ARACNE:

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

Funded by the European Union

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Deliverable 1.5

Guidance model to collect cocoon samples and list of strains to select for each country

Version 1.0

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Dissemination Level

☒	**PU:**	Public
☐	**SEN:**	Sensitive
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Abstract

The present deliverable is a report on the processes and procedures agreed by all partners of the ARACNE project on A) The methodological framework, guidelines and analytical approaches to be employed for the analysis of samples of historical cocoons (available in Museums participating in the project, especially Lepl State Silk Museum (SSM) and Esapolis Museum) so as to recreate phylogenetic relationships among these old cocoon specimens for historical narration, and B) The selection of a typical silkworm strain from local collections or from the CREA/ Nauchen Tsentar Po Bubarstvo Vratsa (SCS) germplasm collections for each willing partner country to recreate typical local silk productions well-characterized and different among them, for future sericulture production. These activities will cover at least Italy, Spain, Bulgaria, Greece, Georgia.

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	X
2	Iniziativa Cube S.r.l.	INI	
3	Lepl State Silk Museum	SSM	X
4	Nauchen Tsentar Po Bubarstvo Vratsa	SCS	X
5	Piraeus Bank Group Cultural Foundation	PIOP	
6	Univerza V Mariboru	UM	
7	Ethniko Kai Kapodistriako Panepistimio Athinon	NKUA	X
8	Instituto Murciano de Investigacion y Desarrollo Agrario y Medioambiental (IMIDA)	IMIDA	X
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 CCIs' 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 CCIs 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 CCIs 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 CCIs 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 CCIs;

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

Europe is working hard to protect, conserve and restore its important **cultural heritage**, in order to make it one of its main resources. The exploitation of cultural heritage helps to strengthen social cohesion and improve social welfare. However, climate change, pollution

and lack of attention towards the valorization of this common inheritance represents an urgent challenge to be managed by the European citizens and representatives.

ARACNE focuses on cultural heritage preservation and protection; in addition, it bets on the enhancement of traditional skills also through the adaptive reuse of built cultural heritage. To address the present challenges, innovative and cutting-edge technologies will be employed in the cultural ecosystem considered, in order to create new sustainable business and governance models, which will be able to promote economic and social growth, and to strengthen the reputation of the European countries abroad.

The goal is to develop new creative ideas in various sectors, improve the skills and competitiveness of the **cultural and creative industries** and enhance the perception of the **artistic and cultural heritage** not only locally but also in an international scenario. The project aims to contribute to the macro-objectives of the European Commission, such as meeting the **Green Deal** and **sustainable development** initiatives, making the economy increasingly attentive to social welfare and contributing to the development of the **New European Bauhaus**.

ARACNE project has the ambitious objective of identifying, consolidating and promoting the silk heritage that is able to shape a silk-linked European cultural identity and the valorisation of a common cultural and artistic heritage. The production and, more in general, the past and present development of silk sector in the countries involved is the common thread for a future European Silk Route as a cultural itinerary across Europe.

The main intention is therefore to create a wide and well-connected network 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 centuries that followed, which aims to:

- Enhance silk arts and cultural heritage, bringing back silk production in vogue by reconstructing a resilient and innovative silk ecosystem that retraces the concerned European countries and promotes traditions, architecture, both tangible and intangible heritages within them. At the same time, the consolidation of an European Silk Route will encourage links and shared activities among European cities and regions, specifically among their museums, study and research centres able to strengthen the preservation and protection of their common heritage and to promote innovations in their production and trade;
- Contribute to improve skills and competitiveness of silk-related European Cultural and Creative Industries (CCIs) through the renewal, the co-development and the implementation of human-centered and place-specific silk-based cultural products, processes and service innovations. It will be done mainly by leveraging on the application of digital solutions and cutting-edge technologies, in order to create and foster the transition to more sustainable business models, able to promote economic and social growth and to strengthen the reputation of European countries abroad.

2.1 Objective of the deliverable

This report aims to inform all partners involved in the ARACNE project about the activities of Task 1.3 of the project, so that they can participate, collaborate with each other and be given clear instructions on the actions that they need to take within the framework of Task 1.3.

The second aim of the present report is to showcase the representative local silkworm races that are currently reared and maintained in European silkworm germ-banks.

It has been agreed by all partners of the ARACNE project, based on historical and archival documents, that each of these presented and described European silkworm races have historical and cultural links with each country where they are designated to be reared and maintained.

By showcasing the historical and cultural links of each silkworm race with each country, this report becomes the stepping stone for the adaptation and continuous maintenance of these races in their respective country of origin, an activity that will be assessed and described by the deliverable D1.6 (Silkworm strain selection rearing report) due on 28/2/2024. The adaptation of each silkworm race in each of the countries described herein has a symbolic status at the moment, but aims to re-unite silk production and sericulture with the cultural identity and the cultural heritage of each site of the country in which each silkworm race will be reared and maintained.

To aid such a "re-union", actions will be taken to provide manuals, instructions and educational kits to museums where these local silkworm races will be maintained and displayed, and historical narratives will be created to highlight their importance as cultural symbols of the local identity. Actions will be taken to encourage farmers to adopt these silkworm races for the local production of silk and related arts. By doing so, the overarching aims of the ARACNE project will be fully materialized and substantiated.

2.2 Document structure

The present report is divided into two parts:

1. Part A: guidance model to collect cocoon samples;
2. Part B: list of strains to select for each country.

Therefore, in the main body of this report these two activities will be described in detail providing justifications for the choices and decisions that have been taken by all partners of the ARACNE project. These two activities are interlinked in their overarching aim since the historical narrative that will be highlighted by activity A will bolster the cultural significance, social identity and biodiversity conservation aim of activity B.

2.3 Part A: Guidance model to collect cocoon samples

2.3.1 Introductory Notes

To re-trace the cultural heritage and cultural identity of Europe in relation to silk and to thematically substantiate the European Silk Route, it is imperative to illustrate and examine the different silkworm races that have been used in the 19th and 20th century in Europe to produce silk that, in turn, was a major trading item and helped improve the economic and trading status of many European countries (Giorgio Riello et al., 2020).

By examining and analysing biological specimens of silkworm races in Europe one can create association maps between silkworm races and human activities, agricultural activities and trade routes and links between European countries that, in turn, influenced the culture and identity of many European countries and shaped the history, art and architecture of many European cities and regions (Cornalia, 1856; Lee et al., 2022; Rusudan, 2021; Φιλιππίδης, 1890). Tracing the relationships and familiarities between the European silkworm races is akin to creating a virtual European silk route map that depicts the cultural exchanges and trade links between European countries (Lee et al., 2022).

To achieve this we have gathered, and deliver herein, a substantial amount of data, resources and information on how to collect and analyse specimens of European silkworm races as scattered and preserved in various European museums, exhibition sites and silkworm germ-bank collections.

Being an invertebrate, an insect, the silkworm, *Bombyx mori* L., does not leave any trace of its past existence, therefore analysing archaeological remains of the silkworm is impossible. The silkworm, however, leaves its cocoon, the protective shell that the larva spins and into which it metamorphoses to a pupa and from which emerges the adult moth. The silkworm cocoon is an elaborate structure composed of about 286 proteins (Zhang et al., 2015) but mostly it is composed of two groups of proteins, fibroins and sericins (Lee et al., 2022). The cocoon as a structure is fairly rigid and durable and can be kept, protected from pests, for many centuries without decay in sealed display screens. Unfortunately, the rule is that the pupa inside the cocoon has to be removed from the cocoon so as not to soil the cocoon when it is placed inside the display screen. This is done by cutting the cocoon, opening and removing the pupa before placing the cocoon in the display screen for preservation. Therefore, the only tangible trace one can use in the analysis of old silkworm specimens is the contents of the cocoon shell. Due to the above-mentioned rule, the preferred use of ancient genomic DNA to trace the phylogenetic relationships of the European silkworm races is also impossible.

Thus, there are two more scientific approaches left to study the phylogenetic relationships and genealogy of the European silkworm races.

One is proteomics, the study of proteins of a given organism on a large scale. Using proteomics, one can analyse the composition, nature and quantity of all the proteins in a biological specimen (Cleland & Schroeter, 2018; Dallongeville et al., 2016; Lee et al., 2022; Zhang et al., 2015). In lieu of using genomic DNA for the study of old cocoon specimens,

proteomics will be used to analyse the old cocoon specimens that will be collected as part of the ARACNE project (Zhang et al., 2015).

The other is metabolomics, the study of unique chemical traces that are left on old cocoon samples before their preservation. With metabolomics one creates a chemical "fingerprint" a profile of small and large molecules that exist in a given biological specimen that can be unique for this specimen and differentiate from similar but geographically distant biological specimens (Dallongeville et al., 2016; Tzachristas et al., 2021).

For the record, a third approach is radiocarbon dating, a technique by which the age of a given specimen can be accurately determined. This technique is of limited value within the realm of the ARACNE project since the oldest preserved European cocoon specimens that exist in Europe are those used by Louis Pasteur to study the occurrence of pebrine in Europe that date back to 1865-1970 and are preserved in the Science Museum in London, UK (https://wellcomecollection.org/works/cdtkrkhw). This date (i.e., 1865) is too close to present for radiocarbon dating to provide any meaningful data.

2.3.2 Methodology

To achieve our goal of analysing old cocoon samples of European silkworm races, in the first month of the ARACNE project we have gathered information about the sites (museums, exhibition halls, germ-bank collections) when old cocoon samples are maintained. This information is listed as follows:

1) The Silk Museum of Tbilisi in Georgia has provided a list of old cocoon specimen from European races that are maintained as display items in the museum. The oldest of these cocoon specimens dates back to 1885 (see Appendix #1).
2) The Laboratory of Sericulture (CREA) in Padova, Italy has a large collection of cocoons from old European silkworm races and non-European silkworm races. The oldest of cocoon specimens dates back to 1881 (see Appendix #2).
3) The Esapolis Museum in Padova, Italy has an extensive collections of old cocoon samples in display screens. All of them also belongs to the Laboratory of Sericulture (CREA) and were lent for exhibition. The oldest of these cocoon specimens dates back to 1870.
4) The Instituto Murciano de Investigación y Desarrollo Agrario y Alimentario (IMIDA) in Murcia, Spain has identified some old cocoon samples maintained in display exhibitions in and around Murcia of unknown date.
5) The University of Maribor (UM) has identified some old cocoon samples in a private collection in Slovenia of unknown date.
6) The Association Chemin de la Soie des Cévennes aux Alpujarras (ASSOIE) in Florac-Trois-Rivières, France has identified some old cocoon specimen at the Saint Hippolyte du Fort's Museum of unknown date.

7) The National and Kapodistrian University of Athens (NKUA) has identified some old cocoon samples at display screens of the Laboratory of Sericulture, Agricultural University of Athens in Athens, Greece. The oldest of these cocoon specimens dates back to 1932.

It is notable that there are no existing cocoon samples that are older than 1860 an indication of the tremendous impact that the silkworm pebrine disease had on European sericulture and silk production. It can, also, be suggested that the impact of this disease led to the realization that biodiversity preservation is of paramount importance in the various European nations at the time which, in turn, led to the creation of various silk museums in Europe.

Beyond the above-mentioned list of old cocoon samples within Europe, there are several European races that are still maintained in countries such as China and Japan. Data about these races has been published in open access journals in the past (Tong et al., 2022; Xiang et al., 2018).

2.3.3 Sample Collection Guidelines

For each of the identified sites where old cocoon samples are maintained, the following instructions will be given to those that will sample, collect and deliver the cocoons to the place of analysis:

1) The exhibition screen (container, jar, or display screen) should be clearly photographed before cocoon sample removal.
2) Any accompanying information (archival documents, labels or scripts) relating to the exhibition screen or the exhibited items, such as origin, date of display's installation, date of move to the present location, should be clearly recorded to accompany the specimen.
3) Cocoon specimens should be removed using latex gloves to avoid contamination by human hands and should be placed and covered in protective tissue paper without coming to contact with other surfaces.
4) When old cocoons are kept in a heap inside a contained, cocoon samples should be taken from the centre of the heap to avoid or minimize possible contaminants from the container or exposure of the cocoons to the sunlight. No instruments should be used to remove the cocoons in this case, only gloved hands.
5) Upon removal, the cocoon specimens should be examined for any traces of pupa remains such as dried biological fluids or stains inside and outside of the cocoon shell. Specimen with traces of pupa remains should or stains not be sampled.
6) At least two (2) cocoons should be sampled from each lot of cocoons. These two cocoons should be, thereupon, considered as perishable and non-refundable.

7) The two cocoons of each sample should be placed in protective tissue paper before they come to contact with any other material such as plastic or paper envelops. Each cocoon sample should be clearly described on an accompanying document and separate records of each label should be maintained.
8) When only 1 to 4 cocoons constitute a lot from which samples are to be removed, sampling should not be done and only photographs should be taken.
9) Care should be taken so that the exhibition screen should not be destroyed or in any way damaged during cocoon sample removal. If suspected that such a thing may happen during sampling, sampling should be avoided.
10) When silkworm cocoons from various races are mixed on exhibition screens or are in close physical proximity within the exhibition screen with cocoons from other lepidopteran species, sampling should be avoided.

Samples of cocoons will be mailed to the Department of Biology, National and Kapodistrian University of Athens where the proteomics and metabolomics analysis of each sample will take place.

2.3.4 Analytical techniques

Upon receiving the cocoon samples, one of the two cocoons will be cut into small pieces that will be used as technical replicates for the analysis (half of the pieces for proteomics and half for metabolomics analysis. This will be done by using sterile scissors and wearing later gloves for each sample. The small pieces will be placed in sterile plastic tubes for further analytical techniques

Biometric measurements will be taken from the other cocoon which will also serve as a backup sample, in case repeat analysis is required. All cocoon samples will be photographed for publication purposes upon arrival.

For the proteomic analysis we will use the Trapped Ion Mobility-Time of Flight Flex (tims TOF Flex) mass spectrometer (Maxis Impact, Bruker Daltonics, Bremen, Germany) that is housed at the core facility of the Faculty of Sciences, National and Kapodistrian University of Athens, Greece. Using already established experimental protocols, the protein number, nature and abundance of a small sample of each cocoon will be analysed (Cleland & Schroeter, 2018; Dallongeville et al., 2016; Hendy, 2021; Lee et al., 2022; Zhang et al., 2015). The generated data will be used to identify counts of spectra or signal intensity and thereby estimate protein abundance as well as other protein modifications. Analytical software such as MaxQuant coupled with statistical analysis with the Perseus software (Moulos et al., 2016). Moreover, there are multiple software pipelines that have been developed to analyse possible protein modifications in an old or ancient biological sample (Dallongeville et al., 2016).

Using such experimental analysis, it has been documented that ancient proteins can be reliably identified from ancient silk garments because the proteome and genome of the silkworm, *Bombyx mori* L. is known (Moulos et al., 2016; Waizumi et al., 2023) and publicly available and in addition the proteins of interest in the cocoon shell are quite stable.

This methodological approach is, however, destructive for the sample although it requires a limited amount of the sample.

For the metabolomics approach, we will use an Ultra high Resolution Quadrupole Time of Flight Mass spectrometer (Maxis Impact, Bruker Daltonics, Bremen, Germany) housed at the core facility of the Faculty of Sciences, National and Kapodistrian University of Athens, Greece (Tzachristas et al., 2021). Using this methodology and associated software packages (Data Analysis 4.4 and TASQ 1.4 software packages from Bruker Daltonics Bremen, Germany), we will attempt to identify small molecules that are present on the cocoon shell samples. Such an approach, by identifying the mixture of the different molecules that are present on cocoon shell, can potentially differentiate and formulate groups of cocoon samples that have a common ancestry or common origin. This is done most simply by using principal components analysis (PCA) but more elaborate statistical analysis tools are currently available (Tzachristas et al., 2021).

Both proteomics and metabolomic analyses will also be carried out in modern day cocoon samples that will be produced by the bioresources centres of the Laboratory of Sericulture (CREA) in Padova, Italy and the Scientific Center on Sericulture (SCS) in Vratsa, Bulgaria. Both centres have extensive collections of silkworm races that will be produced in June or July 2023 and will be mailed to the Department of Biology, National and Kapodistrian University of Athens (NKUA), Greece. These fresh cocoon samples will be used to corroborate and match the identity of the old cocoon samples. Matches and mismatches will be documented and presented in any relevant publications of the analysis results. By the term “match” is meant herein that an old cocoon sample sent from location Z in Europe with the label as silkworm race X is matched in its proteomic profile with the modern-day silkworm race Y that was sent for analysis from CREA or SCS.

All documented data on sampling as well as all the analytical techniques that will be used will be meticulously documented and submitted to the Coordinator of the ARACNE project.

2.4 Part B: List of silkworm races (strains) to select for each country

2.4.1 Introductory Notes

Despite the existence of silkworms in Europe for many centuries, the notion of naming a silkworm race and describing its characteristic has not been used by Europeans before 1850 as attested by several historical manuals on sericulture. Before that date, silkworm larvae and cocoons were described in historical and archival texts or depicted in images but there was no mention of names for the locally reared and bred races. This has changed since 1850

and gradually, possible due to the advent and devastating impact of the pebrine disease and the need to import silkworm eggs from foreign countries but most probably due to the advances in science, names for local races started to appear in scientific textbooks and manuals. This scientific tendency called onomatology soon proliferated and by the introduction of silkworm hybrids in early 1920's became imperative to use when silkworm hybrids had to be described and approved for silk production. In the 20th century onomatology for silkworm races became overwhelmingly extensive and soon reached extremes such as using code names for silkworm races, a practice that was creating more confusion that clarity. As a result, currently throughout the world there are thousands of silkworm races that are reared and bred as biodiversity germ-banks and this number does not include the hundreds of silkworm mutants that are also maintained. Despite this overwhelming production of names for different silkworm races, historical names have been maintained for certain races and these names can be clearly and undisputedly identified in textbooks and manuals dating back to the 19th century.

The partners of the ARACNE project have discussed and agreed, based on tracing historical and archival documents that the silkworm races listed below are, indeed, pure races that are still existing to date. Furthermore, based on such historical and archival references, the partners of the ARACNE project have agreed that the silkworm races listed below have historical links and have been reared in each country that are selected to be re-introduced since the 19th century. For example, the name "Brianza" is selected as a silkworm race for Slovenia because historical records and text report the race "Little Brianza" as being reared in the state of Styria since the mid-19th century. It is precisely due to the evidence on historical archives that this silkworm race should be re-introduced to Slovenia as part of the historical and cultural heritage. Hereupon, efforts will be undertaken by the Slovenian partner (University of Maribor) to encourage the local rearing and maintenance of this silkworm race in museums and exhibitions related to silk within Slovenia, to be distributed to farmers and encourage their rearing as part of ecotourism activities of supplied to schools as education material.

2.4.2 Silkworm race for Italy: Bianco Italia

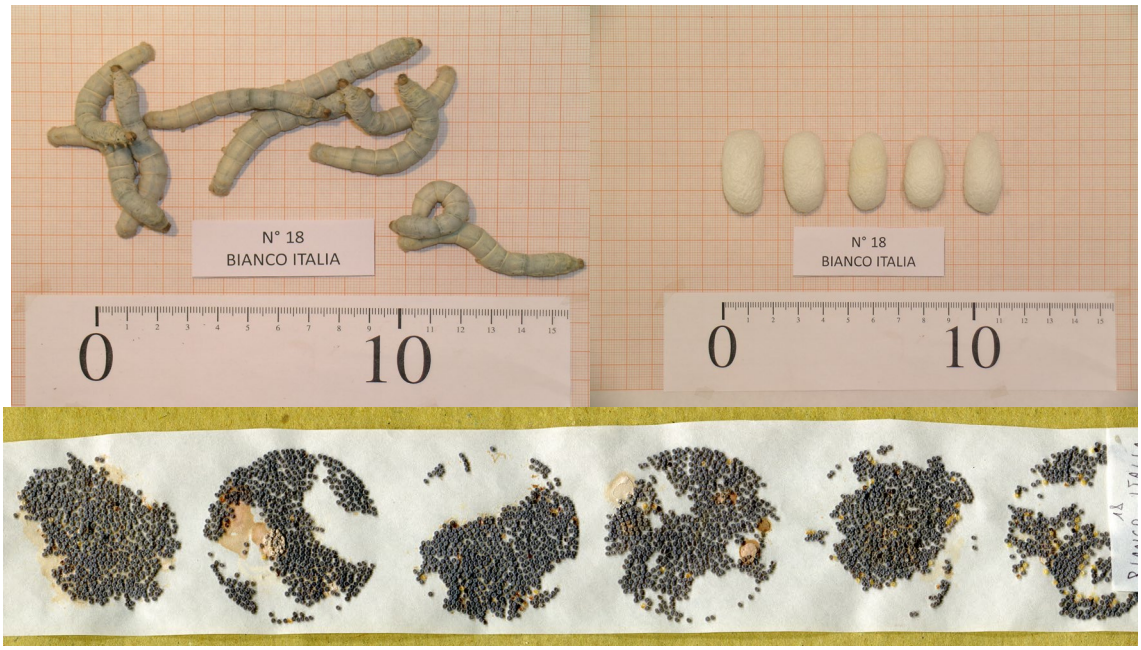

Figure 1 - Pictures of the silkworm race "Bianco Italia". The 5th instar larvae (upper left), the cocoons (upper right) and deposited fertilized eggs by the female moth (below). Courtesy of Dr. S. Cappellozza.

The silkworm race that was selected for Italy is called "Bianco Italia" which translates to English as "Italian White" with reference to the cocoon color of this race. The cocoon of this race is medium in size and represents the standard white type of cocoon that is commercially traded. Dr. S. Cappellozza reports that this is a very old isolate from a yellow cocoon race that is called "Brianzola". The appearance of yellow or white cocoon is quite a complex molecular process (Lu et al., 2023; Ma et al., 2016; Sakudoh et al., 2007; Tsuchida & Sakudoh, 2015) in which the white cocoon color is recessive and forms upon mutation of gene(s) that are responsible for the color of the cocoon.

The "Bianco Italia" race is a typical European univoltine race. The larvae are white-gray with no crescent color (it can only be slightly visible), have white pseudolegs and slightly light head capsule pigmentation. The eggs are grey in colour and adherent to the substrate. The existence of this "Bianco Italia" race has been extensively documented in textbooks dating back to the 19th century.

2.4.3 Silkworm race for Slovenia: Brianza

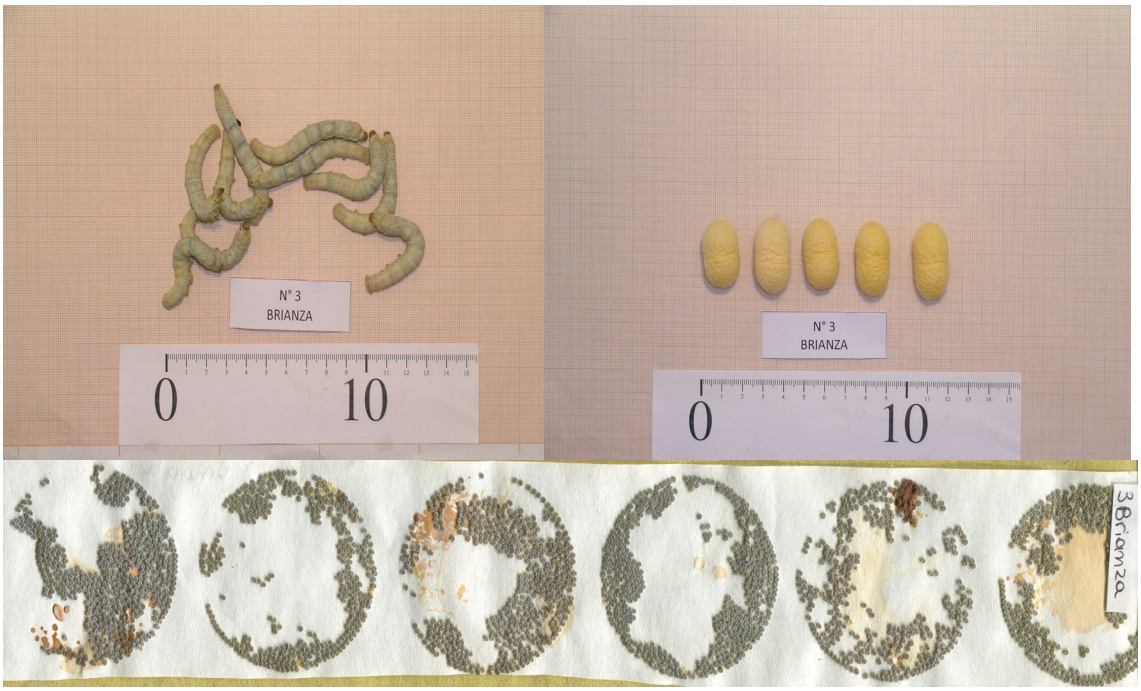

Figure 2 - Pictures of the silkworm race "Brianza". The 5th instar larvae (upper left), the cocoons (upper right) and deposited fertilized eggs by the female moth (below). Courtesy of Dr. S. Cappellozza.

The silkworm race that was selected for Slovenia is called "Brianza". The cocoon of this race is peanut shaped, flesh-yellow on the outside and yellow in its inner layer an indication that it contains a large amount of carotenoid pigments (Lu et al., 2023; Ma et al., 2016; Sakudoh et al., 2007; Tsuchida & Sakudoh, 2015). The larvae colour is beige with slightly visible crescents and yellow pseudolegs and indication that the larvae haemolymph is yellow. This is a univoltine race that produces grey eggs that are adherent to the substrate. It has originated in the Brianza region of Lombardy in Italy.

Archival research by the Slovenia partner (University of Maribor) identified that by 1840 the Styrian Sericultural Association was founded (Hlubek, 1860; Joanneum, 1840) and began to supply Lower Styria (present day Slovenia) with sericultural facilities in the towns of Plevno, Celje, Maribor, Ptuj, Radgona. Among other activities was the provision of silkworm races called "Little Brianza" (Slovenian Brianza) "Great Udineser" possibly a name for the silkworm race "Friulana" and the "Common Strain" with no further identification (Reiter, 1996).

2.4.4 Silkworm race for France: Var

Figure 3 - Pictures of the silkworm race "Var". The 5th instar larvae (upper left), the cocoons (upper middle), a closer view of the cocoon (upper right) and deposited fertilized eggs by the female moth (below). Courtesy of Dr. S. Cappellozza.

The silkworm race that was selected for France is called "Var". The name Var refers to the department of Var in south-eastern France. This is a very well-documented silkworm race in various archival texts and most notably in the classic text by Prof. E. Maillot (Maillot, 1885). The cocoon of this race is medium to long shaped with distinct golden yellow colouration. The larvae are pale grey with crescent coloration typical of most of the modern-day hybrid coloration. The haemolymph has red colour and the pseudolegs are yellow in colour. This is a univoltine race that produces grey eggs adherent to the substrate. This race corresponds to the Italian race called "Varo" The "Var" silkworm race is currently maintained at the Laboratory of Sericulture (CREA) in Padova, Italy and was transferred by INRA to Italy when the silkworm germ-bank in Lyon (France) was closed. It is interesting to note that the name of this race as a European race is mentioned even in textbooks published during the Ottoman Empire (Φιλιππίδης, 1890).

2.4.5 Silkworm race for Spain: Sierra Morena

Figure 4 - Pictures of the silkworm race "Sierra Morena". The 5th instar larvae (upper left), the cocoons (upper right), a closer image of the cocoon (middle left), or the adult male moth (middle right) and deposited fertilized eggs by the female moth (below). Courtesy of Dr. S. Cappellozza.

The silkworm race that was selected for Spain is called "Sierra Morena". The name "Sierra Morena" refers to the mountain range in Southern Spain. This silkworm race was donated by Prof. Jose Cenis Anadon of the Spanish partner (IMIDA) to the Laboratory of Sericulture (CREA) in Padova, Italy in 2010 and is still maintained by CREA under the name "Baco Moro". This race is still maintained in the region of Murcia by IMIDA.

This race has a distinct larvae colouration with "moricaud" brownish body colour, well-tanned crescents and yellow haemolymph. The cocoon size is medium, its shape is oval with the inside layer of the shell being white and the outside layer being golden yellow. The eggs are grey and adherent to the substrate.

2.4.6 Silkworm race for Greece: Baghdad

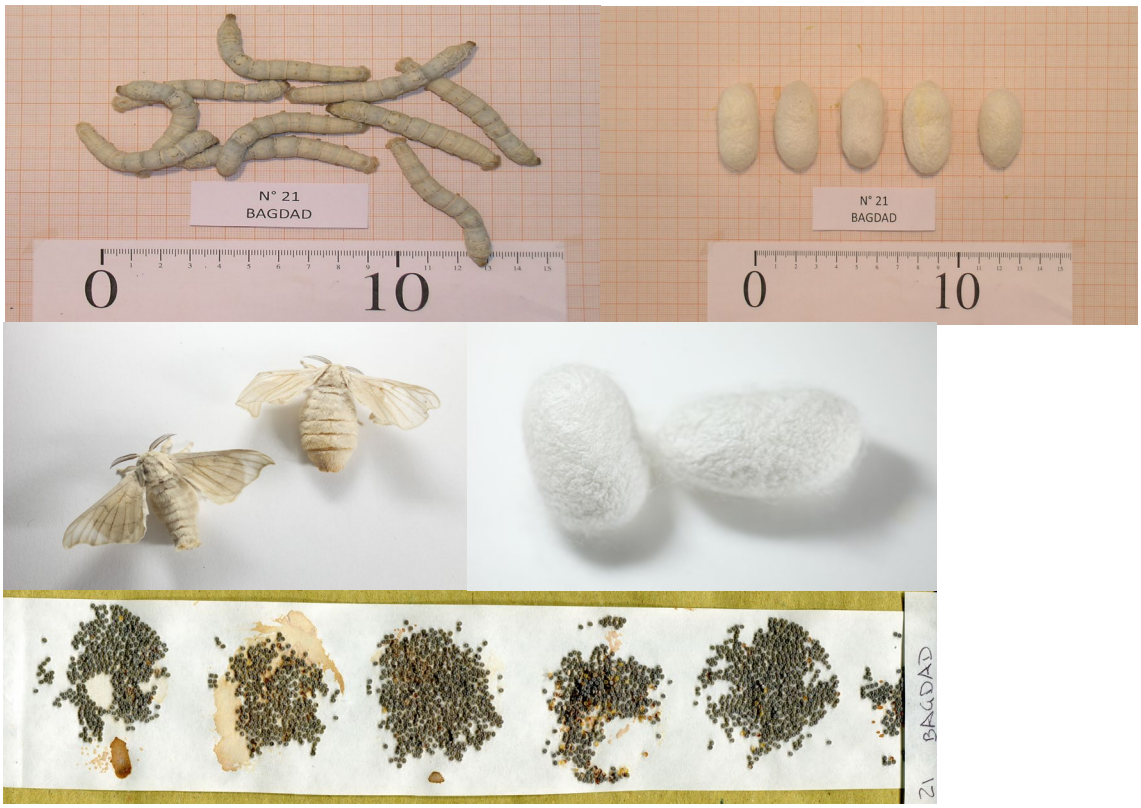

Figure 5 - Pictures of the silkworm race "Baghdad". The 5th instar larvae (upper left), the cocoons (upper right), the male and female moths (middle left), a closer picture of the cocoons (middle right) and deposited fertilized eggs by the female moth (below). Courtesy of Dr. S. Cappellozza.

The silkworm race that was selected for Greece is called "Baghdad". This silkworm race is very-well documented as being reared in the north of Greece since the mid-19th century (Φιλιππίδης, 1890). Its name "Βαγδάτης" appear in several textbooks written in Greek until the 1950's and was abandoned in 1960 upon the introduction of silkworm hybrids. Its name as "Μπαϊντάτια" appears also in traditional songs.

The larvae have slightly visible crescent colouration and are white in colour. This is a univoltine silkworm race. The cocoon is big to medium, oval and long in size. The eggs are grey and occasionally they may be non-adherent to the substrate. Judged by the name, the origin of this silkworm race is from the middle east and one particular reference (Cornalia, 1856) mentions the import of a race in Italy from Persia (modern day Iran) and Burse, Turkey that produced non-adherent eggs.

2.4.7 Silkworm race for Bulgaria: Yellow Local

Figure 6 - Pictures of the silkworm race "Yellow Local". The 5th instar larvae (left) and the cocoons (right). Courtesy of Dr. P. Tzenov.

The silkworm race that was selected for Bulgaria is called "Yellow Local". This silkworm race is very-well documented in Annual Reports of the State Sericulture Experimental and Control Station in Vratsa, Bulgaria in records dating back to 1914. Numerous other references to this race as being reared in Bulgaria also exist.

The larvae of this race have a characteristic stripped body colouration that is referred to as "Zebra". Larval body crescents are marginally pigments. The eggshell colour is green. The cocoon colour is yellow. The cocoon size is medium and its shape elongated.

2.4.8 Silkworm race for Georgia: Telavi, Georgian white; Kakheti green or Kutaisi orange

The decision for the silkworm race to be selected for Georgia is still under deliberation at the time of writing of this report. This is because the Silk Museum in Tbilisi, Georgia which is a partner in the ARACNE project does not maintain and breed any silkworm races. There are about 50 silkworm races maintained and bred at the Laboratory of Sericulture, Agricultural University of Georgia which is not a partner of the ARACNE project and, therefore, access to its resources is limited. Dr. I. Gujabidze, who is the Head of the Laboratory of Sericulture, Agricultural University of Georgia kindly provided information about 4 local silkworm races that will be decided at the later date which of the 4 will be named as representative of Georgia.

According to Dr. I. Gujabidze, the "Telavi" silkworm race has a white cocoon and its name derives from a city of the same name in eastern Georgia. The "Kakheti green" race has a green cocoon and its name derives from a region in eastern Georgia. The "Kutaisi orange" race derives its name from the second largest city in Georgia. No further information was given for the "Georgian white" silkworm race. All of these silkworm races have been reared and bred by local farmers (Rusudan, 2021). According to Dr. I. Gujabidze, the "Telavi" and "Georgian white" are not easily adaptable to modern rearing conditions, while the "Kakheti green" and "Kutaisi orange" are low silk producing varieties.

To achieve the aims of Task 1.3, it was decided to wait until the spring rearing season of 2023 when further information can be provided by Dr. I. Gujabidze, whereupon a decision to transfer one of the 4 above-mentioned silkworm races to the Scientific Center on Sericulture (SCS) in Vratsa, Bulgaria based on the recommendation of Dr. I. Gujabidze and further research on archival documents.

ACRONYMS

[PCA] Principal components analysis

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Appendix I

List of old cocoon samples maintained at the Silk Museum in Tbilisi, Georgia.		
Sample Number	**Sample Name**	**Sample Data**
1	Gran-Sasso	1894; 1896
2	Italian Yellow	1886; 1894; 1895; 1899
3	Italian Green	1894
4	Italian White	1895
5	Italian Yellow “Karpinetti”	1894-1895
6	Sevenien White	1894; 1896
7	Yellow Vari	1894; 1895; 1896
8	French	1886;
9	French White	1887; 1894-1896
10	French Yellow	1887; 1894 -1895
11	Yellow Vartanzy	1894
12	Iberian Yellow	1894; 1896; 1899
13	Bagdad	1895
14	Bursa’s White	1896
15	Corsican Yellow	1896
16	Alpine Yellow	1899
17	European Yellow	1885
18	Italian	1886

Appendix II

List of old cocoon samples collected at the Laboratory of Sericulture (CREA) in Padova, Italy. Each sample contains >3 cocoons in excellent condition.	
Sample Number	**Sample Name**
1	Switzerland
2	Bione (Italy)
3	Ryazan (Russia)
4	Iran
5	Murcia (Spain)
6	Galata (Romania)
7	Korea
8	Thailand
9	Corsica (France)
10	Greece
11	Bursa (Turkey)
12	Schwarzwald (Germany)
13	Bagdad (Iraq)
14	Shangai 1881
15	Iran
16	Shirahine (Japan)
17	Eravan (Armenia)
18	Dehra-Dun (India)
19	Makedonia
20	Uzbekistan

21	Tiflis (Georgia)
22	Cosenza (Italy)
23	Var (France) (VARO?)
24	Suzhou (Soochow?)
25	Bombyx Croesi (India)
26	France
27	Nistri (India)
28	Sarota (Russia) (Volga?)
29	Bombyx textor (China)
30	Ascoli (Italy)
31	Almeria (Spain)
1	Switzerland
2	Bione (Italy)
3	Ryazan (Russia)
4	Iran
5	Murcia (Spain)