

# Diversity of the MHC class II *DRB* alleles in chamois populations

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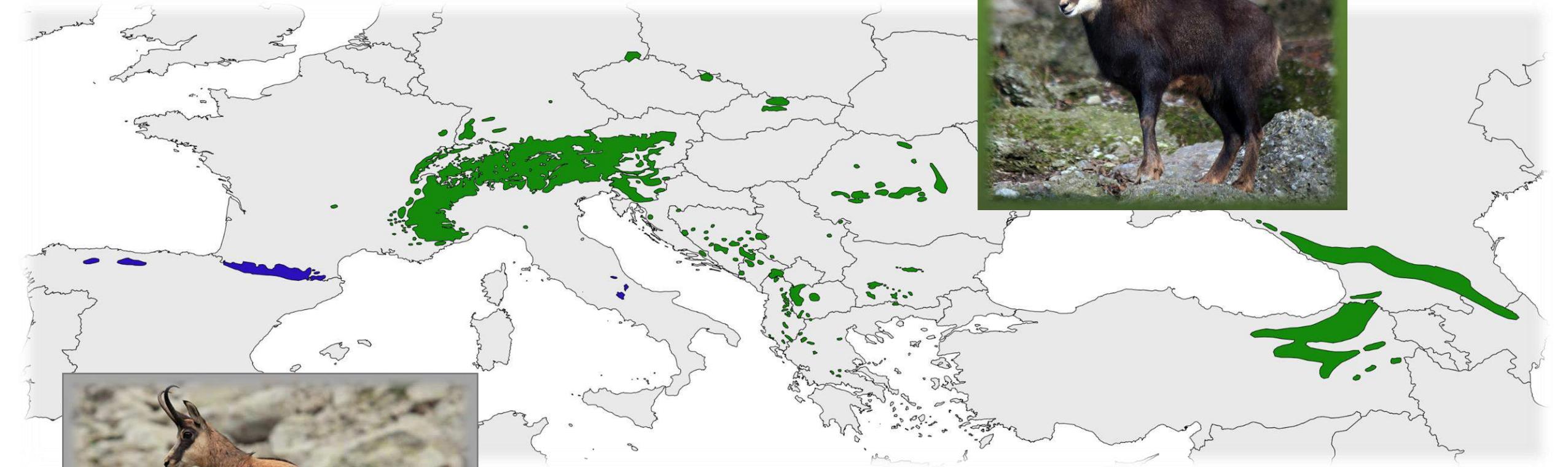
# Genus *Rupicapra*

Introduction

## *Rupicapra rupicapra*

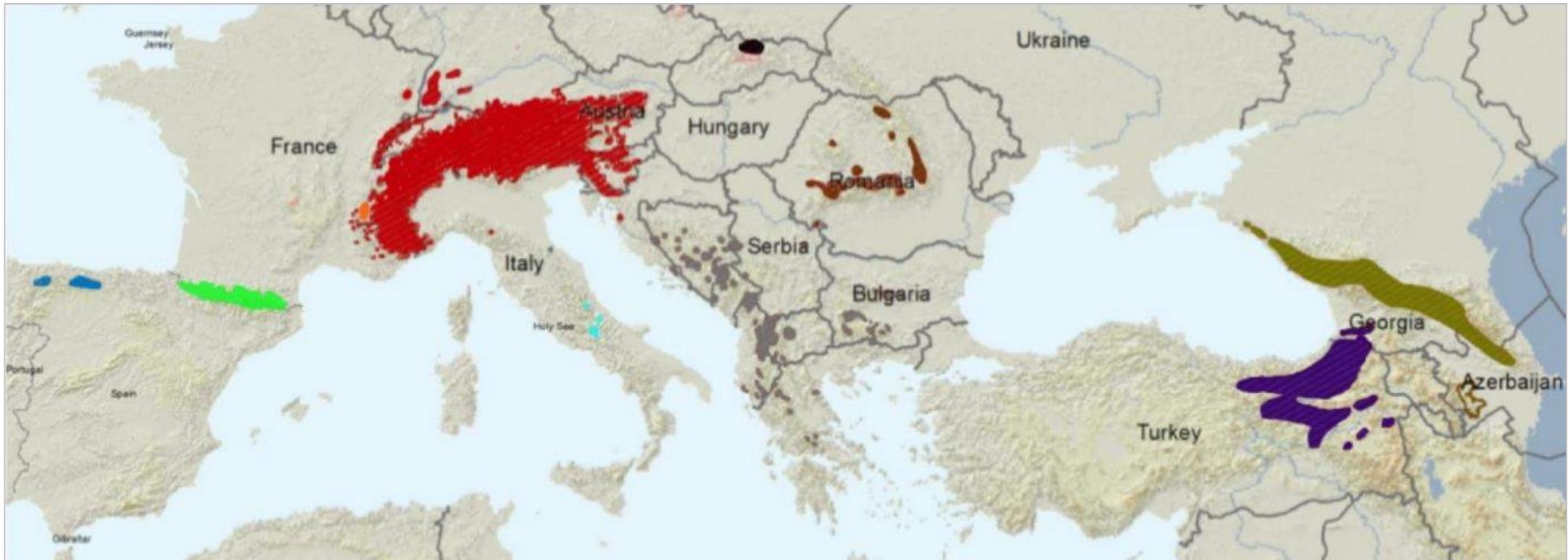


## *Rupicapra pyrenaica*



# Geographic distribution of the genus *Rupicapra* subspecies

Introduction



*R. pyrenaica*

- *ornata*
- *pyrenaica*
- *parva*

*R. rupicapra*

- *balcanica*
- *rupicapra*
- *tatrica*
- *cartusiana*
- *carpathica*
- *asiatica*
- *caucasica*

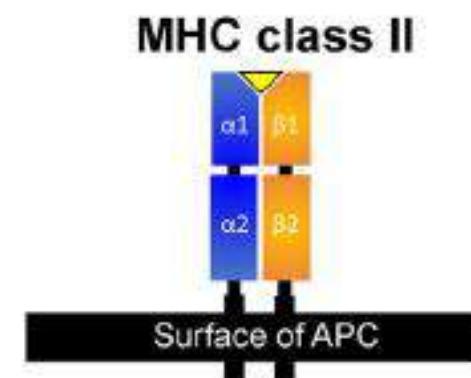
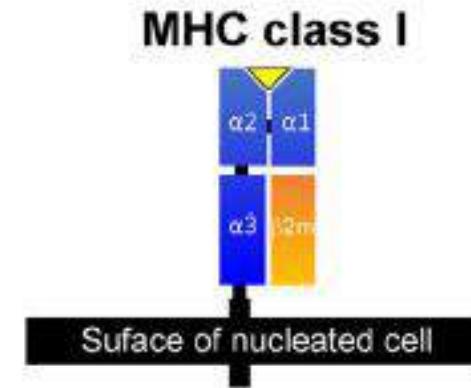
# Major-histocompatibility complex (MHC)

adaptive immunity

gene duplication – balancing selection

genetic markers for adaptive evolution

**High Throughput Sequencing  
(=Next Generation Sequencing)**



RESEARCH ARTICLE

# Extremely low genetic variation in endangered Tatra chamois and evidence for hybridization with an introduced Alpine population

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<https://doi.org/10.1038/s41437-018-0070-5>



ARTICLE



## A new hybrid approach for MHC genotyping: high-throughput NGS and long read MinION nanopore sequencing, with application to the non-model vertebrate Alpine chamois (*Rupicapra rupicapra*)

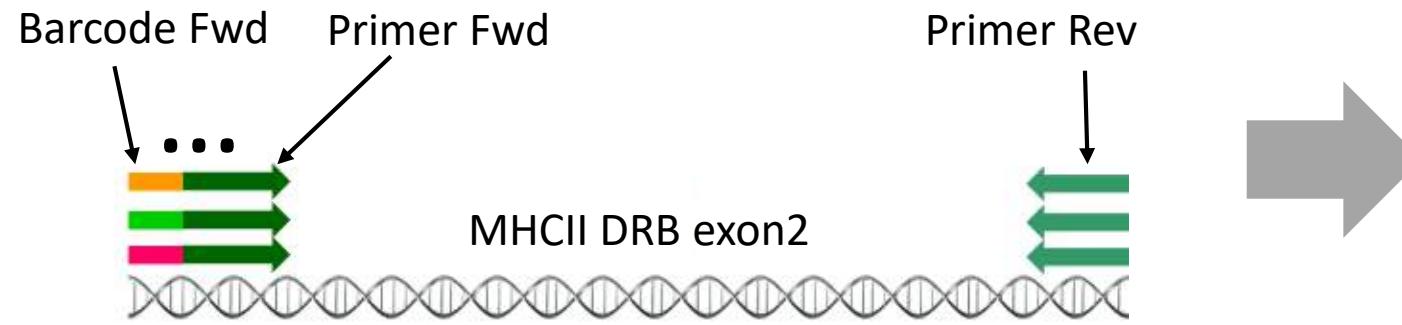
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# Ion Torrent Sequencing

## Materials and methods

### PCR amplification



### DNA sequencing



### Sequence data analysis

**MOLECULAR ECOLOGY RESOURCES**  
Molecular Ecology Resources (2015) doi: 10.1111/1755-0998.1245

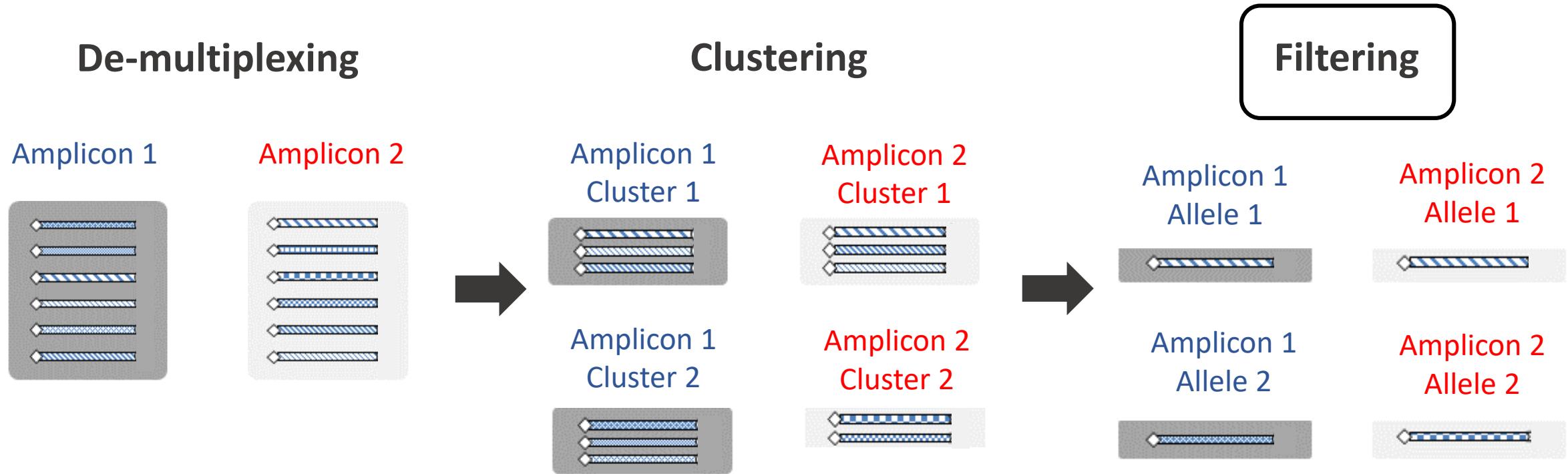
**AMPLISAS: a web server for multilocus genotyping using next-generation amplicon sequencing data**

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```
5;6;5;:=B???CCBB;;6;<<<AAACC>??C;;;C1;7;A>@:>;AAAAC??@CCC@@@?CBACC1<<<7;0666;:::::CD1;:::/;:7;:>:::B@;:::BB;
@015E3:00340:00224
TGAGCGGAACGATGATCCTCTCTGCAGCACATTCTGGTGTATGCTAAAGAGCGAGTGTCAACGGGACGCAGCGGGTGGGGTCTGGACAGATACTTCTA1+
<C????ACACCCCC>><>?:CD?@0@;@B;;1;6;CBAC@A@FCCCACCCDD?<<<6666,6666=666,666;:::CC;:>BB;/7;7;B7;:CD>:>BB6:@CE
@015E3:00340:00226
CGGAAGAACCTCGATATCCTCTCTGCAGCACATTCTGGAGTATGTAAGAGCGAGTGTCAACGGGACCGAGCGGGTGCGGTTCTGGACAGATACTTCC+
??AEACD>?>CCCCCCACCDDDCCC?@DDCCC=CA??>?C???FFCCACCCCCCCC??>CCC>CCACCADDE@CCACCCCC?CCC@6;6;7?DDCCCCC<6;
@015E3:00340:00227
CTTCCATAACGATATCCTCTCTGTAGCACATTCTGGAGTATGTAAGAGCGAGTGTCAACGGGACCGAGCGGGTGCGGTTCTGGACAGATACTTCTA+
BC=A=B=>>?CCCCCC@CCDCDDCCCCD@?CD?C@CDACCCCCCCC?<>CCCCC>>6;<>1>C@CDACCC?@=@CCCCC?CCCDAC6;>:6;CCCCCCCC6;6;6
@015E3:00340:00228
TGCAGTTCGATGATCCTCTCTGCAGCACATTCTGGAGTATGCTAAAGAGCGAGTGTCAACGGGACGCAGCGGGTGCGGTTCTGGACAGATACTTCTATA+
-----<>6CCC<@???:C;:::1;?CCACC@@CCC@@@?B@;:::::6C,><D>A7<,6,666B;:@@1;:>ACACACCAC?>>CCC@C???CF
@015E3:00340:00230
TCTCATCTCGATATCCTCTCTGCAGCACATTCTGGAGTATGTAAGAGCGAGTGTCAACGGGACGCAGCGGGTGCGGTTCTGGACAGATACTTCTATA+
```

# AMPLISAS algorithm

## Methods



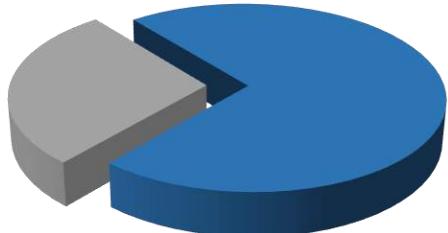
Galan *et al.* BMC Genomics 2010

Sommer *et al.* BMC Genomics 2013

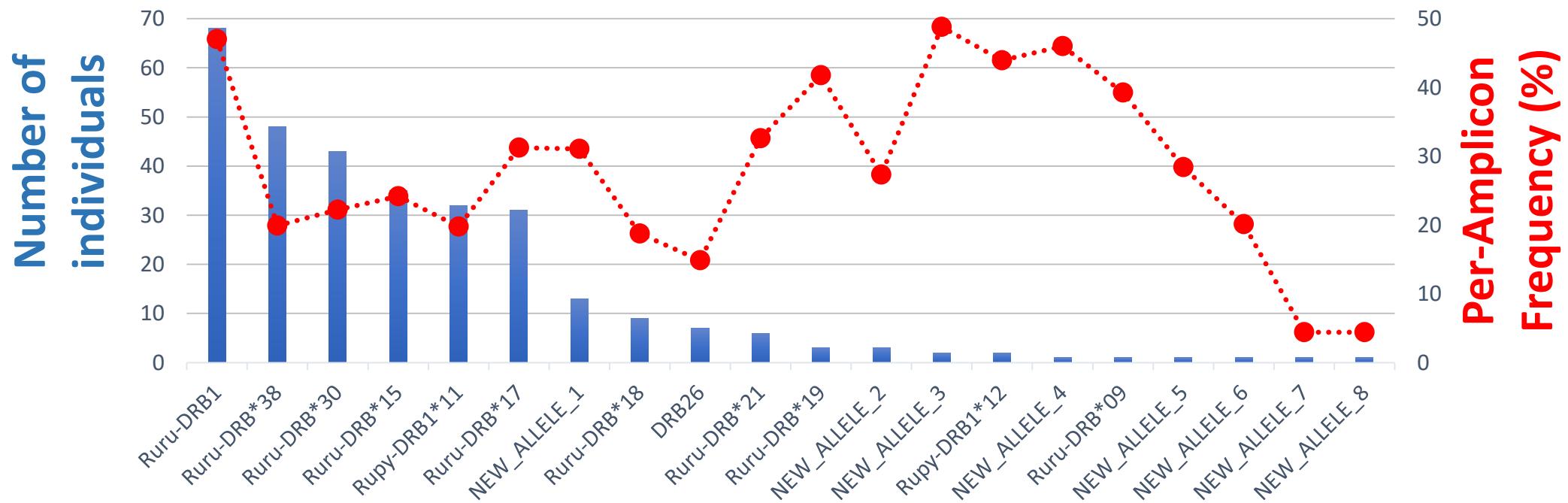
Lighten *et al.* Molecular Ecology 2014

Grogan *et al.* BMC Genomics 2016

depth amplicons: 89175 reads



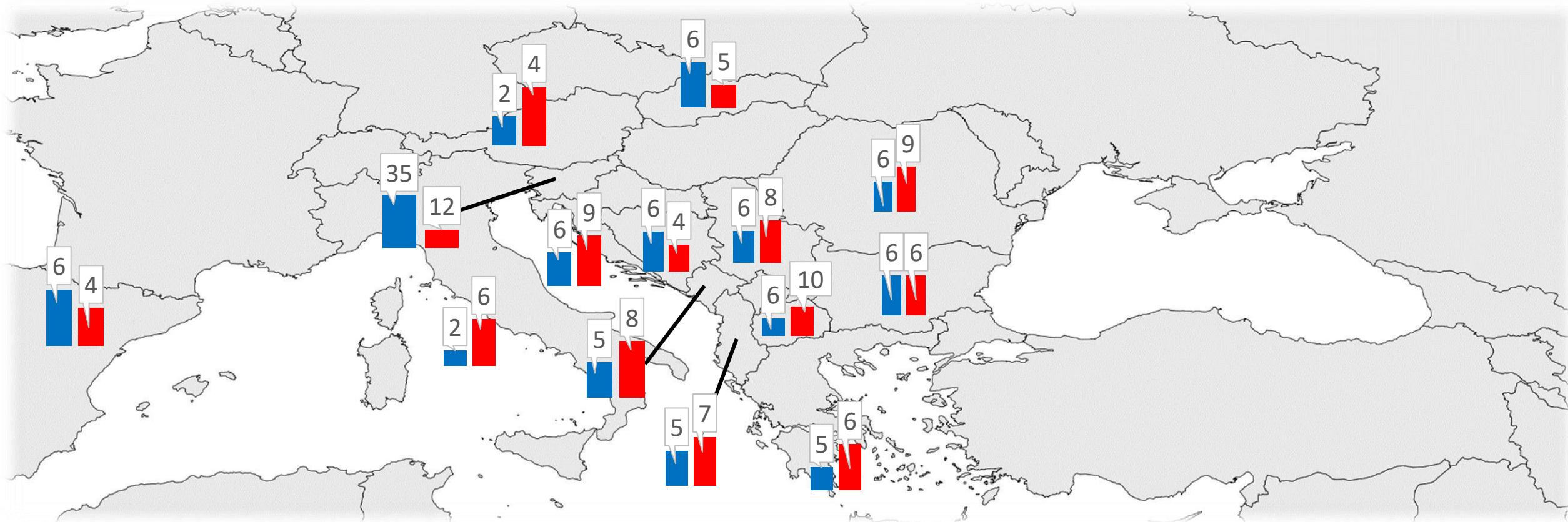
total number of alleles: 20  
previously described in chamois: 12  
new alleles: 8



# MHC diversity

Results

sample number      allele number

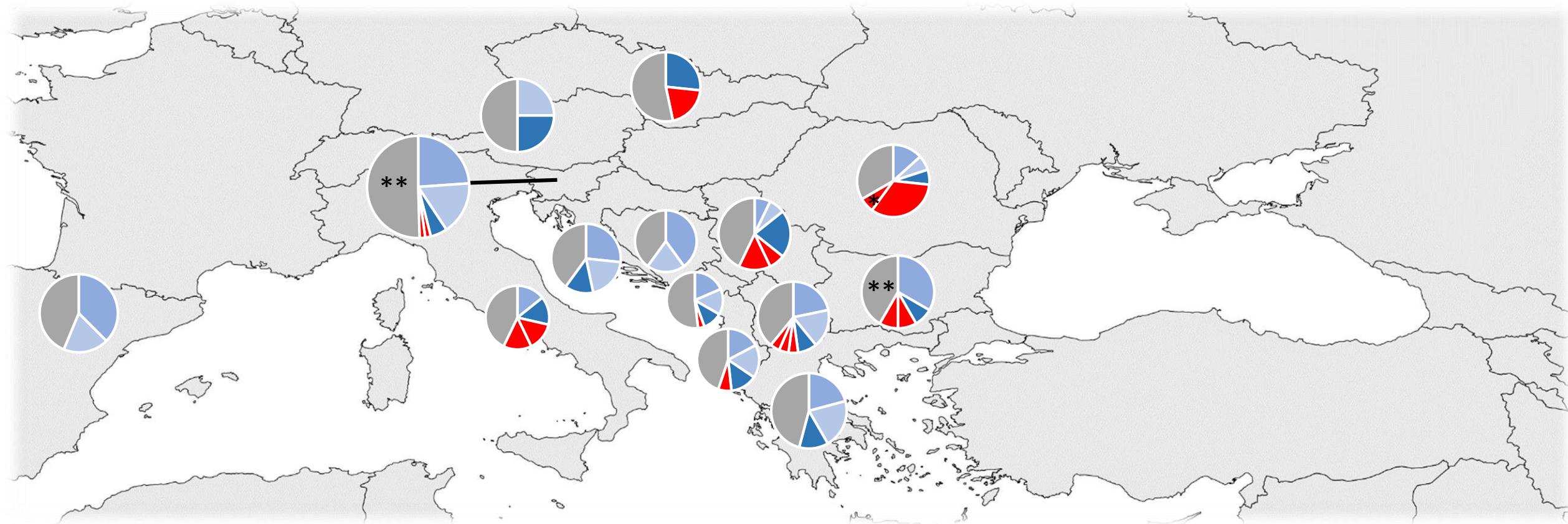


the average number of alleles per individual

Albania	5,6	Italy	3,5	Slovakia	2,5	Bulgaria	2
Montenegro	5,4	Slovenia	3,2	Croatia	2,5		
North Macedonia	4,8	Spain	2,7	Bosnia and Herzegovina	2,1		
Greece	4,8	Romania	2,7	Serbia	2		

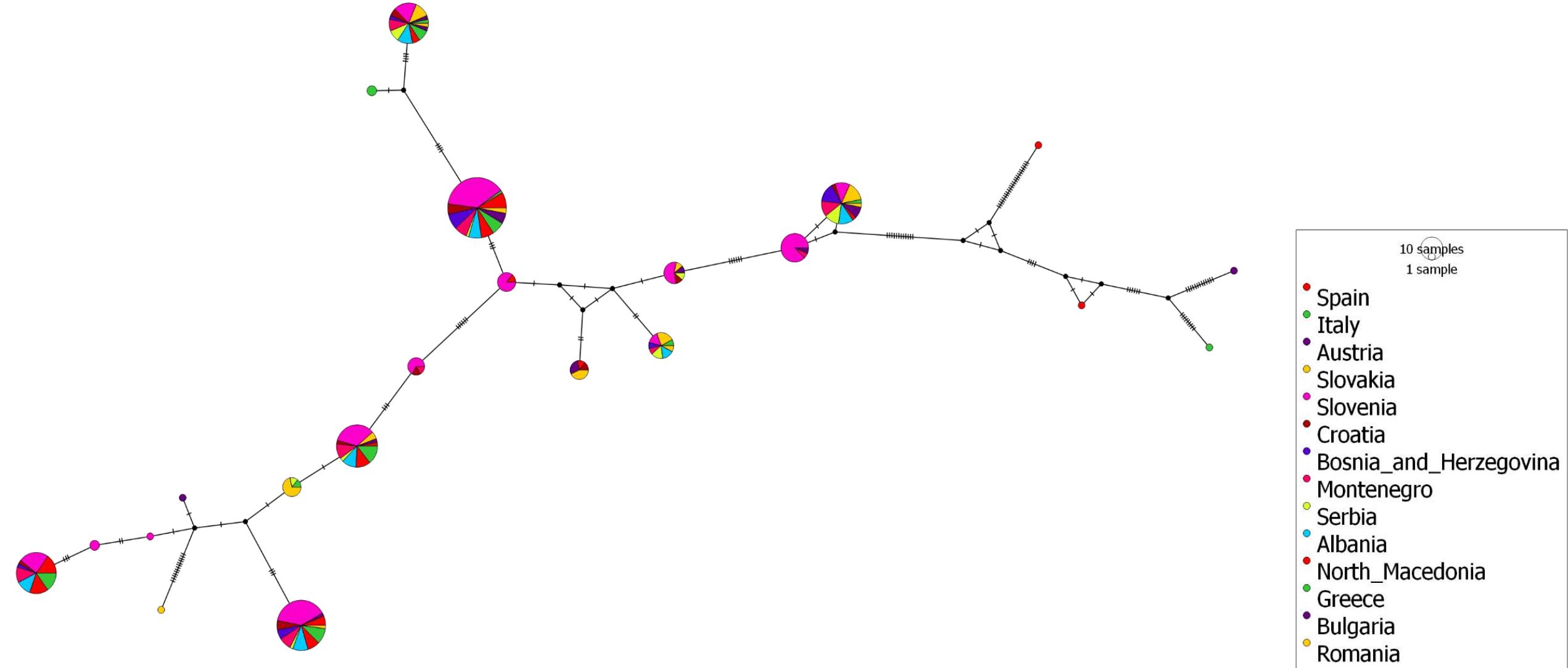
# Frequency distribution of MHC alleles per sampled region

Results



# Median Joining Network

Results



# Conclusions

MHC class II *DRB* is a complex multilocus system with a high level of polymorphism in chamois

evidence of multiple co-amplifying copies

presence of at least 3 loci resulting from gene duplication events





*Dear Colleagues,  
Thank You for Your attention!*

#### ACKNOWLEDGEMENTS

**DNA as a evidence of distribution and vitality of endangered Balkan chamois**

– BalkCham IP-2016-06-5751