine density data

- 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 & Microsatelites

- COI: 10 sequences obtained from 1936 to 1981.
  - Three unique haplotypes
- Microsatelites: 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*

Lees et al (2011) Frontiers in Ecology and Evolution

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.

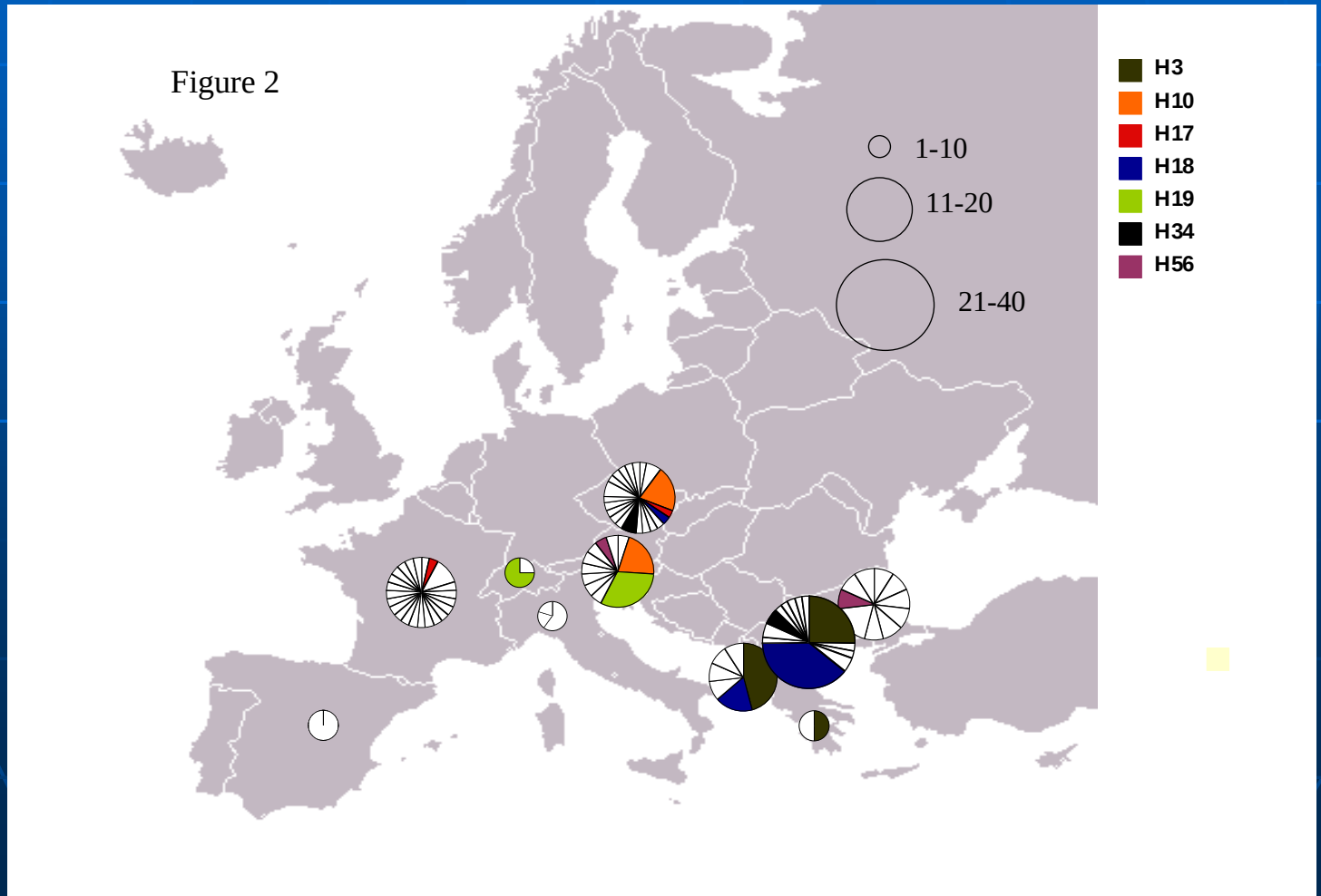


Orchestes spp.



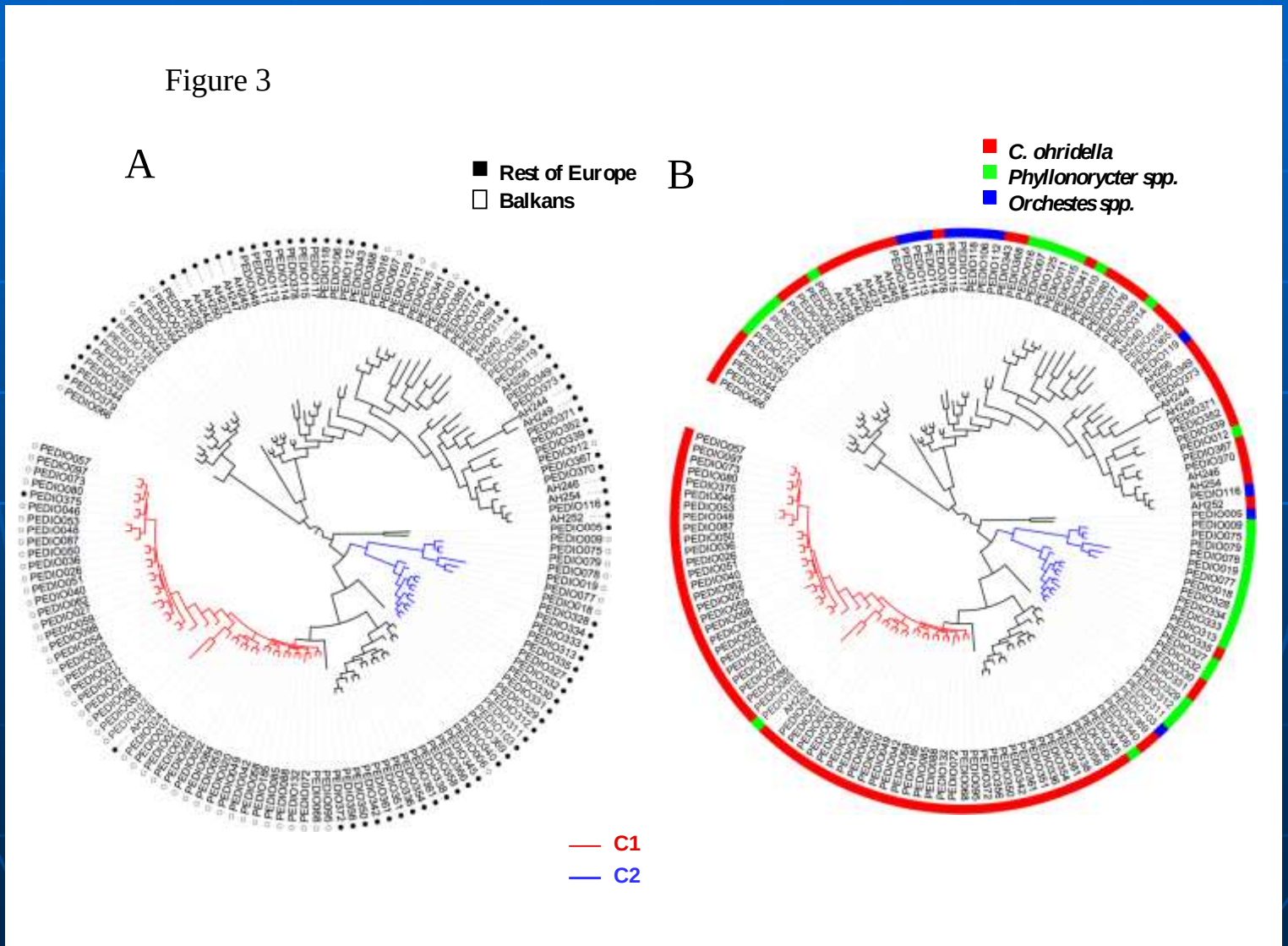
C. ohridella

→ 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



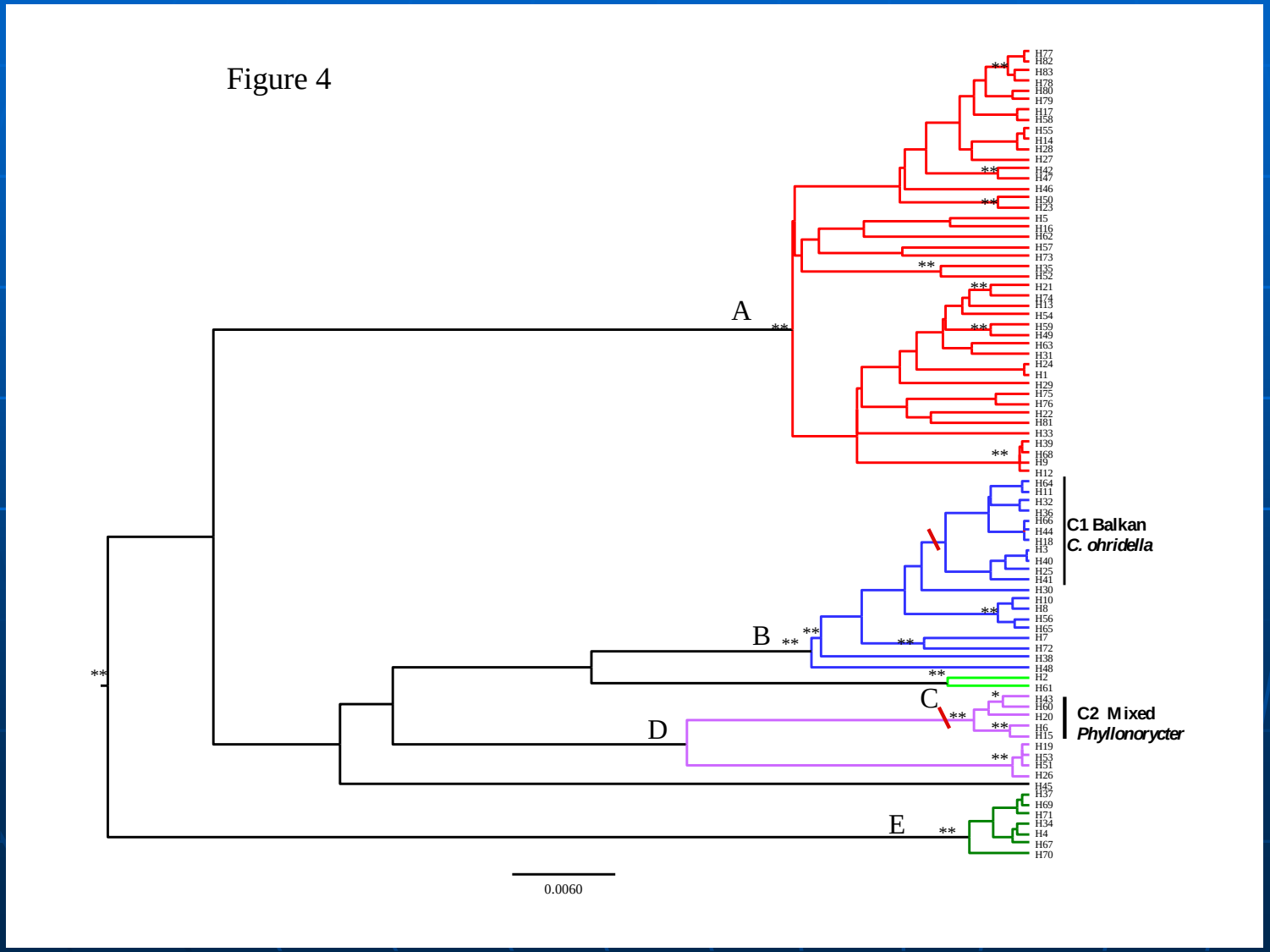
- Most of the Balkan individuals (45 out of 63) are grouped in a monophyletic clade (C1)
- All individuals in C1 apart rom one were reared from *C. ohridella*

Figure 3

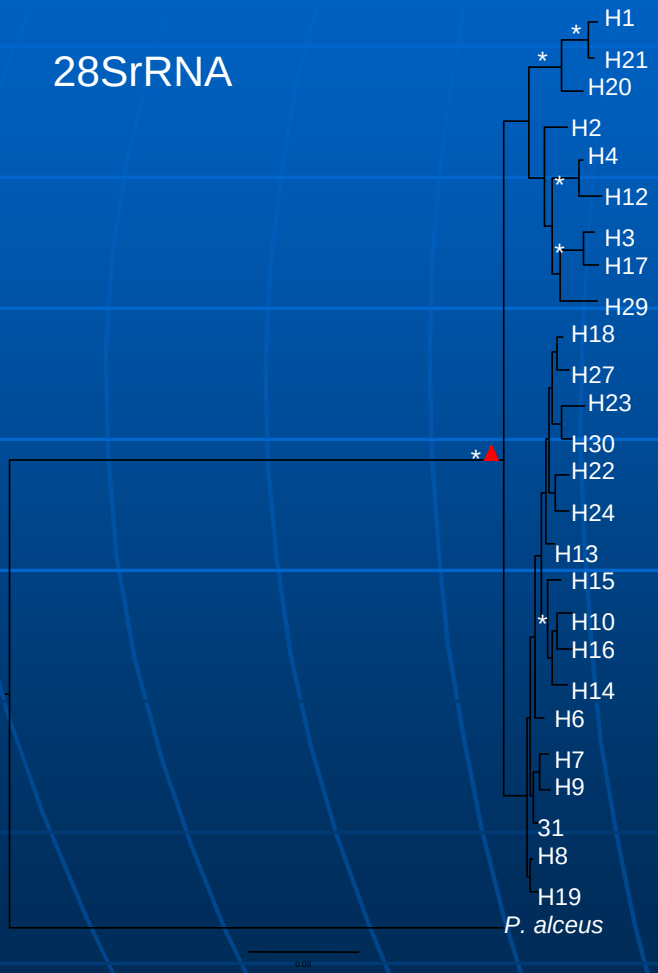


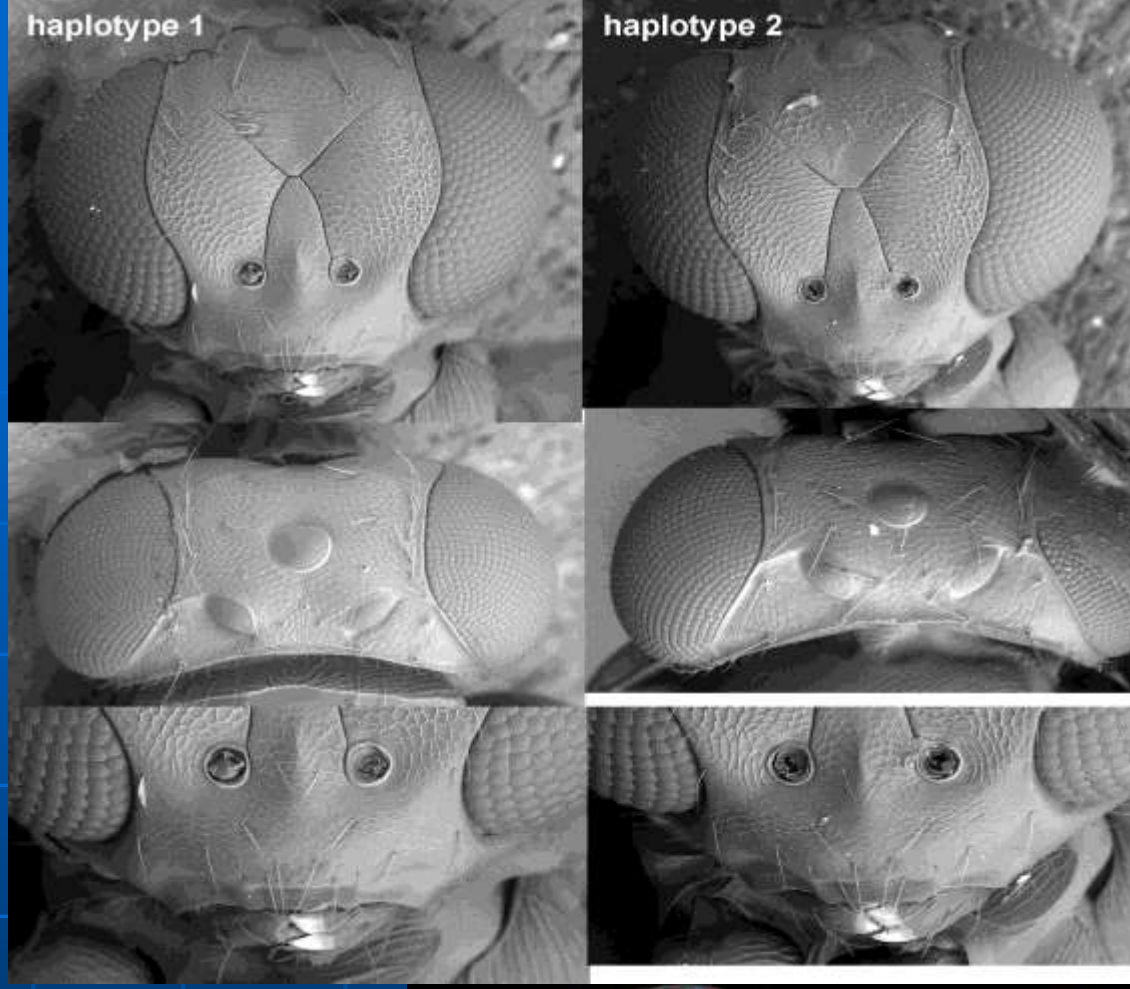
High mitochondrial divergence (up to 7.6%)

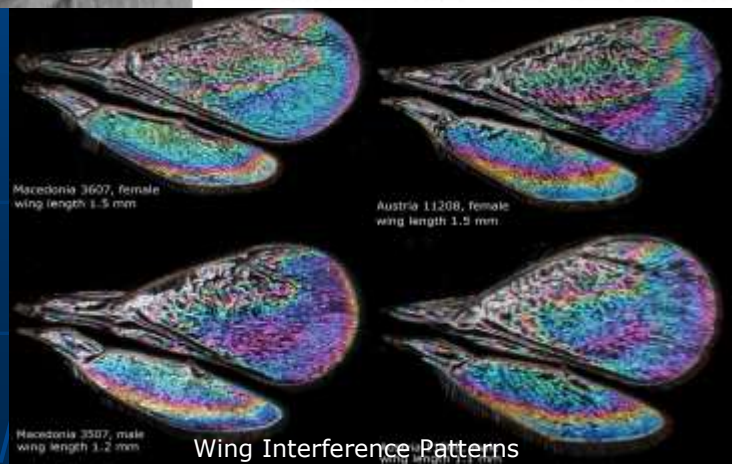
GMYC model revealed five independent coalescent ML clusters



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







Wing Interference Patterns

Hernandez et al (in press) Evolutionary Applications

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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