

Genetic and functional traits variability of Sardinian populations of strawberry tree (*Arbutus unedo* L.)

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The strawberry tree (*Arbutus unedo* L.) is an evergreen shrub belonging to Ericaceae family representing a common element of the flora and vegetation of the Mediterranean basin. It is a highly vigorous resprouting species that can form forests, particularly in wildfire prone regions. At the genetic level, the species is diploid ($2n = 26$) with an estimated genome size of 450 Mb approximately. Presently, only few papers dealing with the characterisation of the genetic variability and the determination of the population structure of strawberry tree are available from the scientific literature, usually based on medium throughput approaches or on a defined number of markers, and no in-depth researches have been carried out so far in Italy.

Aim of the present research is to carry out an in-depth analysis at the DNA level and the determination of functional traits of 113 Strawberry tree plants belonging to 8 wild populations of strawberry tree from different sites in Sardinia (Figure 1). The different sampling sites were selected in areas where strawberry trees grow spontaneously and they were characterised by different altitude, climate and geography in terms of sun exposition, temperature, water availability, soil types and composition.

From all the sampled plants, DNA was extracted from young leaves by using commercially available kits and standardized protocols. DNA analysis were carried out by using a Genotyping By Sequencing (GBS) approach in order to develop a high number of SNP markers to be used for analysing the genetic variability and structure of the strawberry tree populations.

The Raw dataset contained 113 samples and 315,190 SNPs. After the filtering steps and LD pruning a final number of 10,930 SNPs have been considered for the analyses. Each filtering step was processed with PLINK v.1.9 software.

In order to assess the population structure, a Principal Component Analysis (PCA) was carried out using PLINK v.1.9 software. The result obtained was plotted using the software R v.4.1.3.

On the same individuals, samples were collected to assess four Functional Traits (FT), namely: Leaf Mass per Area (LMA), a form factor (FF), Leaf Dry Matter Content (LDMC) and the Leaf Area to Sapwood Area ratio (LA/SA). SLA and LDMC are well known to vary with environmental factors and in general increase in more water scarce environments. LA/SA reflects a plant's hydraulic strategy on the spectrum of efficiency (High LA/SA) versus safety (Low LA/SA).

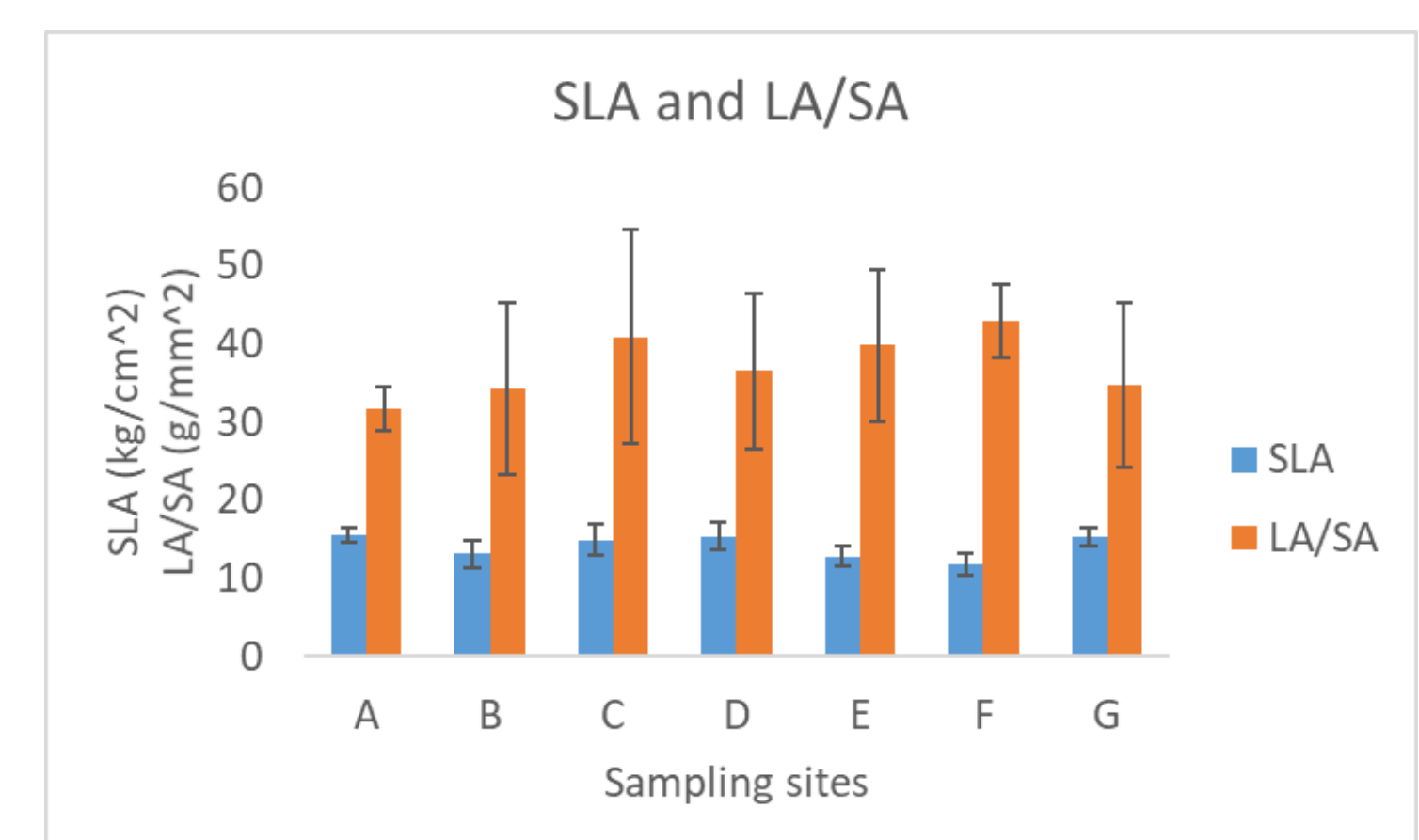
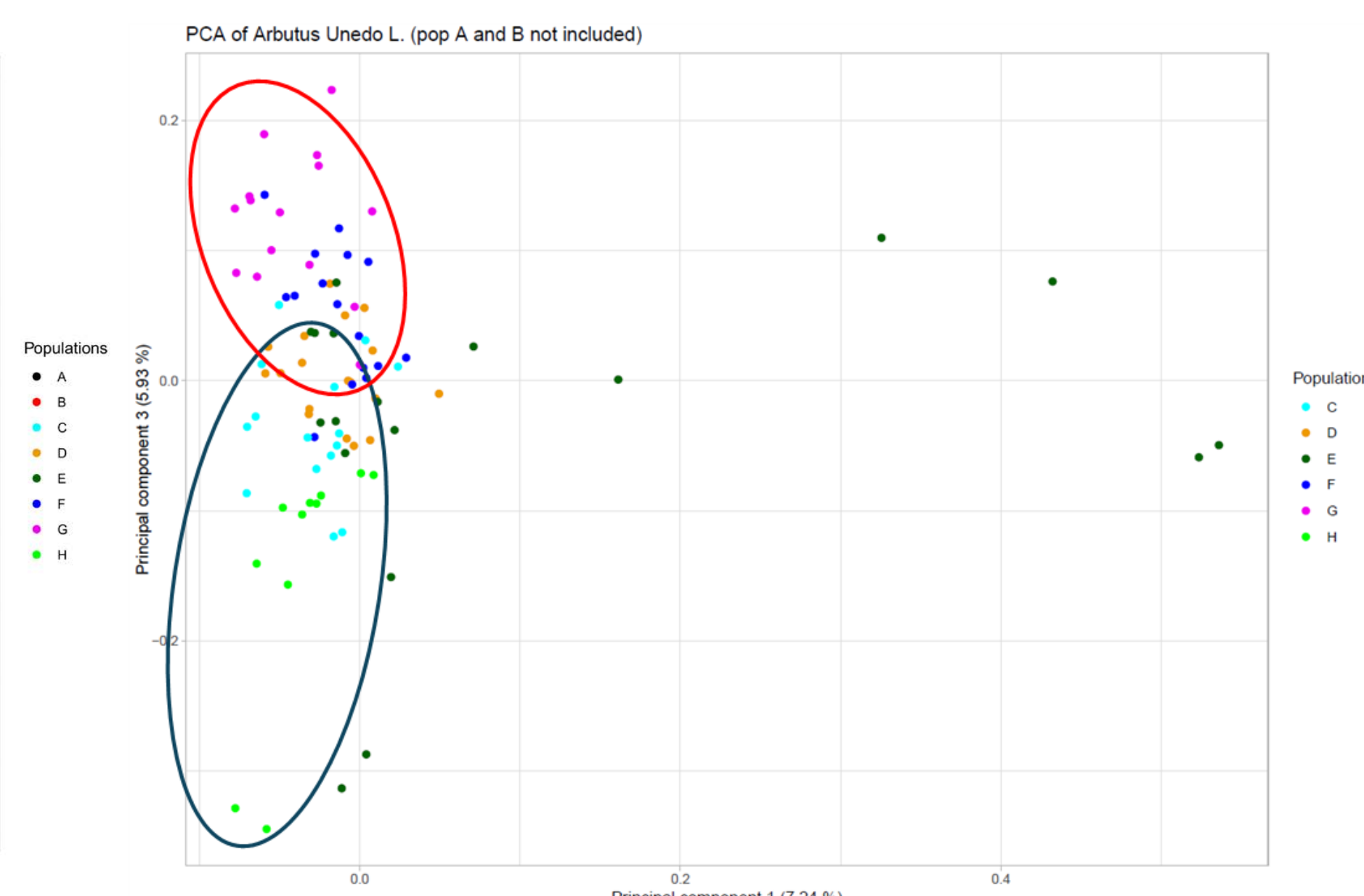
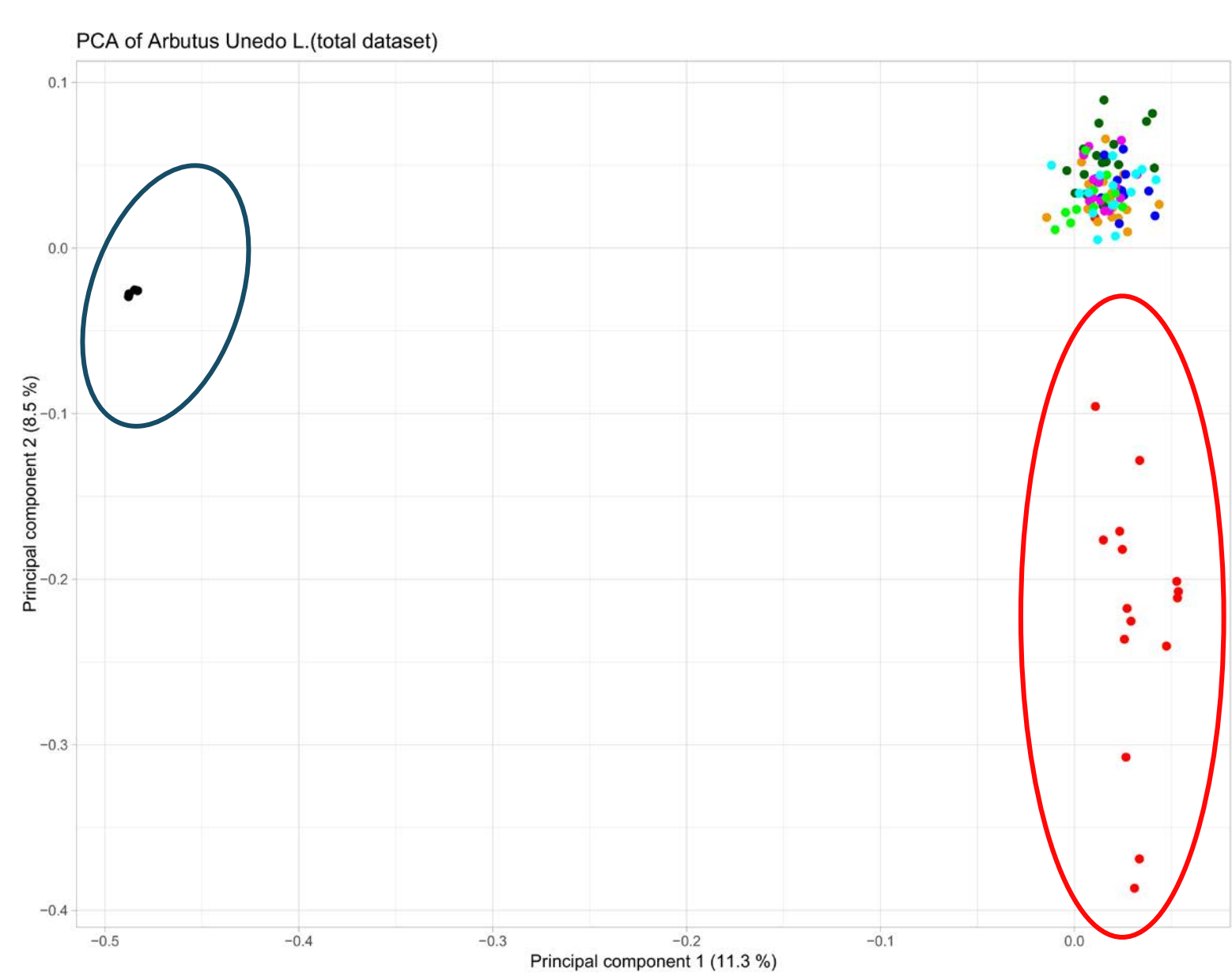
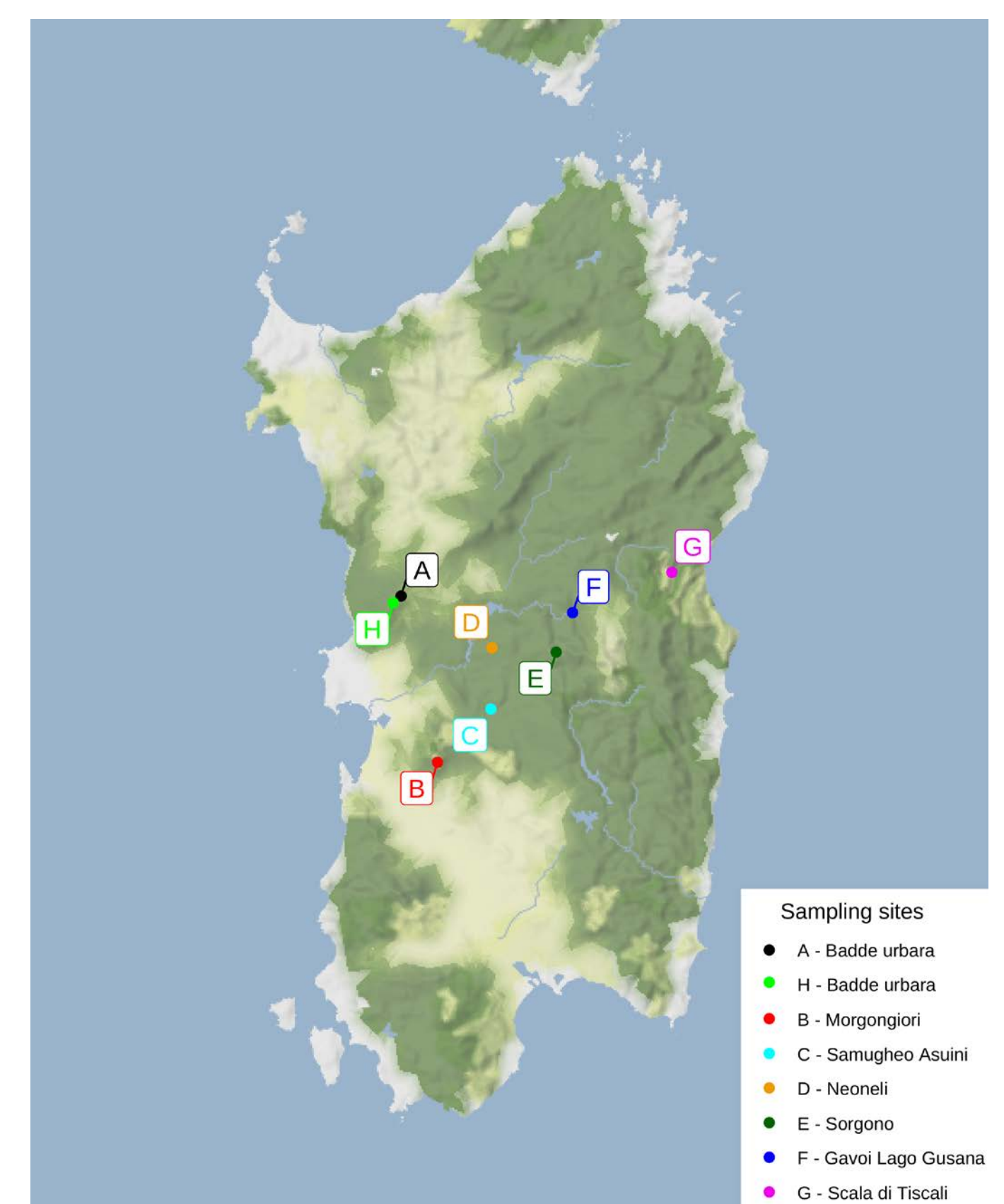


Figure 2. PCA analysis of all the 113 individuals from the 8 populations.

Figure 3. PCA analysis of 6 populations without populations A and B.

Figure 4. Specific Leaf Area (SLA) and Leaf Area to sapwood area ratio (LA/SA) as averages for the sampled sites. Error bars indicate standard deviation.

A high level of genetic variability was observed, with each plant being unique and different from the others. Concerning the 8 populations, two of them were clearly different, population A and population B, while all the remaining population do not seem to have enough variability to be clearly separated (Figure 2). Surprisingly, population from sampling site H, geographically very close to sampling site A, is very different from population A and not so different from other individuals from geographically more distant populations (populations C, D, E, F and G). Removing populations A and B, it was possible to separate better the resting individuals (Figure 3). In this second case, plants from population E appeared to be more dispersed and it seemed possible to define two groups, the first one (red circle) grouping mainly populations F and G, the second one (blue circle) grouping mainly populations C and H. Population D appeared to be more intermediate.

At the phenotypic level, it is possible to see that all four traits vary between sites (Figure 4 and Figure 5). Concerning samples from site H, the evaluation of functional traits is still ongoing.

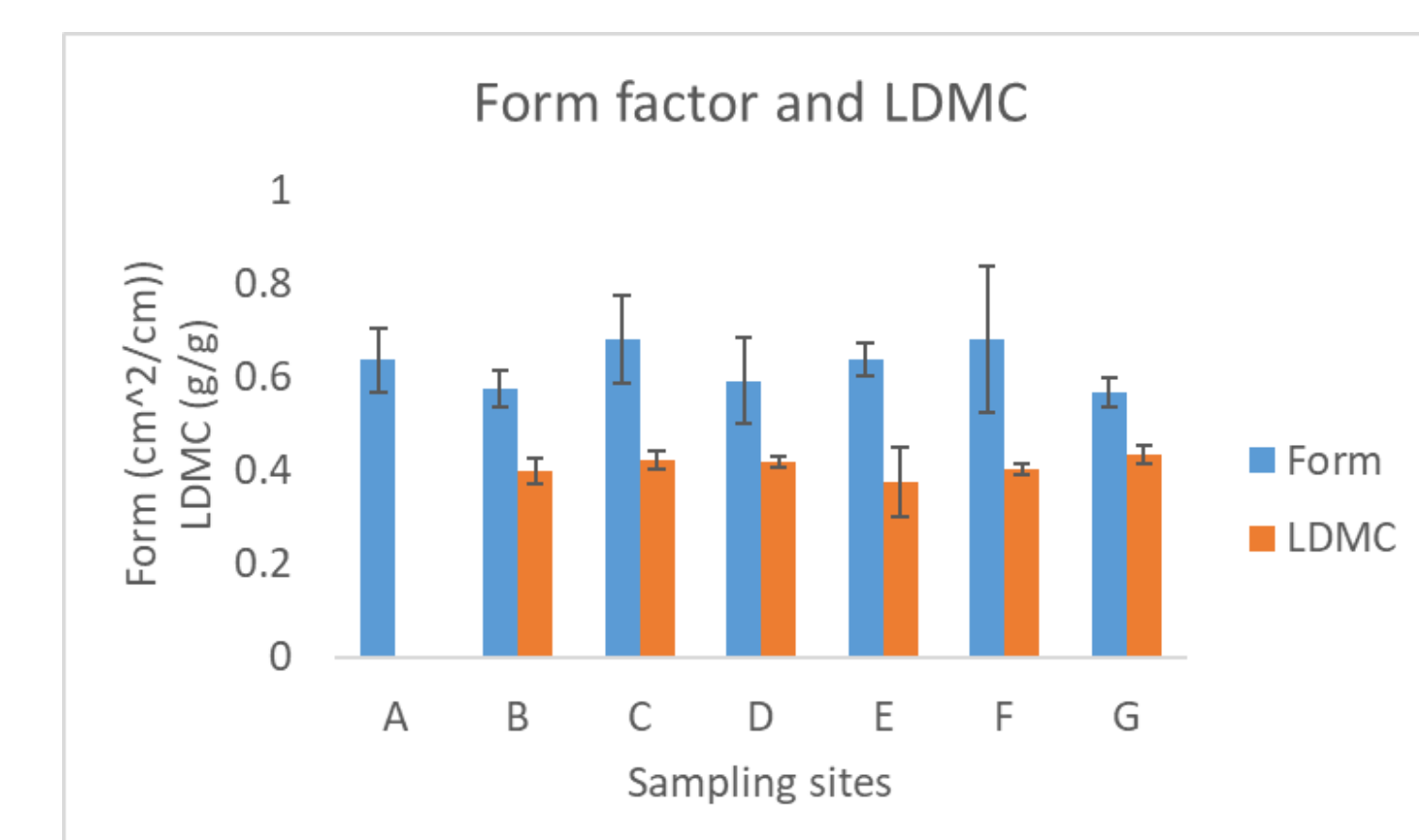


Figure 5. Ratio between leaf area and leaf perimeter (form) and Leaf Dry Matter Content (LDMC) as averages for the sampled sites. Error bars indicate standard deviation.

