



ALMA MATER STUDIORUM
UNIVERSITÀ DI BOLOGNA



Animal and Food Genomics Group

The effect of a regional legislative act on the distribution of honey bee mitochondrial DNA lineages

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Conservation of *Apis mellifera*

Apis mellifera ligustica



Across Europe

- protected conservation areas
- mating stations
- maintaining of pure-bred lines

Italy

- *Since 2017* current Italian legislation takes initiatives to safeguard the subspecies of *A. m. ligustica* by limiting or banning other subspecies used in beekeeping practice, including the use of hybrid (if not natural) in some regions

Law n.2 of March 2019 art.7
preservation of *Apis mellifera ligustica*



Law n.17 October 2022 art.3
measures for the conservation
and valorisation of regional
beekeeping heritage



Apis mellifera – Italy

4 subspecies

Italian honeybee (*A. m. ligustica*)

Carniolan honeybee (*A. m. carnica*)

} C

Black honeybee (*A. m. mellifera*)

M →

Sicilian honeybee (*A. m. siciliana*)

A →



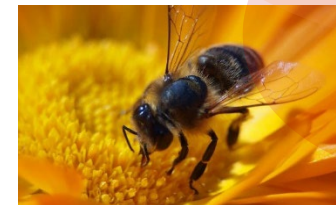
mellifera



carnica



ligustica
C1/M7

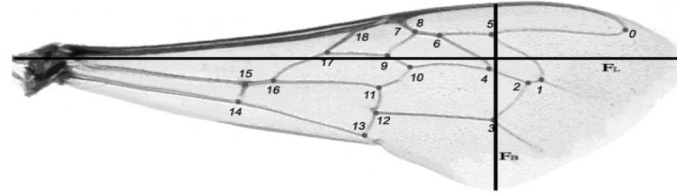
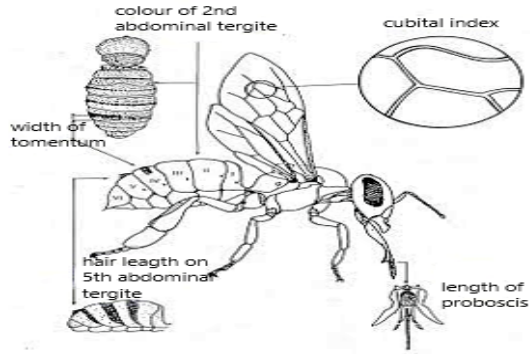


siciliana



Methods for the identification

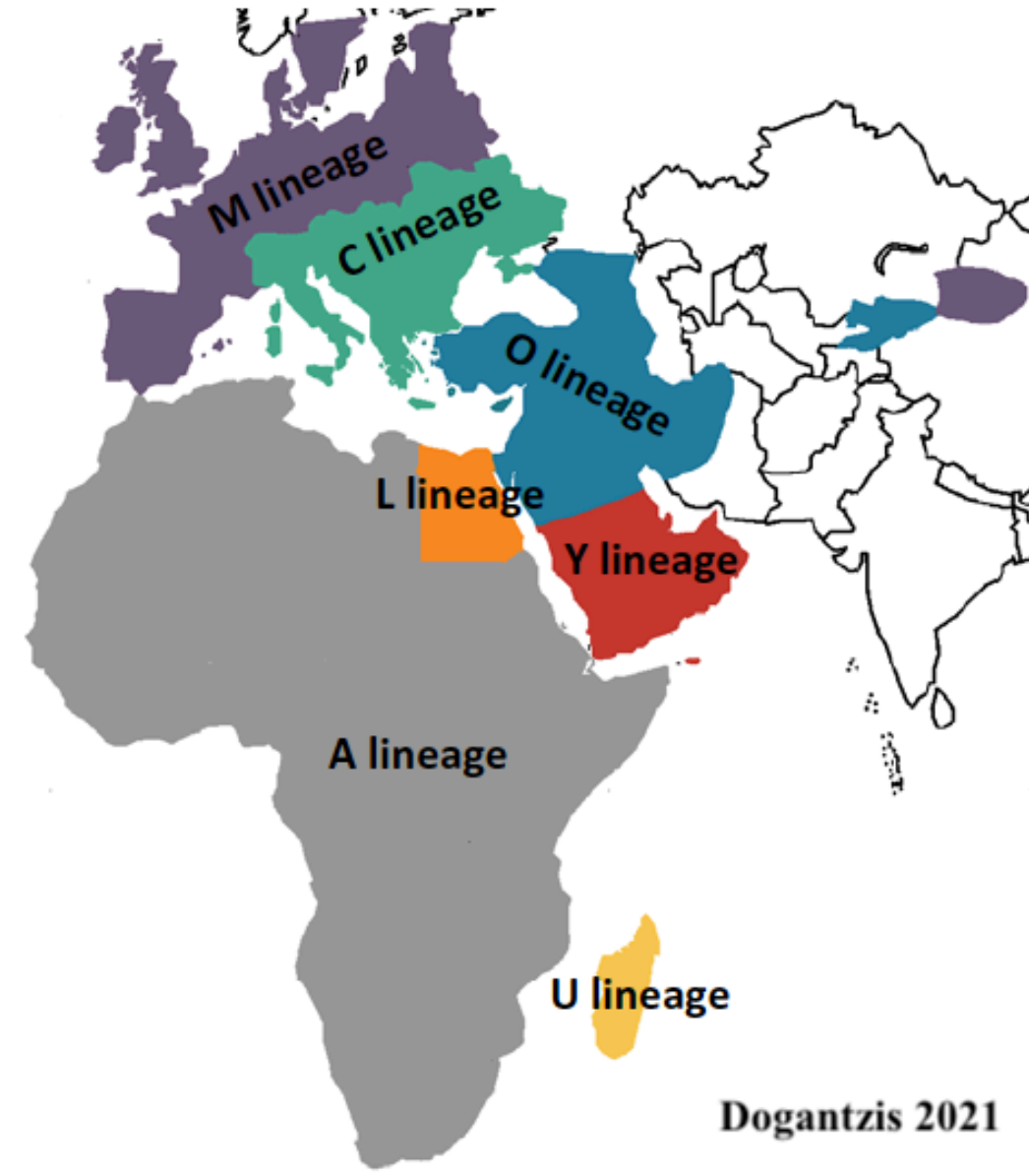
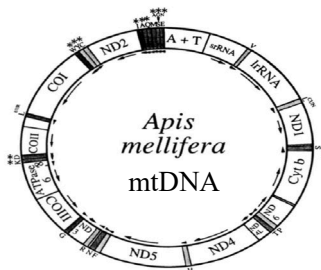
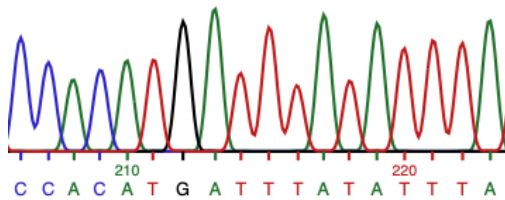
Morphometry



Molecular

Mitochondrial DNA

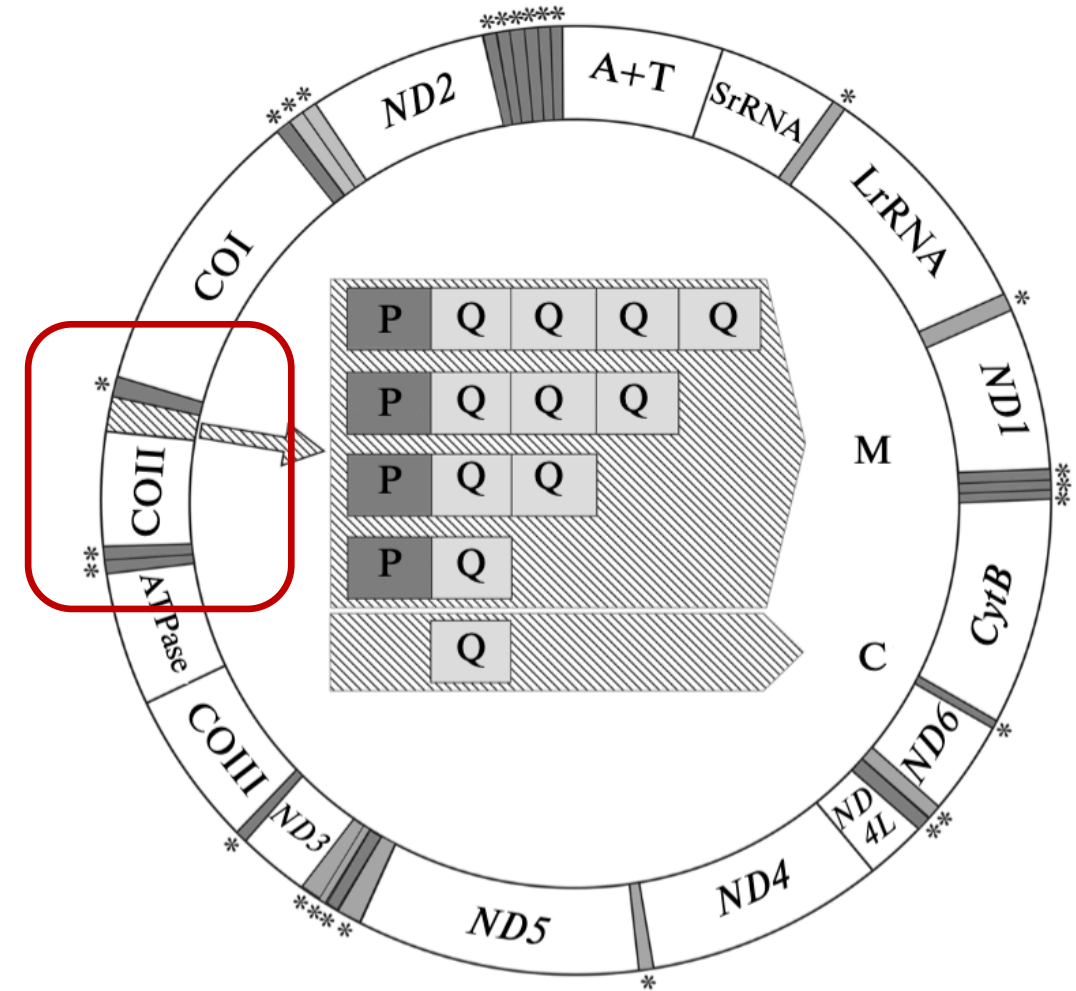
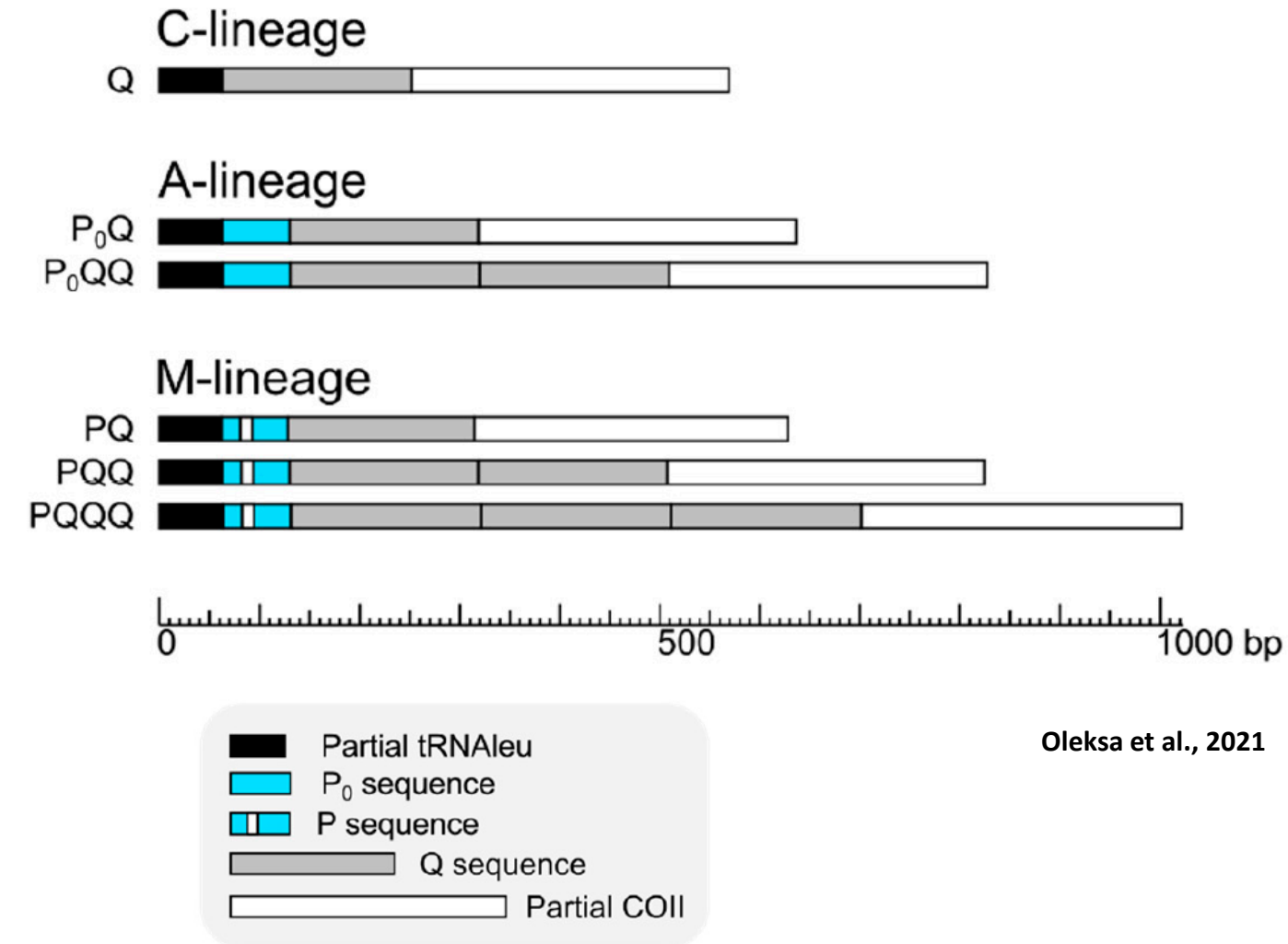
Nuclear genome



Dogantzis 2021

Molecular methods

Mitochondrial DNA analysis (mtDNA) - tRNA^{Leu} - COX2



Ilyasov et al., 2015



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Aim

To investigate the distribution and diffusion of mtDNA haplotypes of the
Apis mellifera population managed in Emilia Romagna region

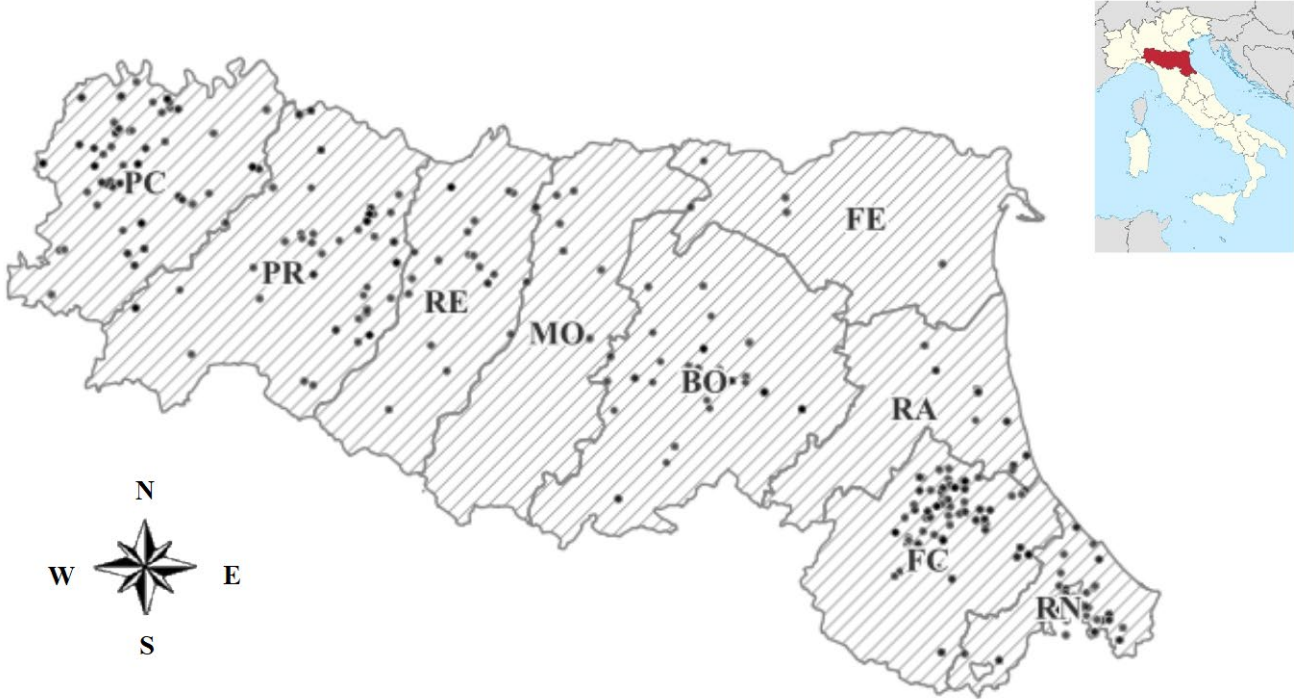


Baseline for monitoring the trend in the frequency of *Apis mellifera ligustica*
mtDNA haplotypes after the application of the regional law



Material and Methods

Administrative province	Years of sampling			Total number
	2020	2021	2022	
Piacenza (PC)	13	48	115	176
Parma (PR)	19	107	87	213
Reggio Emilia (RE)	5	36	25	66
Modena (MO)	3	30	0	33
Bologna (BO)	29	65	56	150
Ferrara (FE)	11	0	5	16
Ravenna (RA)	4	67	6	77
Forlì – Cesena (FC)	3	166	145	314
Rimini (RN)	7	69	22	98
Total number	94	588	461	1143

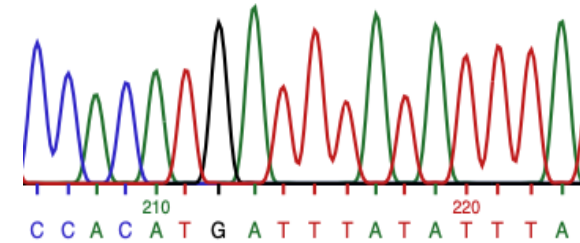
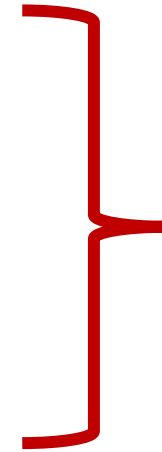


206 beekeepers
 ~ 4 colonies per apiary

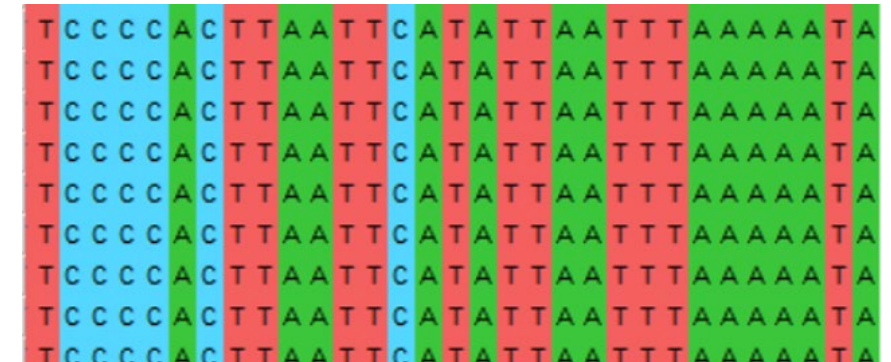
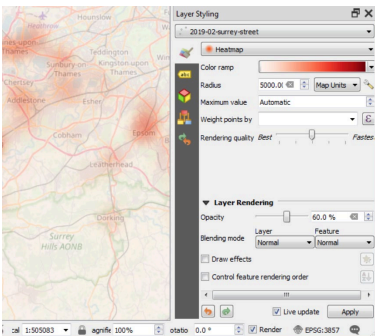
Material and Methods



Sequence data analyses and phylogenetic analyses

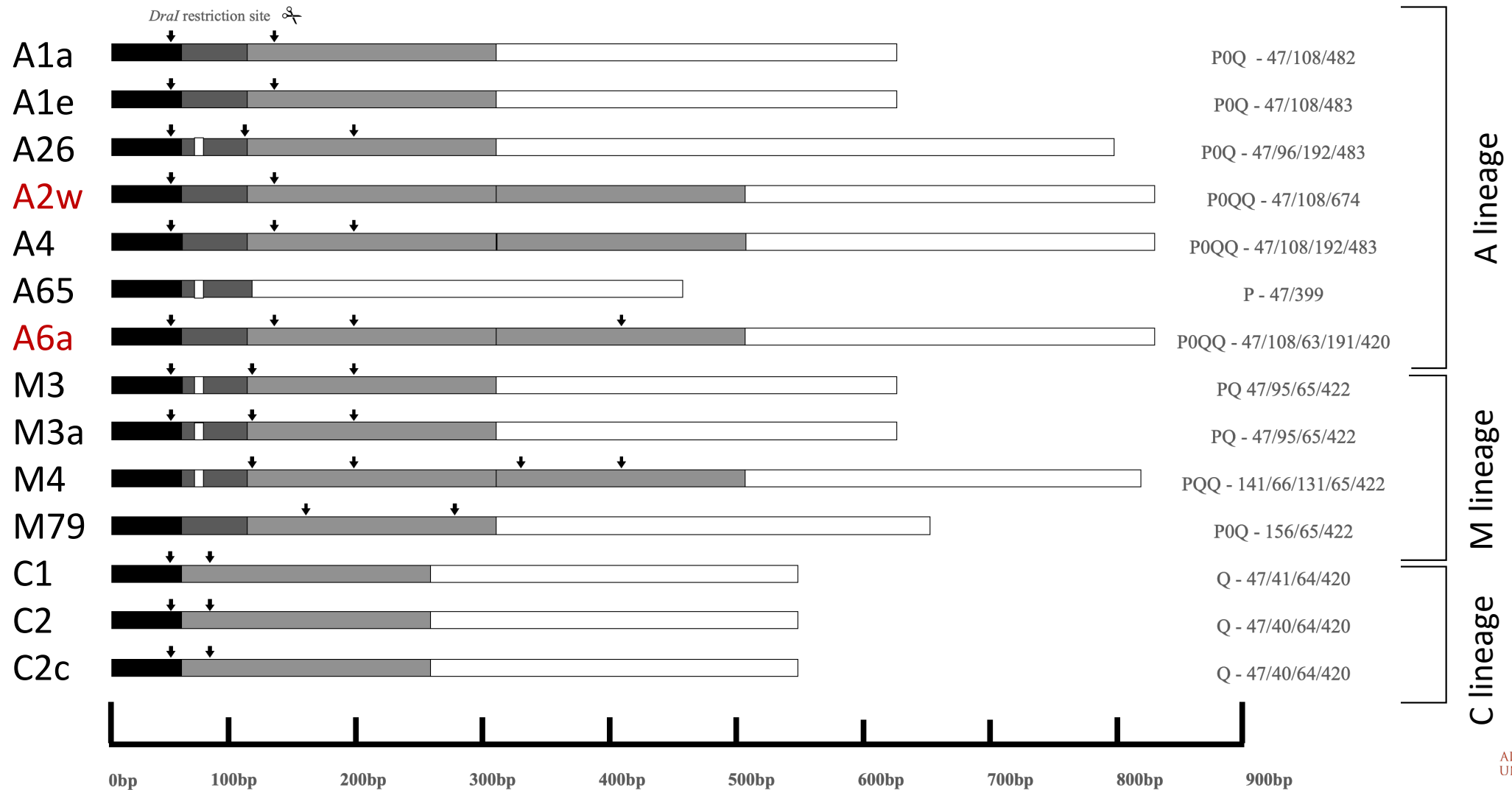


Geographical representation and density map



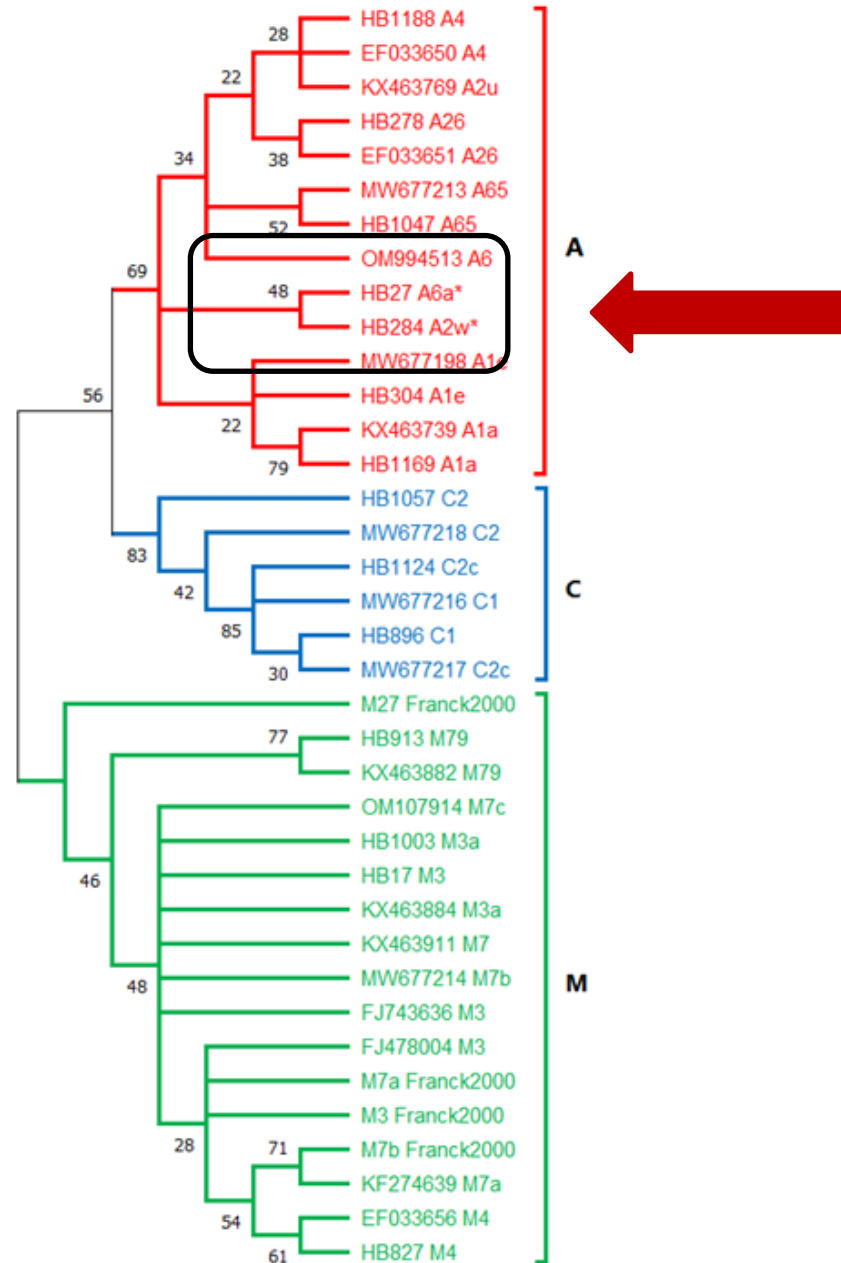
Results -1

- 500-900bp fragments
- 14 different mitochondrial haplotypes
- 2 novel haplotypes



Results -2

Phylogenetic analyses



Results -3

		mtDNA haplotypes			
Year	N. of honey bees	C1	C2	M	A
2020	94	80 (0.860)	9 (0.090)	3 (0.040)	2 (0.010)
2021	588	511 (0.869)	65 (0.111)	6 (0.010)	6 (0.010)
2022	461	398 (0.863)	52 (0.113)	4 (0.009)	7 (0.015)
Total	1143	989 (0.866)	126 (0.110)	13 (0.011)	15 (0.013)

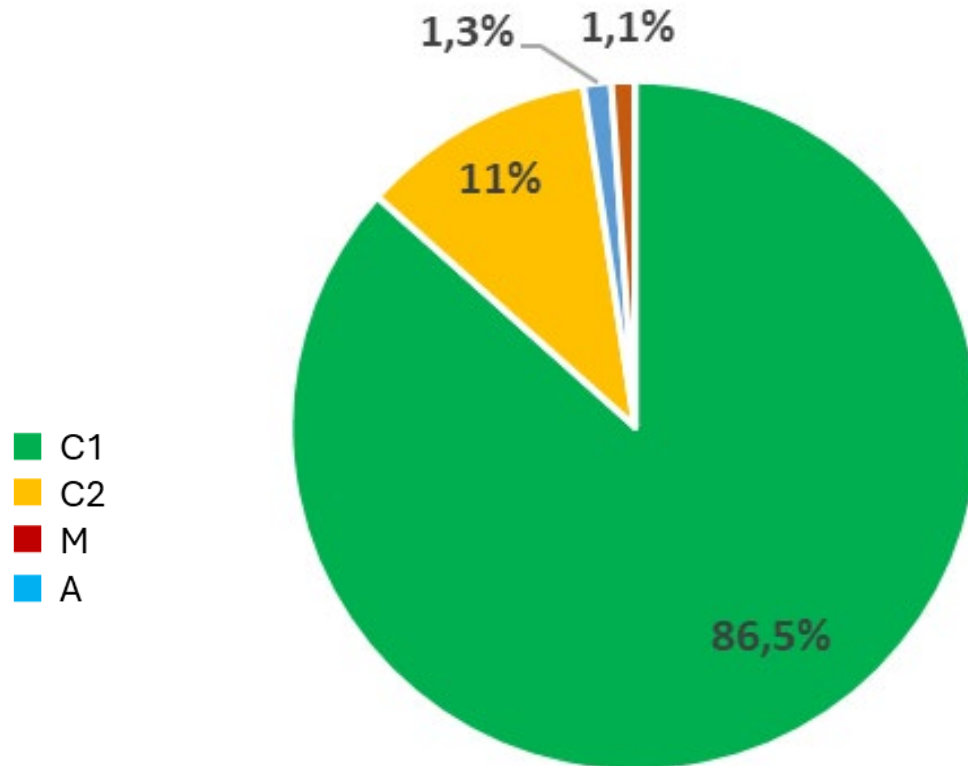
Haplotype Diversity (H) 0.239

Relatively low level -> high frequency of C1



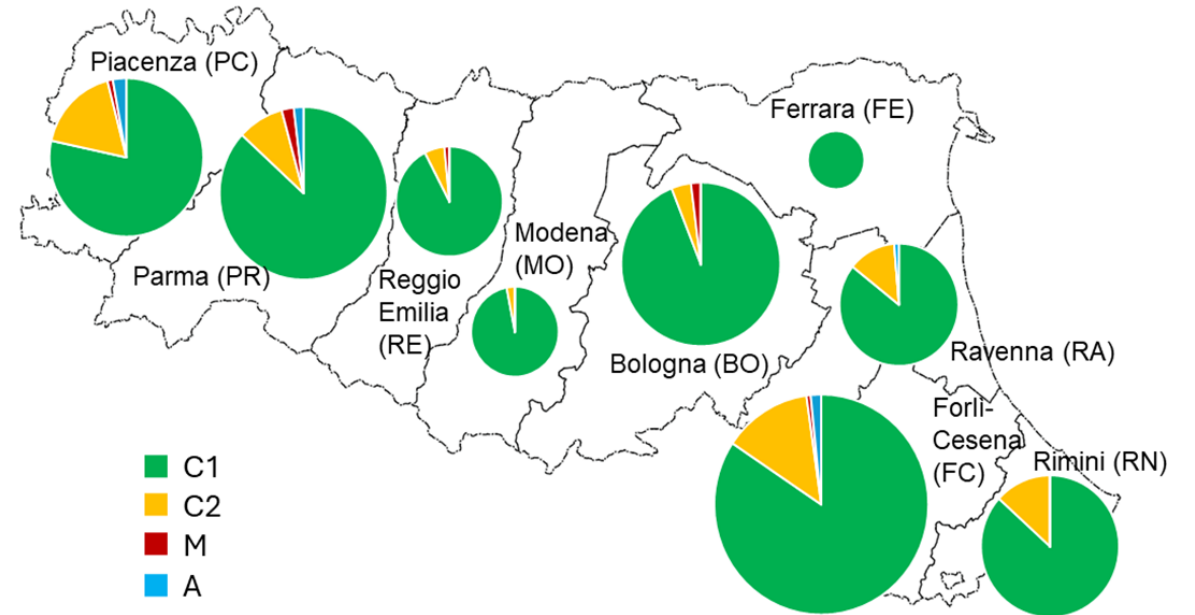
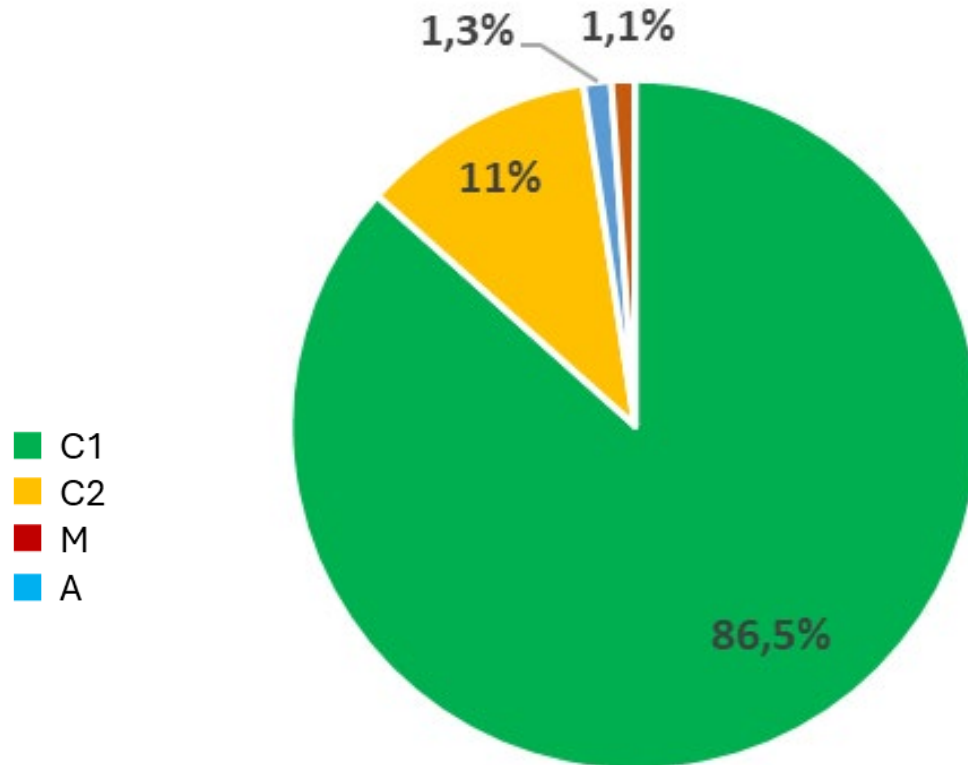
Results - 4

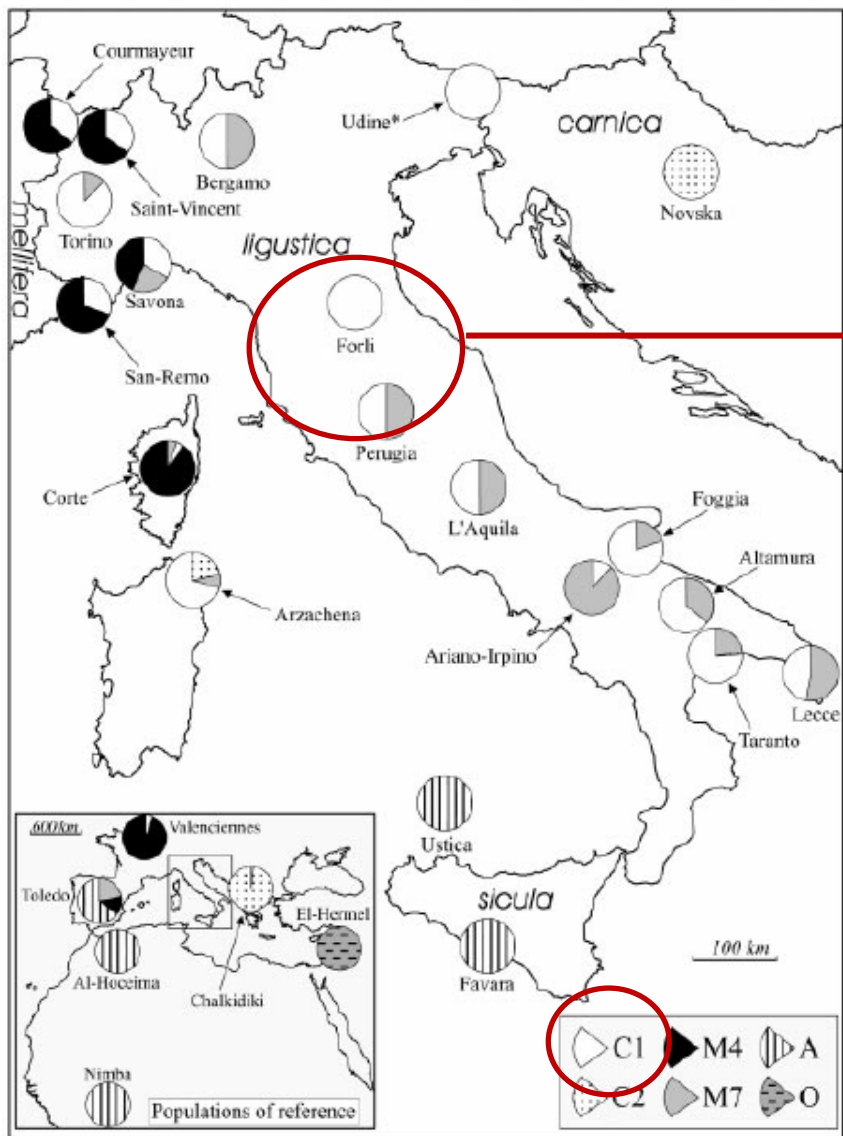
mtDNA haplotypes frequency



Results - 4

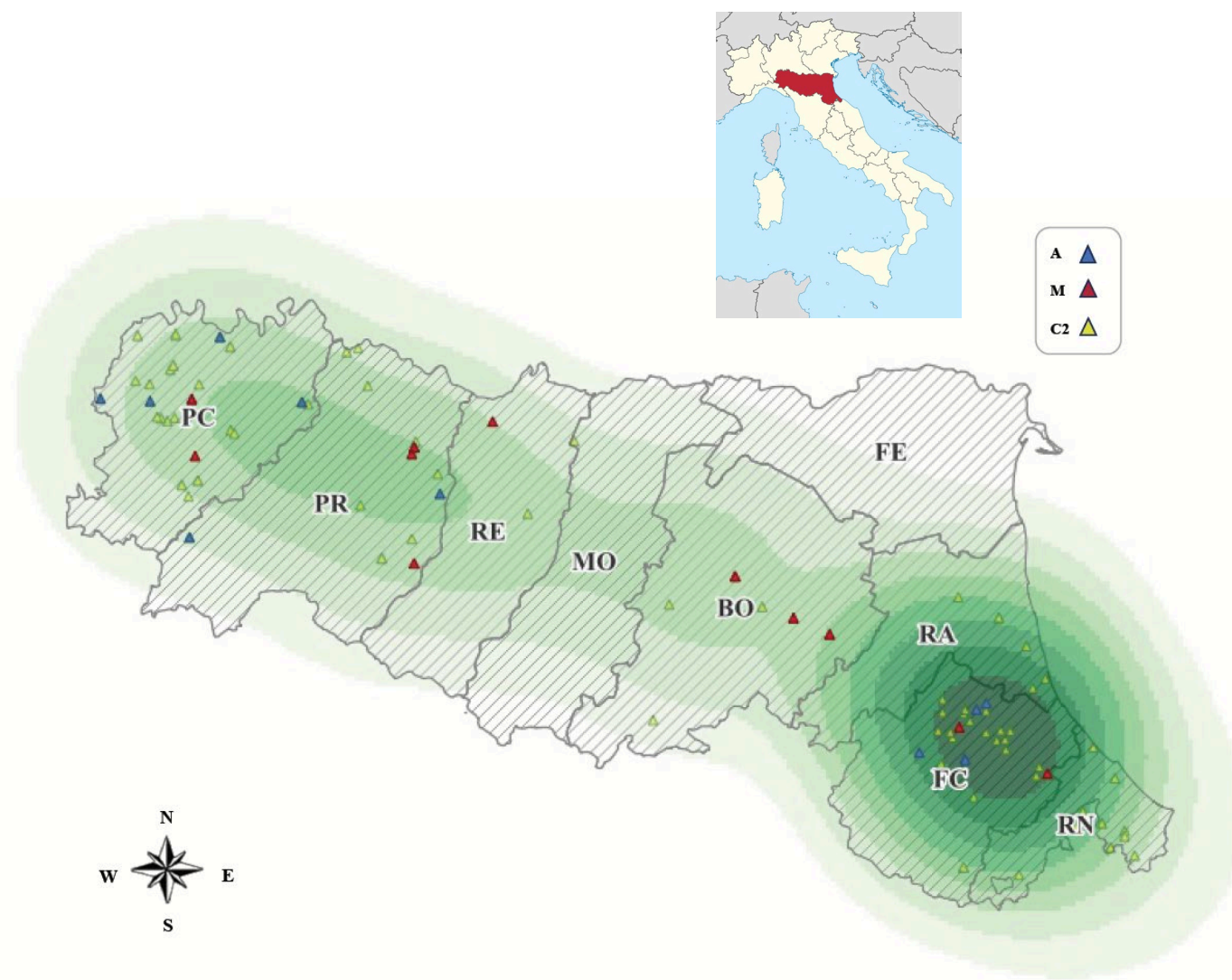
mtDNA haplotypes frequency





Franck et al., 2000

18 sampling site
38 honey bee sampled in Emilia-Romagna region
1 Province Forlì Cesena



1143 sampling site
314 honey bee sampled in Forlì Cesena
9 Administrative Provinces



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Conclusions

- this is the largest study on mtDNA variability conducted thus far to monitor the honey bee populations of the Emilia-Romagna region and one of the most dense in terms of mtDNA data points obtained over a geographic area
- we identified mtDNA haplotypes from the three main lineages: A, M and C: C1 is the most frequent haplotype but other alien haplotypes have been identified, including two novel haplotypes of the A lineage



Conclusions

- starting point for monitoring the effect of the regional law
- design and evaluate the potential effectiveness of conservation policies and action addressed to maintain the integrity of honey bee genetic resources in Italy
- based on the results we can recommend a more stringent policy to prevent diffusion of foreign mitotypes

Next steps ->

- complement the information obtained with nuclear genomic data



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Luca Fontanesi
Full Professor



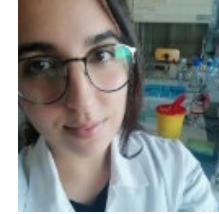
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Acknowledgments

BEE-RER-1-2-3 RESEARCH PROJECT

Thank you !
Questions ?



mipaaf
ministero delle politiche
agricole alimentari e forestali

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Regione Emilia-Romagna



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