Populations research of *Hylurgus ligniperda* and *Hylurgus micklitzi* in Eurasia (mainly in South of France)

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4th IFOPE Conference on pest invasions in Eurasian Forests

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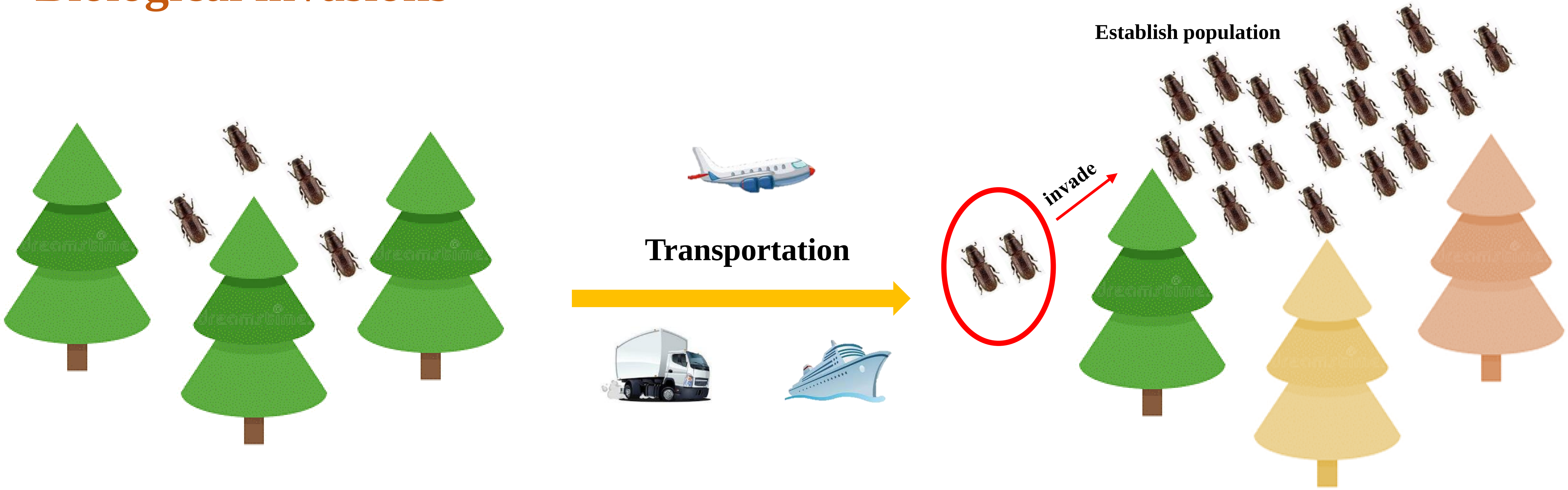
INRAE URZF UNITÉ DE RECHERCHE DE ZOOLOGIE FORESTIÈRE BEIJING FORESTRY UNIVERSITY

Background

Biological invasions



Establish population

invade

Transportation

Native range

Non-Native range

Where dose it come from?

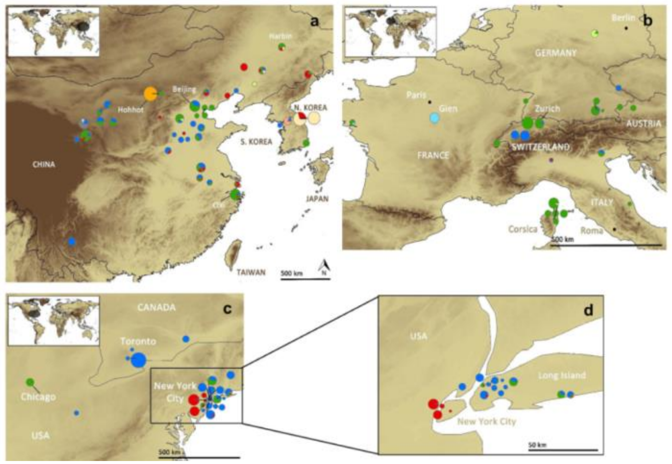
Methods for reconstructing routes of invasion

- ***Direct methods***
  - Based on ***historical and observational data*** which are often sparse, incomplete and, sometimes, misleading

- ***Genetic methods***
  - **Population genetics** has proved a useful approach for reconstructing routes of introduction, highlighting the complexity and the often counterintuitive nature of the true story

Published global invasion routes of beetle species

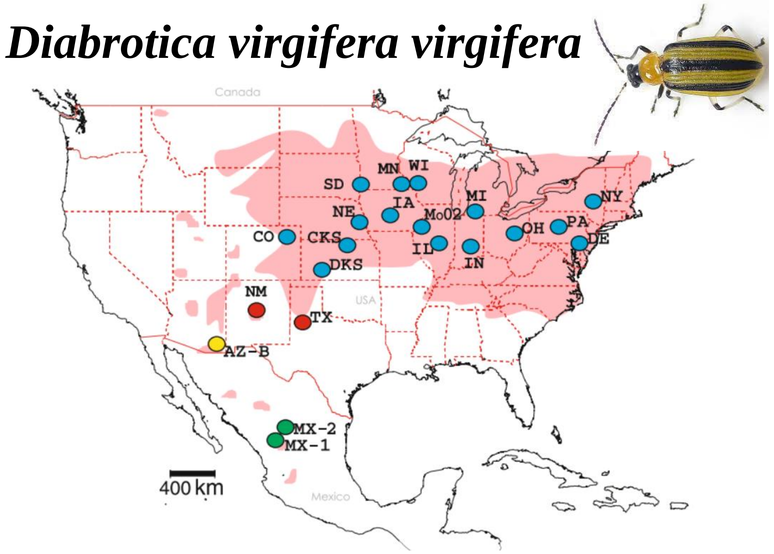
Anoplophora glabripennis



Europe
(Marion Javal, 2019)

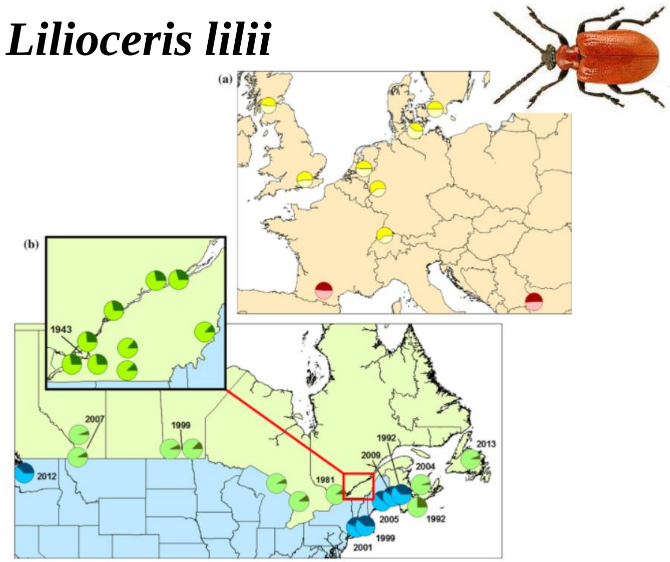
Background

Diabrotica virgifera virgifera



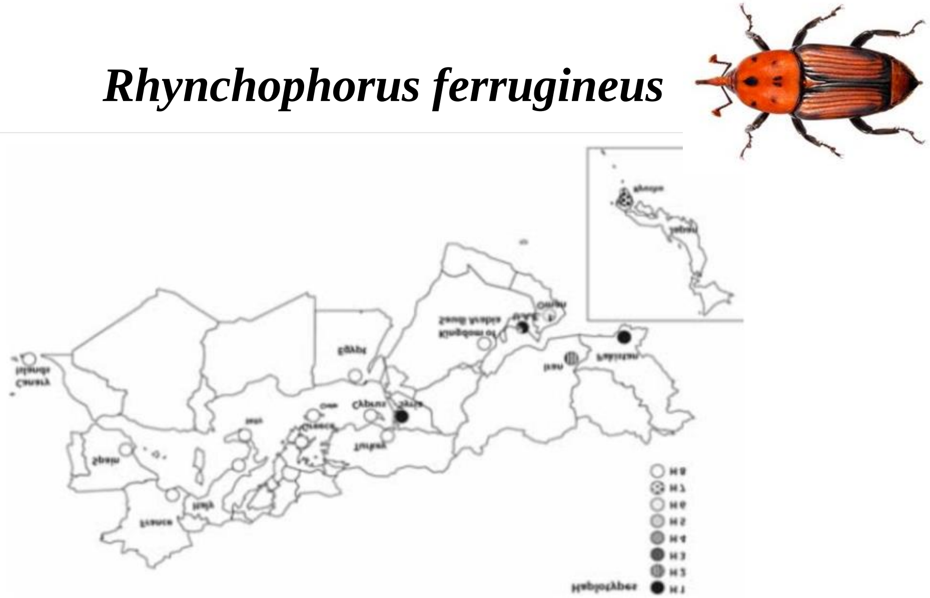
North America
(Eric Lombaert, 2018)

Lilioceris lilii



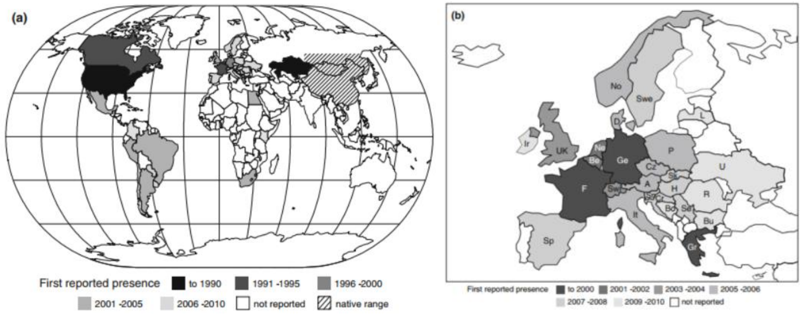
Europe, North America
(Marion Javal, 2016)

Rhynchophorus ferrugineus



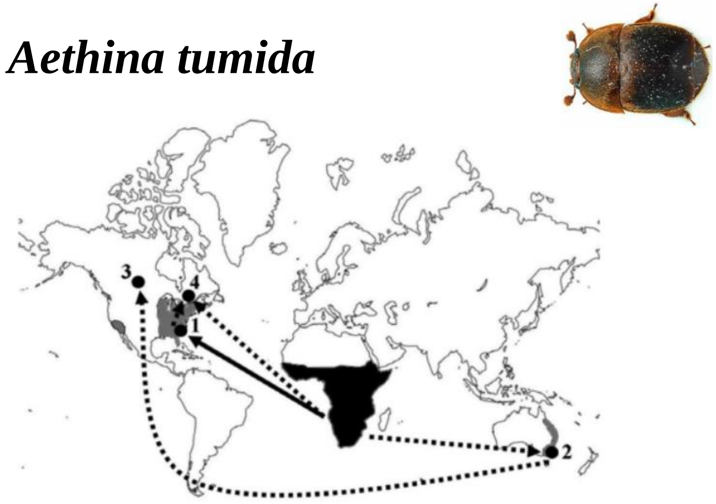
Middle East, the Mediterranean Basin
(Rabab A.A.M. El-Mergawy, 2011)

Harmonia axyridis



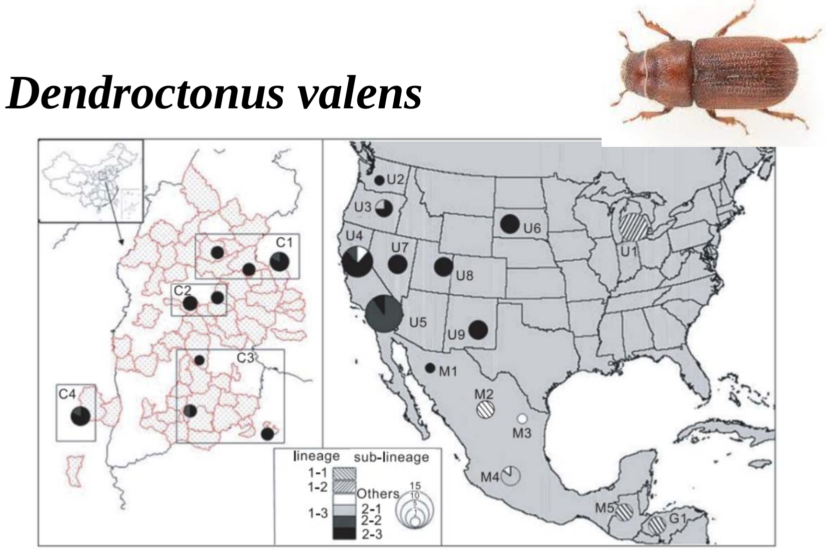
Asia, North America, South America, Europe, Africa
(Peter M. J. Brown, 2011)

Aethina tumida



Australia, Burkina Faso, Cameroon, North America, South Africa, Zimbabwe
(Zachary Lounsberry, 2010)

Dendroctonus valens



China, USA, Mexico
(Yan-Wen Cai, 2008)

Object: *Hylurgus ligniperda* (invasive pest) and *Hylurgus micklitzi*

Taxonomy: **Order:** Coleoptera **Family:** Curculionidae **Subfamily:** Scolytinae **Tribe:** Tomicini **Genus:** *Hylurgus*

Native and invasive ranges:

Hylurgus ligniperda

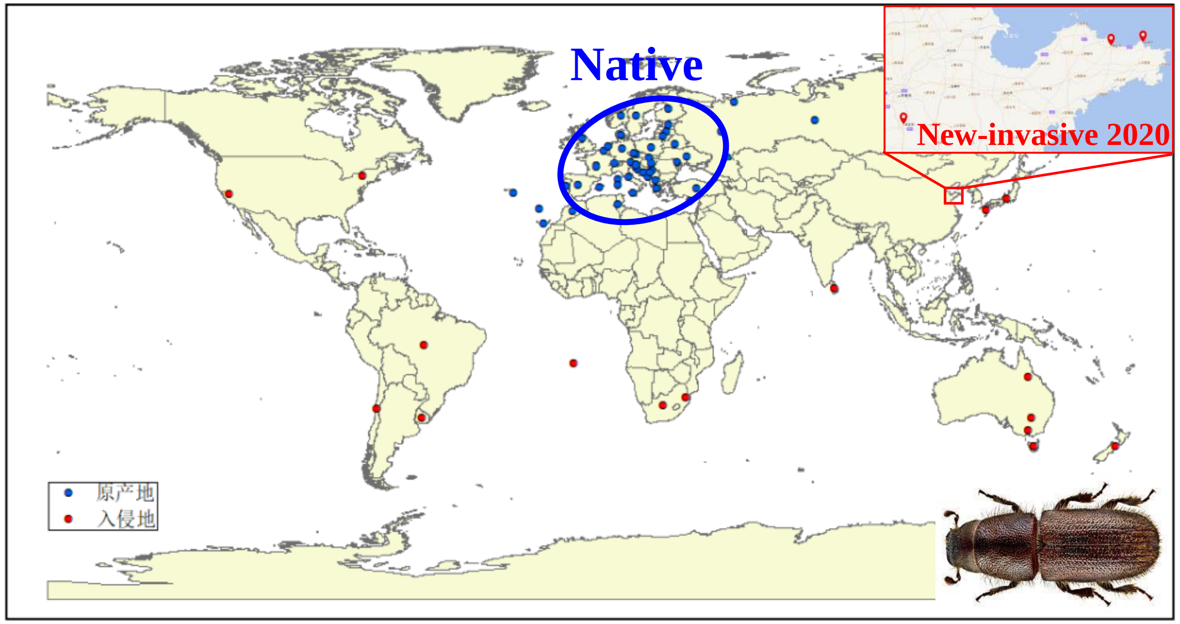
Native region: Mediterranean coast of southern Europe and northern Africa

Invasive region: On almost every continent

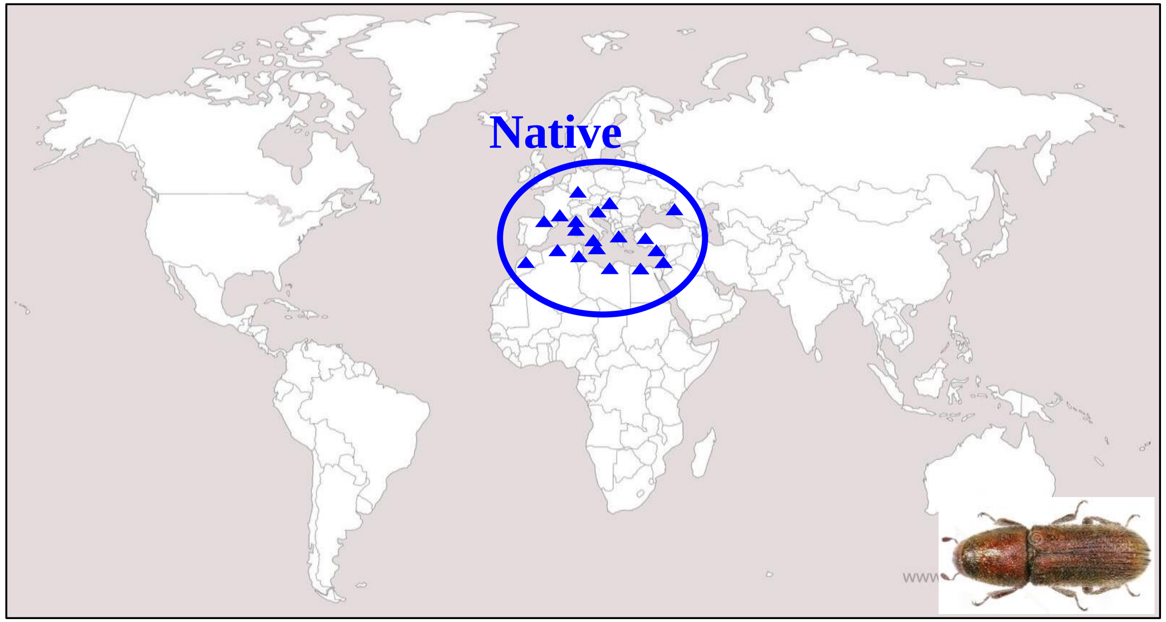
Hylurgus micklitzi

Native region: Mediterranean coast of southern Europe and northern Africa

Invasive region: Not reported until now



Native and invasive ranges of *Hylurgus ligniperda* (Team of Prof. LUO, 2021)



Native and invasive ranges of *Hylurgus micklitzi* (El Khoury Y *et al*., 2019)

Biology

Hylurgus ligniperda

Couple: monogamous

Generation: **2**(France) or **3**(Chile) /year with sister generation

Host: **weak(sub-health pine)**, dying or newly dead trees
(Ren *et al.*, 2021)

Polyphagous

***Pinus* spp. :** *P. sylvestris, P. brutia, P. halepensis, P. halepensis* var. *brutia*, *P. nigra*, *P. nigra* subsp. *pallasiana*, *P. nigra* var. *austriaca*, *P. nigra* var. *caramanica*, *P. pinaster*, *P. thunbergii*, *P. densiflora*, *P. elliottii*, *P. canariensis*, *P. patula*, *P. radiata*, *P. montezumae*, *P. pinea*, *P. strobus*, ***Abies* spp.**, ***Larix* spp.**, ***Picea* spp.**, ***Pseudotsuga* spp.**

Ecological niche: **prefer the lower section of the tree tending to infest the main root system and wood in contact with the ground**

Hylurgus micklitzi

Couple: monogamous

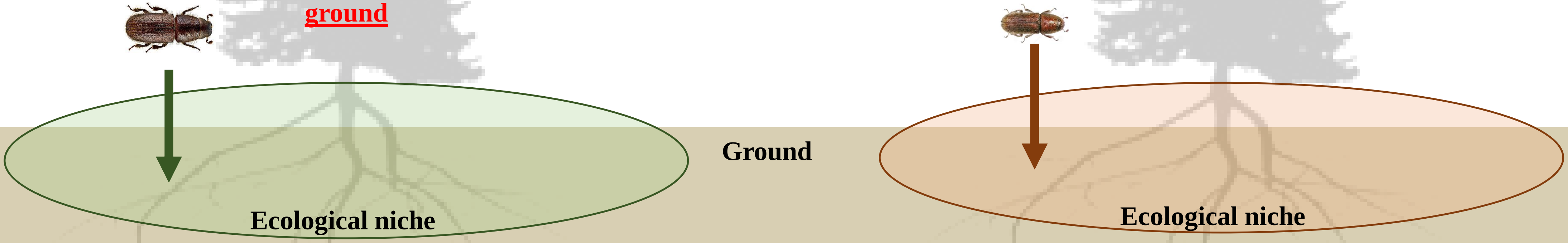
Generation: **1**(Israel) /year with sister generation

Host: dying or newly dead trees

Oligophagous

***Pinus* spp. :** *P. halepensis, P. pinaster*, *P. pinea, P. nigra subsp. pallasiana*

Ecological niche: it's similar to that of *H. ligniperda*
(Z. Mendel *et al.*, 1985)



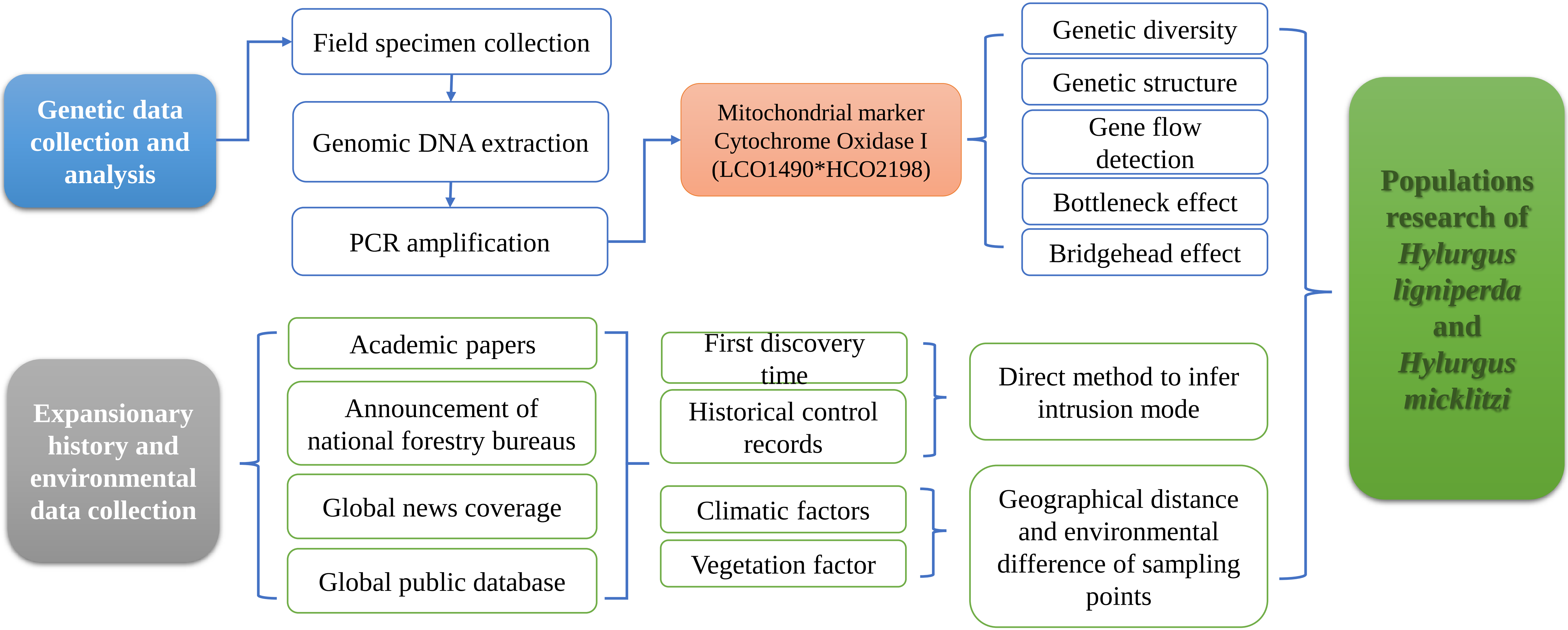
Objectives

① **Define population genetic variability and phylogeographic structure** of the two species *Hylurgus ligniperda* and *Hylurgus micklitzi* in sympatry in South of France

② **Reconstruct invasion routes** by using genetic data

③ **Advise** about developing **effective management strategies** and **sustainable science** based policies and **prevent** new accidental disseminations of alien organisms

Method



Genetic data collection and analysis

Field specimen collection

Genomic DNA extraction

PCR amplification

Mitochondrial marker Cytochrome Oxidase I (LCO1490*HCO2198)

Genetic diversity

Genetic structure

Gene flow detection

Bottleneck effect

Bridgehead effect

Expansionary history and environmental data collection

Academic papers

Announcement of national forestry bureaus

Global news coverage

Global public database

First discovery time

Historical control records

Climatic factors

Vegetation factor

Direct method to infer intrusion mode

Geographical distance and environmental difference of sampling points

Populations research of *Hylurgus ligniperda* and *Hylurgus micklitzi*

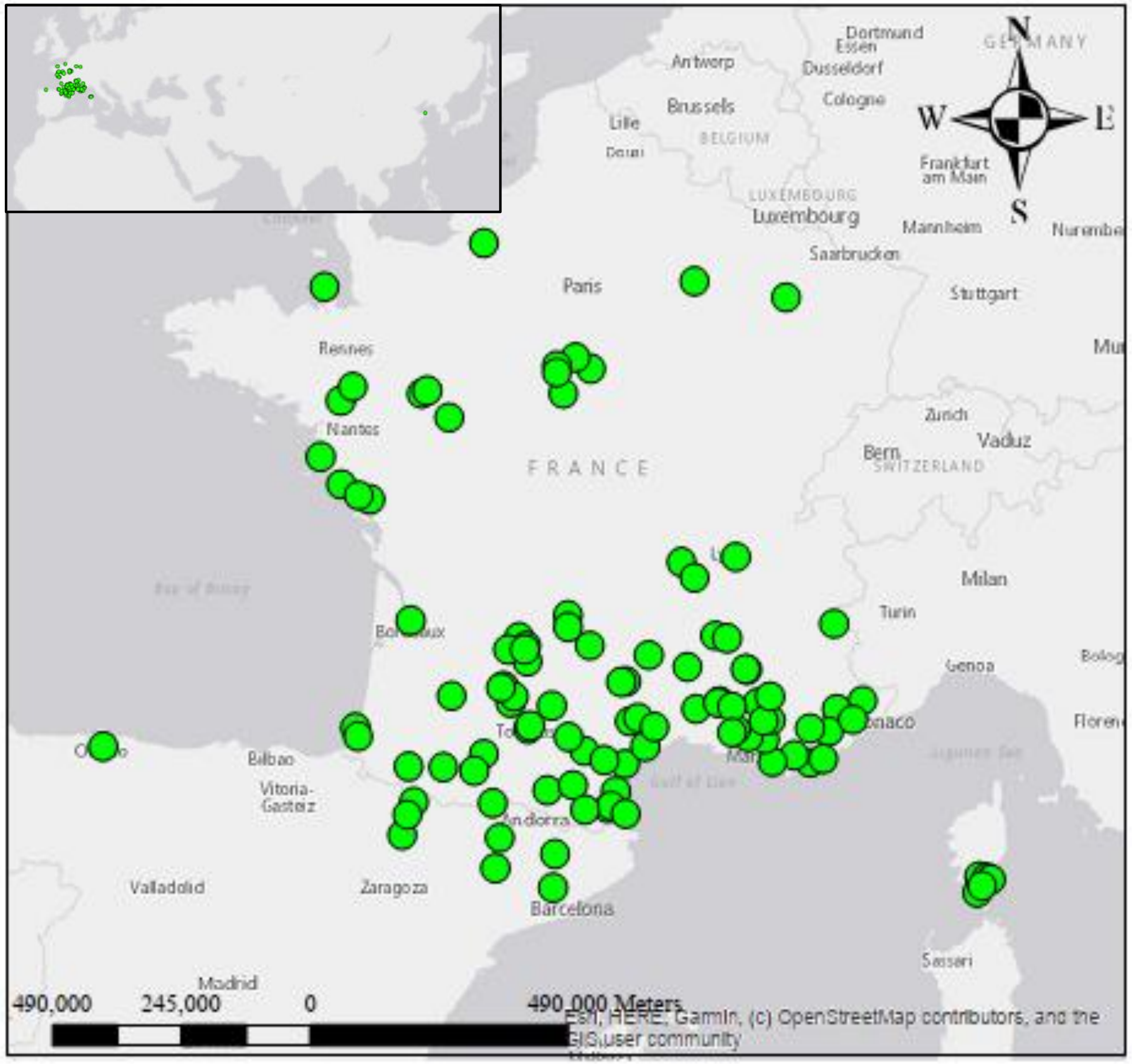
Method Beetle Sampling

Hylurgus ligniperda

2012~2021:

France: 88sites; France Corse: 6sites; Spain: 9sites;

China: 1site

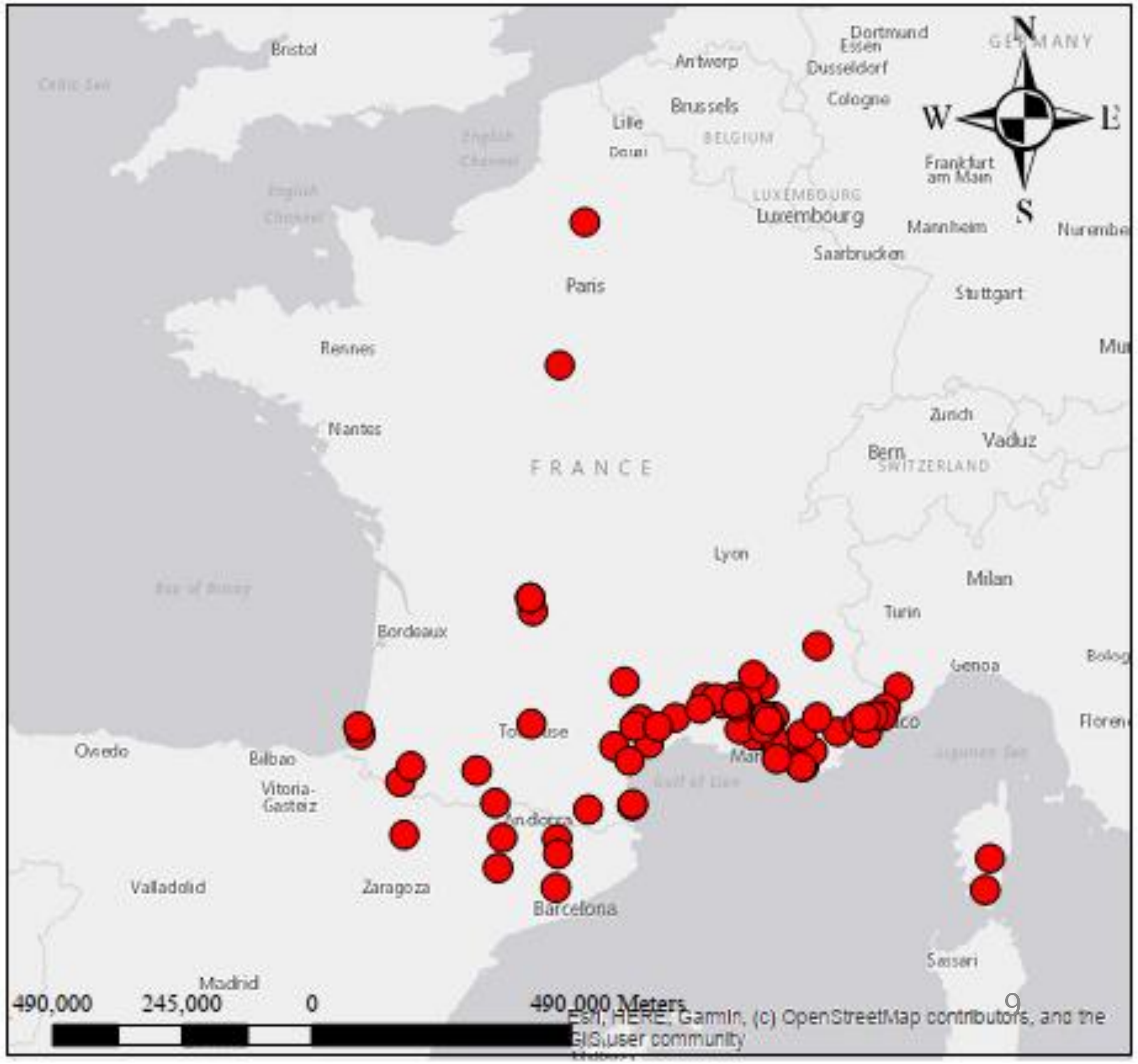


Hylurgus micklitzi

2012~2021:

France: 52sites; France Corse: 2sites; Spain: 7sites;

Italy: 1site



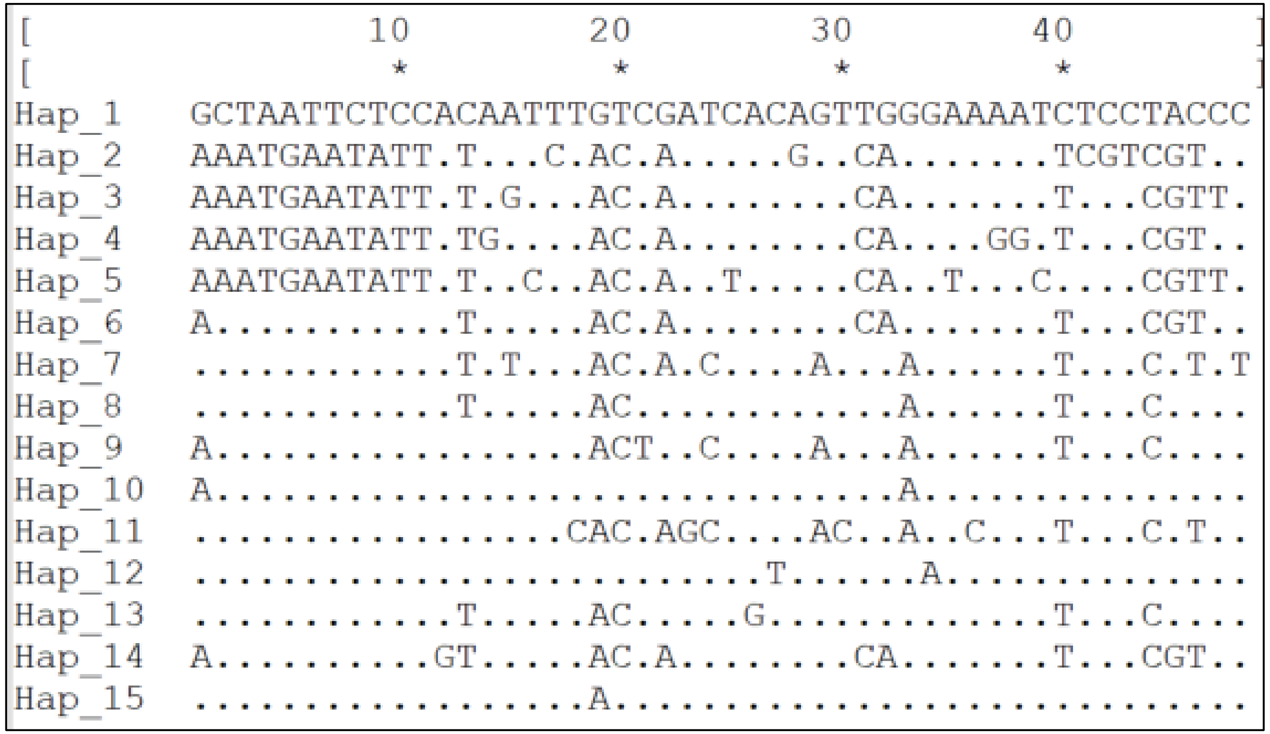
Results Sequence alignment

Hylurgus ligniperda
Obtain a final alignment of **76** individuals;
Sequences were **578bp** long;
A total of **15** mitochondrial haplotypes were identified with
48 polymorphic sites

Haplotypes found in each population and population parameters

Population	Parameter of genetic diversity				
	N	S	H	Hd	π
South Korea	1	0	1	0	0
China	4	0	1	0	0
America	4	15	4	1	0.01326
France Corse	5	16	3	0.7	0.01349
France06	3	0	1	0	0
France11	5	7	2	0.4	0.00484
France12	2	10	2	1	0.01730
France13	14	14	4	0.39560	0.00451
France30	5	11	2	0.4	0.00761
France31	5	1	2	0.4	0.00069
France32	2	13	2	1	0.02249
France34	5	8	5	1	0.00761
France46	4	11	2	0.5	0.00952
France83	9	12	3	0.41667	0.00634
France84	5	0	1	0	0
France85	1	0	1	0	0

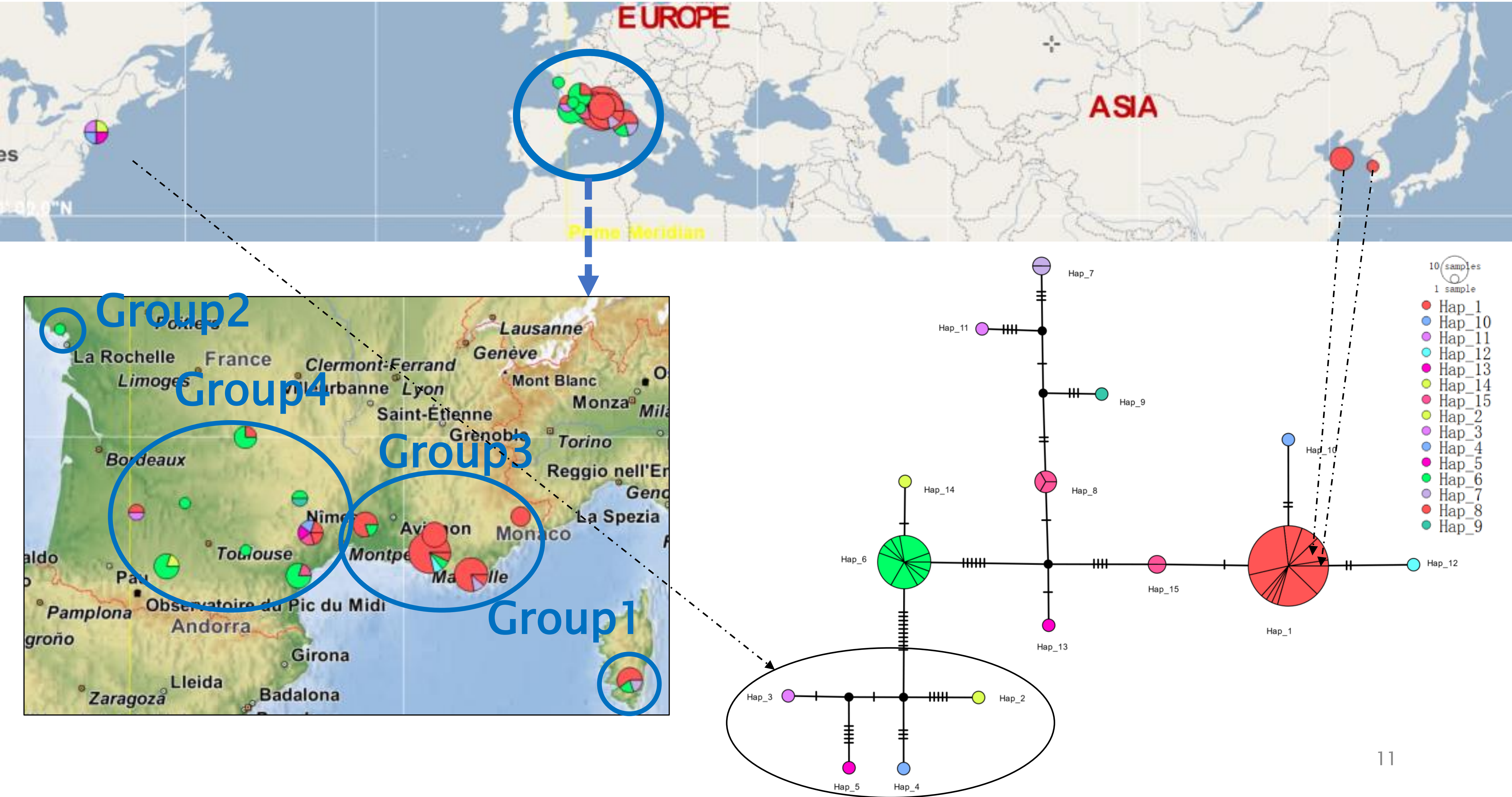
N, number of specimen; S, number of variable sites; H, number of haplotypes;
Hd, haplotype diversity; π, nucleotide diversity (per site)



```
[               10        20        30        40        ]
[                *         *         *         *        ]
Hap_1   GCTAATTCTCCACAATTTGTCGATCACAGTTGGGAAAATCTCCTACCC
Hap_2   AAATGAATATT.T...C.AC.A.....G..CA.......TCGTCGT..
Hap_3   AAATGAATATT.T.G...AC.A........CA.......T...CGTT.
Hap_4   AAATGAATATT.TG....AC.A........CA....GG.T...CGT..
Hap_5   AAATGAATATT.T..C..AC.A..T.....CA..T...C....CGTT.
Hap_6   A...........T.....AC.A........CA.......T...CGT..
Hap_7   ............T.T...AC.A.C....A...A......T...C.T.T
Hap_8   ............T.....AC............A......T...C....
Hap_9   A.................ACT..C....A...A......T...C....
Hap_10  A...............................A...............
Hap_11  .................CAC.AGC....AC..A..C...T...C.T..
Hap_12  ..........................T......A..............
Hap_13  ............T.....AC.....G.............T...C....
Hap_14  A..........GT.....AC.A........CA.......T...CGT..
Hap_15  ..................A.............................
```

Results Haplotype parsimony network

Hylurgus ligniperda



Results Haplotype parsimony network

Hylurgus ligniperda

#Group1 FranceCorse

#Group2 France85

#Group3 France6
France13
France30
France83
France84

#Group4 France11
France12
France31
France32
France34
France46
France81
France82

Analysis of molecular variance(AMOVA) among populations

Source of variation		d.f.	Sum of squares	Variance components	Percentage of variation
Grouping by region	**Among groups**	3	65.562	1.46156 Va	40.59***
	Among pops within groups	11	37.821	0.39018 Vb	10.84*
	Within populations	52	90.945	1.74895 Vc	48.57**

*P<0.05**P<0.01***P<0.001

Results Sequence alignment

Hylurgus micklitzi

Obtain a final alignment of **53** individuals

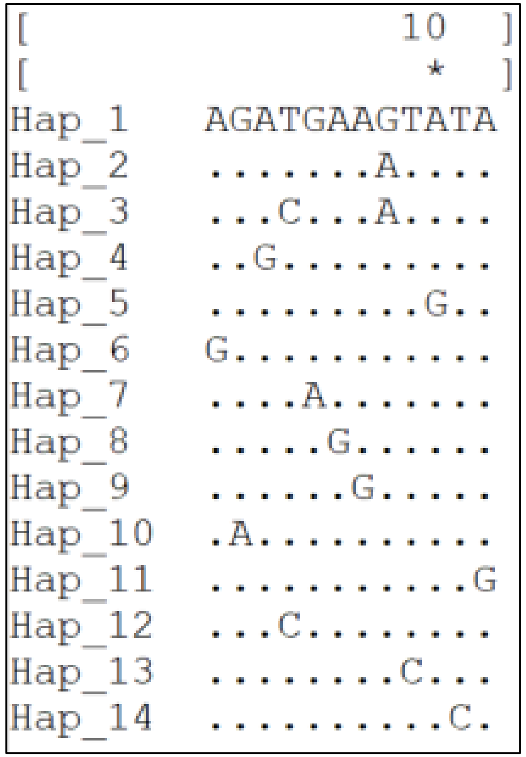
Sequences were **578**bp long

A total of **14** mitochondrial haplotypes were identified with **12** polymorphic sites

Haplotypes found in each population and population parameters

Population	Parameter of genetic diversity				
	N	S	H	Hd	π
Spain	3	2	3	1	0.00231
Italy	1	0	1	0	0
France06	2	0	1	0	0
France11	3	2	3	1	0.00231
France13	12	3	4	0.45455	0.00087
France30	4	2	3	0.83333	0.00173
France31	4	1	2	0.50000	0.00087
France34	11	4	5	0.76364	0.00189
France45	1	0	1	0	0
France66	1	0	1	0	0
France83	7	3	4	0.71429	0.00148
France84	4	1	2	0.50000	0.00087

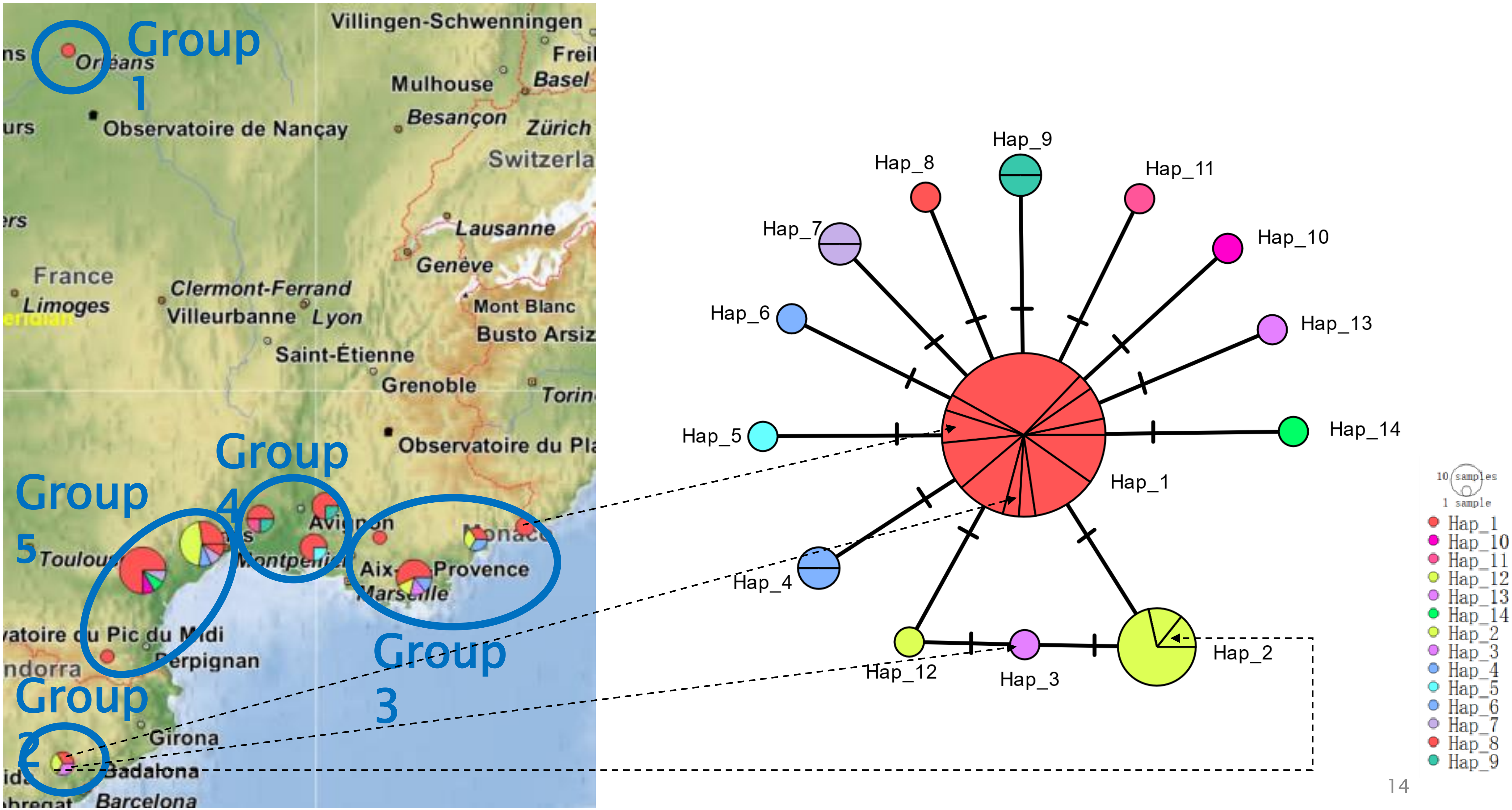
N, number of specimen; S, number of variable sites; H, number of haplotypes; Hd, Haplotype diversity; π, Nucleotide diversity (per site)



```
[                 10   ]
[                  *   ]
Hap_1     AGATGAAGTATA
Hap_2     .......A....
Hap_3     ...C...A....
Hap_4     ..G.........
Hap_5     .........G..
Hap_6     G...........
Hap_7     ....A.......
Hap_8     .....G......
Hap_9     ......G.....
Hap_10    .A..........
Hap_11    ...........G
Hap_12    ...C........
Hap_13    ........C...
Hap_14    ..........C.
```

Results Haplotype parsimony network

Hylurgus micklitzi



Results Haplotype parsimony network

#Group1 France45

#Group2 Spain

#Group3 Italy
France6
France13
France83

#Group4 France30
France31
France84

#Group5 France11
France34
France66

Analysis of molecular variance(AMOVA) among populations

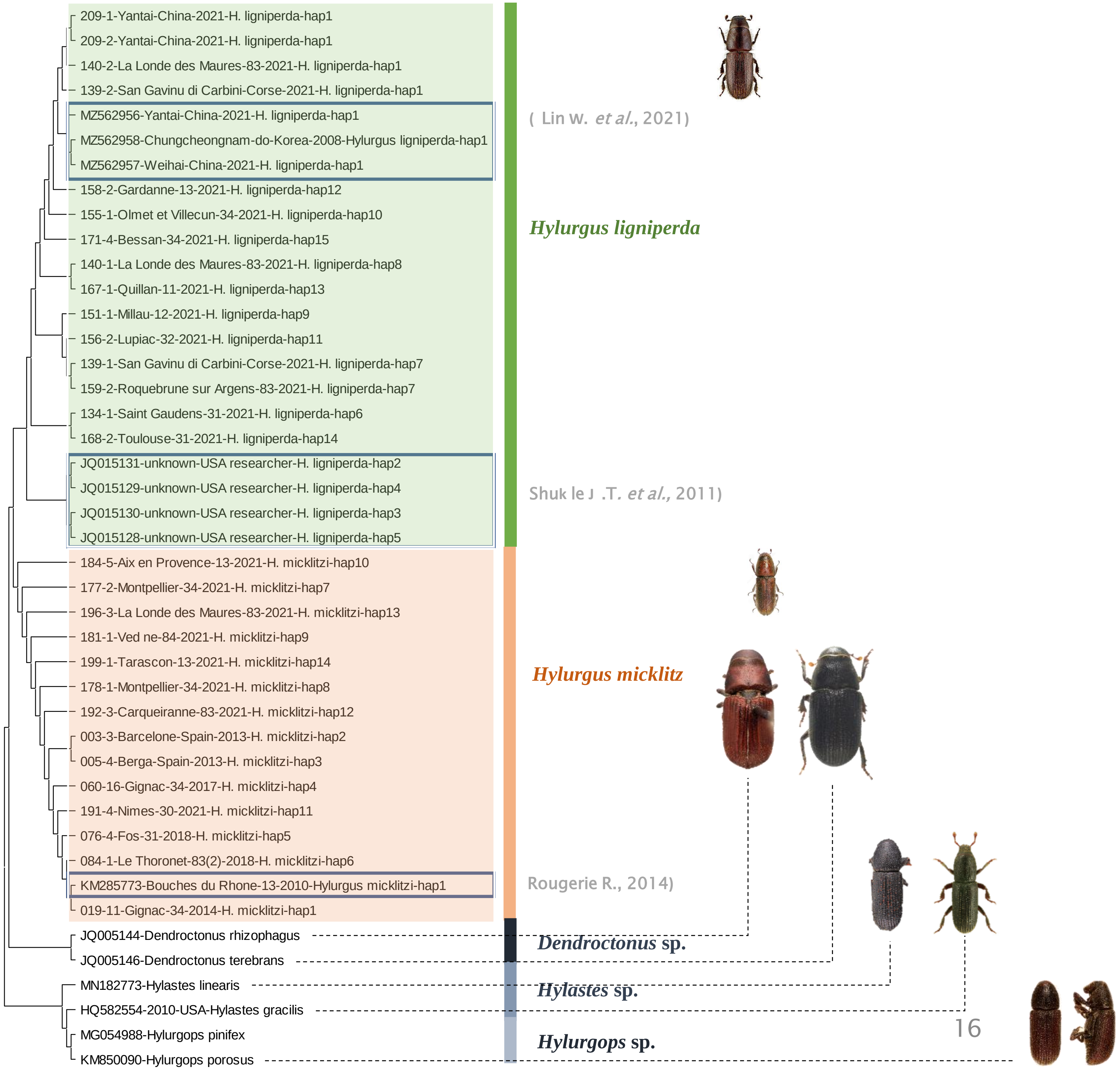
Source of variation		d.f.	Sum of squares	Variance components	Percentage of variation
Grouping by region	**Among groups**	4	3.598	0.08172Va	19.09
	Among pops within groups	7	1.318	-0.05462Vb	-12.76
	Within populations	41	16.443	0.40104Vc	93.67*

*P<0.05**P<0.01***P<0.001

Results Phylogenetic Tree and genetic distances

Maximum Likelihood Tree
Find Best DNA Models: GTR+G+I

Pairwise distance	*Hylurgus ligniperda*	*Hylurgus micklitzi*
Hylurgus ligniperda	0.17%~4.48%	
Hylurgus micklitzi	16.09%~17.47%	0.17%~0.52%
Dendroctonus rhizophagus	17.65%~19.90%	19.03%~19.38%
Dendroctonus terebrans	16.61%~17.82%	17.47%~17.82%
Hylastes gracilis	20.59%~21.80	19.20%~19.72%
Hylastes linearis	16.61%~17.99%	18.69%~19.20%
Hylurgops pinifex	19.72%~20.93%	21.28%~21.63%
Hylurgops porosus	19.55%~20.93%	19.72%~20.24%



Conculsion

- **Contrasted genetic structure** between the two species
- *Hylurgus ligniperda* : **high genetic variability with two main structured haplotypes and several distantly related haplotypes.**
- *Hylurgus micklitzi* : **One main haplotype present in all sites sampled, no phylogeographic structure.**

- Seek cooperation to collect more populations in other continents.
- Exclude the effects of mitochondrial fragments in the nuclear genome (Numts)

Thanks for your listening!