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Characterization of *Apis mellifera* populations in Italy is important for biodiversity conservation and sustainable beekeeping. The objective of this study was conducted within the BeeNet and GENAPIS.IT projects to enhance knowledge on the distribution and genetic status of subspecies and to identify potential signs of genetic introgression. Honey bee samples were obtained from 368 colonies distributed across 20 Italian regions, and analysed using morphometric and molecular methods. For morphometric analysis, 18 right forewings per sample were dissected, and 16 wing parameters were acquired. Subspecies assignment was performed through discriminant analysis with a posterior probability threshold of $\geq 90\%$. In parallel, genetic analysis was conducted by sequencing the tRNA^{Leu}-COX2 intergenic region of the mitochondrial DNA (mtDNA), using E2 and H2 primers. The obtained sequences were analysed with the PHYLIP 3.65 software to construct phylogenetic trees based on lineages A, M, and C, using reference sequences deposited in GenBank. Morphometric analysis showed that 77% of the analysed samples were consistent with native Italian subspecies (68% to *Apis mellifera ligustica* and 9% to *A. m. siciliana*). 13% of the samples exhibited characteristics not attributable to any subspecies in the reference database, suggesting the presence of intraspecific hybrids (e.g., Buckfast) or genetic introgression. An additional 10% of samples were classified as belonging to other crosses or subspecies, including *A. m. carnica* (1%), *A. m. carnica*-*A. m. ligustica* hybrid (7%), and *A. m. siciliana*-*A. m. ligustica* hybrid (2%). Results of molecular analyses showed that 75% of samples carried haplotypes compatible with *A. m. ligustica* (C1, M7) and *A. m. siciliana* (A). The remaining 25% exhibited haplotypes belonging to not native subspecies, confirming the presence of genetic introgression or genetic variability. The data obtained from this study provide a detailed overview of the distribution and conservation status of Italian *A. mellifera* populations. The results highlight the predominance of native subspecies but also the occurrence of hybridization phenomena, emphasizing the need for targeted safeguarding strategies. The integration of morphometric and genetic analyses represents an effective approach for monitoring population status and supporting the sustainable management of Italian beekeeping.

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Distribution and conservation status of *Apis mellifera* populations in Italy

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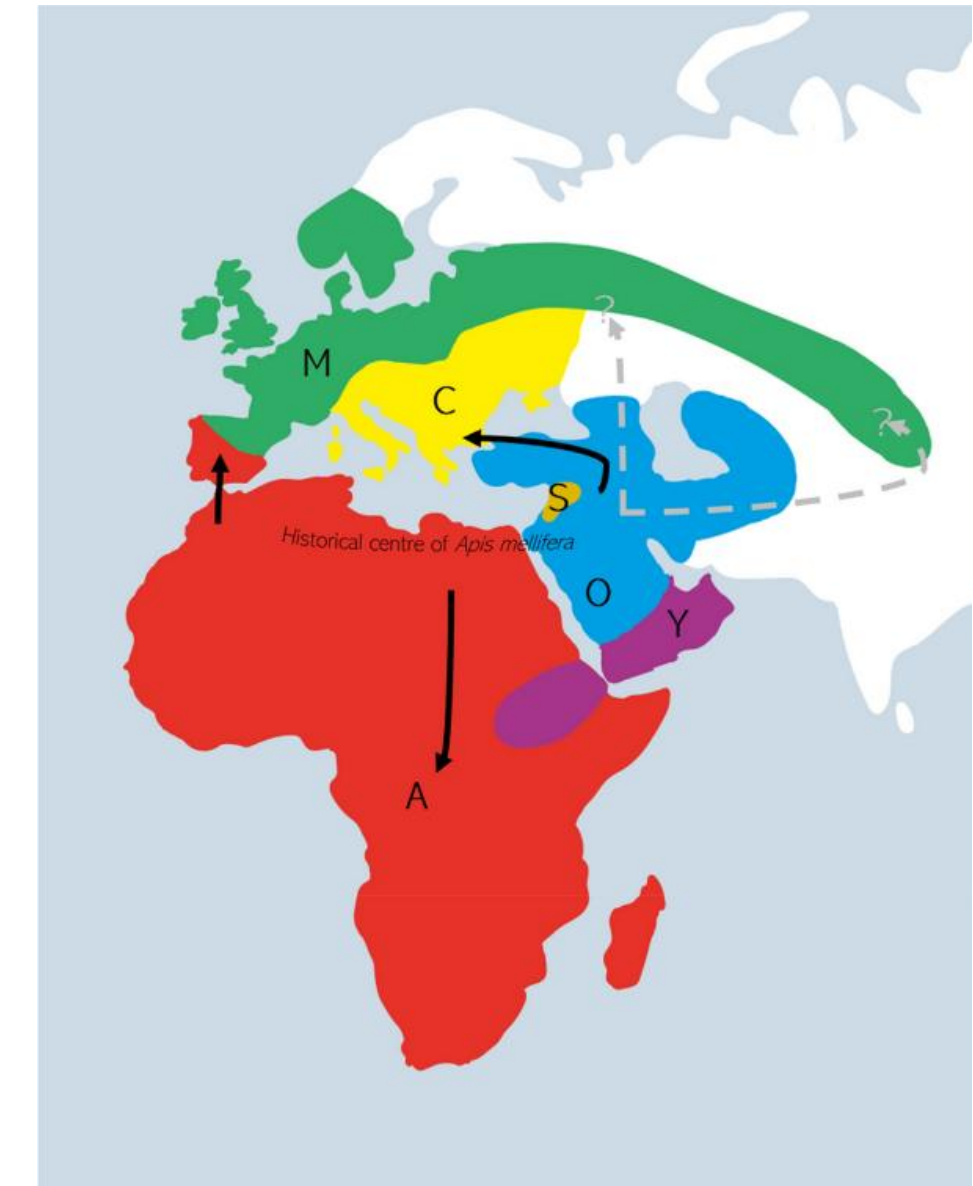
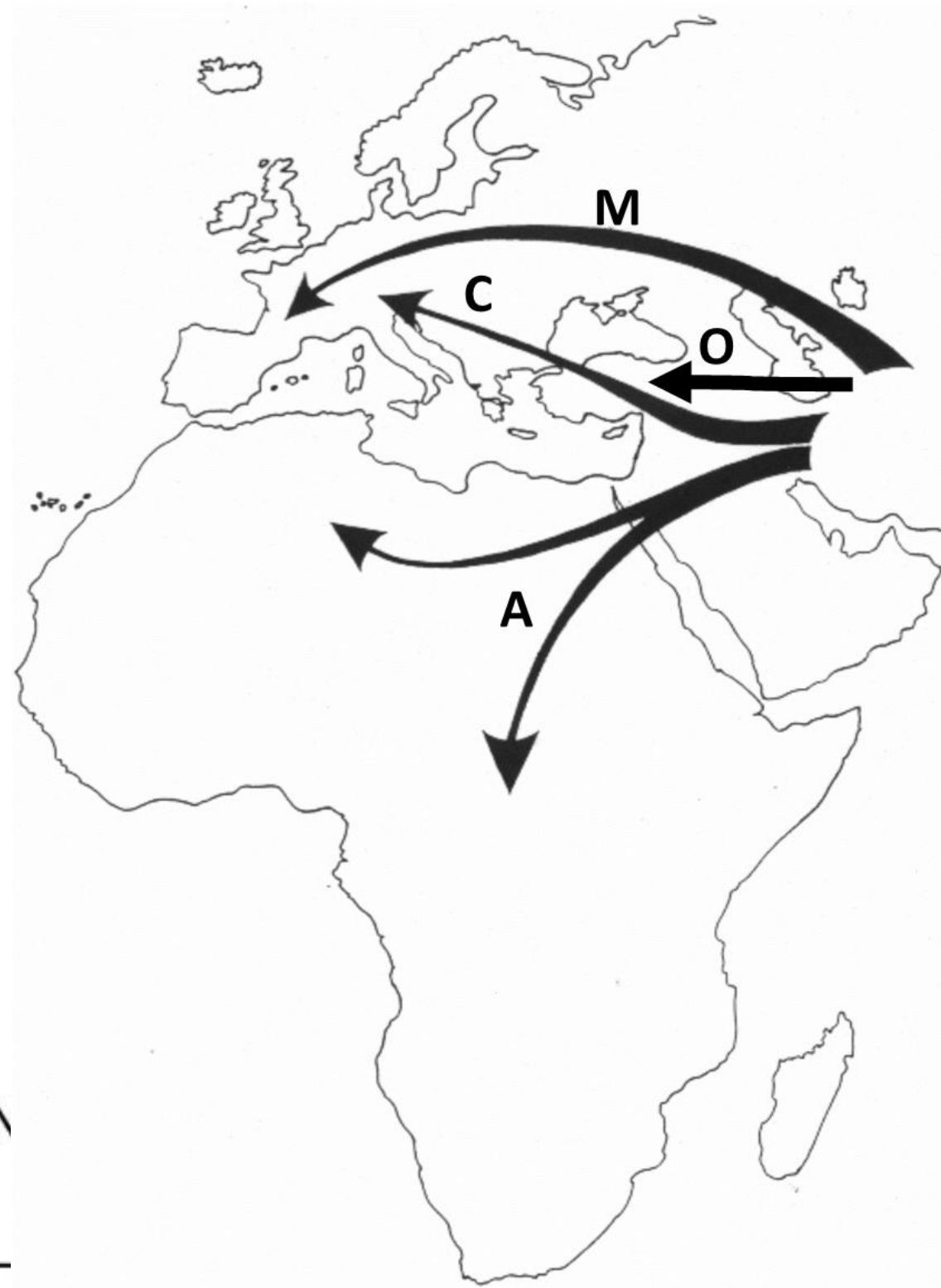
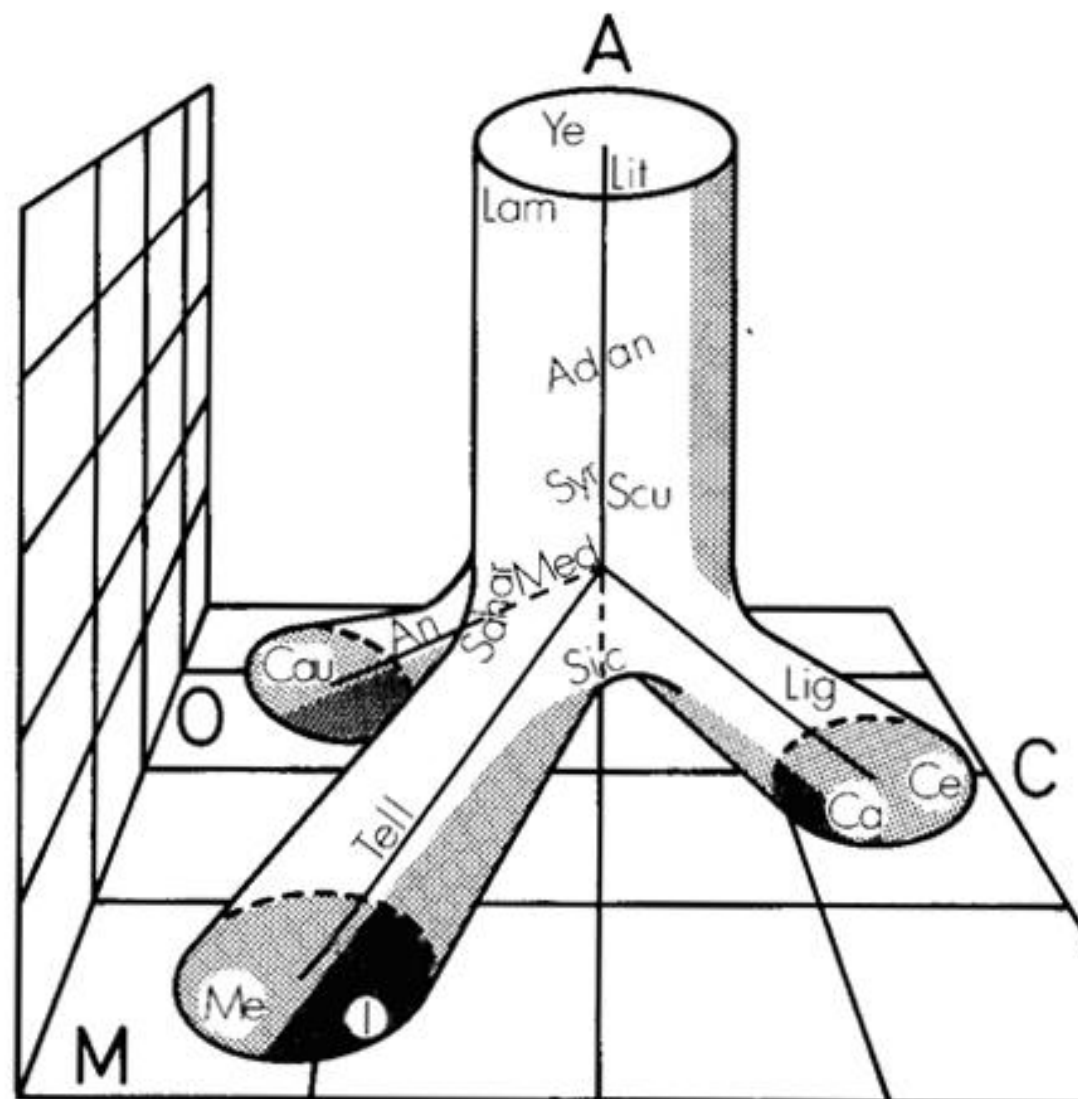
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Subspecies of *Apis mellifera*



Evolutionary lineage of *Apis mellifera*



Article [Open Access](#) | Published: 03 September 2020

Mitochondrial genomes illuminate the evolutionary history of the Western honey bee (*Apis mellifera*)

Erik Tihelka , Chongyang Cai , Davide Pisani & Philip C. J. Donoghue

Scientific Reports 10, Article number: 14515 (2020) | [Cite this article](#)

Evolutionary lineage of *Apis mellifera*

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Available online at:
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Review article

Biodiversity, conservation and current threats to European honeybees*

Pilar DE LA RÚA^{1**}, Rodolfo JAFFÉ^{2**}, Raffaele DALL'OLIO^{3**}, Irene MUÑOZ¹,
José SERRANO¹

- Italian peninsula: C and M lineage
- Sicilia: A lineage



Italian subspecies

Apis mellifera ligustica

Spinola 1806



Apis mellifera siciliana

Dalla Torre 1896



Neighboring subspecies

Apis mellifera carnica

Pollman 1879



Apis mellifera mellifera

Linnaeus 1758



GENAPIS.IT project

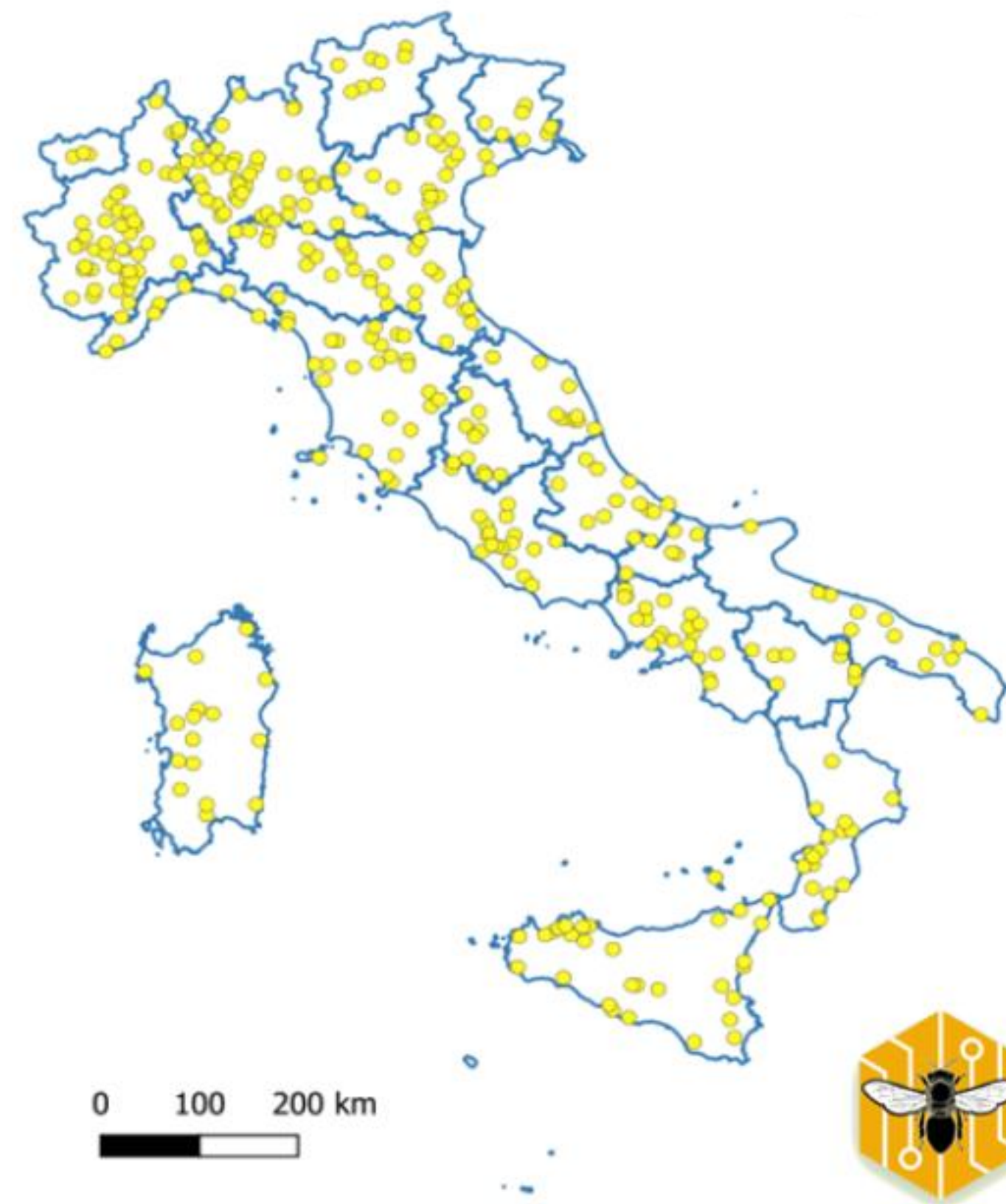


Study of morphometric and genetic characterisation of honey bees subspecied across Italy

Material and Methods

Samples

- One slot collected within the monitoring project BeeNet (only from FAI e MiC apiaries)
- New samples collected from the beekeepers to reach all geographic area



Distribution of samples

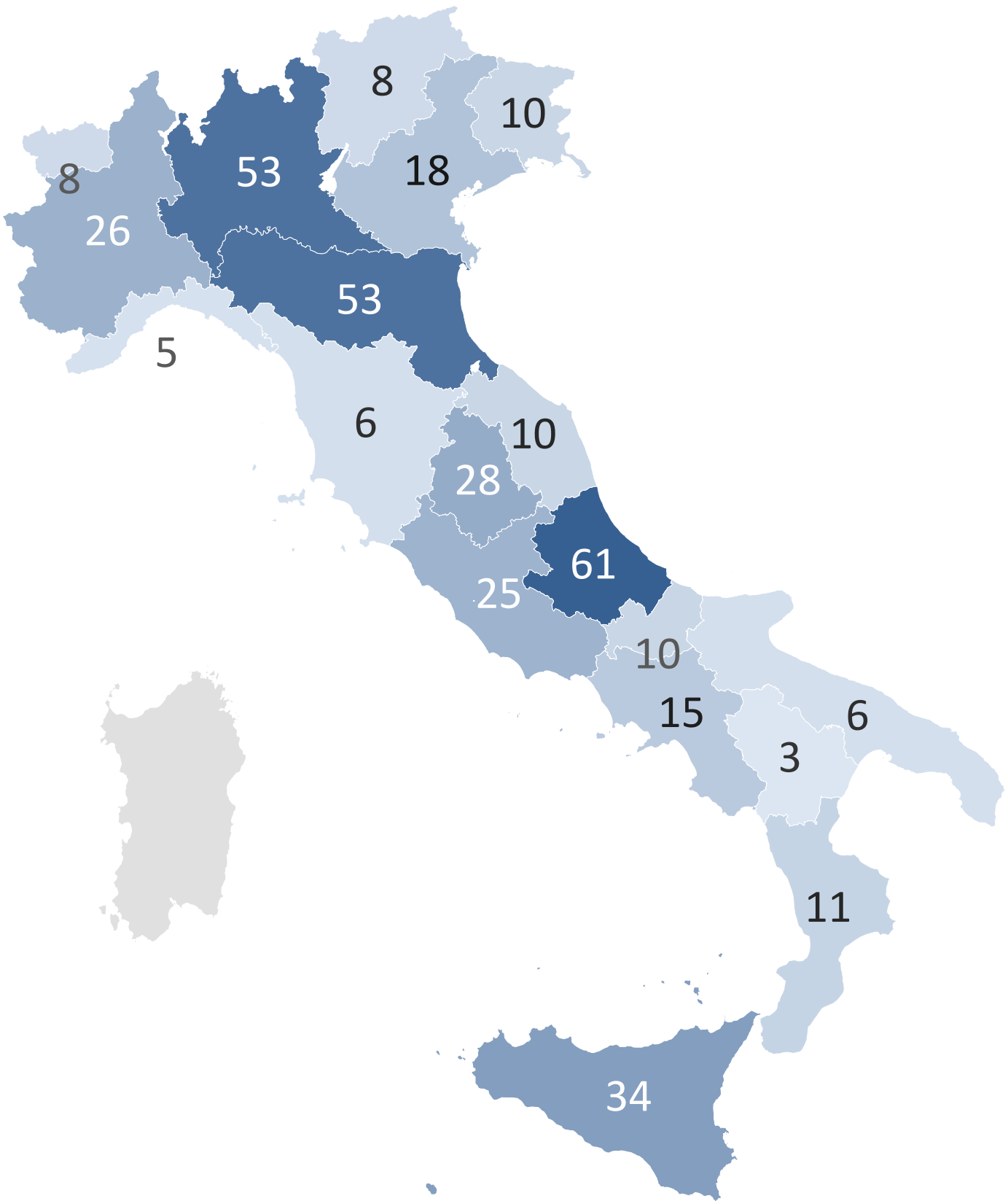
Samples origin



BeeNet = 188



GENAPIS.IT = 200



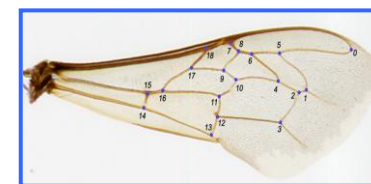
Material and Methods

➤ Analyses

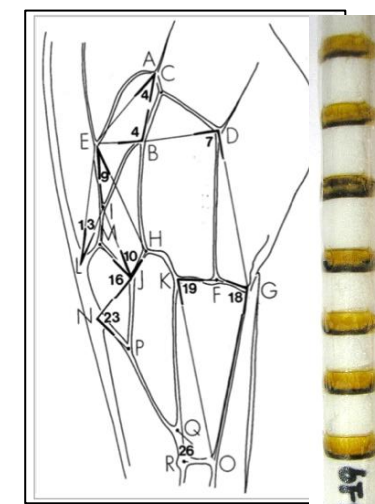
- morphometrics
- genetics



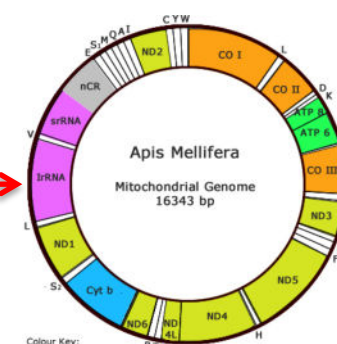
Morphometric database with 178 samples belonging to 5 subspecies (*A.m.carnica*, *A.m.caucasia*, *A.m.ligustica*, *A.m.mellifera*, *A.m.siciliana*)



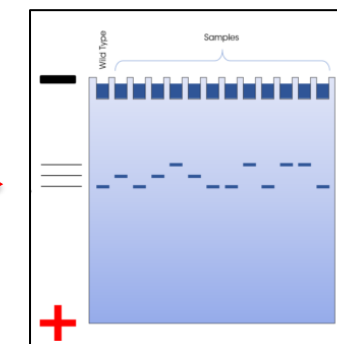
Wings and tergites



Morphometric analysis



mtDNA



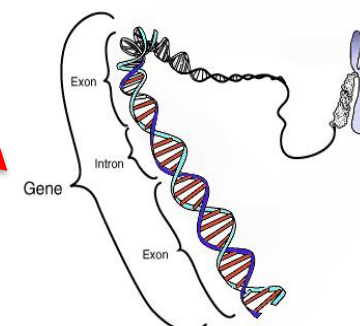
tRNA^{leu}-cox2 region sequencing

Apidologie (2017) 48:533–544
© INRA, DIB and Springer-Verlag France, 2017
DOI: 10.1007/s13592-017-0498-2

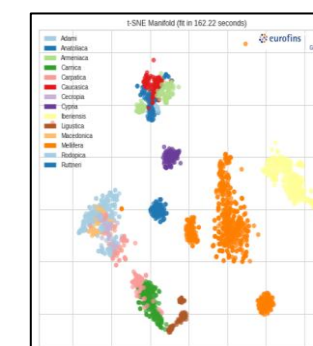
Original article

Mitochondrial DNA variation of *Apis mellifera iberiensis*: further insights from a large-scale study using sequence data of the tRNA^{leu}-cox2 intergenic region

Julio CHÁVEZ-GALARZA^{1,2}, Lionel GARNERY^{3,4}, Dora HENRIQUES^{1,2}, Cátia J. NEVES¹, Wahida LOUCIF-AYAD⁵, J. Spencer JONISTON⁶, M. Alice PINTO¹



nDNA

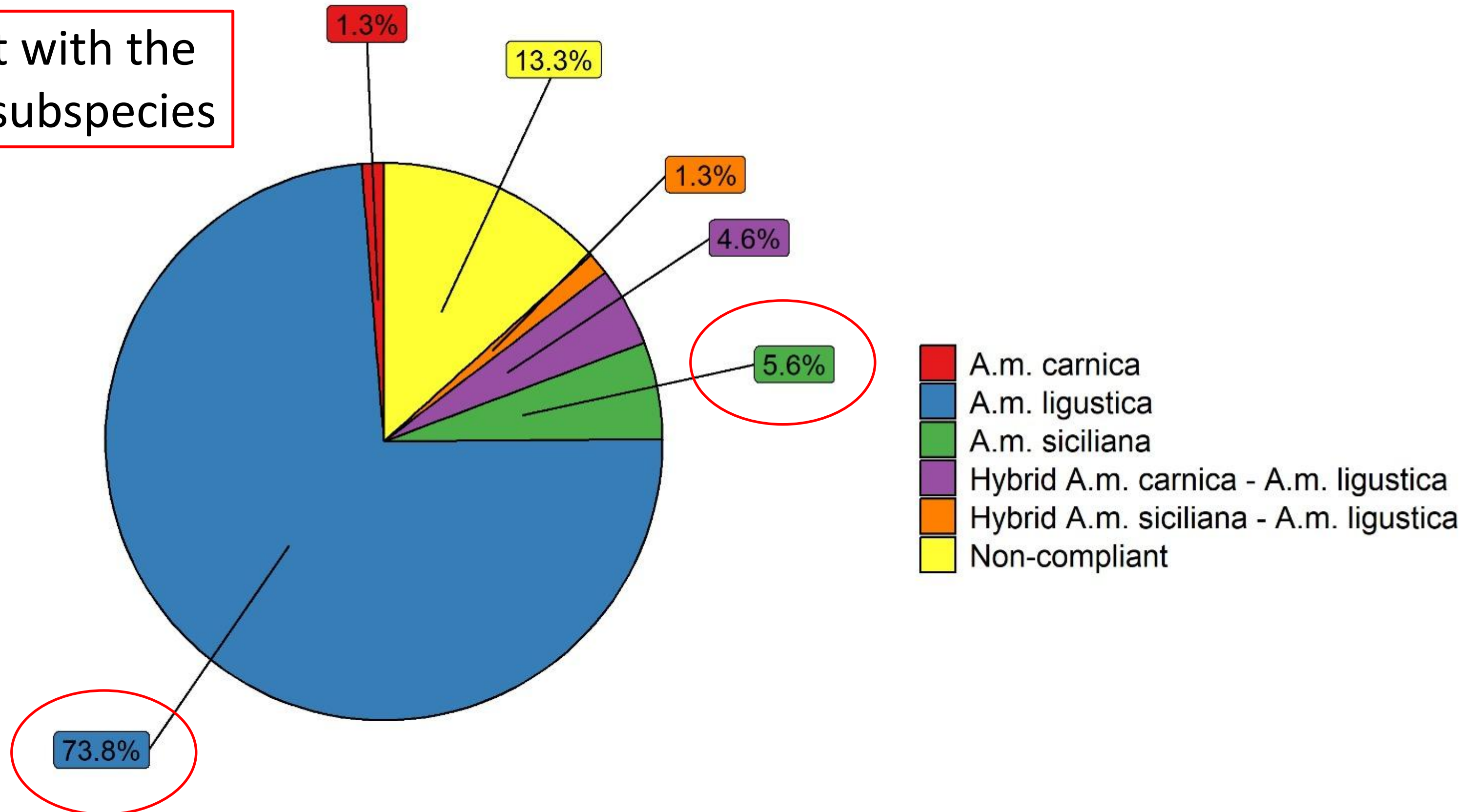


SNPs analysis (SmartBees)

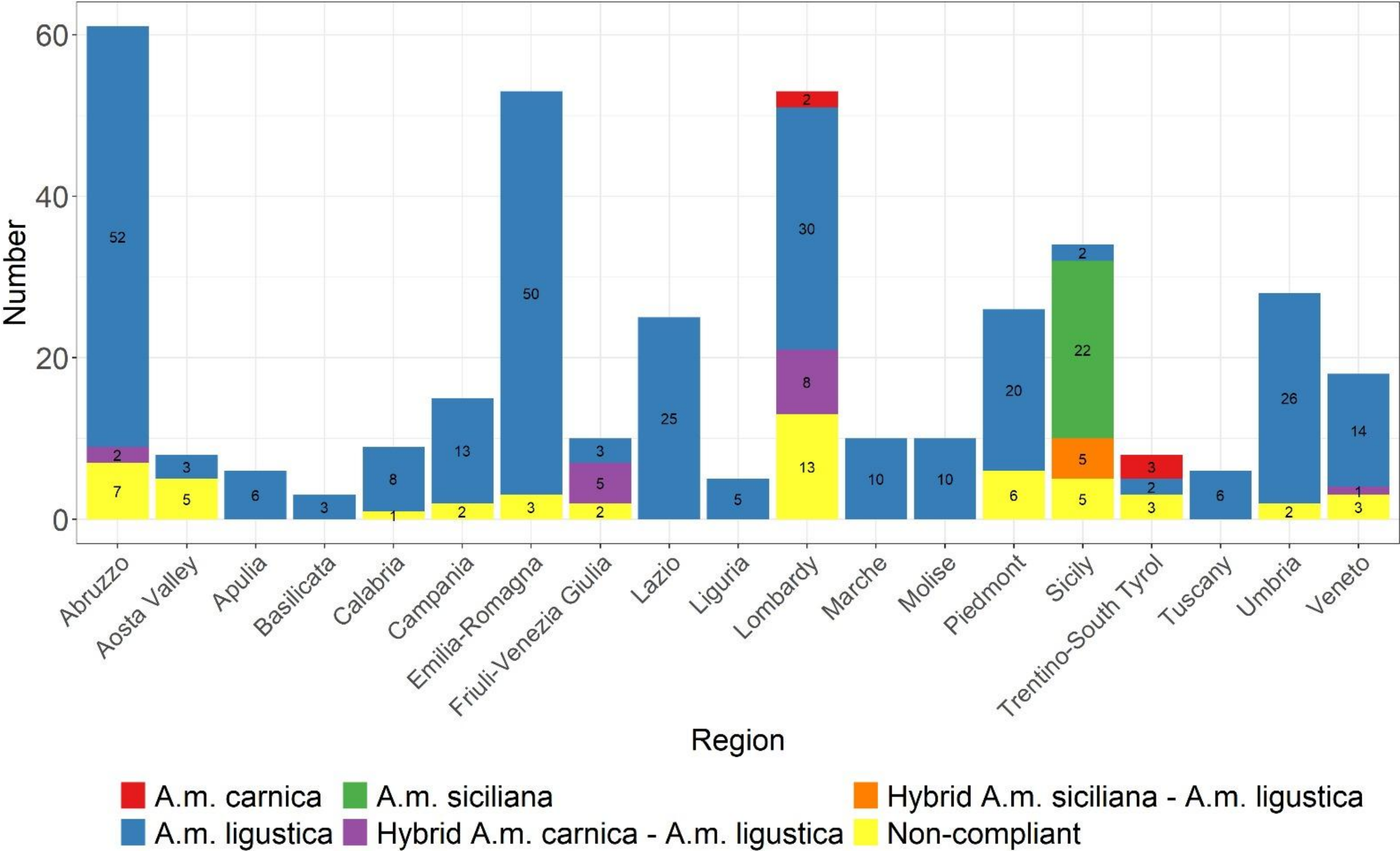
Morphometric analysis



79.4% consistent with the endemic Italian subspecies

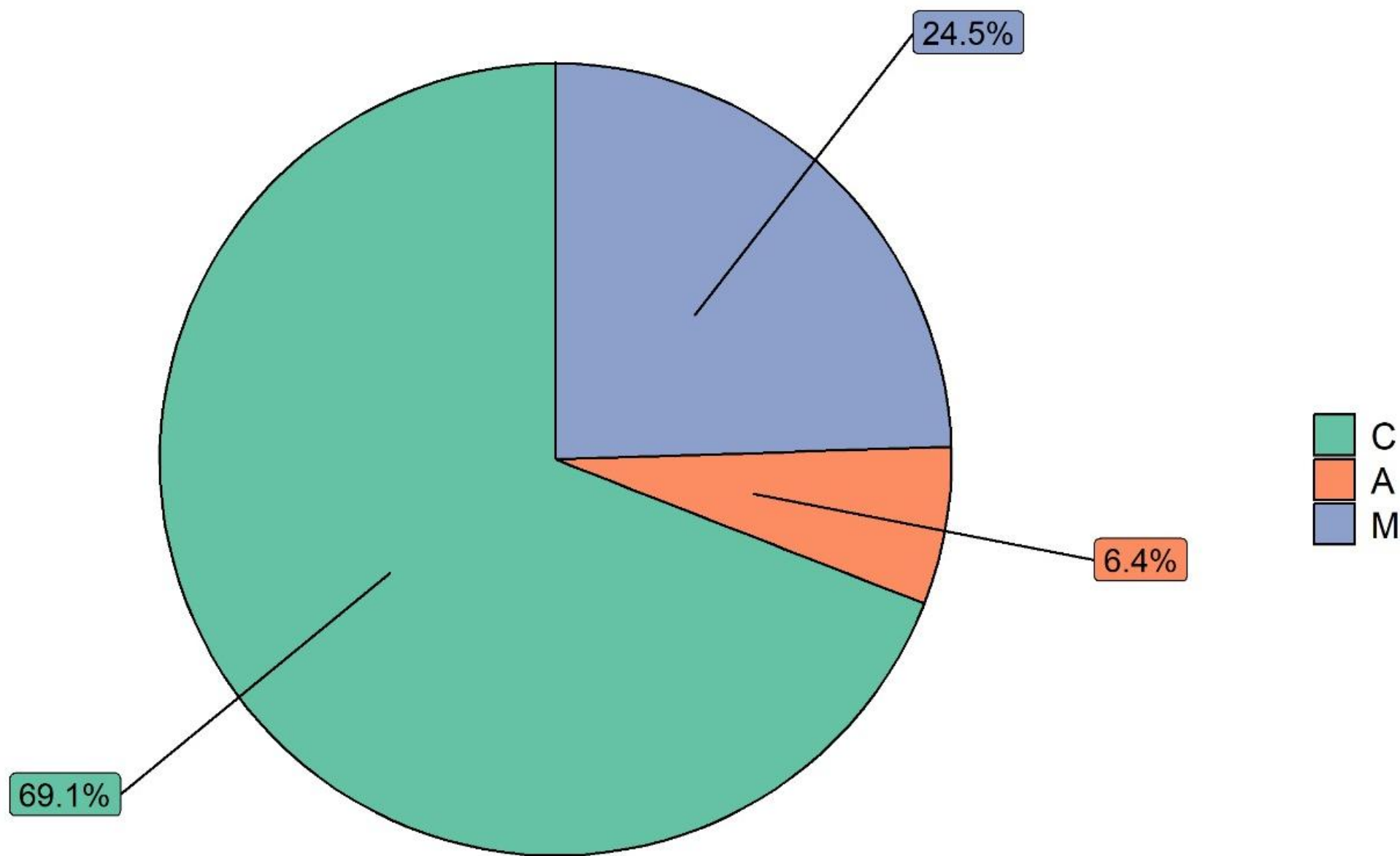


Morphometric analysis per region

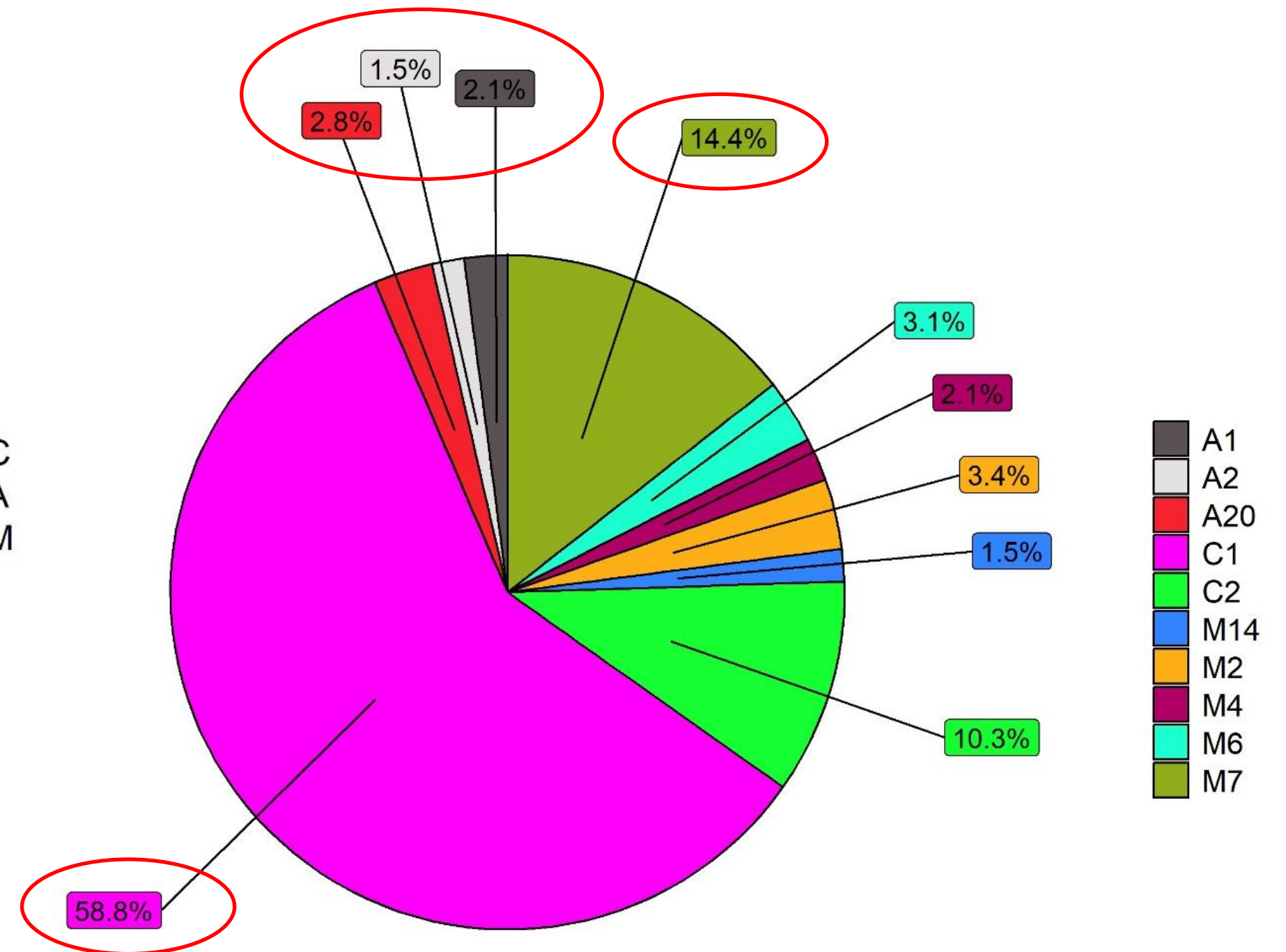


Mithochondrial analysis

Frequency of lignage ($\text{tRNA}^{\text{leu}}\text{-cox2}$)

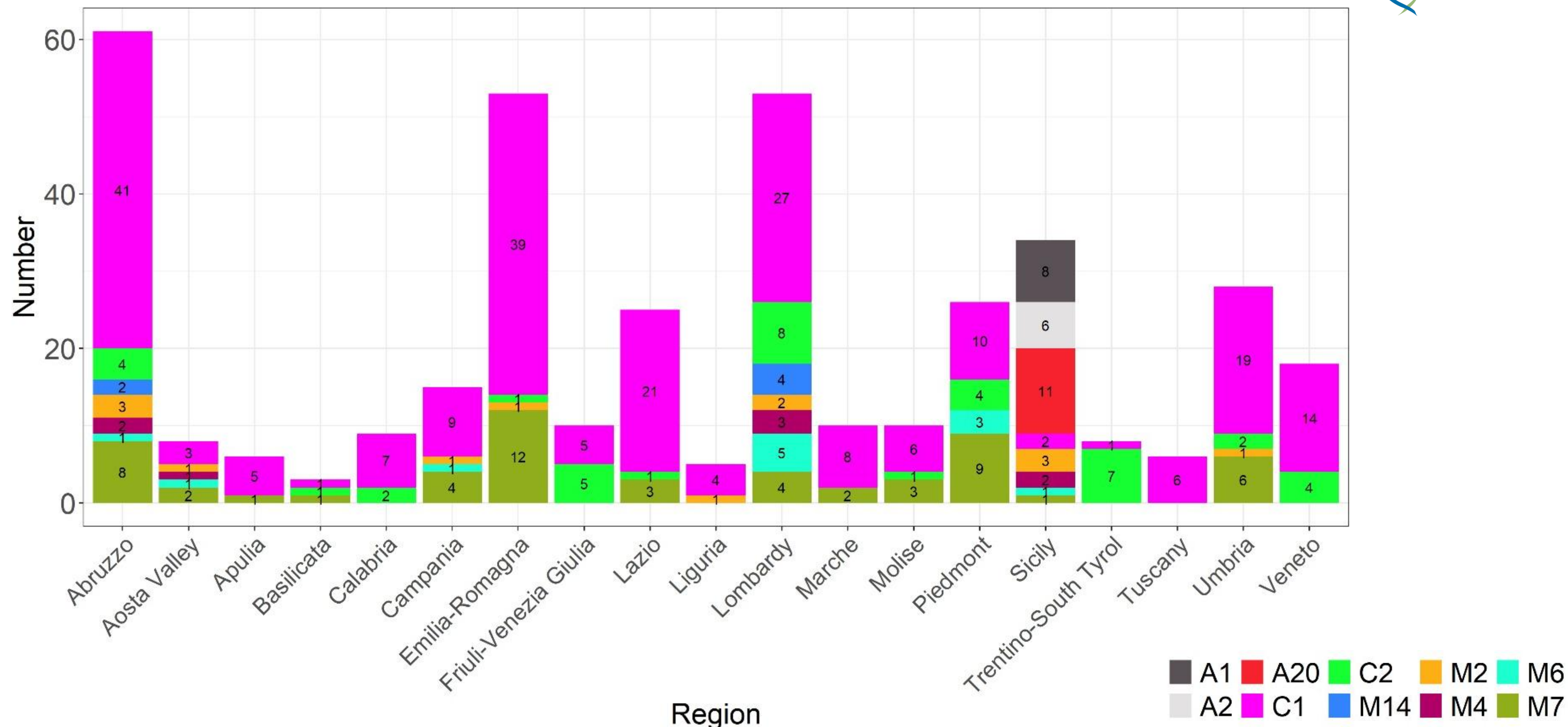


10 haplotypes

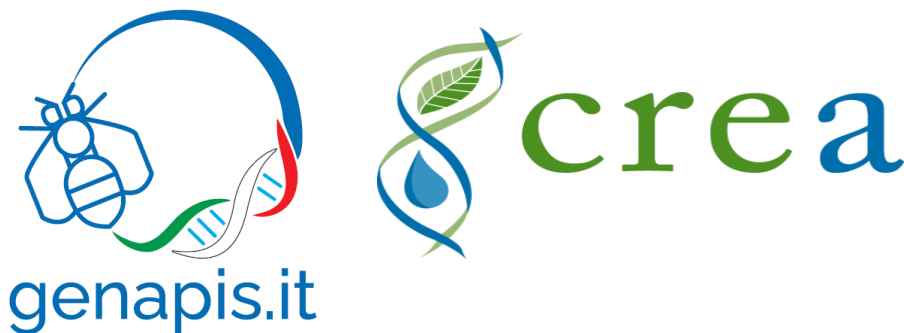


79.6% consistent with the endemic Italian subspecies

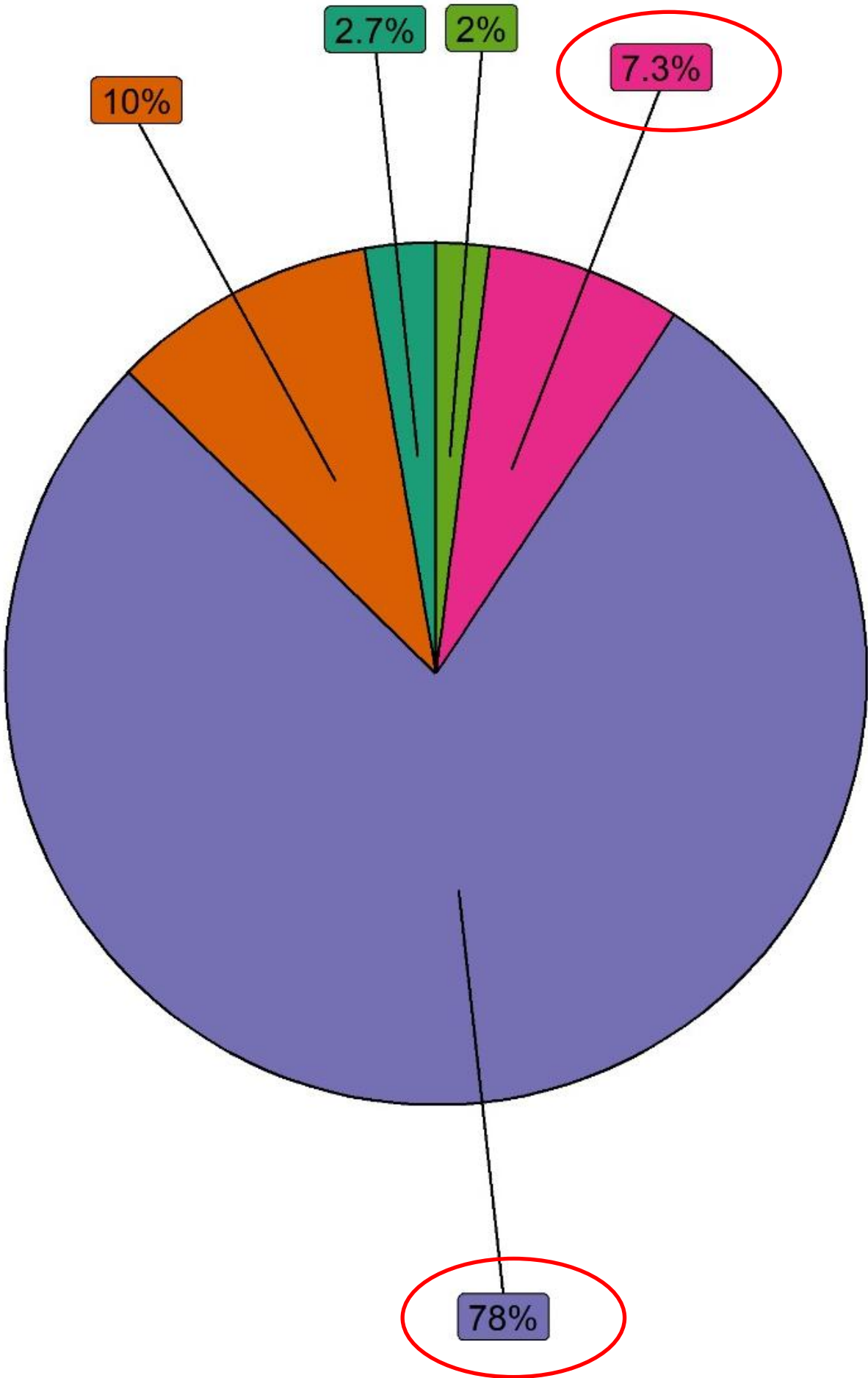
Distribution of haplotypes per region



Nuclear DNA (SNPs) analyses



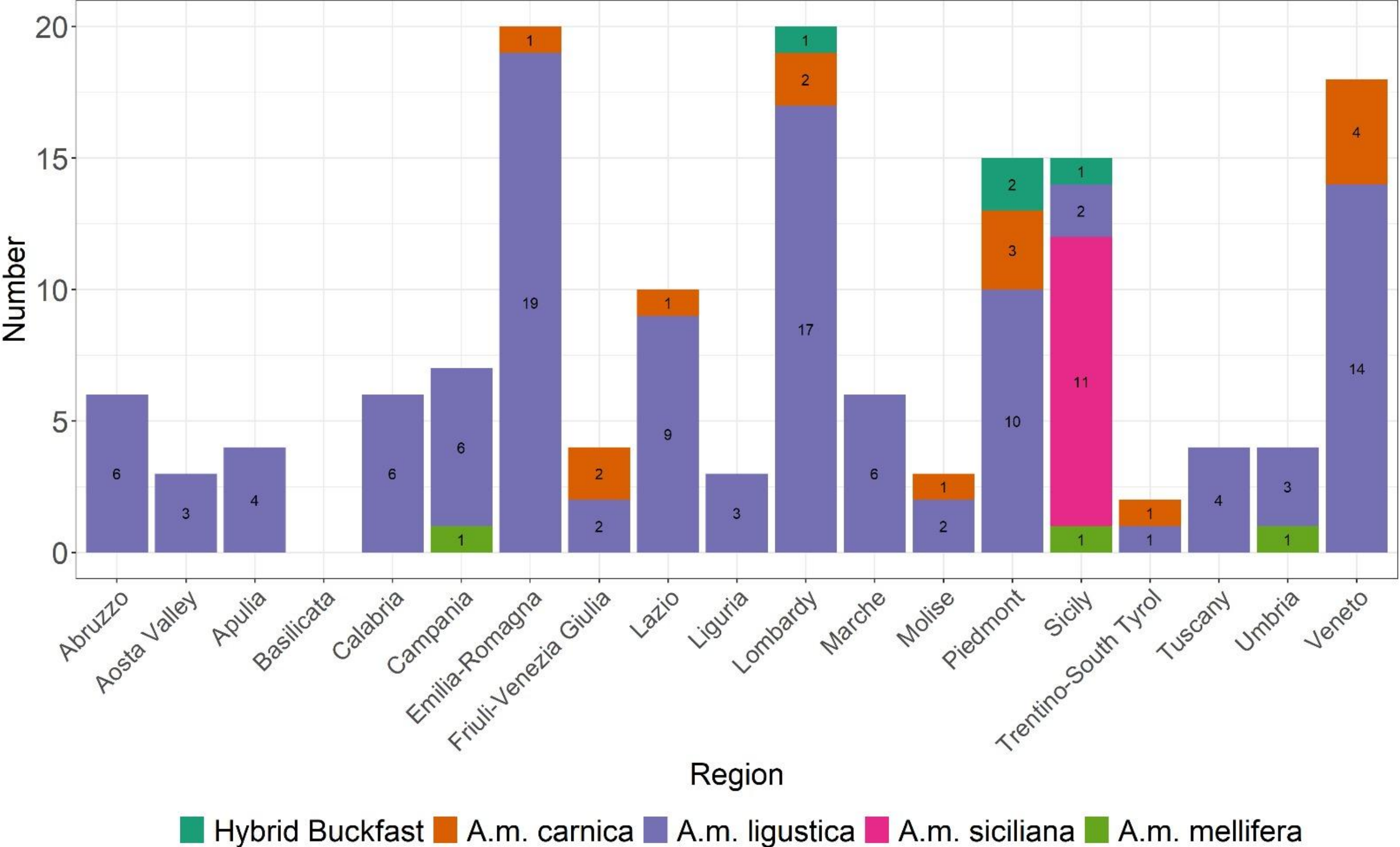
Only 150 samples were analysed out of 388



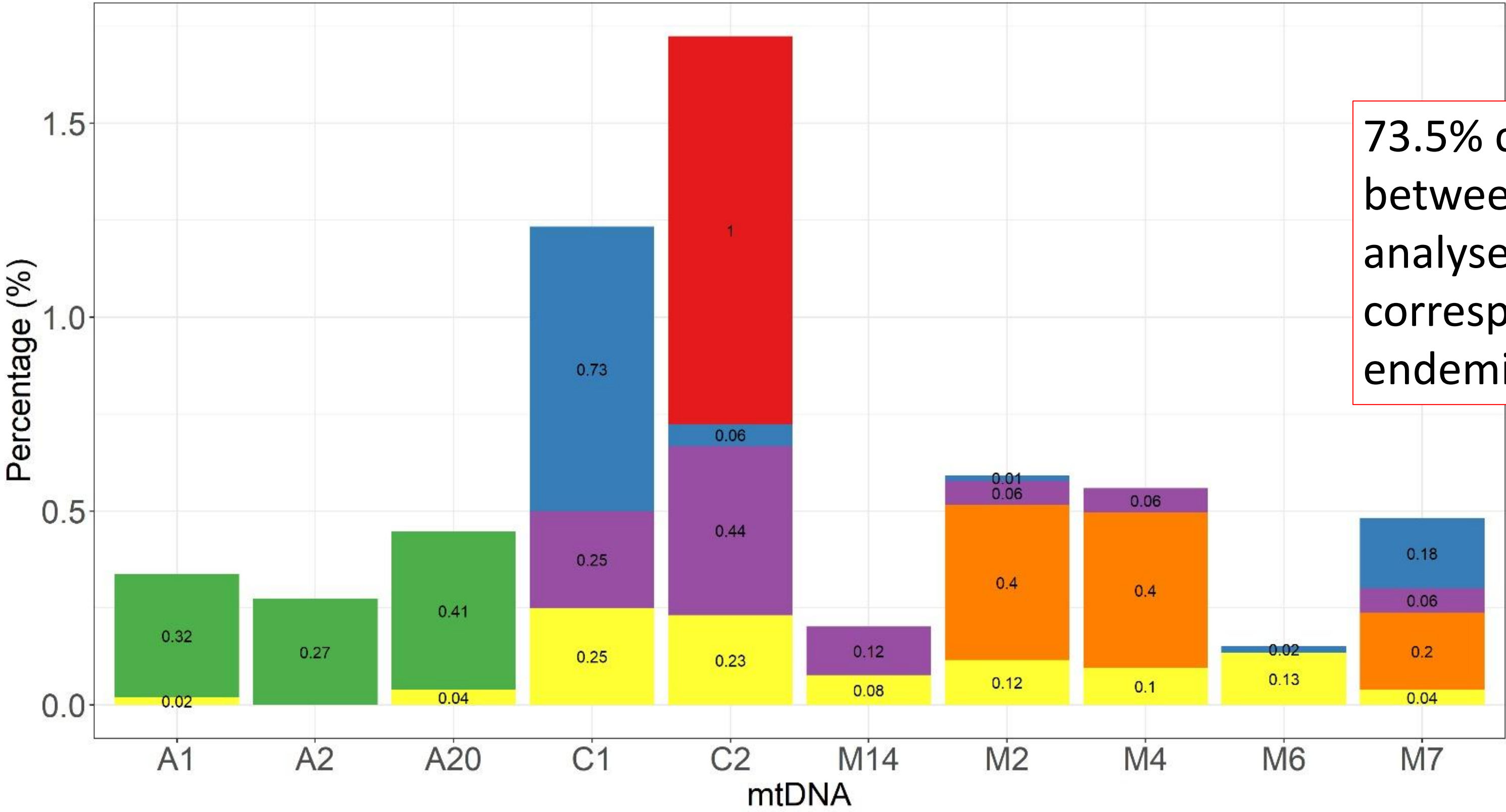
- Hybrid Buckfast
- A.m. carnica
- A.m. ligustica
- A.m. siciliana
- A.m. mellifera

85.3% consistent with the endemic Italian subspecies

Distribution of nDNA oer region



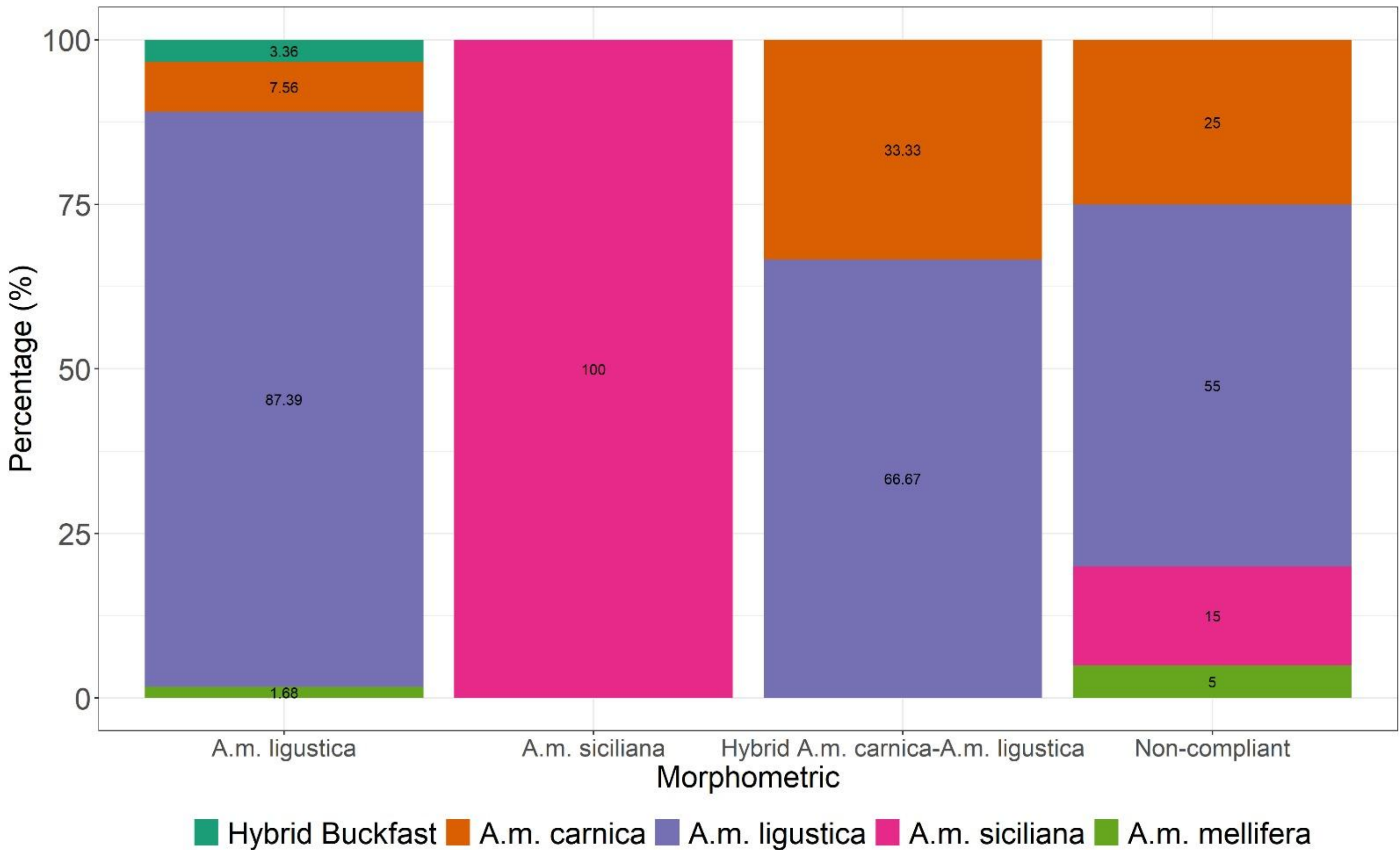
Consistency between morphometric and mtDNA



73.5% congruence between morphometric analyses and mtDNA corresponding to the endemic subspecies

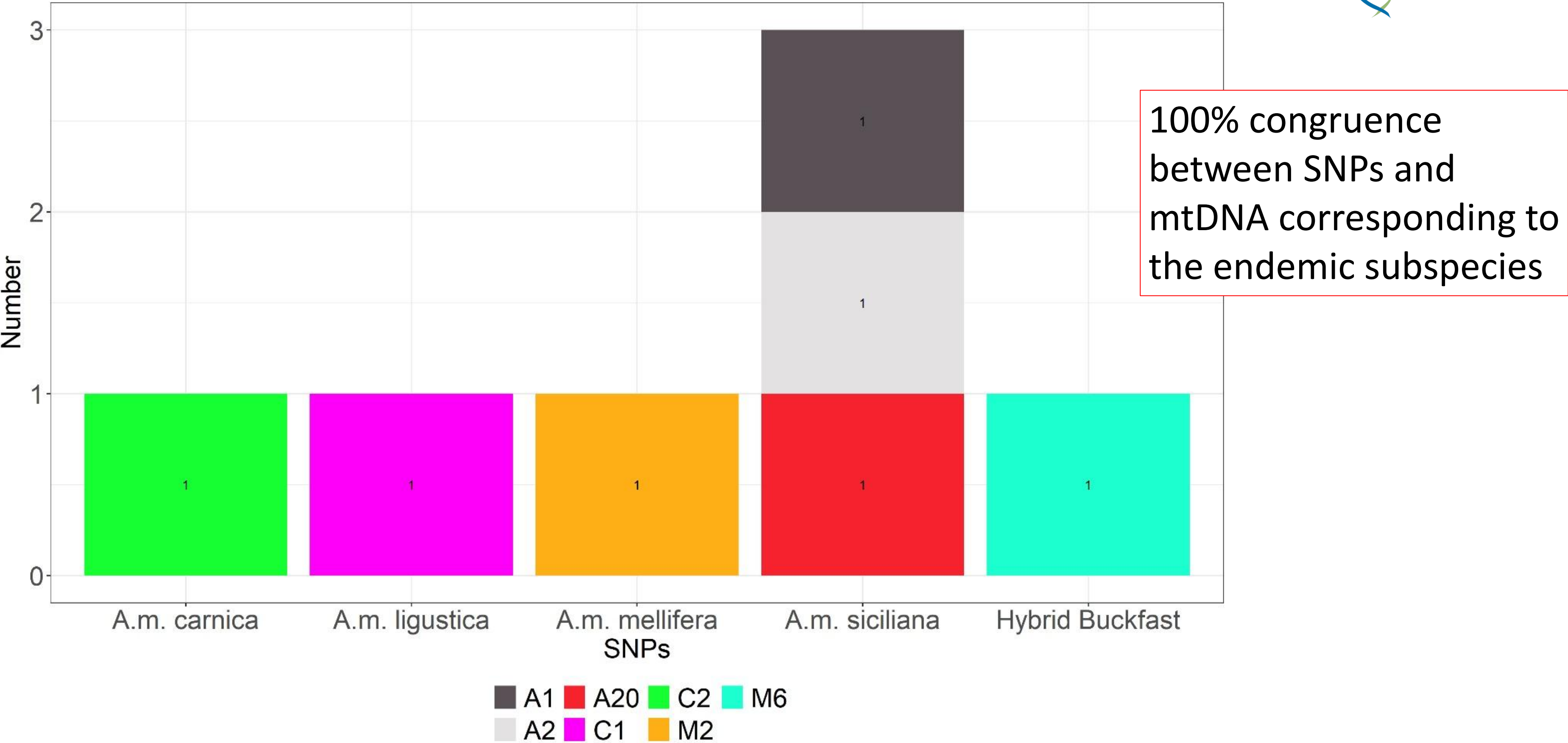
- A.m. carnica
- A.m. siciliana
- Hybrid A.m. siciliana - A.m. ligustica
- A.m. ligustica
- Hybrid A.m. carnica-A.m. ligustica
- Non-compliant

Consistency between morphometric and SNPs

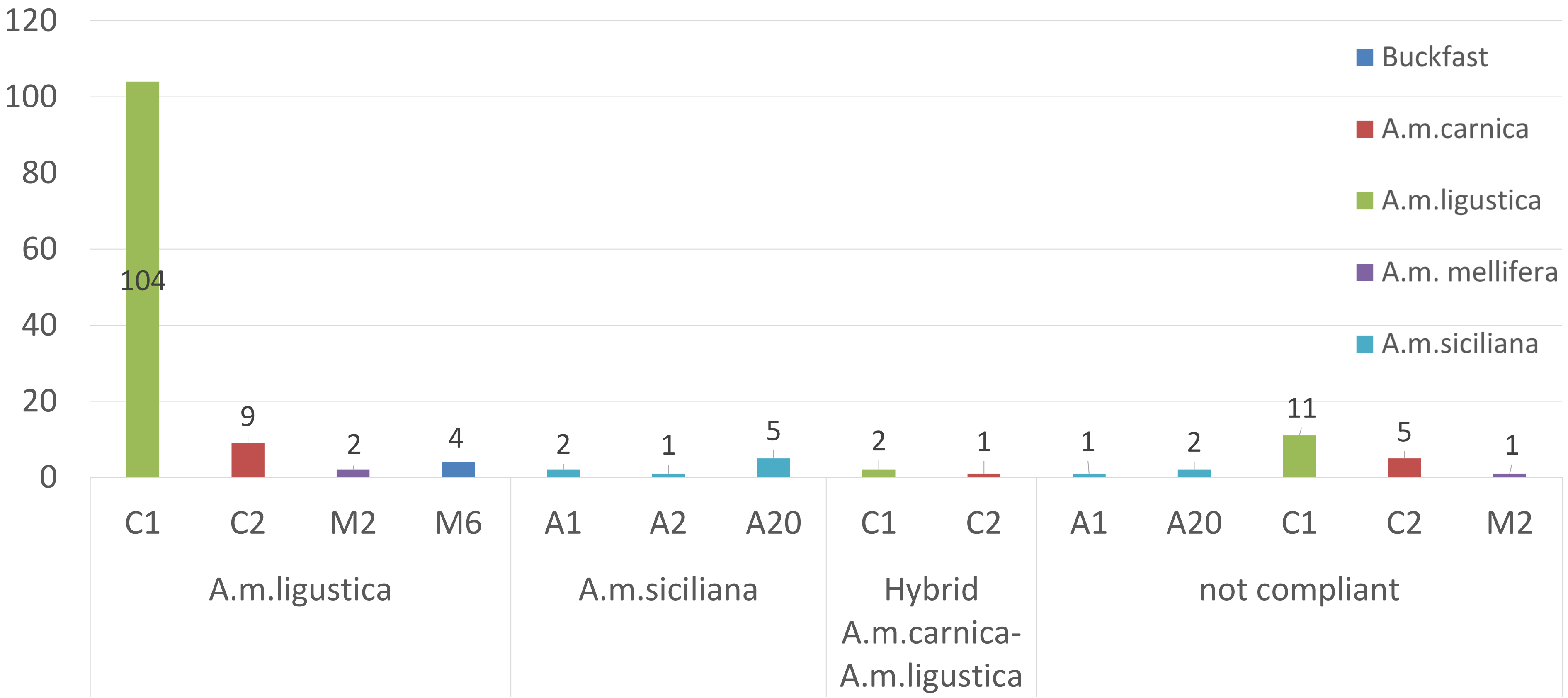


74.7% congruence between morphometric analyses and SNPs corresponding to the endemic subspecies

Consistency between SNPs and mtDNA



Consistency between morphometric, SNPs and mtDNA



Conclusioni

- The endemic subspecies *A. m. ligustica* and *A. m. siciliana* are widely present throughout the country
- The C1 and M7 haplotypes were the most frequent
- The C2 haplotype, characteristic of *A. m. carnica*, was found most frequently in the north-eastern regions.
- Sicily remains mainly characterised by haplotype A.
- The results obtained show that:
 - The current populations of *A. mellifera* in Italy retain the characteristics described in scientific literature.
 - highlight introgression phenomena, both in the North and in the South

Take-home message

An integrated approach combining the three techniques (morphometry, mtDNA and SNPs) improves the accuracy of the analyses and provides a detailed and realistic picture of the genetic distribution of the subspecies.



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COOPERATIVA



Thanks for
your attention!

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