



# ARACNE

## ADVOCATING THE ROLE OF SILK ART AND CULTURAL HERITAGE AT NATIONAL AND EUROPEAN SCALE



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### Deliverable 1.4

#### Report on the collected mulberry samples

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## Abstract

The present deliverable is a report on the results of a comprehensive study of ARACNE participating countries aimed at the identification, characterisation and preservation of old mulberry tree varieties, pivotal for sericulture and ecosystem services. The scope of this study encompasses:

### A. Identification of mulberry tree varieties in European germplasm collections

The report outlines the assortment of mulberry tree varieties catalogued in germplasm collections across participating ARACNE countries, providing an overview of the collections and detailing the varieties available. Specifically, the collections from CREA, the Vratsa Sericultural Centre, and the University of Maribor were evaluated using morphological and phenological descriptors to gauge the diversity and characteristics of the mulberry varieties.

### B. Inventory of Historical Local Mulberry Trees in Sericultural Regions of Participating Countries

To record old local mulberry trees in sericulture regions of the participating partners, the *MorusAPP* was developed. This application has been instrumental in creating a comprehensive database cataloguing the locations, basic information, and morphological descriptors of historical mulberry trees, offering insights into their morphological relationship and possibilities for their preservation.

### C. Genetic analysis of the mulberry species relationships

Sophisticated genetic analyses utilizing SSR (simple sequence repeat) and SNP (single nucleotide polymorphism) markers are currently performed, aimed at defining the relationships among different mulberry varieties. This analysis is critical for tracing the ancient route of the mulberry from Asia to Western Europe, shedding light on the genetic relationship of these trees.

### D. Recreation of Landscapes

The project has embarked on pioneering attempts to incorporate mulberries into various agroecosystems. These efforts are directed towards the recreation of distinctive landscapes, demonstrating the versatile role of mulberries in enhancing biodiversity and ecosystem service. The results are the starting point of the deliverable 4.3 in order to preserve mulberry trees and to maintain them in the germplasm collections, with the aim of having genetic material available for further sericulture development and for the restoration of landscapes highlighting the rich heritage and ecological value of mulberry trees.

## Partners involved in the document

Participant n.	Participant organisation name	Short name	Check if involved
1 Coordinator	Consiglio per la Ricerca in Agricoltura e l'Analisi dell'Economia Agraria	CREA	✓
2	Iniziativa Cube S.r.l.	INI	
3	Lepi State Silk Museum	SSM	
4	Nauchen Tsentar Po Bubarstvo Vratsa	SCS	✓
5	Piraeus Bank Group Cultural Foundation	PIOP	
6	Univerza V Mariboru	UM	✓
7	Ethniko Kai Kapodistriako Panepistimio Athinon	NKUA	✓
8	Instituto Murciano de Investigacion y Desarrollo Agrario y Medioambiental (IMIDA)	IMIDA	✓
9	D'orica S.r.l. Società Benefit	DOR	
10	Chemins De La Soie - Des Cevennes aux Alpujarras	ASSOIE	✓
11	Sericyne	SER	
12	Universita degli Studi di Padova	UNIPD	
13	Council Of Europe - Conseil de L'europe	COE	
14	Mouseio Technis Metaxiou	ASMS	

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## 1. Description of the project

ARACNE project focuses on the cultural heritage of the European silk production and its preservation, protection, and valorisation; it aims at reinvigorating traditional skills through the adaptive reuse of the common cultural and artistic legacy and at shaping a silk-linked European cultural identity.

The production and the past and present development of the silk sector can be again the common basis for a future European Silk Route intended as a cultural itinerary across Europe. To create a wide and well-connected network that, starting from the historical path followed by Marco Polo in his travels to East, even includes the routes of production and commercialization of silk in Europe in the following centuries, we aim to:

- ❖ Bring back silk production in vogue by reconstructing a resilient and innovative silk ecosystem that retraces the concerned European countries and promotes traditions, architecture, and both tangible and intangible heritages. The consolidation of a European Silk Route will encourage links and shared activities among European cities and regions to strengthen the preservation and protection of their culture and promote innovations in production and trade;
- ❖ Contribute to improve skills and competitiveness of silk-related European Cultural and Creative Industries through the renewal, co-development and the implementation of human-centered and place-specific silk-based cultural products, processes and service innovations, leveraging on digital applications and cutting-edge technologies, to foster the transition to more sustainable business models, and promote economic and social growth, and strengthen the reputation of European countries abroad.

### 1.1 ARACNE specific objectives

The overarching goal of ARACNE is to create a wide and well-connected Silk Innovation Ecosystem that, starting from the historical path followed by Marco Polo in his travels to East, also includes the routes of production and commercialization of silk in Europe in the following centuries. An innovation ecosystem is an interconnected network of quadruple helix stakeholders, including academia, industry and different levels of the public sector and civil society. This multi-level approach applies a systemic and bottom-up approach to creating research, innovation and knowledge. Silk Innovation Ecosystem includes every stakeholder and innovator in the cultural silk value chain even if not participating directly in the project activities. The production and, more in general, the past and present development of the silk sector in the ARACNE Consortium countries represent the common thread for the future 'European Silk Route' as a cultural itinerary across Europe, to boost the European values in

relation to the silk arts and CH for the benefit, prosperity, peace of our societies. To this aim, the project will explore the CCI's capacities to create a cultural and artistic niche market where silk produced within EU boundaries will be valued as a distinct immaterial asset; on the other hand, the ambition is to contribute to stop the loss of technical, traditional and cultural know-how and skills that accompanied the decline of this fiber production and that is detrimental exactly to those CCI's which might be active in fashion, art, design and product communication. In fact, the so-called 'Silk Road' is generally associated to its Asian origin; however, its European ramifications were fundamental for the development of Europe as we know it today. More in general, the silk production (silkworm rearing, mulberry cultivation, silk reeling), originated from Asia but subsequently spread to Europe and developed strongly in the Mediterranean and Balkan regions. Bringing back silk production in vogue by reconstructing a resilient and innovative Silk Route that retraces the European countries and enhances traditions, architecture, tangible, and intangible heritage will demonstrate that silk, as a cultural legacy, can contribute to develop the European economy and enrich our society. In this context, ARACNE covers several sectors linked to content creation, conservation, exploitation, management, fruition, diffusion related to the silk historical, artistic and environmental resources and assets. The ambition of ARACNE will be reached through a set of specific, measurable, achievable, realistic and time-constrained (SMART) specific objectives:

Objective 1: Enhancement of knowledge and memory for the renaissance of a European Silk Innovation Ecosystem;

Objective 2: Co-creation of human-centred and place specific creative silk-based solutions leveraging on digital and cutting-edge technologies;

Objective 3: Implementation of innovative strategies and business, governance and financing models for the involved CCI's organisations and SMEs, building on previous research;

Objective 4: Support the establishment of a cultural European Silk Route, based on the tangible and intangible silk cultural heritage and landscapes;

Objective 5: Raise awareness of ARACNE results and impacts among different stakeholders of the territories and CCI's of the silk sector and raise the expectation for the constitution of a European Silk Route in support to the European silk CH and silk CCI's;

Objective 6: Enhance the European cultural identity and strengthen European competitiveness for a more resilient post-crisis society;

Objective 7: Contribution to the European Green Deal, the New European Bauhaus and the Sustainable Development Goals.

## 2. Introduction

The white mulberry *Morus alba* L. originates from the Central and Western provinces of China and adjacent regions of Central Asia (western Tien Shan range, the region between the Amu Darya and Syr Darya rivers), where it has been widely cultivated for more than 4500 years, mainly for leaf yield in sericulture to feed the larvae of the silk producing Lepidopteran insect *Bombyx mori* L. (Kim et al. 2010).

The black mulberry *Morus nigra* L. thrived in the Near East and in Europe, at least since the Iron Age and Roman times, which is documented both literarily and with archaeo-botanical records. This is much earlier than *M. alba* L., which was introduced into this area (together with silkworms) in late Byzantine times or even later. Some *M. alba* forms have black fruits. Thus, contrary to what the botanical names imply, fruit colour (black vs. white) is not a reliable trait to identify the two mulberry species. Indeed, considerable confusion has been caused by its use. Instead, *M. nigra* and *M. alba* can be distinguished from each other easily by their leaf morphology. *M. nigra* is rarely found in Central Europe, as the leaves were not preferred for silkworm rearing, whereas genuinely wild populations of *M. nigra* are quite common in the Aegean and Adria region (Browicz 2000).

In contrast to black mulberry, white mulberry trees can be found all over Europe, especially in southern former silk-producing countries where they became over centuries an integral part of the landscaping, bearing witness to the sericultural past activity. European countries retained several centuries-old mulberry varieties, which are outstanding for their natural heritage, bearing the former socio-economic and political history. Furthermore, the old varieties are a valuable genetic resource best adapted to specific climate conditions, which can significantly contribute to a sustainable mulberry cultivation to meet the increasing demand of the silk industry. To our knowledge, the information on the number of remaining old mulberry varieties in European countries is incomplete and, in general, poorly investigated, and the genetic structure has not been part of any investigation, yet.

The available information (literature, herbarium collections, germplasm collections) seems to be concerned only with cultivated forms, the varieties created by mulberry breeders and/or sub-spontaneous individuals.

The classification of mulberry cultivars is very difficult and unreliable when based solely on morpho-phenological traits. Wide variations in leaf morphology are observed among different species and accessions. Furthermore, the shape of the leaf may vary according to the age of the plant, growth, position in the branches, and period of growth (Urbanek Krajnc et al. 2019, Šelih 2020, Urbanek Krajnc et al. 2023). The phylogeny of mulberries is further difficult as they bear different sex types, monoecious or dioecious, with sex expression varying among species and varieties. Furthermore, different cytomorphs (from diploids to

docosaploids) are available in mulberry, though diploids and triploids dominate mostly (Vijayan et al. 2014).

Genotype is the most important factor in determining the morphological and chemical characteristics of mulberries. The very long period of mulberry cultivation has led to the emergence of genotypes with different characteristics through agronomic selection, because mulberries were mainly propagated by gamic propagation in the past, which increased diversity. The grafting of high-yielding varieties was more common in the warmer regions of Italy and Austria-Hungary (Hlubek, 1850; Bolle, 1896; Bolle, 1908).

Black mulberry trees (*M. nigra*), found favour with ancient civilisations like the Persians, Romans, and Greeks, and their presence in Europe evolved with the dissemination of culture from these regions. The origin of white mulberry (*M. alba*) in Europe, crucial for silkworm rearing, is a complex historical narrative spanning hundreds of years. There is no precise information on when white mulberry was introduced into Europe and spread in cultivation. It is believed that the mulberry species predominant in Europe until the Middle Ages was *M. nigra*, with *M. alba* making its appearance alongside sericulture in Byzantium, Italy and Bulgaria between the ninth and twelfth centuries.

We have found that white mulberry is undergoing genetic erosion of local available gene pool due to the abandonment of sericulture and arable farming, the reconstruction of agro-ecosystems and associated afforestation of agricultural landscapes, and the abandonment of sericultural cultural heritage.

Compared to other horticultural crops, where great progress is being made in breeding new varieties using chemotype markers, research on mulberries is insufficient worldwide. The novelty of this study is that we evaluate the existing gene pool of the sericultural regions of ARACNE partners' countries, which we compare with introduced varieties maintained in different germplasm collections. The report underlines the need to preserve mulberries as historical remnants of sericulture in the context of the traditional and rational use.

## 2.1 Objective of the deliverable

This report aims to inform about the results of the activities of Task 1.2, Recreation of European mulberry heritage, of the project aimed at the identification of mulberries in different germplasm collections and inventories of old local mulberry varieties of sericultural regions of partner countries that were used for sericulture in the past.

The further objective is to showcase the representative local mulberry genotypes as living monuments of sericultural activities. Each of the presented and described mulberry trees has historical and cultural links with each country, where they are designated to be propagated



and maintained to be further available for recreation of landscapes and local stakeholders in order to establish a local sericulture.

The creation of maps of monumental trees for each country, along with basic information and morphological characteristics, aims to raise awareness of the cultural identity as well as the natural and cultural heritage of each country. In order to meet the objectives, the application *MorusAPP* (*MorusAPP*, 2023) was created with the aim of identifying old mulberry (*Morus* sp.) varieties in the different European countries by entering visual observations of individual mulberry trees, which were used to characterise these varieties in sufficient detail, allowing project partners to determine morphotypes of the same species and providing the data required to perform advanced statistical analyses. In the application, taxonomic and phytogeographical information, accessibility and number of trees, tree growth habit, tree vigour, pruning practices, trunk shape, morphological characteristics of shoots, leaves, reproductive structures, and observations on diseases and pests are recorded according to mulberry descriptors.

Based on the database generated of the mulberry varieties available in the mulberry germplasm collections and the database of old local mulberry varieties of partner countries and by evaluation of their morphological characteristics, the most significant varieties were chosen for genetic analyses by using the SRR (simple sequence repeat) and SNP (single nucleotide polymorphism)-based genotyping methods.

The study enabled us to reconstruct the origin and spread of mulberries among silkworm rearers in the past. By reviewing the existing genetic resources and the recreation of mulberry preservation sites, we highlighted the natural, cultural, and scientific value of the white mulberry. The project underlines the necessity of preserving the historical remains of sericulture, particularly mulberries, in the context of genetic erosion, which represents a general tendency among cultivated plants.



## 2.2 Document structure

The present report is divided into four parts:

1. Part A: Identification of the old mulberry tree varieties scattered in various germplasm collections in different European countries;
2. Part B: Inventory of historical local mulberry trees in sericultural regions of the participating partners;
3. Part C: Genetic analysis of the mulberry varieties relationships to trace the antique itinerary of the plant from Asia to Western Europe;
4. Part D: Recreation of landscapes;

Therefore, in the main body of this report, these four activities will be described in detail, providing results that have been taken by all partners of the ARACNE project. The first two activities are interlinked in their overarching aim to select samples for genetic analysis of mulberry relationships to trace the antique itinerary of the plant from Asia to Western Europe. The general goal is to bolster cultural significance, social identity, and biodiversity conservation.

## 3. Part A: Identification of mulberry tree varieties in European germplasm collections

### 3.1 Introductory notes

The collection and conservation of mulberry genetic resources are crucial for supporting diverse breeding programs. The characterisation of genetic resources is vital as it helps determine how the collected material differs from existing varieties. Evaluating the genetic potential of mulberry varieties is essential for effectively utilising the available gene pool. Without a systematic evaluation of existing germplasm, its effective utilisation remains unattainable.

For the efficient use of germplasm, it is imperative to accurately characterise, evaluate, and catalogue it based on valuable genetic traits. Subsequent systematic documentation and information exchange play a critical role in maximising the use of these resources. Documentation is key, providing rearers with the descriptive information necessary for informed decision-making. The sharing of mulberry germplasm information is vital not only for sericulture-practicing countries but also for those utilising mulberry leaves as animal fodder, mulberry fruits as food, and other by-products for various purposes.

The growth and yield of mulberry plants are significantly influenced by soil and climatic conditions. A variety that excels in one region or country may perform poorly in another. For instance, popular Japanese varieties like 'Kairyō Nezumigaeshi' and 'Ichinose' do not give good performance in Bulgaria, likely due to the drier Bulgarian summers. Conversely, the 'Kinryū' variety, less favoured in Japan, thrives in Bulgaria, although it is sensitive to bacteriosis in wet summers. Tropical mulberry varieties are unsuitable for temperate climates like Bulgaria's due to their sensitivity to cold winter weather. For example, a variety introduced to SCS-Vratsa from Egypt (analogue to 'Indian Kanva 2') experiences about 95 % shoot freeze annually, with new shoots sprouting from underground buds in spring.

Mulberry genetic resources form the cornerstone of effective crop management. The practices of collection, introduction, and exchange of these resources can significantly enrich the existing gene pool, offering breeders extensive opportunities for improvement.

Characterising germplasm is crucial for identifying individual genotypes, indicating the extent of variability among accessions, and facilitating comparisons for further investigation and use. This process involves systematically recording highly heritable traits, aiding in the identification of diverse germplasm. The detailed information on variability supports breeders in achieving efficient crop improvement. Enriching the mulberry germplasm bank with high variability and characterising these resources are ongoing processes that contribute to a robust database for research and development.

In the forthcoming chapters, the history of the development of mulberry germplasm resources is presented for Italy, Bulgaria, and Slovenia, countries whose germplasm collections are relevant in terms of variety conservation and selection and serve as a backbone for other countries. The conservation status of mulberry resources in other partner countries will be detailed in the Results section, highlighting the global efforts to preserve and enhance this valuable genetic heritage.

### 3.1.1 History of the development of mulberry germplasm resources in Italy

The first species of mulberry to be introduced to Italy was *M. nigra*, which originated in what is now Iran and was already known in Roman times. *M. alba* arrived in Italy later, between the ninth and twelfth centuries, from the Far East and underwent a significant expansion around the 15th century.

Despite the great importance of mulberries for sericulture, they were never the subject of significant scientific research or genetic selection programmes. It was only in the 19th century, with the transition to the industrialisation of sericulture, that specialised facilities began to be set up with selected varieties distributed throughout the country. Until then, local genotypes were used on a large scale. Between the 1930s and 1950s, new varieties were imported from abroad, particularly from the Far East (mainly Japan). With the decline of sericulture during the 20<sup>th</sup> century, the specialised plantations also disappeared, with the survival of only sporadic solitary trees and spontaneous progeny in marginal areas of the countryside.

Documented activities of mulberry genetic improvement are not known in Italy at least until the eighteenth century. For a long time, farmers predominantly multiplied the mulberry by seed, believing that this would result in more robust and more productive plants, and over time this led to the differentiation of local populations of 'wild mulberry' ('Selvatico' in Italian). Grafting was also used to multiply certain prized lines or those that could hardly be propagated by seed, and even for these, distinct populations were formed at a local or regional level.

Only towards the end of the eighteenth and the beginning of the nineteenth century did Italians begin to introduce new varieties, mostly imported from abroad. This was due to the need to renew the genetic basis of the plantations because of the incidence of new diseases and parasites that affected the mulberry, and on the other hand, to create specialised mulberry orchards to follow the trend towards the industrialization of sericulture.

The most extensive and oldest list of varieties is that reported by Jacopo Alberti in 1773, which encloses 22 varieties of white mulberry. Shortly thereafter, Italy began to import and

study exotic varieties, which include the 'Morettiana' mulberry, obtained in 1780 in Milan from seeds coming from India, while the importation of the 'Filippine' variety from the Philippines (primary origin is North China), which had very wide distribution, dates back to the beginning of 1820s. Another variety to mention is the 'Cattaneo', released in 1865 and recommended for repopulating cultivations decimated by pests and diseases. The 'Restelli' variety dates back to the early 1900s, introduced to combat infestations of *Pseudaulacaspis pentagona* (Alberti, 1773; Verson & Quajat, 1896)

In general, in these years, the selection criteria were based essentially on productivity, which also depended on disease resistance, and on the quality of the leaf, considered essential for the production of quality silk. In more recent years, importance was also given to the earliness of sprouting, a sign of sericulture conducted with planning and specialisation criteria.

After the decline of sericulture in the course of twentieth century, in Italy, there were no more significant genetic improvement activities concerning the mulberry tree. At the Padua Station, the mulberry has been essentially seen as a forage tree for silkworm rearing, and it has never been the subject of dedicated research activities, apart from agronomic experiments, targeting cultivation and propagation techniques rather than the improvement of the genetic basis.

However, an activity has been initiated to recover genotypes widespread across the national territory, through field exploration, with the goal of expanding the genetic base of the collection, and mainly aimed at reconstituting populations particularly adapted to pedoclimatic conditions. For this reason, particular attention has been paid to the recovery of clones obtained from centuries-old specimens, which, precisely because of their long-term resilience, present high probabilities of adaptation to climate variations and environmental stresses.

In Italy, the most relevant mulberry germplasm collection is the one belonging to the Sericulture Laboratory of CREA, the Research Centre of Agriculture and Environment located in Padua (latitude 45.399080°, longitude 11.834279°). The institute was founded in 1871 in the very centre of the town and was later moved to the suburbs in 1923. It was around that time that the first mulberry plantation was established in the new location, next to the institute, but most of the plants in the current collection were planted after 1958, when the Ascoli Piceno Sericulture Station ceased its activities, and its former director, Porzia Lorenza Lombardi, transferred its collection to Padua, thus making the Padua Station the only research hub in Italy on silkworms and mulberries. Thus, the majority of the accessions was inherited from the Ascoli Piceno Sericulture Station. Some were received from the INRA's Sericulture Station of Lyon, when it closed in 2009, while others came from private

collections. Some of the accessions consist of finds that were made in the national territory and come from former plantations.

The collection is also part of a national network for the conservation of genetic resources of food agricultural crops in Italy, as part of a multi-year project of the Italian Ministry of Agriculture, Food Sovereignty and Forestry in collaboration with the FAO (RGV-FAO project). Descriptors are reported in Planta Res (CREA Planta res, 2013).

### 3.1.2 History of the development of mulberry germplasm resources in Slovenia

The very long period of mulberry cultivation in Slovenia has led to the emergence of genotypes with different fruit characteristics through agronomic selection, as mulberries were mainly propagated by gamic propagation in the past, which increased diversity. Documented activities of mulberry genetic improvement in the form of mulberry grafting were not known in Slovenia at least until the end of the eighteenth century (Macflot 1776). The grafting of high-yielding varieties was more common in the warmer regions of Italy (Macflot 1776, Bolle 1896; Bolle 1908, Zimmermann 2016).

At the beginning of the nineteenth century, significant attempts in mulberry cultivation and selection were made by the Sericulture Station of Gorizia. The Gorizia Station was favoured by its direct connection to the Venetian sericulture. The rapid increase in the production of silk in Gorizia caused them to be serious competitors to the Viennese sericulture. Gorizia's sericulture prospered, due to its natural advantages, although the companies did not benefit from loans, as did the Viennese ones.

When the Schools for Viticulture and Fruit Cultivation in Klosterneuburg and Maribor were established in the mid-nineteenth century, sericulture was taught both in theory and practice. Furthermore, in the middle of the nineteenth century, the Styrian Sericultural Society was founded, which provided practical courses for farmers. Furthermore, the society established a large mulberry nursery in Graz and began to supply Lower Styria and Carniola with lots of mulberry trees.

The history of mulberry cultivation and selection in Lower Styria is intricately connected with the fascinating agricultural rise in production of horticultural plants of the Novo Celje mansion, known as Styrian Eden, which was significantly promoted by Archduke John of Meran (1782–1859, brother to the Austro-Hungarian emperor Francis II). In 1843, the owner embarked on a remarkable endeavour by planting an initial 13,000 mulberry trees at the Plevna mansion. The favourable conditions and robust growth of the existing plantations prompted the addition of another 10,000 trees. Dr. Anton Perinello contributed to the

plantation's diversity by acquiring mulberry trees of semi-low and medium cutting form from Lombardy and Rome.

The entire plantation at Novo Celje comprised ten large plantations, with five adhering to the Italian model. Notably, all medium- and high- cutting forms of Italian mulberry trees were grafted above the root crown, demonstrating advanced horticultural techniques. These trees were mainly of the 'Giazza' variety, followed by 'Filippine' variety, renowned, at that time, for their exceptional leaf yield and quality (Zimmermann, 2016).

Economic considerations played a crucial role in shaping sericulture in Novo Celje. Precise calculations determined that only ten adult mulberry trees were needed to yield 100 pounds of silkworm cocoons. This discovery demonstrated the efficiency of the approach, as opposed to relying on a larger number of trees with underdeveloped productivity. With this insight, the plantation adopted a strategic model. It was reported that 40 ten-year-old mulberry trees could produce 100 pounds (approx. 45.4 kg) of cocoons, an impressive 800 mulberry trees could generate a substantial 2,000 pounds (approx. 907.2 kg) of cocoons, making it economically viable (Zimmermann, 2016).

The entire plantation at Novo Celje comprised ten large plantations, with five adhering to the Italian model. The Venetian-style plantations known as 'La Piantata Veneta' were thoughtfully arranged to ensure protection from winds and ample sunlight. Straight rows featured alternating mulberry trees in semi-low and high cutting form, accompanied by high-quality vine varieties, including 'Burgundian' and 'Moselle' (Figure 1, Figure 2). This systematic approach ensured a balance between high and semi-low formed trees and a harmonious integration of vines (Zimmermann, 2016).



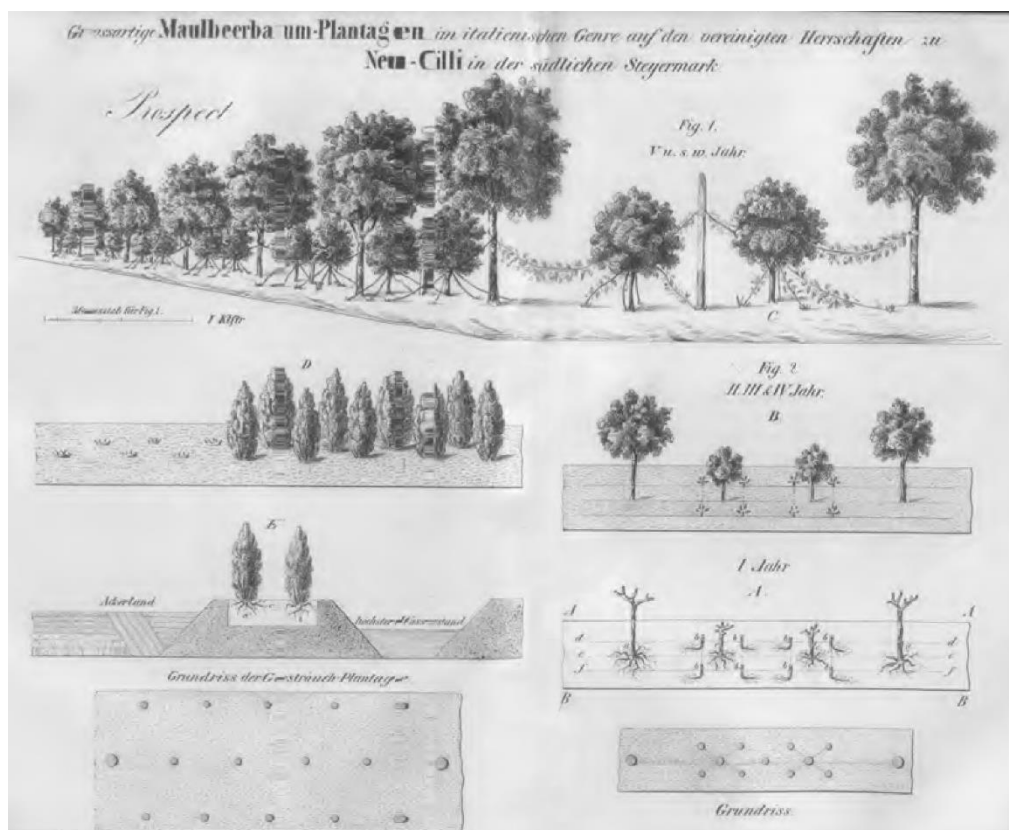


Figure 1 - Scheme of mulberry plantation and tree arrangement at the Novo Celje mansion. (Source: Zimmerman 2016)



Figure 2 - Mulberry trees used as a structural support for grapevines. (Source: Rudolf 1891)

Fertilization was not ignored. Records indicate that a range of organic and mineral fertilisers were used on the plantation, including bovine hair, hooves, waste from the reeling industry,

and bone meal from Graz. The aim was to supply the plantation with nutrients in the long-term. Abundant amounts of livestock manure and compost were transported from neighbouring areas, which further increased the fertility of the soil (Zimmermann, 2016).

The success of Novo Celje's sericulture project was highlighted by the expert Antonio de Marco Paolino, who marvelled at the lush growth and bright foliage of the mulberry trees and vines. He attributed this success to the favourable location, thorough tillage, generous fertilization, the quality of trees, and protective measures against harsh winters.

In the spring of 1845, Novo Celje expanded their plantations to the Turnišče mansion at Ptuj, by importing 2,000 mulberry trees from Rome using Perinell's method. The plantation at the Turnišče mansion, thereby, signified a continued commitment to the further development of sericulture (Zimmermann, 2016).

In 1896, Ivan Bolle's book 'Teachings about moriculture (Murvoreja)' provided valuable insights into mulberry cultivation in Slovenia. Since in the 19<sup>th</sup> century the rural population was predominant, arable land was of great importance; therefore, the mulberry represented a valuable advantage for farmers, due to its adaptability to peripheral areas, roadsides, field edges and less fertile areas. Bolle recommended planting mulberries in 1.3-meter-wide pits and taking soil quality into account. Fertilisers were utilized to promote robust growth (Bolle, 1896).

The need for grafting arose due to the limitations of wild mulberries, which had small and lobed leaves less suitable for silkworms as they matured. Unfortunately, specific variety names were lost in history, and only the most silkworm-friendly varieties prevailed.

Bolle's (1896) detailed account covered the propagation and grafting techniques used in Slovenia. Seeds were extracted from fully ripe mulberry fruits, dried, and stored for spring sowing in the following year. After two years, the seedlings were robust enough for grafting. Grafting was carried out on dry days from mid-March to late April, favouring the bark- or the bark-inlay grafting method, which enabled rapid wound healing. Grafted mulberries were pruned at a height of 1 metre after one year and then replanted. However, Bolle noted that most farmers lacked the knowledge and skills of grafting, which led them to purchase grafted mulberries (Bolle, 1896).

Mulberries were pruned in a manner similar to the Friulian approach, creating a multi-branched vase pruning shape that allowed light and air to penetrate, and made it easier to collect the leaves. The three-year pruning process aimed at obtaining a uniform branch size, a horizontal crown, and maximum leaf production with fewer fruits. Pruning also promoted the accelerated growth and sustainability of the trees. The primary goals of pruning were to



maintain a horizontal canopy, prioritise high-quality foliage over fruit production, and promote tree growth and longevity (Bolle, 1896).

Sericulture and the cultivation of mulberry trees experienced a significant decline after World War II, with no sericultural activities practiced for 70 years. Historical collections from the Sericulture Station of Gorizia, the plantations of Novo Celje, Plevno and Turnišče did not survive this period. The revival of sericulture began with the joint Hungarian-Slovenian Research Project (N1-0041) in 2015 and the establishment of a mulberry germplasm collection at the Faculty of Agriculture and Life Sciences, University of Maribor. This research institution, tracing its roots back to the School of Viticulture founded by Archduke John in 1822, had incorporated sericulture into its curriculum since 1870, aiming to integrate sericultural theory and practice into the study programme. Initially located in the town centre, the original school site was later repurposed by the agricultural institute, with its garden being replaced by fruit trees.

The mulberry germplasm collection at the University of Maribor was established in 2015 and spans 0.6 hectares. It is divided into three main sections. The first section maintains traditional sericultural mulberry varieties sourced from the germplasm collections of the Sericulture Laboratory CREA Padua and SCS Vratsa, including varieties such as 'Florio', 'Morettiana', 'Kokusou 20', 'Kokusou 21', 'Kokusou 60', 'Giazza', 'Muki', 'Restelli', 'Kiuryu', and 'No 25'. The second section comprises vegetatively-propagated trees from local historical Slovenian and Hungarian specimens, collected during a detailed inventory of the mulberry gene pool from 2015 to 2018 as part of the joint research project. This effort aimed to reconstruct and preserve the genetic heritage of ancient mulberry trees, by selection of local varieties optimally adapted to the region's pedoclimatic conditions and available to farmers.

The third section of the collection focuses on species and hybrids primarily cultivated for fruit production, originally aimed at preserving varieties from all major *Morus* species suitable for the geographic region.

### 3.1.3 History of the development of mulberry germplasm resources in Bulgaria

Established in 1896, the Scientific Centre on Sericulture in Vratsa (SCS) has grown to become the largest centre in Bulgaria dedicated to the advancement of the sericulture industry. The Research Department at SCS comprises four specialised laboratories: the Mulberry Selection and Agronomy Laboratory, the Silkworm Genetics Laboratory, the Silkworm Selection and Egg Production Laboratory, and the Silkworm Rearing and Cocoon Processing Laboratory. These laboratories focus on a range of research activities, including genetic studies on breeding technologies for the development of new silkworm lines, breeds, and hybrids; technology for silkworm egg production; prevention of silkworm diseases and pest control; selection and cultivation of mulberry; cocoon and silk processing; as well as sericulture economics and management.

The SCS prides itself on maintaining an extensive collection of more than 200 mulberry varieties and over 100 silkworm strains and lines. These have been gathered from both domestic and international sources and are continuously bred to enhance their genetic traits. This comprehensive approach ensures that SCS remains at the forefront of sericulture research and development, contributing significantly to the industry's growth and sustainability.

In Bulgaria, mulberry has grown naturally since ancient times, and now the following species are available in the country: *M. alba* (syn. *M. multicaulis*), *M. indica* (syn. *M. latifolia*, *M. bombycis*, *M. kagayamae*), *M. rubra* and *M. nigra*. At the beginning of the 20<sup>th</sup> century, almost all the mulberry trees and plantations in Bulgaria were of the local 'wild type' variety, characterised by excellent adaptation to the climatic and soil conditions, but a low leaf yield. 12 mulberry varieties were introduced for the first time from Italy in 1930. After that mulberry varieties have been imported many times from the Soviet Union (in 1956; 1958; 1965; 1967), Japan (in 1963; 1970; 1974), Romania (1964), China (1967; 1982), Egypt (1998), Ukraine (1998) and Azerbaijan (2004).

For the enrichment of mulberry germplasm, SCS Vratsa has organised several field trips for collection of accessions. The exotic accessions imported from Italy and those collected in Bulgaria until 1932 were characterised and evaluated and the accessions N3, N21, N24, N26, N101, N106 and N112 were recommended for use at the field level. From indigenous accessions, collected in 1950 and 1953 the accessions N 59, N51, N33 and N53 having higher leaf yields were selected. In the period 1940-1950 several mulberry hybrids between the female varieties N24, N103, N106 and the male varieties N3, N21, N62, N112 were obtained. As the best combinations, N24 x N3, N103 x N3 and N106 x N3 were recommended.

During the period of 1957 – 1965 the varieties N3, N24, N59, N101, N106, 'Adreuli', 'Pobeda', 'Tbilisuri' and 'Kokusou 70' were tested in the main sericulture regions of Bulgaria. The varieties N106, 'Kokusou 70', 'Pobeda' and 'Tbilisuri' gave the best results and were approved by the government for commercial sapling production.

In the period 1967-1977 the hybrid offsprings of the varieties 'Georgia', 'Kinryu', 'Kokusou 20', 'Kokusou 27', N24 and N106 under natural pollination were investigated. It was found that the offsprings of 'Georgia', 'Kinryu' and N24 had the largest percentage of unlobed leaves, 89%, 67 % and 63 % respectively.

During the period 1976-1978 the mulberry varieties 'Kokusou 21', 'Kokusou 27' and 'Kinryu' were introduced from Japan and new local varieties 'Vratsa 1' and 'Vratsa 18' were selected at SCS Vratsa, tested and approved for commercial use.

New mulberry accessions were created by using the methods of experimental polyploidy and hybridization. Penkov (1980) made a hybridisation between the accessions N3, N116, N118, and N120 belonging to the species *M. alba* and *M. indica* (*M. kagayamae* and *M. latifolia*). A high percentage of trees with unlobed leaves manifested in the hybrids N24 x N116 – 90 %, N117 x N3 – 85 %, and N119 x N3 – 75%. These hybrids were approved by the government as hybrid varieties (Hybrid 50, Hybrid 78 and Hybrid 96) for commercial use in 1980.

The research work completed in 1981 proved that the F<sub>1</sub> mulberry offsprings had very high variation in leaf lobation type and the mother variety influenced the leaf lobation type in the progeny to a greater extent. The leaf size was inherited intermediately in F<sub>1</sub>.

In 1995 a methodology for characterisation and evaluation of mulberry accessions was developed by Petkov (1998). Now the research work on mulberry selection is considered a very important activity at SCS Vratsa. It is necessary to continue the collection of new mulberry varieties both exotic and indigenous. In the period of 1998 – 2005 more than 2500 hybrid seedlings have been obtained, planted and evaluated.

The future direction of the breeding work should be the so called 'far' hybridization between varieties from different geographical regions, different species and especially between varieties having different chromosome numbers. As a result of the breeding work the highly productive mulberry varieties 'Vratsa 1', 'Vezletz' and 'Vratsa 18' were selected at SCS Vratsa which could be of interest for introduction in other countries as well.

## 3.2 Methodology

### 3.2.1 Identification and taxonomical evaluation of the germplasm resources

To achieve the ARACNE goal of identifying old mulberry varieties in different mulberry germplasm collections of the partner countries, we collected information on the locations (gene bank collections, botanical gardens, individual collectors) where mulberry varieties are maintained. This information is listed in **Table 1**.

**Table 1** - List of germplasm collections that provided the list of mulberry (*Morus*) varieties to be included in the morphological and genetic analyses.

Mulberry germplasm collection	Basic information	List of varieties
CREA Padua, <b>Italy</b>	The current collection consists of 68 varieties. 50 varieties belonging to the ingroup <i>M. alba</i> s.l., 14 varieties assigned <i>M. indica</i> s.l., 3 varieties <i>M. rubra</i> cf. or hybrids between <i>M. alba</i> and <i>M. rubra</i> . <i>M. nigra</i> is represented by one genotype. The varieties originate from Italy, France, Spain, China, Japan, Brazil.	See Appendix I
SCS Vratsa, <b>Bulgaria</b>	The germplasm maintains 180 accessions. 96 accessions are selections of the SCS Vratsa breeding programme. 14 accessions are categorised as <i>M. alba</i> s.l., 80 genotypes are regarded as hybrids between <i>M. alba</i> and <i>M. indica</i> with no clearly defined genetic background ( <i>Morus</i> sp.). 2 varieties are regarded as <i>M. indica</i> .	See Appendix II
UM Maribor, <b>Slovenia</b>	The collection is divided into 3 sections. The 1. section comprises sericultural varieties of different origin (8 varieties, 272 trees), the 2. section is represented by old local Slovenian (80 genotypes) and Hungarian varieties (127 genotypes), the 3. section is represented by varieties for fruit production (20).	See Appendix III
IMIDA Murcia, <b>Spain</b>	The collection is represented by 24 varieties. 20 varieties belong to <i>M. alba</i> s.l. <i>M. indica</i> s.l. ingroup is represented 3 varieties of 'Kokusou', namely Kokusou 20, 21 and 27 and 1 local <i>M. nigra</i> .	See Appendix IV
Murier de Porquerolles, Conservatoire Botanique National Méditerranéen, <b>France</b>	The collection maintains 28 varieties of <i>M. alba</i> s.l., 5 varieties of <i>M. indica</i> s.l., 5 varieties of <i>M. nigra</i> and 9 varieties of <i>M. rubra</i> cf.	See Appendix V
Orchard-conservatory of Mercoire, Gard, <b>France</b>	The collection maintains 52 varieties, 39 of <i>M. alba</i> s.l., one of <i>M. boninensis</i> , 6 of local	See Appendix V

Mulberry germplasm collection	Basic information	List of varieties
	<i>M. nigra</i> , 3 varieties of <i>M. rubra</i> cf. and one variety is of unknown taxonomical status.	
Agricultural Research Station of Komotini, <b>Greece</b>	4 ha mulberry field with 32 mulberry varieties	-
University of Athens, Laboratory of Sericulture and Apiculture, <b>Greece</b>	The collection is divided into two plantations, it maintains 2 varieties of <i>M. alba</i> s.l., 1 variety of <i>M. indica</i> and several local varieties	See Appendix VI
Agricultural University of Georgia, Laboratory of Sericulture, Tbilisi, <b>Georgia</b>	Some local varieties are maintained, most of the collection was destroyed due to the mulberry dwarf disease and the absence of sufficient state support during the period 1990 – 2015	-

\*Abbreviations: s.l. -sensu lato, cf. - confer

The curators of the germplasm collections provided us with a list of the varieties maintained, which we revised into the current accepted classification according to the IPNI-The International Plant Names Index (International Plant Names Index 2024), WPO Plant List (WFO Plant list 2024), POWO Plants of the World Online (Kew 2024).

The following 17 species of the genus *Morus* are currently accepted: *M. alba* L., *M. boninensis* Koidz., *M. cathayana* Hemsl., *M. celtidifolia* Kunth, *M. indica* L., *M. koordersiana* J.-F.Leroy, *M. liboensis* S.S.Chang, *M. macroura* Miq., *M. microphylla* Buckley, *M. miyabeana* Hotta, *M. mongolica* (Bureau) C.K.Schneid, *M. nigra* L., *M. notabilis* C.K.Schneid., *M. rubra* L., *M. serrata* Roxb., *M. trilobata* (S.S.Chang) Z.Y.Cao and *M. wittiorum* Hand.-Mazz.

The main issue was that most varieties are labelled with old traditional taxonomic names, synonyms (syn.), that apply to a taxon that now goes by a different scientific name. Moreover, most modern sericultural varieties originate from simple or complex spontaneous and/or artificial crosses between *M. alba*, *M. indica* and *M. rubra*. Therefore, most of the varieties belonging to *M. alba* and *M. indica* have been concurrently defined as sensu lato (s.l.), which means "in the broad sense", whereas *M. rubra* varieties have been concurrently assigned as confer (cf.). This qualifier indicates that most of the diagnostic characters correspond to *M. rubra*, but some characters are unclear. The identification is provisional but is likely to be definitive after comparing with reference material. Varieties that are noted as *M. rubra* cf. could originate from simple or complex spontaneous and/or artificial crosses involving *M. alba*. Their genetic relationship is going to be analysed under the scope of ARACNE mulberry research. The collections also include forms (f.) of *M. alba* (*M. alba* f. *pendula*, *M. alba* f. *pyramidalis*), which denote a taxonomic category below the species level and refer to specific morphological differences. Variety (var.) is a taxonomic category that

ranks between the subspecies and forma level, its members differing from others in minor but permanent or heritable characteristics (Batič et al. 2023).

According to the currently accepted classification of varieties that were identified, evaluated and sampled in germplasm were taxonomically organised according to **Table 2** with respect to the synonyms.

**Table 2** - Current taxonomical status of *M. alba* s.l., *M. indica* s.l. and *M. rubra* cf. along with synonyms.

Current taxonomical status	synonyms
<i>M. alba</i> s.l.	<i>M. multicaulis</i> , <i>M. alba</i> var. <i>multicaulis</i> <i>M. atropurpurea</i> , <i>M. alba</i> var. <i>atropurpurea</i> <i>M. planifolia</i> , <i>M. multicaulis</i> var. <i>planifolia</i>
<i>M. alba</i> f. <i>pendula</i>	<i>M. pendula</i>
<i>M. alba</i> f. <i>pyramidalis</i>	<i>M. pyramidalis</i>
<i>M. indica</i>	<i>M. australis</i> <i>M. latifolia</i> <i>M. kagayamae</i> <i>M. rotundiloba</i> . <i>M. bombycis</i>
<i>M. rubra</i> cf.	Varieties that are noted as <i>M. rubra</i> cf. could originate from simple or complex spontaneous and/or artificial crosses involving <i>M. alba</i> . Their genetic relationship is going to be analysed under the scope of ARACNE mulberry research.

### 3.2.2 Methodology for maintenance of mulberry accessions

Conservation of mulberry accessions begins with their collection, multiplication, and subsequent establishment in *ex-situ* conservation sites. Different maintenance practices are essential to this process, including propagation methods, pruning techniques, and fertilisation strategies. The upcoming chapters will detail the maintenance methodologies employed by the germplasm collections of CREA, SCS Vratsa, and UM. These institutions serve as foundational models for other partners, providing a backbone for the effective conservation and maintenance of mulberry collections.

#### 3.2.2.1 Methodology for maintenance of mulberry accessions at CREA germplasm collection

The main core of the collection at the experimental field of the Sericulture Laboratory of CREA-AA, in Padua (latitude 45.399080°, longitude 11.834279°) consists of a plantation established in unknown dates, but mainly after 1958. The current collection consists of 68 varieties, mainly including varieties of Italian, French, Chinese, and Japanese origin, as detailed in **Appendix I, Supplementary Table 1**.

The core collection is concentrated in a parcel where an average of 5-10 trees for each accession is maintained. The rest of the area is occupied by a larger plantation, with plots of 100-500 m<sup>2</sup>, where trees intended for the production of leaves for silkworm rearing are grown.

The accessions have been propagated by grafting and planted with a spacing of 2.25 × 2.25m. The plants have a vase-shaped cut and have been allowed to grow to a height of about 4-5 meters. Pruning is carried out annually, maintaining the expanded shape with back cuttings and thinning. The plot is fertilised every year exclusively with organic fertilisers, and weeds are controlled mechanically. Sometimes foliar fertilisers for microelements are distributed. The mulberry accessions are grown according to organic agriculture standards. No insecticide, weedicide or fungicide treatments are performed. The parcels of the mulberry collection are rainfed. No irrigation is carried out for old plants, which have a very well-developed root apparatus. For the newly planted cuttings irrigation and plastic mulching are carried out.

During the period 2020-2023, some plots with old mulberries were uprooted, to be gradually replanted with young plants, in order to refresh the collection. In 2023, after a suitable resting period for the soil, the first replanting began. In the new collection, the number of plants conserved per accession increased from 5 to 10.



### 3.2.2.2 Methodology for maintenance of mulberry accessions at UM germplasm collection

The mulberry trees of the UM collection obtained by vegetative propagation from local mulberry varieties are cultivated in a vase shape of medium cutting form (approx. 90-120 cm height). The distance between trees within the row is 1.5-2.5 m, between the rows it is 3.4 m. In the third part of the collection the trees are maintained extensively at a distance of 7 m. Young trees are irrigated manually, and pruning is generally carried out at the end of winter and beginning of spring, as well as at the beginning of summer and autumn for leaf harvest. Mineral fertilization is practiced twice a year, while weeds are mechanically controlled.

### 3.2.2.3 Methodology for maintenance of mulberry accessions at SCS Vratsa germplasm collection

All the accessions at SCS Vratsa were planted in the base collection. At present the plantation is maintained as tree form, bottom pruned once a year, namely either in the winter or at the end of May/beginning of June and used as an active collection for recording data on morphology, anatomy, reproductive and growth parameters, as well as for the production of saplings by hardwood cutting method. The recommended cultivation practice is described in Penkov, 1981. The prevailing shape of the mulberry crown is columnar-shaped (46%) followed by paraboloid-shaped (45%) and pyramid-shaped crown.

### 3.2.2.4 Methodology for germplasm maintenance of mulberry accessions in IMIDA germplasm collection

The core of the collection consists of a plantation established in uncertain dates, between the 1910s and the 1930s with the aim of researching and getting high productivity varieties with excellent adaptation to the climatic and soil conditions. The accessions have been propagated by grafting and planted with a spacing of 2.2 × 2.2 m. These trees have a vase-shaped cut, and pruning is carried out annually, maintaining the expanded shape. The mulberry accessions are grown according to organic agriculture standards and weeds are controlled mechanically. No insecticide, weedicide, fungicide treatments are performed. The parcels of the mulberry collection are rainfed. No irrigation is carried out for old plants, which have a very well-developed root apparatus. For the newly planted cuttings irrigation is carried out with drip irrigation.



### 3.2.3 Methodology for mulberry germplasm characterisation and evaluation

CREA and SCS Vratsa have been active in the evaluation of mulberry using specific descriptors for several years, maintaining a comprehensive database that records both qualitative and quantitative characteristics for most accessions. UM has been evaluating the collection since 2018 based on morphological descriptors. For the ARACNE mulberry research project, the methodology for characterising and evaluating mulberry germplasm was adapted and significantly revised to align with the UPOV descriptors, as described in deliverable D1.2.

To standardise the database of morphological descriptors across all partners, a template for an Excel file pivot table was developed. This template has been utilized by CREA, SCS Vratsa, IMIDA, and UM to ensure consistency in data recording and analysis. The list of evaluated descriptors is summarized

**Table 3** - The list of mulberry descriptors employed for the evaluation of the mulberry genetic resources maintained in the germplasm collections of ARACNE partners.

Category	Descriptor	Options
Taxonomical and phytogeographical information	Identification/ Accession n.	Data entry
	Species	<i>M. alba</i> , <i>M. indica</i> , <i>M. cathayana</i> , <i>M. celtidifolia</i> , <i>M. insignis</i> , <i>M. kordesiana</i> , <i>M. loboensis</i> , <i>M. macroura</i> , <i>M. mesozygia</i> , <i>M. microphylla</i> , <i>M. miyabeana</i> , <i>M. nigra</i> , <i>M. notabilis</i> , <i>M. rubra</i> , <i>M. serrata</i> , <i>M. trilobata</i> , <i>M. wittiorum</i> , interspecific hybrid
	Varietal name	Data entry
	Date of sampling	Data entry
	Date of sampling	Data entry
	Number of individuals in the repository	Individual, Mulberry plantation, Mulberry row
	Method of observation	Single measurement of a group of plants or part of plants, Measurement of a number of individual plants or parts of plants, Visual assessment by a single observation of group of plants or part of plants, Visual

Category	Descriptor	Options
		assessment by observation of individual plants
	Mode of origin/biological status	Natural, Traditional cultivar/landrace, Advanced or improved cultivar, Hybrid
	Ploidy level (if known)	Haploid, Diploid, Triploid, Tetraploid, Hexaploid, Docosaploid, Polyploid, Ploidy level not yet determined
	Geographical origin lat.	Data entry
	Geographical origin long.	Data entry
	Availability	Public, Street, Square, Private Garden, Botanical Garden or Gene bank, Agricultural landscape
	Areas of cultivation	Temperate, tropical, sub-tropical
	Trunk circumference (cm)	<180 cm, 180-249 cm, 250-299 cm, >300 cm
Morphological characteristics	Exact measurement of trunk circumference (cm)	Data entry
	Tree growth habit	Upright, Semi-upright, Weeping
	Tree vigour	Bad condition, Good condition
	Pruning practices	Unpruned tree, Frequently pruned, Yearly pruned tree
	Trunk colour	Greyish brown, Light brownish-grey, Reddish brown
Trunk	Trunk irregularities/damage	Curved, Hollow (pipe tree), Longitudinally cracked, External split
	Current years shoots: length (cm)	Data entry
Shoots	Current years shoot: number of lateral shoots	Data entry
	Current years shoot: length of internode	Data entry
	Colour of one-year old shoot	Greyish brown, Greenish brown, Yellowish brown, Medium brown, Reddish brown, Dark brown
	Lenticel density	High, Medium, Sparse
	Lenticel shape	Elliptical, Oval, Round

Category	Descriptor	Options
Buds	Bud shape	Broad triangular, Medium triangular, Narrow triangular, Ovate
	Bud colour	Greyish brown, Yellowish brown, Reddish brown, Medium brown, Dark brown
	Bud size	Small, medium, large
	Phyllotaxis	Alternate spiral, Alternate distichous, Opposite decussate
Leaves	Leaf shape	Simple, Lobed
	Peduncle length (cm)	Data entry
	Average leaf size: length (cm)	Data entry
	Average leaf size: width (cm)	Data entry
	Average leaf size: area (cm <sup>2</sup> )	Data entry
	Leaf blade	Low (<1.2 broad leaves), Medium (1.3-1.5), High (>1.6 long)
	Petiole range	Absent or very short (< 10 mm), Short (11-20 mm), Medium (21-40mm), Long (41-70 mm), Very long (>71 mm)
	Petiole size (cm)	Data entry
	Shape of leaf base	Cuneate, Truncate, Retuse, Cordate
	Shape of leaf apex	Acute, Obtuse, Obcordate
	Leaf blade tip	Absent, Caudate, Acuminate
	Leaf blade margin	Repand, Crenate, Dentate, Serrulate, Biserrate, Serrate, Aristate
	Hairiness	Glabrous, Midrib and veins, Evenly pubescent
	Glossiness	Glossy, Matte
Phenological descriptors	Date of swollen bud (week)	Data entry
	Date of bud burst (week)	Data entry
	Flowering date (week)	Data entry
	Date of first leaf (week)	Data entry
	Date of first leaf (week)	Data entry
	Early yellowing due to disease (week)	Data entry

Category	Descriptor	Options
	Abundant autumn colouring (week)	Data entry
	Leaf fall (week)	Data entry
	Time of infructescence ripening	Early, Early to medium, Medium, Medium to late, Late
	Uniformity of infructescence ripening	Early, Early to medium, Medium, Medium to late, Late
	Sexual dimorphism	Monoecious, Dioecious
Reproductive structures	Inflorescence type	Male, Female, Predominantly female with some male flowers at the base, Predominantly male with some female flowers at the apex, Predominantly male with some female flowers at the base, Predominantly female with some male flowers at the apex
Inflorescence size	Female inflorescence length (mm)	Data entry
	Female inflorescence diameter (mm)	Data entry
	Female inflorescence stalk length (mm)	Data entry
	Male inflorescence length (mm)	Data entry
	Male inflorescence length (mm)	Data entry
	Male inflorescence stalk length (mm)	Data entry
	Hermaphrodite inflorescence length (mm)	Data entry
	Hermaphrodite inflorescence diameter (mm)	Data entry
	Hermaphrodite inflorescence diameter (mm)	Data entry
Infructescence	Fruit weight (g)	Data entry
	Length (mm)	Data entry
	Width (mm)	Data entry
	Length of peduncle	Short, Medium, Long
	Colour	Yellowish white, Light pink, Purple brown, Reddish black, Black
	Taste	Acidic, Sweet, Balanced

Category	Descriptor	Options
Growth and yield attributes	Infructescence shape	Ovoid/globose, Ellipsoid, Cylindrical, Irregular
	Growth	Slow, Medium, Fast
	Length of primary branch (cm)	Data entry
	Internodal distance (cm)	Data entry
	Leaf No./meter	Data entry
	Weight of 100 fresh leaves (g)	Data entry
Chemical composition of leaves	Total protein (g/100 g)	Data entry
	Mineral (g/100 g)	Data entry
	Fibre (g/100 g)	Data entry
Response to different physiological conditions	Response to drought	Resistant, Tolerant, Susceptible
	Response to salinity	Resistant, Tolerant, Susceptible
	Response to chilling stress	Good, Medium, Poor
	Response to repeated pruning	Good, Medium, Poor
Diseases and pests	Disease option	Fungal leaf spot, Bacterial leaf spot ( <i>Pseudomonas syringae</i> pv. <i>mori</i> ), Soft rot ( <i>Pectobacterium carotovorum</i> ), Ringspot virus
	Pest option	Mulberry moth ( <i>Hyphantria cunea</i> ), Thrips (5 species), Mites ( <i>Tetranychus</i> sp.), Scale insects (Hemiptera), Mealy bugs ( <i>Maconellicoccus hirsutus</i> ), Red scale ( <i>Aonidella aurantii</i> ), Hairy caterpillar ( <i>Spilarctia obliqua</i> ), Jassids ( <i>Empoasca Flavescens</i> )
	Any other disease or pest	Data entry
	Leaf necrotic spots	Low, medium, frequent
	Bark lesions	Frequent, Few
	Additional remarks	Optional
		Data entry

Creating an integrated database and preparing descriptors for mulberry genetic resources was crucial for the involved partners, enabling the availability of data for advanced statistical studies, including multivariate analyses. These analyses are aimed at identifying distinct

morphotypes, which will then be compared with genetic analyses to gain deeper insights into the origin and distribution of mulberry across Europe in the past.

For the evaluation of morphological descriptors, varieties were meticulously selected from each germplasm collection to ensure a comprehensive and comparable analysis:

- ❖ CREA Germplasm: The evaluation included 28 varieties from diverse taxonomical and geographical origins, providing a broad spectrum for analysis.
- ❖ Vratsa Germplasm: A total of 70 mulberry varieties were evaluated. The selection focused on local Bulgarian varieties and those from Eastern Europe and Central Asia (Armenia, Azerbaijan, Georgia, Romania, Ukraine, and Uzbekistan) to trace the possible spread of mulberries (and sericultural activity) from the East to the West. These varieties, introduced and planted at the Mulberry Gene Bank at SCS-Vratsa between 1965 and 2004, offer a unique insight into regional diversity. Observations were made on 1 to 10 trees per variety, providing a rich dataset for analysis.
- ❖ UM Germplasm: 109 distinct varieties of white mulberry (*M. alba* s.l.) were included in the analyses, derived from 91 local historical Slovene trees and 18 Hungarian trees. For each local tree variety, 3-5 trees were observed, ensuring a detailed and representative sampling.

This structured approach to evaluating morphological descriptors across different germplasm collections lays the foundation for a thorough understanding of mulberry genetic diversity. It also facilitates the exploration of historical and geographical patterns in sericulture, contributing to the global knowledge base and supporting future breeding and conservation efforts.

### 3.3 Results on characterisation of mulberry genetic resources

In the following chapters, we present a comprehensive evaluation of the germplasm collections from countries participating in the ARACNE project. This assessment covers the taxonomical characterisation of varieties in each collection and summarises the current state of research associated with them. Institutions such as CREA, SCS Vratsa, and UM have contributed results related to leaf descriptors and phenological evaluation, assessed during the first year of the ARACNE project.

The initial phase of the project focused on evaluating specific leaf descriptors, which were documented in the first year. These evaluations offer preliminary insights into the diversity and characteristics of the plant material within the germplasm collections. Additionally, a phenological assessment was conducted to observe and record the seasonal changes and developmental stages of the plants, providing a foundational understanding of their growth cycles and reproductive patterns.

To enhance the robustness of our findings, it is imperative to extend the monitoring of these descriptors over the next two years. This extended observation period will allow to provide multi-year averages and advanced statistical analyses that will enable a deeper understanding of the data, facilitating the identification of significant correlations and morphotypes which, along with genetic analyses, will mediate future research and conservation strategies.

In addition to the descriptor evaluations carried out by CREA, SCS Vratsa, and UM, this section also provides identification of varieties within the germplasm collections of other countries involved in the ARACNE project. This effort aims to catalogue and characterise the genetic diversity present across the participating countries, underscoring the project's commitment to characterising and preserving European mulberry genetic resources.



### 3.3.1 Evaluation of mulberry genetic resources in Italy

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Italy



Figure 3 - Mulberry germplasm collection, CREA-AA Padua, Italy.

#### 3.3.1.1 General information about the conservation status of mulberry germplasm resources in Italy

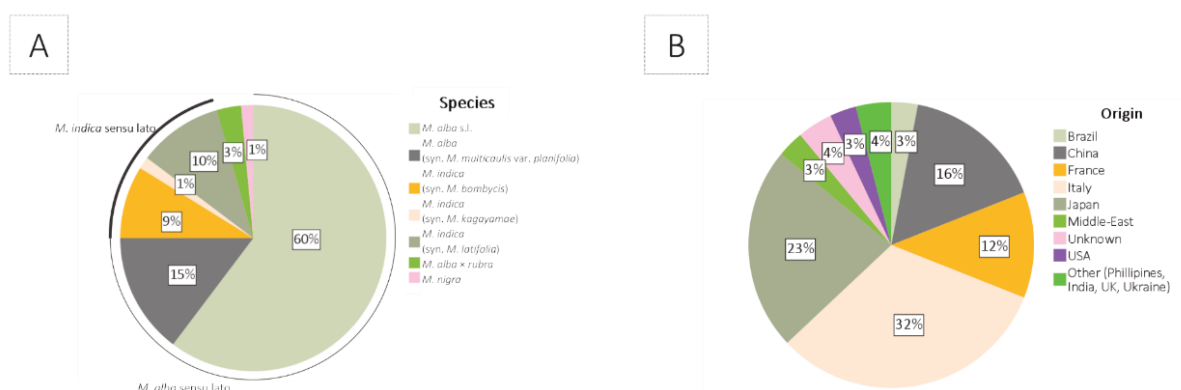
In Italy, the varieties of mulberry are preserved in the experimental field of the Laboratory of Sericulture of CREA-AA, in Padua (latitude 45.399080, longitude 11.834279). The current collection extends over about two and a half hectares, conserving overall 68 varieties of mulberries, of which 21 are of Italian origin **Figure 3**. Furthermore, the collection includes varieties of French, Chinese, and Japanese origin, as detailed in **Supplementary Table 1**.

The most widely cultivated varieties (> 20 trees) are 'Florio', 'Morettiana', 'Kokusou 20', 'Kokusou 21', 'Ichinose', 'Arancina', 'Limoncina', and 'Restelli'. Initially, the creators of the

collection were concerned with preserving varieties belonging to all the then known major species of *Morus*, and so the following species were represented: *M. alba* (L.), *M. nigra* (L.), *M. multicaulis* (Perr.), *M. latifolia* (Poir.), *M. bombycis* (Koidz), *M. kagayamae* (Koidz). However, according to the most recent studies, which employ molecular methods for phylogenetic reconstruction (Zeng et al. 2015), it can be said that all accessions actually fall within the scope of *M. alba* s.l., *M. indica* s.l., *M. nigra* and *M. rubra* cf. **Supplementary Table 1** represents the list of the maintained varieties along with the origin and the number of trees.

The distribution of the mulberry accessions according to their species and geographical origin is presented in **Figure 4**. Most of mulberry varieties (65 accessions, 60 %) belong to *M. alba* s.l. In addition, 15 % of *M. alba* s.l. varieties were, according to the older taxonomical classification, revised with synonymous name *M. multicaulis* var. *planifolia*. 17 varieties (20 %) belong to the ingroup of *M. indica* s.l. Nine *M. indica* varieties (10 %) were previously listed with the synonymous name *M. latifolia*, six varieties (9%) as *M. bombycis* and one variety as *M. kagayamae*. In addition, the collection comprises other species, of which four represent hybrids of *M. alba* × *M. rubra* (*M. rubra* cf.). *M. nigra* is represented by one genotype of local origin (Figure 4A). The data, shown in Figure 4B manifests that there are accessions from nearly all countries practicing sericulture from the temperate and sub-tropical zone. 32 % of the varieties are Italian breeds, 23 % represent the Japanese varieties, 16 % are Chinese varieties, 12 % are from France, 4 % varieties are from the Middle East, the other from Ukraine, the Philippines, UK, USA and 4 % are of unknown origin.

## CREA



**Figure 4** - The percentage of A) species and B) origin of *Morus* sp. maintained in the germplasm collection of CREA Padua.

### 3.3.1.2 Morphological characterisation of mulberry genetic resources in Italy

A comprehensive, contemporaneous and systematic morphological characterisation of the accessions preserved in the Padua mulberry collection has never been carried out, due to the large number of varieties and the fact that the mulberry vegetation season coincides with the silkworm rearing season, so that the technical staff is already busy with reproduction of the silkworm strains and egg production. Therefore, apart from the Planta Res database (CREA Planta res, 2013) some mulberry characteristics for several varieties have not been recorded to date, although the data collected for the more popular varieties has nonetheless been disseminated in the form of scientific publications.

However, a collection and cataloguing activity for taking photos of the leaves and buds of all the accessions began two years ago and should conclude at the end of the current year. The photographic documentation of other descriptor traits, such as the appearance of the shoots and the bark is ongoing in the current year.

**Table 4** represents the list of descriptors that were currently evaluated for the 28 varieties listed in **Appendix I, Supplementary Table 1**.

**Table 4** - List of descriptors that were monitored for 28 varieties in year 2023 at the CREA germplasm collection.

Current years shoot:		length of internode	
	Colour of one-year old shoot	<input checked="" type="checkbox"/>	
	Lenticel density	<input checked="" type="checkbox"/>	
	Lenticel shape	<input checked="" type="checkbox"/>	
Buds	Bud shape	<input checked="" type="checkbox"/>	
	Bud colour	<input checked="" type="checkbox"/>	
	Bud size	<input checked="" type="checkbox"/>	
Leaves	Phyllotaxis	<input checked="" type="checkbox"/>	
	Leaf shape	<input checked="" type="checkbox"/>	
	Peduncle length (cm)		
	Average leaf size: length (cm)		
	Average leaf size: width (cm)		
	Average leaf size: area (cm <sup>2</sup> )		
	Leaf blade	<input checked="" type="checkbox"/>	
	Petiole range	<input checked="" type="checkbox"/>	
	Petiole size (cm)		
	Shape of leaf base	<input checked="" type="checkbox"/>	
	Shape of leaf apex	<input checked="" type="checkbox"/>	
	Leaf blade tip	<input checked="" type="checkbox"/>	
	Leaf blade margin	<input checked="" type="checkbox"/>	
	Hairiness	<input checked="" type="checkbox"/>	
	Glossiness	<input checked="" type="checkbox"/>	
Phenological descriptors	Date of swollen bud (week)	<input checked="" type="checkbox"/>	*
	Date of bud burst (week)	<input checked="" type="checkbox"/>	*
	Flowering date (week)	<input checked="" type="checkbox"/>	*
	Date of first leaf (week)	<input checked="" type="checkbox"/>	*
	Date of fruit ripening (week)	<input checked="" type="checkbox"/>	*
	Early yellowing due to disease (week)	<input checked="" type="checkbox"/>	*
	Abundant autumn colouring (week)	<input checked="" type="checkbox"/>	*
	Leaf fall (week)	<input checked="" type="checkbox"/>	*

Current years shoot:		length of internode	
	Time of infructescence ripening	<input checked="" type="checkbox"/>	*
	Uniformity of infructescence ripening	<input checked="" type="checkbox"/>	*
Reproductive structures	Sexual dimorphism	<input checked="" type="checkbox"/>	
	Inflorescence type	<input checked="" type="checkbox"/>	
Inflorescence size	Female inflorescence length (mm)		
	Female inflorescence diameter (mm)		
	Female inflorescence stalk length (mm)		
	Male inflorescence length (mm)		
	Male inflorescence diameter (mm)		
	Male inflorescence stalk length (mm)		
	Hermaphrodite inflorescence length (mm)		
	Hermaphrodite inflorescence diameter (mm)		
	Hermaphrodite inflorescence stalk length (mm)		
Infructescence	Fruit weight (g)	<input checked="" type="checkbox"/>	
	Length (mm)	<input checked="" type="checkbox"/>	
	Width (mm)		
	Length of peduncle		
	Colour	<input checked="" type="checkbox"/>	
	Taste	<input checked="" type="checkbox"/>	
	Infructescence shape	<input checked="" type="checkbox"/>	
Growth and yield attributes	Growth		
	Length of primary branch (cm)		
	Internodal distance (cm)		
	Leaf No./meter		
	Weight of 100 fresh leaves (g)		
Chemical composition of leaves	Total protein (g/100 g)		
	Mineral (g/100 g)		
	Fibre (g/100 g)		
Response to different physiological conditions	Response to drought		
	Response to salinity		

Current years shoot:	length of internode	
	Response to chilling stress	
	Response to repeated pruning	
DISEASES and PESTS	Disease option	?
	Pest option	?
	Any other disease or pest	<input checked="" type="checkbox"/>
	Leaf necrotic spots	<input checked="" type="checkbox"/>
	Bark lesions	<input checked="" type="checkbox"/>

\*Requires a multi-year average, at least three-years

57% of varieties have simple leaves, 32 % are characterised by both simple and lobed leaves whereas 11 % of the represented varieties have lobed leaves (**Figure 5A**). The majority of varieties (46 %) have a medium leaf size ratio, 36 % varieties are characterised by long leaves (a high leaf size ratio), whereas only 18 % have broad leaves (low leaf size ratio) as displayed in **Figure 5B**.

**Figure 5C** depicts the petiole size range. 43 % of varieties represent medium petioles (21 mm-40 mm) and long petioles (41 mm-70 mm), respectively. 14 % of varieties have a very long (>71 mm) petiole. The variability of leaf base shapes that exhibit the varieties of CREA germplasm collection are visually presented in **Figure 5D**. Among the evaluated varieties, 27 % feature retuse leaf base, 11 % represent intermediate truncate-retuse form, whereas 4 % were characterised by cordate-retuse leaf base. Furthermore, 21 % of varieties had cordate leaves and 4 % an intermediate truncate-cordate leaf base. 21 % of varieties featured a truncate leaf base, whereas the intermediate truncate-cuneate form was represented by 4%. Cuneate leaf form was rare or represented as intermediate cuneate-truncate form (4 % each).

**Figure 5E** illustrates the blade tip shapes. The majority, accounting for 68 % of the recorded trees, featured an acuminate blade tip. A smaller proportion, 32 % of the trees, exhibited a caudate blade tip. The prevalence of the acuminate blade tip aligns with the dominance of the acute leaf apex shape.

The leaf blade margin revealed various shapes. The predominant types were serrate (39 %) and crenate (36 %) leaf blade margins, followed by 21 % of mulberries with serrulate leaf margins, while 4 % featured dentate margins (**Figure 5F**). The assessment of leaf hairiness of the abaxial leaf epidermis indicated that 58 % exhibited hairiness on midrib and veins, whereas 42 % were glabrous (**Figure 5H**). Furthermore, 61% of the observed varieties



exhibited glossy leaves, which is a characteristic feature of white mulberry while the remaining 39 % had matt leaves.

### 3.3.1.3 Phenological evaluation

From the vegetative and reproductive point of view, the most studied characteristic is the phenological behaviour, which allows for the assessment of adaptability to various climatic environments of the peninsula, with a view to reviving activities at an entrepreneurial level.

The phenological stages observed are budburst, flowering, fruit ripening, and leaf fall. All are recorded using the BBCH scale adapted for mulberry (Sánchez-Salcedo et al. 2017). Considering budburst as phase 9 BBCH, in recent years it has been concentrated in the last ten days of March, with an overall variability (from the earliest to the latest budburst) of about 4 weeks (second and third ten days of March, first ten days of April). Flowering (BBCH 65) occurs immediately after budburst and extends over an even more concentrated period, from the last week of March to the first week of April, with little differentiation among the accessions. Fruit ripening, using BBCH 85 as a reference, typically occurs between the last ten days of May and the first half of June, a bit longer than the flowering period and with some variability among accessions. The fall of the leaves (BBCH 97), an interesting characteristic to determine the duration of the tree productive cycle, extends over a period from mid-October, for the earliest accessions, to the end of November for the latest ones.

### Mulberry descriptors of CREA germplasm



**Figure 5** - Evaluation of the leaf morphology descriptors of CREA germplasm collection. A) Leaf shape. B) Leaf size ratio. C) Petiole size. D) Shape of leaf base. E) Leaf blade tip. F) Leaf blade margin. G) Hairiness. H) Glossiness.

### 3.3.1.4 Sensory and biochemical evaluation of the infructescence of mulberry accessions

In the last two years, a sensory evaluation of the fruits has been undertaken, whose valorisation is seen as an opportunity to relaunch this food supply chain as a secondary product of sericulture. The soroses of *M. alba* are usually less prized than those of *M. nigra*, as they typically lack acidity, resulting in a sweet but cloying taste. However, there is considerable range of variability among varieties, as some have an acceptable balance between sugar content and acidity, resulting in a pleasant taste, which also depends on the degree of ripeness. The fruits are classified with a qualitative empirical scale from 1 to 3, where the highest value represents the greatest palatability. Varieties that are appreciable and interesting from this point of view include, for example, 'Illinois Everbearing', 'Aoba Nezumi', 'Okaraguwa', 'Kayriou Rosou', 'Platanoide', 'Lhou'. It should be noted that palatability can also vary with the degree of ripeness, therefore, for some varieties it may be better in the early stages of ripening, for others, in later stages.

### 3.3.1.5 Evaluation of bioactive compounds in woody/bark tissue

Another area of activity is the evaluation of accessions for their ability to produce bioactive compounds used in the pharmaceutical, cosmetic, or nutraceutical fields. A varietal characteristic currently under study is the content of stilbenoids in the woody/bark tissues of mulberries. This class of substances includes molecules characterised by interesting properties, such as anti-inflammatory, anti-tyrosinase, and antioxidant actions. As for the content of stilbenoids, the varieties show a high variability, ranging from low levels of <0.3 mg/g (e.g., 'Restelli', 'Kokusou 21', 'Kayriou Nezumigaeshi') to contents >2.0 mg/g (e.g., 'Morettiana', 'Cattaneo (female)', 'Rosa di Lombardia'). With the currently available data, it appears that native varieties are superior to the exotic ones in the Italian environment.

### 3.3.2 Evaluation of mulberry genetic resources in Slovenia

Faculty of Agriculture and Life Sciences	University of Maribor	Chair of Botany and Plant Physiology
Curator:	Andreja Urbanek Krajnc	<a href="mailto:andreja.urbanek@um.si">andreja.urbanek@um.si</a>
Postal Address:	Pivola 8, 2311 Hoče	



**Figure 6** - Mulberry germplasm collection, UM Maribor, Slovenia.

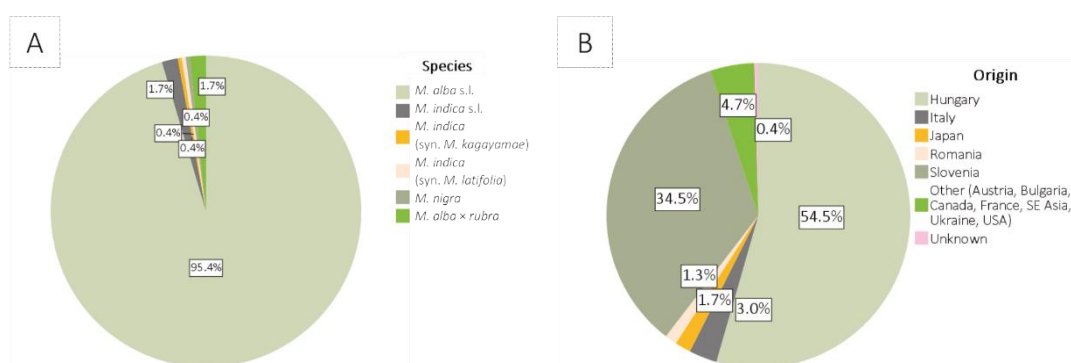
#### 3.3.2.1 General information about the conservation of mulberry germplasm resources in Slovenia

In Slovenia, the relevant mulberry germplasm collection is maintained at the Faculty of Agriculture and Life Sciences University of Maribor (UM). It extends over 0.6 ha and is organised into three sections. The first is represented by the traditional sericultural mulberry varieties obtained from the germplasm collections of the Sericultural Institutes CREA Padua and SCS Vratsa. Those varieties are 'Florio', 'Morettiana', 'Kokusou 20', 'Kokusou 21', 'Kokusou 60', 'Giazza', 'Muki', 'Restelli', 'Kiuryu', 'No. 25'. In the continuation of this collection, we grow vegetatively-propagated trees derived from the local historical Slovene and Hungarian trees, which were obtained during the inventory of the mulberry gene pool, which was the main task of the Slovene partner of the joined Slovenian-Hungarian research project between 2015-2018 (ARIS N1-0041), in an effort to reconstruct a genetic heritage based on ancient populations, with a view to selecting local varieties best adapted to the pedo-climatic environment of the region, for the variability of plant material available for farmers, even for applications other than silkworm rearing.

The first varieties were planted in 2015. Most of the Slovenian varieties were propagated in 2016 and planted in 2017, whereas most of the Hungarian varieties were propagated in 2017 and planted in 2018. The third part of the collection maintains species and hybrids mainly

grown for fruit production. Initially, the founders of the collection were concerned with preserving varieties belonging to all the major species of *Morus*, that can be cultivated in our geographical region. Currently, the following species are represented: *M. alba*, *M. nigra*, *M. alba* x *M. rubra*, *M. rubra* cf., whereas the species *M. maclura*, *M. boninensis* are maintained in pots overwintering in a cold house.

**Appendix III, Supplementary Table 4-** represent the lists of the maintained varieties along with their origin and circumference of parent tree.



**Figure 7** - The percentage of species and origin of *Morus* sp. maintained in the germplasm collection of UM.

In the collection, a significant majority of 95.4% of the varieties are classified under *M. alba* s.l. and are mainly of local origin, while *M. indica* s.l. accounts for 2.5% of the varieties. Additionally, 1.7% of the varieties in the third section of the collection are hybrids between *M. rubra* and *M. alba* (**Figure 7A**). The collection predominantly features old local Hungarian varieties, constituting 54.5% of the collection, followed by Slovenian varieties, which make up 34.5%. Varieties of Italian origin, sourced from the CREA germplasm collection, represent 3% of the collection. Furthermore, 1.7% of the collection consists of Japanese varieties, and 1.3% are varieties that were obtained from a collector in Romania (**Figure 7B**). This diverse assortment underscores the collection's broad geographical and genetic range, highlighting its importance as a resource for morphological and genetic studies.

### 3.3.2.2 Morphological characterisation of mulberry genetic resources in Slovenia

#### Leaf descriptors of mulberry germplasm in Slovenia

Within the UM germplasm collection, a range of descriptors were closely monitored from March, marking the onset of bud burst, through mid-December 2023, by observing leaf fall and bud descriptors. This comprehensive monitoring period captures the full cycle of vegetative growth and dormancy indicators, providing valuable insights into the phenological patterns and adaptability of the collection's varieties to their growing conditions.

Leaf descriptors were monitored between the 10th and 14th July 2023. The records include 109 distinct varieties of white mulberries (*M. alba* L.) originating from 91 local historical Slovene and 18 Hungarian trees. Notably, we excluded monitoring of the leaf descriptors for the traditional sericultural mulberry varieties obtained from the germplasm collections of the Sericulture Laboratory CREA Padua and SCS Vratsa, as they were already accounted for in the observations of CREA and Vratsa.

The majority of mulberries (92%) exhibited alternate distichous phyllotaxis, while only 8 % displayed alternate spiral phyllotaxis. It is important to note that describing this descriptor can be challenging, as in most trees, one form of phyllotaxy, typically alternate distichous, was observed up to the first few nodes before transitioning to alternate spiral phyllotaxis.

**Figure 8A** shows that the prevailing leaf morphology among mulberries was characterised by both simple and lobed shapes, constituting 60 %. Specifically, 33 % of the mulberries displayed exclusively a simple leaf shape, while 7 % exhibited solely lobed leaves.

The majority (96 %) of recorded mulberries had a medium leaf blade, while only 4 % had a high leaf size ratio. Notably, there were no mulberry varieties with broad leaves in our observations (**Figure 8B**).

**Figure 8C** shows the distribution of petiole ranges among the recorded mulberries, indicating that 88 % of mulberries had a medium petiole size range, while 12% exhibited a long petiole size range. Our findings imply constrained variability, as no mulberries in our dataset exhibited very short (< 10 mm), short (11-20 mm), or very long (>71 mm) petiole ranges.

The diversity of leaf base shapes is visually represented in **Figure 8D**. Out of the 110 observed trees, 48 % exhibited a retuse leaf base, 37 % displayed a cordate leaf base, and a minority of 15 % featured a truncate leaf base. Importantly, there were no recorded instances of mulberries with a cuneate leaf base.

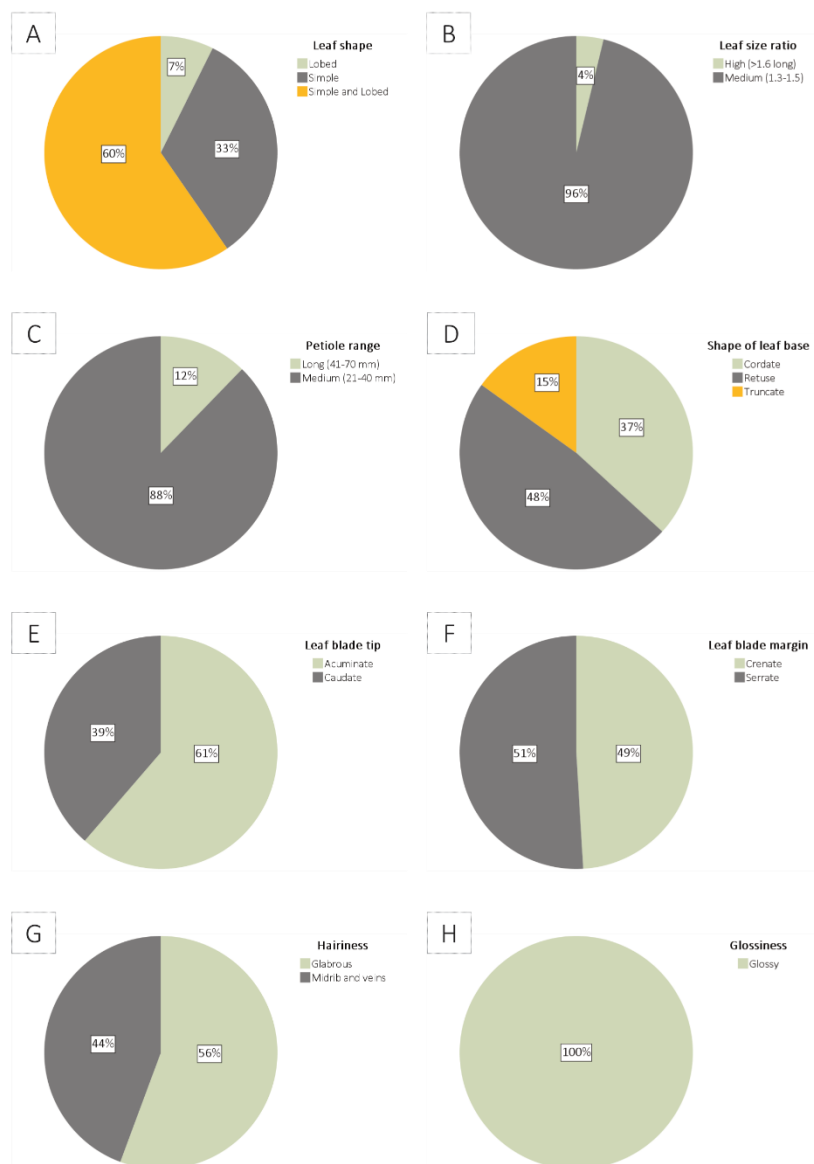
All mulberries in the dataset exhibited an acute leaf apex. 61 % of the recorded mulberries featured an acuminate leaf blade tip, while 39 % displayed a caudate tip. Notably, none of the recorded mulberries had an absent blade tip **Figure 8E**.

**Figure 8F** shows that 51 % of mulberries had a serrate and 49 % had crenate leaf blade margin. Notably, no mulberries in our records exhibited a repand, dentate, serrulate, biserrate, or aristate leaf blade margin.

The assessment of leaf hairiness indicated that 56 % had glabrous leaves and 44 % featured hairiness on midrib and veins of the abaxial epidermis layer (**Figure 8G**). Additionally, **Figure 8H** illustrates that every recorded mulberry had glossy leaves as characteristic for *M. alba* s.l.



### Mulberry descriptors of UM germplasm



**Figure 8** - Detailed descriptors of leaf morphology for germplasm collection at the Faculty of Agriculture and Life Sciences University of Maribor. Revaluation of local Slovenian and Hungarian white mulberries (*M. alba* L.). Detailed descriptors of leaf morphology. A) Leaf shape. B) Leaf size ratio. C) Petiole size. D) Shape of leaf base. E) Leaf blade tip. F) Leaf blade margin. G) Hairiness. H) Glossiness.

### 3.3.2.3 Phenological evaluation

Examining phenology is a crucial aspect of assessing adaptability to diverse climatic conditions from a vegetative-productive perspective. By detailing phenological stages, we can pinpoint the vegetative period, a key determinant for mulberry cultivators. We began our phenological evaluation in the 12th week (20.3-26.3.2023) with the phenological phase 'swollen bud' and concluded in week 50 (11.12-17.12.2023) with leaf fall. This implies that the longest vegetative period spanned 266 days, while the shortest was observed in black mulberry, lasting 231 days.

In the UM germplasm collection, we included 22 mulberry fruit varieties into our phenological evaluation, primarily comprising hybrids of white and red mulberry (*M. alba* × *rubra*), along with one black mulberry (*M. nigra*). Additionally, we observed phenology in six traditional sericultural mulberry varieties: 'Florio', 'Morettiana', 'Giazzola', 'Muki' and 'Restelli'. Predominantly, our focus was on recording phenological descriptors in white mulberries sourced from local historical Slovene and Hungarian trees.

The observed phenological stages included swollen bud, bud burst, first leaf, flowering, fruit ripening, abundant autumn colouring, and leaf fall. These stages were documented using the BBCH scale specifically adapted for mulberry (Sánchez-Salcedo et al., 2017).

In our study, the swollen bud phase (03 BBCH) occurred in 114 white mulberry trees during the 12th week, in 78 trees during the 15th week, and for black mulberry, this phase was observed in the 17th week.

Between weeks 16 and 17, frostbite occurred, resulting in damage to the buds of 20 trees. Subsequently, our study revealed that the bud burst phase (09 BBCH) occurred in 93 white mulberry trees during the 16th week, in 79 trees during the 17th week, and for black mulberry, this phase was observed in the 18th week.

We observed the emergence of the first leaf (BBCH 11) for 120 mulberry trees during the 18th week, while 57 mulberries grew their first leaf during the 19th week. A minority of 6 mulberries had their first leaf emergence during the 20th week.

Flowering (BBCH 65) occurred during the 19th week for 30 trees, on the 20th week for 85 trees, including black mulberry, and 69 mulberries flowered during the 21st week.

As a result of frostbite and the need for inflorescence measurement, we could only ascertain fruit ripening (BBCH 85) in 9 white mulberry trees. Among these, fruit ripened during the 27th week for 8 of them, while for black mulberry, fruit ripening transpired during the 28th week. Our observation indicates that fruit ripening occurred unusually late (3.7-16.7.2023), given that it typically takes place between the last ten days of May and the first half of June.

We introduced the parameter for early yellowing due to disease, considering the notable prevalence of such conditions. Among the 213 trees observed, 83 mulberries displayed premature yellowing in the 38th week, and three exhibited it during the 40th week.

Abundant autumn colouring (BBCH 95) was observed in 123 trees during week 46, in 51 mulberries during week 47, and in 39 mulberries during week 48.

The leaf fall (BBCH 97), an intriguing characteristic crucial for determining the duration of the tree's productive cycle, occurred later than usual for our climate in Slovenia. For the majority of 105 mulberries leaves fell on week 46, for 41 trees this occurred on week 47, for 18 mulberries on week 48 and for 94 mulberries leaves fell as late as in week 50.

### 3.3.2.4 Phytochemical evaluation of mulberry leaves

Mulberry leaves are rich in proteins, with a unique amino acid composition dominated by threonine, arginine, asparagine, serine, and glutamine. They also contain significant levels of phenolic compounds, including caffeoylquinic acid derivatives, quercetin malonyl-hexoside, rutin, kaempferol acetyl-hexoside, quercetin-3-glucoside, and p-coumaric acid derivatives. Since 2016 the working group of botany of UM is focused on the evaluation of the existing genetic resources of historical mulberries in Slovenia and Hungary, collection establishment, identification of important metabolites in leaves and their nutritional and biochemical significance, especially in the context of sericulture and environmental factors. Screening of leaves of local mulberry varieties from different regions provided comprehensive insights into how pruning, eco-geographical regions and morphotypes influence the biochemical profile of mulberry leaves (Urbanek Krajnc et al. 2019; Šelih et al. 2020; Urbanek Krajnc et al. 2022). These findings underscore the potential of selected local varieties for high-quality silk cocoon and raw silk production. Key findings from these studies include:

**Influence of pruning:** Pruning practices, particularly yearly pruning, have a profound effect on the nutritional quality of mulberry leaves. Pruning management increases the levels of specific amino acids (asparagine, alanine, serine) and phenolics (rutin, quercetin malonyl-hexoside, quercetin-3-glucoside), enhancing the leaf's nutritional profile for sericulture.

**Eco-geographical impact:** The geographical location and traditional pruning methods (e.g. pollarding in the Submediterranean region) influence the biochemical composition of mulberry leaves. Variations in total proteins, total phenolics, caffeoylquinic acid derivatives, and flavonols were observed across different regions, indicating that local environmental conditions and cultural practices contribute to the leaf's chemical composition.

Sericulture relevance: The content of proteins, specific amino acids, and phenolic compounds in mulberry leaves is crucial for silkworm nutrition, affecting larval growth and the quality of silk produced. The studies identified certain metabolites, such as caffeoylquinic acid, quercetin malonylhexoside, rutin, kaempferol acetylhexoside, and isoquercetin, as valuable feed markers for their beneficial effects on silkworm larvae growth and cocoon quality.

Chemotype classification: Through multivariate analysis, we were able to classify local mulberry genotypes into distinct chemotypes based on their biochemical traits. This classification helps in identifying varieties with superior nutritional profiles for sericulture purposes.

Overall, the studies underscore the importance of selecting mulberry varieties with optimal biochemical profiles for sericulture, considering the effects of pruning and eco-geographical factors. The research highlights the potential for enhancing silk production through targeted agricultural and breeding practices, emphasising the natural, cultural, and scientific value of mulberry trees.

### Infructescences

The recently published article by Urbanek Krajnc et al. (2023) presents a comprehensive evaluation of the primary metabolites and phenolics in mulberry fruits (soroses) across different colour types, highlighting the distinct chemotype characteristics associated with each colour. The research focused on local mulberry genotypes and varieties within a mulberry collection, assessing their chemical profiles to understand how colour influences the fruit's nutritional and beneficial health properties.

The predominant sugars found across all infructescence colour types were glucose and fructose, with citric and malic acids being the major organic acids in darker varieties, contributing to their acidity and flavour profile. In contrast, fainter varieties had higher levels of fumaric and tartaric acids, known for their more astringent taste.

A total of 42 phenolic compounds were identified, with chlorogenic acid being the most prevalent. The most abundant flavonoids in mulberry fruits are anthocyanins, followed by flavonols (quercetin and kaempferol glycosides), flavanols (catechin, epicatechin and procyanidin), flavanons, which are represented by naringenin derivatives, laricitrin hexoside, and quercetin rhamnosyl-hexoside. The diversity of colours of white mulberry soroses is an interplay of qualitative and quantitative differences of anthocyanins, which were below the detection level in yellowish-white infructescences, and increased gradually by more than 100

times from light pink to black coloured genotypes. The predominant anthocyanins were cyanidin-3-glucoside and cyanidin-3-rutinoside.

The research illustrated a clear link between mulberry colour and its chemical profile. Fainter coloured (yellowish-white) soroses were sweeter due to higher fructose and glucose content, while darker (black) varieties had a sour taste attributed to higher organic acid content. Phenolic acids and anthocyanins were significantly higher in darker varieties, correlating with their colour intensity. The detailed analysis of sugars, organic acids, and phenolic compounds across colour types provides valuable insights into the potential health benefits of mulberries, reinforcing the importance of these fruits in diet and medicine.

### Ongoing research

Recently, we have been focusing on the evaluation of the chemical composition of the bark of various local, sericultural and fruit varieties with regard to the inhibitory effect against certain bacteria and fungi. The research is ongoing and will be published as part of the ARACNE project activities.

### 3.3.3 Evaluation of mulberry genetic resources in Bulgaria

#### Scientific Center on Sericulture

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Figure 9 - Mulberry germplasm collection SCS Vratsa, Bulgaria.

#### 3.3.3.1 General information about the conservation status of mulberry germplasm resources in Bulgaria

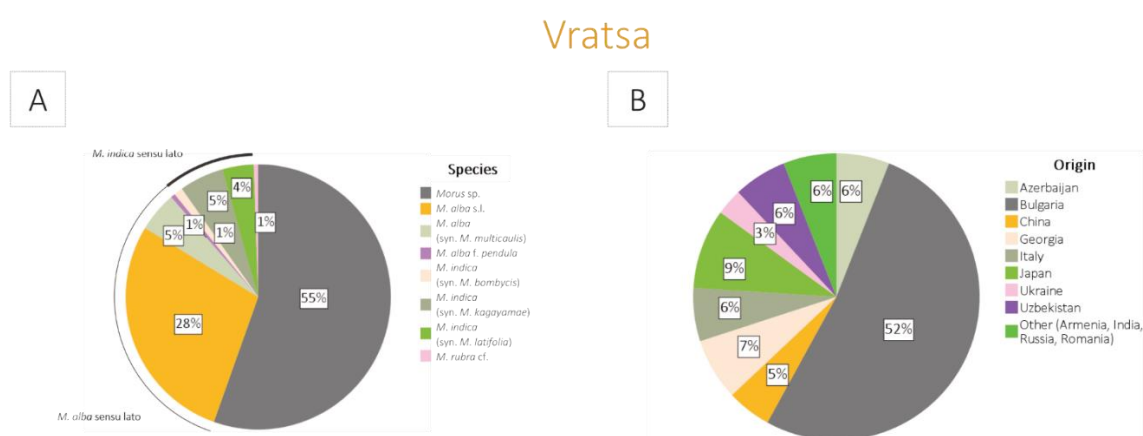
There are presently more than 190 mulberry accessions in the germplasm, maintained at SCS-Vratsa (Figure 9), but only 113 out of them have been studied in detail (Petkov et al., 1994). The list of accessions, taxonomical status, origin, propagation date and number of individuals is presented in Appendix II, Fehler! Verweisquelle konnte nicht gefunden werden..

The distribution of the mulberry accessions according to their species and geographical origin is presented in Figure 10. The collection comprises 106 accessions (55 % of the total), which were assigned to *Morus* sp., because they are hybrid selections of *M. alba* s.l. and *M. indica* s.l. with no clearly defined genetic background.



Of the 190 accessions in total, 54.28 % are of the ingroup *M. alba* s.l., which is widely spread in Bulgaria, along with nine accessions (5 %) which are, according to the traditional classification, evidenced under the synonymous *M. multicaulis*. One genotype of the *M. alba* s.l. ingroup is the weeping form of *M. alba* f. *pendula*. In addition, the collection comprises 19 varieties of the ingroup *M. indica* s.l., of which ten accessions (5 %) were, after traditional classification, regarded as the synonymous *M. kagayamae*, seven (4 %) as the synonymous *M. latifolia*, and two accessions were listed as the synonymous *M. bombycis*. Furthermore, *M. rubra* is represented by one accession (1 %).

100 accessions are selections of the SCS Vratsa breeding programme. 14 accessions are categorised as *M. alba* s.l., 84 genotypes are regarded as hybrids between *M. alba* and *M. indica* with no clearly defined genetic background (*Morus* sp.). ‘Vesletz’ is according to the traditional classification a variety regarded as the synonymous *M. latifolia*, whereas ‘Vratsa 1’ is regarded as the synonymous *M. kagayamae*.



**Figure 10** - The percentage of species and origin of *Morus* sp. maintained in the germplasm collection of SCS Vratsa.

The types of accessions that are prevailing are hybrids and vegetatively propagated plants obtained by rooting of woody shoots. Hybridisation (genetic recombination, mostly associated with dioecy - separated male and female individuals) is crucial in genetic breeding of mulberries. When we discuss the origin of individual clones, we have to assume that, from an evolutionary point of view, all clones originate from seeds and most seeds of mulberries originate from hybridization, which is enhanced by dioecy. Neglecting the small, almost



insignificant role of mutations, seed propagation is crucial in inducing genetic and thus phenotypical variation.

The largest number of the accessions are represented by vegetatively propagated clones obtained during the initial stages of mulberry selection. There are also 3 accessions, selected by the method of polyploidy, namely 'Kairyu' improved, 'Tadgikian' without seeds and 'Uzbekian'.

The data, shown in **Figure 10B** manifest that there are accessions from nearly all countries practicing sericulture in the temperate and sub-tropical belt like Italy, Russia, Georgia, Japan, Armenia, China, Azerbaijan, Uzbekistan, Egypt and Ukraine.

Bulgarian varieties are represented by 97 (52 %), 17 varieties (9 %) originate from Japan, 13 (7 %) from Georgia, 11 (6 %) from Italy, Uzbekistan and Azerbaidjan; 10 (5 %) from China, 5 (3 %) from Ukraine and 6 % from other countries.

Nine varieties have been recognized by the Bulgarian State Executive Agency for Variety Testing and allowed to be propagated for commercial use. These are 'Vratsa 1', 'Vesletz', 'Vratsa 18', 'N106', 'N24', 'Kinryu', 'Kokusou 20', 'Kokusou 27' and 'Tbilisuri'. The last introduction of exotic mulberry varieties was in 2004 from Azerbaijan.

### 3.3.3.2 Morphological characterisation of mulberry genetic resources in SCS-Vratsa

In the mulberry germplasm maintained at SCS Vratsa there are accessions from nearly all countries dealing with sericulture from the temperate and sub-tropical climatic zones and the germplasm is yearly screened for morphological and phenological parameters. In general, most of the varieties maintained have curved shoots with different degrees of the curvature. Regarding the phyllotaxy grade, most of the accessions have a 1/3 grade, but there are some accessions with grades 2/5 and 2/7. The distance between two neighbouring leaves on one shoot is called internodal distance and is not only a qualitative character but also a very important commercial trait determining the leaf yield by one tree or unit land. The value of this character is different in different parts of the shoot; therefore, it is measured at the shoot base part, middle part and top part. Most of the accessions had medium internodal distance (63%), followed by those with short internodal distance (31%). Most of the mulberry accessions maintained are female, followed by male and bisexual varieties. In most of the varieties the flowering has medium power.

70 mulberry varieties of different origins were included in the morphological study according to the ARACNE proposed descriptor database (**Appendix II, Supplementary Table 2**). Most of

them are Bulgarian varieties of local origin or selections of the breeding programme (24), introduced varieties that were included in the study are from Georgia (13), followed by those from Uzbekistan (12), from Azerbaijan (11), Ukraine (6), Armenia (4) and from Romania (1).

The cultivars belong to different mulberry taxonomic groups. The varieties of *M. alba* s.l. prevail by 35 accessions out of which 8 varieties were assigned *M. multicaulis*. *M. indica* s.l. is represented by 6 varieties, 5 varieties are labelled as *M. kagayamae* and one as *M. latifolia*. *M. rubra* cf. is represented by one variety ('Nerrosa'). So far 28 varieties are of undetermined species affiliation, as a result of selection of hybrids between *M. alba* × *M. indica*.

Except for C1 accession in which the leaves are lobed in the other varieties the leaves are simple, with single lobe in some varieties. The mulberry varieties studied are characterised by relatively large leaves. The size of the mulberry leaves is a basic economic and selection trait, as larger and heavier leaves determine higher leaf yield, and hence the efficiency of silkworm rearing.

The distribution of the mulberry varieties studied according to the values of their leaf ratio index, as well as the ratio of the leaf length to the width, revealed that the varieties with a medium leaf blade and an index between 1.3 and 1.5 prevail (52 varieties, 75 %). Three varieties (4%) have highly elongated leaves with an index above 1.6, namely 'Kutaturi', 'Vratsa 18' and 'Harkovska 3'. On the other hand, 15 varieties (21 %) are characterised by relatively broad heart-shaped leaves (**Figure 11A**).

The distribution of the mulberry varieties studied according to leaf size is shown in **Figure 11B**. There was a great variation in the length of the mulberry leaf among the varieties tested, from 11.32 cm in 'P 23' to 29.44 cm in 'Kamil tut'. Varieties with medium leaves between 20.02 cm and 22.91 cm prevail (28 varieties), followed by those between 22.92 cm and 25.82 cm (18 varieties) and those between 17.12 cm and 20.01 cm (12 varieties). Only 6 varieties are characterised by small leaves, less than 17 cm length. The tendency for a strong variation established for leaf length character, as well as a predominance of varieties with medium-wide leaves was observed. Varieties with medium-wide (from 13.96 to 19.96 cm) prevail (56 varieties). The width of the leaf varieties from 9.96 cm in 'P 7' to 22.12 cm in 'Kamil tut' with an average of 15.87 cm.

With regard to the length of the leaf petiole the following was found (**Figure 11C**): varieties with a long petiole prevail (50 varieties, 71 %), followed by those with a medium petiole (18 varieties, 26 %) and varieties (No. 24 and No. 26) with a short petiole (2 varieties, 3 %).

A strong variation in the shape of the leaf base morphological character was also found. Varieties with varying degrees of cordate base predominate (56 varieties, 80 %). 11 varieties are characterised by a retuse base (16 %), and only three have a truncate base (4 %) (**Figure**

11D). All the cultivars tested have an acute shape of their leaf apex. There is some variation of the leaf blade tip morphological character. The varieties with caudate leaf blade type prevailed (43 varieties, 66 %) compared to 27 varieties (37 %) with acuminate leaf tip type (Figure 11E).

A great diversity was reported in terms of leaf blade margin morphological character. The most varieties are characterised by crenate leaf blade margin (28 varieties, 39 %), followed by 25 varieties with serrate margin (37 %), and 12 varieties with biserrate leaf margin (17 %). Two varieties have aristate and dental leaf blade margin (3 %), and one variety, 'Azniish 7', is characterised by a serrulate leaf margin (Figure 11F). 84 % of leaves had glossy adaxial epidermis, whereas 16 % were characterised by matte surface (Figure 11H).

### 3.3.3.3 Phenological evaluation

There is a high variety-specific variation regarding the phenological stages, namely the beginning of 1<sup>st</sup> - 2<sup>nd</sup> leaf sprouting, 4<sup>th</sup> - 5<sup>th</sup> leaf sprouting and flowering. The lowest variation was shown by the phenological stages: beginning of bud swelling, fruit ripening, leaf yellowing and leaf fall. The necessary sum of the effective temperatures for mass bud swelling was 17.9 °C. The duration of the whole vegetation period varied from 206 days in *M. indica* (syn. *M. latifolia*) to 216 days in *M. alba* s.l.

According to Petkov (1998) the minimum temperature necessary for the beginning of mulberry vegetation in the region of Vratsa is 5 °C. The necessary sums of the effective temperatures for the varieties of *M. alba* are 160 °C for buds swelling, 236 °C for buds sprouting, 271 °C for 1<sup>st</sup> - 2<sup>nd</sup> leaves sprouting, 333 °C for 4<sup>th</sup> - 5<sup>th</sup> leaf sprouting, 343 °C for flowering, and 674 °C for fruit ripening. The duration of the whole vegetation period varied from 206 days in *M. indica* (syn. *M. latifolia*) to 216 days in *M. alba* s.l.

## Mulberry descriptors of SCS Vratsa germplasm



**Figure 11** - Evaluation of leaf morphology descriptors of the SCS Vratsa germplasm collection. A) Leaf shape. B) Leaf size ratio. C) Petiole size. D) Shape of leaf base. E) Leaf blade tip. F) Leaf blade margin. G) Hairiness. H) Glossiness.

### 3.3.3.4 Evaluation of the leaf yield and biochemical evaluation of leaves

Previously, leaf yield from individual trees, the proportion of leaves in the total vegetation yield, and the annual leaf yield per hectare in a mulberry garden, utilising 66 mulberry accessions from the genetic collection at SCS-Vratsa were studied (Petkov 1998). The research highlighted significant varietal differences in mulberry productivity. On average, each tree produced 6.21 kg of leaves, with yields ranging from 4.54 kg in the 'Kairyou Ichinose' variety to 8.11 kg in the 'Emin tut' variety. Most accessions yielded between 6 and 7 kg per tree (40%). Notably, *M. alba* and *M. multicaulis* exhibited the greatest variation in productivity.

An additional key factor affecting productivity was the percentage of leaves relative to the total vegetation yield. The study found significant deviations from the mean in 12 accessions, with varieties such as 'Tbilisuri', 'Georgia', 'Husan 1', 'Husan 39', 'Saniish 5', 'Shintso 2', and 'Siozisu' having leaf percentages above 50%. Variability was the highest in *M. alba*, *M. kagayamae*, and *M. multicaulis*, while *M. rubra* had a lower leaf percentage, and *M. bombycis* showed exceptionally high percentages.

The annual leaf yield per hectare in rainfed mulberry plantations revealed that 16 accessions had relatively low productivity (below 10 t/ha), while 7 accessions demonstrated significantly higher yields. Most accessions had yields ranging from 10.50 t to 12.00 t per hectare (62%). The leaf yield of some Bulgarian varieties is shown in **Appendix II, Supplementary Table 3**.

SCS Vratsa also explored the biochemical composition of mulberry leaves in 13 accessions from the gene bank (Petkov 1998). The study found that varieties 'Husan 5', 'Firudin tut', 'Husan 1', 'Kartli', and 'Digmuri' contained significantly less crude protein compared to the 'N 106' control variety. Conversely, the 'Uhvi', 'P 7', and 'P 9' varieties exhibited higher protein content. The dry matter content of the leaves varied from 27.12% to 30.27%, crude protein from 23.57% to 26.25%, crude fats from 3.05% to 4.77%, and crude fibres from 10.59% to 12.40%.

### 3.3.4 Evaluation of mulberry genetic resources in France

In France two mulberry germplasm collections were identified, namely the National Mediterranean Botanical Conservatory of Porquerolles and the Orchard-Conservatory of Mercoire (Gard).

The INRA (Institut National de la Recherche Agronomique) mulberry germplasm collection in France was a significant repository of genetic diversity for mulberry plants. It played an essential role in the conservation and study of mulberry varieties, contributing to research on genetics, breeding, and cultivation practices. However, in 2008, this collection was abandoned due to various reasons, such as resource constraints, changes in research priorities, and the reorganisation of agricultural research institutions in France.

Following the discontinuation of the INRA collection, a portion of the mulberry varieties were transferred and are now maintained at the CREA germplasm collection in Padua, Italy.

#### 3.3.4.1 The National Mediterranean Botanical Conservatory of Porquerolles (CBNMED)

Murier de Porquerolles, Conservatoire Botanique National Méditerranéen		
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The National Mediterranean Botanical Conservatory of Porquerolles was created in 1979. It is approved by the Ministry of the Environment. It is attached to the public administrative establishment of Port-Cros National Park which provides the support functions of the conservatory. It studies the flora across all continental Mediterranean regions, i.e. nine departments of Languedoc-Roussillon, Provence and Côte d'Azur. It also works transversally with other national botanical conservatories and the OFB.

Its first vocation was to enhance the agricultural plains of the island of Porquerolles, creating Provençal landscapes by planting orchards, which also act as a firebreak. From the 1980s onwards, the conservatory brought together old, forgotten varieties of Provençal fruit species on the island and established conservatory orchards of olive trees, fig trees and mulberry trees in order to safeguard all this cultivated biodiversity: a genetic heritage to be preserved for future generations. The Conservatory orchards serve as a support for scientific research.

The collection of Porquerolles maintains the following fruit trees:

- 250 varieties of fig trees
- 200 varieties of French and Mediterranean olive trees
- 90 oleasters
- 50 varieties of mulberries
- 50 varieties of date palms

Planting began as soon as the conservatory was created to have the best possible representation of the cultivated biodiversity of different varieties of the Mediterranean territory. They are managed by the conservatory in collaboration with Port-Cros National Park which ensures their maintenance. The objective of these collections is to contribute to the preservation of the identity heritage of the different territories of the French Mediterranean region and to have, for future generations, a gene bank.

These collections serve as support for scientific research. Thus, work on the genetic characterisation of the varieties was carried out in the collection of olive and fig trees, establishing for each their relationships with the basis of domestication and the origin of their selection (relationships between cultivars and wild parents). These collections are also valuable for studying adaptation capacities to climate change. The ClimOlivMed programme brings together scientific organizations such as INRAE, CEFE CNRS, CIRAD, INRA and the University of Morocco, the life traits of different olive tree varieties are studied for an approach on adaptation characteristics to climate change. The Porquerolles olive collection is at the centre of the system.

The 50 varieties of mulberry trees planted in Porquerolles originate mainly from old silkworm factories in the Var and the Cévennes. **Appendix V, Supplementary Table 8** represents the variety list along with the number of individuals. The ingroup of *M. alba* is represented by 28 varieties, 4 varieties are assigned as the synonymous *M. multicaulis*. The ingroup of *M. indica* is represented by 5 varieties, 2 are assigned as the synonymous *M. kagayamae*, two of them as the synonymous *M. latifolia* and one as the synonymous *M. rotundiloba*. Furthermore, the collection maintains 5 varieties of *M. nigra* and 9 different varieties of *M. rubra* cf. which could originate from simple or complex spontaneous and/or artificial crosses involving *M. alba*. Their genetic relationship is going to be analysed under the scope of ARACNE mulberry research.



### 3.3.4.2 Orchard-conservatory of Mercoire (Gard)

Orchard-conservatory of Mercoire (Gard)	Association Mercoire (Commune de Peyremale)
Curator:	Anya Cockle-Betian (Mercoire Association) <a href="mailto:anya.cockle@orange.fr">anya.cockle@orange.fr</a>
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Most of the trees in the orchard in Mercoire originate from the National Botanical Conservatory of Porquerolles (via Jean-Paul ROGER) and have been planted between 2002 and 2016.

The list of varieties maintained in the Orchard-conservatory Marcoire is shown in **Appendix V, Supplementary Table 10**. Out of 52 varieties, 39 belong to the *M. alba* s.l. ingroup and comprise sericultural varieties obtained from France, Italy, Japan, China. The garden also maintains the rare *M. boninensis* and 6 *M. nigra* trees, among them 2 ancient local trees 'Mercoire Haut' and 'Mercoire Bas', along with other varieties. Furthermore, three varieties belong to *M. rubra* cf. and one variety 'hybride de Saint-Christol' is of unknown taxonomical status.

The plant names originate from the labels provided by the nursery personnel, as commissioned by the National Botanical Conservatory of Porquerolles. However, the accuracy of some names remains uncertain. Varieties no longer present in the orchard represent trees that were initially planted but subsequently perished for various reasons. Two ancient *M. nigra* trees, known as 'Mercoire Haut' (Upper Mercoire) and 'Mercoire Bas' (Lower Mercoire), predate the establishment of the orchard. These venerable specimens, likely planted by the hamlet's original settlers over a century ago, stand near the current orchard boundaries—one nestled between two houses and the other situated just below the formerly cultivated terraces. Despite not being officially catalogued as part of the orchard, their historical significance and proximity suggest they should be recognised alongside the conserved orchard trees. A third ancient black mulberry once stood at the hamlet's opposite end but was removed in the 1990s during a property transfer.

In addition to the Mercoire orchard, the INRA orchard at Saint-Christol-les-Alès has been long neglected, though a few trees have survived. The Mercoire orchard curator obtained the original layout, which detailed the tree identities, from Bertrand Limier, who collaborated with Jean Fosset at INRA. Another significant source of mulberries was the Jardin d'Aristée, established by Jean Fosset, a retired INRA researcher from Saint-Christol-les-Alès. Fosset propagated several of INRA's mulberry varieties within his personal garden. Following

Fosset's passing, the Jardin d'Aristée was sold and is currently facing partial or complete destruction for residential development.

The potted mulberries are propagated from cuttings sourced from the Mercoire orchard and two other local collections in Saint-Christol-les-Alès—approximately 30 km north of Gard, France. These include the now abandoned and dilapidated former INRA orchard and the Jardin d'Aristée. The latter, once a vibrant testament to Fosset's dedication to mulberry conservation, is now threatened by the construction of a housing estate, marking the end of an era for these invaluable botanical resources.



**Figure 12** - The *M. nigra* de Mercoire Haut is one of two ancient black mulberry trees that date back to long before the creation of the orchard.

### 3.3.1 Evaluation of mulberry genetic resources in Greece

#### 3.3.1.1 University of Athens

University of Athens	Laboratory of Sericulture and Apiculture	
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Curator of the collection:	Dr. Antonios Tsagkarakis	
correspondence	Myrto Aglaia Stamouli	<a href="mailto:myrtostamouli@aia.gr">myrtostamouli@aia.gr</a>
Postal Address:	75 Lera Odos Street 118 55 Athens, Greece	

The Laboratory of Sericulture at the University of Athens plays a pivotal role in advancing the study of sericulture, the cultivation of silkworms for the production of silk. This institution has contributed significantly to research and education in sericulture, underpinned by its maintenance of two mulberry orchards that serve both as living laboratories for scientific investigation and as educational resources for students. The first of these orchards was established between 1990 and 1992, specifically to support doctoral research in sericulture. It includes three identified varieties of mulberry trees, along with several local varieties that are pending genetic identification. Experiments were conducted mainly in the 1990s, aimed at enhancing sericulture practices and understanding the biological and environmental factors that influence silk production. The list of varieties is represented in **Appendix VI, Supplementary Table 10**).

The second orchard dates back to the period between 1940 and 1960, and it primarily features *M. alba*, the white mulberry, which is widely recognized for its importance in sericulture. This orchard not only serves as a resource for breeding and cultivation studies but also plays a crucial role in the training and education of students in the field of sericulture. Efforts are currently underway to genetically identify the trees in this orchard, which will further enrich the research capabilities and educational offerings of the Laboratory of Sericulture. The Laboratory's scientific articles reflect a wide range of interests within the field of sericulture, including genetics, plant breeding, pest management, and the nutritional needs of silkworms. These publications contribute to the global body of knowledge on sericulture, offering insights into optimizing mulberry cultivation, improving silk yield and quality, and ensuring the sustainability of silk production practices.



### 3.3.1 Evaluation of mulberry genetic resources in Spain

Instituto Murciano de Investigación y Desarrollo Agrario y Medioambiental (IMIDA)		
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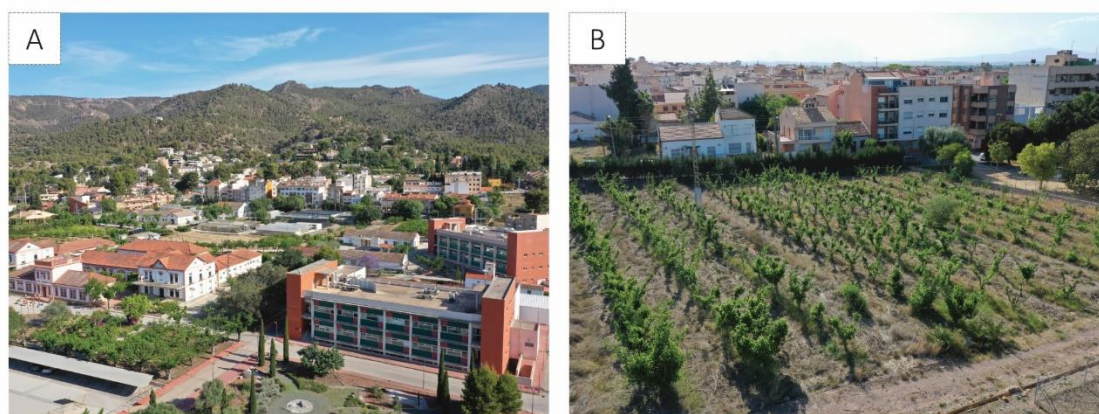


Figure 13 - Mulberry germplasm collection, IMIDA Murcia, Spain.

#### 3.3.1.1 General information about the conservation status of mulberry germplasm resources in Spain

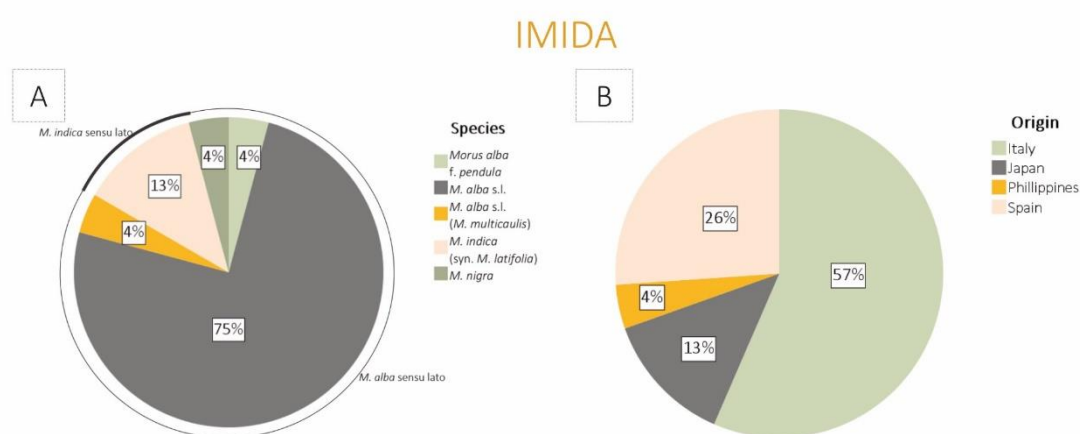
In Murcia, Spain, *M. nigra* has grown naturally since ancient times, before the Arabs arrived in the Iberian Peninsula. Until the 15th century, the whole silkworm rearing of this area was based on this species. In the 12th century, *M. alba*, of Asian origin, was introduced, and its cultivation began to displace *M. nigra* in the rearing of the silkworm.

In Murcia, Spain, diverse varieties of mulberry are preserved in the facilities of IMIDA (Institute of Agricultural and Environmental Research and Development of Murcia), at the old premises of the ancient Sericulture Station of Murcia (latitude 37.939636°, longitude -1.133487°). The collection is therefore located in an urbanised area, and consists of 24 varieties, including *M. nigra* and mainly *M. alba* varieties of Italian, Spanish (local) and Japanese origin, as detailed in **Supplementary Table 7**. The Italian accessions were received from the Experiment Sericulture Station of Padua around the 1920s, which is also the

approximate time of arrival of the Japanese varieties. Regrettably, precise information of the origin of the collection has been lost along the years.

The distribution of the mulberry accessions according to their species and geographical origin is presented in **Figure 14A**. The collection is represented by 24 varieties. Most of the mulberry varieties (20 accessions, 75 %) belong to *M. alba* s.l. Out of them, one variety was according to the former taxonomical classification revised with the synonymous name *M. multicaulis* 'Filippine'. One variety represents the weeping form *M. alba* f. *pendula*.

*M. indica* s.l. ingroup is represented by 3 varieties of 'Kokusou', namely Kokusou 20, 21 and 27. Furthermore, the collection maintains one local black mulberry variety (*M. nigra*). The data, shown in **Figure 14B**, manifest that most of accessions are from Italy, followed by Spain varieties and Japanese Kokusou varieties.



**Figure 14** - The percentage of A) species and B) origin of *Morus* sp. maintained in the germplasm collection of IMIDA Murcia.

### 3.3.1.2 Morphological characterisation of mulberry genetic resources at IMIDA

A comprehensive, contemporaneous, and systematic morphological characterisation of the accessions preserved in the IMIDA mulberry collection has never been carried out, due to the lack of technical staff involved in this task. Therefore, mulberry characteristics for the different varieties have not been recorded to date. Now, with the aim of ARACNE, cataloguing activity and the photographic documentation of descriptor traits of the accessions will be ongoing in the current and the next year.

In addition to the supply of leaves for silkworm rearing, the biotechnology team of IMIDA is carrying out diverse experimental activities with the aim of revaluing the cultivation of mulberries. In the last two years, an experimental evaluation of the fruits has been undertaken, whose valorisation is seen as an opportunity to relaunch this food as a new market and cultivation option for mulberry, in a region with optimal agricultural conditions like Murcia, as well as a secondary product of sericulture industry. Another area of activity is the evaluation of mulberry samples for their ability to produce bioactive compounds to be used in the biomedical, cosmetic, or nutraceutical fields. A varietal characteristic currently under study is the content of antioxidants and anti-inflammatory compounds of both mulberry leaves and fruits.

### 3.3.1 Evaluation of mulberry genetic resources in Georgia

Agricultural University of Georgia	Laboratory of Sericulture
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#### 3.3.1.1 General information about the conservation status of mulberry germplasm resources in Georgia

The history of sericulture in Georgia, a country in the Trans-Caucasian region, is deeply rooted and extensive. It traces back to the 5th century A.D., linked with King Vakhtang Gorgasali. By the mid-19th century, Georgia achieved a remarkable feat in sericulture, producing annually around 6.4-6.5 thousand tonnes of fresh cocoons.

Regarding mulberry cultivation, the sericultural sector faced a significant setback due to a mycoplasma mulberry disease (mulberry dwarf disease), which led to the destruction of 16 million mulberry trees. Despite efforts to rehabilitate mulberry plantations, sericulture in Georgia has encountered numerous challenges. By 1995, fresh cocoon production had plummeted by 98% compared to 1964. The decline was further exacerbated by the complete halt of production in silk factories, the widespread destruction of mulberry plantations, and the collapse of the silkworm egg production system.

The downturn in sericulture was primarily due to factors like the reduction in mulberry trees, the loss of traditional silk markets in Russia and the Baltic countries, challenges in exploring new markets, low-quality raw silk due to outdated reeling machines and technology, and inefficient silkworm egg production systems.

To revive and develop sericulture, Georgia relies on its 15-century-old sericultural traditions, favourable climate for producing high-quality bivoltine cocoons, over 4 million mulberry trees nationwide, and the potential to produce significant quantities of raw silk. Future strategies for revitalising mulberry cultivation include to propagate mulberry saplings resistant to dwarf leaf disease, transferring scientific knowledge and methods to control the disease, testing foreign mulberry varieties for disease resistance, early diagnostics for dwarf leaf disease, and implementing modern cocoon reeling technologies with high economic efficiency.

### 3.3.1.2 Mycoplasma-Like Organism – Causal Agent of Mulberry Dwarf Disease

Dwarf leaf disease was first discovered in Far East Asia, but has also affected plantations in Georgia, where the disease has been very well studied. Dwarf leaf disease was first discovered in 1963 on a small number of trees at the regional sericulture research centre in Kutaisi in western Georgia. By 1968, the disease had already spread to 20 provinces and then infected mulberry trees throughout western Georgia, destroying more than 10 million of the total 12.7 million trees. The rapid spread of the disease was helped by the fact that the mulberry variety 'Georgia' at the time appeared to be very susceptible to the disease. The disease affects the entire crown of the tree and is spread by infected seedlings or cuttings via the mulberry cicada *Hishimonus sellatus* Uhler.

The disease manifests itself in the following symptoms: The leaves become smaller, the infected leaves have an elongated and protruding shape, the leaves look wrinkled. The infected leaves turn pale green or yellow-green. The infected shoots have a shorter internode distance and are also much shorter than the healthy shoots. Sometimes both infected and healthy shoots can be observed on a tree. The symptoms of the disease are not present at the beginning of the growing season. The first symptoms appear in May on the uppermost leaves of the shoot. In severely infected trees, the shoots and branches dry out and later even the whole tree dries out. The disease significantly reduces the leaf yield of infected trees and the harvest of seedlings and young plants in the nurseries. Some mulberry varieties are very sensitive to the disease, but some are comparatively tolerant. On the tolerant varieties the symptoms of this disease appear to a much less extent, mainly as wrinkles on the leaf surface.

Measures to control the disease: 1. In the regions where the disease occurs for the first time, the infected trees must be eradicated and burnt. 2. The production of mulberry seedlings and cuttings in the infected regions must be prohibited. 3. Only mulberry varieties that are tolerant to the disease may be planted in the infected regions. 4. Earlier start of spring rearing and prohibition of summer-autumn rearing in the infected regions. 5. Insecticide treatment



of the trees and seedlings in the nurseries against the mulberry cicada 3 times a year (second half of May; July; September).

### 3.3.1.3 Current germplasm conservation status in Georgia

In fact, in Georgia the scientists comparatively quickly succeeded in finding mulberry varieties, naturally resistant to the dwarf leaf disease, thus the mulberry plantations were recovered by them in 1970s. In 2003 the most popular varieties in Georgia at the field level were the Georgian 'Imereti 90', 'Saamo 91', 'Racha 9', 'Racha 10', 'Egrisi', 'Novogruzinskii 2', the Japanese 'Oshima', 'Ichinose', 'Kayriou Rosou', 'Rokoko Jaso' and the Chinese 'Rosou'.

In 2003 about 4 million mulberry trees were available in Georgia. Nearly 50 % of them are of highly productive and thus good varieties which has been a very good achievement for the country. The growth form is middle to high stem form (1.5 – 2 m height). Most of the single trees are situated along the fences of the farmer's yards. The average leaf yield per one single tree is about 10-12 kg. Only about 20 % of the trees were planted as intensive plantations. It has been proved by Sericulture Institute that the most suitable type of plantation for Georgia is medium-height stem- 40-60 cm, 3/1.5 m and 2200 trees per ha. In each 4-5 years, one year is practised without picking mulberry leaves for autumn rearing for giving rest to the mulberry trees.

### 3.3.1.4 Characterisation of mulberry genetic resources in Georgia

The main characteristics of some high-yielding Georgian mulberry varieties are presented in **Table 5**. Variety with the highest leaf size and yield is 'Iverija', those of lowest is 'Gurija'.

**Table 5 - Characteristics of leaf size (cm) in leaf yield (ha/kg) of some Georgian mulberry varieties.**

Parameters	'Iverija'	'Gruzniish 4'	'Imeruli 1'	'Imeruli 2'	'Gurija 10'	'Gurija 20'	'Kolheti 85'	'Rioni'	'Digomskaja 125'	'Tbilisuri 2'
Leaf size(cm)	32X20	20X18	17X20	21X17	20X15	15X11	20X18	20X16	19X16	21X17
Leaf yield per ha in kg	12000	11000	9800	9800	6500	8000	7300	9300	9500	7500

## 4. Part B: Inventory of historical local mulberry trees in sericultural regions of the participating partners

### 4.1.1 Origin and distribution of *M. alba*

The origin of white mulberries in Europe is a complex historical narrative spanning hundreds of years. There is no precise information on when white mulberry was introduced into Europe and spread in cultivation. The mulberry species widespread in Europe until the Middle Ages was *M. nigra*, with *M. alba* making its appearance alongside sericulture in Italy between the ninth and twelfth centuries (Cappelozza, 2002).

Historically, it is believed that moriculture was introduced to Italy around the mid-12th century by King Ruggero II of Sicily. Silbermann 1870 reported on the tax on the mulberry leaves in Bologna in 1364. Further statement came from Pescia, where Francesco Bonvicino showed some mulberry plants, he had brought with him after his return from the Orient in 1434 (Cappelozza, 2002). Italy had a thriving silk industry from the 13th century onward, while France also developed its silk production, utilizing leaves of both black and predominantly white mulberry trees as silkworm feed (Masera, 1968).

In The United Kingdom, King James I (who reigned from 1603 to 1625) made efforts to establish a silk industry by urging the planting of mulberry trees, although it did not gain significant attractiveness, possibly due to the choice of black mulberries and the unsuitable climate. In Virginia (U.S.), white mulberries (which is not native of USA) thrived, but colonial silk production did not flourish (The Fruit of Broken Dreams, 2000; The Mulberry Tree, 2016; Time line of the mulberry in London, 2016).

Sericulture is believed to have been introduced in Bulgaria during the first Bulgarian Empire in the late 10th century AD, with records of extensive silk production in the Balkan Peninsula during the 12th century. In the 19th century, several million mulberry trees were planted in Bulgaria.

In Poland, the first documented instances of white mulberry cultivation date back to the 17th century, with growing interest in the species in the early 20th century, leading to the establishment of the Experimental Silk Station in Milanówek in 1924. Subsequently, white mulberries have become popular in schoolyards and workplaces, and the Polish variety 'Żółwińska wielkolistna' was developed in the 1950s. This historical account highlights the diverse and intricate journey of white mulberries in Europe, intertwining with the history of silk production and cultural exchanges (Blitek et. al, 2022).

#### 4.1.2 Origin and distribution of *M. nigra*

*M. nigra* originated in the Near East. It was known by the Greeks and Romans before the Christian era. It was also cultivated in ancient Egypt (Ahlawat et al. 2016). However, the exact origin of *M. nigra* is still unknown. Both literary and archaeo-botanical records indicate that *M. nigra* thrived in the Near East and in Europe, at least since the Iron Age and Roman times. This is much earlier than *M. alba*, which is native to China and was introduced into this area (together with silkworms) in late Byzantine times or even later (Browicz, 2000). For historians, there is still a controversy regarding the origin and time of the first introduction of mulberry in Italy. Most historians agree that *M. nigra* came to Italy from Persia (Cappelozza, 2000). The black mulberry was introduced to Europe and Britain more than 500 years ago and cultivated as food for silkworms. Although this venture did not prove successful, its fruit is still eaten in various forms today. It is cultivated for its fruits in southern Europe and southwest Asia and is the most important mulberry species in the Mediterranean countries (Hojjatpanah et al. 2011). *M. nigra* was rapidly adopted and cultivated for its fruits in the Mediterranean world and also for its pharmaceutical and cosmetic properties (Cappelozza, 2000). In antiquity, Pliny the Elder (77 AD) described the mulberry tree as 'sapientissima arborum', the wisest of trees (Naturalis Historia, book XVI, cap LXI, v 102). According to his findings its berries were frequently eaten and used by Greeks and Romans as ingredients for a delicious, sweet wine, called 'moretum'. In European medieval times black mulberries were still popular for making drinks, such as fruit juice, cider or wine, and for sweetening dishes as a sauce or syrup (Gelorini & Bourgeois, 2005). In the mid-eighteenth century, Carl Linneaus believed that *M. nigra* was indigenous to Southern Italy and had certainly been cultivated there for over 2000 years. In France and Spain, Archebotanist Marie-Pierre Ruas and her colleagues have presented evidence that the Romans introduced *M. nigra* as a fruit tree to southern France in 1st century BC and it began to be cultivated in Languedoc in the fourth century AD again for its fruit. The black mulberry also came to Britannia (Southern England), as preserved fruit (or seed) with the Romans in the second century AD. (Coles, 2022).

The black mulberry (Turkish name 'Kara Dut') is widely grown in Turkey (Yaltirik, 1982). Along with Mediterranean conditions, Northeast part of Turkey, in particular the Coruh valley has notable populations of black mulberry, which have been cultivated in gardens for their delicious edible fruits. (Ercisil&Orhan, 2008).

### 4.1.3 Mulberry cultivation

#### 4.1.3.1 History of mulberry cultivation in Italy

In Italy, *M. nigra* was the first species of mulberry introduced. *M. alba* arrived in Italy later, between the ninth and twelfth centuries, from the Far East. By 1364, the significance of mulberry cultivation was underscored by the imposition of a tax on mulberry leaves in Bologna, highlighting its economic importance. The oldest reliable testimony on the presence of *M. alba* in Italy came from Francesco Bonvicino in 1434 in Pescia, who had brought white mulberry plants from the Orient (Petrus, 1477; Silberman, 1870; Masera, 1968; Cappellozza, 2002).

Even at the end of the 15th century, mulberry-growing seems to have been scarcely practised in Lombardy, Liguria and Piedmont, while it had already taken hold in Veneto and Emilia-Romagna, in the inland areas of Tuscany, Marche and Umbria and, of course, in Sicily, Calabria and the area between Naples and Salerno. In the late Renaissance, mulberry cultivation was concentrated in hilly and foothill areas, with much rarer and sporadic presences in the lower plains and was almost completely absent from coastal or excessively humid areas (Battistini, 2003). Mulberries are mostly cultivated as isolated plants, placed near farmhouses or along boundary hedges, with only a few trees per hectare, which are perhaps allowed to grow to a much larger size than was usual in the 19th and 20th centuries.

During the 17th century the diffusion of mulberry trees and silkworm rearing continued to grow, becoming inextricably linked to promiscuous cultivation and partial contracts, and at the same time exports of raw silk to the major traffic centres and manufactures in northern Europe grew. The mulberry tree, however, remained mainly concentrated in hilly and foothill areas. In the 18th century, on the other hand, its spread accelerated, with an increasingly dense presence of this tree cultivation even in areas of damp plains and large estates. From this period onwards, the mulberry is increasingly found combined with vines in the planting rows.

The second half of the 18th century and above all the 19th century was the period of maximum expansion of mulberry cultivation, which expanded even in areas with little vocation such as irrigated areas, e.g. the Polesine, and saw the multiplication of plants on the dry plain farms. This process is accompanied by progressive changes in the cultivation of the plants, which are subjected to more intense pruning to limit their growth and make it easier to harvest leaves during the periods of faster growth of the worms, while new varieties are introduced that are more productive or considered more suitable than the traditional ones for damp and clayey soils.

#### 4.1.3.2 History of mulberry cultivation in Slovenia

The first attempts to promote the cultivation of mulberry trees in Slovenia date back to the 16<sup>th</sup> century, when sericulture was introduced to the Gorizia region from Friuli-Venezia. This influence has later spread throughout the former Austro-Hungarian Empire.

In the 17<sup>th</sup> century, mulberries were widely planted in Primorska region, especially in Koper and in Venetian Istria. The Dukes of Eggenberg significantly encouraged sericulture, promoted the weaving of silk fabrics and socks. In Primorska region, Henrik pl. Raigerfeld and Ivan Seifrid Count Herberstein tried to develop silk production.

In the Goriška region in 1716 Karel VI gave privileges to silk processors and manufacturers. Shortly after that, they opened four hand reeling mills in Gorica and three in Krmin. Thirty weavers of silk fabrics also worked in the country. Among the priority tasks was the planned expansion of mulberry plantations. The economic magistrate for Gorizia and Gradisca wanted to heighten the status of sericulture in the regions to the level it was in Italy and France. In the year 1756 all farmers were divided in three categories according to their land size and were ordered to plant mulberry tree saplings.

In 1723, the Carniola provincial estates decided to introduce sericulture and asked the government to issue a patent to encourage farmers in Carniola to start planting mulberries. A French wigmaker from Ljubljana, started manufacturing silk stockings in 1725. In order to become independent from the purchase of expensive silk from the Goriška region, he introduced mulberry trees and sericulture in Carniola. He planted them by the city moat in Ljubljana, near the city brickworks in Trnovo and on the castle hill, but this plantation was destroyed in the same year.

Among the attempts to plant more mulberries, it is worth highlighting the Ljubljana merchant Franc Perro, who in 1743 started planting larger nurseries in Ljubljana. He planted around 20,000 stronger trees in his own gardens in Tranča, on the meadows in Prule, Friškovec and near the city's brickworks. He also planted them in Lamberger's avenue (later Zois' garden). In Carniola, interest in sericulture grew in the 19<sup>th</sup> century. For these purposes, the Carniola agricultural association (Kranjska kmetijska družba) arranged a mulberry nursery on its property and sent many seedlings to Dolenjska, where landowners expanded mulberry plantations.

In 1740, the government recommended the planting of mulberries and the introduction of sericulture in Lower Styria (in the Savinjska region) following the example of Friuli. They granted the right to plant on all lands that were not used (*jus planti*). In Styria in 1764, the commercial consensus appointed Karl Josip Stehausen as supervisor of mulberry plantations.

He arranged a mulberry nursery in the village of Ješovec and gave away around 200,000 mulberries from it.

The French wars greatly exhausted sericultural activity and severely damaged the mulberry plantations. At the last retreat the troops cut down and burned many old mulberry trees. The wars were followed by an agricultural crisis as a result of falling grain, livestock and wine prices, which led to a resurgence of interest in sericulture. During this time, agricultural companies arranged nurseries with mulberries. In addition, exotic varieties were imported from Asia which included 'Morettiana' (obtained from seeds from India in 1780 in Milan, named after Moretti, the director of the botanical garden in Pavia) and 'Filippine' variety imported from the Philippines began to be cultivated. In the middle of the 19th century, there were around 2 million mulberry trees in Goriša region and Gradišče.

In 1843, the Sericultural Society was founded in Graz, Styria, which, among many other activities, organised a large tree nursery in Baiernhof, which sold the saplings to the Lower Styria. Large number of mulberry trees was planted in Lower Styria as well as Prekmurje region.

The history of sericulture in Styria is intricately connected with the fascinating story of mansion Novo Celje. Count Petazzi and Baron Wertenburg tried to expand mulberry plantations in Celje. In 1843, the owner embarked on a remarkable endeavour by planting an initial 13,000 mulberry trees at the mansion Plevna. The favourable conditions and robust growth of the existing plantations prompted the enlargement of the collection. Dr. Anton Perinello contributed to the plantation's diversity by acquiring mulberry trees of semi-low and medium cutting form from Lombardy and Rome. The entire plantation at Novo Celje comprised ten large plantations, with five following the Italian model.

In the spring of 1845, Novo Celje expanded their plantations to the Turnišče mansion at Ptuj, by importing 2,000 mulberry trees from Rome using Perinell's method. The plantation at the Turnišče mansion, signified a continued commitment to the further development of sericulture (Zimmermann, 2016).

Around 200,000 trees were counted along the line from Frohnleiten to Ljubljana in 1867, because the railway guards reared silkworms, but when the Austrian state sold the Southern Railway to French financiers, the railway workers were forbidden to cultivate silkworms and the mulberries were gradually cut down.

The decrease of sericulture was apparent from the third quarter of the 19th century onward. The First World War and the new boundaries with Italy were finally the causes of the decline of sericulture in Slovenia. Additionally, the low prices of cocoons and silk, which were not

economically acceptable for long-term silk production, led to the loss of interest in the silk industry.

After the Second World War, in the warmest regions of Slovenia, the promotion of sericulture was included in a five-year development plan, intending to promote the textile industry sector. The last rearers ended with the silkworm rearing at the beginning of 60-ies. Sericulture survived the longest in the plane between the Soča and Vipava rivers, on the so-called Goriška Ravna and the Lower Vipava Valley. Typical sericultural villages were Rupa, Peč, Sovodnje, Gabrje, Miren, Orehovlje, Bilje, Vrtojba, Šempeter, and partly Renče. The rearing season took place mainly in the 'dead time' before the seasons of haymaking and cereal harvesting.

#### 4.1.3.3 History of mulberry cultivation in Bulgaria

In Bulgaria, mulberry trees have traditionally been planted predominantly as tall, single-stem trees along roads in villages and towns. Many of the old local mulberry varieties, which can still be found today have small, lobed leaves, though some trees with larger, unlobed leaves also exist. These trees typically originate from seedlings produced through open pollination.

As of 1990, approximately 1.6 million local trees were recorded across the country. Currently, their number has decreased to about one million. In addition to these individual trees, several mulberry plantations have been established. These plantations usually contain about 3,330 trees per hectare, with a planting distance of 3 x 1 meters and a stem height of 0.6 meters. The first official introduction of mulberry varieties into Bulgaria occurred in 1930, with the importation of 12 varieties from Italy. In 1932, staff from the SCS-Vratsa undertook sampling excursions in the South-Eastern and Vratsa regions to identify promising local mulberry trees. This effort led to the collection and creation of numerous mulberry genotypes, resulting in the development of highly productive Bulgarian accessions such as 'No. 24', 'Vratsa 1', 'Vratsa 18', 'Vesletz', 'Trakia 6', among others. Today, there are 97 Bulgarian mulberry accessions preserved at the Scientific Center on Sericulture in Vratsa, ensuring the conservation and continuation of this valuable genetic heritage.

#### 4.1.3.4 History of mulberry cultivation in Spain

The beginnings of sericulture during the Arab rule were carried out by feeding the silkworms with mulberry leaves of *moral* or black mulberry (*M. nigra*). The progressive spread of sericulture to the rest of the country led to the cultivation of this species. In the 16th century, however, the white mulberry *M. alba* began to be cultivated in Valencia and Murcia. This has



a more tender and palatable leaf for the worms, and it is more nutritious. As a result, it produces better quality silk. The harder-leaved *M. nigra*, on the other hand, is a very resistant species that is well adapted to the altitude and aridity of the mountainous region of Las Alpujarras in Granada where it was grown. This led to a conflict, as the silk growers of the Alpujarras region, of Moorish origin (Muslims who converted to Christianity after the conquest of Granada in 1492), clung to the cultivation of the *moral*, as they considered its quality to be superior. The Christian authorities also defended the use of *moral* against the mulberry, issuing decrees banning it and ordering its uprooting. This led to the so-called 'mulberry conflict', which lasted throughout the century. In reality, however, this conflict over the quality of the silk was a measure to protect Granada silk from competition from cheaper, higher quality silk from Murcia and Valencia. Eventually, the cultivation of *M. alba* was imposed and *M. nigra* became marginal, being cultivated only on the island of La Palma in the Canary Islands until now.

We do not know exactly which varieties of mulberry were cultivated in the distant past. Over time, however, two types of mulberry trees have been selected as native to Spain. One is the 'Valenciana' type, predominant in Valencia, which is not a single genotype but a family of genotypes with different characteristics (*Valenciana* curly, *Valenciana* early). Another type that predominates in Murcia is the 'Cristiana', very palatable and adapted to semiarid conditions. After the creation of the Sericulture Station, these two types and their hybrids spread all over Spain, as one of its tasks was to maintain nurseries and distribute free seedlings to all growers who requested them. During decades, 20,000 seedlings a year were distributed.

The predominant method of silkworm rearing was integrated into an agricultural system of smallholders or tenant farmers working on very small plots of vegetable or cereal crops. Mulberry trees were planted on the edges of the plots or along the irrigation ditches to provide leaves for the rearing, which was carried out by the whole family in the spring. In Valencia and Murcia, this was the case in the systems known as *Huerta*, which were very fertile plains irrigated by the rivers Jucar and Segura respectively, with a hydraulic system that was very well developed by the Muslim population. A second method, more common in Murcia, was to plant mulberry trees in large mulberry groves owned by the nobility or the Church, who sold the leaves to the growers. Alternatively, the owners of the mulberry groves ('lords of the leaf') could provide a home for a farmer and his family and supply him with the eggs of the silkworms, the implements, and the leaf in exchange for two thirds of the harvest for the landlord.

#### 4.1.3.5 History of mulberry cultivation in France

The black mulberry (*M. nigra*) was probably introduced to France during the Roman period, with the sole purpose of consuming its fruit. The earliest mention of the white mulberry (*M. alba*) in France was found in the Corsican site of Ortole and dates to the end of the 14th century or the beginning of the 15th century. In fact, although mulberry cultivation developed in the Cévennes from 1296, according to the archives of notaries in the Languedoc, it is not clear whether white mulberry had already been introduced or whether the silkworms were fed with leaves of black mulberry. In the 15th century, the white mulberry was identified in Occitania, in Carcassonne (Languedoc) and it spread because of its resistance to cold and its hardiness (Durand et al. 2016)..

Olivier de Serres, recognized as the father of French agronomy, pioneered the method of planting mulberries during the reign of Henry IV in the 16th century. At Henry IV's behest, Olivier de Serres undertook the task of establishing large-scale silk production in France. This initiative aimed to retain the expenditures on fine fabrics within the country. Legend says that the mulberry saplings originated from a tree brought to France during the last Crusade, which thrived notably in the south and other regions. Some mulberry trees from this era, known as the "Sully" mulberries, still stand near Saint Hippolyte du Fort. These trees were often planted as boundary markers for plots of land, and there were strict prohibitions against cutting them down.

There is not much information about the varieties of mulberry that were planted but there were some that stood out, such as 'Sauvageon', 'Collombasse', 'Morettiana', 'Lhou' (of Chinese origin) and 'Tartarie' mulberry. The most productive département in terms of mulberry leaf production between 1924 and 1938 was Gard, with a peak of 37,000 tonnes in 1930. In the same year, 15,000 tonnes were produced in the Ardèche and 10,000 in the Drôme. In 1821, the 'Filippine' mulberry (*M. multicaulis*) was introduced from Philippines to France although it was actually native to China; this variety had the advantage of being able to grow quickly and sprouting earlier. For this reason this variety was very popular for several decades. Japanese varieties such as "Kokusou 21" were also introduced in the 20th century and are still used today.

#### 4.1.3.6 History of mulberry cultivation in Greece

The cultivation of mulberry trees in Greece traces its roots back to ancient times. The mulberry tree was first mentioned by Theophrastus, the ancient Greek philosopher and botanist (371–287 BC), who mentioned the black mulberry in his book 'Περὶ φυτῶν ἱστορία,'

(350-287 BC) highlighting the value of its wood and marking the mulberry's first recorded presence in Greek history.

Sericultural history takes a significant turn in 553-554 AD, during the Byzantine Empire under Emperor Justinian I, when monks introduced a silk-producing insect, likely *Bombyx mori*, from Syrinda (Σηρίνδα) to Byzantium. This event, is documented for its serendipitous introduction of silk production to the region and underscored the mulberry's crucial role as a food source for the silkworm, thereby elevating the tree's agricultural and economic importance.

Until the early 14<sup>th</sup> century, mulberry is mentioned in various texts under names like Συκαμηνιά and Συκαμιά, though without clear classification into black mulberry (*M. nigra*) or white mulberry (*M. alba*). This period reflects a continued recognition of the mulberry, albeit with a lingering ambiguity about its specific varieties.

The 18th century saw a more structured approach to mulberry cultivation, particularly in the island of Chios, then under Ottoman rule. Between 1760 and 1770, Joseph Guichard provided detailed accounts of mulberry planting for leaf production.

By 1846, new mulberry varieties were pursued beyond Greek borders with Stefanus Marcella's description of six mulberry varieties in France and the assertion that the best yield was achieved when mulberries were propagated by grafting, reflecting the growing scientific and agricultural knowledge of mulberry cultivation.

The year 1927 marked a milestone in the documentation of Greek mulberry varieties with the publication of a textbook on silkworm rearing. This work, detailing nine distinct varieties, from wild and semi-wild morphotypes with expressed lobation to those with simple heart-shaped leaves best suited for silkworm rearing and fruit production. Furthermore, sycamore mulberry classified as *M. alba* var. *platanifolia* as well as introduced high-yielding varieties from Japan and Italy ('Cattaneo') were mentioned.

The latter half of the 20th century to the present day has been characterised by an increase in the diversity of introduced mulberry varieties in Greece, driven by massive imports from Italy, Japan, China, and other countries. This introduction has transformed the mulberry landscape in Greece, with varieties now serving primarily as ornamentals in landscape planning within the urban and rural environment. In regions like Evros, the cultivation of mulberries for sericulture persists, blending traditional practices with modern varieties.

#### 4.1.3.7 History of mulberry cultivation in Georgia

The black mulberry has been widespread in Georgia since the middle of the Tertiary period. There is no precise information on when the white mulberry was introduced to Georgia. The

tradition of sericulture in Georgia is indeed ancient. Historical accounts suggest that it dates back to the 5th century of the reign of King Vakhtang Gorgasali, a seminal figure in Georgian history, known for founding Tbilisi, the capital of Georgia.

The white mulberry is characterised by its genotypic diversity. There are many small-leaved, fruiting endemic species that are considered to be the ancestors of modern varieties and are particularly valuable for the nutritiousness of the leaf and the flavour of the fruit.

Mulberry leaves were widely used for sericulture throughout the country until the plants became seriously infected by the dwarf leaf disease first detected in 1963 at the Kutaisi Regional Sericulture Experiment Station in western Georgia. By 1968, the disease had spread to 20 provinces and infected mulberry trees throughout the whole of western Georgia, destroying more than 10 million of the total 12.7 million trees. In fact, scientists in Georgia were relatively quick in suggesting to adopt new mulberry varieties that were resistant to the dwarf leaf disease, allowing mulberry plantations to be restored in 1970s.

In 2003, the most popular varieties in Georgia, at the field level, were those bred in the country: 'Imereti 90', 'Saamo 91', 'Racha 9', 'Racha 10', 'Egrisi', 'Novogruzinskii 2'; among the Japanese ones: 'Oshima', 'Ichinose', 'Kairiou Rosou', 'Rokoko Jaso' and the Japanese 'Rosou'. Additionally, there are local mulberry accessions in Georgia including 'Tbilisuri', 'Iveria', 'Kutaturi', 'Gruzniish-4', 'Hybrid-2', 'Imeruli-1', 'Imeruli-2', 'Guria-10', 'Guria-20', 'Kolkheti-85' and 'Nata', which are relatively resistant to the dwarf leaf disease.

In 2003, there were about 4 million mulberry trees in Georgia. Nearly 50% of them are highly productive varieties, which is a very good achievement for the country. The cultivation shape is medium to high stem (1.5-2 m height). Only about 20% of the trees have been planted as compact plantations. Most of the individual trees are situated near the fences of the farmers' yards.

## 4.2 Methodology

- Development of *MorusAPP*
- Sampling procedure of mulberry trees

The application – ***MorusAPP*** (MorusAPP, 2023) was created with the aim of identifying old mulberry (*Morus* sp.) varieties in the different European countries by entering visual observations of individual mulberry trees. In the application, taxonomic and phytogeographical information, accessibility and number of trees, tree growth habit, tree vigour, pruning practices, trunk shape, morphological characteristics of shoots, leaves and reproductive structures, and observations on diseases and pests will possibly be recorded.

The application is conceived to enable the editor to use it in a simple, transparent and at the same time comprehensive way. The application allows the user to enter and list specimens of mulberry trees found in the field throughout Europe. Supporting schematic and pictorial information helps the user choose which specific parameters to enter. Descriptors, which are designed to create a comprehensive representation of an individual specimen, are provided with images / schemes and remarks for each option. The user can also make decisions with the aid of prebuilt schemes with supporting images.

### ❖ 1 Mandatory descriptors to be entered in the application

1.1 Species; 1.2 Identification number; 1.3 Accessibility; 1.4 Geographical origin; 1.5 Number of individuals at the location; 1.6 Tree growth habit; 1.7 Pruning management; 1.8 Tree vigour

### ❖ 2 Non-mandatory descriptors

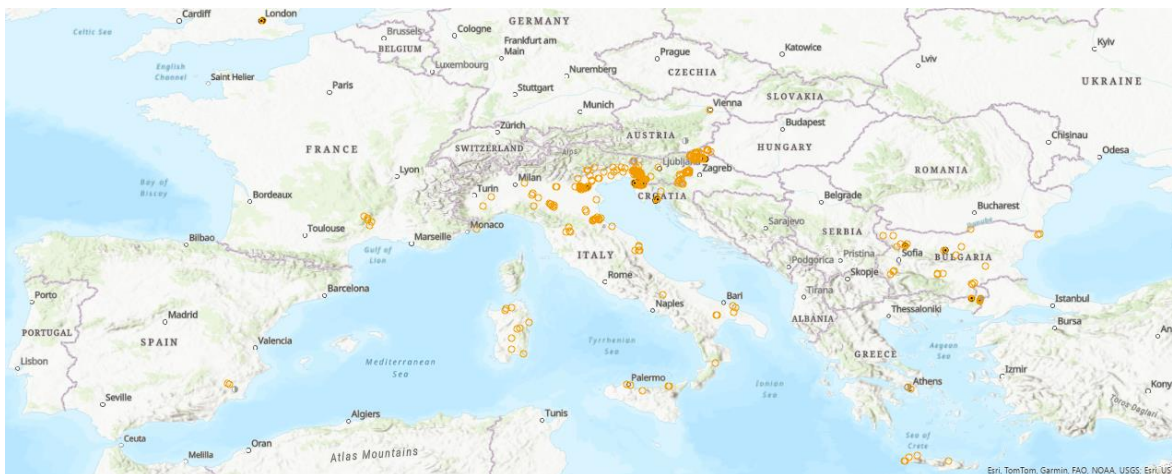
2.1 Varietal name; 2.2 Trunk circumference; 2.3 Tree growth habit; 2.4 Trunk, 2.4.1 Trunk colour, 2.4.2 Trunk irregularities / damage; 2.5 Shoots, 2.5.1 Colour of one-year-old shoot, 2.5.2 Lenticel density, 2.5.3 Lenticel shape, 2.5.4 Buds, 2.5.4.1 Shape, 2.5.4.2 Colour; 2.6 Leaves, 2.6.1 Phyllotaxis, 2.6.2 Leaf shape (lobation / heterophylly), 2.6.3 Leaf blade (ratio; length:width), 2.6.4 Petiole, 2.6.5 Shape of leaf base, 2.6.6 Shape of leaf apex, 2.6.7 Leaf blade tip, 2.6.8 Leaf blade margin, 2.6.9 Leaf hairiness (abaxial surface), 2.6.10 Leaf hairiness (adaxial surface); 2.7 Reproductive structures, 2.7.1 Sexual dimorphism, 2.7.2 Inflorescence shape, 2.7.3 Stigma persistency; 2.8 Infructescence, 2.8.1 Infructescence peduncle length; 2.8.2 Colour of infructescence; 2.8.3 Taste of infructescence, 2.8.4 Shape of infructescence, 2.8.5 Uniformity of infructescence ripening; 2.9 Diseases, 2.9.1 Fungal leaf spot, 2.9.2 Bacterial leaf spot/mulberry blight (*Pseudomonas syringae* pv. *mori*), 2.9.3 Soft rot (*Pectobacterium carotovorum*), 2.9.4 Ringspot virus; 2.10 Pests, 2.10.1 Mulberry moth (*Hyphantria cunea*), 2.10.2 Thrips (five species), 2.10.3 Scale insects (*Hemiptera*), 2.10.4

Mealy bugs (*Maconellicoccus hirsutus*), 2.10.5 Hairy caterpillar (*Spilarctia obliqua*), 2.10.6 Jassids (*Empoasca flavescens*).

Field excursions were performed to sericultural regions of ARACNE partners on clear days from the beginning of June to mid-November 2023. We recorded the exact GPS locations and collected leaf samples of local *M. alba* trees, with a stem diameter at breast height (CBH >300 cm) or, on pollarded trees, below the crown to define their approximate age. In addition to morphological evaluation a short shoot tip with young leaf preserved in a tube with silica gel was picked for further genetic analysis (see **Part C: Genetic analyses**).

## 4.3 Results on the inventory of mulberry trees in different European countries

### 4.3.1 General inventory report

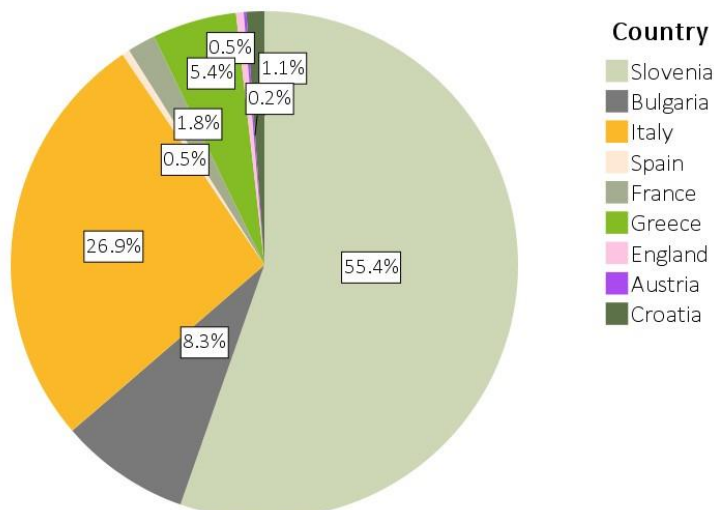


**Figure general\_1** - Mulberry distribution map. Trees were recorded in Austria, Croatia, France, Greece, Italy, Slovenia, Spain and the United Kingdom using *Morus*App. *M. alba* (orange circles), *M. nigra* (orange circles with black center), *Morus* sp. (half-filled grey circles)

Currently, **67 users from seven countries** (Italy, Slovenia, Spain, Greece, France, Bulgaria, Austria) are registered to the ***Morus*APP**. Silvia Cappellozza (CREA, Italy), Gianni Fila (CREA, Italy) and Andreja Urbanek Krajnc (UM, Slovenia) are designated superadministrators, 13 users act as administrators and other editors. See [MorusAPP](#) for updated numbers.

In 2023, a total of 614 mulberry trees from participating countries were recorded via the *Morus*APP (Fehler! Verweisquelle konnte nicht gefunden werden.). The majority of mulberry trees recorded (95 %, 586) were identified as *M. alba*, a minority of 4 % (23) as *M. nigra*. Five trees (1 %) were identified only at the genus level (*Morus* sp). More than half of *M. alba* were recorded in Slovenia (340, 55.4 %), followed by Italy (165, 26.9 %) and Bulgaria (8.3 %), Greece (33, 5.4 %), France (11, 1.8 %) Spain (3, 0.5 %) and 11 trees from other countries. 43 % of *M. nigra* was recorded in Slovenia, followed by Croatia (22 %) and Greece (13 %) see Fehler! Verweisquelle konnte nicht gefunden werden. and Fehler! Verweisquelle konnte nicht gefunden werden.A.





**Figure general\_2**– Percentage of *Morus* trees sampled in different countries using *MorusAPP*.

Fehler! Verweisquelle konnte nicht gefunden werden.**B** shows the accessibility options of mulberries. The majority were grown in private gardens (35 %), public areas (24 %), agricultural areas (17 %) and along streets (15 %). Only 6 % were found in botanical gardens or collections, and 3 % in squares. 72 % of mulberries grow individually, while 17 % reported mulberry locations were represented by mulberry rows and 11 % plantations (Fehler! Verweisquelle konnte nicht gefunden werden.**C**).

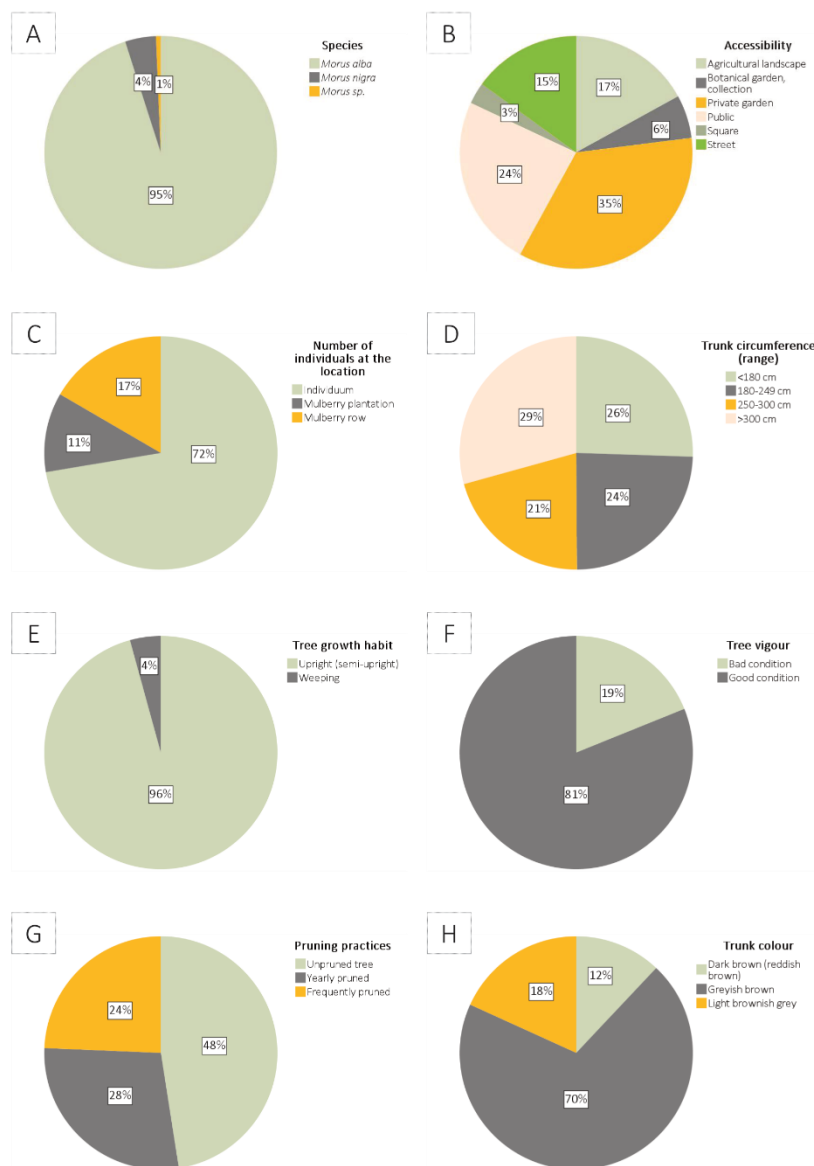
In the distribution of mulberry trees according to their circumference, one third of the trees (29 %) had a circumference of more than 300 cm, followed by trees with a circumference of less than 180 cm (26 %). 24 % of the trees had a circumference of 180 to 249 cm and 21 % of the trees had a circumference of 250 to 300 cm (Fehler! Verweisquelle konnte nicht gefunden werden.**D**).

The inventory of mulberries by tree growth habit indicated that almost all (96 %) of the recorded trees grew upright or semi-upright, while only one tree (4 %) had a weeping growth habit (Fehler! Verweisquelle konnte nicht gefunden werden.**E**). Eighty-one percent of mulberries were in good condition, while 19 % were in bad condition (Fehler! Verweisquelle konnte nicht gefunden werden.**F**).

Fehler! Verweisquelle konnte nicht gefunden werden.**G** shows pruning practices of the mulberries. Forty-eight percent of trees were unpruned, 28% were pruned yearly and 24% frequently. This means that half of the trees are pruned. The predominant trunk colour among the recorded mulberries, representing 70 %, was greyish-brown. Additionally, 18 %

showed a light brownish-grey trunk colour, while 12 % featured a dark brown (reddish-brown) trunk colour (Fehler! Verweisquelle konnte nicht gefunden werden.H).

### General inventarisation report



**Figure general\_3**— Basic information on the recorded mulberry trees from all countries (Austria, Bulgaria, Croatia, France, Greece, Italy, Spain, Slovenia, United Kingdom). A) Percentage of recorded *M. alba* and *M. nigra* trees. B) Accessibility options of trees. C) State on the number of individuals at location. D) Percentage of trees at certain trunk circumference range. E) Percentage of trees of upright and weeping growth habit. F) Percentage of trees in bad and good condition. G) Report on the pruning frequency of the trees. H) The percentage of trees of different trunk colours.—

### 4.3.2 General report on pest and disease

Besides collecting the taxonomic and phytogeographical information, accessibility and number of trees, tree growth habit, tree vigour, pruning practices, trunk shape, morphological characteristics of shoots, leaves and reproductive structures, we also performed observations on diseases and pests, which we report in general below.

During the sampling excursion we were focused on the presence of any leaf necrotic symptoms and bark lesions as, for an advanced determination of the disease cause, the symptoms must be observed under a microscope or further genetic analyses of infecting microorganisms are required.

Among bacterial diseases, bacterial blight caused by *Pseudomonas syringae* pv. *mori* (Pseudomonadaceae) is a common ailment of mulberry (Wulandari et al., 2018), commonly recorded during sampling excursions in various agroclimatic conditions around the world, including all participating countries. However, leaf necrosis can also be caused by other pathogens, such as *Pectobacterium carotovorum* (Enterobacteriaceae), ringspot virus (Secoviridae), and others. Bark lesions are typically associated with *Pseudomonas* disease, whereas, in the case of leaf necrosis, differentiation can be challenging. Morphological and genetic analyses confirmed the presence of two pathogenic fungi, namely *Epicoccum nigrum* (Didymellaceae) and *Alternaria alternata* (Pleosporaceae), on mulberry trees in Slovenia (Sever 2019). Another common fungal disease, *Phloeospora maculans* (Mycosphaerellaceae), causes small dark brown dead spots about the size of a pinhead to develop, surrounded by a halo of light green to yellow tissue. As the symptoms of fungal diseases are quite similar and not easily determined during sampling, our observations focused on documenting the occurrence of leaf necrosis and bark lesions, rather than attempting to identify the specific type of disease.

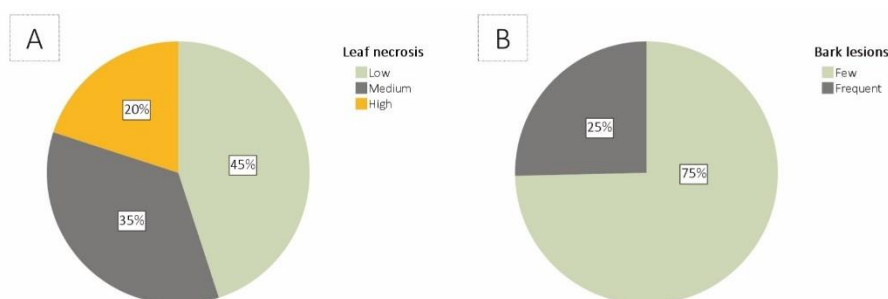
Furthermore, there is a problem with the mulberry yellow dwarf disease (MYD, *Candidatus Phytoplasma asteris*) in Georgia. Dwarf leaf disease was first discovered in 1963 on a small number of trees at the regional sericulture research centre in Kutaisi in western Georgia. By 1968, the disease had already spread. The disease affects the entire crown of the tree and is spread by infected seedlings or cuttings via the mulberry cicada *Hishimonus sellatus* (Cicadellidae). The disease manifests itself in the form of smaller leaves that are elongated and protruding; the leaves look wrinkled (Luo et al. 2022).

The most common pest observed on mulberry trees is *Maconellicoccus hirsutus* (Pseudococcidae – Pink Mealybug), which affects the bark of younger trees. In Greece, a severe infestation of *Xylotrechus chinensis* has been reported, affecting the stems of

mulberries, particularly those with a diameter > 15 cm. *X. chinensis* (Cerambycidae – Tiger Longicorn Beetle) is an Asian wood borer that has recently been found in two EPPO countries (Spain, Greece), causing the mortality of *Morus* trees. In its area of origin, other reported hosts include *Malus* spp. (apple), *Pyrus* spp. (pear), and *Vitis vinifera* (grapevine). Considering the importance of the host plants in the EPPO region and their recent introduction in several places almost simultaneously, the EPPO Secretariat considered that *X. chinensis* should be added to the EPPO Alert List (Eppo, 2024)

In Hungary we faced an infestation with the American moth, *Hypanthria cunea* (Arctiidae) which is native to North America, where it is widespread. It was introduced into Europe (Hungary, then Austria and Yugoslavia) immediately after the Second World War and has since spread further CABI/EPPO (1998 No. 79) (Eppo, 2024).

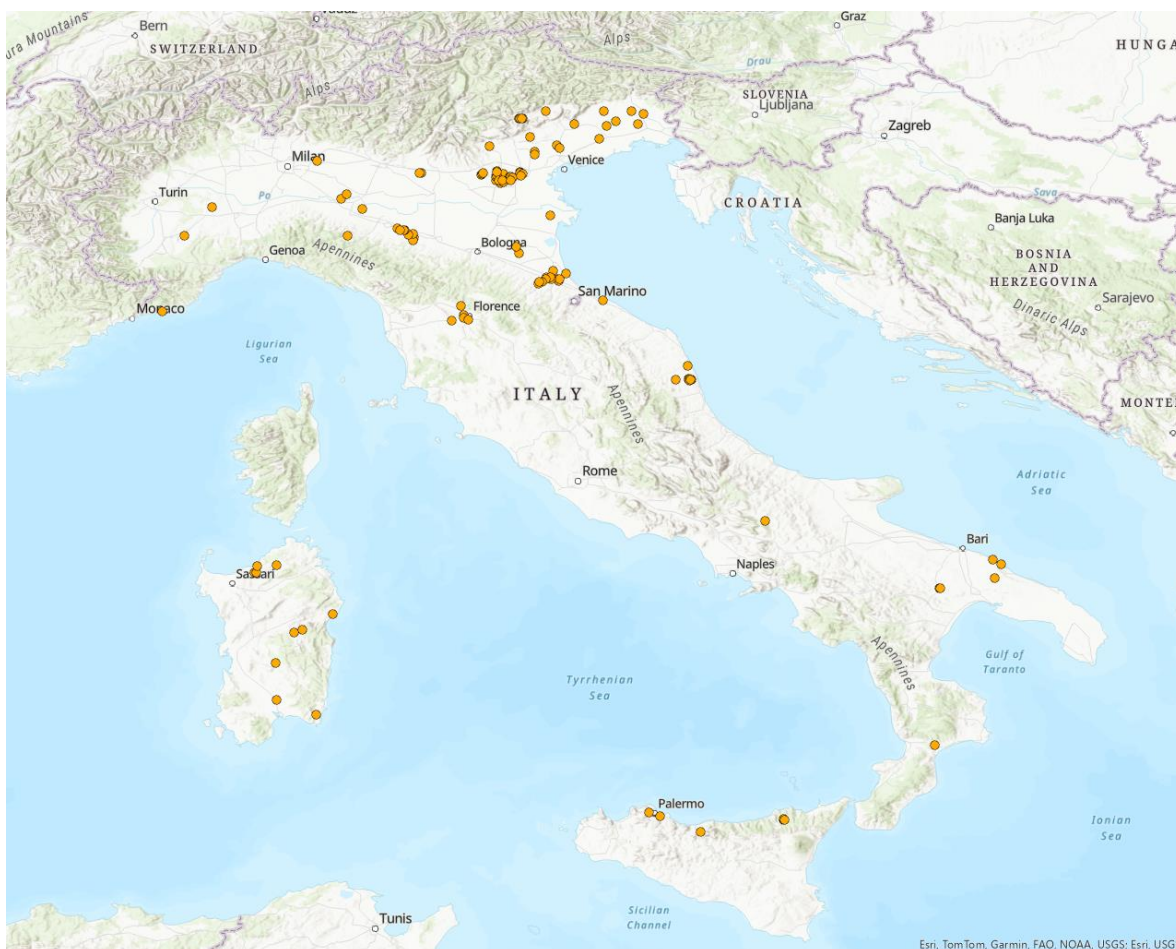
Fehler! Verweisquelle konnte nicht gefunden werden. **A** shows the incidence of leaf necrosis among a total of 119 observed mulberries from all countries (Austria, Bulgaria, Croatia, France, Greece, Italy, Spain, Slovenia, UK). The majority (45 %) of leaves exhibited low leaf necrosis, while 35 % displayed medium necrosis, and 20 % showed a high frequency of leaf necrosis. **Figure general\_ 4B** shows the frequency of bark lesions in 67 observed mulberries from all countries (Austria, Bulgaria, France, Greece, Italy, Spain, Slovenia, UK). The majority, comprising 75 %, exhibited few bark lesions, while 25 % displayed a frequent occurrence of bark lesions.



**Figure general\_ 4-** Inventory of mulberries from all countries (Austria, Bulgaria, Croatia, France, Greece, Italy, Spain, Slovenia, UK). Diseases. A) Leaf necrotic spots. B) Bark lesions.

### 4.3.3 Report on the inventory of mulberries in Italy

#### 4.3.3.1 Basic Information



**Figure IT\_1** - Distribution map of mulberries in Italy using *MorusAPP*

In 2023, a total of 165 mulberry trees from Italy were recorded via the *MorusAPP* (**Figure IT\_1**). Inventory of Italian mulberries (**Figure IT\_ 2**) by species indicates that a majority of recorded trees were white mulberries (*M. alba*) with a total of 163 (98.8 %), while there was only 1 (0.6 %) black mulberry (*M. nigra*) and one (0.6 %) undefined *Morus* sp. (**Figure IT\_ 2A**). Compared to other countries, Italy recorded the second highest number of black mulberry (**Figure IT\_ 2A**).

**Figure IT\_ 2B** shows the accessibility options of mulberries recorded in Italy. The majority were grown in private gardens (30 %). The second most common were mulberries in public

areas (21 %), and the third most common were mulberries in agricultural areas and along streets (15 %), while 12 % were found in botanical gardens or collections, and only 7 % in squares. Half of the mulberries in Italy grew individually (52 %), while we recorded one third (33 %) in mulberry rows and 15 % in plantations (**Figure IT\_ 2C**).

The distribution of mulberries based on circumference was predominated by more than half by trees (54 %) featuring a very large circumference, exceeding 300 cm, followed by trees ranging from 250 to 300 cm (22 %), trees ranging from 180 to 249 cm circumference (18 %) and those with a circumference less than 180 cm (6%) (**Figure IT\_ 2D**).

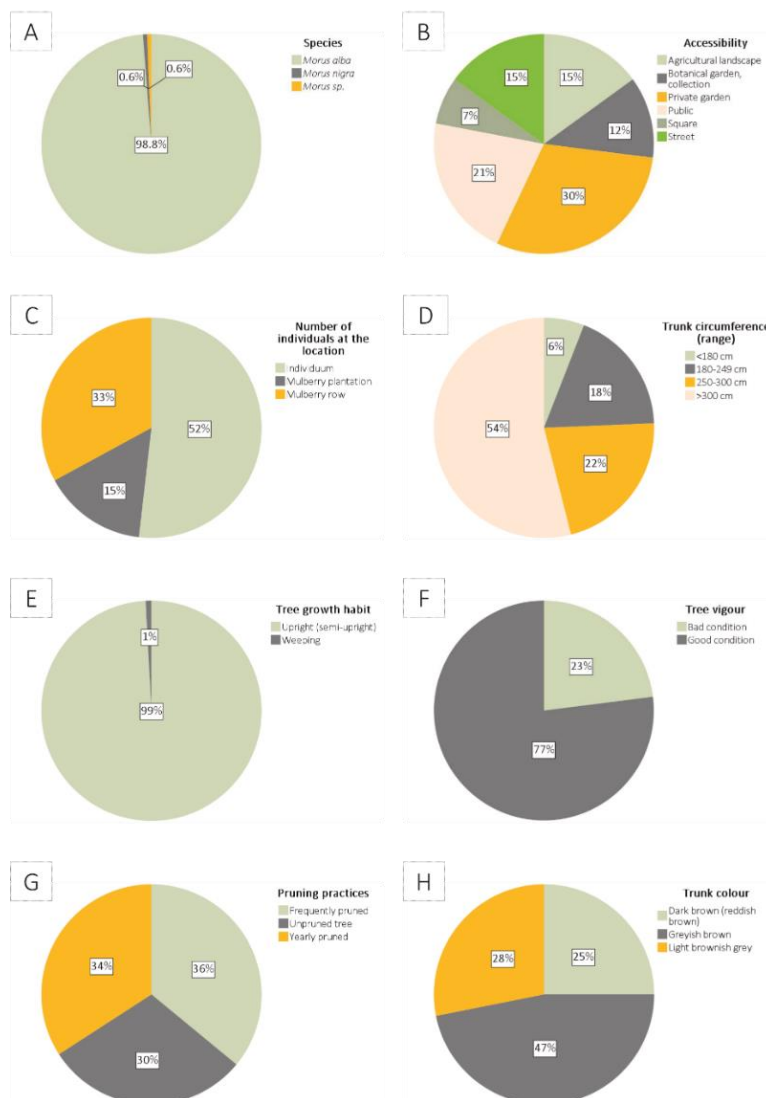
The inventory of mulberries by tree growth habit indicated that almost all (99 %) of the recorded trees grew upright or semi-upright, while only one tree (1 %) had a weeping growth habit (**Figure IT\_ 2E**). Seventy-seven percent of mulberries recorded in Italy were in good condition, only a minority of trees (23 %) were in bad condition (**Figure IT\_ 2F**).

Figure **Figure IT\_ 2G** shows the pruning practices of the mulberries recorded in Italy. Pruning practices were distributed fairly equal between frequently pruned (36%), yearly pruned (34 %) and unpruned (30 %) trees. This means, a significant portion, comprising more than two thirds of the trees is getting pruned.

The predominant trunk colour among the recorded mulberries, representing 47 %, was greyish-brown. Additionally, 28 % showed a light brownish-grey trunk colour, while 25 % featured a dark brown (reddish-brown) trunk colour (**Figure IT\_ 2H**).



## Inventarisation of mulberries in Italy

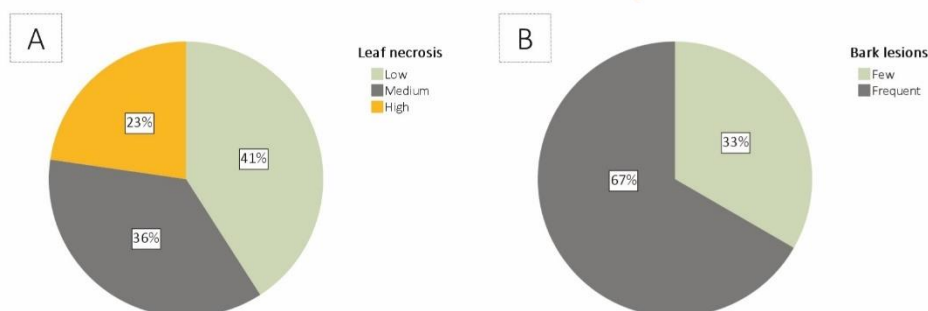


**Figure IT\_2** - Basic information on the inventory of mulberry trees in Italy. A) Percentage of recorded *M. alba* and *M. nigra* trees. B) Accessibility options of trees. C) State on the number of individuals at location. D) Percentage of trees at certain trunk circumference range. E) Percentage of trees of upright and weeping growth habit. F) Percentage of trees in bad and good condition. G) Report on the pruning frequency of the trees. H) The percentage of trees of different trunk colours.



#### 4.3.3.2 Report on mulberry diseases in Italy

**Figure IT\_3A** shows the incidence of leaf necrosis among a total of 22 observed mulberries. The distribution of leaf necrosis occurrence is nearly even; 41 % had low, 36 % had medium, and 23 % had a high leaf necrosis occurrence. In **Figure IT\_3B**, the frequency of bark lesions in six observed mulberries is depicted. The majority, comprising 67 %, exhibited frequent bark lesions, while 33 % displayed a few occurrences of bark lesions.



**Figure IT\_3** - Inventory of mulberries in Italy. Diseases. A) Leaf necrotic spots. B) Bark lesions.

#### 4.3.3.3 Monumental mulberry trees of Italy

In Italy, 83 trees with a circumference of more than 300 cm were recorded. The inventory also revealed that ten trees have a circumference of between 500 and 600 cm, followed by 53 trees with a circumference of between 300 and 500 cm.

The highest density of mulberry trees was found in the north-east Italian provinces of Veneto, Friuli-Venezia Giulia and Emilia-Romagna. Other trees were recorded in Toscana, Marche, Puglia, Calabria and Liguria.

In Sicilia, 5 trees from the Palermo region and 5 trees from Tortorici with a circumference of less than 300 cm were monitored. In Sardegna, 4 trees were reported in the northern part and 2 in the south of the island. **IT23\_00724** is the largest tree in central Sardegna near Laconi with a circumference of more than 318 cm.

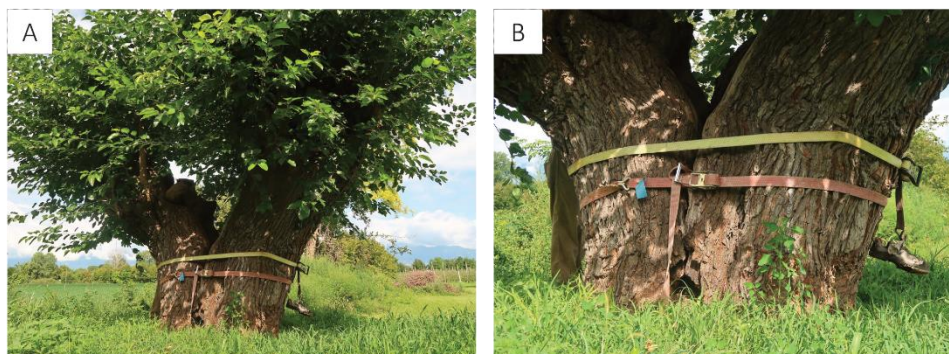
The trees with the largest circumference are the monumental trees of the private plantation of Mauro Rizzotti, a dedicated farmer who rediscovered centuries-old mulberry trees after many years of neglect and replanted them in the plantation in Vivaro (province of Pordenone). This collection represents a sort of Noah's Arch of mulberry trees in terms of genetic treasure. Mauro Rizzotti's mulberry tree collection maintains more than 700 mulberry trees that are between 70 and 400 years old (Benedetti & Fila, 2023). A tree called

'The God's Hand' has a circumference of 880 cm (IT23\_00443). Another tree called 'The King' (IT23\_00442) has a circumference of 700 cm, while the 'Napoleon Tree' (IT23\_00439) has a circumference of 560 cm. The collection maintains further 10 trees with a circumference of more than 300 cm, which originate from different locations and have been transplanted into the plantation. 'Petrolio' has a circumference of 500 cm and comes from Malavicina. 'Nunzio' is 470 cm in circumference and comes from Resegaferro. 'Dario' has a circumference of 440 cm and comes from Valeggio sul Mincio.

In addition, a monumental tree with a circumference of 880 cm was found in the mulberry plantation in Thiene (IT23\_00444). Another monumental tree (IT23\_00725) can be found in the garden of the Corte Bottegal company in Lonigo. It has a circumference of 570 cm and was planted before 1735.

Some magnificent rows are shown in the Figures below. IT23\_00541 shows a row of mulberries in Montefiore dell' Aso with trees of 320 cm in circumference. IT23\_00688 shows a row of trees in Albinea with a circumference of 370 cm. IT23\_00690 represents a plantation in Codemondo with trees of 270 cm in circumference.

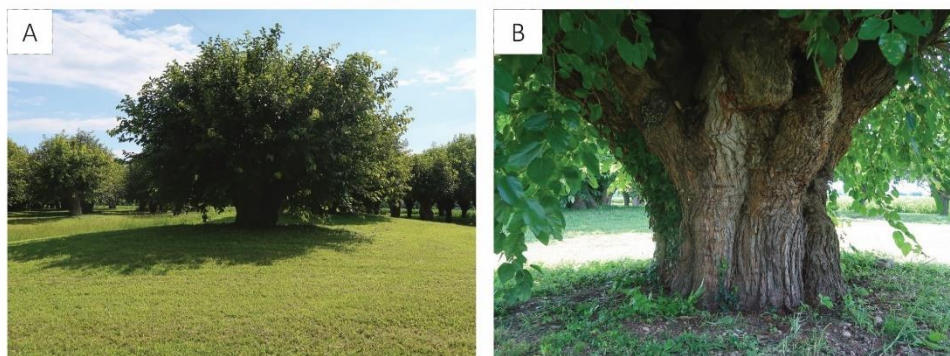
## IT23\_00439 "Napoleon"



**Figure IT23\_00439:** A) Tree habitus; B) Trunk aspect.

Basic descriptors	Species: <i>Morus alba</i> L.
	Location: Italy, Vivaro
	GPS coordinates: 46.091065,12.783650
	Accessibility: Botanical garden, collection
	Number of individuals at the location: Mulberry plantation
Trunk	Trunk circumference (cm): 590 cm
	Tree growth habit: Mulberry plantation
	Tree vigour: Good condition
	Pruning practices: Yearly pruned
	Trunk colour: Light brownish grey
Disease	Trunk irregularities/damage: Split
	Leaf necrotic spots: /
	Bark lesions: /

## IT23\_00442 "The King"



**Figure IT23\_00442:** A) Tree habitus; B) Trunk aspect.

### Basic descriptors

Species: *Morus alba* L.  
 Location: Italy, Vivaro  
 GPS coordinates: 46.092659, 12.784179  
 Accessibility: Botanical garden, collection  
 Number of individuals at the location: Mulberry plantation  
 Trunk circumference (cm): 700 cm  
 Tree growth habit: Upright (semi-upright)  
 Tree vigour: Good condition  
 Pruning practices: Yearly pruned

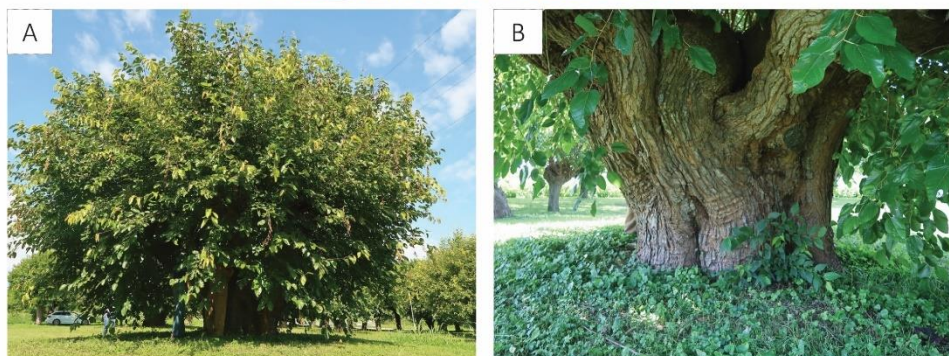
### Trunk

Trunk colour: Light brownish grey  
 Trunk irregularities/damage: Curved

### Disease

Leaf necrotic spots: /  
 Bark lesions: /

### IT23\_00443 "The God's hand"



**Figure IT23\_00443:** A) Tree habitus; B) Trunk aspect.

#### Basic descriptors

Species: *Morus alba* L.  
 Location: Italy, Vivaro  
 GPS coordinates: 46.092689, 12.784199  
 Accessibility: Botanical garden, collection  
 Number of individuals at the location: Mulberry plantation  
 Trunk circumference (cm): 877 cm  
 Tree growth habit: Upright (semi-upright)  
 Tree vigour: Good condition  
 Pruning practices: Yearly pruned

#### Trunk

Trunk colour: Light brownish grey  
 Trunk irregularities/damage: Curved

#### Disease

Leaf necrotic spots: /  
 Bark lesions: /



## IT23\_00444



**Figure IT23\_00444:** A) Tree habitus; B) Trunk aspect.

Basic descriptors	Species: <i>Morus alba</i> L.
	Location: Italy, Thiene
	GPS coordinates: 45.692401, 11.4839019
	Accessibility: Botanical garden, collection
	Number of individuals at the location: Mulberry plantation
Trunk	Trunk circumference (cm): 880 cm
	Tree growth habit: Upright (semi-upright)
	Tree vigour: Good condition
	Pruning practices: Yearly pruned
	Trunk colour: Light brownish grey
Disease	Trunk irregularities/damage: Split
	Leaf necrotic spots: /
	Bark lesions: /

## IT23\_00541

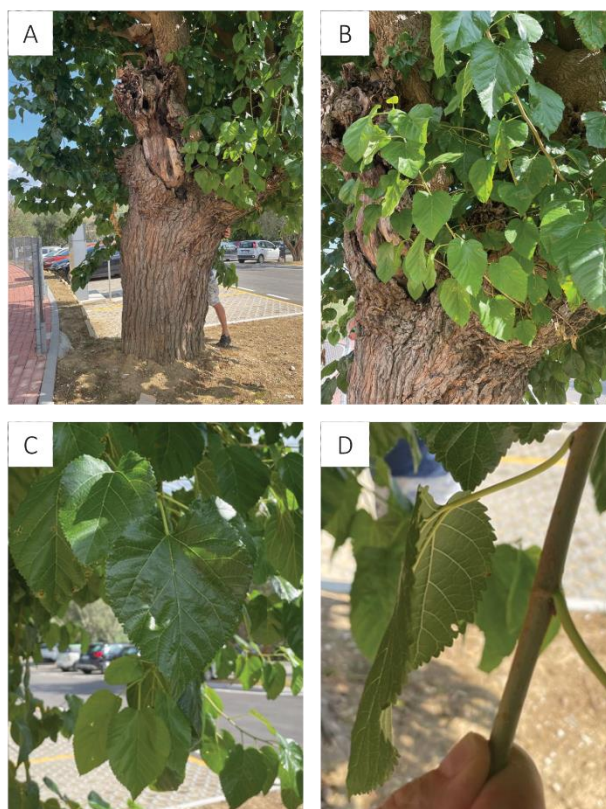


**Figure IT23\_00541:** A) Tree habitus; B) Trunk aspect; C) Simple leaf adaxial; D) One-year old shoot.

Basic descriptors	Species: <i>Morus alba</i> L. Location: Italy, Montefiore dell'Aso GPS coordinates: 43.03560376, 13.75875976 Accessibility: Private garden Number of individuals at the location: Mulberry row Trunk circumference (cm): 320 cm Tree growth habit: Upright (semi-upright) Tree vigour: Good condition Pruning practices: Frequently pruned
Trunk	Trunk colour: Greyish brown Trunk irregularities/damage: /
Leaves	Phyllotaxis: Predominantly alternate spiral Leaf shape: Simple Leaf blade: Medium (1.3-1.5) Petiole range: Long (41-70mm) Shape of leaf base: Cordate Shape of leaf apex: Acute Leaf blade tip: Acuminate Leaf blade margin: Crenate



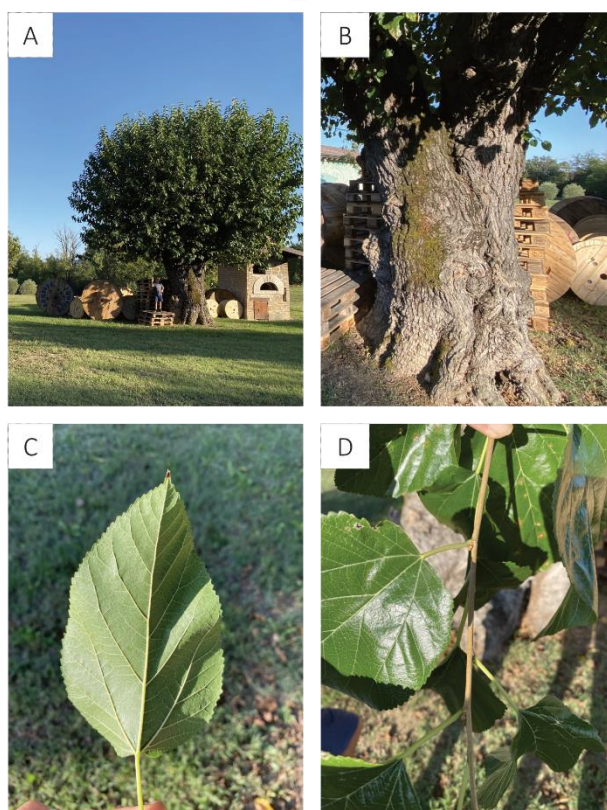
## IT23\_00565



**Figure IT23\_00565:** A) Tree habitus; B) Trunk aspect; C) Simple leaf adaxial; D) One-year old shoot.

Basic descriptors	Species: <i>Morus alba</i> L. Location: Italy, Gradara GPS coordinates: 43.94080937, 12.7727928 Accessibility: Public Number of individuals at the location: Individuum Trunk circumference (cm): 400 cm Tree growth habit: Upright (semi-upright) Tree vigour: Good condition Pruning practices: Frequently pruned
Trunk	Trunk colour: Light brownish grey Trunk irregularities/damage: /
Leaves	Phyllotaxis: Predominantly alternate spiral Leaf shape: Simple Leaf blade: Medium (1.3-1.5) Petiole range: Long (41-70mm) Shape of leaf base: Cordate Shape of leaf apex: Acute Leaf blade tip: Acuminate Leaf blade margin: Serrate

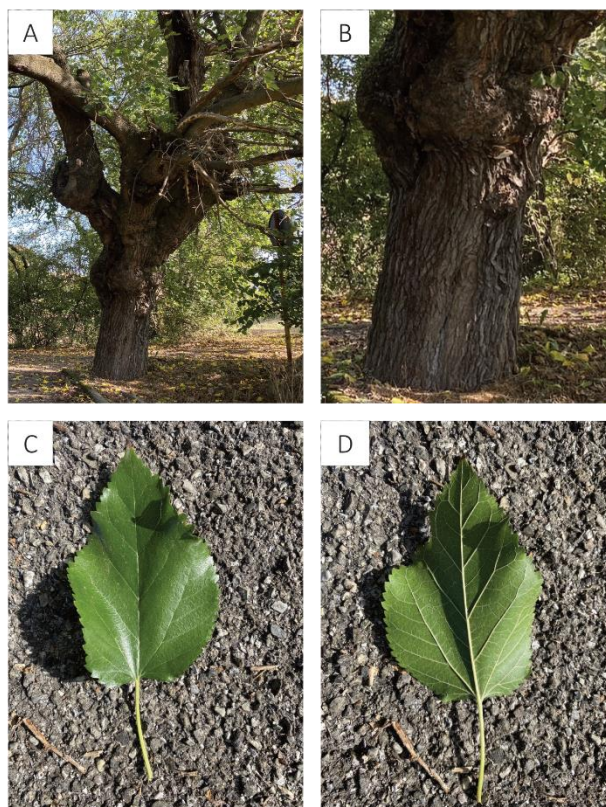
# IT23\_00688



**Figure IT23\_00688:** A) Tree habitus; B) Trunk aspect; C) Simple leaf abaxial; D) One-year old shoot.

Basic descriptors	<p>Species: <i>Morus alba</i> L.</p> <p>Location: Italy, Albinea</p> <p>GPS coordinates: 44.6270708480, 10.61205834407</p> <p>Accessibility: Private garden</p> <p>Number of individuals at the location: Mulberry row</p> <p>Trunk circumference (cm): 370 cm</p> <p>Tree growth habit: Upright (semi-upright)</p> <p>Tree vigour: Good condition</p> <p>Pruning practices: Frequently pruned</p>
Trunk	<p>Trunk colour: Greyish brown</p> <p>Trunk irregularities/damage: Curved</p>
Leaves	<p>Phyllotaxis: Predominantly alternate distichous</p> <p>Leaf shape: Simple</p> <p>Leaf blade: High (&gt;1.6, long leaves)</p> <p>Petiole range: Medium (21-40mm)</p> <p>Shape of leaf base: Truncate</p> <p>Shape of leaf apex: Acute</p> <p>Leaf blade tip: Acuminate</p> <p>Leaf blade margin: Serrate</p>

## IT23\_00690

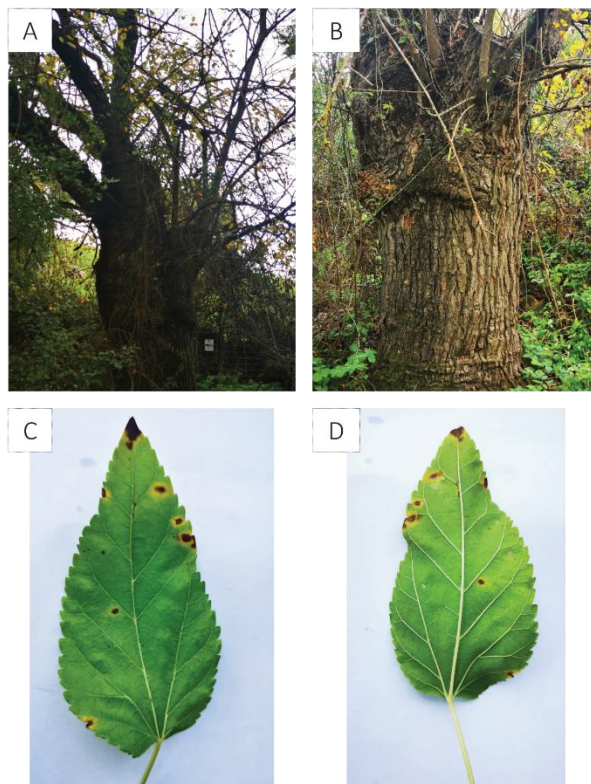


**Figure IT23\_00690:** A) Tree habitus; B) Trunk aspect; C) Simple leaf adaxial; D) Simple leaf abaxial.

Basic descriptors	Species: <i>Morus alba</i> L. Location: Italy, Codemondo GPS coordinates: 44.6911441298, 10.55630443804 Accessibility: Public Number of individuals at the location: Mulberry plantation Trunk circumference (cm): 270 cm Tree growth habit: Upright (semi-upright) Tree vigour: Good condition Pruning practices: Unpruned tree
Trunk	Trunk colour: Dark brown (reddish brown) Trunk irregularities/damage: /
Leaves	Phyllotaxis: Predominantly alternate spiral Leaf shape: Simple Leaf blade: High (>1.6, long leaves) Petiole range: Long (41-70mm) Shape of leaf base: Truncate Shape of leaf apex: Acute Leaf blade tip: Acuminate Leaf blade margin: Serrate



## IT23\_00724



**Figure IT23\_00724:** A) Tree habitus; B) Trunk aspect; C) Simple leaf adaxial; D) Simple leaf abaxial.

Basic descriptors	Species: <i>Morus alba</i> L.
	Location: Italy, Laconi
	GPS coordinates: 39.818236, 9.052635
	Accessibility: Public
	Number of individuals at the location: Individuum
Trunk	Trunk circumference (cm): 318
	Tree growth habit: Upright (semi-upright)
	Tree vigour: Good condition
	Pruning practices: Unpruned tree
	Trunk colour: Dark brown (reddish brown)
Leaves	Trunk irregularities/damage: /
	Phyllotaxis: Predominantly alternate spiral
	Leaf shape: Simple
	Leaf blade: High (>1.6, long leaves)
	Petiole range: Medium (21-40mm)
	Shape of leaf base: Truncate
	Shape of leaf apex: Acute
	Leaf blade tip: Acuminate
	Leaf blade margin: Serrate

## IT23\_00725

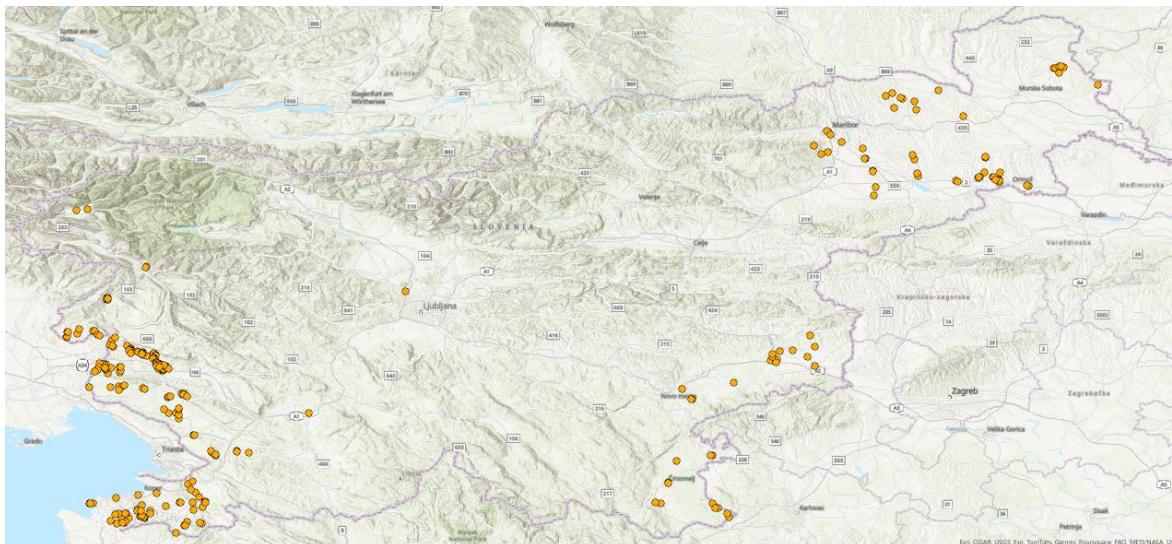


**Figure IT23\_00725:** A) Tree habitus; B) Trunk aspect; C) Simple leaf adaxial; D) One-year old shoot.

Basic descriptors	Species: <i>Morus alba</i> L. Location: Italy, Lonigo GPS coordinates: 45.373100, 11.386317 Accessibility: Private garden Number of individuals at the location: Individuum Trunk circumference (cm): 570 cm Tree growth habit: Upright (semi-upright) Tree vigour: Good condition Pruning practices: Yearly pruned
Trunk	Trunk colour: Dark brown (reddish brown) Trunk irregularities/damage: Hollow (pipe tree)
Leaves	Phyllotaxis: Predominantly alternate distichous Leaf shape: Simple Leaf blade: High (>1.6, long leaves) Petiole range: Long (41-70mm) Shape of leaf base: Cordate Shape of leaf apex: Acute Leaf blade tip: Acuminate Leaf blade margin: Crenate

### 4.3.4 Report on the inventory of mulberries in Slovenia

#### 4.3.4.1 Basic Information



**Figure SI\_1** - Distribution map of mulberries in Slovenia using *MorusAPP*

From 2nd June to October 29, 2023, six active Slovenian users recorded 340 mulberries within different Slovenian ecogeographical regions (**Figure SI\_1**) and an additional 46 mulberries abroad [in Austria (1), Croatia (6), United Kingdom (3), Italy (8), and Greece (28)]. Most of the trees (274) were recorded during the sampling excursions in June in order to monitor also the infructescence descriptors. Basic descriptors were recorded for all of the 340 mulberries, while other descriptors were recorded according to the possibilities of sampling.

Inventory of mulberries by species (**Figure SI\_2**) indicates that a majority of recorded trees were white mulberries (*M. alba*) with a total of 329 (96.8 %), while there were only 10 (2.9 %) black mulberries (*M. nigra*) and one (0.3 %) undefined *Morus* sp. (**Figure SI\_2A**). Compared to other countries, Slovenia recorded the highest number of both white and black mulberries (**Figure SI\_2A**).

**Figure SI\_2B** shows the accessibility options of mulberries recorded in Slovenia. The majority were grown in private gardens (43 %). The second most common were mulberries in public areas (27 %), and the third most common were mulberries in agricultural areas (19 %), while 8 % were found along the streets, 2 % in botanical gardens or collections, and only 1 % in squares. The majority of mulberries in Slovenia grew individually (83 %), while we recorded 23 mulberry rows and 16 plantations, collectively accounting for 17% (**Figure SI\_2C**).



The distribution of mulberries based on circumference was relatively even; however, those with the smallest circumference, i.e., less than 180 cm, still prevailed, constituting 37 % of all recorded mulberries. They were predominated by mulberries with diameters ranging from 180 to 249 cm. Sixty-seven percent of all recorded mulberries in Slovenia had a diameter less than 249 cm. It was concluded that the majority of recorded trees in Slovenia had a smaller circumference compared to neighbouring Italy. This could be explained by the decimation of mulberry trees during the post-war period (mid-20th century) in Slovenia. Meanwhile, there was an equal percentage (18 %) of trees with diameters ranging from 250 to 300 cm and those exceeding 300 cm (Figure SI\_2D).

The inventory of mulberries by tree growth habit indicated that a majority of the recorded trees grew upright or semi-upright (94 %), while only 6 % of trees had a weeping growth habit (Figure SI\_2E). The majority of mulberries recorded in Slovenia were in good condition, only a small minority of trees were in bad condition (Figure SI\_2F).

Figure SI\_2G shows the pruning practices of the mulberries recorded in Slovenia. A significant portion, comprising 54 %, appeared to be unpruned. In contrast, 30 % were subjected to yearly pruning, while 16 % underwent frequent pruning. This distribution provided insight into the varied mulberry maintenance practices.

The predominant trunk colour among the recorded mulberries, representing 69 %, was greyish-brown. Additionally, 15 % showed a dark brown (reddish-brown) trunk colour, while 16 % featured a light brownish-grey trunk colour (Figure SI\_2H).

#### 4.3.4.2 Detailed descriptors of the trunk and one-year old shoots

Mulberries can be distinguished by trunk irregularities. Out of the 340 recorded mulberry trees in Slovenia, 61 showed some form of trunk irregularities or damage, constituting 17.9 % of the total. Figure SI\_3A shows that the most prevalent irregularity among Slovenian trees was a longitudinally cracked trunk, accounting for 46 % and involving 28 trees. Additionally, 21 % (14 trees) featured a curved trunk, while 13 trees featured a hollow trunk, also known as a pipe tree. Only 10 % (6 trees) featured a split trunk (Figure SI\_3A).

Descriptors of one-year-old shoots weren't recorded in many trees, as this parameter is highly dependent on the time of sampling, and it is best to sample in winter or early spring. The colour of one-year old branches is quite a diverse descriptor. It was recorded in only 36 out of 340 Slovenian mulberries. Almost half (47 %) of the trees had a greyish-brown colour of the one-year shoot, the second most represented colour was greenish-brown (25 %), and the third was medium brown (19 %). A minority of the trees had yellowish-brown (4 %), reddish-brown (3 %), and dark brown (2 %) colour of the one-year-old shoot (Figure SI\_3B).



While the colour of one-year-old shoot was difficult to determine in summer, lenticels could be more easily recorded. Among the 68 recorded trees, 53 % featured medium lenticel density, 32 % had sparse, and 15 % had high lenticel density (Figure SI\_3C). Examining the lenticel shape revealed that nearly half were round, 27 % were oval, and 24 % were elliptical (Figure SI\_3D).

The shape of buds is typically determined in winter or early spring, so we could only ascertain the shape of buds for three, or in the case of bud colour, two trees. Two of the mulberries had a medium triangular bud shape, and one had ovate buds (Figure SI\_3E). One mulberry had dark brown bud colour, and the other had greyish buds (Figure SI\_3F).

In Figure SI\_3G, we show that 81 % of the 146 recorded trees predominantly had alternate spiral phyllotaxis, and the rest predominantly had alternate distichous phyllotaxis. Leaf shape was recorded in 151 trees, of which 48 % featured pronounced heterophylly. These trees had both simple and lobed leaves, while 48 % had only simple leaves, and only 4 % had only lobed leaves (Figure SI\_3H).

#### 4.3.4.3 Detailed descriptors of leaf morphology

Leaf blade was determined in 132 mulberries, with the majority (71 %) having a medium leaf blade, 21 % having a high leaf blade, which corresponds to oblong leaves, and only 8 % having broad leaves (low leaf blade) as shown in **Figure SI\_4A**.

**Figure SI\_4B** shows the petiole range, determined in 129 mulberries. The majority (58 %) featured a medium petiole length (21 mm-40 mm), followed by long petioles (41 mm-70 mm) in 33 % of the mulberries. A minority of mulberries had a short (11-20 mm) petiole (5 %), while a small proportion featured a very long (>71 mm) petiole (4 %).

The variety of leaf base shapes is presented in **Figure SI\_4C**. Among the observed 134 trees, 37 % featured a truncate leaf base, an equal percentage (37 %) featured a retuse leaf base, 22 % featured a cordate leaf base, and a minority of 4 % featured a cuneate leaf base. This distribution highlights the diversity of different, but evenly distributed leaf base shapes within the recorded mulberry population in Slovenia.

**Figure SI\_4D** shows the distribution of leaf apex shapes in the 132 recorded trees. Strikingly, the majority, comprising 95 % of the trees, featured an acute leaf apex shape. In contrast, only a few, (5 %) of the trees featured an obtuse leaf apex shape. None of the recorded mulberries had an obcordate leaf shape apex. This distribution emphasizes the predominance of the acute leaf apex shape within the observed mulberry population.

**Figure SI\_4E** shows the distribution of blade tip shapes among the 134 observed mulberries. The majority, accounting for 79 % of the recorded trees, featured an acuminate blade tip, characterised by a tapering and pointed end. A smaller proportion, 13 % of the trees, featured a caudate blade tip. Additionally, 8 % of the observed mulberries had an absent blade tip. The prevalence of the acuminate blade tip aligns with the dominance of the acute leaf apex shape.

The leaf blade margin was assessed in 134 observed mulberries, revealing various shapes. The predominant types were crenate (47 %) and serrate (29 %) leaf blade margins, followed by 12 % of mulberries with dentate leaf margins. A minority of trees featured biserrate (8 %), while only 2 % featured aristate and serrulate margins (**Figure SI\_4F**).

The assessment of leaf hairiness in 131 mulberries indicated that 60 % had glabrous leaves, 35 % featured hairiness on midrib and veins, and 5 % were evenly pubescent (**Figure SI\_4G**). **Figure SI\_4H** shows that 95 % of the 142 recorded trees featured glossy leaves, while the remaining 5 % featured matte leaves. This was expected, as glossy leaves are a characteristic feature of white mulberry.

#### 4.3.4.4 Detailed descriptors of infructescence and inflorescence

Mulberry trees are unique in their sexual expression as they are both monoecious and dioecious, their inflorescences being male or female catkins, appearing also in combination of both male and female flowers. The small, multiple sweet infructescences (soroses) are a consolidation of drupelets with remnants of perianth. Determining the sexual expression of mulberries is extremely challenging. It is most reliable to ascertain monoecy or dioecy when the plants are in flower. Therefore, we determined the sexual expression in only 24 plants, of which 54 % were monoecious and 46 % were clearly dioecious (**Figure SI\_5A**).

We determined inflorescence types in 24 recorded mulberries, a slight majority (46 %) was female, while 42 % were male, 4 % were male and female and 8 % were predominantly female with some male flowers at the base (**Figure SI\_5B**).

In **Figure SI\_5C**, stigma persistency on the infructescence of 28 mulberries is shown. The data revealed that most (97 %) of the observed mulberries featured a non-persistent stigma, while a very small percentage (3 %), showed a persistent stigma. This distribution underscored the prevalence of non-persistent stigma in the recorded mulberry population, with only a minor proportion featuring persistent stigma on their infructescence.

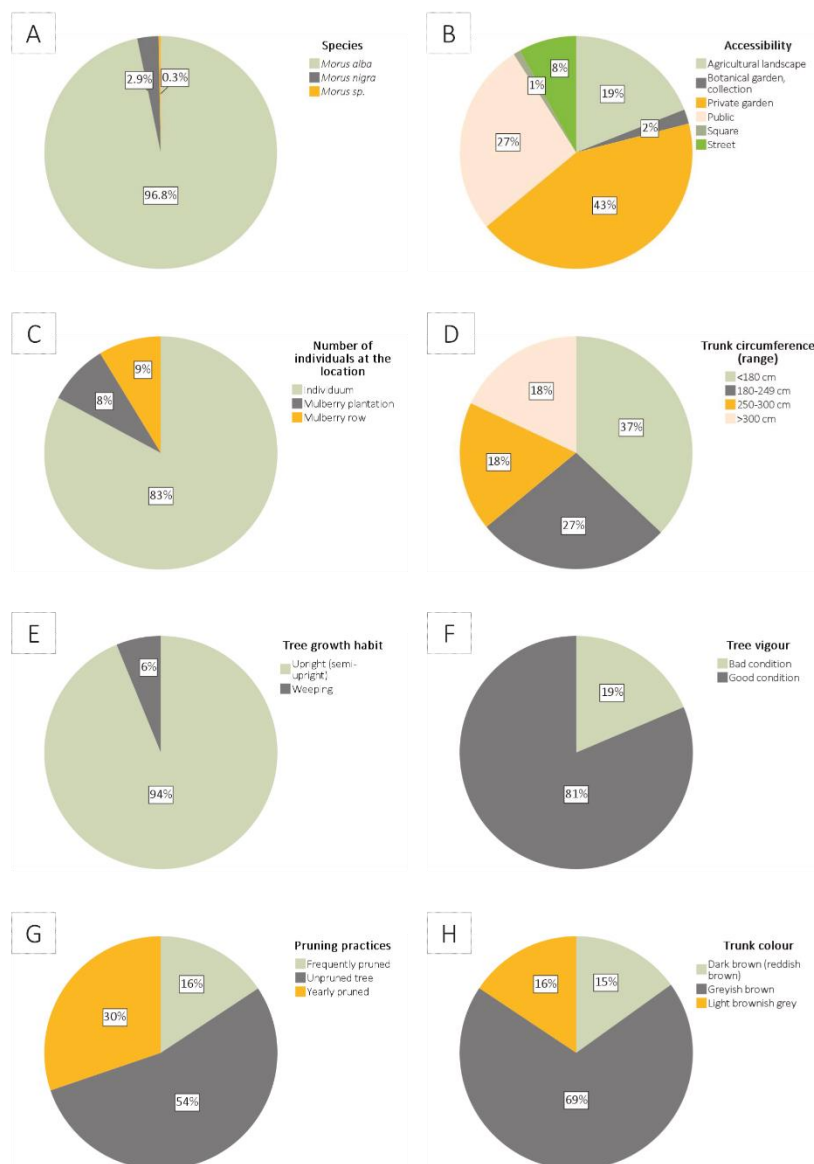
We assessed peduncle length in 83 mulberry trees, revealing that the majority (66 %) of infructescences had a medium-sized peduncle, while 24 % had a short-sized peduncle, and 10 % had a long-sized peduncle (**Figure SI\_5D**).

Mulberries featured a diverse range of infructescence colours, ranging from yellowish white, through light pink, and all the way to purple brown, reddish black and black. **Figure SI\_5E** shows the frequency of the varied colours observed in the infructescences of 83 recorded mulberries. The majority (69 %) were yellowish white, 12 % were purple brown, 8 % were black, 7 % were light pink, and 4 % were reddish black.

The evaluation of infructescence taste in 67 mulberries revealed that the majority (96 %) of the recorded mulberries tasted sweet, while an equal proportion (2 %) had a balanced and acidic taste (**Figure SI\_5F**).

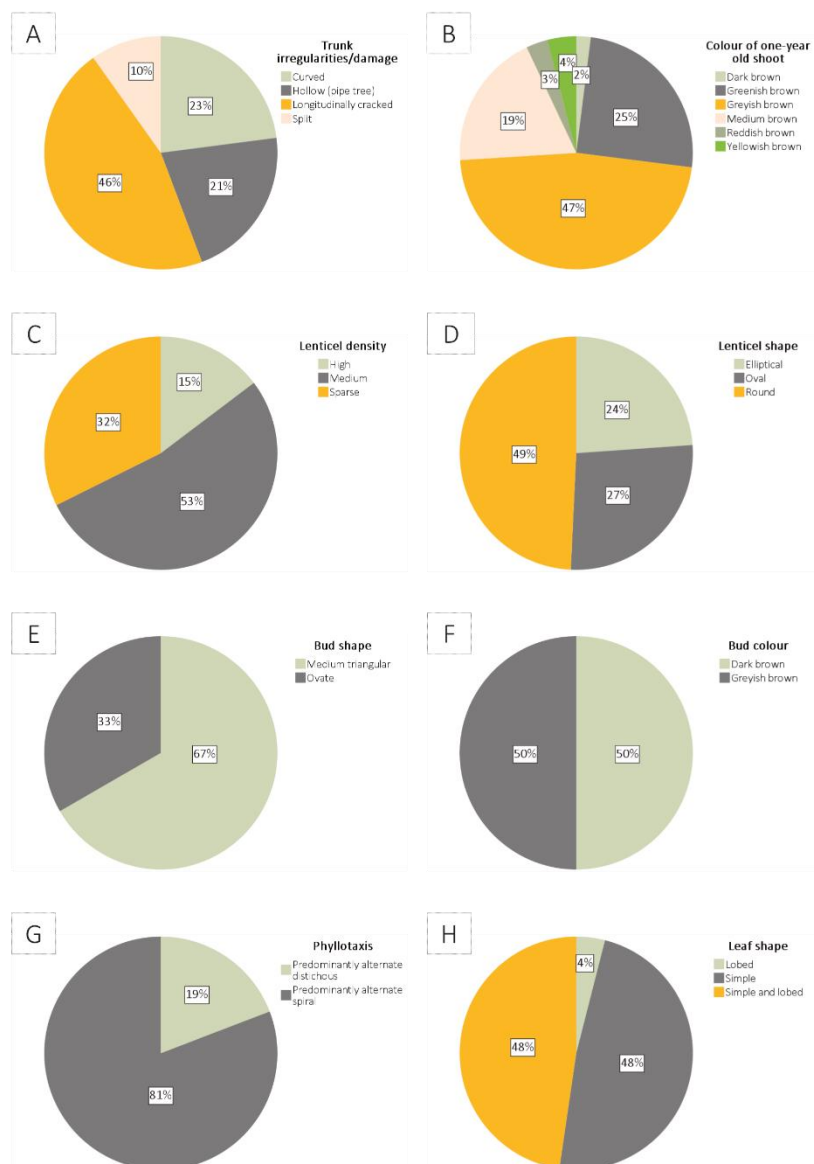
We assessed the infructescence shape in 83 recorded mulberries, observing various shapes. The majority (67 %) of infructescences were ellipsoid, followed by cylindrical (15 %), ovoid or globose (13 %), and only 5 % were irregular in shape (**Figure SI\_5G**). The uniformity of infructescence ripeness was assessed in 69 recorded mulberries. The majority (74 %) showed prolonged uniformity of ripeness, while 26 % showed uniform ripeness. This highlighted another unique characteristic of mulberry species (**Figure SI\_5H**).

## Inventarisation of mulberries in Slovenia



**Figure SI\_2** - Basic information on the inventory of mulberry trees in Slovenia. A) Percentage of recorded *M. alba* and *M. nigra* trees. B) Accessibility options of trees. C) State on the number of individuals at location. D) Percentage of trees at certain trunk circumference range. E) Percentage of trees of upright and weeping growth habit. F) Percentage of trees in bad and good condition. G) Report on the pruning frequency of the trees. H) The percentage of trees of different trunk colours.

## Inventarisation of mulberries in Slovenia



**Figure SI\_3** - Inventory of mulberries in Slovenia. Detailed descriptors of one-year old shoots and leaf morphology. A) Trunk irregularities or damage. B) Colour of one-year old shoot. C) Lenticel density. D) Lenticel shape. E) Bud shape. F) Bud colour. G) Phyllotaxis. H) Leaf shape.

## Inventarisation of mulberries in Slovenia



**Figure SI\_4** - Inventory of mulberries in Slovenia. Detailed descriptors of leaf morphology. Inventarisation of mulberries in Slovenia. Detailed descriptors of leaf morphology. A) Leaf blade. B) Petiole range. C) Shape of leaf base. D) Shape of leaf apex. E) Leaf blade tip. F) Leaf blade margin. G) Leaf hairiness. H) Leaf glossiness.

## Inventarisation of mulberries in Slovenia

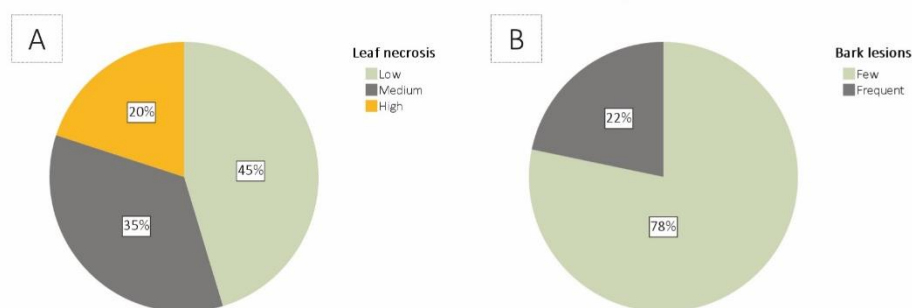


**Figure SI\_5** - Inventory of mulberries in Slovenia. Infructescences and inflorescence descriptors. Inventarisation of mulberries in Slovenia. Infructescences and inflorescence descriptors. A) Sexual dimorphism. B) Inflorescence types. C) Stigma persistency. D) Peduncle length. E) Infructescence colour types. F) Infructescence taste. G) Infructescence shape types. H) Uniformity of infructescence ripeness.



#### 4.3.4.5 Report on mulberry diseases in Slovenia

**Figure SI\_ 6A** shows the incidence of leaf necrosis among a total of 75 observed mulberries. Almost half (45 %) of the mulberries exhibited a low leaf necrosis frequency, while 35 % displayed medium leaf necrosis, and a minority (20 %) of trees had a high leaf necrosis frequency. In **Figure SI\_ 6B**, the frequency of bark lesions in 46 observed mulberries is depicted. The majority, comprising 78%, exhibited few bark lesions, while 22% displayed a frequent occurrence of bark lesions. Therefore, we can conclude that the majority of mulberries observed in our study did not exhibit significant leaf necrosis or bark lesions.



**Figure SI\_ 6** - Inventory of mulberries in Slovenia. Diseases. A) Leaf necrotic spots. B) Bark lesions.

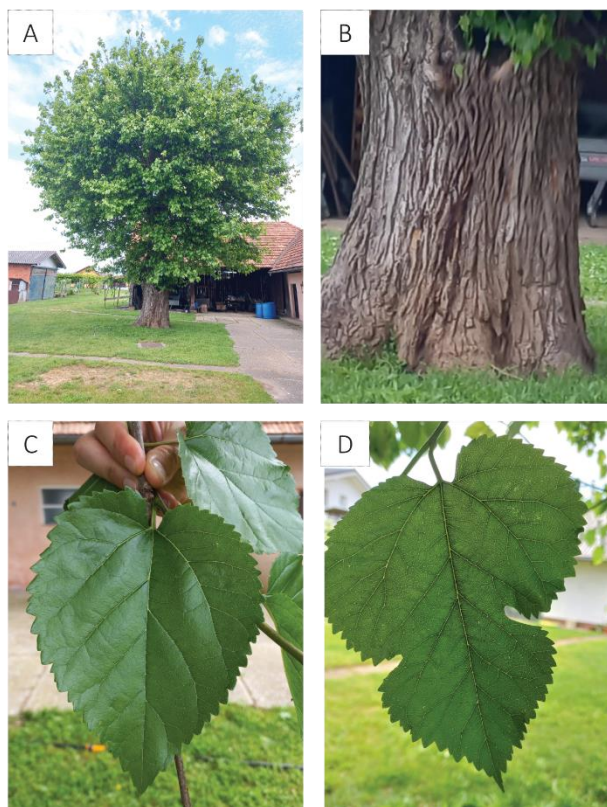
#### 4.3.4.6 Monumental mulberry trees of Slovenia

Based on previous inventarisations in Slovenia over three vegetation periods, we have inventoried 645 mostly historical trees (with circumferences > 180 cm). Last year, 46 trees with a circumference between 300 and 400 cm were recorded with *MorusAPP*. The highest density of mulberry trees was found in the Sub-Mediterranean region, followed by the Sub-Pannonian region, the South-Eastern region and Central Slovenia. The oldest mulberry tree in Slovenia (**SI23\_00356**) is located on the Fabiani homestead in Kobdilj and has a circumference at breast height of 752 cm. The second largest tree (**SI23\_00234**) with a circumference of 447 cm is located in Miren in the Gorizia region and is part of a row of 45 trees that were historically used for sericulture. This collection includes several varieties of different ages, including the *M. alba* f. *platanifolia* variety. Notable tree rows include those in Loka (**SI23\_00199**; N=4, CBH >300 cm), near Školj Castle, Famlje (**SI23\_00260**, N=13, CBH 130-225 cm) and at the Benedictine monastery in Krog nad Sečovljami (**SI23\_00177**, N=9, CBH 130-289 cm; **SI23\_00180**, N=6, CBH 308 cm) and in front of the church in Bertoki (**SI23\_00191**, N=30, CBH 180-249 cm). **SI23\_00076.2** shows one of the 4 trees in front of Dobrovo Castle in Goriška Brda. Our previous study showed that in the SM region mulberry trees are traditionally pruned (77% pruned), while in other regions most mulberry trees are not pruned (Urbanek Krajnc et al., 2019).

In the SP region, the most magnificent tree grows in a meadow in Destrnik (**SI23\_00059**). The tree has an unusual morphotype characterised by long leaves with dentate leaf margin. Another tree with a circumference of 347 cm is located in Prepolje (**SI23\_00053**) and a tree with a circumference of 321 cm is located in Sv. Ana (**SI23\_00305**).

In the central region, we found a magnificent tree at the foot of Rašica Mountain with a circumference of 410 cm (**SI23\_00392**). Other monumental trees in the coastal region are **SI2300160** from Šmarje with a circumference of 400 cm, **SI23\_00166** with a circumference of 359 cm from Koštabona, **SI23\_00175** with a circumference of 330 cm from Fieroga and **SI23\_00193** with a circumference of 382 cm from Marežige.

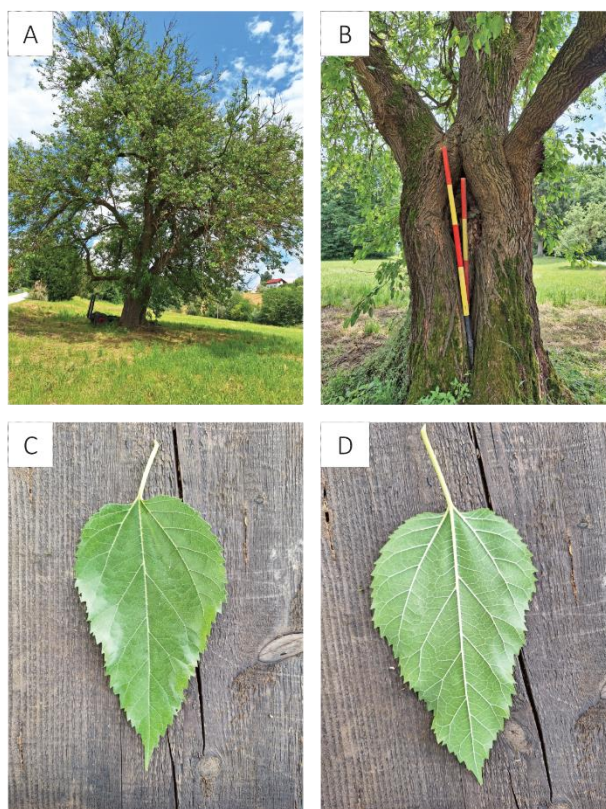
## SI23\_00053



**Figure SI23\_00053:** A) Tree habitus; B) Trunk aspect; C) Simple leaf adaxial; D) Lobed leaf adaxial.

Basic descriptors	Species: <i>Morus alba</i> L. Location: Slovenia, Prepolje GPS coordinates: 46.4456557, 15.7647189 Accessibility: Private garden Number of individuals at the location: Individuum Trunk circumference (cm): 347 cm Tree growth habit: Upright (semi-upright) Tree vigour: Good condition Pruning practices: Unpruned tree
Trunk	Trunk colour: Greyish brown Trunk irregularities/damage: /
Leaves	Phyllotaxis: Predominantly alternate spiral Leaf shape: Simple and lobed Leaf blade: Medium (1.3-1.5) Petiole range: Medium (21-40mm); 35 mm Shape of leaf base: Cordate Shape of leaf apex: Acute Leaf blade tip: Acuminate Leaf blade margin: Serrate

SI23\_00059

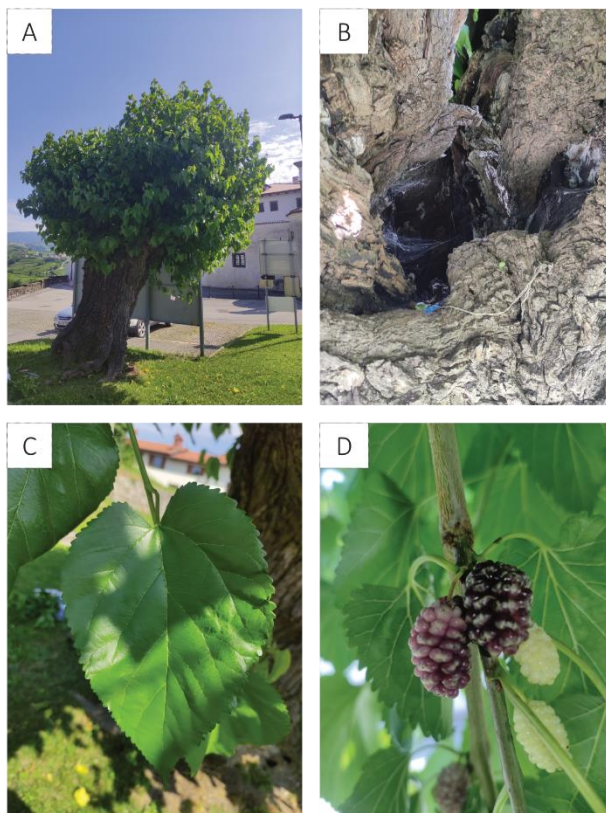


**Figure SI23\_00059:** A) Tree habitus; B) Trunk aspect; C) Simple leaf adaxial; D) Simple leaf abaxial.

Basic descriptors	<p>Species: <i>Morus alba</i> L.</p> <p>Location: Slovenia, Destrnik</p> <p>GPS coordinates: 46.4768028, 15.881215</p> <p>Accessibility: Agricultural landscape</p> <p>Number of individuals at the location: Individuum</p> <p>Trunk circumference (cm): 401 cm</p> <p>Tree growth habit: Upright (semi-upright)</p> <p>Tree vigour: Good condition</p> <p>Pruning practices: Unpruned tree</p>
Trunk	<p>Trunk colour: Dark brown (reddish brown)</p> <p>Trunk irregularities/damage: Longitudinally cracked</p>
Leaves	<p>Phyllotaxis: Predominantly alternate spiral</p> <p>Leaf shape: Simple</p> <p>Leaf blade: High (&gt;1.6, long leaves)</p> <p>Petiole range: Medium (21-40mm)</p> <p>Shape of leaf base: Retuse</p> <p>Shape of leaf apex: Acute</p> <p>Leaf blade tip: Caudate</p> <p>Leaf blade margin: Dentate</p>



## SI23\_00076.2



**Figure SI23\_00076.2:** A) Tree habitus; B) Trunk aspect; C) Simple leaf adaxial; D) Infructescence.

Basic descriptors	Species: <i>Morus alba</i> L. Location: Slovenia, Dobrovo GPS coordinates: 45.9966774, 13.5232529 Accessibility: Public Number of individuals at the location: Mulberry plantation Trunk circumference (cm): 358 cm Tree growth habit: Upright (semi-upright) Tree vigour: Good condition Pruning practices: Yearly pruned
Trunk	Trunk colour: Light brownish grey Trunk irregularities/damage: Hollow (pipe tree)
Leaves	Phyllotaxis: Predominantly alternate spiral Leaf shape: Simple and lobed Leaf blade: Low (<1.2, broad leaves) Petiole range: Very long (>71mm); 80 mm Shape of leaf base: Retuse Shape of leaf apex: Acute Leaf blade tip: Caudate Leaf blade margin: Biserrate

S123\_00160



Basic descriptors	Species: <i>Morus alba</i> L.
	Location: Slovenia, Šmarje
	GPS coordinates: 45.4997585, 13.71845
	Accessibility: Agricultural landscape
	Number of individuals at the location: Individuum
	Trunk circumference (cm): 400 cm
	Tree growth habit: Upright (semi-upright)
	Tree vigour: Good condition
	Pruning practices: Yearly pruned
Trunk	Trunk colour: Dark brown (reddish brown)
	Trunk irregularities/damage: /
	Colour of one-year old shoot: Greenish brown
	Lenticel density: Medium
	Lenticel shape: Oval
Leaves	Phyllotaxis: Predominantly alternate spiral
	Leaf shape: Simple and lobed
	Leaf blade: Medium (1.3-1.5)
	Petiole range: Medium (21-40mm); 30 mm
	Shape of leaf base: Cordate
	Shape of leaf apex: Acute
	Leaf blade tip: Acuminate
	Leaf blade margin: Serrate
	Hairiness: Glabrous
	Glossiness: Glossy
Inflorescence	Stigma persistency: No inflorescence
	Peduncle length: /
	Inflorescence colour: /
	Inflorescence taste: /
	Inflorescence shape: /
	Uniformity of inflorescence ripeness: /
Disease	Leaf necrotic spots: Low
	Bark lesions: Few

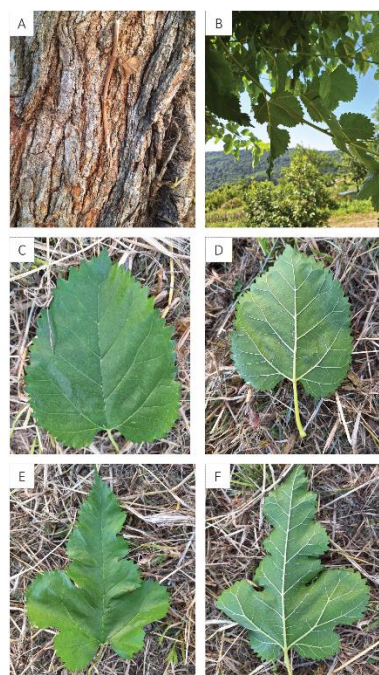
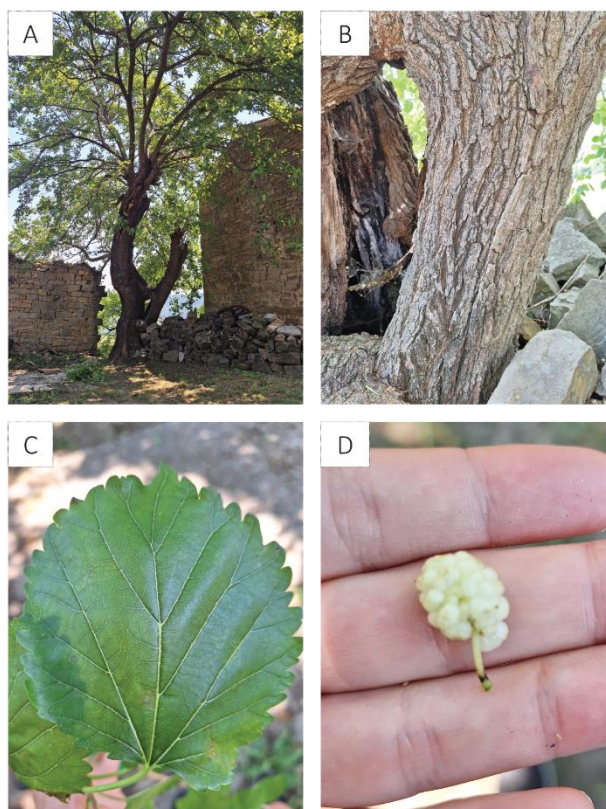


Figure S123\_00160: A) Trunk aspect; B) One-year old shoot; C) Simple leaf adaxial; D) Simple leaf abaxial; E) Lobed leaf adaxial; F) Lobed leaf abaxial.



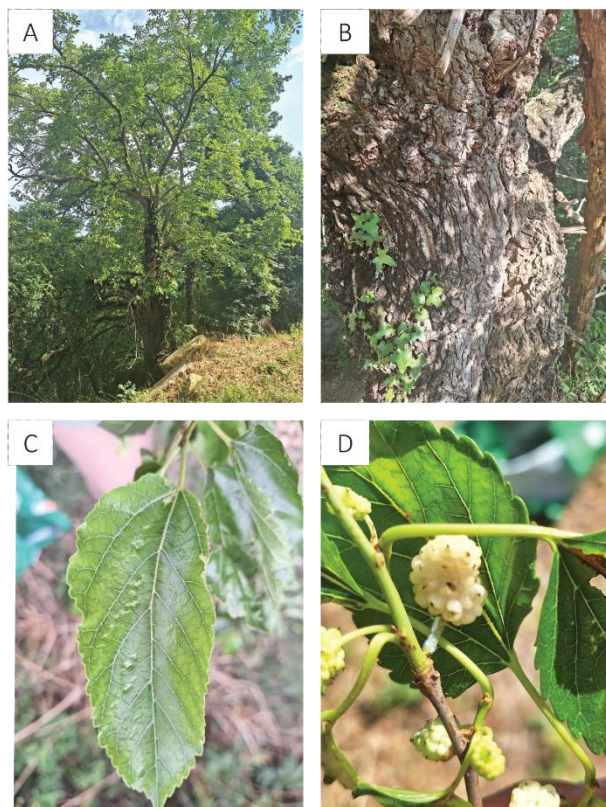
SI23\_00166



**Figure SI23\_00166:** A) Tree habitus; B) Trunk aspect; C) Simple leaf adaxial; D) Infructescence.

Basic descriptors	Species: <i>Morus alba</i> L. Location: Slovenia, Koštabona GPS coordinates: 45.479241, 13.7370314 Accessibility: Agricultural landscape Number of individuals at the location: Individuum Trunk circumference (cm): 359 cm Tree growth habit: Upright (semi-upright) Tree vigour: Good condition Pruning practices: Unpruned tree
Trunk	Trunk colour: Greyish brown Trunk irregularities/damage: Hollow (pipe tree)
Leaves	Phyllotaxis: Predominantly alternate spiral Leaf shape: Simple Leaf blade: Low (<1.2, broad leaves) Petiole range: Medium (21-40mm); 35 mm Shape of leaf base: Truncate Shape of leaf apex: Obtuse Leaf blade tip: Absent Leaf blade margin: Crenate

## SI23\_00175



**Figure SI23\_00175:** A) Tree habitus; B) Trunk aspect; C) Simple leaf adaxial; D) Infructescence.

Basic descriptors	<p>Species: <i>Morus alba</i> L.</p> <p>Location: Slovenia, Fijeroga</p> <p>GPS coordinates: 45.4995444, 13.7276222</p> <p>Accessibility: Street</p> <p>Number of individuals at the location: Individuum</p> <p>Trunk circumference (cm): 330 cm</p> <p>Tree growth habit: Upright (semi-upright)</p> <p>Tree vigour: Bad condition</p> <p>Pruning practices: Unpruned tree</p>
Trunk	<p>Trunk colour: Greyish brown</p> <p>Trunk irregularities/damage: Curved</p>
Leaves	<p>Phyllotaxis: Predominantly alternate spiral</p> <p>Leaf shape: Simple</p> <p>Leaf blade: High (&gt;1.6, long leaves)</p> <p>Petiole range: Medium (21-40mm); 30 mm</p> <p>Shape of leaf base: Cuneate</p> <p>Shape of leaf apex: Acute</p> <p>Leaf blade tip: Acuminate</p> <p>Leaf blade margin: Serrate</p>

SI23\_00177



**Figure SI23\_00177:** A) Tree habitus; B) Trunk aspect; C) Simple leaf adaxial; D) Infructescence.

Basic descriptors	<p>Species: <i>Morus alba</i> L.</p> <p>Location: Slovenia, Sečovelje</p> <p>GPS coordinates: 45.4616707, 13.6594748</p> <p>Accessibility: Public</p> <p>Number of individuals at the location: Mulberry row; 9 trees</p> <p>Trunk circumference (cm): 180-249 cm</p> <p>Tree growth habit: Upright (semi-upright)</p> <p>Tree vigour: Good condition</p> <p>Pruning practices: Unpruned tree</p>
Trunk	<p>Trunk colour: Greyish brown</p> <p>Trunk irregularities/damage: /</p>
Leaves	<p>Phyllotaxis: Predominantly alternate spiral</p> <p>Leaf shape: Simple</p> <p>Leaf blade: High (&gt;1.6, long leaves)</p> <p>Petiole range: Medium (21-40mm); 32 mm</p> <p>Shape of leaf base: Truncate</p> <p>Shape of leaf apex: Acute</p> <p>Leaf blade tip: Acuminate</p> <p>Leaf blade margin: Serrate</p>



SI23\_00191

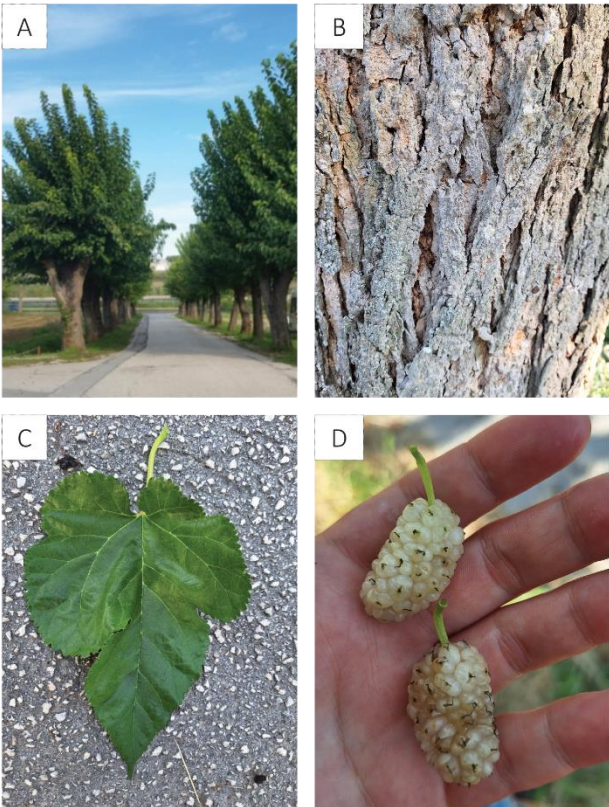
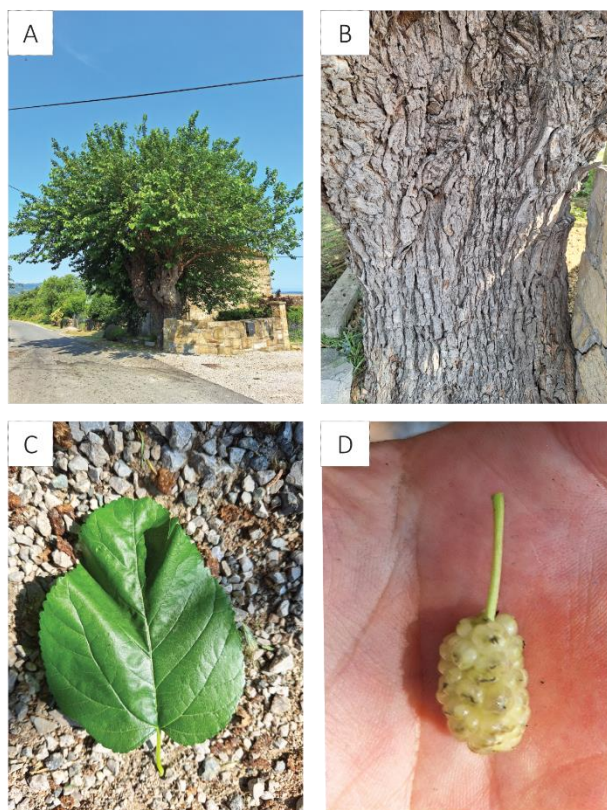


Figure SI23\_00191: A) Tree habitus; B) Trunk aspect; C) Lobed leaf adaxial; D) Infructescence.

Basic descriptors	Species: <i>Morus alba</i> L.
	Location: Slovenia, Bertoki
	GPS coordinates: 45.5482187, 13.7716604
	Accessibility: Public
	Number of individuals at the location: Mulberry row, 30 trees
Trunk	Trunk circumference (cm): 180-249 cm
	Tree growth habit: Upright (semi-upright)
	Tree vigour: Good condition
	Prunning practices: Yearly pruned
	Trunk colour: Greyish brown
Leaves	Trunk irregularities/damage: /
	Phyllotaxis: Predominantly alternate spiral
	Leaf shape: Simple and lobed
	Leaf blade: Medium (1.3-1.5)
	Petiole range: Long (41-70mm); 57 mm
	Shape of leaf base: Cordate
	Shape of leaf apex: Acute
	Leaf blade tip: Acuminate
	Leaf blade margin: Serrate

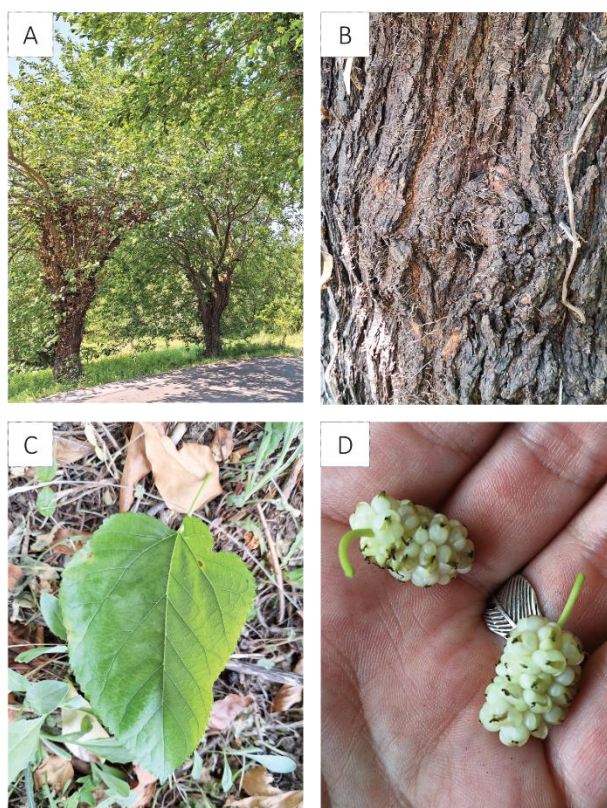
## SI23\_00193



**Figure SI23\_00193:** A) Tree habitus; B) Trunk aspect; C) Simple leaf adaxial; D) Infructescence.

Basic descriptors	<p>Species: <i>Morus alba</i> L.</p> <p>Location: Slovenia, Marezige</p> <p>GPS coordinates: 45.5112749, 13.7966352</p> <p>Accessibility: Private garden</p> <p>Number of individuals at the location: Individuum</p> <p>Trunk circumference (cm): 382 cm</p> <p>Tree growth habit: Upright (semi-upright)</p> <p>Tree vigour: Good condition</p> <p>Pruning practices: Yearly pruned</p>
Trunk	<p>Trunk colour: Greyish brown</p> <p>Trunk irregularities/damage: /</p>
Leaves	<p>Phyllotaxis: Predominantly alternate spiral</p> <p>Leaf shape: Simple</p> <p>Leaf blade: Medium (1.3-1.5)</p> <p>Petiole range: Medium (21-40mm)</p> <p>Shape of leaf base: Retuse</p> <p>Shape of leaf apex: Obtuse</p> <p>Leaf blade tip: Absent</p> <p>Leaf blade margin: Serrate</p>

## SI23\_00199

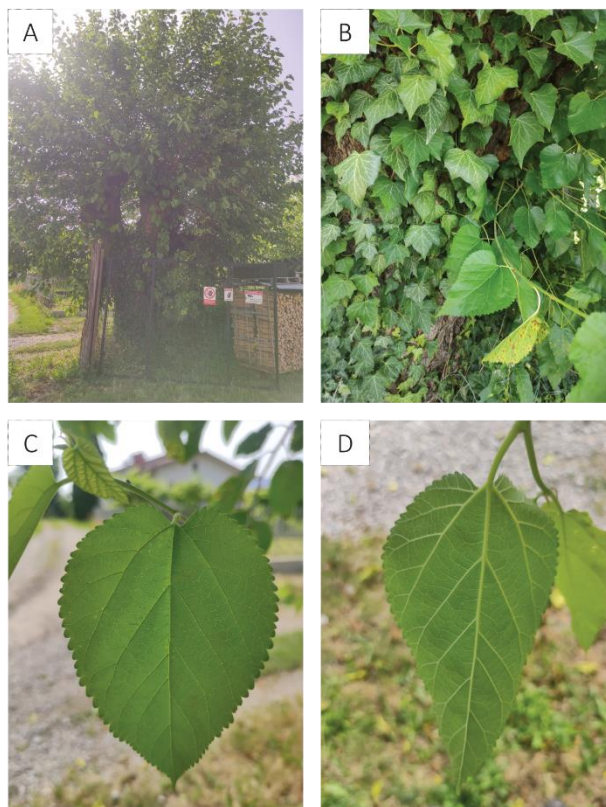


**Figure SI23\_00199:** A) Tree habitus; B) Trunk aspect; C) Simple leaf adaxial; D) Inflorescence.

Basic descriptors	<p>Species: <i>Morus alba</i> L.  Location: Slovenia, Loka  GPS coordinates: 45.5373534, 13.8882535  Accessibility: Street  Number of individuals at the location: Mulberry row, 4 trees  Trunk circumference (cm): &gt;300 cm; the biggest 310 cm  Tree growth habit: Upright (semi-upright)  Tree vigour: Good condition  Pruning practices: Unpruned tree</p>
Trunk	<p>Trunk colour: Dark brown (reddish brown)  Trunk irregularities/damage: /</p>
Leaves	<p>Phyllotaxis: Predominantly alternate spiral  Leaf shape: Simple and lobed  Leaf blade: Medium (1.3-1.5)  Petiole range: Long (41-70mm); 45 mm  Shape of leaf base: Retuse  Shape of leaf apex: Acute  Leaf blade tip: Acuminate  Leaf blade margin: Serrate</p>



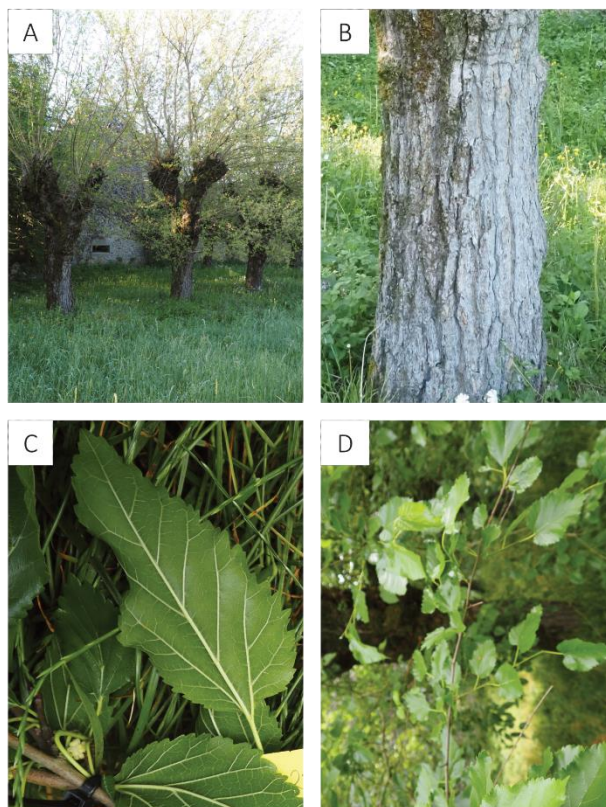
SI23\_00234



**Figure SI23\_00234:** A) Tree habitus; B) Trunk aspect; C) Simple leaf adaxial; D) Simple leaf abaxial.

Basic descriptors	<p>Species: <i>Morus alba</i> L.</p> <p>Location: Slovenia, Miren</p> <p>GPS coordinates: 45.9106684, 13.6118158</p> <p>Accessibility: Private garden</p> <p>Number of individuals at the location: Mulberry row, 45 trees</p> <p>Trunk circumference (cm): &gt;300 cm; the biggest tree 447 cm</p> <p>Tree growth habit: Upright (semi-upright)</p> <p>Tree vigour: Good condition</p> <p>Pruning practices: Yearly pruned</p>
Trunk	<p>Trunk colour: Greyish brown</p> <p>Trunk irregularities/damage: Curved</p>
Leaves	<p>Phyllotaxis: Predominantly alternate distichous</p> <p>Leaf shape: Simple</p> <p>Leaf blade: Medium (1.3-1.5)</p> <p>Petiole range: Medium (21-40mm); 37 mm</p> <p>Shape of leaf base: Truncate</p> <p>Shape of leaf apex: Acute</p> <p>Leaf blade tip: Caudate</p> <p>Leaf blade margin: Crenate</p>

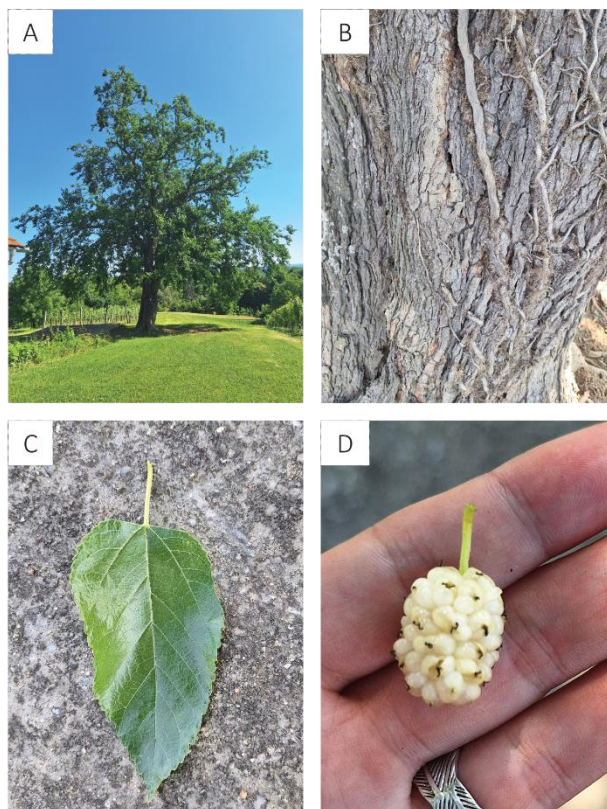
## SI23\_00260



**Figure SI23\_00260:** A) Tree habitus; B) Trunk aspect; C) Simple leaf abaxial; D) One-year shoot

Basic descriptors	<p>Species: <i>Morus alba</i> L.</p> <p>Location: Slovenia, Školj</p> <p>GPS coordinates: 45.6595637, 14.0287067</p> <p>Accessibility: Public</p> <p>Number of individuals at the location: Mulberry plantation</p> <p>Trunk circumference (cm): 180-249 cm</p> <p>Tree growth habit: Upright (semi-upright)</p> <p>Tree vigour: Bad condition</p> <p>Pruning practices: Unpruned tree</p>
Trunk	<p>Trunk colour: Greyish brown</p> <p>Trunk irregularities/damage: Hollow (pipe tree)</p>
Leaves	<p>Phyllotaxis: Predominantly alternate spiral</p> <p>Leaf shape: Simple</p> <p>Leaf blade: High (&gt;1.6, long leaves)</p> <p>Petiole range: Long (41-70mm)</p> <p>Shape of leaf base: Truncate</p> <p>Shape of leaf apex: Acute</p> <p>Leaf blade tip: Acuminate</p> <p>Leaf blade margin: Crenate</p>

## SI23\_00305



**Figure SI23\_00305:** A) Tree habitus; B) Trunk aspect; C) Simple leaf adaxial; D) Infructescence.

Basic descriptors	<p>Species: <i>Morus alba</i> L.</p> <p>Location: Slovenia, St. Ana</p> <p>GPS coordinates: 46.6471967, 15.8483705</p> <p>Accessibility: Private garden</p> <p>Number of individuals at the location: Individuum</p> <p>Trunk circumference (cm): 321 cm</p> <p>Tree growth habit: Upright (semi-upright)</p> <p>Tree vigour: Good condition</p> <p>Pruning practices: Unpruned tree</p>
Trunk	<p>Trunk colour: Greyish brown</p> <p>Trunk irregularities/damage: /</p>
Leaves	<p>Phyllotaxis: Predominantly alternate spiral</p> <p>Leaf shape: Simple</p> <p>Leaf blade: High (&gt;1.6, long leaves)</p> <p>Petiole range: Medium (21-40mm)</p> <p>Shape of leaf base: Truncate</p> <p>Shape of leaf apex: Acute</p> <p>Leaf blade tip: Acuminate</p> <p>Leaf blade margin: Crenate</p>



SI23\_00356 "Fabiani's mulberry"



Basic descriptors	Species: <i>Morus alba</i> L.
	Location: Slovenia, Kobdilj
	GPS coordinates: 45.8161155, 13.8558821
	Accessibility: Private garden
	Number of individuals at the location: Individuum
	Trunk circumference (cm): 752 cm
	Tree growth habit: Upright (semi-upright)
	Tree vigour: Good condition
	Pruning practices: Yearly pruned
	Trunk colour: Light brownish grey
Trunk	Trunk irregularities/damage: Longitudinally cracked
	Colour of one-year shoot: Greenish brown
	Lenticel density: Medium
	Lenticel shape: Oval
Leaves	Phyllotaxis: Predominantly alternate distichous
	Leaf shape: Simple and lobed
	Leaf blade: Medium (1.3-1.5)
	Petiole range: Long (41-70mm); 53 mm
	Shape of leaf base: Cordate
	Shape of leaf apex: Acute
	Leaf blade tip: Acuminate
	Leaf blade margin: Serrate
	Hairiness: Midrib and veins
	Glossiness: Glossy
Inflorescence	Stigma persistency: Nonpersistent
	Peduncle length: Medium
	Inflorescence colour: Yellowish white
	Inflorescence taste: Sweet
	Inflorescence shape: Ellipsoid
	Uniformity of inflorescence ripeness: Prolonged
Disease	Leaf necrotic spots: High
	Bark lesions: Few

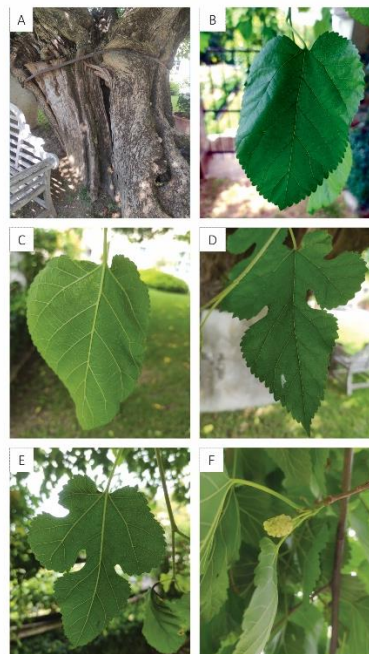
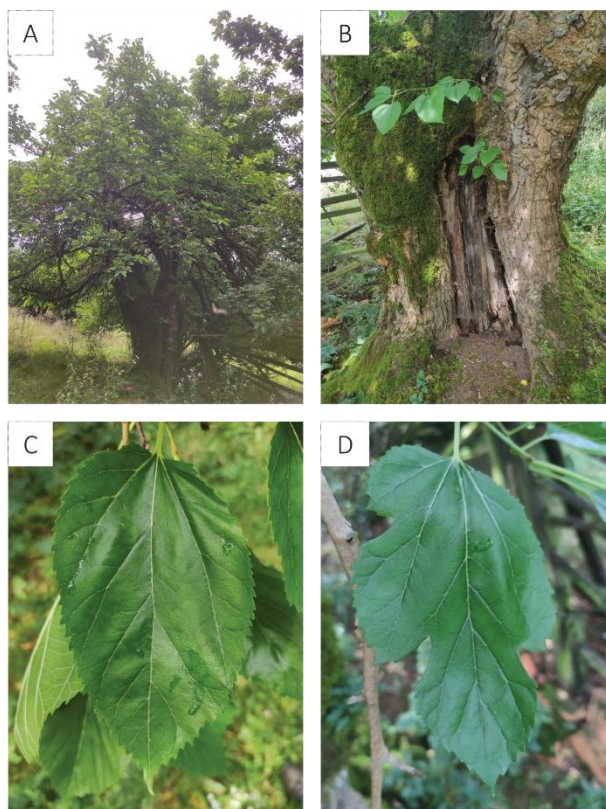


Figure SI23\_00356: A) Trunk aspect; B) Simple leaf adaxial; C) Simple leaf abaxial; D) Lobed leaf adaxial; E) Lobed leaf abaxial; F) Inflorescence.

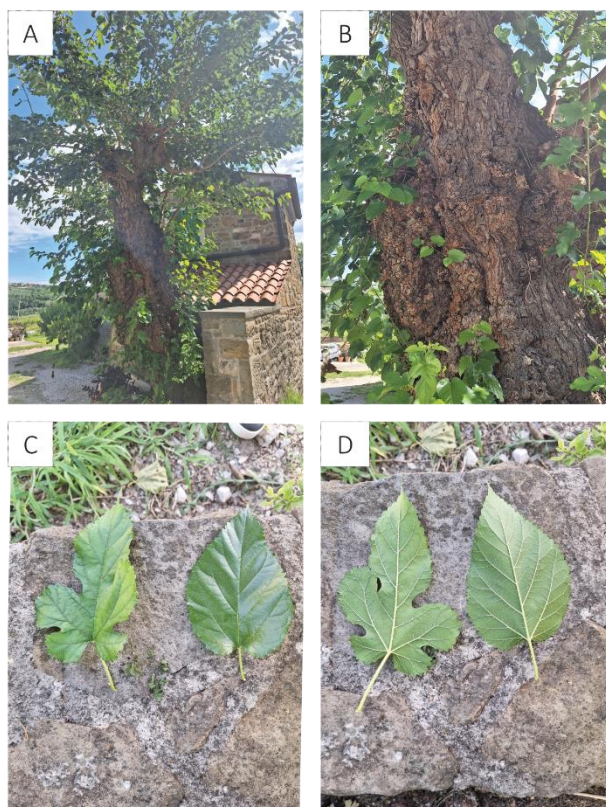
SI23\_00392



**Figure SI23\_00392:** A) Tree habitus; B) Trunk aspect; C) Simple leaf adaxial; D) Lobed leaf abaxial.

Basic descriptors	Species: <i>Morus alba</i> L. Location: Slovenia, Rašica GPS coordinates: 46.1090716, 14.4650436 Accessibility: Agricultural landscape Number of individuals at the location: Individuum Trunk circumference (cm): 410 cm Tree growth habit: Weeping Tree vigour: Bad condition Pruning practices: Unpruned tree
Trunk	Trunk colour: Greyish brown Trunk irregularities/damage: Longitudinally cracked
Leaves	Phyllotaxis: Predominantly alternate spiral Leaf shape: Simple and lobed Leaf blade: High (>1.6, long leaves) Petiole range: Long (41-70mm); 44 mm Shape of leaf base: Truncate Shape of leaf apex: Acute Leaf blade tip: Caudate Leaf blade margin: Serrate

# SI23\_00433



**Figure SI23\_00433:** A) Tree habitus; B) Trunk aspect; C) Simple and Lobed leaf adaxial; D) Simple and Lobed leaf abaxial.

Basic descriptors	Species: <i>Morus alba</i> L. Location: Slovenia, St. Anton GPS coordinates: 45.4671246, 13.6834905 Accessibility: Private garden Number of individuals at the location: Individuum Trunk circumference (cm): 260 cm Tree growth habit: Upright (semi-upright) Tree vigour: Good condition Pruning practices: Yearly pruned
Trunk	Trunk colour: Dark brown (reddish brown) Trunk irregularities/damage: Curved
Leaves	Phyllotaxis: Predominantly alternate distichous Leaf shape: Simple and lobed Leaf blade: Medium (1.3-1.5) Petiole range: Medium (21-40mm) Shape of leaf base: Cordate Shape of leaf apex: Acute Leaf blade tip: Acuminate Leaf blade margin: Crenate



## 4.3.5 Report on inventory of mulberries in Bulgaria

### 4.3.5.1 Basic information

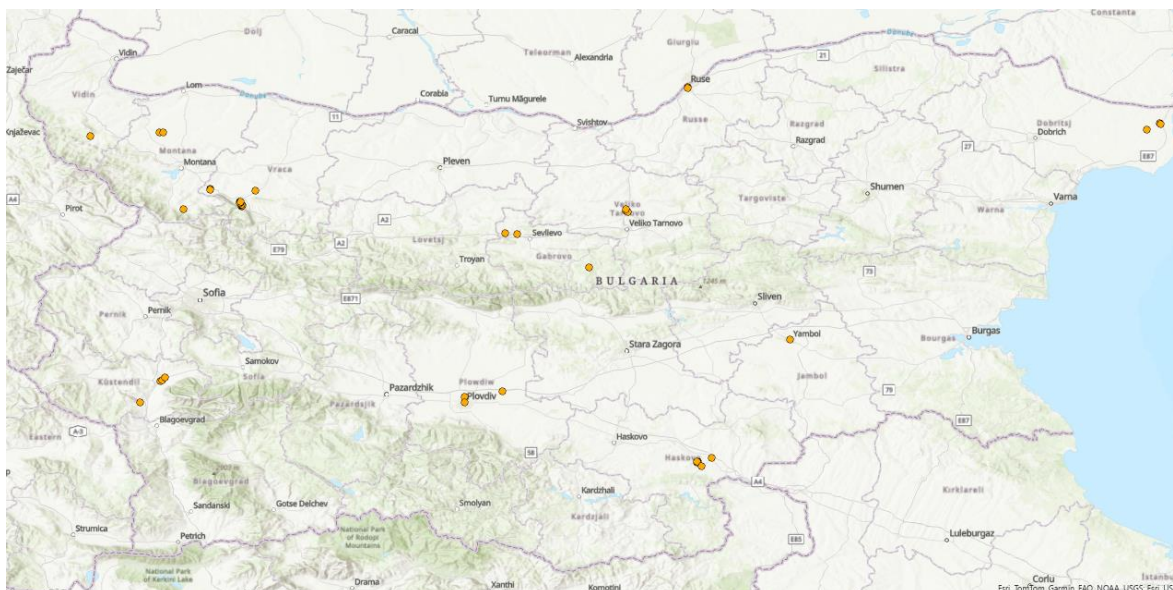


Figure BG\_1 - Distribution map of mulberries in Bulgaria using *MorusAPP*.

In 2023, a total of 51 mulberry trees from Bulgaria were recorded via the *MorusAPP* (

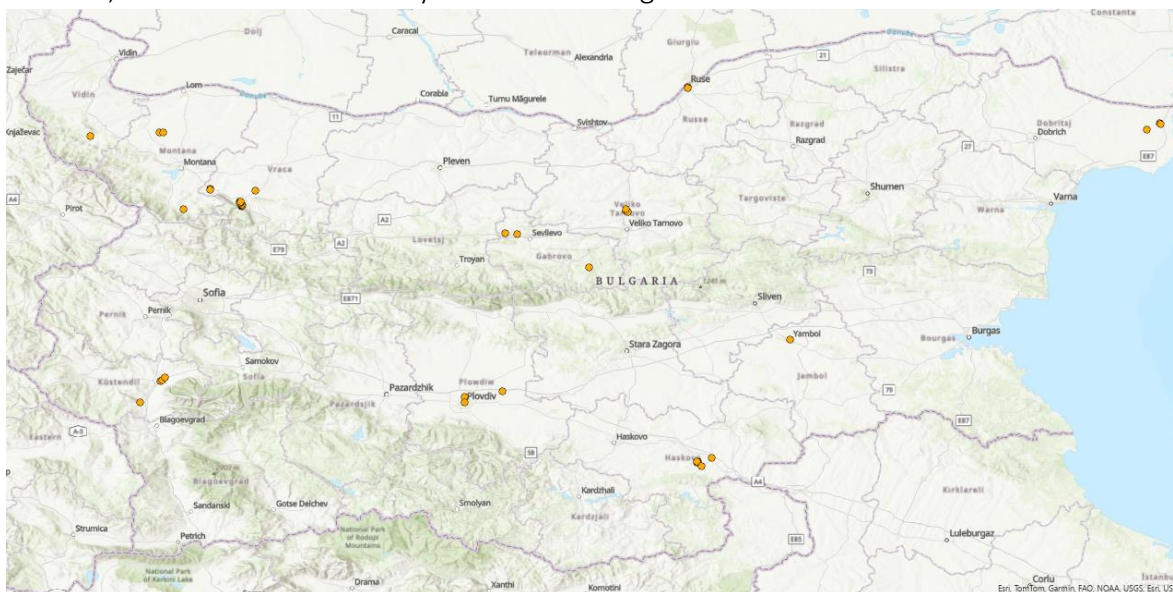


Figure BG\_1). Inventory of Bulgarian mulberries (Figure BG\_2) by species indicates that a majority of recorded trees were white mulberries (*M. alba*) with a total of 50 trees, while there was only one black mulberry tree (*M. nigra*) (Figure BG\_2A).

Figure BG\_2B shows the accessibility options of mulberries recorded in Bulgaria. The majority were found along the streets (52 %). The second most common were mulberries in public areas (16 %), and the third most common were mulberries grown in private gardens (14 %). Only 6 % were equally found in agricultural areas, in botanical gardens or collections, and in squares. The majority of mulberries in Bulgaria grew individually (88 %), while we recorded 10 % in mulberry rows and only 2 % in plantations (Figure BG\_2C).

The distribution of mulberries based on circumference was almost equally represented by trees with a large circumference of 250 to 300 cm (41 %) and trees with a very large circumference of more than 300 cm (35%); twenty-two percent of all recorded mulberries in Bulgaria had a diameter less than 249 cm and only two percent featured a circumference below 180 cm (Figure BG\_2D).

The inventory of mulberries by tree growth habit indicated that a majority of the recorded trees grew upright or semi-upright (98 %), while only 2 % of trees had a weeping growth habit (Figure BG\_2E). The majority of mulberries recorded in Bulgaria were in good condition (80 %), the minority of trees (20 %) were in bad condition (Figure BG\_2F).

Figure BG\_2G shows the pruning practices of the mulberries recorded in Bulgaria. Unfortunately, two thirds of the recorded mulberries were unpruned (61 %). One third of the trees (33 %), were frequently pruned and only a minority (6%) were yearly pruned. This distribution provided insight into the varied mulberry maintenance practices. The predominant trunk colour among the recorded mulberries, representing 90 %, was greyish-brown, while 10 % featured a light brownish-grey trunk colour (Figure BG\_2H).

#### 4.3.5.2 Detailed descriptors of the trunk and one-year old shoots

Mulberries can be distinguished by trunk irregularities. Out of the 51 recorded mulberry trees in Bulgaria, 45 showed some form of trunk irregularities or damage, constituting 88 % of the total. Figure BG\_3A shows that the most prevalent irregularity among Bulgarian trees was a hollow trunk, also known as a pipe tree, accounting for 60 % and involving 27 trees. Additionally, 27 % (12 trees) featured a curved trunk, while 6 trees (13 %) featured a longitudinally cracked trunk. None of the recorded trees featured a split trunk (Figure BG\_3A).

The colour of one-year old branches is quite a diverse descriptor. It was recorded in all Bulgarian mulberries. Two-thirds (62 %) of the trees had a greyish-brown colour of the one-year shoot, the second most represented colour was medium brown (18 %), and the third was in equal proportion yellowish-brown and greenish-brown (8 %). A minority of the trees

had reddish-brown (4 %). None of the recorded trees featured a dark brown colour of the one-year-old shoot (Figure BG\_3B).

Among the 45 trees of which lenticel features were recorded, 64 % had medium lenticel density, 24 % had sparse, and 12 % had high lenticel density (Figure BG\_3C). Examining the lenticel shape revealed that two-thirds (60 %) were round, 21 % were oval, and 19 % were elliptical (Figure BG\_3D).

The shape of buds is typically determined in winter or early spring, so we could only ascertain the shape and colour of buds for ten trees. Two-thirds (60 %) of the mulberries had an ovate bud shape, and 20 % each featured medium triangular or narrow triangular buds and (Figure BG\_3E). Bud colour was almost equally distributed, with 40 % for medium brown and 30 % each for dark brown and greyish brown (Figure BG\_3F).

In Figure BG\_3G, we show that half (49 %) of the 51 recorded trees predominantly had alternate spiral phyllotaxis, 35 % predominantly had combined alternate with opposite decussate and 16 % predominantly had alternate distichous phyllotaxis. Eighty percent of the recorded mulberries featured a simple leaf shape, while 10 % featured pronounced heterophyly. These trees had both simple and lobed leaves and 8 % had lobed leaves (Figure BG\_3H).

#### 4.3.5.3 Detailed descriptors of leaf morphology

Leaf blade was determined in all Bulgarian mulberries, with the majority (55 %) having a medium leaf blade, 25 % having broad leaves (low leaf blade) and 20 % having a high leaf blade, which corresponds to oblong leaves, as shown in **Figure BG\_4A**.

**Figure BG\_4B** shows the petiole range, determined in 49 mulberries. The majority (53 %) featured a long petiole (41 mm-70 mm), followed by 31 % of the mulberries which featured a medium petiole length (21 mm-40 mm). A minority of mulberries had either a very long (>71 mm) (8 %) or a short (11-20 mm) petiole (6 %), while a very small proportion featured a petiole (2 %).

The variety of leaf base shapes is presented in **Figure BG\_4C**. Among the observed 51 trees, 35 % featured a truncate leaf base, 25 % featured a cordate leaf base and 20 % featured either a retuse or cuneate leaf base. This distribution highlights the diversity of different, but fairly evenly distributed leaf base shapes within the recorded mulberry population in Bulgaria.

**Figure BG\_4D** shows the distribution of leaf apex shapes in the 49 recorded trees. Strikingly, the majority, comprising 84 % of the trees, featured an acute leaf apex shape. In contrast,

only 16 % of the trees featured an obtuse leaf apex shape. None of the recorded mulberries had an obcordate leaf shape apex. This distribution emphasizes the predominance of the acute leaf apex shape within the observed mulberry population.

**Figure BG\_4E** shows the distribution of blade tip shapes among the 50 observed mulberries. A majority, accounting for 72 % of the recorded trees, featured an acuminate blade tip, characterised by a tapering and pointed end. A smaller proportion, 14 % of the trees, featured a either an absent or caudate blade tip. The prevalence of the acuminate blade tip aligns with the dominance of the acute leaf apex shape.

The leaf blade margin was assessed in 51 recorded mulberries, revealing various shapes. The predominant type was the crenate (48 %) leaf blade margin, followed by 16 % of mulberries with repand, 10 % each with serrate or biserrate and 8 % each with serrulate or dentate leaf margins (**Figure BG\_4F**).

The assessment of leaf hairiness in 51 mulberries indicated that 49 % had either glabrous leaves or featured hairiness on the midrib and veins, and only a minority of 2 % were evenly pubescent (**Figure BG\_4G**). **Figure BG\_4H** shows that slightly more (54 %) of the recorded trees featured glossy leaves, while the remaining 46 % featured matte leaves. This was expected, as glossy leaves are a characteristic feature of white mulberry.

#### 4.3.5.4 Detailed descriptors of infructescence and inflorescence

Mulberry trees are unique in their sexual expression as they are both monoecious and dioecious, their inflorescences are male or female catkins, appearing also in combinations of both male and female flowers. The small, multiple sweet infructescences (soroses) are a consolidation of drupelets with remnants of perianth. Determining the sexual expression of mulberries is extremely challenging. It is most reliable to ascertain monoecy or dioecy when the plants are in flower. Therefore, we determined the sexual expression in only 17 plants, of which two-thirds (65 %) featured a combination of monoecious and dioecious, one-third (29 %) were clearly monoecious and only 6 % were clearly dioecious (**Figure BG\_5A**).

We determined inflorescence types in 17 recorded mulberries, the majority (76 %) was male, while 18 % were male and female and 6 % were predominantly female with some male flowers at the base (**Figure BG\_4B**).

In **Figure BG\_5C**, stigma persistency on the infructescence of three mulberries is shown. The data revealed that two of the recorded mulberries featured a non-persistent stigma, while one featured a persistent stigma.

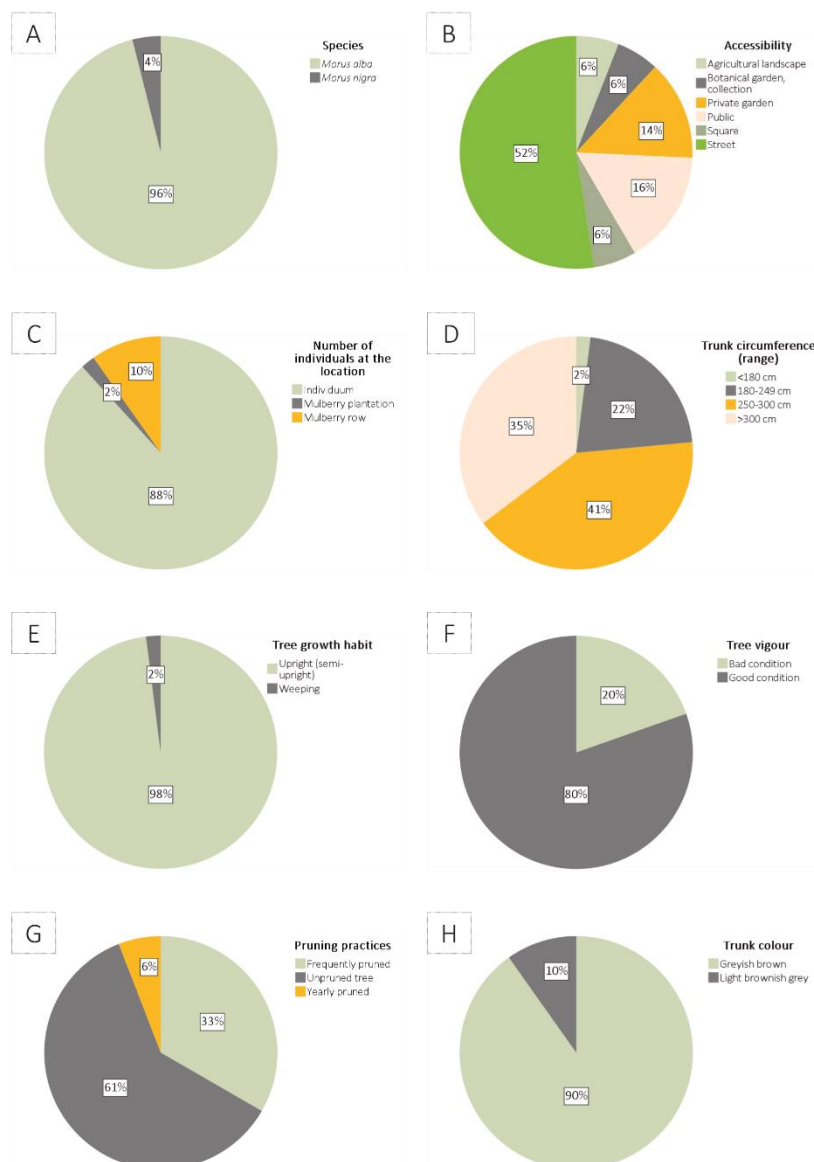
We assessed peduncle length in four mulberry trees, revealing that three infructescences had a medium-sized peduncle, one had a short-sized peduncle, and none had a long-sized peduncle (**Figure BG\_5D**).

Among the four mulberries recorded for infructescence colours, two had yellowish-white, the other two had black infructescences **Figure BG\_5E**. Out of four recorded mulberries, the infructescence taste was sweet in two, while the other two had a balanced taste (**Figure BG\_5F**).

We assessed the infructescence shape in four recorded mulberries; three had an ellipsoid shape and one had a cylindrical shape (**Figure BG\_5G**). The uniformity of infructescence ripeness was assessed in four recorded mulberries. Three mulberries showed prolonged uniformity of ripeness, while one showed uniform ripeness (**Figure BG\_5H**).



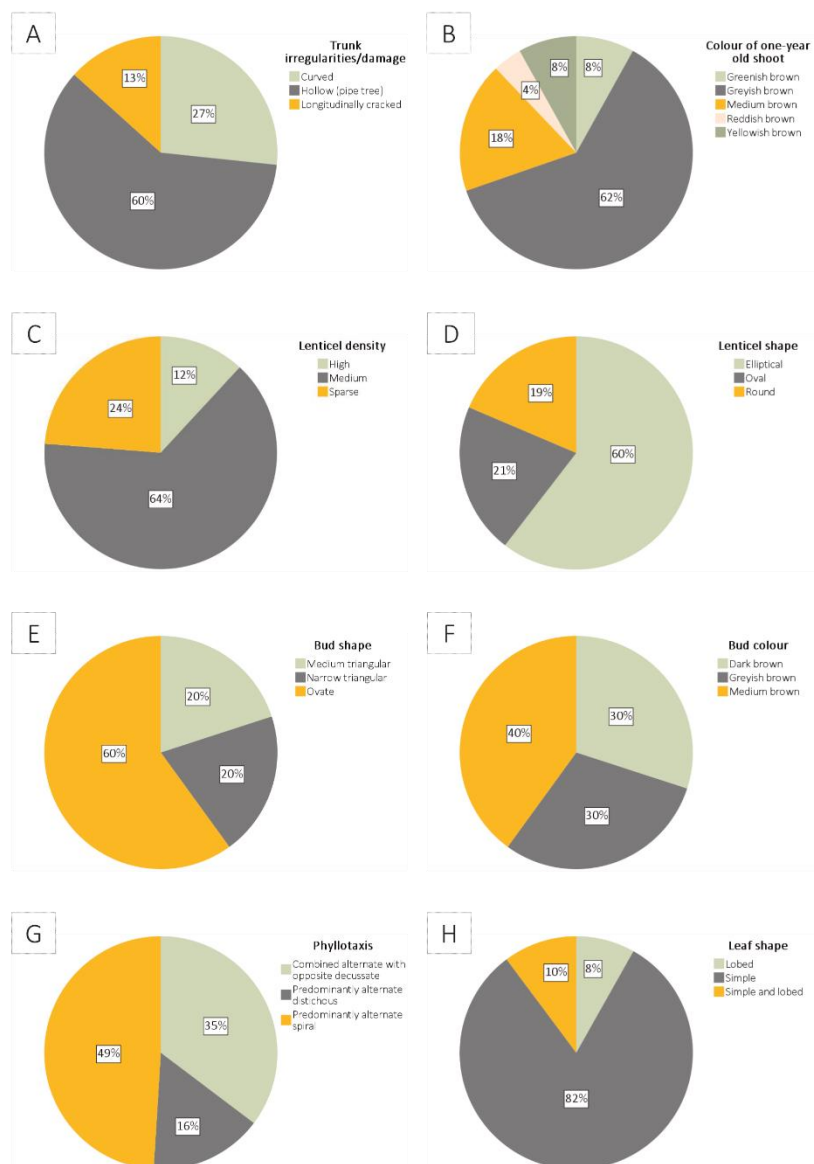
## Inventarisation of mulberries in Bulgaria



**Figure BG\_2** – Basic information on the inventory of mulberry trees in Bulgaria. A) Percentage of recorded *M. alba* and *M. nigra* trees. B) Accessibility options of trees. C) State on the number of individuals at location. D) Percentage of trees at certain trunk circumference range. E) Percentage of trees of upright and weeping growth habit. F) Percentage of trees in bad and good condition. G) Report on the pruning frequency of the trees. H) The percentage of trees of different trunk colours.



## Inventarisation of mulberries in Bulgaria



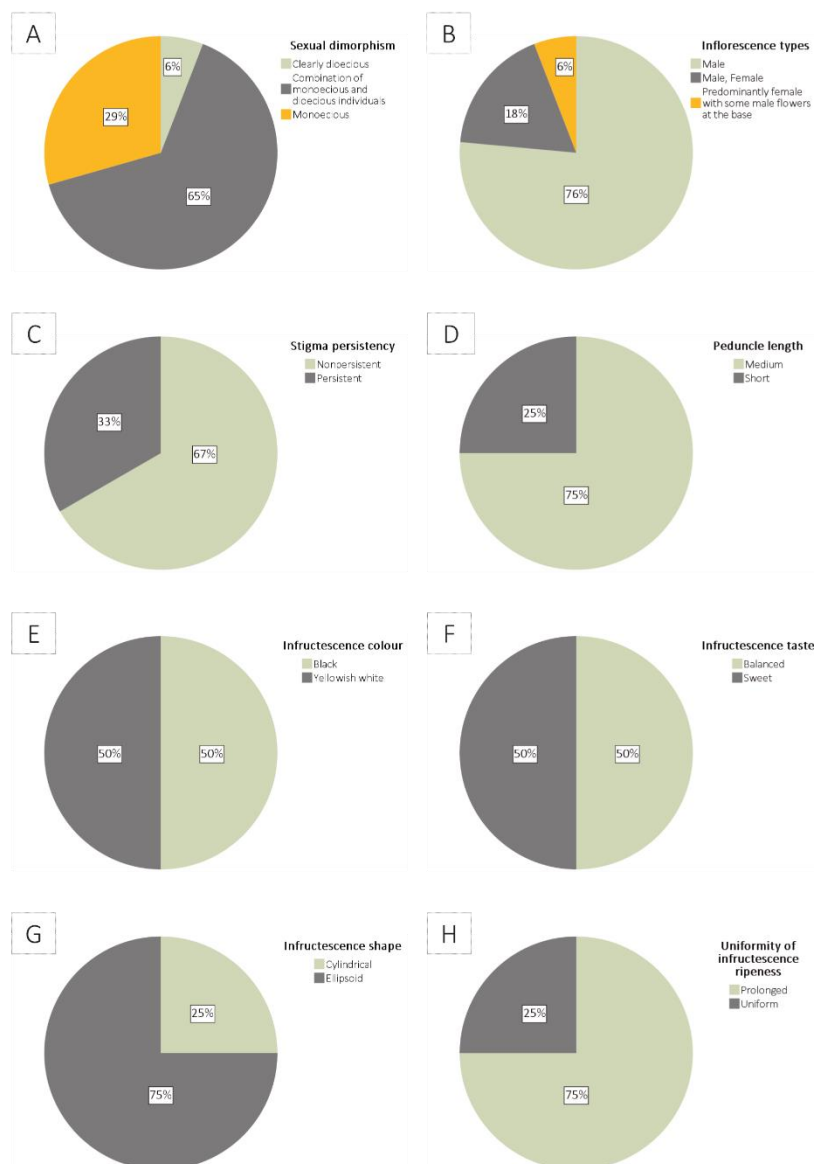
**Figure BG\_3** - Inventory of mulberries in Bulgaria. Detailed descriptors of one-year old shoots and leaf morphology. A) Trunk irregularities or damage. B) Colour of one-year old shoot. C) Lenticel density. D) Lenticel shape. E) Bud shape. F) Bud colour. G) Phyllotaxis. H) Leaf shape.

## Inventarisation of mulberries in Bulgaria



**Figure BG\_4** - Inventory of mulberries in Bulgaria. Detailed descriptors of leaf morphology. A) Leaf blade. B) Petiole range. C) Shape of leaf base. D) Shape of leaf apex. E) Leaf blade tip. F) Leaf blade margin. G) Leaf hairiness. H) Leaf glossiness.

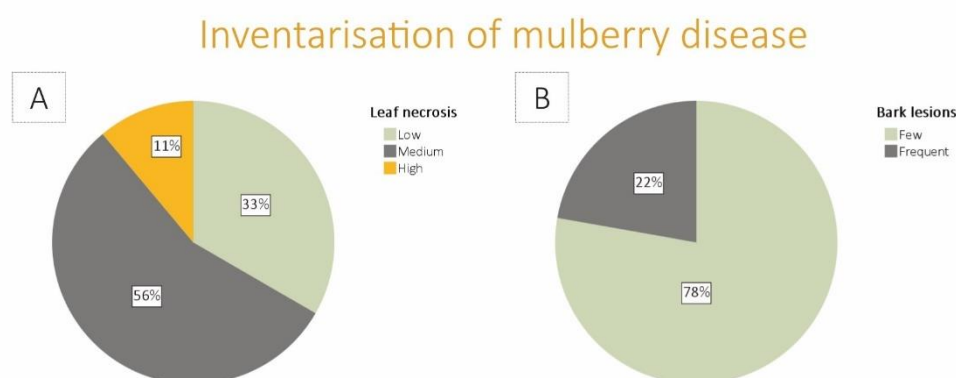
## Inventarisation of mulberries in Bulgaria



**Figure BG\_5** - Inventory of mulberries in Bulgaria. Infructescences and Inflorescence descriptors: A) Sexual dimorphism. B) Inflorescence types. C) Stigma persistency. D) Pedicel length. E) Infructescence colour types. F) Infructescence taste. G) Infructescence shape types. H) Uniformity of infructescence ripeness.

#### 4.3.5.5 Report on mulberry diseases in Bulgaria

**Figure BG\_6A** shows the incidence of leaf necrosis among a total of nine observed mulberries. The majority (56 %) of leaves exhibited medium leaf necrosis, while 33 % displayed medium necrosis, and a minority showed a high frequency of leaf necrosis. **Figure BG\_6B** shows the frequency of bark lesions in nine observed mulberries. The majority, comprising 78 %, exhibited few bark lesions, while 22 % displayed a frequent occurrence of bark lesions.



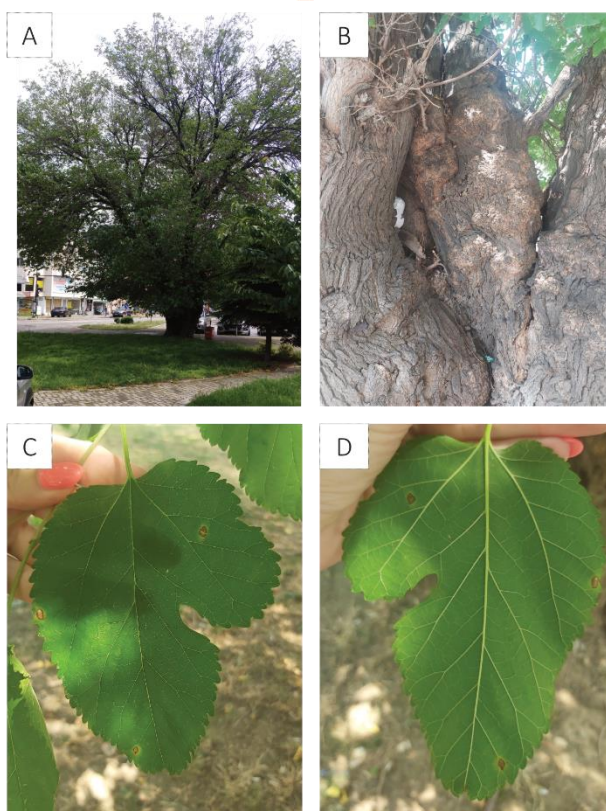
**Figure BG\_6** - Inventory of mulberries in Bulgaria. Diseases. A) Leaf necrotic spots. B) Bark lesions.

#### 4.3.5.6 Monumental mulberry trees of Bulgaria

Among the surveyed trees, sixteen boast circumferences ranging from 300 to 490 cm. Additionally, three trees feature circumferences between 400 and 500 cm. Notably, one tree (**BG23\_00672**) stands out with a circumference of 630 cm, forming part of three very old trees in Plovdiv. Another tree (**BG23\_00641**), with a circumference of 530 cm, graces the square of Sevlievo. In Vratsa, nine monumental trees with circumferences exceeding 300 cm can be found. **BG23\_00655** has a circumference of 357 cm and is characterized by a very long leaf and high length/width ratio. Further trees located in Vratsa and listed in the figures are **BG23\_00659** (CBH 440 cm), **BG23\_00661** (CBH 362 cm) and **BG23\_00667** (CBH: 433 cm), while Slivovik is home to two such remarkable specimens (**BG23\_00673**, CBH 340 cm).

Further tree **BG23\_00626** (CBH 414 cm) is located in Dupnica, **BG23\_00628** (CBH 380 cm) is located in Kormyansko and **BG23\_00669** (CBH 390 cm) is located in Glavatski.

## BG23\_00626



**Figure BG23\_00626:** A) Tree habitus; B) Trunk aspect; C) Lobed leaf adaxial; D) Lobed leaf abaxial.

Basic descriptors	Species: <i>Morus alba</i> L.
	Location: Bulgaria, Dupnitsa
	GPS coordinates: 42.266458,23.122608
	Accessibility: Public
	Number of individuals at the location: Individuum
Trunk	Trunk circumference (cm): 414 cm
	Tree growth habit: Upright (semi-upright)
	Tree vigour: Good condition
Leaves	Pruning practices: Unpruned tree
	Trunk colour: Greyish brown
	Trunk irregularities/damage: Hollow (pipe tree)
Leaves	Phyllotaxis: Predominantly alternate spiral
	Leaf shape: Simple
	Leaf blade: Medium (1.3-1.5)
	Petiole range: Medium (21-40mm); 40 mm
	Shape of leaf base: Truncate
	Shape of leaf apex: Acute
	Leaf blade tip: Acuminate
	Leaf blade margin: Crenate



## BG23\_00628

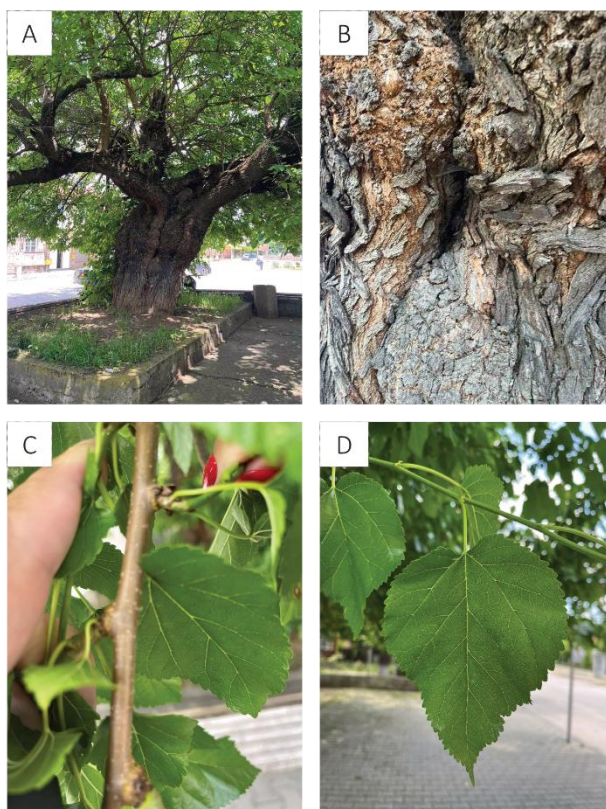


**Figure BG23\_00628:** A) Tree habitus; B) Trunk aspect; C) Simple leaf adaxial; D) One year-old shoot.

Basic descriptors	Species: <i>Morus alba</i> L. Location: Bulgaria, Kormyansko GPS coordinates: 43.05367,25.03492 Accessibility: Public Number of individuals at the location: Individuum Trunk circumference (cm): 380 cm Tree growth habit: Upright (semi-upright) Tree vigour: Good condition Pruning practices: Unpruned tree
Trunk	Trunk colour: Greyish brown Trunk irregularities/damage: Hollow (pipe tree)
Leaves	Phyllotaxis: Combined alternate with opposite decussate Leaf shape: Simple Leaf blade: Medium (1.3-1.5) Petiole range: Long (41-70mm); 60 mm Shape of leaf base: Truncate Shape of leaf apex: Acute Leaf blade tip: Acuminate Leaf blade margin: Serrate



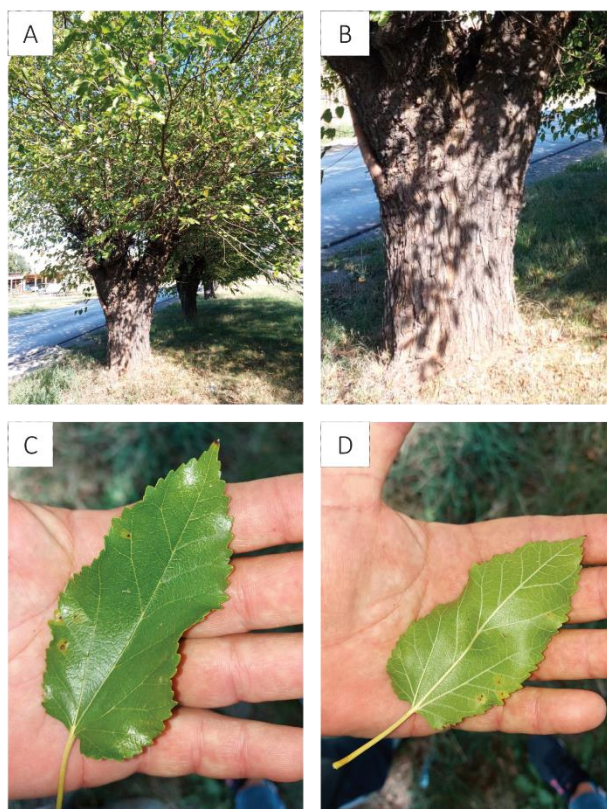
BG23\_00641



**Figure BG23\_00641:** A) Tree habitus; B) Trunk aspect; C) One-year old shoot; D) Simple leaf abaxial.

Basic descriptors	Species: <i>Morus alba</i> L. Location: Bulgaria, Sevlievo GPS coordinates: 43.05613,24.97117 Accessibility: Square Number of individuals at the location: Individuum Trunk circumference (cm): 530 cm Tree growth habit: Upright (semi-upright) Tree vigour: Good condition Pruning practices: Unpruned tree
Trunk	Trunk colour: Greyish brown Trunk irregularities/damage: Hollow (pipe tree)
Leaves	Phyllotaxis: Predominantly alternate distichous Leaf shape: Simple Leaf blade: Low (<1.2, broad leaves) Petiole range: Long (41-70mm) Shape of leaf base: Retuse Shape of leaf apex: Acute Leaf blade tip: Acuminate Leaf blade margin: Crenate

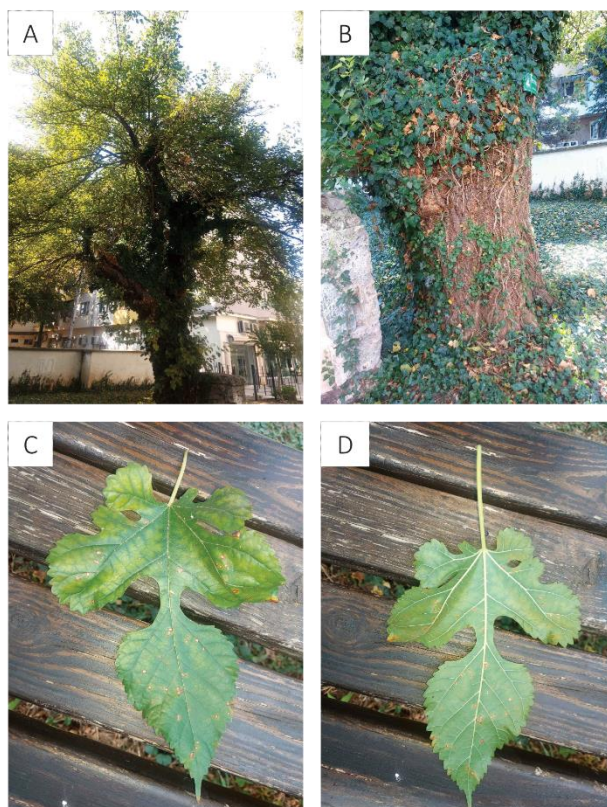
BG23\_00655



**Figure BG23\_00655:** A) Tree habitus; B) Trunk aspect; C) Simple leaf adaxial; D) Simple leaf abaxial.

Basic descriptors	Species: <i>Morus alba</i> L. Location: Bulgaria, Vratsa GPS coordinates: 43.2253579,23.5422082 Accessibility: Street Number of individuals at the location: Mulberry row Trunk circumference (cm): 357 cm Tree growth habit: Upright (semi-upright) Tree vigour: Good condition Pruning practices: Frequently pruned
Trunk	Trunk colour: Greyish brown Trunk irregularities/damage: Hollow (pipe tree)
Leaves	Phyllotaxis: Predominantly alternate distichous Leaf shape: Simple Leaf blade: High (>1.6, long leaves) Petiole range: Long (41-70mm); Shape of leaf base: Truncate Shape of leaf apex: Acute Leaf blade tip: Acuminate Leaf blade margin: Dentate

BG23\_00659

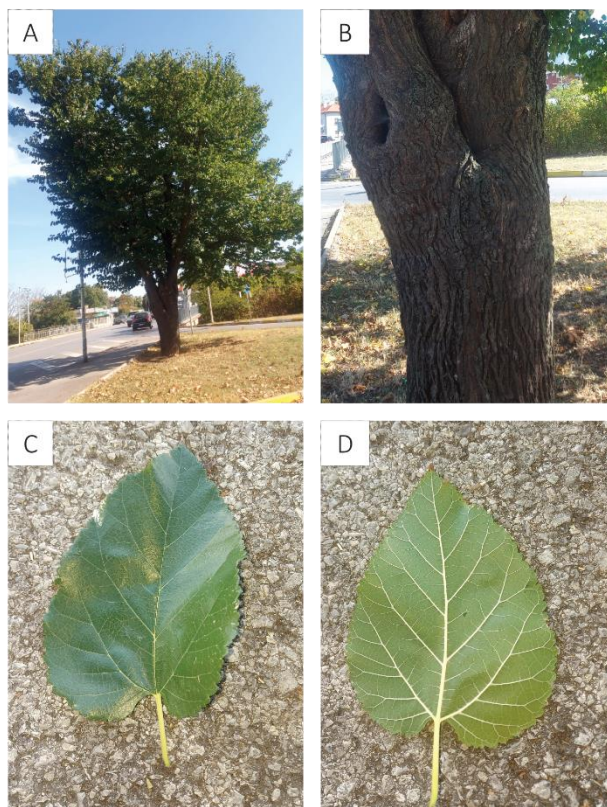


**Figure BG23\_00659:** A) Tree habitus; B) Trunk aspect; C) Lobed leaf adaxial; D) Lobed leaf abaxial.

Basic descriptors	<p>Species: <i>Morus alba</i> L.</p> <p>Location: Bulgaria, Vratsa</p> <p>GPS coordinates: 43.204199,23.551574</p> <p>Accessibility: Botanical garden, collection</p> <p>Number of individuals at the location: Individuum</p> <p>Trunk circumference (cm): 470 cm</p> <p>Tree growth habit: Upright (semi-upright)</p> <p>Tree vigour: Good condition</p> <p>Pruning practices: Unpruned tree</p>
Trunk	<p>Trunk colour: Greyish brown</p> <p>Trunk irregularities/damage: Curved</p>
Leaves	<p>Phyllotaxis: Combined alternate with opposite</p> <p>Leaf shape: Lobed</p> <p>Leaf blade: High (&gt;1.6, long leaves)</p> <p>Petiole range: Very long (&gt;71mm); 80 cm</p> <p>Shape of leaf base: Retuse</p> <p>Shape of leaf apex: Acute</p> <p>Leaf blade tip: Caudate</p> <p>Leaf blade margin: Serrate</p>



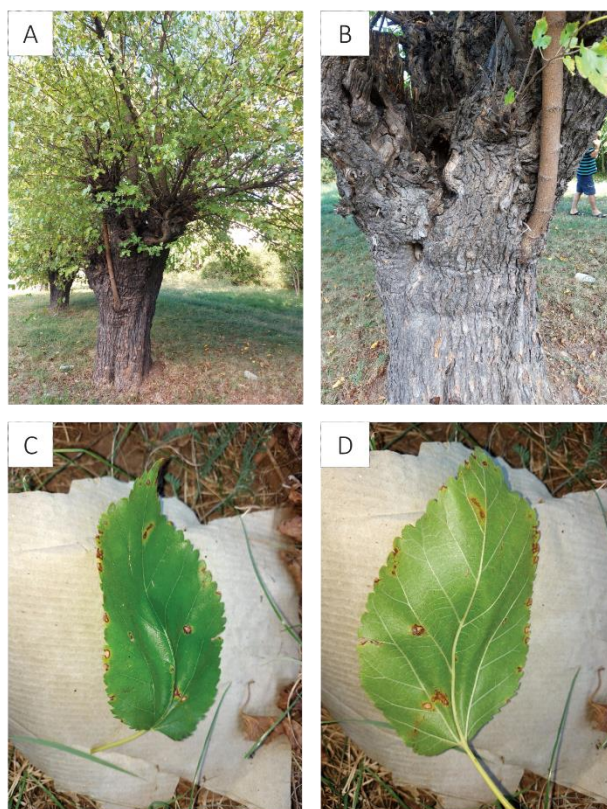
BG23\_00661



**Figure BG23\_00661:** A) Tree habitus; B) Trunk aspect; C) Simple leaf adaxial; D) Simple leaf abaxial.

Basic descriptors	Species: <i>Morus alba</i> L. Location: Bulgaria, Vratsa GPS coordinates: 43.210352,23.552472 Accessibility: Street Number of individuals at the location: Individuum Trunk circumference (cm): 362 cm Tree growth habit: Upright (semi-upright) Tree vigour: Good condition Pruning practices: Frequently pruned
Trunk	Trunk colour: Greyish brown Trunk irregularities/damage: Hollow (pipe tree)
Leaves	Phyllotaxis: Combined alternate with opposite decussate Leaf shape: Simple Leaf blade: Medium (1.3-1.5) Petiole range: Medium (21-40mm); 40 mm Shape of leaf base: Cordate Shape of leaf apex: Acute Leaf blade tip: Acuminate Leaf blade margin: Seratte

BG23\_00667

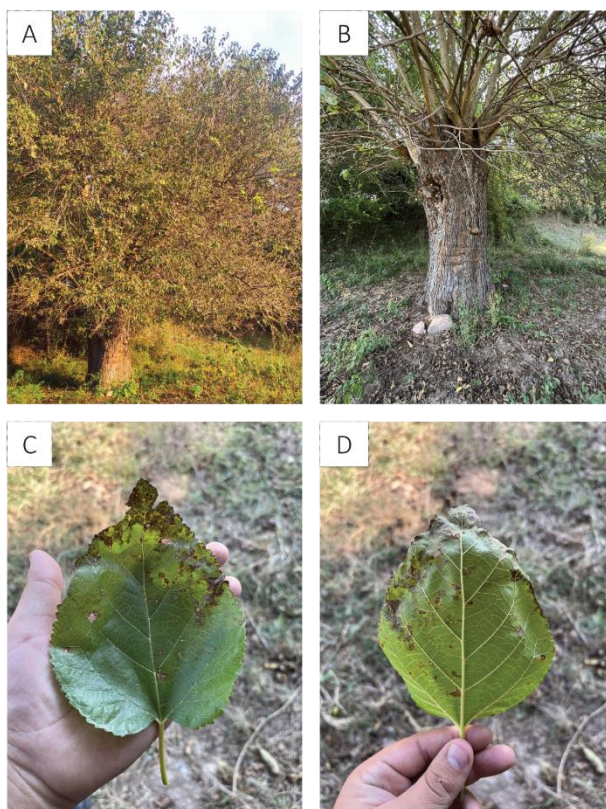


**Figure BG23\_00667:** A) Tree habitus; B) Trunk aspect; C) Simple leaf adaxial; D) Simple leaf abaxial.

Basic descriptors	Species: <i>Morus alba</i> L. Location: Bulgaria, Vratsa GPS coordinates: 43.224682,23.542958 Accessibility: Street Number of individuals at the location: Individuum Trunk circumference (cm): 433 cm Tree growth habit: Upright (semi-upright) Tree vigour: Good condition Pruning practices: Frequently pruned
Trunk	Trunk colour: Greyish brown Trunk irregularities/damage: Curved
Leaves	Phyllotaxis: Combined alternate with opposite decussate Leaf shape: Simple Leaf blade: High (>1.6, long leaves) Petiole range: Medium (21-40mm); 30 cm Shape of leaf base: Cuneate Shape of leaf apex: Acute Leaf blade tip: Caudate Leaf blade margin: Crenate



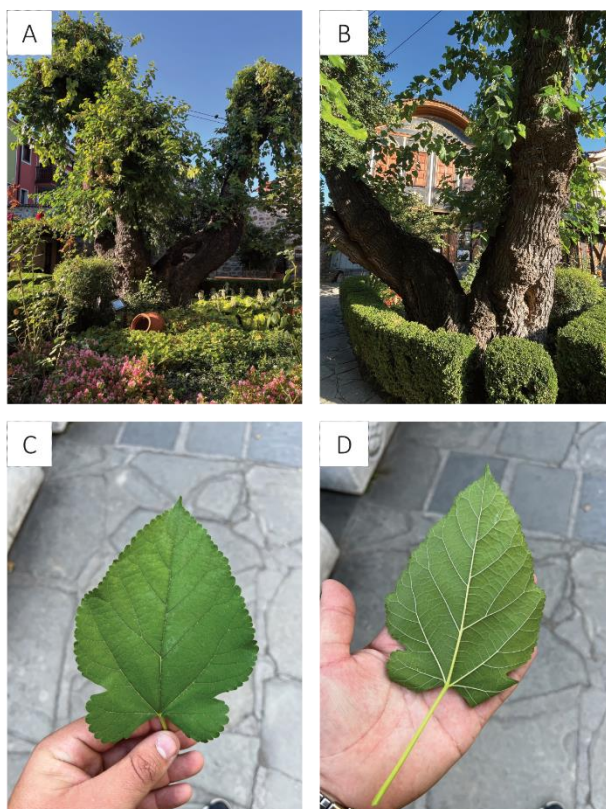
BG23\_00669



**Figure BG23\_00669:** A) Tree habitus; B) Trunk aspect; C) Simple leaf adaxial; D) Simple leaf abaxial.

Basic descriptors	Species: <i>Morus alba</i> L. Location: Bulgaria, Glavatsi, Krivodol GPS coordinates: 43.2936718,23.3802634 Accessibility: Public Number of individuals at the location: Individuum Trunk circumference (cm): 390 cm Tree growth habit: Upright (semi-upright) Tree vigour: Good condition Pruning practices: Unpruned tree
Trunk	Trunk colour: Greyish brown Trunk irregularities/damage: /
Leaves	Phyllotaxis: Predominantly alternate spiral Leaf shape: Simple Leaf blade: Low (<1.2, broad leaves) Petiole range: Long (41-70mm); 42 mm Shape of leaf base: Cordate Shape of leaf apex: Acute Leaf blade tip: Absent Leaf blade margin: Crenate

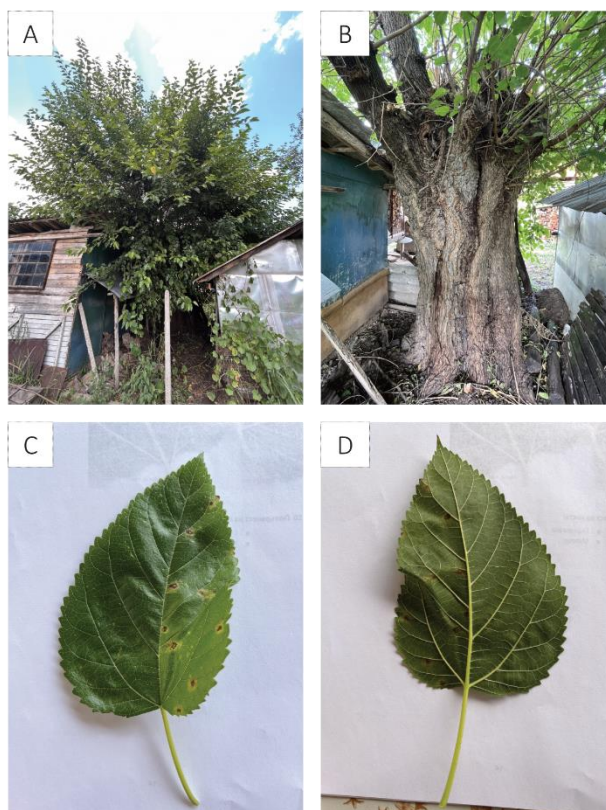
## BG23\_00672



**Figure BG23\_00672:** A) Tree habitus; B) Trunk aspect; C) Simple leaf adaxial; D) Lobed leaf abaxial.

Basic descriptors	Species: <i>Morus alba</i> L.
	Location: Bulgaria, Plovdiv
	GPS coordinates: 42.1501165,24.7531096
	Accessibility: Public
	Number of individuals at the location: Mulberry row
Trunk	Trunk circumference (cm): 630 cm
	Tree growth habit: Upright (semi-upright)
	Tree vigour: Good condition
	Pruning practices: Yearly pruned
	Trunk colour: Greyish brown
Leaves	Trunk irregularities/damage: Curved
	Phyllotaxis: Predominantly alternate distichous
	Leaf shape: Simple
	Leaf blade: Medium (1.3-1.5)
	Petiole range: Long (41-70mm); 70 mm
	Shape of leaf base: Cordate
	Shape of leaf apex: Acute
	Leaf blade tip: Acuminate
	Leaf blade margin: Crenate

BG23\_00673



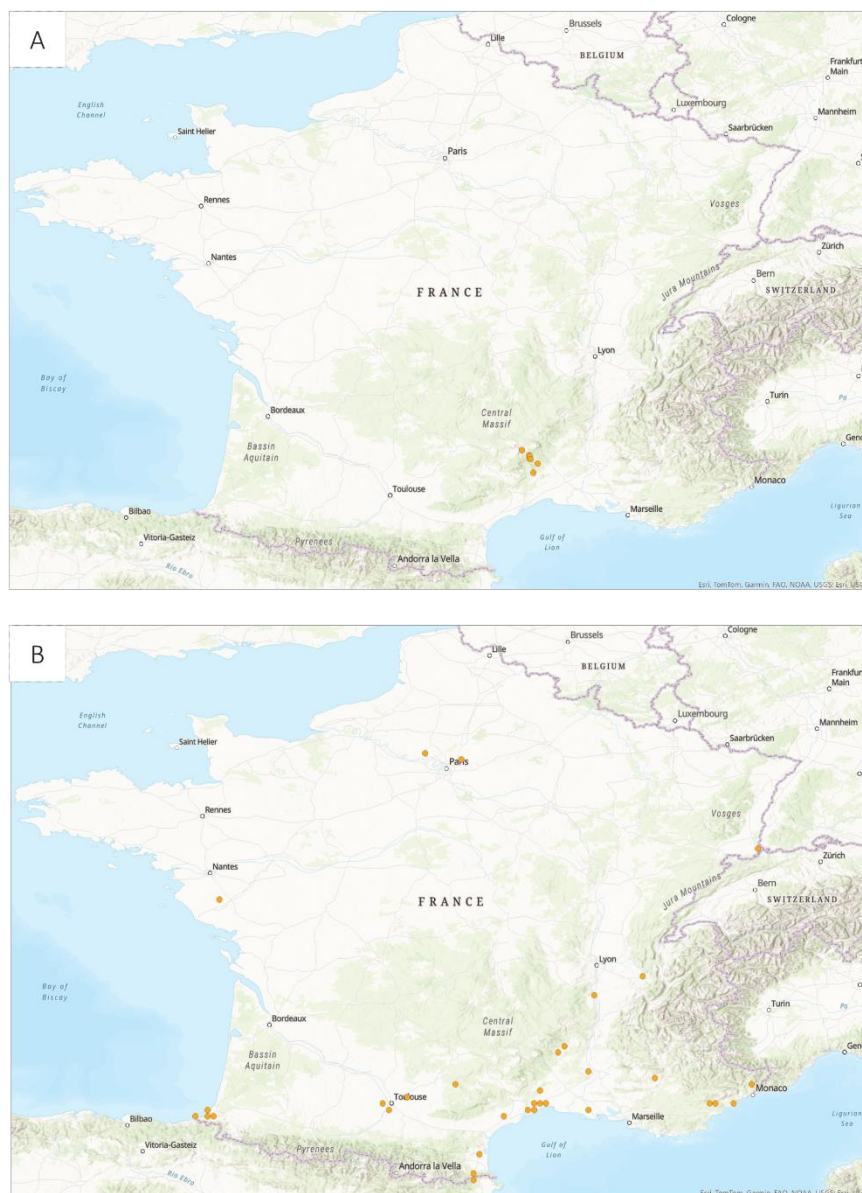
**Figure BG23\_00673:** A) Tree habitus; B) Trunk aspect; C) Simple leaf adaxial; D) Simple leaf abaxial.

Basic descriptors	<p>Species: <i>Morus alba</i> L.</p> <p>Location: Bulgaria, Slivovik, Medkovets</p> <p>GPS coordinates: 43.5984472,23.1102743</p> <p>Accessibility: Private garden</p> <p>Number of individuals at the location: Individuum</p> <p>Trunk circumference (cm): 340 cm</p> <p>Tree growth habit: Upright (semi-upright)</p> <p>Tree vigour: Good condition</p> <p>Pruning practices: Frequently pruned</p>
Trunk	<p>Trunk colour: Greyish brown</p> <p>Trunk irregularities/damage: Hollow (pipe tree)</p>
Leaves	<p>Phyllotaxis: Predominantly alternate spiral</p> <p>Leaf shape: Simple</p> <p>Leaf blade: High (&gt;1.6, long leaves)</p> <p>Petiole range: Long (41-70mm); 53 mm</p> <p>Shape of leaf base: Truncate</p> <p>Shape of leaf apex: Acute</p> <p>Leaf blade tip: Acuminate</p> <p>Leaf blade margin: Serrate</p>



## 4.3.6 Report on inventory of mulberries in France

### 4.3.6.1 Basic information



**Figure FR\_1** - Distribution map of mulberries in France A) using *MorusAPP*, B) using *in-silico* analysis *GBIF* (2019-2024). For France, *in-silico* analysis via GBIF (Global Biodiversity Information Facility) revealed that 34 mulberry trees were recorded between 2019 and 2024.

In 2023, a total of 11 mulberry trees from France were recorded via the *MorusAPP* (**Figure FR\_1A**) and an *in-silico* analysis via GBIF (Global Biodiversity Information Facility) revealed that 34 mulberry trees were recorded between 2019 and 2024 (**Figure FR\_1B**). Inventory of

French mulberries (**Figure FR\_2**) by species indicates that all of the 11 recorded trees were white mulberries (*M. alba*, **Figure FR\_2A**). **Figure FR\_2B** shows the accessibility options of mulberries recorded in France. A slight majority (36 %) were found in public areas. The second most common with 27 % each, were mulberries, grown in botanical gardens or collections and in private gardens. Only 10 % were found along the streets. None were found in squares. A slight majority of mulberries in France grew either individually or in mulberry rows (36 %), while we recorded 28 % in plantations (**Figure FR\_1C**).

The distribution of mulberries based on circumference was dominated by trees with a circumference below 180 cm (73 %), while a small percentage (9 %) had either a trunk circumference between 180 and 249 cm, a large circumference of 250 to 300 cm or a very large circumference of more than 300 cm (**Figure FR\_2D**).

The inventory of mulberries by tree growth habit indicated that all of the recorded trees grew upright, none grew semi-upright or had a weeping growth habit (**Figure FR\_2E**).

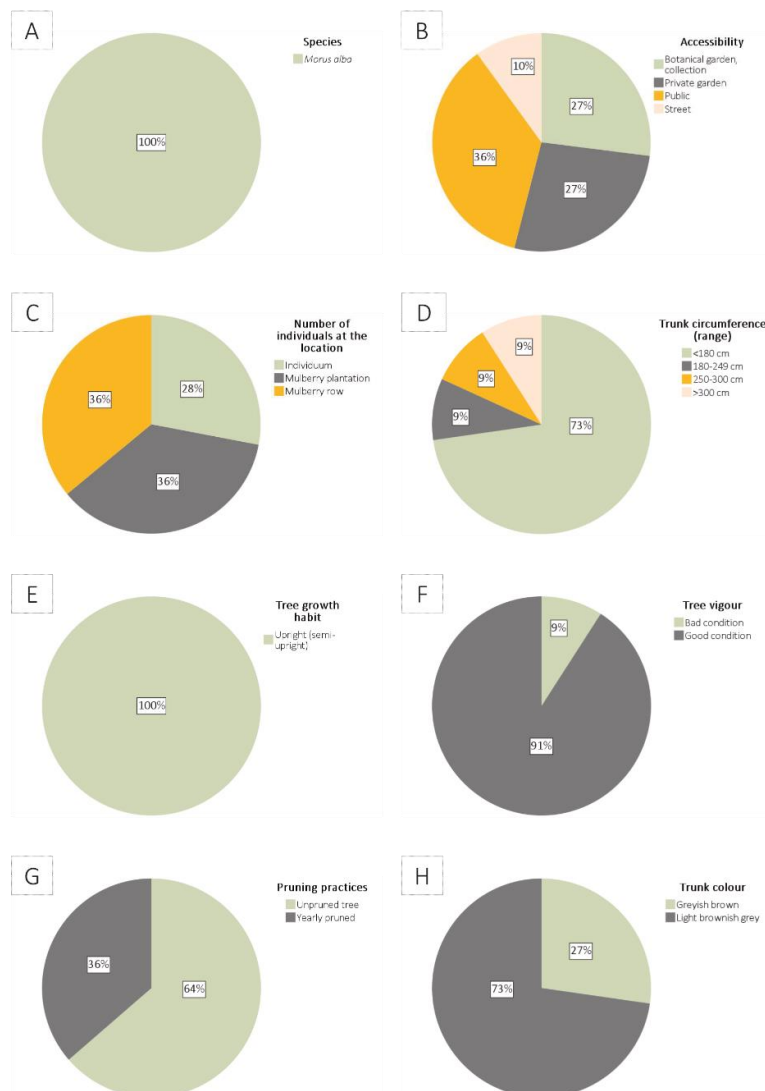
A large majority of mulberries recorded in France was in good condition (91 %), some trees (9 %) were in bad condition (**Figure FR\_2F**).

**Figure FR\_2G** shows the pruning practices of the mulberries recorded in France. Unfortunately, two-thirds of the recorded mulberries were unpruned (64 %). One third of the trees (36 %), was yearly pruned.

The predominant trunk colour among the recorded mulberries, representing 73 %, was light brownish-grey, while 27 % featured a greyish brown trunk colour (**Figure FR\_2H**).



## Inventarisation of mulberries in France



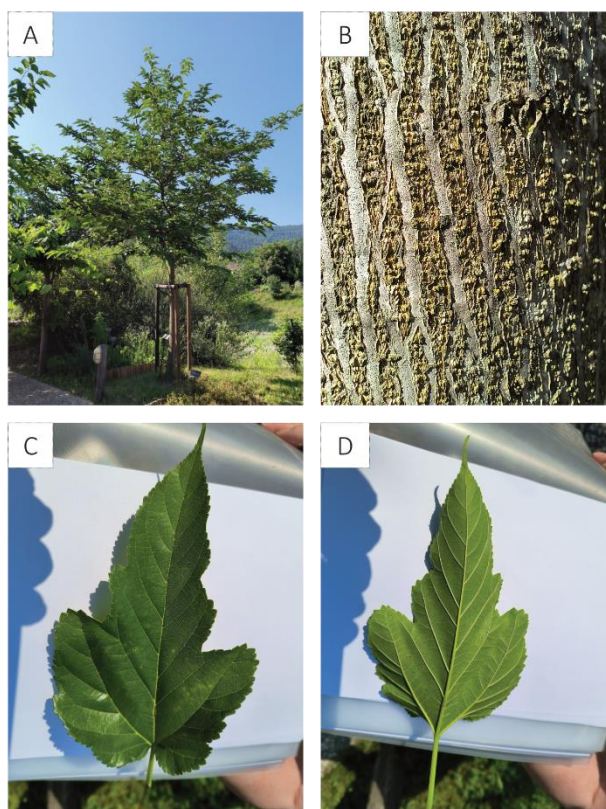
**Figure FR\_2** - Basic information on the inventory of mulberry trees in France. A) Percentage of recorded *M. alba* trees. B) Accessibility options of trees. C) State on the number of individuals at location. D) Percentage of trees at certain trunk circumference range. E) Percentage of trees of upright and weeping growth habit. F) Percentage of trees in bad and good condition. G) Report on the pruning frequency of the trees. H) The percentage of trees of different trunk colours.

#### 4.3.6.2 Monumental mulberry trees of France

The largest mulberry tree (**FR23\_00601**) with a circumference of 302 cm is one of the trees in a row in Saint-Martin de Lansuscle. Another tree, **FR23\_00594**, with a circumference of 251 cm, was recorded in La Cadere-et-Cambo. **FR23\_00599** is one of the tree in a row with circumference of 135 cm in Sainte-Croix-Vallee Francaise.

The trees **FR23\_00297**, *M. indica* s.l. (syn. *M. multicaulis*), and **FR23\_00298**, variety 'Colombassette', are part of the collection of the Maison Rouge - Musée des Vallées Cévenoles in Saint-Jean-du-Gard, France, which is dedicated to the history and culture of the Cévennes valleys, with a particular focus on traditional industries, including sericulture. Beside the collection of historical tools and equipment used in the cultivation of mulberry trees, the care and feeding of silkworms, and the processing of silk threads the museum exhibits 7 trees of circumference 30-60 cm of different varieties.

FR23\_00297



**Figure FR23\_00297:** A) Tree habitus; B) Trunk aspect; C) Lobed leaf adaxial; D) Lobed leaf abaxial.

Basic descriptors	Species: <i>Morus alba</i> L. Location: France, Saint-Jean-du-Gard GPS coordinates: 44.1039537, 3.8872684 Accessibility: Botanical garden, collection Number of individuals at the location: Mulberry plantation Trunk circumference (cm): 37 cm Tree growth habit: Upright (semi-upright) Tree vigour: Good condition Pruning practices: Yearly pruned
Trunk	Trunk colour: Light brownish grey Trunk irregularities/damage: /
Leaves	Phyllotaxis: Predominantly alternate distichous Leaf shape: Lobed Leaf blade: Medium (1.3-1.5) Petiole range: Medium (21-40mm); 35 mm Shape of leaf base: Cordate Shape of leaf apex: Acute Leaf blade tip: Caudate Leaf blade margin: Crenate

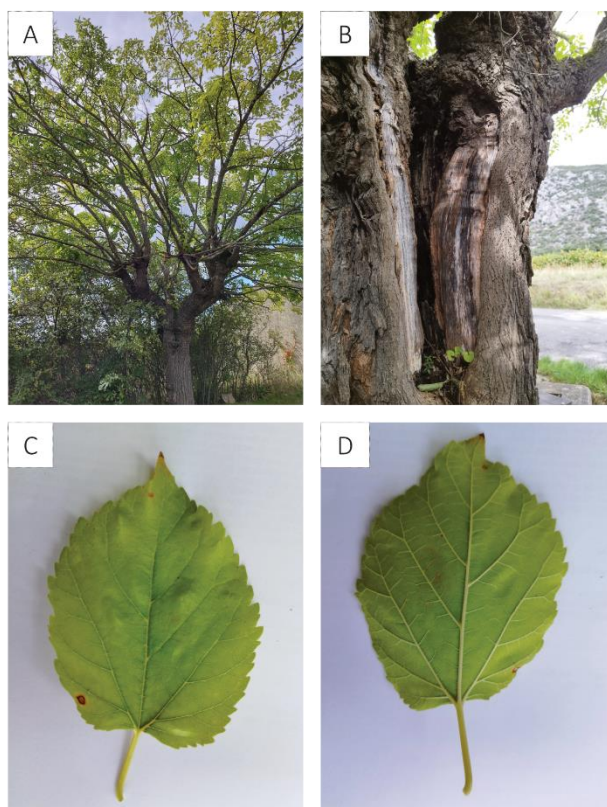
FR23\_00298



**Figure FR23\_00298:** A) Tree habitus; B) Trunk aspect; C) Simple leaf adaxial; D) Simple leaf abaxial.

Basic descriptors	<p>Species: <i>Morus alba</i> L.</p> <p>Location: France, Saint-Jean-du-Gard</p> <p>GPS coordinates: 44.1037362, 3.8860879</p> <p>Accessibility: Botanical garden, collection</p> <p>Number of individuals at the location: Mulberry plantation</p> <p>Trunk circumference (cm): 32 cm</p> <p>Tree growth habit: Upright (semi-upright)</p> <p>Tree vigour: Good condition</p> <p>Pruning practices: Yearly pruned</p>
Trunk	<p>Trunk colour: Light brownish grey</p> <p>Trunk irregularities/damage:</p>
Leaves	<p>Phyllotaxis: Predominantly alternate spiral</p> <p>Leaf shape: Simple</p> <p>Leaf blade: Medium (1.3-1.5)</p> <p>Petiole range: Long (41-70mm); 52 mm</p> <p>Shape of leaf base: Retuse</p> <p>Shape of leaf apex: Acute</p> <p>Leaf blade tip: Acuminate</p> <p>Leaf blade margin: Crenate</p>

FR23\_00594

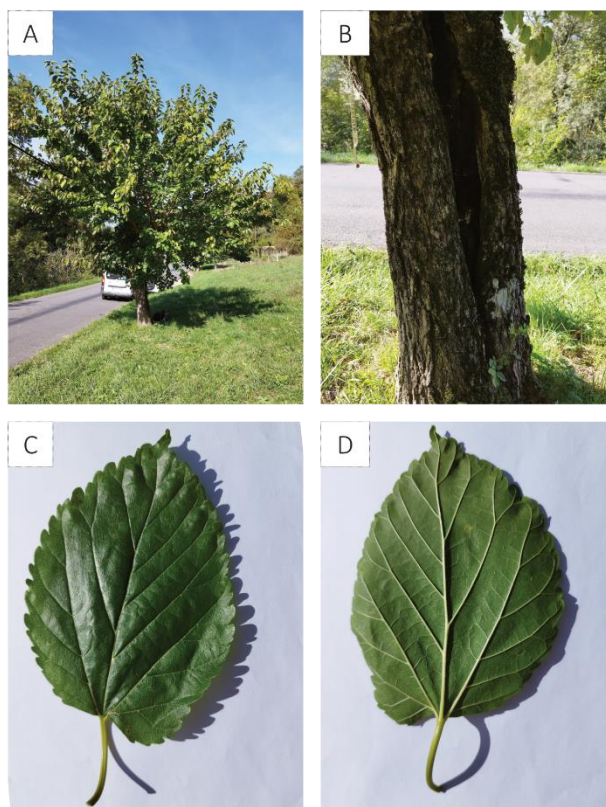


**Figure FR23\_00594:** A) Tree habitus; B) Trunk aspect; C) Simple leaf adaxial; D) Simple leaf abaxial.

Basic descriptors	Species: <i>Morus alba</i> L. Location: France, La Cadière-et-Cambo GPS coordinates: 44.1806782, 3.7430329 Accessibility: Street Number of individuals at the location: Individuum Trunk circumference (cm): 251 cm Tree growth habit: Upright (semi-upright) Tree vigour: Good condition Pruning practices: Unpruned tree
Trunk	Trunk colour: Greyish brown Trunk irregularities/damage: Hollow (pipe tree)
Leaves	Phyllotaxis: Predominantly alternate spiral Leaf shape: Simple Leaf blade: Medium (1.3-1.5) Petiole range: Medium (21-40mm); 27 mm Shape of leaf base: Truncate Shape of leaf apex: Acute Leaf blade tip: Acuminate Leaf blade margin: Dentate



FR23\_00599



**Figure FR23\_00599:** A) Tree habitus; B) Trunk aspect; C) Simple leaf adaxial; D) Simple leaf abaxial.

Basic descriptors

Species: *Morus alba* L.

Location: France, Sainte-Croix-Vallée-Française

GPS coordinates: 44.1806782, 3.7430329

Accessibility: Public

Number of individuals at the location: Mulberry row

Trunk circumference (cm): 135 cm

Tree growth habit: Upright (semi-upright)

Tree vigour: Bad condition

Pruning practices: Unpruned tree

Trunk

Trunk colour: Greyish brown

Trunk irregularities/damage: Hollow (pipe tree)

Leaves

Phyllotaxis: Predominantly alternate spiral

Leaf shape: Simple

Leaf blade: Medium (1.3-1.5)

Petiole range: Medium (21-40mm); 40 mm

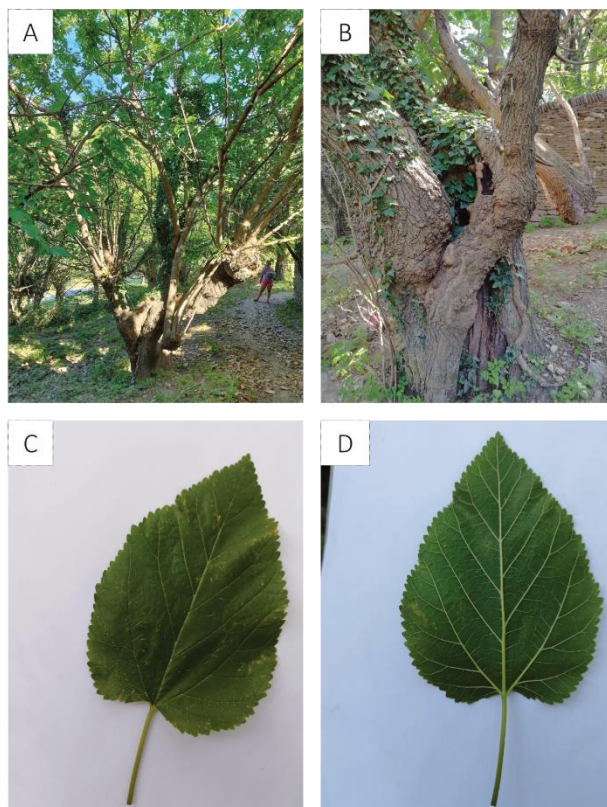
Shape of leaf base: Retuse

Shape of leaf apex: Acute

Leaf blade tip: Acuminate

Leaf blade margin: Crenate

FR23\_00601

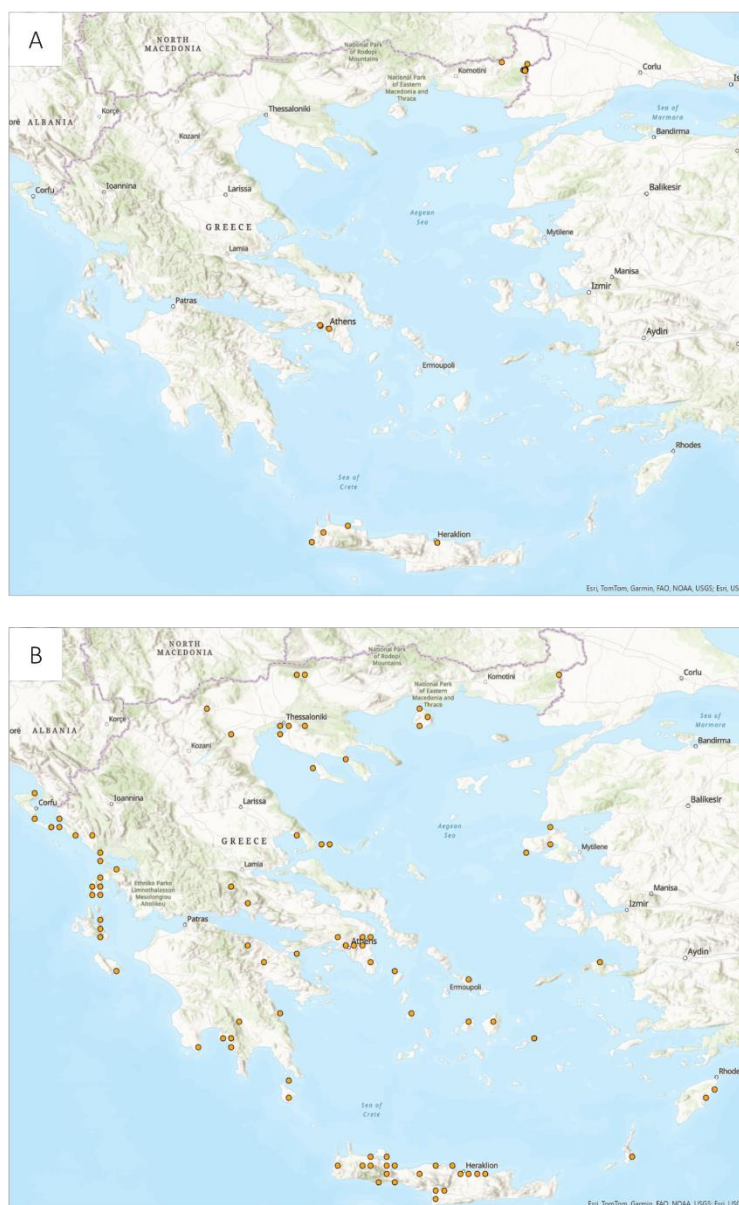


**Figure FR23\_00601:** A) Tree habitus; B) Trunk aspect; C) Simple leaf adaxial; D) Simple leaf abaxial.

Basic descriptors	Species: <i>Morus alba</i> L. Location: France, Saint-Martin de Lansuscle GPS coordinates: 44.2172764, 3.753392 Accessibility: Public Number of individuals at the location: Mulberry row Trunk circumference (cm): 302 cm Tree growth habit: Upright (semi-upright) Tree vigour: Good condition Pruning practices: Unpruned tree
Trunk	Trunk colour: Greyish brown Trunk irregularities/damage: Curved
Leaves	Phyllotaxis: Predominantly alternate spiral Leaf shape: Simple Leaf blade: Medium (1.3-1.5) Petiole range: Long (41-70mm); 46 mm Shape of leaf base: Retuse Shape of leaf apex: Acute Leaf blade tip: Acuminate Leaf blade margin: Crenate

## 4.3.7 Report on inventory of mulberries in Greece

### 4.3.7.1 Basic information



**Figure GR\_1** - Distribution map of mulberries in Greece A) using *MorusAPP*, B) using *in-silico* analysis *GBIF* (2019-2024). For Greece, *in-silico* analysis via GBIF (Global Biodiversity Information Facility) revealed that 116 mulberry trees were recorded between 2019 and 2024.

In 2023, a total of 33 mulberry trees from Greece were recorded via the *Morus*APP (**Figure GR\_ 1A**) and an *in-silico* analysis via GBIF (Global Biodiversity Information Facility) revealed that 116 mulberry trees were recorded between 2019 and 2024 (**Figure GR\_ 1B**). Inventory of Greek mulberries by species represented in **Figure GR\_ 2** indicates that most recorded trees were white mulberries (*M. alba*) with a total of 28 (85 %), while there were only three (9 %) black mulberries (*M. nigra*) and two mulberries identified at genus level (*Morus* sp.). Compared to other countries, Greece recorded the third highest number of black mulberries (**Figure GR\_ 2A** and **Table 6**).

**Figure GR\_ 2B** shows the accessibility options of mulberries recorded in Greece. A slight majority were found in private gardens (34%). The second most common were mulberries along the streets (27 %), and the third most common were mulberries grown in agricultural areas (21 %). Only 9 % were equally found in botanical gardens or collections and in public areas. None were found in squares. Most mulberries in Greece grew individually (49 %), while we recorded 27 % in mulberry rows and 24 % in plantations (**Figure GR\_ 2C**).

The distribution of mulberries based on circumference was almost equally represented by trees with a circumference below 180 cm (40 %) and trees with a circumference below 249 cm (33 %). Twenty percent of all recorded mulberries in Greece had a very large circumference of more than 300 cm and only 7 % had a large circumference of 250 to 300 cm (**Figure GR\_ 2D**).

The inventory of mulberries by tree growth habit indicated that a majority of the recorded trees grew upright or semi-upright (91 %), while 6 % had a weeping growth habit and only 3 % of trees grew upright (**Figure GR\_ 2E**). A large majority of mulberries recorded in Greece was in good condition (90 %), a minority of trees (10 %) were in bad condition (**Figure GR\_ 2F**).

**Figure GR\_ 2G** shows the pruning practices of the mulberries recorded in Greece. Two thirds of the recorded mulberries were frequently pruned (64 %), 24% were yearly pruned, and a minority (12 %) were yearly pruned. This distribution provided insight into the varied mulberry maintenance practices. The predominant trunk colour among the recorded mulberries, representing 78 %, was greyish-brown, while 22 % featured a light brownish-grey trunk colour (**Figure GR\_ 2H**).

## Inventarisation of mulberries in Greece



**Figure GR\_ 2-** Basic information on the inventory of mulberry trees in Greece. A) Percentage of recorded *M. alba* and *M. nigra* trees. B) Accessibility options of trees. C) State on the number of individuals at location. D) Percentage of trees at certain trunk circumference range. E) Percentage of trees of upright and weeping growth habit. F) Percentage of trees in bad and good condition. G) Report on the pruning frequency of the trees. H) The percentage of trees of different trunk colours.



#### 4.3.7.2 Monumental mulberry trees of Greece

In Greece, the cultivation of mulberry trees and the practice of sericulture are especially connected with regions such as Thessaly, Macedonia, and the Peloponnese. Mulberry trees are planted near homes and sericulture facilities.

In the public park in Athens, a noteworthy tree (**GR23\_00770**) with a circumference of 346 cm was catalogued. Additionally, the Diomedes Botanical Garden is home to three white mulberry trees (**GR23\_00466**, CBH <180 cm; **GR23\_00468**, CBH <180 cm).

In the region of Soufli, known for its sericultural heritage, several mulberry trees from historic plantations were documented. These trees predominantly boast circumferences ranging from 150 to 250 cm. Notably, the widest tree (**GR23\_00031**), originating from one such private garden, measures 331 cm in circumference. **GR23\_00032** is one of the trees in plantation with a circumference of 171 cm. A mulberry row along the street was characterised by trees with a circumference of 250-300 cm (**GR23\_00038**). Mulberries also adorn the main thoroughfare of Soufli (**GR23\_00028**).

On the island of Crete, ten mulberry trees were identified as significant, with most circumferences falling between 180 and 249 cm. In particular, three distinguished rows of mulberry trees were located in Chrisoskalitissa, Topolia, and Knossos (**GR23\_00608**), marking these sites with their historical and botanical significance.

## GR23\_00028



**Figure GR23\_00028:** A) Tree habitus; B) Trunk aspect.

### Basic descriptors

Species: *Morus alba* L.

Location: Greece, Soufli

GPS coordinates: 41.1942349, 26.3052175

Accessibility: Street

Number of individuals at the location: Mulberry row

Trunk circumference (cm): 223 cm

Tree growth habit: Upright (semi-upright)

Tree vigour: Good condition

Pruning practices: Yearly pruned

### Trunk

Trunk colour: Greyish brown

Trunk irregularities/damage: Longitudinally cracked

### Disease

Leaf necrotic spots: /

Bark lesions: /

## GR23\_00031



**Figure GR23\_00031:** A) Tree habitus; B) Trunk aspect.

Basic descriptors	Species: <i>Morus alba</i> L.
	Location: Greece, Soufli
	GPS coordinates: 41.2001833, 26.3075665
	Accessibility: Private garden
	Number of individuals at the location: Mulberry plantation
	Trunk circumference (cm): 331 cm
	Tree growth habit: Upright (semi-upright)
	Tree vigour: Good condition
	Pruning practices: Yearly pruned
Trunk	Trunk colour: Greyish brown
	Trunk irregularities/damage: Split
Disease	Leaf necrotic spots: /
	Bark lesions: /



## GR23\_00032



**Figure GR23\_00032:** A) Tree habitus; B) Trunk aspect.

Basic descriptors	Species: <i>Morus alba</i> L. Location: Greece, Soufli GPS coordinates: 41.2009817, 26.2990539 Accessibility: Agricultural landscape Number of individuals at the location: Mulberry plantation Trunk circumference (cm): 171 cm Tree growth habit: Upright (semi-upright) Tree vigour: Good condition Pruning practices: Frequently pruned
Trunk	Trunk colour: Greyish brown Trunk irregularities/damage: Hollow (pipe tree)
Disease	Leaf necrotic spots: / Bark lesions: /

## GR23\_00038

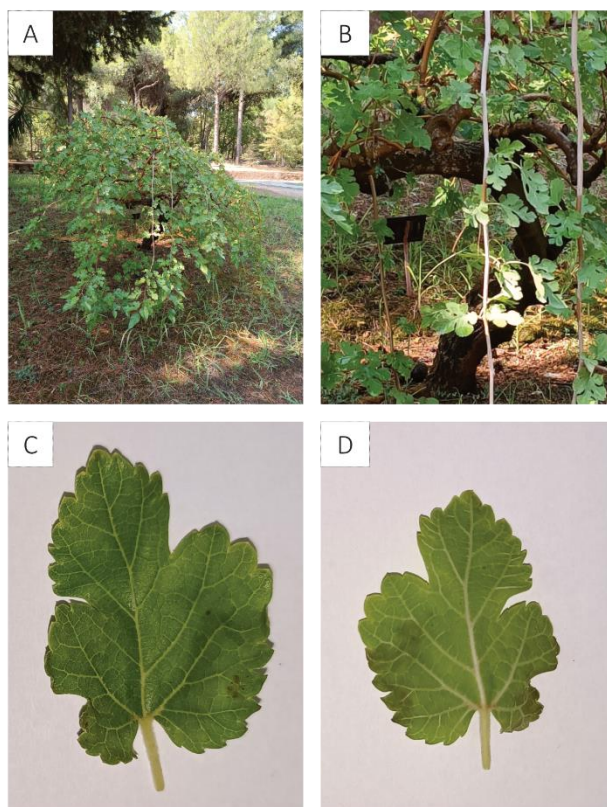


**Figure GR23\_00038:** A) Tree habitus; B) Trunk aspect.

Basic descriptors	Species: <i>Morus alba</i> L.
	Location: Greece, Soufli
	GPS coordinates: 41.191445, 26.2974
	Accessibility: Street
	Number of individuals at the location: Mulberry row
Trunk	Trunk circumference (cm): 250-300 cm
	Tree growth habit: Upright (semi-upright)
	Tree vigour: Good condition
	Pruning practices: Frequently pruned
Disease	Trunk colour: Greyish brown
	Trunk irregularities/damage: /
	Leaf necrotic spots: /
	Bark lesions: /



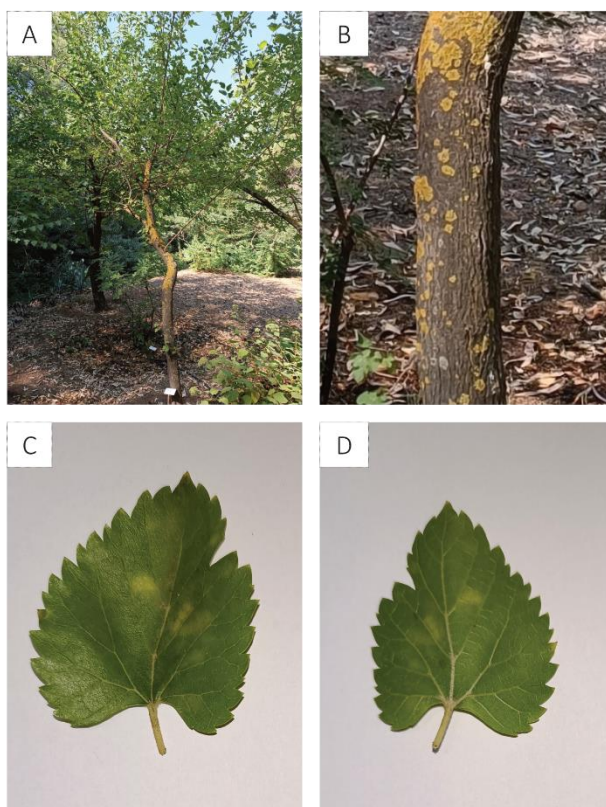
GR23\_00466



**Figure GR23\_00466:** A) Tree habitus; B) Trunk aspect; C) Lobed leaf adaxial; D) Lobed leaf abaxial.

Basic descriptors	Species: <i>Morus alba</i> L. Location: Greece, Haidari GPS coordinates: 38.00902, 23.64831 Accessibility: Botanical garden, collection Number of individuals at the location: Individuum Trunk circumference (cm): <180 cm Tree growth habit: Weeping Tree vigour: Good condition Pruning practices: Frequently pruned
Trunk	Trunk colour: Dark brown (reddish brown) Trunk irregularities/damage: /
Leaves	Phyllotaxis: Predominantly alternate distichous Leaf shape: Lobed Leaf blade: Medium (1.3-1.5) Petiole range: Medium (21-40mm) Shape of leaf base: Cordate Shape of leaf apex: Obtuse Leaf blade tip: Absent Leaf blade margin: Crenate

## GR23\_00468



**Figure GR23\_00468:** A) Tree habitus; B) Trunk aspect; C) Lobed leaf adaxial; D) Lobed leaf abaxial.

Basic descriptors	Species: <i>Morus</i> sp. L. Location: Greece, Haidari GPS coordinates: 38.01182, 23.64133 Accessibility: Botanical garden, collection Number of individuals at the location: Mulberry row Trunk circumference (cm): <180 cm Tree growth habit: Upright (semi-upright) Tree vigour: Good condition Pruning practices: Unpruned tree
Trunk	Trunk colour: Dark brown (reddish brown) Trunk irregularities/damage: /
Leaves	Phyllotaxis: Predominantly alternate distichous Leaf shape: Simple and lobed Leaf blade: Medium (1.3-1.5) Petiole range: Medium (21-40mm) Shape of leaf base: Cordate Shape of leaf apex: Obtuse Leaf blade tip: Absent Leaf blade margin: Serrate

## GR23\_00608



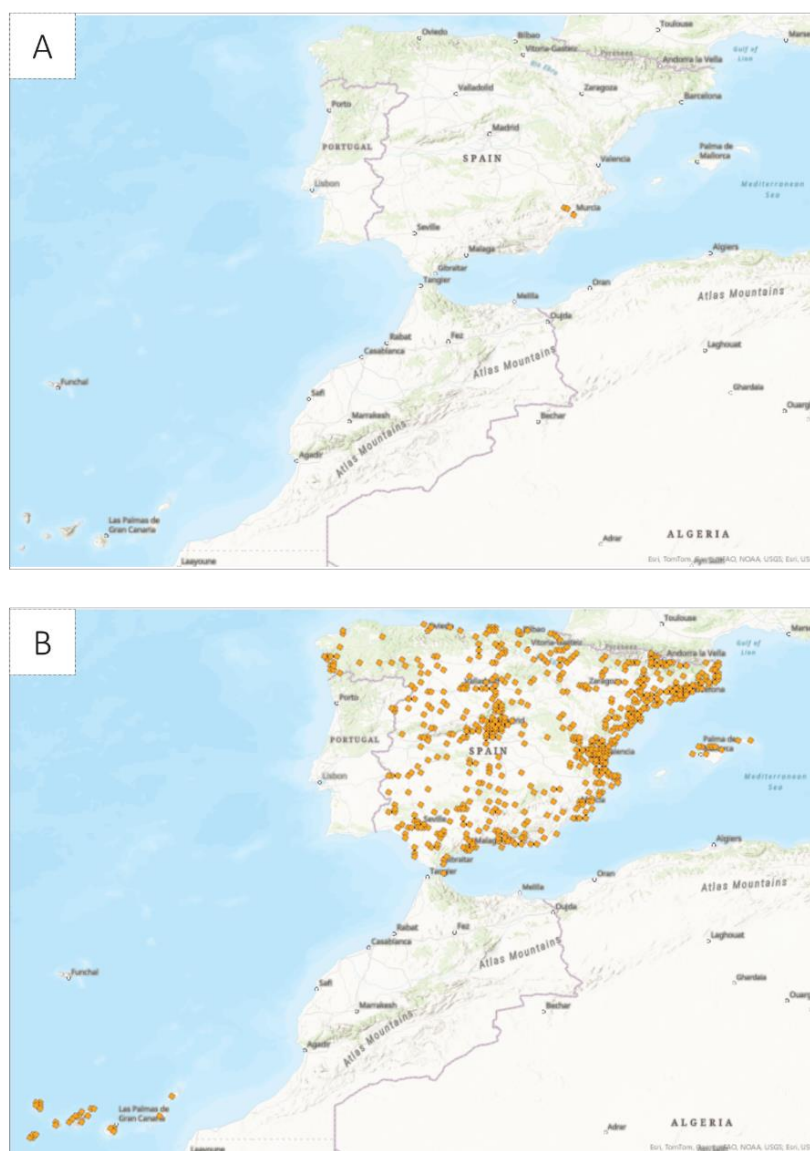
**Figure GR23\_00608:** A) Tree habitus; B) Trunk aspect.

Basic descriptors	Species: <i>Morus alba</i> L.
	Location: Greece, Knosos
	GPS coordinates: 35.2991, 25.1609
	Accessibility: Street
	Number of individuals at the location: Mulberry row
Trunk	Trunk circumference (cm): 180-249 cm
	Tree growth habit: Upright (semi-upright)
	Tree vigour: Good condition
	Pruning practices: Frequently pruned
Disease	Trunk colour: Greyish brown
	Trunk irregularities/damage: /
	Leaf necrotic spots: /
	Bark lesions: /



### 4.3.8 Report on inventory of mulberries in Spain

#### 4.3.8.1 Basic information



**Figure ES\_1** - Distribution map of mulberries in Spain A) using *MorusAPP*, B) using *in-silico* analysis *GBIF* (2019-2024). For Spain, *in-silico* analysis via GBIF (Global Biodiversity Information Facility) revealed that 1331 mulberry trees were recorded between 2019 and 2024.

In 2023, a total of three mulberry trees from Spain were recorded via the *MorusAPP* (**Figure ES\_1A**) and an *in-silico* analysis via GBIF (Global Biodiversity Information Facility) revealed that 1394 mulberry trees were recorded between 2019 and 2024 (**Figure ES\_1B**). Inventory

of Spanish mulberries (**Figure ES\_2**) by species indicates that two out of the three recorded trees were white mulberries (*M. alba*), while there was one mulberry identified at genus level (*Morus* sp.). Black mulberries (*M. nigra*) have not been recorded so far in Spain (**Figure ES\_2A**).

**Figure ES\_2B** shows the accessibility options of mulberries recorded in Spain. The majority were found in botanical gardens or collections (96 %). A few were found in private gardens (4 %) (**Figure ES\_2C**). The majority of mulberries in Spain grew individually (96 %), while we recorded 4 % in plantations (**Figure ES\_2C**).

All mulberry trees had a very large circumference of more than 300 cm (**Figure ES\_2D**). The inventory of mulberries by tree growth habit indicated that all of the recorded trees grew upright or semi-upright (**Figure ES\_2E**). All of the mulberries recorded in Spain were in good condition (**Figure ES\_2F**).

**Figure ES\_2G** shows the pruning practices of the mulberries recorded in Spain. Seventy percent of the recorded mulberries were frequently pruned, 26 % were yearly pruned and only 4 % were unpruned. This distribution provided insight into the varied mulberry maintenance practices. All of the recorded mulberries featured a greyish brown trunk colour (**Figure ES\_2H**).



## Inventarisation of mulberries in Spain



**Figure ES\_2** - Basic information on the inventory of mulberry trees in Spain. A) Percentage of recorded *M. alba* and *M. nigra* trees. B) Accessibility options of trees. C) State on the number of individuals at location. D) Percentage of trees at certain trunk circumference range. E) Percentage of trees of upright and weeping growth habit. F) Percentage of trees in bad and good condition. G) Report on the pruning frequency of the trees. H) The percentage of trees of different trunk colours.

#### 4.3.8.2 Monumental mulberry trees of Spain

Three trees with a circumference of more than 250 cm have been recorded as individual trees in public areas and private gardens in surrounding of Murcia (**ES23\_00708**, CBH 300 cm; **ES23\_00766.002**, CBH 250 cm). More than 500 entries of mulberries are recorded in the GBIF database, which should be visited for detailed descriptor observations.

## ES23\_00708



**Figure ES23\_00708:** A) Tree habitus; B) Trunk aspect; C) Simple leaf adaxial; D) One-year old shoot.

Basic descriptors	<p>Species: <i>Morus alba</i> L.</p> <p>Location: Spain, Murcia</p> <p>GPS coordinates: 37.9389,-1.13538</p> <p>Accessibility: Private garden</p> <p>Number of individuals at the location: Individuum</p> <p>Trunk circumference (cm): 300 cm</p> <p>Tree growth habit: Upright (semi-upright)</p> <p>Tree vigour: Good condition</p> <p>Pruning practices: Unpruned tree</p>
Trunk	<p>Trunk colour: Greyish brown</p> <p>Trunk irregularities/damage: /</p>
Leaves	<p>Phyllotaxis: Predominantly alternate spiral</p> <p>Leaf shape: Simple</p> <p>Leaf blade: High (&gt;1.6, long leaves)</p> <p>Petiole range: Medium (21-40mm)</p> <p>Shape of leaf base: Cordate</p> <p>Shape of leaf apex: Acute</p> <p>Leaf blade tip: Acuminate</p> <p>Leaf blade margin: Serrate</p>

ES23\_00766.002

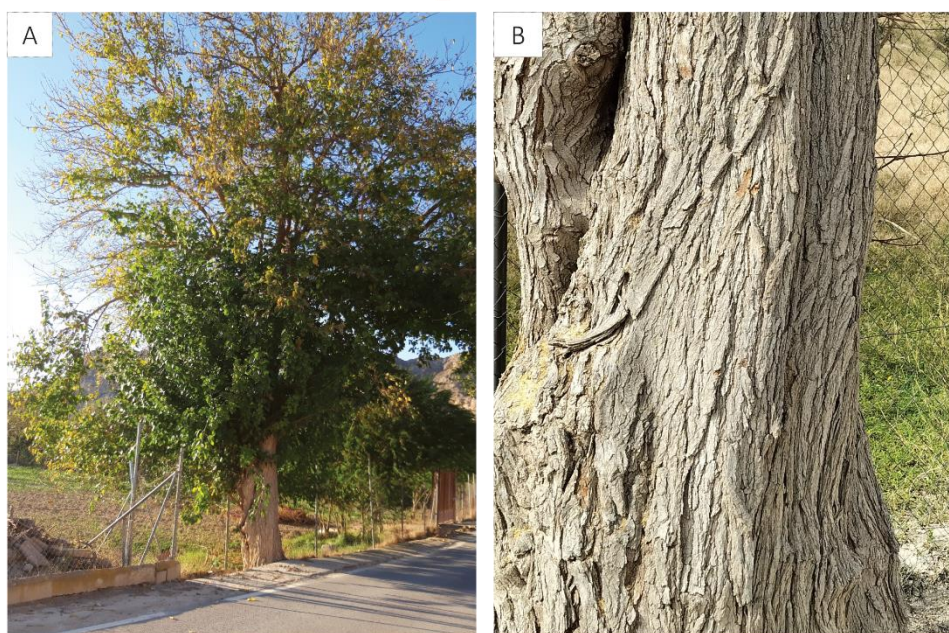


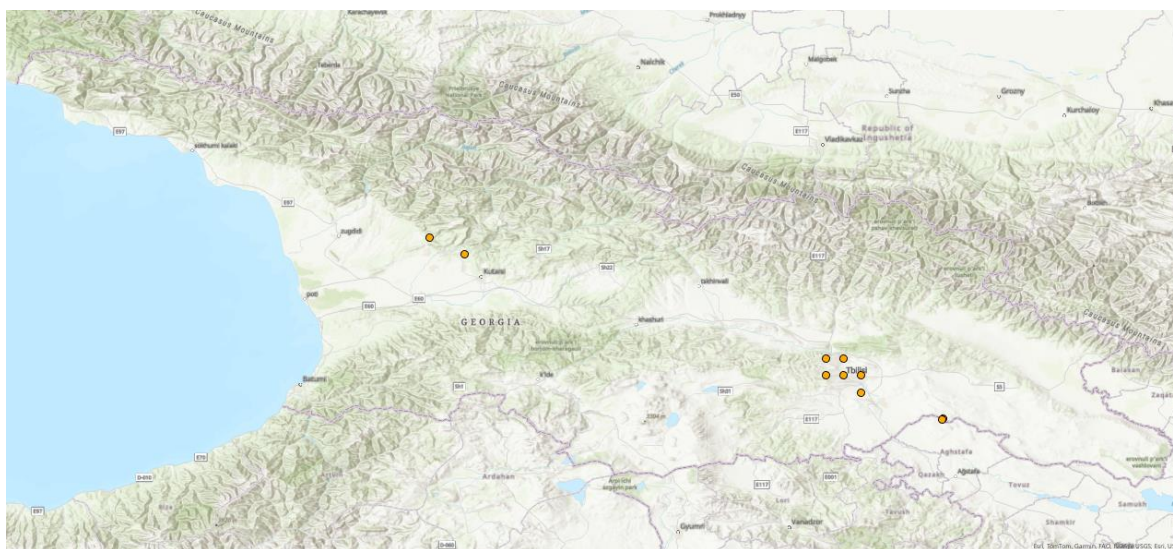
Figure ES23\_00766.002: A) Tree habitus; B) Trunk aspect.

Basic descriptors	Species: <i>Morus alba</i> L.
	Location: Spain, Ulea Archena
	GPS coordinates: 38.135777,-1.316768
	Accessibility: Street
	Number of individuals at the location: Individuum
Trunk	Trunk circumference (cm): 250 cm
	Tree growth habit: Upright (semi-upright)
	Tree vigour: Good condition
	Pruning practices: Unpruned tree
Disease	Trunk colour: Greyish brown
	Trunk irregularities/damage: /
	Leaf necrotic spots: /
	Bark lesions: /



### 4.3.9 Report on inventory of mulberries in Georgia

#### 4.3.9.1 Basic information



**Figure GE 1** - Distribution map of mulberries in Georgia (*in-silico* analysis, GBIF records 2019-2024)

For Georgia *in-silico* analysis via GBIF revealed that 21 old local *M. alba* trees and 3 *M. nigra* trees were recorded between 2019 and 2024 (**Figure GE 1**). In addition, personal recording revealed 4 *M. nigra* trees from different locations. Two trees were recorded at the monastery Dawit Garetscha, Udabno. This year sampling excursions, accompanied by the Laboratory of Sericulture of the Agricultural University of Georgia, are planned.



#### 4.4 Report on the inventory of *M. nigra* trees

Inventory of black mulberries (*M. nigra*) by origin indicates that the majority of trees were recorded in Slovenia with a total of 10 trees (43 %), followed by 5 trees in Croatia (22 %), 3 trees in Greece (13 %) 2 trees each in Bulgaria and the United Kingdom (9 %) and 1 tree in Italy (4 %) (**Table 6, Figure 15, Figure 16**).

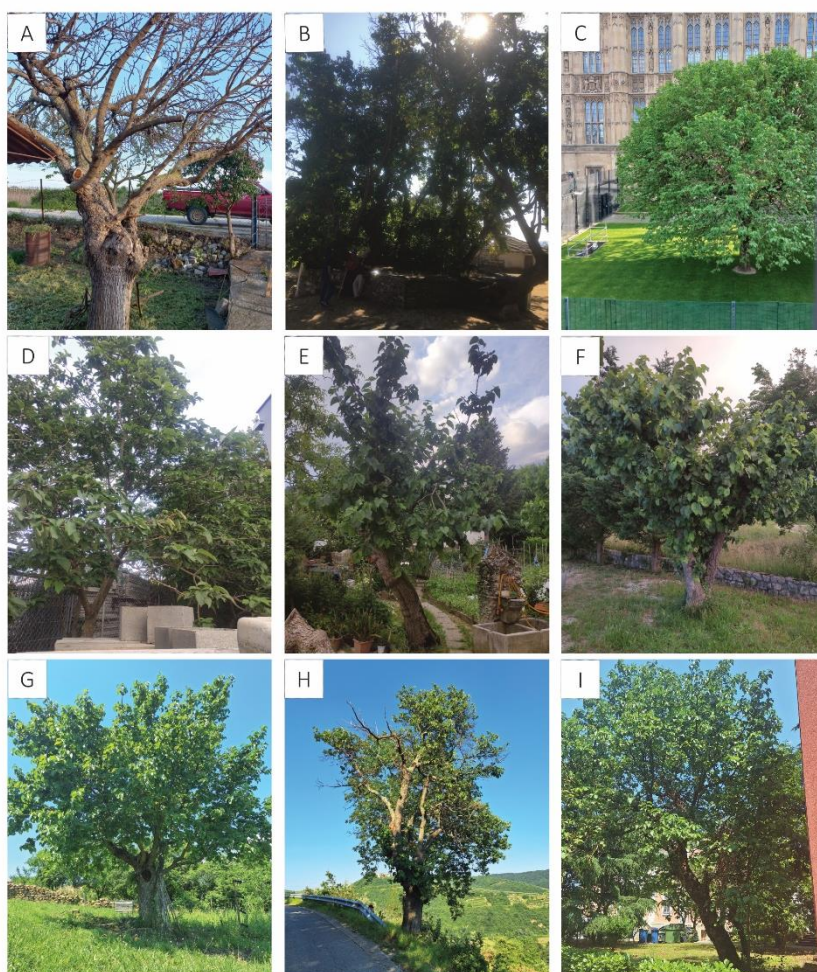
The majority of recorded black mulberries (9 trees) were grown in private gardens. The second most common were black mulberries in public areas and along the streets (5 trees each), and the third most common were black mulberries in botanical gardens or collections and in agricultural areas, none were found in squares. All of the black mulberries grew individually (**Table 6**).

The distribution of black mulberries based on circumference was predominated by 15 trees with a circumference below 180 cm, followed by 3 trees with a circumference below 249 cm. A large tree circumference of 250 to 300 cm and very large circumference of more than 300 cm was equally represented by 2 trees. The most monumental black mulberries had circumferences of 380 and 602 cm, respectively (**Table 6**).

**Table 6** - Information on the inventarised black mulberry trees (*M. nigra*) in Europe (CBH=circumference at breast height in cm)

ID NO.	Date of sampling	Location	Geographical origin	Accessibility	No. individuals at the location	Circumf. range	CBH (cm)
<b>Italy</b>							
IT23_00469	1.09.2023	CREA gene bank	45.398002, 11.833734	Public	Individuum		
<b>Slovenia</b>							
SI23_00126	15.06.2023	Crnice 2	45.9113258, 13.7700184	Private garden	Individuum	<180 cm	
SI23_00132	15.06.2023	Crnice 9	45.9072484, 13.7800351	Private garden	Individuum	<180 cm	
SI23_00153	15.06.2023	Gojace 1	45.8995071, 13.7965318	Public	Individuum	<180 cm	
SI23_00172	16.06.2023	Koštabona	45.4850947, 13.7305084	Agricultural landscape	Individuum	<180 cm	173
SI23_00190	17.06.2023	Street to Korte	45.4915456, 13.6652616	Street	Individuum	<180 cm	174
SI23_00197	18.06.2023	Osp	45.5721989, 13.8589273	Public	Individuum	<180 cm	105
SI23_00201	18.06.2023	Izola	45.5353119, 13.6580143	Street	Individuum	<180 cm	95
SI23_00217	20.06.2023	Zazid (grafted on <i>M. alba</i> )	45.5222986, 13.9076464	Private garden	Individuum	250-300 cm	250
SI23_00393	16.07.2023	Natural reserve Ormož Lagune	46.4039327, 16.1935983	Agricultural landscape	Individuum	<180 cm	
SI23_00569	9.09.2023	Sentjane	45.520257, 13.5944904	Private garden	Individuum	<180 cm	70
<b>Bulgaria</b>							
BG23_00628	13.10.2023	Kormyansko	43.05367,25.03492	Public	Individuum	>300 cm	380

ID NO.	Date of sampling	Location	Geographical origin	Accessibility	No. individuals at the location	Circumf. range	CBH (cm)
BG23_00657	16.10.2023	Vratsa	43.200317,23.5523552	Botanical garden, collection	Individuum	<180 cm	170
<b>Greece</b>							
GR23_00034	25.04.2023	Soufli 6	41.20085, 26.2988467	Private garden	Individuum	<180 cm	40
GR23_00035	25.04.2023	Soufli 7	41.1953733, 26.28097	Private garden	Individuum	<180 cm	251
GR23_00774	29.08.2023	GR-Roussa 17	41.2927615, 25.9989805	Private garden	Individuum	>300 cm	602
<b>Croatia</b>							
HR23_00345	2.07.2023	Cres	44.96389, 14.4105233	Private garden	Individuum	180-249 cm	200
HR23_00346	4.07.2023	Mali Podol	44.8786417, 14.3581233	Street	Individuum	180-249 cm	200
HR23_00347	4.07.2023	Mali Podol 2	44.8787253, 14.357305	Private garden	Individuum	<180 cm	
HR23_00348	4.07.2023	Loznati 1	44.9229398, 14.4384518	Street	Individuum	180-249 cm	245
HR23_00349	4.07.2023	Lozati 2	44.9230138, 14.4369426	Street	Individuum	250-300 cm	
<b>United Kingdom</b>							
EN23_00044	24.05.2023	Big Ben	51.5014366, -0.1241482	Public	Individuum	<180 cm	180
EN23_00045	25.05.2023	Chelsea hospital	51.4874701, -0.1560741	Public	Individuum	<180 cm	180



**Figure 15-** Inventory of *M. nigra*. A) Soufli Greece (SI23-00035), B) Greece (to be reported), C) Big Ben, London, Great Britain (SI23-00044), D) Submediterranean region, Slovenia (SI23\_00126), E) SI23\_00132, F) SI23\_00153, G) SI23\_00172, H) Korte, Slovenia (SI23\_00190), I) SI23\_00201. For detailed information of trees see Suppl. Table 3.





**Figure 16** – Inventory of *M. nigra*. A) Chelsea, London, Great Britain (GB23-00045), B) Osp, Slovenia (SI23\_00197), C) Cres, Croatia (HR23\_00345), D) Podol, Cres (HR00346), E) Podol, Cres (HR23\_00347), F) Cres (SI23\_00348), G) Cres (SI23\_00349), H) Soufli (GR23\_00034). For detailed information of trees see Suppl. Table 3.

## 4.5 Conclusion and planned activities

The evaluation of mulberry germplasm of participating countries, according to the proposed methodology, and an integrated database, will be continued. Furthermore, biochemical analyses of the leaves, fruits and bark (chlorophyll; total proteins; individual phenolics, soluble carbohydrates) will be performed along with molecular characterisation in order to select genotypes with a specific chemotype profile and tolerance/resistance to cold, drought and diseases.



## 5. Part C: Genetic analysis of the mulberry species relationships to trace the antique itinerary of the plant from Asia to Western Europe

### 5.1 Introductory notes

Mulberries are deciduous trees of the genus *Morus*, which belongs to the plant family *Moraceae*. *Moraceae* exhibit a complex array of inflorescence architectures, breeding systems, and pollination syndromes, which form the basis of traditional taxonomic classification (Clement & Weiblen, 2009). The family *Moraceae* contains 37 genera and about 1,100 species that are found in temperate, tropical, and subtropical regions of the world in both wild and cultivated forms (Hussain et al. 2023). *Moraceae* plants are of important economic and medicinal value and are rich in prenylated phenolics with structural and biological diversity, which have attracted a lot of attention as an important source for drug discovery (Hou, 2015). The *Moraceae* family among others includes the following genera: *Morus*, *Artocarpus*, *Maclura*, *Broussonetia*, *Milicia* and *Ficus* (Burlando et al. 2017).

The genus *Morus* is widely distributed in tropical, subtropical and temperate areas (Yuan et al. 2015). The disjunct distribution in the Northern Hemisphere makes the genus biogeographically interesting. Most of the Asian species (*M. alba*, *M. indica*, *M. cathayana*, *M. mongolica*) are found in East and Southeast Asia, while Western Asia has an endemic species, *M. nigra* L. The disjunct distribution between eastern Asia and eastern North America is a major biogeographic pattern in the Northern Hemisphere and has been studied in many taxa. This disjunction is considered to comprise the remnants of once-widely distributed temperate forests during the Tertiary. The tribe Artocarpeae, which belongs to the *Moraceae* family, also has an Asian and American disjunction, and biogeographic reconstruction indicates that *Moraceae* likely originated in the Americas, and the Asian clade split from the American clade during the Paleocene (55.24–65.03 million years ago), when the North Atlantic Land Bridge was available for migration (Yang et al. 2023).

In history the taxonomy of *Morus* has been unstable, with great variation in the numbers of species recognised by different researchers (Nepal, 2008). The taxonomic position of the genus and the number of species have undergone many modifications as the system of classification evolved from the sexual systems (eighteenth century) to the modern molecular phylogenetic system (twenty-first century) (Vijayan et al. 2011).

Linnaeus first established the genus *Morus* and described seven species including *M. alba* L., *M. rubra* L., *M. nigra* L., *M. indica* L., *M. tartarica*, *M. papyrifera* and *M. tinctoria* (Venkatesh, 2021; Yang et al. 2023). Over the last 200-plus years, the number of recognised species, varieties, and subvarieties has changed significantly. For example, Bureau, recognized six

species, 20 varieties (16 under *M. alba* L.), and 12 subvarieties (11 under *M. alba*). Koidzumi (1917) elevated many of these varieties to species and reduced others to synonymy, resulting in 24 species that were divided into two sections based on the length of the style: section *Macromorus* Koidz. (=sect. *Morus*) and section *Dolichostylae* Koidz. Leroy (1949) classified 18 *Morus* species into three subgenera: subg. *Eumorus* J.F.Leroy (= subg. *Morus*) distributed in Asia and North America; subg. *Gomphomorus* J.F.Leroy distributed in South America; and subg. *Afromorus* A.Chevalier distributed in tropical Africa. Hotta (1954) recognised 35 *Morus* species, and Chang et al. (1998) recognized 16 *Morus* species, 11 of which are distributed in China (Yang et al. 2023). Takhtajan (1980) based on phylogenetic relationships deduced from morphological characters placed *Morus* under the family Moraceae of the order Urticales, which is considered an advanced order among the woody flowering plants. Recently, the angiosperm phylogenetic group (AGP II, 2003) based on evidences from molecular phylogenies, placed the family Moraceae in the order Rosales (Vijayan et al. 2011). Even today, it is indicated that the borderline between different species in mulberry is very thin and the species status of many of the genotypes is a matter of great debate (Vijayan, 2004). The classification of the *Morus* genus based on morphology did not truly reflect phylogenetic relationships (Zeng et al. 2022). Wide variations in leaf morphology are observed among different species and accessions. *Morus* sp. expresses the phenomenon called heterophylly, which means that simple and lobed leaves appear on the same plant. The tendency toward heterophylly may depend on the genotype, developmental period, the age of the plant, and may vary according to the position of the branches within the canopy, pruning practices and environmental conditions (Brus 2012, Urbanek Krajnc et al., 2019). Morphology and colour of mulberry infructescences are also not reliable for distinguishing among *Morus* species, because they vary greatly from white to black with different colour shades upon ripening (Urbanek Krajnc et al. 2022). Furthermore, *M. alba* fruit colour differ, ranging from white, dark red and black, even in the trees of the same species (Kadri et al. 2021; Urbanek Krajnc et al. 2023)

The phylogeny of mulberries is further difficult as they bear different sex types, monoecious or dioecious, with sex expression varying among species and varieties. Furthermore, different cytormorphs are available in mulberry, in the case of *M. alba* diploids and triploids dominate mostly (Vijayan et al. 2004). The ploidy of mulberry varies from haploid with 14 somatic chromosomes to decaploid *M. nigra* with 308 chromosomes (Vijayan, 2023). Diploid *Morus* species such as, *M. acidosa*, *M. alba*, *M. atropurpurea*, *M. bombycs*, *M. indica*, *M. kagayamae*, *M. latifolia* and *M. rotundiloba* have  $2n = 2x = 28$ . Although fertile diploid individuals form the large majority of these species some infertile or poorly fertile triploids occur in their population. Triploid form ( $2n = 3x = 42$ ) and tetraploid form ( $2n = 4x = 56$ ) have been identified in *M. laevigata*. *M. boninensis* is also a tetraploid. *M. serrata* and *M. tiliaefolia* are hexaploid ( $2n = 6x = 84$ ) species. As mentioned, *M. nigra* is docosaploid ( $2n = 22x = 308$ )

(Yamanouchi, 2017). Leaves with a larger leaf area are more suitable for the use in sericulture (Ruiz et al.). It was observed that the cell size increases in autotetraploid compared with diploid but the cell number does not reveal an apparent difference. On the transcriptome level, it was found that up-regulated expression of cytokinin and gibberellin genes promotes plant growth and affects cell size. Moreover, a series of photosynthesis related genes, including several chloroplast-specifically expressed genes, are up-regulated in autotetraploid mulberry (Dai et al. 2015).

In recent phylogenetic research, different molecular markers have been used for determining genetic diversity, genotype identification and genetic mapping (Banerjee et al. 2016). The most commonly used molecular methods for genetic diversity in plants are: random amplified polymorphic DNA (RAPD), DNA amplification fingerprinting (DAF), arbitrarily primed polymerase chain reaction (AP-PCR), intersimple sequence repeat (ISSR), amplified fragment length polymorphisms (AFLP), restriction fragment length polymorphisms (RFLP), microsatellites (SSR), sequence characterised amplified regions (SCAR3), cleaved amplified polymorphic sequence (CAPS), expressed sequence tag (EST), single nucleotide polymorphism (SNP) and sequence tagged sites (STS) (Idrees & Irshad, 2014).

Based on the evaluation of the highly conserved ITS markers Zeng et al. (2015) proposed acceptance of only 8 species, namely: *M. alba* L., *M. nigra* L., *M. notabilis* C.K. Schneid., *M. serrata* Roxb., *M. rubra* L., *M. celtidifolia* Kunth, *M. insignis* Bureau and *M. mesozygia* Stapf. The authors recognized 4 major clades. In the polyphyletic A group, two Asian species (*M. nigra* and *M. serrata*) and two American species (*M. rubra* and *M. celtidifolia*) were placed. The A clade also included two genotypes that were a red mulberry parent of a hybrid (*M. rubra* x *M. alba*). The B clade was the largest monophyletic clade, comprising only Chinese white mulberry (*M. alba*). The C clade consisted of *M. notabilis* and *M. yunnanensis* as close relatives to *M. mongolica*, that contained 14 chromosomes. The D clade consisted of two species including *M. insignis* and *M. mesozygia* (Zeng et al. 2015).

The phylogenetic analyses based on the complete chloroplast genome showed that *M. alba* is closely clustered with *M. cathayana* and *M. mongolica* (Luo et al. 2019, He et al. 2020; Yang et al. 2022). Furthermore, the complete nucleotide sequence of the *M. mongolica* chloroplast (cp) genome was also characterised by Kong & Yang (2016). Comparative analysis with *M. indica* cp genome indicated 22 SSRs with length polymorphisms and 1 SSR with nucleotide content polymorphism. The phylogenetic analysis of 60 PCGs from 62 cp genomes provided strong support for the monophyletic, single origin (Kong & Yang, 2016).

Xuan et al. (2023) performed genomic *in situ* hybridization (cGISH) and self-GISH to illustrate the chromosome constitution and genetic relationships of 40 mulberry accessions belonging

to 12 species. They defined five mulberry sections, namely *M. notabilis*, *M. nigra*, *M. wittiorum*, *M. cathayana* and *M. alba*, the last containing seven closely related taxa and three varieties, further divided into two subsections.

Muhonja et al. (2020) investigated the genetic relationships among 54 mulberry accessions from eight species using genome-wide single nucleotide polymorphism markers (SNP) and proposed three monophyletic species. There were no clear monophyletic clades within *M. alba* and *M. latifolia* varieties, which could be a result of several hybridization events after their introductions from China to Japan.

Another study by Vijayan et al. (2004b) used inter-simple sequence repeat (ISSR) and random amplified polymorphic DNA markers to explore the population structure of 19 mulberry genotypes from five species, and concluded that *M. laevigata* was a separate species and *M. latifolia*, *M. bombycis*, *M. alba*, and *M. indica* should be considered as a single species.

The dendrograms which were based on ISSR analysis of 20 mulberry genotypes from India, realised from these markers clustered the genotypes into three groups. The outermost group was *M. serrata* Roxb., which was followed by the group of *M. macroura* Miq. And the innermost group contained genotypes of *M. indica* L. and *M. alba* L. This intermixing of genotypes of *M. indica* and *M. alba* supports the view that *M. indica* is merely a synonym of *M. alba* (Vijayan et al. 2006). The results about genetic similarity between *M. alba* and *M. indica* of Vijayan et al. (2006) study were also in agreement with the results of the study conducted by Kar et al. (2008). Genetic similarity between *M. alba* and *M. nigra* in the aforementioned study was shown by ISSR markers method (Kar et al. 2008).

In the last decade, the technique of simple sequence repeat (SSR) (Powell et al., 1996; Venkateswarlu et al., 2006; Vijayan et al., 2006) based on the characterisation of variation in genomic microsatellite regions is usually preferred. The application of SSR markers requires the previous characterisation of a set of microsatellite loci of the genome of the species under analysis. They allow for a complete description of *Morus* variability, genetic relationship and pedigrees.

Wani et al. (2013) assessed genetic relatedness with the SSR method among 17 mulberry genotypes from the germplasm bank of the Temperate Sericulture Institute, SKUAST Kashmir. Clustering of the genotypes was performed with the unweighted pair group method using arithmetic average (UPGMA) which generated five clusters.

The objective of the work presented by Garcia-Gomez et al. (2019) was to make a genetic characterisation by 12 SSR markers of a collection of 37 *M. alba* genotypes from nine different countries including Cuba, Costa Rica, Brazil, South Korea, China, Japan, Italy, Ethiopia and Spain, to clarify the distribution of this cultivated species though the world. The

whole dendrogram could be roughly subdivided in three main groups. The Japanese and Italian cultivars formed separated subgroups, whereas the rest of the accessions from the same country were clustered in different subgroups. This study provides valuable insights into the genetic diversity, relationships, and dissemination patterns of mulberry cultivars worldwide, emphasizing the importance of SSR markers in understanding these aspects. The study highlights the possibility of hybridization events and misclassification in the varietal denominations, especially in the context of long historical cultivation and adaptation.

Orhan et al. (2020) analysed 26 genotypes of *M. alba*, 26 genotypes of *M. nigra* and 21 genotypes of *M. rubra* with the SSR method. All the analysed samples belonged to different local genotypes from the Eastern Anatolia region of Turkey. In their study, clustering based on Jaccard's similarity coefficient and an unweighted pair group method with a UPGMA revealed three main clusters that corresponded with the aforementioned species (Orhan et al. 2020).

Identification of mulberry on lower taxonomical levels continues to be a point of great debate among scientists despite the number of morphological criteria such as floral characters, wood, bark, buds and leaf anatomical and biochemical characters, used to identify the species within this genus. However, no consensus system of classification has yet emerged. For conservation and breeding purposes, it is essential to characterise and clarify the relationships between species and lower taxonomical levels.

The current accepted classification according to the IPNI-The International Plant Names Index (<https://www.ipni.org/>), WPO Plant List (<https://wfoplantlist.org/taxon/>), POWO Plants of the World Online (Kew, 2024) comprises the following 17 species: *M. alba* L., *M. boninensis* Koidz., *M. cathayana* Hemsl., *M. celtidifolia* Kunth, *M. indica* L., *M. koordersiana* J.-F.Leroy, *M. liboensis* S.S.Chang, *M. macroura* Miq., *M. microphylla* Buckley, *M. miyabeana* Hotta, *M. mongolica* (Bureau) C.K.Schneid, *M. nigra* L., *M. notabilis* C.K.Schneid., *M. rubra* L., *M. serrata* Roxb., *M. trilobata* (S.S.Chang) Z.Y.Cao and *M. wittiorum* Hand.-Mazz .

The ARACNE mulberry research group is basically focused on morphological variability and the taxonomical concepts of the white mulberry ingroup (*M. alba* s.l.) as well as with *M. indica* s.l. ingroup, from which sericultural varieties were selected for high leaf yield. Both ingroups represent taxonomically complex groups with wide morphological diversity and close genetic relationships among the taxa on a lower taxonomical level. The taxonomical rank and position of taxa are inconsistent among the mulberry research groups. In the case of cultivars, the status is even more complicated as they are a result of selection over longer periods of time and there can even be a larger genetic difference within a cultivar among different countries if plants were obtained by seed propagation.



The taxa and cultivars are morphologically poorly defined, the descriptions are often contradictory and they actually merit the rank of a subspecies, variety or form. The taxonomic issue is being explored by several research groups at molecular phylogenetic level. Their results are difficult to integrate into a precise phylogenetic tree, as researchers most often address few local varieties and few specific cultivars using a variety of more or less supportive molecular markers. Consequently, the genetic distance among the cultivars and local varieties versus *M. alba* and *M. indica* sensu stricto has been proven difficult to resolve.

In the proposed genetic studies, we focused on two challenging ingroups, i.e. *M. alba* s.l. and *M. indica* s.l., by selecting a subset of varieties from ARACNE partners germplasm collections of different origins (Japan, China, Italy, Spain, Romania, Azerbaijan, ...) along with local varieties available in partners countries. Detailed molecular analysis based on SSR and SNP will in the end be integrated in multivariate analysis combining morphological descriptors. In the end of the project, multivariate analyses such as principal component analysis (PCA), discriminant analysis (DA) and principal coordinate analysis (PCoA) will be performed by combining both morphometric and molecular data to determine the relationships with higher certainty and to reconstruct the dissemination of the cultivated mulberry species via the silk road to Europe.

## 5.2 Methodology

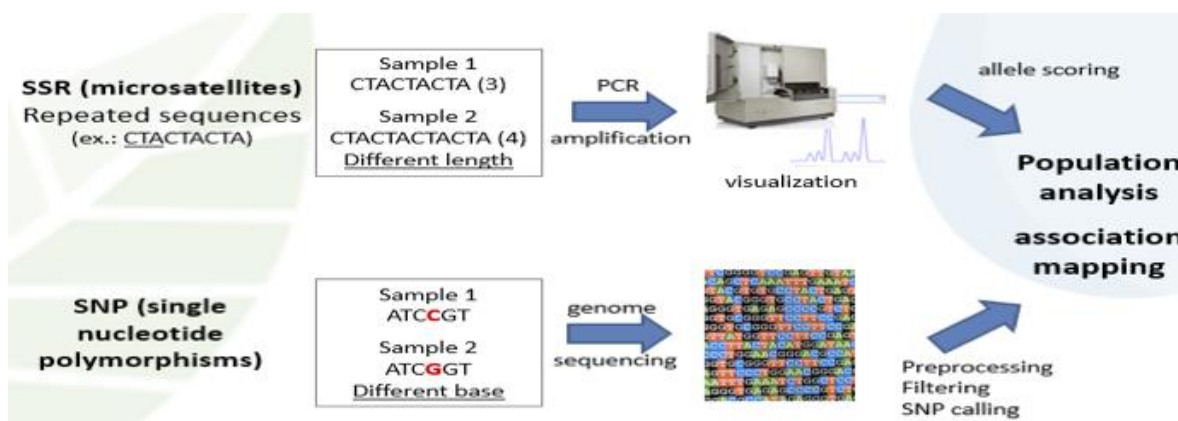
Selected mulberry varieties that were identified in germplasms (PART A) and by inventory in different sericultural regions of European countries (PART B) were sampled for genotypisation as follows:

Young apex with first young leaf was sampled, wrapped in a moist towel, placed in the plastic bag, and kept in the cool box during sampling. In order to avoid DNA degradation and allow conservation of the samples, they were desiccated using silica gel to a ratio 1:10 (leaves: silica gel) in weight and stored in airtight containers away from light. This method was chosen with respect to liquid nitrogen and -80°C storage or freeze-dry as such facilities were not accessible in many sampling sites.



**Figure 17** - Sampling of young shoot apex (arrow, A) in tubes with silica beads (B) for molecular analyses.

DNA isolation was performed according to a modified CTAB method by Maguire et al (1994). In addition, samples of the isolated DNA (1.5 mL vial, 10  $\mu$ L) of the Slovenian local mulberry varieties were sent to CREA. A preliminary analysis was performed using SSR markers on 12 loci on a subset of samples to assess their genetic discriminating power. To provide a more thorough and in-depth insights into the genetic architecture of germplasm collections (part A) and local isolates (part B) it was opted to use the 'Double digest restriction-site associated sequencing' (ddRADseq) approach, a SNP-based genotyping method, for its reliability, scalability and cost effectiveness (**Figure 18**). For this aim, high quality DNA is being extracted from all the samples identified in the frame of the project (currently 576) and will be shipped for library preparation and sequencing to a specialised company. Bioinformatic analyses will be performed on the samples to analyse the genetic diversity and the structure of the population.



**Figure 18 -** Schematic illustration of the analyses of SSR and SNP markers involved in the genetic evaluation of mulberries.

### 5.3 Results and discussion on the preliminary genetic evaluation

In general, information on genetic diversity and relationships between European genotypes is sparse compared to the abundance of published studies on populations from India and China cited in the literature. Moreover, the lack of cross analyses between European and Asian varieties present in different germplasm collections prevents the perception of a global context of the diversity of the genus.

Traditional classification methods, such as morphological and agronomic traits, have led to *Morus* being divided into varieties of different species. The difficulty in classifying genotypes is also due to the continuous variation in phenotypic traits and the potential for artificial and spontaneous hybridisation. In addition, the limitations of using phenotypic traits for the identification of interspecific hybrids, the evaluation of introgression patterns or defining genetic variation structure at the species level must be emphasised.

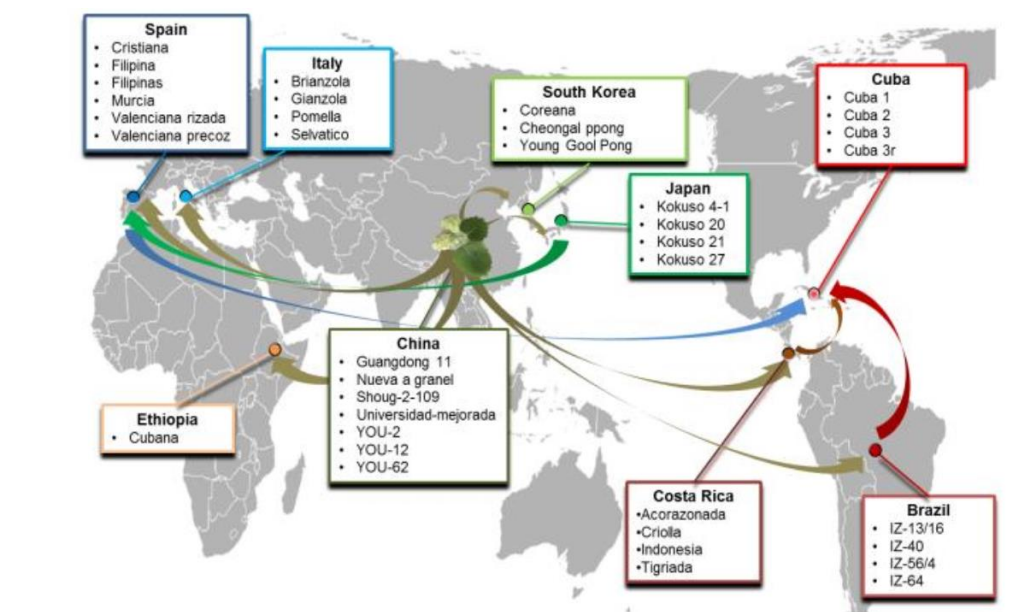
Molecular studies of the varieties belonging, according to the latest taxonomic classification, to *M. alba* s.l. and *M. indica* s.l. show a complex structure with relatively high genetic similarity, potential natural hybridisation and challenges in the accurate classification of varieties, all of which have important implications for the taxonomy, conservation and management of genetic resources in mulberry.

Previously, 48 mulberry varieties from the collection of the CREA Sericultural Station in Padua were analysed with AFLP markers (Botton et al. 2005). The cultivars were classified according to distinct phenotypic characteristics and labelled *M. alba*, *M. nigra*, *M. bombycis*, *M. latifolia* and *M. kagayamae*. The last three taxa are synonyms of *M. indica*, according to current taxonomy. The study revealed a high degree of genetic similarity within each mulberry species (with the exception of *M. nigra*), ranging from 0.845 to 0.884. Further, the study

found that some accessions that were considered different taxonomic groups, based on morphological traits or original classification, were genetically identical. This suggests that some accessions may be hybrids or introgressors and that environmental and anthropological influences may have led to phenotypic variation resulting in misclassification.

Over the last decade, microsatellite markers (SSRs), have become the marker of choice for the molecular characterisation of *Morus* species. Twelve SSR primer pairs developed for mulberry, representing different regions of its genome, were tested on 37 accessions of *M. alba* from the IMIDA and CENSA collections, originating from Cuba, Costa Rica, Brazil, South Korea, China, Japan, Italy, Ethiopia, and Spain, to clarify the distribution of this cultivated species in the world (Garcia-Gomez et al. 2019). The following varieties were included from the IMIDA collection: Brianzola, Filipina, Giazza, Kokusou 4-1 20, 21, 27; Pomella, Selvatica, Valenciana precoz, Valenciana rizada. In particular, there were no studies on Spanish germplasm prior to this work.

The dendrogram obtained by the NJ cluster analysis could be roughly divided into three main groups, structured from A to C and subdivided into seven subgroups. The group furthest from the rest (C2) is formed by three Brazilian accessions. The other 30 accessions are divided into two large subgroups, one of which comprises groups A1 and A2 and the other groups B1, B2 and B3. Group A1 includes accessions that are thought to have been cultivated in Murcia since ancient times: 'Valenciana rizada', 'Cristiana' and 'Filipina', as well as the Italian group ('Brianzola', 'Giazza', 'Selvatico' and 'Pomella'), the Japanese group ('Kokusou 21', 'Kokusou 4-1' and 'Kokusou 20') and the Chinese cultivar 'Young-Gool-Pong'. Group B consists of only three subgroups (Cobean, Ethiopia, South Korean accessions and the Spanish accessions 'Filipinas' and 'Murcia'). The Japanese and Italian cultivars formed separate subgroups, while the other accessions from the same country were summarised in different subgroups. The mulberry cultivars and genotypes from Murcia form a distinct genetic group compared to the Japanese and even Italian genotypes (apart from some hybridisation events). The cultivars and genotypes of Spanish origin, after centuries of selection, may have interesting traits for the palatability of the leaves for feeding the silkworm, as well as for rusticity and tolerance to dry and hot Mediterranean climates. It is therefore a genetic material that is worth analysing for its genetic relationship with a broader collection of genotypes of ARACNE partner countries.



**Figure 19** - Schematic distribution of dissemination of mulberry varieties around the world (from Garcia-Gómez et al. 2019).

The analysis also revealed that Chinese germplasm is present in most of the identified clusters, indicating the major influence of the native germplasm on the other globally distributed mulberry cultivars. These molecular results demonstrate the spread of cultivated mulberry species from China around the world, the introduction to South Korea and Japan in the first step of dispersal, and the subsequent wide spread of modern mulberry to the Mediterranean region and later to South America and the Caribbean (**Figure 19**). However, our results, in which germplasm from many countries was analysed, complement the earlier results of Vijayan et al. (2011), which show additional dispersal routes from the Mediterranean region and later to South America and the Caribbean, a first route from China to Korea and Japan and a second route from China, India, Bangladesh, Pakistan and others. In addition, alternative pathways from Japan to Spain and from Spain to Cuba were also observed, which were not described by Vijayan et al. (2011). This study provides valuable insights into the genetic diversity, relationships and distribution patterns of mulberry cultivars worldwide and emphasises the importance of SSR markers for understanding these aspects. The results have implications for the conservation, breeding and sustainable utilisation of mulberry genetic resources.

During the previous basic research project of the Slovenian partner (ARIS N1-0041), the variability of local mulberry varieties was assessed by analysing morphological traits and at the molecular level by using 6 SSR microsatellite markers. Genetic characterisation proved to be challenging, mainly due to the different degree of ploidy, sexual dimorphism, past distribution, and natural genetic recombination between trees of different origins. The



genotypes were categorised into 4 main clusters using the clustering method, with the central cluster branching into three subclusters. The study enabled us to reconstruct the origin and distribution of mulberry among silkworm rearers of different Slovenian regions in the past (data not yet published).

So far, there has been no comparative study on the genetic relationship of mulberries within the genetic pool of mulberries present in the landscapes and germplasms of different European countries. SSR have been studied for cultivated mulberries only in the Indian subcontinent (Krishnan et al., 2014). There is an urgent need to further investigate the introduction of mulberry in the eastern Mediterranean region and southern Italy and its subsequent spread to western European countries, as well as its impact on the native germplasm and subsequent spread from sericulture centres.

For this reason, a comprehensive, Europe-wide genetic characterisation of mulberry samples is being performed that combines varieties identified in germplasms collections and local samples, concentrating on areas where sericulture was practised.

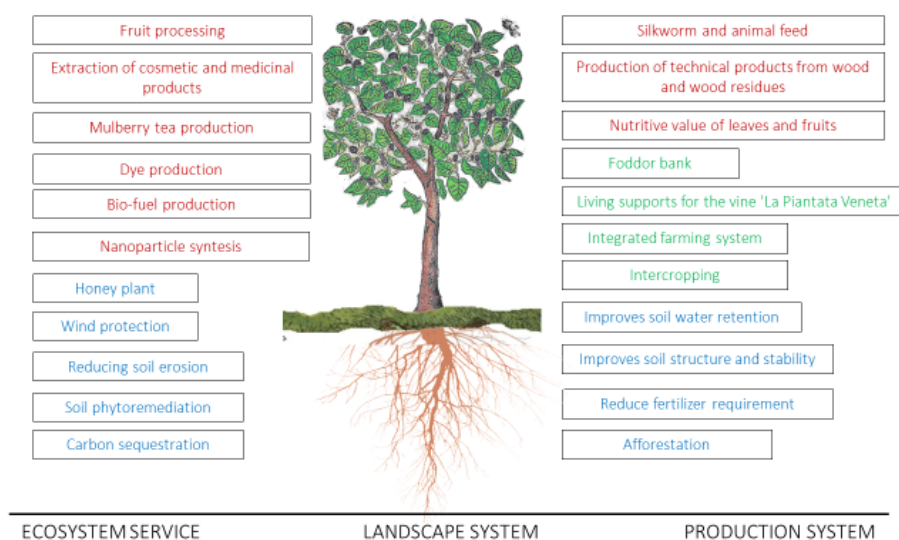
In the first step a precise selection was done by obtaining the lists of varieties of the germplasm collections held at CREA, IMIDA, Vratsa, UM (partners of the project) along with the previous morphological evaluations. A detailed revision was done followed by two meetings evaluating the morphological descriptors and photographic material in order to classify and optimise the material to be included in the analyses. In parallel, field research was done to collect accessions in different sericultural regions of European countries that could represent on one hand a remnant of the original introduction of mulberries in those areas and specific genotypes adapted to these peculiar regions. Hence, during the inventarisation local mulberries of circumference > 350 cm and monumental trees were sampled and later selected for genetic analyses by evaluation of the morphological traits as described in **Part B** (Inventory of historical local mulberry trees in sericultural regions of the participating partners). The collection of literacy sources, field research and morphological evaluation, which was done in the first year of the project were crucial and represent the cornerstone to select samples for the genetic analyses.

A preliminary analysis to assess the material was previously conducted on the CREA germplasm collection held at Padua using SSR markers (Pietrella et al, 2019). This exploration, conducted on over 40 varieties and further reanalysed, evidenced that such approach was not sufficient to fully discriminate the accessions and provide a clear population structure analysis. Moreover, given the paucity of the markers used, there could be the possibility of incorrect groupings or assignments to non-true groups; this is sometimes evident when different branches originate from dendrograms built by analysing the same samples with different markers. In this sense, increasing the number of markers in the analysis decreases

incorrect assignments and lineages; for this reason a SNP-based genotyping method has been chosen. The genetic analyses of selected mulberry cultivars from the collections of CREA, IMIDA, SES Vratsa and UM as well as of the local trees of the participating countries are still in progress and need to be subjected to a multivariate statistical approach involving the morphological evaluation (PART A) of selected cultivars over a period of at least the three years of the project duration.

## 6. Part D: Recreation of landscapes

Ancient mulberry trees are ecologically very important organisms. They affect the water regime, carbon storage, nutrient cycling in the environment, and the microclimate regime. Old trees are also habitats for many animal species. They are also related to the ethnological, historical, and socio-cultural heritage of a certain region. The mulberry is an extremely sustainable and versatile tree species, which, in addition to growing leaves to feed silkworms and livestock, can be used for many other lesser-known purposes, such as the production of mulberry tea and the extraction of cosmetic and medicinal products from the leaves, the production of technical products from wood and wood residues, fruit processing, production of dyes, soil phytoremediation, planting against erosion, and spatial planning in the agricultural landscape in an urban environment. Furthermore, mulberry trees are suitable for planting in polluted areas, around highways and factories and for landscaping in urban areas. An additional value is the reduction of noise and improvement of the living environment. Furthermore, cut branches can be used as firewood, for paper production and other technical purposes, (Sánchez, 2000; Jian *et al.*, 2012; Liu and Willison, 2013; Nikolova, 2015; Xueping *et al.*, 2016; Sfiligoj Smole *et al.*, 2019; Tikader and Vijayan, 2017; Zeng *et al.* 2020). The importance of mulberry trees in providing ecosystem services and their role in landscape and production systems is summarised in **Figure 20**.



**Figure 20** - Overview of the ecosystem service, landscape, and production system of mulberry trees (according to Urbanek Krajnc *et al.* 2024).

Below the range of the key ecosystem services provided by mulberry trees are summarised:

**Biodiversity Support:** Mulberry trees are known to support a diverse range of wildlife. They provide food for birds, insects, and other animals through their fruits and leaves. This in turn helps in maintaining and enhancing biodiversity in their surroundings. Furthermore, mulberry was also reported for the production of root exudates which nurture and increase the soil microflora and fauna (Chen et al., 2006).

**Ecorestoration, soil enrichment and protection:** The roots of mulberry trees help in stabilizing the soil and preventing erosion. They can also contribute to soil fertility through leaf litter and other organic matter that decomposes and enriches the soil. Mulberry trees can grow in a range of soil types, from fertile to barren, coarse, and nutrient-poor soils making them an effective choice for natural restoration projects. They are resilient to extreme weather conditions, including dry spells, sand storms, waterlogging, drought, and salinity, as well as extreme temperatures ranging from -30°C to over 40°C (Rohela et al. 2020). This resilience is largely due to their deep and wide root system, which enables them to establish in varied environments.

Furthermore, they can be cultivated under irrigated as well as rain fed conditions with a range of 600–2500 mm annual rainfall. Desert mulberry is an ecotype of mulberry that was successfully planted in desert areas of Xinjiang parts of China. This ecotype enables the mulberry plants to be cultivated in deserted conditions with minimum levels of irrigation, hence it is recommended for natural restoration of empty deserted lands and desertified grasslands (Jian et al., 2012 ). Mulberry plants are also utilized and successfully demonstrated for ecorestoration of salinated lands and stony deserted areas (Jian et al., 2012).

**Afforestation:** As a fast-growing and highly heterozygous species, mulberry is ideal for afforestation. It adapts well to different altitudes, soil types, temperatures, and pH conditions. It is used for landscaping in urban areas due to its resistance to floods, drought, and wind. Mulberry trees support afforestation efforts by restoring soil carbon, maintaining soil water holding capacity, preventing soil erosion, improving soil nutrients, nurturing soil microflora, and improving air quality. They can also be intercropped with other plants, enhancing biodiversity and economic benefits.

**Phytoremediation:** Mulberry plants have been identified as effective agents for the phytoremediation of heavy metals, offering a sustainable and eco-friendly solution to environmental issues. Heavy metals, unlike organic pollutants, do not degrade over time and can be highly toxic and persistent in the environment, posing severe risks to human health if they enter the body through water or other means (Xu et al., 2015; Nazarian et al., 2016) Phytoremediation, the use of plants to remediate pollutants from the environment, is

recognized for its cost-effectiveness and environmental friendliness, with plants capable of taking up water-soluble heavy metals and accumulating them in structures like phytoliths and vacuoles (Olson and Fletcher et al., 1990; Cunningham et al., 1995; Raskin and Ensley, 2000).

The phytoremediation potential is correlated with the characteristics of mulberries to deposit opal silica ( $\text{SiO}_2$ ) within the cell wall of the epidermal layer in solid form creating amorphous structures commonly known as phytoliths or silica bodies (Nitta et al. 2006, Katayama et al. 2008, Katayama et al. 2013, Tsutsui et al. 2016). Silica bodies can occlude harmful metal ions, reducing the stress caused by these metals in many plants. They reduce soil-soluble metals, mainly in contaminated areas and, most importantly, without the risk of contamination of the food chain due to their stability. Phytoliths can contribute to the immobilization and subsequent inactivation of the plant's tolerance to toxic metals (Farnezi et al. 2022). Previously, *M. alba* has been regarded as a potential Cd extractor (Zeng et al. 2020) and is also suitable for phytoextraction of Ni (Rafati et al. 2012). Furthermore, mulberry exhibited a considerable adaptability to soil Pb, and no significant decrease in biomass was observed across the various soil Pb treatments (Wang & Ji, 2021). *M. alba* is also described as a good phytoextractor of Cu, Fe, Co, Cd and Ca, and as a phytostabiliser of Mg, Zn and Mn (Rohela et al. 2020, Ahmad et al. 2023).

In addition, a study by Hashemi and Tabibian (2018) on the accumulation of mercury (Hg) in *M. nigra* highlighted the plant's capability to absorb Hg, particularly in its roots, when grown in soil treated with mercury nitrate at varying concentrations. This study showed that Hg absorption was the highest in the root parts compared to the leaves and stems, indicating that mulberry is particularly effective in the phytoremediation of Hg-contaminated soils.

The success of mulberry in the phytoremediation of soils contaminated with a broad spectrum of heavy metals underscores its value in environmental restoration efforts. Its ability to thrive in various conditions, coupled with its significant potential to absorb and accumulate heavy metals, makes mulberry a promising candidate for mitigating soil and water pollution, thereby safeguarding human health and the environment (Jiang et al., 2017).

**Carbon Sequestration:** Like other trees, mulberries play a role in carbon sequestration. Mulberries are rapidly growing trees with high photosynthetic activities, and therefore can reduce atmospheric carbon dioxide regarded as a good carbon sink, thereby mitigating the impact of climate change (Nepal & Purintun, 2021). According to Ghosh et al. (2017), one mulberry tree is able to absorb 4162 kg of carbon dioxide and release 3064 kg of oxygen each year.



**Air Quality Improvement:** The leaves of mulberry plants also have a strong ability to absorb air pollutants such as carbon monoxide, hydrogen fluoride, sulphur dioxide and chlorine from the atmosphere (Lu et al., 2004; Ghosh et al., 2017). Mulberries can absorb 5.7 g of sulphur dioxide per kg of dry leaves and mulberry forests can annually absorb large amounts of sulphur dioxide gas (Lu and Jiang, 2003). Therefore, mulberry is categorised as a tree species resistant to sulphur dioxide pollution (Jian et al., 2012). Mulberry trees were likewise considered to be naturally resistant to chlorine, as their leaves were not damaged even when exposed to higher levels of chlorine (Lu et al., 2004). These abilities have led to the mulberry plant being used efficiently for bioremediation of air pollutants in urban areas and phytoremediation of carbon pollutants in contaminated soils around industries and chemical factories (Olson and Fletcher, 1999; Rohela et al. 2020).

**Microclimate Regulation:** The presence of mulberry trees can influence the local microclimate. They provide shade, which can reduce temperatures in urban areas, and their transpiration process can increase humidity, making the local climate more comfortable.

**Water Regulation:** Mulberry trees can help in regulating water cycles. Their root systems can enhance groundwater recharge and reduce surface runoff, helping to prevent flooding and maintaining water levels in nearby water bodies. The deep and wide spreading rooting system of mulberry has enabled the soils to retain and hold water at increased rates in comparison with other plant species. A deep and densely tangled network of root systems of mulberry is reported to improve the shear strength of soils and thereby increase the anti-erosion capacity of lands planted with mulberry. The annual runoff in mulberry plantation sites of plain lands can be reduced by 38% under 5 years old and by 91% under 10 years old bush plantations respectively in comparison with slope lands (Du et al., 2001).

**Animal husbandry:** Besides their primary use as feed for the silkworms, mulberry trees play a significant role in animal husbandry, contributing to the nutritional diet of livestock and offering economic benefits to the farming community. Recognised for their adaptability across varied agro-climatic zones, mulberry plants can grow in tropical to temperate conditions, either as shrubs or trees. While primarily cultivated for silkworm rearing, the leaves and branches of mulberry trees are also utilized as fodder for domestic animals in countries like India, China and Korea (Savithri et al., 2013).

Mulberry leaves are rich in proteins, carbohydrates, and minerals, exhibiting high digestibility, which is crucial for animal feed. The nutritional composition of young mulberry leaves includes 20-23% crude protein, 8-10% total sugar, and 12-18% minerals, though these values can vary dependent on the genotype, pruning practise, with the season and age of the leaf (Yashvant et al., 2015; Urbanek Krajnc et al. 2019, Šelih et al. 2020). This nutritional

profile makes mulberry leaves an excellent supplement to traditional feeds, which may lack balanced nutritional content.

The impact of the mulberry leaves as nutritional supplement on livestock has been studied by several authors (Tateno et al., 1999, b; Sudo et al., 2000, Datta et al. 2002, Yao et al., 2000, Venkatesh Kumar et al., 2015). Mulberry leaves have been shown to increase milk production, with studies indicating enhancements in milk protein, carbohydrate, and lipid content after feeding mulberry leaves to cows and goats for 60 days (Venkatesh Kumar et al., 2015; Datta et al. 2002) also observed increases in milk yield and fat content in cow milk from mulberry feeding. Furthermore, supplementation of mulberry leaves with ammoniated rice straw improved lamb and sheep growth rates (Yao et al., 2000; Rohela et al. 2020).

Mulberry leaves have been reported to increase egg size and improve yolk colour in hens with additional benefits such as increased beta-carotene, and vitamin K content observed in poultry birds (Tateno et al., 1999; Sudo et al., 2000).

The integration of mulberry leaves into the diets of pigs and rabbits has led to economic benefits, including reduced costs of commercial feeding (Trigueros and Villalta, 1997; Sanchez, 2000). Deshmukh et al. (1993) reported that mulberry leaves provide sufficient nutrition for growing rabbits.

The use of mulberry leaves as animal feed presents an opportunity for reducing commercial feed costs while maintaining or enhancing livestock productivity and health. This aligns with integrated farming systems promoted by government agencies, aiming to double the income of the farming community by combining crop production with livestock raising. Mulberry's low maintenance requirements and adaptability make it a sustainable alternative to traditional fodder crops, especially in areas where land resources are limited.

**Cultural and Recreational Services:** Mulberry trees can have cultural significance and contribute to the aesthetic value of landscapes. They are often planted in gardens and parks for their beauty and are appreciated for their fruits.

Each of these services contributes to the overall health and sustainability of ecosystems, highlighting the importance of trees in maintaining ecological balance.

## 6.1 Case studies of a landscape-based regional design approach by including mulberry trees in providing ecosystem service

### 6.1.1 Italy

This traditional agricultural landscape showcases an ancient organization of agrarian space, with roots traceable to Etruscan and Roman times, featuring a diverse cultivation approach known as multi- or poly-cropping. This system harmoniously integrates three types of crops: a shrub-like plant species (commonly grapevines), a supportive tree species (elm, maple, willow, and notably fruit trees such as peaches and mulberries serving as a natural trellis for the vines, while also providing pasture, fruit, or wood), and a ground-level herbaceous crop (such as cereals, vegetables, or a permanent grass meadow). The interplay among these inter-cropped plants creates a synergistic effect, enhancing the ecological efficiency of the system. This not only boosts the biodiversity and health of the ecosystem but also yields significant economic benefits, making it a sustainable model of agriculture that leverages the natural advantages of varied plant interactions.

The unique approach known as the "Piantata Veneta" emerged in Italy during the 18th and 19th centuries, incorporating precise hydraulic systems to enhance water balance efficiency. This method is a prime example of multi-cropping, showcasing the innovative ways in which past civilizations engineered symbiotic relationships between plant species for both mutual and economic gains.

Designed to maximize harvests across different seasons, this technique was particularly beneficial in times when farmers paid their rent through natural produce to landowners. However, the practice was not without its challenges; for instance, poor cereal yields were sometimes a consequence of insufficient manure availability. To mitigate this, legume grasses were cultivated where feasible, enhancing soil fertility and crop productivity. While this approach was economically driven, it arguably offered a more sustainable alternative to some modern intensive farming methods, underlining the historical precedence of ecological and economic balance in agricultural practices.

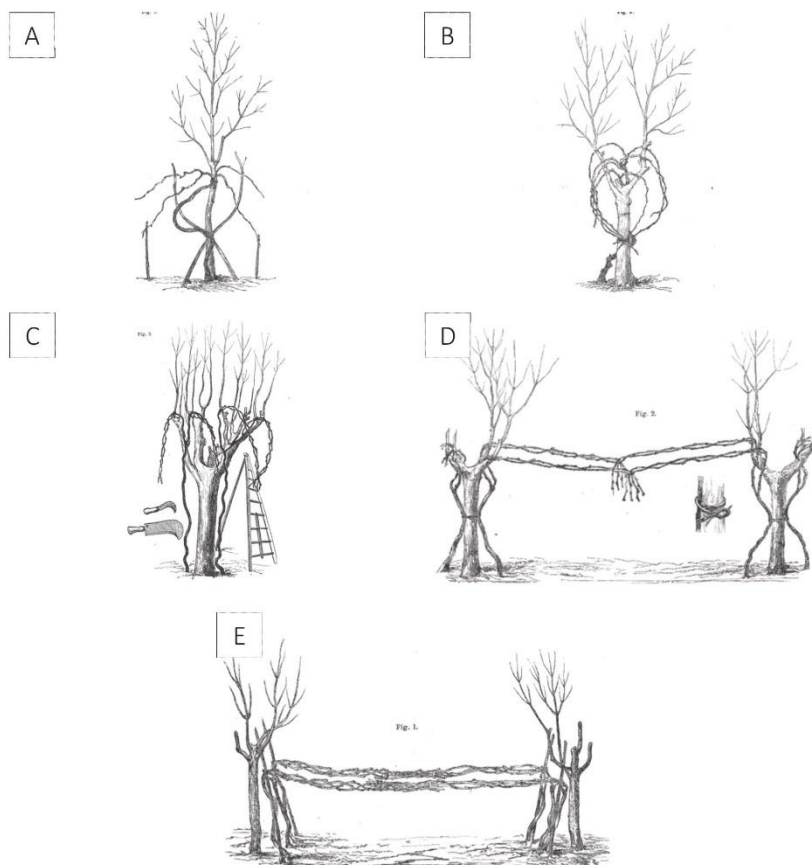
The transition from a variety of tree species to predominantly mulberry trees was significantly influenced by the silk industry's demand for mulberry leaves to feed silkworms. This shift underscores the adaptability of agricultural practices to economic challenges, while maintaining the essence of traditional polyculture systems. Such systems are highlighted in the scientific and technical literature of the 1800s, with Giovanni Bottari's manuals in the Veneto-Friulian territory and the Carpené Vianello viticulture book of 1874

providing detailed methods for vine cultivation on trees, demonstrating the long-standing integration of this poly-culture into the Italian agricultural landscape.

The fact that the Superintendency for Historical, Artistic and Ethno-anthropological Heritage for the provinces of Venice, Belluno, Padua and Treviso, in north-eastern Italy, has protected the historical plantation landscape managed by the Borgo di Baver Onlus Association since February 2014 as a cultural asset of an ethno-anthropological nature, underlines the value of this cultivation system and the preservation of agrobiodiversity. Moreover, the inclusion of 'La Piantata Veneta' of Baver in the National Register of Rural Landscapes of Agricultural Practices and Traditional Knowledge in 2018 highlights the national recognition of this practice as an integral part of Italy's rural heritage (Reterurale, 2020).

The services provided by this poly-cropping systems include wind protection, hydrogeological benefits, wood and fruit production, and the enhancement of recreational and tourism potential. These benefits are anchored in specific agronomic practices, such as strategic planting, pruning, and the avoidance of insecticides, by adopting preventive agronomic measures to ensure the health of both the mulberry trees and the vines.

Taken together, these elements paint a picture of a practice that is deeply interwoven with Italy's agricultural and cultural identity, and demonstrate the enduring importance of traditional knowledge in fostering sustainable, biodiverse, and culturally rich agricultural systems (**Figure 21-23**).



**Figure 21** - Schematic demonstration of planting vine along the mulberry trees (Vianello A. & Carpena A. 1874).

The traditional agricultural practise “La Piantata Veneta” (cultivated plots surrounded by rows of vines planted next to tall trees) was included in the (Italian) National Register of Rural Landscapes with Agricultural Practises and Traditional Knowledge (2018).





**Figure 22** - The 'Ancient Vineyard of Bayer' in Godega di Sant'Urbano serves as a living example of mulberry-vine polyculture.



**Figure 23** - The 'Ancient Vineyard of Baver' in Godega di Sant'Urbano serves as a living example of mulberry-vine polyculture. Examples of mulberry rows between arable land. The ecosystem's service extends to wind protection, hydrogeological benefits, production of wood and fruits, and enhancement of recreational and tourism potential.

A Friulian project stands out in terms of passion for keeping mulberry giants alive. In Vivaro (Pordedone province), Mauro Rizzotti, a dedicated farmer re-discovers centuries-old mulberry trees after many years of neglect. He rescues, re-plants, maintains and protects these monumental trees together with his girlfriend Laura Nassimbeni and small rural municipalities.

Their collective effort created a unique natural biodiversity oasis, to the benefit of the local community, mulberries and endangered species, such as the little shrike (*Lanius collurium*). In an awareness project, Laura Nassimbeni introduces Vivaro primary school children to the rich world of the mulberry giants.

The efforts of generations of farmers, propagating their favourite mulberries by grafting led to a high diversity in local varieties in this collection. This genetic treasure allows for local varieties with specific tolerance to drought, salinity, water logging, insects or disease, depending on their origin.

More than 700 mulberry trees, which are between 70 and 400 years old, thrive in Mauro Rizzotti's 'sacred forest'. Three centuries-old mulberry trees, for instance, were rescued from Villafranca (Verona province) and re-planted in Vivaro. A special skill set and a lot of effort and money are required for successfully uprooting, transporting and replanting such monumental mulberry trees. Mauro Rizzotti's biggest wish is to transform his mulberry orchard into a park. It would certainly be a must-see point of interest on the 'ARACNE Silk-Road' tourist itinerary (Benedetti & Fila, 2023).

### 6.1.2 Slovenia

#### 6.1.2.1 Attempts at planting mulberries in vineyards to provide ecosystem service

In Slovenia, the first attempts at reintroducing mulberry cultivation have been made under the bilateral Slovenian-Hungarian research project ARIS N1-0041, which was based on the evaluation of the existing genetic resources of historical mulberries in Slovenia and Hungary, collection establishment, identification of important metabolites in leaves (SI priorities) and testing their relevance on the development and health of silkworms (HU priorities).

Furthermore, within the EIP project 'Introduction of New Mechanical and Autonomous Automated Technologies for Sustainable Grape Production in Vineyards,' we underscored the practice of preserving biodiversity in vineyards through the introduction of various native fruit species, including mulberries. By experimentally integrating mulberries into the vineyard landscape, specifically on banks and terraces, we aimed to mitigate erosion and explore the phytoremediation capabilities of mulberries in relation to fungicide, herbicide, and insecticide (FFS) residues. Mulberries were identified for their rapid growth and capacity to sequester heavy metals and semimetals within silicon bodies, underscoring their utility as a phytoremediation species. The project also aimed to preserve old local mulberry genotypes and traditional orchards in former sericultural regions (**Figure 24-27**).

Key outcomes and benefits of integrating mulberries into vineyard agroecosystems include:

- Erosion control on banks and terraces.
- Enhanced phytoremediation potential, capturing excess pesticide residues and nutrients.



- Improved nutrient availability and preservation of vital soil microbiota, especially vesicular-arbuscular (VA) mycorrhizae.
- The deep-root system of mulberries promotes soil aeration and indirectly supports soil fertility.
- Mulberries serve as a refuge and feeding ground for beneficial insects, birds, reptiles, and small mammals, enriching local biodiversity.

By reintroducing ancient local mulberry varieties, we significantly contribute to the conservation of the natural and cultural heritage associated with sericulture, reinforcing the symbiotic relationship between agricultural innovation and environmental stewardship.



**Figure 24** - Planting of mulberry trees along with cover crops at the vineyard Simčič, Brda in form of rows along two pilot vineyards in order to establish natural corridors for beneficial animals.



**Figure 25** - Newly established vineyard of the company Merum involved planting of several white and black mulberry trees in form of corridors.





**Figure 26** - Map with marked location of newly established vineyard of the Merum company. Arrow marks the position of the mulberry plantation.

The Institute of Sericulture and Silk Processing (Svila, 2024), under the leadership of Rebeka Lucijana Berčič, has been actively engaged in the revival of sericulture through the planting of mulberry trees and the reintroduction of sericulture practices.

Alongside the agricultural efforts of planting mulberry trees, the institute aims to reintroduce sericulture practices. This includes training programmes for farmers and entrepreneurs on silkworm rearing techniques, silk production processes, and the economic benefits of sericulture. The initiative seeks to provide comprehensive knowledge and support, from mulberry cultivation to silkworm rearing and silk processing.

Currently, the Institute supports several farmers and enthusiasts who plant mulberries in the form of plantations, rows or individual trees. Besides their use for rearing silkworms, they also use the leaves also for feeding other animals (poultry, rabbits, cattle) and for fruit production.

The initiatives led by Rebeka Luciana Berčič and the Institute of Sericulture represent a multifaceted approach to reviving an ancient industry through modern, sustainable practices also in the form of urban farming. By combining agricultural innovation with traditional

knowledge, these efforts aim to establish sericulture as a viable and environmentally friendly source of income and to preserve an important cultural heritage.

#### 6.1.2.2 Mulberry naturalization at the Ormož basins

The Ormož Basins, situated near the town of Ormož in Slovenia, close to the Croatian border, epitomize a remarkable environmental restoration and conservation endeavour. Formerly an industrial site, this area has been remarkably transformed into a crucial habitat for an array of bird species, showcasing the potential for repurposing industrial sites into spaces for nature conservation.

These basins represent an exemplary case of an anthropogenic ecosystem. Spanning 69.59 hectares in the vicinity of the Drava River and Lake Ormoz, the area has seen significant ecological and land-use changes over time. Originally a floodplain forest until 1969, it was later replaced with poplar plantations. The construction of the Varaždin hydroelectric power plant in 1975 led to the creation of an accumulation lake, and by 1977, the area began its industrial chapter with the construction of a sugar factory, leading to the establishment of the wastewater basins known today as the Ormož Basins. Despite the factory's operation, the site was a refuge for many rare birds. The sugar factory ceased operations in 2006, following EU sugar sector reforms. Subsequently, the basins underwent rehabilitation with EU funds, enhancing the area's ecological conditions to support a variety of rare plant and animal species, especially birds. Infrastructure for visitors was developed, and in 2017, the Ormož Basins were officially designated as a nature reserve.

The basins, once pivotal to the sugar factory's operation by serving as settling ponds for wastewater containing lime (calcium carbonate) and other solids, now stand as a testament to environmental restoration. This process not only cleansed the wastewater but also resulted in a carbonate-rich sediment, laying the foundation for the basins' current ecological significance.

Today, the Ormož Basins are celebrated for their ecological significance on both national and international levels. They underscore the positive impact of environmental restoration projects and serve as a paradigm for converting industrial sites into precious natural habitats. The site draws nature enthusiasts, bird watchers, and researchers, bolstering the local economy through eco-tourism and providing essential ecological services.

Intriguingly, within the lime basin characterised by the carbonate-rich sediment a significant number of young mulberry trees have been observed, alongside various pioneer species such as *Sambucus nigra*, and to a lesser extent, *Salix* and *Populus*. This emergence of mulberry

trees, previously not found in the area, is a fascinating instance of naturalisation, likely facilitated by birds from the surrounding villages where old mulberry trees exist as remnants of former sericultural activities. The naturalisation of *M. alba* s.l. in the Ormož Basins reflects the species' adaptability and preference for carbonate-rich soils, showcasing its resilience to both drought conditions and transient wetlands (**Figure 27**). This phenomenon, similarly, observed on a smaller scale in Škocjan Bay, highlights the mulberry tree's ecological versatility.



**Figure 27** - Map of presenting the area of Ormož basins. Within the lime basin 211 mulberry trees have been recorded by local mulberry and silkworm farmer Maja Botulin Vaupotič. The area of the lime basin is approximately 140 x 220 metres.



### 6.1.3 France

While specific references to mulberry use in French agroforestry systems are scarce, the general principles of agroforestry and the known benefits of mulberries suggest that they could be a valuable addition to such systems in France, particularly in regions with suitable climates. Given France's commitment to sustainable agriculture and biodiversity conservation, as well as the increasing interest in agroforestry practices, further research and implementation of mulberry-based agroforestry systems could provide substantial ecological and economic benefits.

The curator of the Orchard-conservatory Mercuire informed us that several people have bought mulberry trees from them for their agroforestry system project. Two of these customers particularly wanted to use white mulberry leaves as a fodder supplement for sheep on the one hand, and as nitrogen fertiliser for the soil on the other hand.

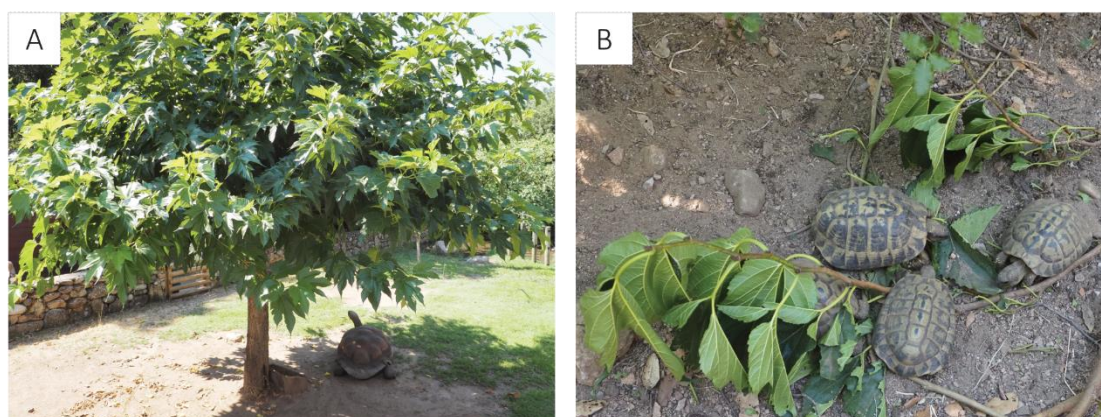
For detailed studies or specific examples of mulberry use in French agroforestry, one would need to consult academic databases, agricultural research institutions in France or specific agroforestry projects within the country that might have documented such applications.

A fascinating example of the practical application of mulberry trees in supporting conservation and animal care efforts is the 'A Cupulatta' turtle park in Corsica ('A cupulatta', 2024), which is dedicated to the protection and study of turtles and tortoises (**Figure 28**). The park showcases an innovative approach to sustainable animal feed through the cultivation of mulberry trees. This practice underscores the versatility of mulberry leaves as a sustainable, nutrient-rich feed option in animal husbandry and conservation settings.

The park's decision to plant mulberry trees aligns with sustainable agricultural practices by providing a renewable, locally sourced food supply for the turtles. This approach not only reduces the carbon footprint associated with the transport of feed but also ensures that the turtles receive a fresh and natural diet by daily harvesting of the shoots. It reflects a broader trend in conservation and animal care towards using environmentally friendly and sustainable resources.

Beyond the practical benefits of mulberry cultivation for turtle feed, the presence of these trees at 'A Cupulatta' likely contributes to the educational experience for visitors. By demonstrating the use of mulberry leaves as turtle feed, the park can highlight the importance of sustainable farming practices and the interconnectedness of plant and animal life within ecosystems. Additionally, it emphasizes the role of innovative feeding strategies in conservation efforts, particularly for species that may have specific dietary needs.

This innovative use of mulberry trees showcases the potential for integrating sustainable agricultural practices within animal care and conservation projects, contributing to the health and wellbeing of captive animals while promoting ecological sustainability.



**Figure 28** - Turtles feeding on mulberry leaves in the ‘A Cupulatta’ turtle zoo (Photo: A. Urbanek Krajnc)

#### 6.1.4 Other ARACNE countries

In Bulgaria most of the mulberry trees are planted along the streets of the villages/small towns. The vines in the villages/small towns, however, are planted inside private yards. Consequently, a polyculture of mulberry and vines is not common.

Detailed information about projects using mulberries for ecosystem services and landscape restoration in Bulgaria is not available in mainstream academic and project literature. However, the potential for such applications is consistent with the known ecological benefits of mulberry trees, which have been documented in various contexts globally.

The cultivation and presence of mulberry trees in Spain, particularly in the Murcia region, highlights an intriguing aspect of agricultural history intertwined with the legacy of silk culture. Traditionally, these trees have been relegated to the peripheries of irrigated plots, serving primarily to provide shade. This arrangement has inadvertently created a distinctive landscape feature characterised by scattered, isolated mulberry trees. While this scenario offers a visual connection to the historical silk culture for those familiar with it, it has not been embraced as a formal cultural practice.



In a fascinating development, the potential of mulberry trees is being explored beyond their historical and aesthetic contributions. A pioneering project focused on leveraging mulberry trees for carbon dioxide (CO<sub>2</sub>) capture, an endeavour that signifies a shift towards environmental and restorative applications of these trees. By establishing a mulberry farm in Valencia and other locations, this project is pioneering in its approach to integrating mulberry trees into the broader context of landscape restoration and environmental sustainability.

### 6.1.5 Attempts at using mulberry in landscape planning in other European countries

The integration of mulberry trees into European agroecosystems and conservation efforts is still in its nascent stages, with limited but promising attempts observed. These endeavours are part of the broader objectives of the ARACNE project, which seeks to explore and expand the role of mulberries in landscape planning and environmental sustainability across Europe. The following sections represent an overview of the current state of mulberry usage in landscape planning across the continent.

#### 6.1.5.1 Germany

Mulberry trees hold a unique place in Germany's environmental and historical tapestry, although their use in contemporary agroforestry practices in Germany is limited, primarily due to policy preferences for native species in projects aimed at enhancing natural biodiversity. This preference impacts the potential for broader integration of mulberry trees into modern landscape planning and conservation efforts.

Historically, mulberry trees were extensively planted in the 18th century, encouraged by royal decrees to support the silk industry through silkworm rearing. These historical plantings, often found in churchyards, schoolyards, and along roadsides, now serve as living relics of a bygone era of silk production. Their persistence in these locations highlights their resilience and the cultural heritage they represent.

Efforts to conserve and celebrate the mulberry tree's legacy in Germany, such as the Zernikow Initiative, which focuses on the conservation of mulberry trees and the broader narrative of mulberry silk production have been made over recent years. This initiative, along with the activities of individuals like Herbert Kolb and organizations such as Udo Krause's initiative, illustrates a continued interest in and dedication to the cultivation, preservation, and appreciation of mulberry trees. These efforts not only aim to preserve the trees

themselves but also to revive and sustain the rich cultural and historical narratives associated with mulberry silk production.

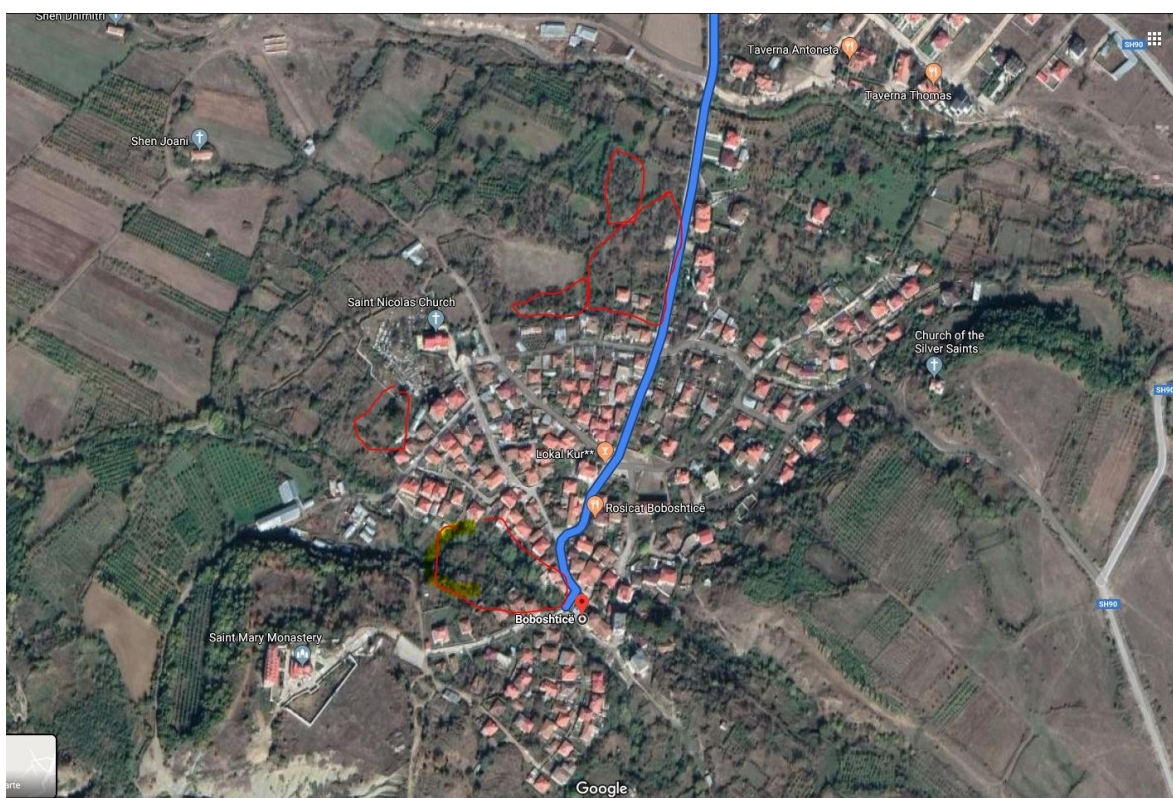
The Mulberry Alley at Zernikow was created between 1746 and 1748. The avenue was protected as a natural monument as early as 1938 and is now also part of the Zernikow estate, a registered cultural monument. The Mulberry Alley in Zernikow in the north of Mark Brandenburg reintroduced silkworm rearing and raw silk production, which were intensively promoted in Prussia in the mid-18th century. Until the middle of the 20th century, there were several phases in which silkworm rearing was practiced in this region, but only with limited economic success. The Mulberry Alley in Zernikow is unique in its characteristics and form and contributes significantly to the diversity, beauty and uniqueness of the cultural and natural landscape. The non-profit association 'Initiative Zernikow e.V.' works to preserve the cultural landscape and its history and organises various cultural events at the Zernikow estate every year. For many years, the association has also been in charge of preserving the mulberry alley, by caring for the remaining old trees and by planting new ones. In addition, a small exhibition, and the annual Mulberry Festival draw attention to the history of the alley (**Figure 29**). In this way, an almost forgotten part of the region's cultural history is preserved (Ines Ronnefhart, Initiative Zernikow e.V. (Initiative Zernikow, 2024).



**Figure 29** - Mulberry alley at the Zernikow estate is protected as natural monument (Source: Initiative Zernikow, 2024).

### 6.1.5.2 Albania

In South-eastern Albania there are a few villages with exceptionally old specimens of the true black mulberry, *M. nigra*. Fruits of some of these trees are collected by locals and processed into high-quality liquor, which is considered medicinal. But some of the trees have already fallen victim to the timber industry because the wood of the slow-growing species is sought after and valuable. In a project led by Marianne Graf (Albania-Austria Partnership), these mulberry trees have been propagated vegetatively and generatively since 2015 by farmers with low-income opportunities under the guidance of biologists. In return, the farmers receive financial support and the plants generated in this way are distributed to schools, farmers, and families, who take special care of further cultivation. This not only passes on the genetics of the old trees, but also promotes awareness of old trees in society. Official protection of the original old trees was also obtained as part of the project (Figure 30, 31).



**Figure 30** - Map showing the village Boboshtice (Albania) with red marked areas of old historical black mulberry trees.





**Figure 31** - In a Austrian-Albanian project led by Marianne Graf (Albania-Austria Partnership), mulberry trees have been propagated vegetatively and generatively since 2015 by farmers with low-income opportunities under the guidance of biologists (Photos: J. Rabensteiner).

### 6.1.5.3 Austria

Sericulture has been practiced in Austria since the 17th century at the latest, and white mulberry (*M. alba*) trees were planted along with it. There were more extensive efforts to introduce silk farming during the time of Empress Maria Theresa. The oldest *M. alba* specimens still alive in Austria originate from this time and are still reminiscent of this time today. Old genotypes of the black mulberry (*M. nigra*) are less common in Austria. However, there are a few trees that are particularly noticeable because of their gnarled growth habits. In an initiative by Johannes Rabensteiner, these trees are recorded in a private database, information is collected from the owners and trees are propagated using air layering (**Figure 32**) in order to further multiply these old genotypes and preserve them in the long term (Urbanek Krajnc & Rabensteiner 2018; Der Kampf, 2023).



**Figure 32** - Replanting of old *M. nigra* mulberry trees and air layering procedure of *M. nigra* tree.



## 7. Conclusions

The present deliverable provides information on the current mulberry germplasm status and mulberry varieties that are maintained in the germplasm collections of partner countries with respect to the current taxonomical classification. Furthermore, the deliverable is a report on the first year inventarisation of the local mulberry varieties in the landscapes of participating countries. The creation of maps of monumental trees for each country along with basic information and morphological characteristics aims to raise awareness of the cultural identity as well as the natural and cultural heritage of each country.

Based on the database of mulberry varieties available in the mulberry germplasm collections and the database of old local mulberry varieties of the partner countries and by evaluating their morphological characteristics, the most important varieties were selected for genetic analyses using the SSR and SNP-based genotyping methods. Further on, the results of the genetic analyses together with the collected information on the morphological characteristics of the trees will allow us to reconstruct the origin and distribution of mulberries among silkworm rearers in the past. In the last part of the report, case studies on landscape-based planning using mulberry trees in are presented for ARACNE and other European countries.

Despite the promising trials described above, the integration of mulberry trees into European landscapes faces several challenges, including limited awareness of the benefits of mulberry trees in agroecosystems and nature conservation, which hinders wider planting. In addition, more scientific research is needed to understand the best practices for growing mulberries in different European climates and soils. Nevertheless, more policy support and incentives are needed to encourage farmers and landowners to incorporate mulberries into their land use practices.

The exchange of information on mulberry germplasm and the utilisation of genetic resources at the global level has so far been slow, as national strategies are not coherent. The current report emphasises the urgent need to establish an international network for the management and utilisation of mulberry germplasm at the global level. At the time of the first year report, ARACNE is ready to take over the scientific research and coordination of the mulberry gene pool inventory in Europe to drive further research and testing of mulberry in the coming years. The objectives of the project include increasing the knowledge base on mulberry cultivation, demonstrating its multiple benefits for agro-ecosystems and nature conservation, and ultimately integrating mulberries into European landscape planning and environmental strategies.

## 7.1 ACRONYMS

[AFLP]	amplified fragment length polymorphisms
[AP-PCR]	arbitrarily primed polymerase chain reaction
[CAPS]	cleaved amplified polymorphic sequence ( )
[cf.]	confer
[DA]	discriminant analysis
[DAF]	DNA amplification fingerprinting
[EST]	expressed sequence tag (EST)
[f.]	form
[IPNI]	IPNI-The International Plant Names Index (International Plant Names Index 2024)
[ISSR]	intersimple sequence repeat
[PCA]	principal component analysis
[POWO]	POWO Plants of the World Online (Kew 2024)
[RAPD]	random amplified polymorphic DNA markers
[RFLP]	restriction fragment length polymorphisms
[SCAR3]	sequence characterised amplified regions
[s.l.]	sensu lato
[SNP]	single nucleotide polymorphism markers
[SSR]	simple sequence repeat markers
[STS]	sequence tagged sites (STS)
[var.]	variety
[WPO]	WPO Plant List (WFO Plant list 2024)

## Appendix I – CREA mulberry germplasm Supplementary Tables

**Supplementary Table 1** - List of *Morus* varieties maintained in the CREA Padua collection.

Species	Variety	Origin (year of introduction)	Obtained	Prop./Planting date	No. of individuals	Descriptor monitoring
<b>Brazil</b>						
<i>M. alba</i> s.l.	'Korin'	Brazil	Brazil (private farmer)	1990s	8	<input checked="" type="checkbox"/>
<i>M. alba</i> s.l.	'Miura'	Brazil	Brazil (private farmer)	1990s	10	
<b>China</b>						
<i>M. alba</i> (syn. <i>M. multicaulis</i> var. <i>planifolia</i> )	'Kasuga'	China	former Sericulture Station of Ascoli Piceno (IT)	1985	18	
<i>M. alba</i> (syn. <i>M. multicaulis</i> var. <i>planifolia</i> )	'Kayriou Rosou'	China	former Sericulture Station of Ascoli Piceno (IT)	1985	20	<input checked="" type="checkbox"/>
<i>M. alba</i> (syn. <i>M. multicaulis</i> var. <i>planifolia</i> )	'Kayriou Nezumigaeshi'	China				
<i>M. alba</i> (syn. <i>M. multicaulis</i> var. <i>planifolia</i> )	'Seijuurou'	China	former Sericulture Station of Ascoli Piceno (IT)	1985	18	

Species	Variety	Origin (year of introduction)	Obtained	Prop./Planting date	No. of individuals	Descriptor monitoring
<i>M. alba</i> (syn. <i>M. multicaulis</i> var. <i>planifolia</i> )	'Tougounishiki'	China	former Sericulture Station of Ascoli Piceno (IT)	1985	5	
<i>M. alba</i> s.l.	'Tago-Wase o Tagowase'	China	former Sericulture Station of Ascoli Piceno (IT)	1985	12	
<i>M. indica</i> (syn. <i>M. bombycis</i> )	'Akagi'	China	former Sericulture Station of Ascoli Piceno (IT)	1985	17	☑
<i>M. indica</i> (syn. <i>M. bombycis</i> )	'Date-Akagi'	China	former Sericulture Station of Ascoli Piceno (IT)	1985	10	☑
<i>M. indica</i> (syn. <i>M. bombycis</i> )	'Enshu-Takasuke'	China	former Sericulture Station of Ascoli Piceno (IT)	1985	20	
<i>M. indica</i> (syn. <i>M. latifolia</i> )	'Daikokusou'	China	former Sericulture Station of Ascoli Piceno (IT)	1985	9	
<i>M. indica</i> (syn. <i>M. latifolia</i> )	'Daikokusou 70'	China	former Sericulture Station of Ascoli Piceno (IT)	1985	13	☑
<b>France</b>						
<i>M. alba</i> (syn. <i>M. multicaulis</i> var. <i>planifolia</i> )	'Planifolia'	France	CBNMed-Porquerolles (FR)	2010	3	
<i>M. alba</i> s.l.	'Badena Tout'	France	CBNMed-Porquerolles (FR)	2009	3	
<i>M. alba</i> s.l.	'Cabassette'	France	CBNMed-Porquerolles (FR)	2009	2	
<i>M. alba</i> s.l.	'Grisetto'	France	CBNMed-Porquerolles (FR)	2009	4	
<i>M. alba</i> s.l.	'Romana lhou'	France	CBNMed-Porquerolles (FR)	2009	1	

Species	Variety	Origin (year of introduction)	Obtained	Prop./Planting date	No. of individuals	Descriptor monitoring
<i>M. alba</i> s.l.	'Romana rabaleire'	France	CBNMed-Porquerolles (FR)	2009	4	
<i>M. alba</i> s.l.	'Rougetto'	France	CBNMed-Porquerolles (FR)	2009	2	
<i>M. alba</i> s.l.	'Tenuifolia seringe	France	CBNMed-Porquerolles (FR)	2010	2	
<b>India</b>						
<i>M. alba</i> s.l.	'Indiana'	India	former Sericulture Station of Ascoli Piceno (IT)	2010	7	<input checked="" type="checkbox"/>
<b>Italy</b>						
<i>M. alba</i> s.l.	'Arancina'	Italy	former Sericulture Station of Ascoli Piceno (IT)	1985	34	
<i>M. alba</i> s.l.	'Ascolana'	Italy	former Sericulture Station of Ascoli Piceno (IT)	1985	10	
<i>M. alba</i> s.l.	'Cattaneo (female)'	Italy	former Sericulture Station of Ascoli Piceno (IT)	1985	9	<input checked="" type="checkbox"/>
<i>M. alba</i> s.l.	'Cattaneo (male)'	Italy	former Sericulture Station of Ascoli Piceno (IT)	1985	10	<input checked="" type="checkbox"/>
<i>M. alba</i> s.l.	'Florio'	Italy	former Sericulture Station of Ascoli Piceno (IT)	1985	30	<input checked="" type="checkbox"/>
<i>M. alba</i> s.l.	'Giazzola'	Italy	former Sericulture Station of Ascoli Piceno (IT)	1990	24	<input checked="" type="checkbox"/>
<i>M. alba</i> s.l.	'Lhou'	Italy	former Sericulture Station of Ascoli Piceno (IT)	1985	5	



Species	Variety	Origin (year of introduction)	Obtained	Prop./Planting date	No. of individuals	Descriptor monitoring
<i>M. alba</i> s.l.	'Limoncina'	Italy	former Sericulture Station of Ascoli Piceno (IT)	1985	25	
<i>M. alba</i> s.l.	'Morettiana'	Italy	former Sericulture Station of Ascoli Piceno (IT)	1985	26	☑
<i>M. alba</i> s.l.	'Muki'	Italy	former Sericulture Station of Ascoli Piceno (IT)	1985	30	
<i>M. rubra</i> cf.	'Nerrosa (narrow/large leaf)'	Italy	former Sericulture Station of Ascoli Piceno (IT)	1980	2	☑
<i>M. rubra</i> cf.	'Nerrosa (wide/large leaf)'	Italy	former Sericulture Station of Ascoli Piceno (IT)	1980	2	☑
<i>M. alba</i> s.l.	'Pendula'	Italy	former Sericulture Station of Ascoli Piceno (IT)	2000	8	☑
<i>M. alba</i> s.l.	'Pyramidalis'	Italy	former Sericulture Station of Ascoli Piceno (IT)	1985	5	☑
<i>M. alba</i> s.l.	'Restelli'	Italy	former Sericulture Station of Ascoli Piceno (IT)	1985	46	☑
<i>M. alba</i> s.l.	'Rosa di Lombardia'	Italy	former Sericulture Station of Ascoli Piceno (IT)	1985	12	
<i>M. alba</i> s.l.	'Selvatico'	Italy	former Sericulture Station of Ascoli Piceno (IT)	1990	15	
<i>M. alba</i> s.l.	'Spagna frutto bianco (Spain white fruit)'	Italy	former Sericulture Station of Ascoli Piceno (IT)	1985	4	

Species	Variety	Origin (year of introduction)	Obtained	Prop./Planting date	No. of individuals	Descriptor monitoring
<i>M. alba</i> s.l.	'Spagna frutto nero (Spain black fruit)'	Italy	former Sericulture Station of Ascoli Piceno (IT)	1985	7	
<i>M. alba</i> s.l.	'unknown (internally nicknamed 'Svizzera')'	Italy	former Sericulture Station of Ascoli Piceno (IT)	1970	21	
<i>M. indica</i> (syn. <i>M. kagayamae</i> )	'Platanoide'	Italy	former Sericulture Station of Ascoli Piceno (IT)	1985	4	<input checked="" type="checkbox"/>
<i>M. indica</i> (syn. <i>M. latifolia</i> )	'Kokusou rosso'	Italy. Mutation in Kokuso_20 observed at CREA-AA, Padoua	mutation of Kokusou 20/21 occurred in Italy (IT)	1970s	5	<input checked="" type="checkbox"/>
<b>Japan</b>						
<i>M. alba</i> (syn. <i>M. multicaulis</i> var. <i>planifolia</i> )	'Goshoerani	Japan	former Sericulture Station of Ascoli Piceno (IT)	1985	12	
<i>M. alba</i> (syn. <i>M. multicaulis</i> var. <i>planifolia</i> )	'Okaraguwa'	Japan	former Sericulture Station of Ascoli Piceno (IT)	1985	10	
<i>M. alba</i> (syn. <i>M. multicaulis</i> var. <i>planifolia</i> )	'Rosou'	Japan	former Sericulture Station of Ascoli Piceno (IT)	1985	10	
<i>M. alba</i> s.l.	'Aoba nezumi'	Japan	CBNMed-Porquerolles (FR)	2010	4	
<i>M. alba</i> s.l.	'Hayate sakari'	Japan	CBNMed-Porquerolles (FR)	2009	4	
<i>M. alba</i> s.l.	'Ichinose'	Japan	Brazil (private farmer)	1990s	15	<input checked="" type="checkbox"/>
<i>M. alba</i> s.l.	'Kayriou Nezumigaeshi'	Japan	Brazil (private farmer)	1990s	20	

Species	Variety	Origin (year of introduction)	Obtained	Prop./Planting date	No. of individuals	Descriptor monitoring
<i>M. alba</i> s.l.	'Kayriou-Wase-Jumonji'	Japan	former Sericulture Station of Ascoli Piceno (IT)	1985	11	
<i>M. alba</i> s.l.	'Nagazaki'	Japan	CBNMed-Porquerolles (FR)	2009	3	
<i>M. indica</i> (syn. <i>M. bombycis</i> )	'Kenmochi'	Japan	former Sericulture Station of Ascoli Piceno (IT)	1985	10	
<i>M. indica</i> (syn. <i>M. bombycis</i> )	'Shimanouchi'	Japan	former Sericulture Station of Ascoli Piceno (IT)	1985	11	
<i>M. indica</i> (syn. <i>M. bombycis</i> )	'Yamanake-Takasuka'	Japan	former Sericulture Station of Ascoli Piceno (IT)	1985	5	
<i>M. indica</i> (syn. <i>M. latifolia</i> )	'Kokka'	Japan	former Sericulture Station of Ascoli Piceno (IT)	1985	9	
<i>M. indica</i> (syn. <i>M. latifolia</i> )	'Kokousou 20'	Japan	former Sericulture Station of Ascoli Piceno (IT)	1985	22	☑
<i>M. indica</i> (syn. <i>M. latifolia</i> )	'Kokousou 21'	Japan	former Sericulture Station of Ascoli Piceno (IT)	1985	20	☑
<i>M. indica</i> (syn. <i>M. latifolia</i> )	'Kokousou 27'	Japan	former Sericulture Station of Ascoli Piceno (IT)	1985	18	
<i>M. indica</i> (syn. <i>M. latifolia</i> )	'Nihou Kokusou 70'	Japan				☑
<i>M. indica</i> (syn. <i>M. latifolia</i> )	'Kokusou' with fruits	Japan				☑
<i>M. indica</i> (syn. <i>M. latifolia</i> )	'Kokusou' fruitless	Japan				☑
Middle-East						

Species	Variety	Origin (year of introduction)	Obtained	Prop./Planting date	No. of individuals	Descriptor monitoring
<i>M. alba</i> × <i>M. nigra</i>	'Chirtut'	Middle-East	CBNMed-Porquerolles (FR)	2009	2	☑
<i>M. alba</i> s.l.	'Herati'	Middle-East	CBNMed-Porquerolles (FR)	2009	4	
Philippines						
<i>M. alba</i> (syn. <i>M. multicaulis</i> var. <i>planifolia</i> )	'Filippine'	Philippines	former Sericulture Station of Ascoli Piceno (IT)	2010	2	
UK						
<i>M. alba</i> (syn. <i>M. multicaulis</i> var. <i>planifolia</i> )	'Queensland black'	UK	CBNMed-Porquerolles (FR)	2009	4	
Ukraine						
<i>M. alba</i> s.l.	'Ukraina 9'	Ukraine	Cluji-Napoca University	2009	4	
USA						
<i>M. rubra</i> × <i>M. alba</i>	'Hicks fancy'	USA	CBNMed-Porquerolles (FR)	2009	2	
<i>M. rubra</i> × <i>M. alba</i>	'Illinois everbearing'	USA	CBNMed-Porquerolles (FR)	2009	4	
Origin is not known						
<i>M. alba</i> s.l.	'Sinuense'	unknown	former Sericulture Station of Ascoli Piceno (IT)	1985	24	
<i>M. alba</i> s.l.	'Sterile'	unknown	former Sericulture Station of Ascoli Piceno (IT)	1985	16	
<i>M. nigra</i>	unknown variety	unknown		1990	2	

## Appendix II – SCS Vratsa mulberry germplasm Supplementary Tables

**Supplementary Table 2-** List of *M.* varieties maintained in the SCS Vratsa collection.

species	variety	origin (year of introduction)	prop./planting date	No.indiv.	
<b>Armenia</b>					
<i>M. alba</i> (syn. <i>M. multicaulis</i> )	No 127-Armenia'	Armenia, 1964	1966,1967	19	✓
<i>M. alba</i> s.l.	No 129-Russkaya'	Armenia, 1964	1966,1967	20	✓
<i>M. alba</i> (syn. <i>M. multicaulis</i> )	No 130-Singetz'	Armenia, 1964	1966	10	✓
<i>M. alba</i> s.l.	No 131-Sujrh'	Armenia, 1964	1966	7	✓
<b>Azerbaijan</b>					
<i>M. alba</i> (syn. <i>M. multicaulis</i> )	No 115-Pobeda'	Azerbaijan	1958,1964,1966,1967	60	✓
<i>M. alba</i> s.l.	No 161-Zariff tut'	Azerbaijan, 1962	1966	10	✓
<i>M. alba</i> s.l.	No 162 – Emin tut'	Azerbaijan, 1962	1966,1973	18	✓
<i>M. alba</i> s.l.	No 163 – Jacub tut'	Azerbaijan, 1962	1966	9	✓
<i>M. alba</i> s.l.	No 164 – Firudin tut'	Azerbaijan, 1962	1966,1973	27	✓
<i>M. alba</i> s.l.	No 165 – Azeri tut'	Azerbaijan, 1962	1966	8	✓
<i>M. alba</i> s.l.	No 166 - Azerbaijan 20'	Azerbaijan, 1962	1966,1973	21	✓
<i>M. sp.</i>	AzNIISH 7'	Azerbaijan, 2004	2004	5	✓
<i>M. sp.</i>	Camil tut'	Azerbaijan, 2004	2004	5	✓



species	variety	origin (year of introduction)	prop./planting date	No.iondiv.	
<i>M. sp.</i>	Gezal tut'	Azerbaijan, 2004	2004,201	20	✓
<i>M. sp.</i>	Hanlar tut'	Azerbaijan, 2004	2004	5	✓
<b>Bulgaria</b>					
<i>M. alba</i> s.l.	Hybrid 2a'	Bulgaria	1951	11	
<i>M. alba</i> s.l.	Hybrid 4b'	Bulgaria	1964	16	
<i>M. alba</i> s.l.	Hybrid 6a'	Bulgaria	1967	101	
<i>M. alba</i> s.l.	Hybrid 7a'	Bulgaria	1952	30	
<i>M. alba</i> s.l.	Hybrid 8b'	Bulgaria	1958	7	
<i>M. alba</i> s.l.	Hybrid 9a'	Bulgaria	1973	432	
<i>M. alba</i> s.l.	Hybrid II'	Bulgaria	1958	20	
<i>M. alba</i> s.l.	Hybrid IV'	Bulgaria	1958	20	
<i>M. alba</i> s.l.	Hybrid VII'	Bulgaria	1990	9	
<i>M. alba</i> s.l.	Hybrid VIII'	Bulgaria	1999	9	
<i>M. alba</i> s.l.	No 24'	Bulgaria	1937,1950,1951,1952,1956,1958,1964,1966,1970	1045	✓
<i>M. alba</i> s.l.	No 26'	Bulgaria	1951,1966	43	✓
<i>M. alba</i> s.l.	No 3'	Bulgaria	1937,1952,1964,1966	607	✓
<i>M. alba</i> s.l.	Vratsa 18'	Bulgaria, 1976	1966,1967,1970,2010	76	✓
<i>M. indica</i> (syn. <i>M. latifolia</i> )	Vesletz'	Bulgaria, 1983	1973,198	36	

species	variety	origin (year of introduction)	prop./planting date	No.iondiv.	
<i>M. indica</i> (syn. <i>M. kagayamae</i> )	Vratsa 1'	Bulgaria, 1976	1966,1967,1970,1980	198	<input checked="" type="checkbox"/>
<i>Morus</i> sp.	117 x local'	Bulgaria	1982	49	
<i>Morus</i> sp.	119 x 119'	Bulgaria	1982	11	
<i>Morus</i> sp.	119 x local'	Bulgaria	1982	88	
<i>Morus</i> sp.	C 1'	Bulgaria	1999	5	
<i>Morus</i> sp.	C 2'	Bulgaria	1973	5	
<i>Morus</i> sp.	C 4'	Bulgaria	1999	5	
<i>Morus</i> sp.	C 6'	Bulgaria	1999	5	
<i>Morus</i> sp.	C 9'	Bulgaria	1999	5	
<i>Morus</i> sp.	Hybrid 12/1 No117'	Bulgaria	1968	64	
<i>Morus</i> sp.	Hybrid 12/10 No24xNo120'	Bulgaria	1968	63	
<i>Morus</i> sp.	Hybrid 12/11 No106xNo120'	Bulgaria	1968	27	
<i>Morus</i> sp.	Hybrid 12/13 119xNo116'	Bulgaria	1968	56	
<i>Morus</i> sp.	Hybrid 12/14 No119xNo118'	Bulgaria	1968	49	
<i>Morus</i> sp.	Hybrid 12/15 No173'	Bulgaria	1968	30	
<i>Morus</i> sp.	Hybrid 12/17 No119xNo3'	Bulgaria	1968	22	
<i>Morus</i> sp.	Hybrid 12/18 No118xNo3'	Bulgaria	1968	18	
<i>Morus</i> sp.	Hybrid 12/2 No155'	Bulgaria	1968	51	
<i>Morus</i> sp.	Hybrid 12/3 No24'	Bulgaria	1968	79	
<i>Morus</i> sp.	Hybrid 12/4 No106'	Bulgaria	1968	31	

species	variety	origin (year of introduction)	prop./planting date	No.iondiv.
<i>Morus</i> sp.	Hybrid 12/5 No119'	Bulgaria	1968	67
<i>Morus</i> sp.	Hybrid 12/6 No120'	Bulgaria	1968	37
<i>Morus</i> sp.	Hybrid 12/7 No117xNo118'	Bulgaria	1968	33
<i>Morus</i> sp.	Hybrid 12/8 No117xNo120'	Bulgaria	1968	31
<i>Morus</i> sp.	Hybrid 12/9 No24xNo116'	Bulgaria	1968	56
<i>Morus</i> sp.	No 14'	Bulgaria	1937,1951,1952	39
<i>Morus</i> sp.	No 15'	Bulgaria	1952	8
<i>Morus</i> sp.	No 16'	Bulgaria	1952	7
<i>Morus</i> sp.	No 17'	Bulgaria	1952	8
<i>Morus</i> sp.	No 20'	Bulgaria	1952	6
<i>Morus</i> sp.	No 21'	Bulgaria	1951,1952	23
<i>Morus</i> sp.	No 31'	Bulgaria	1951	18
<i>Morus</i> sp.	No 34'	Bulgaria	1952	6
<i>Morus</i> sp.	No 4'	Bulgaria	1952	9
<i>Morus</i> sp.	No 5'	Bulgaria	1937, 1952	19
<i>Morus</i> sp.	No 52'	Bulgaria	1958	3
<i>Morus</i> sp.	No 53'	Bulgaria	1958	4
<i>Morus</i> sp.	No 57'	Bulgaria	1958	7
<i>Morus</i> sp.	No 59'	Bulgaria	1952,1958,1964	542
<i>Morus</i> sp.	No 8'	Bulgaria	1952	12

species	variety	origin (year of introduction)	prop./planting date	No.iondiv.	
<i>Morus</i> sp.	No 9'	Bulgaria	1937, 1951, 1952	36	
<i>Morus</i> sp.	No103 x No3'	Bulgaria	1970	22	
<i>Morus</i> sp.	No106 x No3'	Bulgaria	1970	27	
<i>Morus</i> sp.	No117 hybrid'	Bulgaria	1958	102	
<i>Morus</i> sp.	No24 hybrid'	Bulgaria	1970	25	
<i>Morus</i> sp.	No24 x No 3'	Bulgaria	1970	30	
<i>Morus</i> sp.	No24 x No21'	Bulgaria	1970	13	
<i>Morus</i> sp.	No24 x No62'	Bulgaria	1970	23	
<i>Morus</i> sp.	P 10'	Bulgaria, 1963	1966	10	✓
<i>Morus</i> sp.	P 11'	Bulgaria, 1963	1967	38	
<i>Morus</i> sp.	P 12'	Bulgaria, 1963	1966	8	✓
<i>Morus</i> sp.	P 13'	Bulgaria, 1963	1966	8	✓
<i>Morus</i> sp.	P 14'	Bulgaria, 1963	1966	9	✓
<i>Morus</i> sp.	P 15'	Bulgaria, 1963	1967	20	
<i>Morus</i> sp.	P 16'	Bulgaria, 1963	1966	7	✓
<i>Morus</i> sp.	P 17'	Bulgaria, 1963	1966	8	✓
<i>Morus</i> sp.	P 19'	Bulgaria, 1963	1966,1967	19	✓
<i>Morus</i> sp.	P 20'	Bulgaria, 1963	1966	9	✓
<i>Morus</i> sp.	P 21'	Bulgaria, 1963	1966	7	✓

species	variety	origin (year of introduction)	prop./planting date	No.iondiv.	
<i>Morus</i> sp.	P 22'	Bulgaria, 1963	1966	8	✓
<i>Morus</i> sp.	P 23'	Bulgaria, 1963	1966	8	✓
<i>Morus</i> sp.	P 26'	Bulgaria	1980	15	
<i>Morus</i> sp.	P 28'	Bulgaria	1980	13	
<i>Morus</i> sp.	P 29'	Bulgaria	1980	18	
<i>Morus</i> sp.	P 30'	Bulgaria	1980,201	42	
<i>Morus</i> sp.	P 4'	Bulgaria, 1960	1966	10	✓
<i>Morus</i> sp.	P 7'	Bulgaria, 1963	1966,1967	39	✓
<i>Morus</i> sp.	P 9'	Bulgaria, 1963	1966	8	✓
<i>Morus</i> sp.	Pl 1'	Bulgaria	2004	4	
<i>Morus</i> sp.	Pl 2'	Bulgaria	2004	4	
<i>Morus</i> sp.	Pl 3'	Bulgaria	2004	4	
<i>Morus</i> sp.	Pl 4'	Bulgaria	2004	4	
<i>Morus</i> sp.	C1	Bulgaria			✓
<i>Morus</i> sp.	C 12'	Bulgaria	1973	3	
<i>Morus</i> sp.	C 13'	Bulgaria	1973	3	
<i>Morus</i> sp.	C 14'	Bulgaria	1973	4	
<i>Morus</i> sp.	C 19'	Bulgaria	1973	3	
<i>Morus</i> sp.	C 27'	Bulgaria	1973	3	



species	variety	origin (year of introduction)	prop./planting date	No.iondiv.	
<i>Morus</i> sp.	C 3'	Bulgaria	1999	5	✓
<i>Morus</i> sp.	C 5'	Bulgaria	1999	5	✓
<i>Morus</i> sp.	C 6	Bulgaria	1999	5	✓
<i>Morus</i> sp.	C 8	Bulgaria	1999	5	✓
<i>Morus</i> sp.	C 36'	Bulgaria	1973	5	
<i>Morus</i> sp.	C 39'	Bulgaria	1973	4	
<i>Morus</i> sp.	C 41'	Bulgaria	1973	5	
<i>Morus</i> sp.	C 45'	Bulgaria	1973	3	
<b>China</b>					
<i>M. alba</i> s.l.	No 140-Hussang 1'	China, 1961	1966,1973	28	
<i>M. alba</i> s.l.	No 144-Hussang 39'	China, 1967	1966	18	
<i>M. alba</i> s.l.	No 145-Hussang 3'	China, 1967	1966	7	
<i>M. alba</i> (syn. <i>M. multicaulis</i> )	No 143-Hussang 13'	China, 1967	1966,1973	11	
<i>M. indica</i> (syn. <i>M. kagayamae</i> )	No 141-Hussang 2'	China, 1964	1966	8	
<i>M. indica</i> (syn. <i>M. kagayamae</i> )	No 146-Hussang 4'	China, 1967	1966	8	
<i>M. indica</i> (syn. <i>M. kagayamae</i> )	No 147-Hussang 5'	China, 1967	1966,1973	19	
<i>Morus</i> sp.	No 128-Hussang'	China, 1964	1966	6	
<i>Morus</i> sp.	No 188 - Hussang 7'	China, 1982	1982	12	
<i>Morus</i> sp.	No 189 - Hussang 32'	China, 1982	1982	14	

species	variety	origin (year of introduction)	prop./planting date	No.iondiv.	
<b>Georgia</b>					
<i>M. alba</i> s.l.	No 116-Tbilisuri'	Georgia	1958,1964,1966,1970	80	✓
<i>M. alba</i> s.l.	No 150-GRUZNISH 4'	Georgia, 1962	1966,1967	27	✓
<i>M. alba</i> s.l.	No 151-GRUZNISH 5'	Georgia, 1962	1966,1967	24	✓
<i>M. alba</i> s.l.	No 152-GRUZNISH 6'	Georgia, 1962	1966	10	✓
<i>M. alba</i> s.l.	No 153-GRUZNISH 7'	Georgia, 1962	1966	6	✓
<i>M. alba</i> s.l.	No 156-TBILNISH 2'	Georgia, 1962	1966	10	✓
<i>M. alba</i> s.l.	No. 79	Georgia			✓
<i>M. alba</i> s.l.	No 160-No 79'	Georgia, 1962	1966,1973	30	
<i>M. indica</i> (syn. <i>M. kagayamae</i> )	No 154-Kartli'	Georgia, 1962	1966	8	✓
<i>M. indica</i> (syn. <i>M. kagayamae</i> )	No 155-Georgia'	Georgia, 1962	1966	5	✓
<i>M. indica</i> (syn. <i>M. kagayamae</i> )	No 158-Uhli'	Georgia, 1962	1966	8	✓
<i>M. indica</i> (syn. <i>M. latifolia</i> )	No 159-Digmuri'	Georgia, 1962	1966,1973	26	✓
<i>Morus</i> sp.	No 113-Adreuli'	Georgia	1958,1964,1966	39	✓
<i>Morus</i> sp.	No 157-Kutaturi'	Georgia, 1962	1966	9	✓
<b>India</b>					
<i>Morus</i> sp.	India 1'	India, 2000	1999	5	
<i>Morus</i> sp.	India 2'	India, 2000	1999	5	

species	variety	origin (year of introduction)	prop./planting date	No.iondiv.
<i>Morus</i> sp.	India 3'	India, 2000	1999	5
<b>Italy</b>				
<i>M. alba</i> s.l.	No 101-Rosa di Lombardia'	Italy	1950,1951,1952,1958,1964,1967,1973	241
<i>M. alba</i> f. <i>pendula</i>	No 110- Pendula'	Italy	1956	1
<i>M. alba</i> s.l.	No 102-Florio'	Italy	1952,1958,1972	20
<i>M. alba</i> s.l.	No 103- Muki'	Italy	1937,1952,1966,1970	61
<i>M. alba</i> s.l.	No 104-Cattaneo'	Italy	1939,1952,1966,1973	105
<i>M. alba</i> s.l.	No 106-Giazzola '	Italy	1937,1939,1951,1952,1956,1958,1964,1966,1967,1970,1972,1973,1974,1978,1980,1982	5578
<i>M. alba</i> s.l.	No 107-Restelli'	Italy	1952,1973	24
<i>M. alba</i> s.l.	No 108-Arancina	Italy	1951,1952	29
<i>M. alba</i> s.l.	No 109-Morettiana'	Italy	1973	11
<i>M. alba</i> s.l.	No 112-Lhou	Italy	1951,1952,1966	35
<i>Morus</i> sp.	No 111-Colombassa'	Italy	1952	7
<b>Japan</b>				
<i>M. alba</i> s.l.	No 121-Ichinose'	Japan, 1963	1966,1973	15
<i>M. alba</i> s.l.	No 122-Kayriou Ichinose'	Japan, 1954	1966	9
<i>M. alba</i> s.l.	No 123-Kayriou Nezumigaeshi'	Japan, 1954	1966,1973	16
<i>M. alba</i> s.l.	No 126-Kayriou improved'	Japan, 1954	1973	11

species	variety	origin (year of introduction)	prop./planting date	No.iondiv.	
<i>M. indica</i> (syn. <i>M. bombycis</i> )	No 124-Kenmochi'	Japan, 1954	1966	9	
<i>M. indica</i> (syn. <i>M. bombycis</i> )	No 125-Shintso 2'	Japan, 1954	1966	6	
<i>M. indica</i> (syn. <i>M. kagayamae</i> )	No 117-Kinryu'	Japan, 1958	1966,1967,1970,2014	131	
<i>M. indica</i> (syn. <i>M. kagayamae</i> )	No 172 - Siozisu'	Japan, 1960	1966	7	
<i>M. indica</i> (syn. <i>M. latifolia</i> )	No 114-Kokusou70'	Japan	1958,1964,1966	54	
<i>M. indica</i> (syn. <i>M. latifolia</i> )	No 118-Kokusou21'	Japan, 1959	1950,1958,1966,1967,1972,1973	223	
<i>M. indica</i> (syn. <i>M. latifolia</i> )	No 119-Kokusou20'	Japan, 1959	1958,1966,1972	336	
<i>M. indica</i> (syn. <i>M. latifolia</i> )	No 120-Kokusou27'	Japan, 1959	1958,1966,1967,1970,1972,1973,1980	380	
<i>M. indica</i> (syn. <i>M. latifolia</i> )	No 171 - Kokusou 70'	Japan, 1958	1966	8	
<i>Morus</i> sp.	No 173 - Qua 1'	Japan, 1970	1973	21	
<i>Morus</i> sp.	No 174 - Qua 2'	Japan, 1971	1973	21	
<i>Morus</i> sp.	No 186'	Japan, 1974	1973	10	
<i>Morus</i> sp.	No 187'	Japan, 1974	1973	8	
<b>Romania</b>					
<i>Morus</i> sp.	Olteni'	Romania, 2004	2004	1	<input checked="" type="checkbox"/>
<b>Russia</b>					
<i>Morus</i> sp.	Hassak'	Russia	1972	48	
<b>Ukraine</b>					
<i>M. alba</i> (syn. <i>M. multicaulis</i> )	No 168 - Ukraine9'	Ukraine, 1962	1966	10	<input checked="" type="checkbox"/>

species	variety	origin (year of introduction)	prop./planting date	No.iondiv.	
<i>M. alba</i> s.l.	No 167 - Ukraine4'	Ukraine, 1962	1966	9	✓
<i>M. alba</i> s.l.	No 169 - Ukraine107'	Ukraine, 1962	1966,1967	31	✓
<i>M. alba</i> s.l.	No 170 - Harkovska 3'	Ukraine, 1962	1966,1967	29	✓
<i>Morus</i> sp.	Slobodjanskaya 1'	Ukraine, 1998	1999	2	✓
<i>Morus</i> sp.	Slobodjanskaya 2'	Ukraine, 1998	1999	2	✓
<b>Uzbekistan</b>					
<i>M. alba</i> (syn. <i>M. multicaulis</i> )	No 134-SANIISH 5'	Uzbekistan, 1965	1966	9	✓
<i>M. alba</i> (syn. <i>M. multicaulis</i> )	No 135-SANIISH 14'	Uzbekistan, 1965	1966	9	✓
<i>M. alba</i> (syn. <i>M. multicaulis</i> )	No 136-SANIISH 15'	Uzbekistan, 1965	1966	8	✓
<i>M. alba</i> (syn. <i>M. multicaulis</i> )	No 137-SANIISH 17'	Uzbekistan, 1965	1966,1967	30	✓
<i>M. alba</i> s.l.	No 132-Zimostoikii'	Uzbekistan, 1965	1966	10	✓
<i>M. alba</i> s.l.	No 133-Pionerskii'	Uzbekistan, 1965	1966	9	✓
<i>M. alba</i> s.l.	No 138-Tadgik seedlees'	Uzbekistan, 1965	1966	10	✓
<i>M. alba</i> s.l.	No 139-Uzbekskii'	Uzbekistan, 1965	1966	9	✓
<i>M. alba</i> s.l.	No 148-SANIISH 6'	Uzbekistan, 1967	1966	9	✓
<i>M. indica</i> (syn. <i>M. kagayamae</i> )	No 149 SANIISH 7'	Uzbekistan, 1959	1966,1967	28	✓
<i>M. rubra</i> cf	No 142-Nerrosa'	Uzbekistan, 1964	1966	3	✓



● Deliverable 1.4 – Report on the collected mulberry samples

\*Varieties that are noted as *M. rubra* cf. could originate from simple or complex spontaneous and/or artificial crosses involving *M. alba*. Their genetic relationship is going to be analysed under the scope of ARACNE mulberry research.

**Supplementary Table 3-** Leaf yield characteristics of some Bulgarian mulberry varieties.

Accession	Leaf yield by 1 tree ( kg)	Leaves percentage from the whole vegetation yield ( %)	Leaf annual yield per 1 ha (kg)
P 4	6.45±1.06	53.73±2.60	11760.5
P 7	6.37±0.23	56.30±3.28	11363.0
P 9	6.19±0.19	46.17±2.62**	10115.9*
P 10	6.58±0.19	46.17±2.62**	10115.9*
P 12	6.02±0.23	57.13±4.29	11464.1
P 13	6.44±2.36	58.85±3.96*	12628.0**
P14	6.06±0.69	57.80±4.62	11623.9
P 16	5.66±1.50	59.56±6.35*	11227.7
P 17	6.97±0.93	44.72±4.93**	10384.2
P 19	5.00±0.42	63.45±2.57***	105672
P 20	5.44±0.15	53.29±3.23	9657.0**
P 21	6.02±0.45	58.48±2.87	11732.7
P 22	5.25±0.42	54.87±5.51	9592.6**
N 3	7.24±0.37*	46.06±4.81	11102.6
N 24	7.57±3.72**	45.04±4.45**	11358.1
N 26	5.61±0.55	47.88±2.09*	8942.2*
N 106	7.14±1.42*	47.33±3.72*	11805.0
Vratsa1	5.56±0.68	59.07±3.36	11387.8
Vratsa 18	7.25±0.90*	47.28±3.00*	11421.9
Singetz tut	6.90±1.30	48.50±3.63	11138.9
Surh	7.70±1.21**	48.21±2.27	12354.3*
Zarif tut	6.82±0.88	49.83±0.90	11310.9
Emin tut	8.11±1.05***	47.64±3.54*	12873.2***
Jakub tut	6.11±0.65	56.37±4.18	11471.0
Firudin tut	7.43±2.02*	45.20±6.81**	11190.2
Azeri tut	6.32±0.40	49.90±3.45	10500.6
Azerbaidjan	5.50±1.16	56.87±2.09	10416.0
Armenia	5.50±0.92	57.28±2.09	10500.7
Tbilisuri	5.60±0.99	57.29±4.76	10500.9
Gruzniish 4	7.70±0.62**	49.58±3.05**	12707.2**
Gruzniish 5	7.69±0.46**	46.25±5.70**	11851.6

Accession	Leaf yield by 1 tree ( kg)	Leaves percentage from the whole vegetation yield ( %)	Leaf annual yield per 1 ha (kg)
Kartli	5.96±0.50	46.18±4.68**	9156.0***
Georgia	4.78±0.93	44.65±5.71***	9967.8**
Digmuri	6.93±0.42	51.25±4.08	11826.0
N 79	7.00±0.60	48.14±5.48*	11219.0
Adreuli	6.14±0.97	53.13±6.17	10870.7
Muki	7.05±0.68	46.38±4.62**	10893.6
Kataneo	6.04±1.10	55.38±1.89*	11136.9
Husan 1	5.03±1.04	63.93±2.21***	10705.0
Husan 2	7.11±1.15*	53.41±3.08	12415.4*
Husan 4	6.34±0.29	42.45±4.23***	8968.8***
Husan 5	4.79±0.79***	61.45±6.89**	9460.0**
Husan 39	5.14±0.26*	62.86±5.20***	10767.2
Pobeda	4.46±1.02***	63.28±4.80***	9405.5***
Russian	5.70±0.32	47.47±2.50*	8946.6***
Pionerskii	5.39±0.65	53.90±6.81	9686.5**
Saniish 5	5.41±1.24	63.86±3.44***	11516.3
Saniish 7	7.53±1.25**	44.40±7.75***	11535.5
Saniish 14	7.53±1.13**	44.53±3.39***	11272.3
Saniish 17	6.06±0.93	46.16±2.66	9312.4***
Tadgikian seedless	6.76±1.81	52.99±2.29	12144.6
Uzbekian	6.27±0.93	59.23±8.93*	12326.8*
Nerrosa	6.91±1.41	46.93±3.57*	10803.2
Ukrainian 9	7.73±0.45***	44.05±4.84***	11353.1
Ukrainian 107	6.12±0.63	53.46±5.48	10887.5
Harkovska 3	7.23±1.21*	46.21±3.11**	11129.5
Kinryu	7.47±0.78**	47.50±2.75*	11816.0
Kokusou 13	5.63±1.09	59.94±6.41*	11326.0
Kokusou 20	6.80±0.76	52.13±2.17	11864.8
Kokusou 21	6.64±0.58	51.93±1.06	11484.7
Kokusou 27	6.42±0.49	52.45±3.44	11207.2
Ichinose	5.56±0.91	48.40±2.71	8963.5**
Kayriou ichinose	4.54±0.75***	61.42±2.35**	9291.6***

Accession	Leaf yield by 1 tree ( kg)	Leaves percentage from the whole vegetation yield ( %)	Leaf annual yield per 1 ha (kg)
Kenmoshi	6.16±0.76	45.27±6.62**	9286.3***
Shinso 2	5.58±0.81	62.97±9.26***	11703.8
Siuzisu	5.27±0.69*	63.10±2.56***	11077.8
X average	6.203	53.31	11137.8
GD at P 0.05	0.906	5.17	101.92
P 0.01	1.192	6.79	133.90
P 0.001	1.519	8.67	171.08

## Appendix III – UM mulberry germplasm Supplementary Tables

**Supplementary Table 4-** List of local mulberry genotypes from Hungary planted in the mulberry collection of the Faculty of Agriculture University Maribor with location, geographic coordinates and detailed data.

Identification No.	Eco-geographical region	Regionalization (Perko et al. 1998)	Sampling date	Longitude	Latitude	Circumference (cm)	No. of individuals	Descriptor monitoring
<b>Submediterranean region (SM)</b>								
A/29/270515	SM	Vipavska dolina	27/5/2015	45,888090	13,821810		2	☑
A/6/110216	SM	Vipavska dolina	11/2/2016	45,904970	13,780760	250	2	☑
KP/142/060616	SM	Kopraska brda	6/6/2016	45,542390	13,872230	143	1	☑
KP/150/060616	SM	Podgorski Kras	6/6/2016	45,537150	13,888050	600	1	☑
KP/181/140616	SM	Kopraska brda	14/6/2016	45,490810	13,762850	280	1	☑
KP/184/290616	SM	Kopraska brda	29/6/2016	45,479300	13,738340		1	☑
KP/186/290616	SM	Kopraska brda	29/6/2016	45,479130	13,737220	460	2	☑
KP/189/290616	SM	Kopraska brda	29/6/2016	45,490080	13,762820	280	1	☑
KP/192/290616	SM	Kopraska brda	29/6/2016	45,510940	13,799290		1	☑
KP/196/290616	SM	Kopraska brda	29/6/2016	45,518350	13,778810	145	1	☑
KP/205/290616	SM	Kopraska brda	29/6/2016	45,437210	13,825810	290	1	☑



Identification No.	Eco-geographical region	Regionalization (Perko et al. 1998)	Sampling date	Longitude	Latitude	Circumference (cm)	No. of individuals	Descriptor monitoring
KP/208/290616	SM	Kopraska brda	29/6/2016	45,436550	13,825140	130	2	✓
KP/226/130716	SM	Podgorski Kras	13/7/2016	45,578220	14,032110	158	2	✓
KP/228/130716	SM	Podgorski Kras	13/7/2016	45,559430	14,035130	234	5	✓
KP/228/130716	SM	Podgorski Kras	13/7/2016	45,559430	14,035130	234	1	✓
KP/229/130716	SM	Podgorski Kras	13/7/2016	45,561480	14,035160	75	1	✓
KP/295/03072015	SM	Tržaški zaliv	3.7.2015	45,525240	13,599510		1	✓
NG/210/040716	SM	Goriška brda	4/7/2016	45,996790	13,524740	220	3	✓
NG/211/040716	SM	Goriška brda	4/7/2016	45,996580	13,523240	366	2	✓
NG/212/040716	SM	Goriška brda	4/7/2016	45,996560	13,523120	230	1	✓
NG/214/040716	SM	Goriška brda	4/7/2016	45,982020	13,519870	120	5	✓
NG/215/040716	SM	Goriška brda	4/7/2016	45,982360	13,520490	200	1	✓
NG/217/040716	SM	Goriška brda	4/7/2016	45,991770	13,549410	260	1	✓
NG/218/040716	SM	Goriška brda	4/7/2016	45,999460	13,593200	100	2	✓
P/136.11/220216	SM	Kopraska brda	22/2/2016	45,470920	13,643080	184	1	✓
P/136.15/220216	SM	Kopraska brda	22/2/2016	45,470920	13,643080	196	2	✓
P/137.1/220216	SM	Kopraska brda	22/2/2016	45,465650	13,646830	237	1	✓
P/137.2/220216	SM	Kopraska brda	22/2/2016	45,465650	13,646830	270	1	✓

Identification No.	Eco-geographical region	Regionalization (Perko et al. 1998)	Sampling date	Longitude	Latitude	Circumference (cm)	No. of individuals	Descriptor monitoring
S/100.1/220216	SM	Kras	22/2/2016	45,660898	14,006956	189	1	☑
S/100.1/220216	SM	Kras	22/2/2016	45,660898	14,006956	189	2	☑
S/322	SM	Kras		45,816015	13,855863	757	1	☑
S/88.7/220216	SM	Kras	22/2/2016	45,661045	13,924789	139	1	☑
S/88.9/220216	SM	Kras	22/2/2016	45,661045	13,924789	145	1	☑
S/89/220216	SM	Kras	22/2/2016	45,660500	13,925863	275	5	☑
S/90/220216	SM	Kras	22/2/2016	45,665530	13,923370	271	2	☑
<b>Subpannonean region (SP)</b>								
MB/300/080617	SP	Dravska ravan	8.6.2017	46,480133	15,743345	276	4	☑
MB/301/080617	SP	Dravska ravan	8.6.2017	46,479474	15,744551	280	1	☑
MB/302/080617	SP	Dravska ravan	8.6.2017	46,480133	15,743345	103	1	☑
MB/303/080617	SP	Dravska ravan	8.6.2017	46,445965	15,764567	340	4	☑
P/251/20072016	SP	Lendavske gorice	20/7/2016	46,561950	16,478290	125	1	☑
P/252/20072016	SP	Lendavske gorice	20/7/2016	46,562030	16,456050	50	1	☑
P/256/26072016	SP	Murska ravan	26/7/2016	46,655350	16,354410	178	2	☑
P/260/26072016	SP	Goričko	26/7/2016	46,685490	16,393160	268	1	☑

Identification No.	Eco-geographical region	Regionalization (Perko et al. 1998)	Sampling date	Longitude	Latitude	Circumference (cm)	No. of individuals	Descriptor monitoring
P/265/26072016	SP	Goričko	26/7/2016	46,724900	16,335420	126	1	☑
P/267/26072016	SP	Goričko	26/7/2016	46,746930	16,143100	100	1	☑
P/272/26072016	SP	Goričko	26/7/2016	46,744130	16,090650	178	2	☑
P/278/26072016	SP	Goričko	26/7/2016	46,803970	16,070610	347	1	☑
PT/304/080617	SP	Dravska ravan	8.6.2017	46,375654	15,766740	267	6	☑
PT/306/080617	SP	Haloze	8.6.2017	46,273440	15,791595	160	3	☑
SG/04/20072016	SP	Slovenske gorice	20/7/2016	46,647630	15,847630	298	1	☑
SG/08/20072016	SP	Murska ravan	20/7/2016	46,694120	15,834570	172	1	☑
SG/10/20072016	SP	Slovenske gorice	20/7/2016	46,653600	15,799760	270	4	☑
SG/11/20072016	SP	Slovenske gorice	20/7/2016	46,607160	15,871140	187	1	☑
SG/12/20072016	SP	Slovenske gorice	20/7/2016	46,614460	15,887180	281	4	☑
SG/237/20072016	SP	Slovenske gorice	20/7/2016	46,556780	16,012760	145	3	☑
SG/238/20072016	SP	Slovenske gorice	20/7/2016	46,547220	16,034290	267	1	☑
SG/240/20072016	SP	Slovenske gorice	20/7/2016	46,547210	16,022620	152	1	☑
SG/242/20072016	SP	Slovenske gorice	20/7/2016	46,538370	16,006470	90	1	☑
SG/245/20072016	SP	Slovenske gorice	20/7/2016	46,477620	16,230650	60	2	☑
SG/247/20072016	SP	Slovenske gorice	20/7/2016	46,564300	15,795470	287	1	☑

Identification No.	Eco-geographical region	Regionalization (Perko et al. 1998)	Sampling date	Longitude	Latitude	Circumference (cm)	No. of individuals	Descriptor monitoring
SG/249/20072016	SP	Murska ravan	20/7/2016	46,582110	16,117470	70	1	☑
CE/222/060716	SP	Savinjska ravan	4/7/2016	46,234860	15,355200	200	2	☑
CE/223/060716	SP	Srednjesotelsko gričevje	4/7/2016	46,061560	15,539580	230	1	☑
CE/224/060716	SP	Srednjesotelsko gričevje	4/7/2016	46,063720	15,529910	170	1	☑
<b>South-Eastern region (SE)</b>								
BK/282/02082016	SE	Bela krajina	2/8/2016	45,636750	15,218740	110	1	☑
BK/290/02082016	SE	Bela krajina	2/8/2016	45,669010	15,364560	190	1	☑
BK/292/02082016	SE	Bela krajina	2/8/2016	45,653600	15,332960	210	2	☑
BK/5/06072016	SE	Bela krajina	6/7/2016	45,520700	15,159010	122	2	☑
BK/6/06072016	SE	Bela krajina	6/7/2016	45,490500	15,361140		1	☑
BK/7.2/06072016	SE	Bela krajina	6/7/2016	45,506100	15,329540		2	☑
BK/7/06072016	SE	Bela krajina	6/7/2016	45,506100	15,329540	90	1	☑
BK/8/06072016	SE	Bela krajina	6/7/2016	45,506450	15,329100	94	5	☑
BK/9.1/06072016	SE	Bela krajina	6/7/2016	45,506130	15,328900	190	1	☑
BK/9.2/06072016	SE	Bela krajina	6/7/2016	45,506130	15,328900	190	1	☑
DO/12/13072016	SE	Krška ravan	13/7/2016	45,898420	15,464520	160	1	☑

Identification No.	Eco-geographical region	Regionalization (Perko et al. 1998)	Sampling date	Longitude	Latitude	Circumference (cm)	No. of individuals	Descriptor monitoring
DO/17/13072016	SE	Krška ravan	13/7/2016	45,929460	15,575570	123	1	<input checked="" type="checkbox"/>
DO/18/13072016	SE	Krška ravan	13/7/2016	45,919440	15,498780	177	1	<input checked="" type="checkbox"/>
DO/19/13072016	SE	Krška ravan	13/7/2016	45,921120	15,498170	152	1	<input checked="" type="checkbox"/>
DO/24/13072016	SE	Novomeška	13/7/2016	45,856240	15,377380	275	3	<input checked="" type="checkbox"/>
DO/25.2/13072016	SE	Novomeška	13/7/2016	45,809340	15,262840		1	<input checked="" type="checkbox"/>
DO/25/13072016	SE	Novomeška	13/7/2016	45,809340	15,262840	270	1	<input checked="" type="checkbox"/>



**Supplementary Table 5-** List of local mulberry genotypes from Hungary planted in the mulberry collection of the Faculty of Agriculture University Maribor with location, geographic coordinates and detailed data.

Identification No.	Region	Location	Sampling date	Longitude	Latitude	Circumference	No. of individuals	
<b>Baranya</b>								
BA/2106/140617	Baranya	Antalszallas	14.6.2017	46,208210	17,840410	150	1	☑
BA/2111/140617	Baranya	Szuliman	14.6.2017	46,123830	17,819420	265	2	☑
BA/2112/140617	Baranya	Cserto	14.6.2017	46,082162	17,816217	70-90	2	☑
BA/2114/140617	Baranya	Szigetvar	14.6.2017	46,054660	17,810960	160	1	☑
BA/2122/140617	Baranya	Szigetvar	14.6.2017	46,037370	17,781810	170	1	☑
BA/2126/140617	Baranya	Ketujfalu	14.6.2017	45,981790	17,732420	170	2	☑
BA/2132/140617	Baranya	Teklafalu	14.6.2017	45,941620	17,730320	320	1	☑
BA/2151/140617	Baranya	Bogadindszent	14.6.2017	45,916110	18,054140	132	6	☑
BA/2153/140617	Baranya	Gorcsony	14.6.2017	45,967680	18,124050	125	2	☑
BA/2154/140617	Baranya	Ocsard	14.6.2017	45,936840	18,152900	220	1	☑
BA/2157/140617	Baranya	Turony	14.6.2017	45,912940	18,230920	185	2	☑
BA/2159/140617	Baranya	Csarnota	14.6.2017	45,893630	18,231930	160	2	☑
BA/2165/140617	Baranya	Kishasany	14.6.2017	45,849770	18,357270	295	1	☑
BA/2165/140617	Baranya	Kishasany	14.6.2017	45,849770	18,357270	295	1	☑

Identification No.	Region	Location	Sampling date	Longitude	Latitude	Circumference	No. of individuals	
BA/2169/140617	Baranya	Pocsa	14.6.2017	45,918590	18,469130	220	1	✓
BA/2171/140617	Baranya	Szajk	14.6.2017	45,986890	18,539990	100-120	1	✓
BA/2174/140617	Baranya	Mohacs	14.6.2017	46,036720	18,698680	50-90	3	✓
BA/2175/140617	Baranya	Bar	14.6.2017	46,041890	18,708380	243	2	✓
BA/2179/140617	Baranya	Dunaszekso	14.6.2017	46,066690	18,746480		1	✓
BA/2207/150617	Baranya	road Feked - Lovaszheteny	15.6.2017	46,153450	18,478960	210	2	✓
BA/2212/150617	Baranya	road Lovaszheteny - Pecsvarad	15.6.2017	46,167980	18,448600	285	2	✓
BA/2220/150617	Baranya	Pecsvarad	15.6.2017	46,144530	18,404700	270	1	✓
BA/2225/150617	Baranya	Pecsvarad	15.6.2017	46,134550	18,377724	290	2	✓
BA/2231/150617	Baranya	road Pecsvarad - Hird	15.6.2017	46,126890	18,350720	285	1	✓
BA/2259/150617	Baranya	road Kishajmas - Kisbodolja	15.6.2017	46,208080	18,099240		1	✓
<b>Borsod-Abauj-Zemlen</b>								
BAZ/1219/040717	Borsod-Abauj-Zemlen		4.7.2017	47,928330	20,566940	430	2	✓
<b>Bekes</b>								
BE/1258/050717	Bekes	Bekes	5.7.2017	46,761300	21,126900	160-187	1	✓

Identification No.	Region	Location	Sampling date	Longitude	Latitude	Circumference	No. of individuals	
BE/1264/2/050717	Bekes	Bekescsaba	5.7.2017	46,684300	21,087600	182	3	☑
BE/1265/1/050717	Bekes	Kondoros, mulberry tree avenue	5.7.2017	46,770100	20,758900	220	2	☑
<b>Bacs-Kiskun</b>								
BU/1184/030717	Bacs-Kiskun	Martonfa utca	3.7.2017	47,491700	18,998900	481	1	☑
<b>Gyor-Moson-Sopron</b>								
GMS/2286/200617	Gyor-Moson-Sopron	Bosarkany	20.6.2017	47,713730	17,223230	330	3	☑
GMS/2311/200617	Gyor-Moson-Sopron	Dunaszeg	20.6.2017	47,784510	17,521950	286	2	☑
GMS/2326/200617	Gyor-Moson-Sopron	Lebeny	20.6.2017	47,730200	17,403570	270	1	☑
GMS/2329/200617	Gyor-Moson-Sopron	Lebeny	20.6.2017	47,737740	17,386580		2	☑
GMS/2330/200617	Gyor-Moson-Sopron	Lebeny	20.6.2017	47,737280	17,387060		1	☑
GMS/2357/200617	Gyor-Moson-Sopron	Morichida	20.6.2017	47,517870	17,412390	236	2	☑
GMS/2528/200617	Gyor-Moson-Sopron	Peresztég	20.6.2017	47,580280	16,710310	240	2	☑
GMS/2530/200617	Gyor-Moson-Sopron	Nagycentk	20.6.2017	47,606730	16,689200		2	☑

Identification No.	Region	Location	Sampling date	Longitude	Latitude	Circumference	No. of individuals	
GMS/2531/200617	Gyor-Moson-Sopron	Nagycenk	20.6.2017	47,610360	16,672090	270	1	✓
GMS/2532/200617	Gyor-Moson-Sopron	Nagycenk	20.6.2017	47,610360	16,672090	245	2	✓
GMS/2533/200617	Gyor-Moson-Sopron	Nagycenk	20.6.2017	47,610360	16,672090	225	2	✓
GMS/2535/200617	Gyor-Moson-Sopron	Pinny	20.6.2017	47,597740	16,770360	309	1	✓
HU(III) GYORO	Gyor-Moson-Sopron	Gyoro	6.12.2014				1	✓
<b>Pest</b>								
BUDAPEST 6.12.	Pest	Budapest	6.12.2014				1	✓
PE/1194/030717	Pest	Alsonemedi	3.7.2017	47,293200	19,183700	397	1	✓
PE/1195/030717	Pest		3.7.2017	47,292900	19,188100	320	1	✓
PE/1254/1/050717	Pest	Tatárszentgyörgy	5.7.2017	47,102050	19,361240	226	3	✓
<b>Somogy</b>								
SO/1001/300517	Somogy	at the road, seedling, branched	30.5.2017	46,467065	17,127073		3	✓
SO/1003/300517	Somogy		30.5.2017	46,447175	17,183614		2	✓
SO/1007/300517	Somogy	Fő utca, Varazslo	30.5.2017	46,432274	17,217251	126	1	✓

Identification No.	Region	Location	Sampling date	Longitude	Latitude	Circumference	No. of individuals	
SO/1013/300517	Somogy	Fö utca, Nagybjom	30.5.2017	46,395783	17,499668	2x70	3	✓
SO/1015/300517	Somogy		30.5.2017	46,365937	17,597231	233	1	✓
SO/1029/300517	Somogy	Somogyjard, Fo u.	30.5.2017	46,418767	17,596384	360	1	✓
SO/1035/300517	Somogy		30.5.2017	46,516639	17,819101		4	✓
SO/1040/300517	Somogy	Igal	30.5.2017	46,505300	17,887875		1	✓
SO/1042/300517	Somogy	Igal	30.5.2017	46,533231	17,939263	340	3	✓
SO/1043/300517	Somogy	Igal	30.5.2017	46,537941	17,937317		4	✓
SO/1046/300517	Somogy		30.5.2017	46,566011	17,933882		3	✓
SO/1085/140617	Somogy	pred Sagvar	14.6.2017	46,866200	18,076300	121	2	✓
SO/1087/140617	Somogy		14.6.2017	46,865800	18,075200	122	1	✓
SO/1104/140617	Somogy	Petolfi Sanolor utca	14.6.2017	46,834100	18,103100	294	1	✓
SO/1107/1/140617	Somogy	mulberry avenue around cemetery SOM	14.6.2017	46,811548	18,138500	254	1	✓
SO/2004/300517	Somogy	Iharos	30.5.2017	46,339410	17,103510	78	1	✓
SO/2008/300517	Somogy	Csurgo	30.5.2017	46,247080	17,105960	70-120	2	✓
SO/2010/300517	Somogy	Berzence	30.5.2017	46,203990	14,163180	330	1	✓
SO/2011/300517	Somogy	Berzence	30.5.2017	46,202400	17,165780	100	1	✓
SO/2014/300517	Somogy	Vizvar	30.5.2017	46,086480	17,240770	110	1	✓



Identification No.	Region	Location	Sampling date	Longitude	Latitude	Circumference	No. of individuals	
SO/2018/300517	Somogy	Barcs-Dravaszent	30.5.2017	45,985240	17,426230	310	1	✓
SO/2020/300517	Somogy	Barcs	30.5.2017	45,962440	17,488250	210	3	✓
SO/2021/300517	Somogy	Homokszentgyorgy	30.5.2017	46,018320	17,538050	225	1	✓
SO/2023/300517	Somogy	Lad	30.5.2017	46,138320	17,640230	130	1	✓
SO/2025/300517	Somogy	Lad	30.5.2017	46,145460	17,647560	230	2	✓
SO/2026/300517	Somogy	Mike	30.5.2017	46,240330	17,531260	160	5	✓
SO/2027/300517	Somogy	Labod	30.5.2017	46,213300	17,444830	230	1	✓
SO/2028/300517	Somogy	Somogyszob	30.5.2017	46,299530	17,291030	120	3	✓
<b>Tolna</b>								
TO/1126/1/140617	Tolna		14.6.2017	46,750100	18,673500	170	1	✓
TO/1128/140617	Tolna	at the road	14.6.2017	46,646100	18,845330		2	✓
TO/1129/140617	Tolna	Paks Varosi museum	14.6.2017	46,627600	18,867400	561	1	✓
TO/1130/140617	Tolna	Kosseith Nagydóc	14.6.2017	46,601100	18,797500	323	2	✓
TO/1131/140617	Tolna		14.6.2017	46,664900	18,598900	483	3	✓
TO/1142/150617	Tolna	Arany Janos utca	15.6.2017	46,480700	18,664700	320	2	✓
TO/1146/150617	Tolna	Dombon pri Irkosu	15.6.2017	46,425000	18,895500	362	3	✓
TO/1167/150617	Tolna	at the road	15.6.2017	46,459200	18,324100		1	✓

Identification No.	Region	Location	Sampling date	Longitude	Latitude	Circumference	No. of individuals	
TO/1169/150617	Tolna	at the road Agro Kirid	15.6.2017	46,446800	18,302400	341	2	✓
TO/2185/150617	Tolna	Bata	15.6.2017	46,127560	18,752150	268	3	✓
<b>Vas</b>								
VA/1051/060617	Vas	Apatistvanfalva	6.6.2017	46,942500	16,274500	142	2	✓
VA/1056/060617	Vas	Alsojanoshegyi	6.6.2017	46,886200	16,170000		4	✓
VA/1060/060617	Vas		6.6.2017	46,807400	16,390300	172	3	✓
VA/1062/060617	Vas	Csorotnek	6.6.2017	46,876700	16,403900	181	4	✓
VA/1064/060617	Vas	Csorotnek	6.6.2017	46,876300	16,403900	220	2	✓
VA/1065/060617	Vas	Oriszentpeter, Egresszer 87	6.6.2017	46,945300	16,376800	162	1	✓
VA/1069/060617	Vas		6.6.2017	46,966400	16,470700	339	3	✓
VA/1070/060617	Vas		6.6.2017	46,967800	16,470300	272	4	✓
VA/2507/200617	Vas	Szombathely	20.6.2017	47,246510	16,629300	263	1	✓
VA/2563/200617	Vas	Rabapaty, at the church	20.6.2017	47,309870	16,931980	350	1	✓
VA/2564/200617	Vas	Rabapaty, at the church	20.6.2017	47,310280	16,932760	295	1	✓
VA/2568/200617	Vas	Sitke	20.6.2017	47,243470	17,023680	320	2	✓
VA/2569/200617	Vas	Sitke	20.6.2017	47,243470	17,023680	220	1	✓
VA/2570/200617	Vas	Sitke	20.6.2017	47,243470	17,023680	220	3	✓

Identification No.	Region	Location	Sampling date	Longitude	Latitude	Circumference	No. of individuals	
<b>Veszprem</b>								
HU-15	Veszprem	Noszlop	29.11.2014	47,183340	1,745,851		1	☑
HU-2 (I.1)	Veszprem	Noszlop	29.11.2014	47,183340	17,458510		1	☑
HU-4 (I.2)	Veszprem	Noszlop	29.11.2014	47,183340	17,458510		1	☑
VE/2373/200617	Veszprem	Iszkaz	20.6.2017	47,175030	17,295530	257	2	☑
VE/2620/270617	Veszprem	Sumeg	27.6.2017	46,980800	17,290600	170	1	☑
VE/2682/270617	Veszprem	Csopak	27.6.2017	46,974900	17,931000		1	☑
VE/2706/270617	Veszprem	Tihany	27.6.2017	46,922300	17,824900		1	☑
VE/2707/270617	Veszprem	Tihany	27.6.2017	46,922300	17,824900		1	☑
VE/2714/270617	Veszprem	Balatonakali	27.6.2017	46,882000	17,750100	400	1	☑
VE/2727/270617	Veszprem	Revfulop	27.6.2017	46,829015	17,631479	370	1	☑
<b>Zala</b>								
ZA/2001/300517	Zala	Nagykanizsa, Varasdi utca 57	30.5.2017	46,455090	16,960880	cca 130	1	☑
ZA/2041/060617	Zala	Tornyiszentmiklos	6.6.2017	46,529500	16,559300	210	4	☑
ZA/2043/060617	Zala	Lovaszi	6.6.2017	46,543200	16,557300	190	1	☑
ZA/2044/060617	Zala	road Szecsisziget - Paka	6.6.2017	46,592800	16,615050	60	1	☑
ZA/2045/060617	Zala	Paka	6.6.2017	46,593750	16,648510	205	3	☑


Identification No.	Region	Location	Sampling date	Longitude	Latitude	Circumference	No. of individuals	
ZA/2047/060617	Zala	Radihaza	6.6.2017	46,649450	16,780080	110	5	☑
ZA/2052/060617	Zala	Tofej - Sojtor	6.6.2017	46,672460	16,841920	120	1	☑
ZA/2053/060617	Zala	Sojtor, Deak Ferenc Utca 21	6.6.2017	46,685670	16,856180	130	3	☑
ZA/2055/060617	Zala	road Bak-Sojtor	6.6.2017	46,707640	16,854290	145	5	☑
ZA/2057/060617	Zala	Pacsa	6.6.2017	46,727100	16,999770	155	5	☑
ZA/2060/060617	Zala	Zalaapati	6.6.2017	46,723720	17,105250	100-120	4	☑
ZA/2063/060617	Zala	Zalaapati	6.6.2017	46,726960	17,117980	60	3	☑
ZA/2064/060617	Zala	Keszthely	6.6.2017	46,761760	17,213880	100	3	☑
ZA/2081/060617	Zala	Zalaszbár - Orosztong	6.6.2017	46,638120	17,079580		1	☑
ZA/2084/060617	Zala	road Kiliman - Gelse	6.6.2017	46,628430	16,995460	60	7	☑
ZA/2088/060617	Zala	road Ujudvár - Palin	6.6.2017	46,514850	16,981200	70	3	☑
ZA/2090/060617	Zala	Eszteregyhely	6.6.2017	46,472450	16,883980	85	4	☑
ZA/2094/060617	Zala	road Borsfa - Bazakerettye	6.6.2017	46,518930	16,775570	30-70	1	☑
ZA/2095/060617	Zala	Szentmargitfalva	6.6.2017	46,494230	16,656630	190	2	☑

**Supplementary Table 6-** List of sericultural and fruit varieties of *Morus* sp. planted in the mulberry collection of the Faculty of Agriculture University Maribor with location, geographic coordinates and detailed data.

Species	Variety	Origin (year of introduction)	Obtained	Prop./planting date	No. of individuals	Descriptor monitoring
<i>M. alba</i> s.l.	'Florio'	Italy	mulberry gene bank CREA Padua, Italy	3.2015/10.2015	11	✓
<i>M. alba</i> s.l.	'Giazzola'	Italy	mulberry gene bank CREA Padua, Italy	3.2015/10.2016	5	✓
<i>M. indica</i> (syn. <i>M. latifolia</i> )	'Kokusou 20'	Japan	mulberry gene bank CREA Padua, Italy	3.2015/10.2017	11	✓
<i>M. alba</i> s.l.	'Morettiana'	Italy	mulberry gene bank CREA Padua, Italy	3.2015/10.2018	11	✓
<i>M. alba</i> s.l.	'Restelli'	Italy	mulberry gene bank CREA Padua, Italy	3.2015/10.2019	2	✓
<i>M. alba</i> s.l.	'Muki'	Italy	mulberry gene bank CREA Padua, Italy	3.2015/10.2019	2	✓
<i>M. indica</i> (syn. <i>M. kagayamae</i> )	No 117-Kinryu'	Japan	Sericultural Centre Vratsa, Bulgaria	3.2022/3.2023	3	✓
<i>M. alba</i> s.l.	'No. 24'	Bulgaria	Sericultural Centre Vratsa, Bulgaria		3	✓
<b>Fruit varieties</b>						
<i>M. alba</i> s.l.	Galicja	Ukraine		2018/3.2019	1	✓
<i>M. alba</i> s.l.	'No 5'	Bulgaria	Hubmann, Austria (original introduced via hortensis.de)	2018/3.2020	1	✓
<i>M. alba</i> s.l.	'Gelso Rosso'	Italy	Hubmann, Austria	2014/3.2016	1	✓
<i>M. alba</i> s.l.	'Agathe'	/	Rabensteiner J.	23.06.2018	1	✓
<i>M. alba</i> s.l.	'M. 150/N01 = Red'	Hybrid from Ukraine. Poltava (Schell 150)	/	2014/3.2016	1	✓



Species	Variety	Origin (year of introduction)	Obtained	Prop./planting date	No. of individuals	Descriptor monitoring
<i>M. alba</i> s.l.	'Big Ten'	SE Asia	Pucher, Austria	2014/3.2016	1	✓
<i>M. alba</i> s.l.	'Black'	Romania	Hubmann, Austria (collector from Bulgaria)	2014/3.2016	1	✓
<i>M. alba</i> s.l.	'White'	Romania	Hubmann, Austria (collector from Bulgaria)	2014/3.2016	1	✓
<i>M. alba</i> s.l.	'Red'	Romania	Rabensteiner J.	23.06.2018	1	✓
<i>M. alba</i> s.l.	'CREA fruit selection'	Italy	mulberry gene bank CREA Padua. Italy	1.03.2015	1	✓
<i>M. alba</i> × <i>rubra</i>	/	USA	Hubmann, Austria (original from tree in Graz, Leonhardstr., planted by nursery Grinschgl)	2014/3.2016	1	✓
<i>M. alba</i> × <i>rubra</i>	'French Hybrid'	Hybrid from France	Pucher (original <a href="http://www.cochetfrederic.com">http://www.cochetfrederic.com</a> )	2014/3.2016	1	✓
<i>M. alba</i> × <i>rubra</i>	'Illinois Everbearing'	USA	Pucher, Austria	2014/3.2016	1	✓
<i>M. alba</i> × <i>rubra</i>	'Ivory'	Hybrid from Canada	Hubmann, Austria (original introduced via hortensis.de)	2014/3.2016	1	✓
<i>M. indica</i>	'Shin-Tso'	Japan	Hubmann, Austria	2014/3.2016	1	✓
<i>M. indica</i>	'Coree'	France	Hubmann, Austria (originally from a collector in Turkey)	2014/3.2016	1	✓
<i>M. indica</i>	'Japan Fuji Red'	Japan		2014/3.2016	1	✓
<i>M. nigra</i>		local genotype, East Styria, Austria	Pucher, Austria, lat.46.97057256490299, long. 15.70354170496526	2014/3.2016	1	✓



Deliverable 1.4 – Report on the collected mulberry samples



Project: ARACNE - ADVOCATING THE ROLE  
OF SILK ART AND CULTURAL HERITAGE  
AT NATIONAL AND EUROPEAN SCALE

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## Appendix IV – IMIDA mulberry germplasm Supplementary Tables

**Supplementary Table 7**-List of varieties maintained at the Institute of Agricultural and Environmental Research and Development of Murcia.

Species	Variety	Origin (year of introduction)
<b><i>M. alba</i> s.l.</b>		
<i>M. alba</i> s.l.	'Agarena'	Spain
<i>M. alba</i> s.l.	'Cristiana'	Spain
<i>M. alba</i> s.l.	'Macocana'	Spain
<i>M. alba</i> s.l.	'Valenciana type 1'	Spain
<i>M. alba</i> s.l.	'Valenciana type 2'	Spain
<i>M. alba</i> s.l.	'A. Mazzeto'	Italy
<i>M. alba</i> s.l.	'Bresciana'	Italy
<i>M. alba</i> s.l.	'Brianzola'	Italy
<i>M. alba</i> s.l.	'Ciaroca'	Italy
<i>M. alba</i> s.l.	'Fossombrone'	Italy
<i>M. alba</i> s.l.	'Giazzola'	Italy
<i>M. alba</i> s.l.	'Grianzzela'	Italy
<i>M. alba</i> s.l.	'Innesto a frutto nero'	Italy
<i>M. alba</i> s.l.	'Limoncella'	Italy
<i>M. alba</i> s.l.	'Moscatella doppia'	Italy
<i>M. alba</i> s.l.	'Nostrano del Garda'	Italy
<i>M. alba</i> s.l.	'Pomella'	Italy
<i>M. alba</i> s.l.	'Selvatico'	Italy
<i>M. alba</i> f. <i>pendula</i>	'Pendula'	Spain
<i>M. alba</i> s.l. ( <i>M. multicaulis</i> )	'Filippine'	Philippines
<b><i>M. indica</i> s.l.</b>		
<i>M. indica</i> (syn. <i>M. latifolia</i> )	'Kokusou 20'	Japan
<i>M. indica</i> (syn. <i>M. latifolia</i> )	'Kokusou 21'	Japan
<i>M. indica</i> (syn. <i>M. latifolia</i> )	'Kokusou 27'	Japan
<b><i>M. nigra</i></b>		
<i>M. nigra</i>	local variety	

## Appendix V – France mulberry germplasm collections

### Supplementary Tables

**Supplementary Table 8** - List of varieties maintained in the The National Mediterranean Botanical Conservatory of Porquerolles.

species	variety	Origin	No. individuals
<b><i>M. alba</i> s.l.</b>			
<i>M. alba</i> s.l.	'Wellington Mulberry'	UK	3
<i>M. alba</i> s.l.	'Black English'	UK	2
<i>M. alba</i> s.l.	'Chirtout'		5
<i>M. alba</i> s.l.	'Aureifolia'		8
<i>M. alba</i> s.l.	'Badena Tut'		2
<i>M. alba</i> s.l.	'Cattaneo'	Italy	6
<i>M. alba</i> s.l.	'Colombassetta'	Italy	8
<i>M. alba</i> s.l.	'Furcata'		5
<i>M. alba</i> s.l.	'Gamette Hative'		26
<i>M. alba</i> s.l.	'Grisetto'	France	8
<i>M. alba</i> s.l.	'Giazzola'		9
<i>M. alba</i> s.l.	'Herati'		9
<i>M. alba</i> s.l.	'Hort in Steud (Giazzola ?)'		1
<i>M. alba</i> s.l.	'Italica'	Italy	5
<i>M. alba</i> s.l.	'Lhou / Romana'	Italy	2
<i>M. alba</i> s.l.	'Lhou ?'	Italy	2
<i>M. alba</i> s.l.	'Moretti'	Italy	12
<i>M. alba</i> s.l.	'Muki'	Italy	6
<i>M. alba</i> s.l.	'Okka'		1
<i>M. alba</i> s.l.	'Pakistan'		4
<i>M. alba</i> s.l.	'Romana'		50
<i>M. alba</i> s.l.	'Rosea'		1
<i>M. alba</i> s.l.	'Rupps Roumanian'		4
<i>M. alba</i> s.l.	'Silk Hope'		7
<i>M. alba</i> s.l.	'Tago-Wase'	China	6
<i>M. alba</i> s.l.	'Tenuifolia'		10
<i>M. alba</i> s.l.	'Tout Badena'	France	5
<i>M. alba</i> f. <i>pendula</i>	'Pendula'		7

species	variety	Origin	No. individuals
<i>M. alba</i> f. <i>pyramidalis</i>	‘Pyramidalis’		2
<i>M. alba</i> s.l. (syn. <i>M. multicaulis</i> )	‘Bullato’		32
<i>M. alba</i> s.l. (syn. <i>M. multicaulis</i> )	‘Georgeous’		4
<i>M. alba</i> s.l. (syn. <i>M. multicaulis</i> )	‘Large Black’		4
<i>M. alba</i> s.l. (syn. <i>M. multicaulis</i> )	‘Planifolia’		3
<b><i>M. boninensis</i></b>			<b>1</b>
<b><i>M. indica</i> s.l.</b>			
<i>M. indica</i> (syn. <i>M. latifolia</i> )	‘Kokusou’		4
<i>M. indica</i> (syn. <i>M. latifolia</i> )	‘Kokusou 27’		8
<i>M. indica</i> (syn. <i>M. kagayamae</i> )			18
<i>M. indica</i> (syn. <i>M. kagayamae</i> )	male		2
<i>M. indica</i> (syn. <i>M. rotundiloba</i> )	Rotundiloba		18
<b><i>M. nigra</i></b>			
<i>M. nigra</i>	‘Koester’		3
<i>M. nigra</i>	‘Moldenheaven’		2
<i>M. nigra</i>	‘Port-Cros’		6
<i>M. nigra</i>	‘Scott Jumbo’		1
<i>M. nigra</i>	‘Shah tut’		2
<b><i>M. rubra</i> cf.*</b>			
<i>M. rubra</i> cf.	‘Balhi Tout’		1
<i>M. rubra</i> cf.	‘Collier’		7
<i>M. rubra</i> cf.	‘Downalk’		2
<i>M. rubra</i> cf.	‘Far Bibay’		2
<i>M. rubra</i> cf.	‘Hicks fancy’		7
<i>M. rubra</i> cf.	‘Illinois Everbearing’		10
<i>M. rubra</i> cf.	‘Nansii’		1
<i>M. rubra</i> cf.	‘Thomson’		3
<i>M. rubra</i> cf.	‘Townsend’		2
<i>M. rubra</i> cf.	‘Nerrosa’	Ukraine	5



Supplementary Table 9- List of varieties maintained in the Orchard-conservatory Mercoire.

species	variety	Origin	No. individuiums in the Mercoire	No cuttings in pots or containers
<b><i>M. alba</i></b>				
<i>M. alba</i> s.l.	'Aureifolia'		1	1
<i>M. alba</i> s.l.	'Cabanette'		/	2
<i>M. alba</i> s.l.	'Cattaneo mâle'		2	1
<i>M. alba</i> s.l.	'Colomba (e)sse'	Italy	/	4
<i>M. alba</i> s.l.	'Colombassette'	Italy	/	4
<i>M. alba</i> s.l.	'Costo Blanco'		/	1
<i>M. alba</i> s.l.	'Furcata'		3	/
<i>M. alba</i> s.l.	'Gamette Hâtive'		2	1
<i>M. alba</i> s.l.	'Grisetto'	France	2	/
<i>M. alba</i> s.l.	'Guzziola'		1	/
<i>M. alba</i> s.l.	'Herati'	Middle East	/	1
<i>M. alba</i> s.l.	'Ichinose'	Japan	2	/
<i>M. alba</i> s.l.	'Italica'	Italy	1	/
<i>M. alba</i> s.l.	'Langue de Boeuf'	France	1	1
<i>M. alba</i> s.l.	'Lhou'	Italy	2	/
<i>M. alba</i> s.l.	'Mâle du Piémont'	France	1	/
<i>M. alba</i> s.l.	'Moretti'	Italy	/	10
<i>M. alba</i> s.l.	'Muki'	Italy	1	/
<i>M. alba</i> s.l.	'Nagasaki'	Japan	/	/
<i>M. alba</i> s.l.	'Poumaou'		1	3
<i>M. alba</i> s.l.	'Rabalaire'		3	2
<i>M. alba</i> s.l.	'Romana'	France	3	/
<i>M. alba</i> s.l.	'Rosea'		1	9
<i>M. alba</i> s.l.	'Rougeto'	France	/	1
<i>M. alba</i> s.l.	'sauvageon'	France	/	/
<i>M. alba</i> s.l.	'Stérile'	unknown	/	/
<i>M. alba</i> s.l.	'Tagowase'	China	/	1
<i>M. alba</i> s.l.	'Tut Bedana'	France	/	/
<i>M. alba</i> s.l.	'Chirtout'		2	/
<i>M. alba</i> s.l.	'Wellington Mulberry'		1	/
<i>M. alba</i> s.l.	'English Black'		2	/
<i>M. alba</i> s.l. (syn. <i>M. atropurpurea</i> )	/		1	/

species	variety	Origin	No. individuals in the Mercoire	No cuttings in pots or containers
<i>M. alba</i> (syn. <i>M. multicaulis</i> )	'Bullata'		/	8
<i>M. alba</i> (syn. <i>M. multicaulis</i> )	'Gorgeous'		1	/
<i>M. alba</i> (syn. <i>M. multicaulis</i> )	'Large Black'		1	/
<i>M. alba</i> (syn. <i>M. multicaulis</i> )	'Planifolia'	France	/	/
<i>M. alba</i> f. <i>pendula</i>	'Pendula'	Italy	1	/
<i>M. alba</i> f. <i>pyramidalis</i>	var. <i>pyramidalis</i>	Italy	1	1
<i>M. indica</i> (syn. <i>M. latifolia</i> )	'Kokusou 25'	Japan	/	/
<i>M. indica</i> (syn. <i>M. latifolia</i> )	'Kokusou 27'	Japan	2	/
<b><i>M. boninensis</i></b>	<b>/</b>		<b>/</b>	<b>5</b>
<b><i>M. nigra</i></b>				
<i>M. nigra</i>	'3B'		1	/
<i>M. nigra</i>	'de Gleizolles'		1	/
<i>M. nigra</i>	'du Masaout'		1	/
<i>M. nigra</i>	'de Mercoire Bas'		1	/
<i>M. nigra</i>	'de Mercoire Haut'		1	/
<i>M. nigra</i>	'de Mercouret'		2	/
<b><i>M. rubra</i> *</b>				
<i>M. rubra</i> cf.	'Fair Bibay'		1	/
<i>M. rubra</i> cf.	'Illinois Everbearing'		1	/
<i>M. rubra</i> cf.	'Nerrosa'	Italy	2	1
<b><i>Morus</i> sp.</b>				
<i>Morus</i> sp.	'hybride de Saint-Christol'		1	/

\*Varieties that are noted as *M. rubra* cf. could originate from simple or complex spontaneous and/or artificial crosses involving *M. alba*. Their genetic relationship is going to be analysed under the scope of ARACNE mulberry research.

## Appendix VI – Greece mulberry germplasm Supplementary Tables

**Supplementary Table 10-** The list of mulberry varieties maintained in the collection of the University of Athens.

species	variety	origin	number of individuals
<b><i>M. alba</i> s.l.</b>			
<i>M. alba</i> s.l.	'Kayriou Ichinose'	China	13
<i>M. alba</i> s.l.	'Kokerka'		13
<b><i>M. indica</i> s.l.</b>			
<i>M. indica</i> (syn. <i>M. latifolia</i> )	'Kokusou 21'	Japan	21
<b><i>Morus</i> species</b>			
<i>Morus</i> sp.	Local variety 1	Greece	156
<i>Morus</i> sp.	Local variety 2	Greece	97
<i>Morus</i> sp.	Early spring	Greece	54
<i>Morus</i> sp.	Quick growth	Greece	56
<i>Morus</i> sp.	Slow growth	Greece	41
<i>Morus</i> sp.	Large leaves	Greece	26
<i>Morus</i> sp.	Maria	Greece	26
<i>Morus</i> sp.	Wild mulberry	Greece	28
<i>Morus</i> sp.	Different unidentified varieties	Greece	11

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