

# EARLY MEDIEVAL MIGRATIONS IN THE EASTERN ITALIAN ALPS: ANTHROPOLOGICAL, ISOTOPIC AND ANCIENT DNA ANALYSES

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## 1. Background

The decline of the Western Roman Empire, marked the beginning of the Middle Ages of European history (from ~400 AD to ~1000 AD). At this time, germanic tribes, with different origins, settled in Europe by intense migration events. In Early middle age (EMA), some of these groups, but also slavs, reached the Eastern Italian Alps (South Tyrol) entering the territory in different parts (e.g. Franks and Baiuvars northwest; Longobards south; Slavs east). Historical and archaeological data indicate that contacts between local and allochthonous groups led to the mutual cultural exchanges. However, the biological impact of EMA migrations on Eastern Italian Alpine groups is still unknown.

## 3. Approach and methods

- Analyze anthropologically (~200), human remains from 12 archaeological sites (Figure 1) in order to define the state of preservation of the bones, reconstruct the minimum number of individuals, estimate sex and age and to select the petrous bone (*pars Petrosa*) suitable for the molecular analyses.
- Study the genetic variation of the ancient DNA (nuclear and mitochondrial DNA) from a subset of good samples using the approach and methods summarized in Figure 2.
- Analyze the stable isotopes ( $\delta^{13}C$ ,  $\delta^{15}N$ ,  $\delta^{34}S$  and  $\delta^{87}Sr$ ) in human and animal bone collagen samples to reconstruct the origin (local versus not local geographic origin) of the examined individuals.

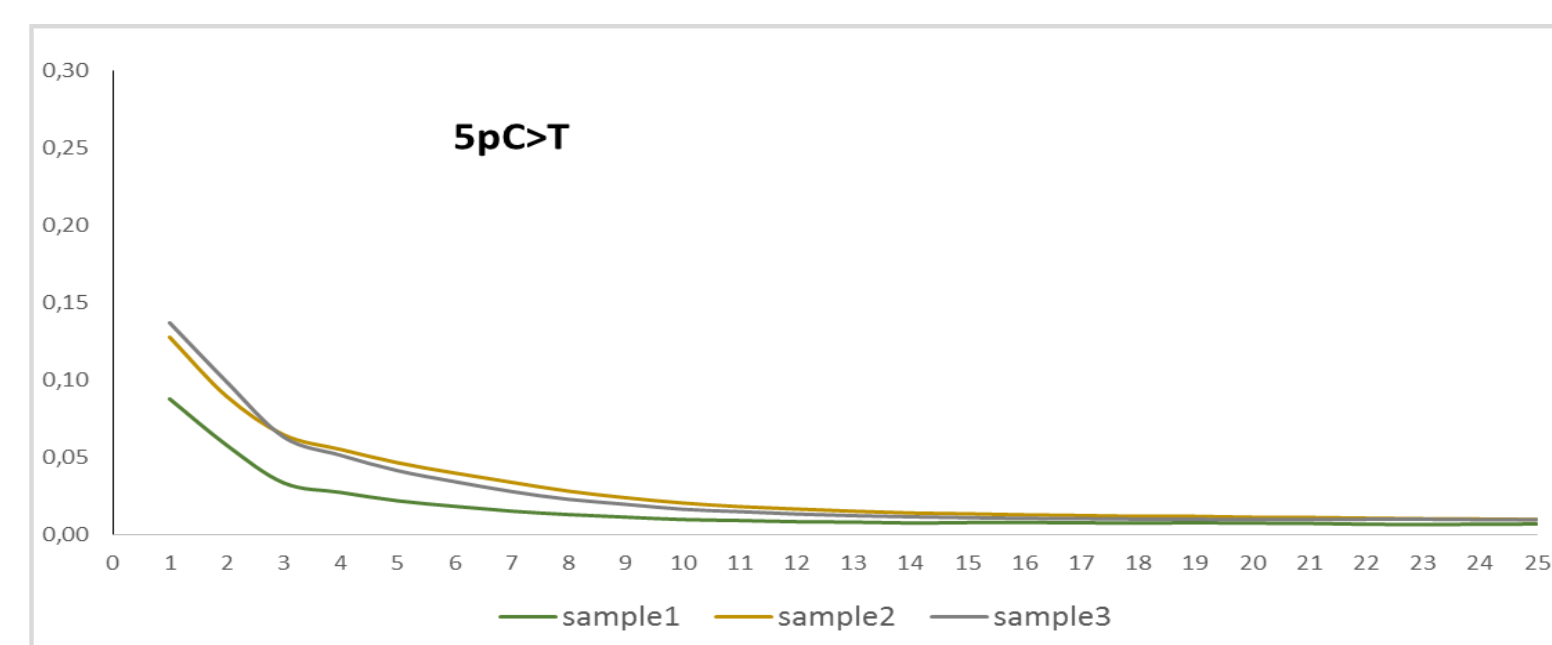


Figure3. Damage pattern profile (mapDamage) for three samples with the highest endogenous DNA content and contamination estimates based on the mitochondrial DNA (Schmutzi; Renaud et al. 2015). DMG 1<sup>st</sup> 5' = C to T changes at the first position in the 5' end of the sequence read.

ID	End. Content (%)	Reads	Mean Cov.	mtDNA Cov.	mtDNA haplog.
sample1	32,8	13867932	0.1215X	15,199	H3b+16129
sample2	67	14394939	0.1121X	32,475	H27+16093
sample3	43,9	17297923	0.2933X	22,7860	I2

Table 1. Percentage of reads mapped to the human reference genome (hg19); Merged reads after quality check; Mean genome coverage; mtDNA coverage and haplogroups.

## 2. Aims

In this project (BioArchEM), we combine genomic, anthropological and isotopic data in order to answer to the following questions: i) are the ancient individuals coming from diverse valleys genetically differentiated? ii) if so, is it possible to relate these differences to the impact of migrants with different origin occurred during EMA? Further comparison with modern European populations can help to answer to this question iii) what are the genetic relationships between medieval alpine individuals and other medieval European samples?

## 4a. Results. quality data

So far, we screened molecularly 61 individuals from 4 valleys (excluded Pusteria, Figure 1). Bioinformatic analyses of molecular data showed good aDNA preservation as showed by the damage pattern profiles, the percentage of human DNA reads (up to 76) and contamination estimates (only three samples showed contamination estimate > than 5%). Examples are reported in Figure3 and Table2.

## 4b. Results

Complete or partial mitochondrial genome sequences were reconstructed in 55 individuals by using shotgun and capture data (Figure 2). Haplogroups frequencies are within the expected overall mitochondrial diversity of western Eurasian mtDNA (H+HV=55%; U5+U8=13%; J1+J2=9%; T1+T2=9%; K1=7%; and I2, M1, N1 and W3=2%). Forty-three samples with best coverage (percentage of nucleotide positions covered  $\geq 3$  times; q=30) were also used for populations genetic analyses. Intra-population genetic analyses showed low level of sharing and high gene diversity within valleys. Additionally, Venosta valley also show significant high negative value of the Fu's parameter suggesting demographic expansion in this group (Table2).

Inter-population genetic analyses (e.g. exact test of sample differentiation and analyses of the genetic distances) showed no significant difference among valleys (and sites). Also the network analyses (Figure 4) showed that mtDNA haplotypes from diverse valleys are distributed in the tree. Overall, analyses of mtDNA data showed high genetic diversity within south-tyrolean valleys but no differentiation between valleys (and sites). However, principal component analyses (PCA) built using genomic data from twenty-three best ancient samples (with mean coverage from 0.10 to 0.37) and comparative data from present-day European populations (Human origin dataset, Lazaridis et al. 2016) highlight some differences among the ancient alpine samples analyzed. In fact, some individuals showed more genetic affinities with present-day populations from south Europe while other individuals are more similar to populations from North-west Europe (data not shown).

valley (n. sites)	N	n. hapl.	n. usable loci	n. polym. loci	gene diversity	Fu's FS test	FS p-value (10.000)
Adige (4)	7	6	16511	81	0,9524 (0,0955)	1,938	0,7454
Isarco (2)	7	7	13484	65	1,0 (0,0764)	-0,343	0,2604
MeranoB. (2)	8	8	16287	89	1,0 (0,0625)	-0,474	0,2411
Venosta (3)	21	20	16321	152	0,9952 (0,0165)	-5,311	<b>0,0187</b>

Table 2. Whole mtDNA data. Intra-populations parameters estimated by using Arlequin software 3.5 (Excoffier and Lischer, 2010). N= number of samples; n.hapl=number of haplotypes; n.polym.loci= number of polymorphic sites; Fu's test (Fu, 1997) and related p values (value marked in bold is significant, p<0.02).

## 5. Further development of the study

Molecular screening of further ~40 samples is in progress. Next step will be to perform a deeper genomic sequencing of some selected samples (with highest coverage and best quality). Genetic data will be compared to isotopic data analysed in the same individuals and from some fauna samples from the same archeological sites. Results will be integrated with archeological and historical information.

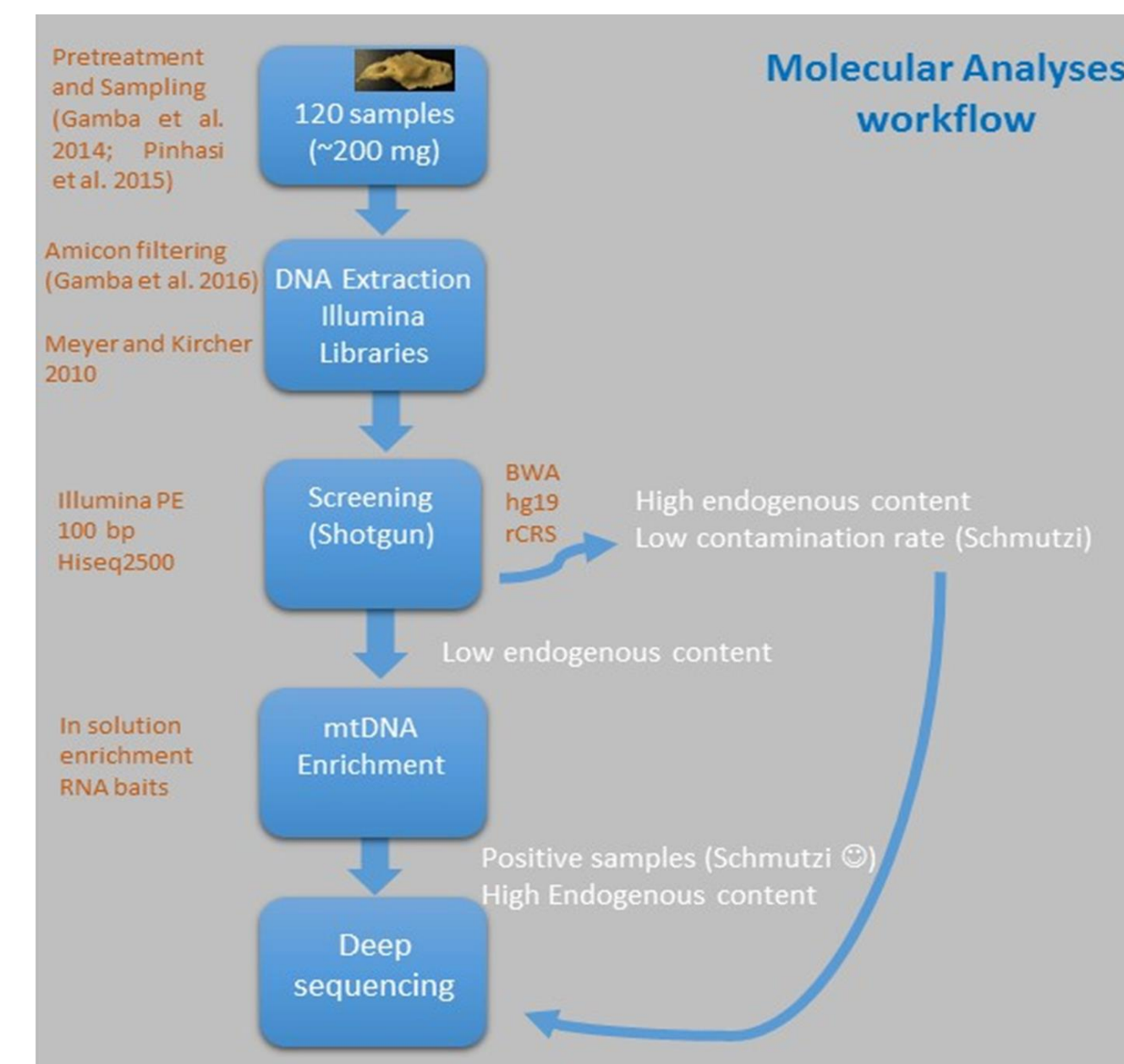


Figure 2. Main steps for the molecular analyses: sampling, DNA extraction, library preparation, sequencing and mtDNA enrichment with relative main references.

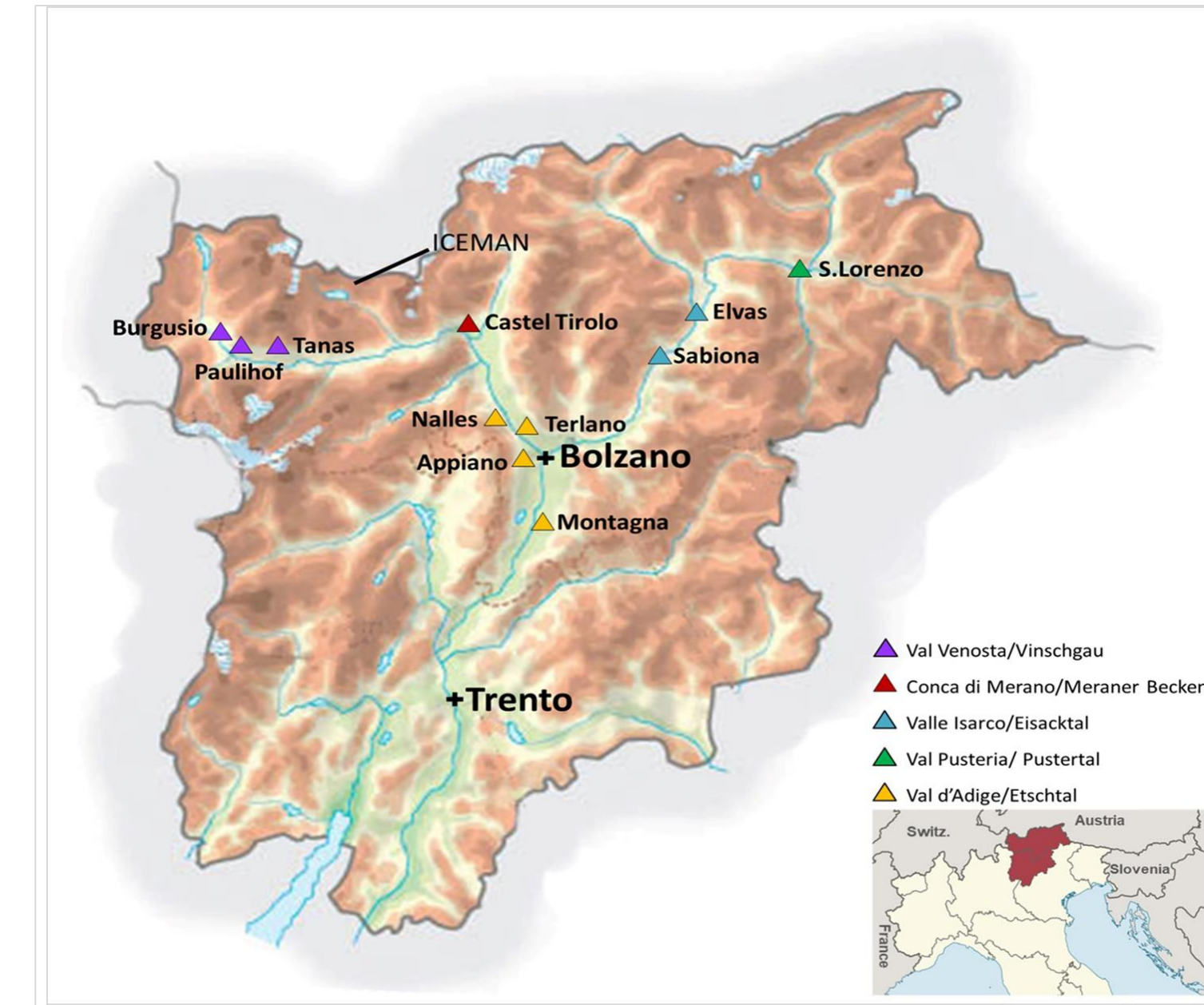


Figure 1. Distribution of the eleven archaeological sites in five valleys in South Tyrol (Trentino-Alto Adige region, Italy) analyzed in this study so far. All the skeletal remains are dated to the Early Medieval time (from 400 to 1100 AD).

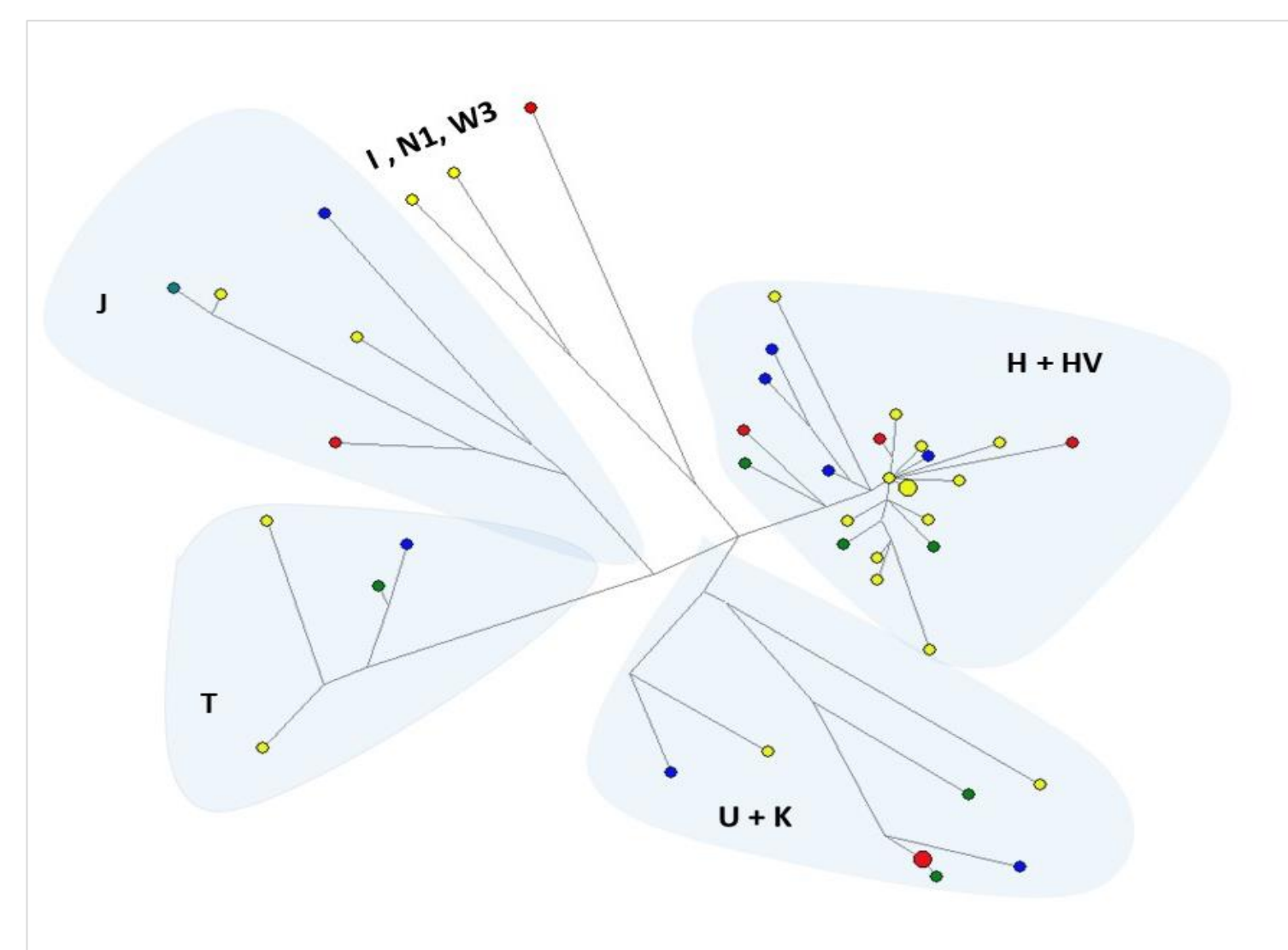


Figure 4. Whole mtDNA data. Median Joining network performed by using Network 5.0 (Bandelt et al. 1999). Circles are proportional to the frequency of haplotypes. Adige valley=red; Merano basin= blue; Isarco= green; Venosta= yellow.