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COCHINEAL, A PRECIOUS SOURCE OF RED

Cochineal Dyes Characterization by High Performance Liquid Chromatography with Diode Array Detection and Principal Component Analysis

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Abstract

The identification of precise cochineal species used to dye historical textiles can provide important information about the provenance and date of these objects. The most widely used method to identify cochineal species in textiles involves quantification of specific minor compounds, after High-performance Liquid Chromatography with Diode Array Detection (HPLC/DAD) analysis. However, there are several factors which are not presently taken in account when characterizing cochineal species on historical textiles. Not only all the species of cochineal are not well studied, but also the current studies, based on a limited number of species, frequently face difficulties with the identification of these on historical textiles, especially due to the analysis conditions and the results treatment.

Therefore, a new approach on the study of cochineal species present in historical textiles was developed. Different parameters for the analysis conditions were undertaken to optimize the results for both insect species and textiles samples. Afterwards, with Principal Components Analysis (PCA), results from textiles samples exhibited a satisfactory correlation when compared with a cochineal reference database. Moreover, High-performance Liquid Chromatography with Diode Array Detector coupled with Mass Spectrometry (HPLC/DAD/MS^n) analysis could offer accurate information on cochineal species and textiles samples. The characterization of six species of cochineal allowed, through PCA and HPLC/DAD/MS^n analysis, the identification of unidentified cochineal insect samples and a group of Islamic and Italian historical dyed-cochineal textiles, dated from 15th to 17th centuries.

This identification contributes to connect the textiles' history, and the trade and dyeing technologies on possible different species of cochineal. This fact regards especially textiles produced in the main textile centres, where, after the 16th century, the traded American cochineal was swiftly adopted, as many historical publications assert. Although this study identified American cochineal in a 17th-century Indian textile for the first time, the results for the other analyzed textiles did not reveal the presence of this species. In this way, the possibility of the prompt spread of the American specie in European and Asian textiles dyeing seems to be more complex than what is emphasized by present publications.

Communications and Publications

Ana Serrano, Micaela Sousa, Jessica Hallett, Maria Fernanda Passos Leite, "Cochineal, A Precious Source of Red" – accepted for oral communication in "Colours" conference, Victoria & Albert Museum, London, November 20, 2010.

Ana Serrano, Micaela Sousa, Jessica Hallett, João Lopes, Conceição Oliveira, Monique Simmonds and Mark Nesbitt – "Cochineal Dyes Identification by High-Performance Liquid Chromatography with Diode Array Detector and Principal Component Analysis", to be submitted, 2010.

Sumário

A identificação precisa de espécies de cochinilha usadas no tingimento de têxteis históricos pode fornecer informação importante para a proveniência e a datação dos mesmos. O método mais usado para a identificação dessas espécies nos têxteis caracteriza-se pela quantificação de compostos minoritários específicos, depois de análises por Cromatografia Líquida de Alta Eficiência por Vector de Díodos (HPLC/DAD). Contudo, existem determinados factores que não são tidos em conta quando as espécies de cochinilha são caracterizadas em têxteis históricos. Para além de nem todas as espécies de cochinilha estarem estudadas, estudos recentes, baseados num limitado número de espécies, encontram dificuldades na identificação das mesmas em têxteis, devido às condições de análise e ao tratamento dos resultados.

Assim, desenvolveu-se um novo método de estudo para caracterizar espécies de cochinilha em têxteis históricos. Diferentes parâmetros para as condições de análise foram realizados, de modo a optimizar os resultados para as amostras de insectos e de têxteis. Posteriormente, com Análises por Componente Principal (PCA), os resultados das amostras têxteis demonstraram uma correlação satisfatória quando comparados com a biblioteca de referência de espécies de cochinilha. Adicionalmente, análises por Cromatografia Líquida de Alta Eficiência por Vector de Díodos acoplado a Espectrometria de Massa (HPLC/DAD/MS^n) permitiram obter informação mais precisa sobre as espécies de cochinilha e as amostras têxteis. A caracterização de seis espécies de cochinilha, através de análises por PCA e HPLC/DAD/MS^n, permitiu identificar insectos de cochinilha de espécie desconhecida e um grupo de têxteis históricos islâmicos e italianos, datados de entre os sécs. XV e XVII.

Esta identificação contribuiu para interligar a história dos têxteis, o comércio e as tecnologias têxteis, com as diferentes espécies de cochinilha. Esta interligação é mais comum em têxteis produzidos nos principais centros têxteis, onde, após o séc. XVI, a cochinilha americana foi rapidamente adoptada, tal como as publicações históricas defendem. Apesar de, neste estudo, se ter identificado pela primeira vez, cochinilha americana num têxtil indiano do séc. XVII, os resultados para os outros têxteis não revelaram a presença desta espécie. Assim, a possibilidade da rápida assimilação da espécie americana nos tingimentos têxteis europeus e asiáticos parece ser mais complexa do que é enfatizado pelas recentes publicações.

Comunicações e Publicações

Ana Serrano, Micaela Sousa, Jessica Hallett, Maria Fernanda Passos Leite, “Cochineal, A Precious Source of Red” – aceite para comunicação oral na conferência “Colours”, Victoria & Albert Museum, Londres, a 20 de Novembro de 2010.

Ana Serrano, Micaela Sousa, Jessica Hallett, João Lopes, Conceição Oliveira, Monique Simmonds and Mark Nesbitt – “Cochineal Dyes Identification by High-Performance Liquid Chromatography with Diode Array Detector and Principal Component Analysis”, a ser submetido em 2010.

Subjects Index

Introduction

1. Cochineal, a Precious Source of Red[1]

Cochineal was one of the most precious natural red dyes, appreciated by Europeans and Asians alike. Cochineal, like kermes and lac, belongs to the group of coccid dyes, which have been appreciated for yielding brilliant and enduring crimson hues. These costly dyestuffs were applied almost exclusively to luxury textiles owing to the large quantities of insects necessary to make sufficient dye, as well as the special skills involved in gathering and preparing the insects and the complex dyeing process [1,2].

Cochineal species belong to two families from the Coccoidea superfamily, Margarodidae and Dactylopiidae [3,4]. Currently, Margarodidae family includes, among others, *Porphyrophora* genus, with 47 species, spread all over the Palearctic region [5]. On the other hand, Dactylopiidae family comprises *Dactylopius genus,* which has 10 species, original from America [6]. Information on dyeing with *Porphyrophora* species is rare, and other species apart from the well-documented *P. polonica*, *P. hamelii*, and the domesticated *D. coccus*, were probably also used for dyeing textiles in the past, especially in regions remote from the main centres of textile manufacturing and international trade routes [4].

From a taxonomical point of view, many insects from the same family are relatively similar to each other, with very slight differences, and so, accuracy should be taken when analyzing species with the same geographical origin. Especially because different species could be gathered in the past and named under the same designation, due to their high similarity, or even due to a dishonestly meaning, like mixture of high-quality *D. coccus* specie with other inferior species of wild *Dactylopius* [4,7]. Consequently, when identifying red cochineal-dyes in historical textiles, great care must be taken when affirming that a dye might belong to *P. polonica, P. hamelii* or *D. coccus* species, since not all the species are studied.

Until 16th century, *Porphyrophora* insects, as well as kermes, were often used by the wealthiest Europeans and Asians to dye textiles. However, soon after the conquest of Mexico by the Spaniards, in 1521, the first shipments of Mexican cochineal (*D. coccus*) began arriving in Europe and spread from there to Asia [2,7]. It provided more vivid crimson colours, as well as the possibility of achieving a wider range of mixed hues, which were not possible with other coccid dyes. But the greatest advantage of this dyestuff was its high dye content, which had important economic implications [4]. As demand for it increased dramatically, American cochineal became a great source of income for the Spanish economy, and became, after silver, the most valuable item traded in the Hispanic empire during the 17th century [7,8].

The identification of cochineal in European and Asian textiles dating from immediately after the Spanish conquest of Mexico is commonly associated with the adoption of *D. coccus* [9, 10]. However, there were many different sources and species of cochineal available for preparing dyes in these regions which have not been well studied, and previous publications have had difficulty distinguishing them [4, 10]. Hence, in this study a diverse group of red European and

[1] Extensive information on cochineal's art historical perspective is described in Appendix 1.

Islamic textiles produced after the documented arrival of Mexican cochineal are analysed, with the intention of examining the penetration of this insect in dyeing practices.

In Ottoman Turkey, Safavid Iran and Mughal India, red coccid dyes were widely employed in the production of luxurious silk textiles, which were conceived as expressions of wealth, status and prestige. Red was often used as a background colour, providing a vivid contrast to designs woven in blue, green and yellow, and embellished with luxurious precious metal thread [9,11]. Five exquisite velvets and a spectacular Persian silk carpet from these regions and dated from 16th to 17th centuries, were analyzed and compared with a database of cochineal species. In addition, two Italian velvets, one dating from prior to 1521 (MNAA 1616Tec) and the other from later in the 16th century (GCM 245) were also analyzed, Appendix 2. These analyses pretended to identify the possible presence of different cochineal species, and hence contribute to the textiles' history and the trade on the species of cochineal.

2. Previous studies on cochineal species identification with HPLC/DAD

So far, only four species of cochineal have been characterized [12-16] and simply three are being characterized in historical textiles through High-performance Liquid Chromatography with Diode Array Detection (HPLC/DAD) [10,12,16-25]. In the pioneering work on cochineal by Wouters and Verhecken [13-14], it was determined that chromatograms representative of cochineal species are characterized by several minor markers in addition to carminic acid (CA), table 1, which are extracted easily from the insect with aqueous and acidic solutions[2]. These markers vary according to each species as well according to the developmental stage of the insect, figure 1 [13, 14]. For instance, in *P. Polonica* it was found that insect cysts contains a higher proportion of fk+ka than mature insects, and that some females displayed a very low content of fk+ka, comparable to *D. coccus* [13], which could complicate cochineal species identification. Moreover, in all of the species analyzed, the recovery of dye from insect specimens using strong acidic solutions resulted in a higher content of fk+ka in the final dye extract [13]. Nevertheless Wouters *et. al* developed a graphical system to distinguish the *Dactylopius coccus* from *Porphyrophora hamelii* and *Porphyrophora Polonica*, based in the relative percentages of dcII and fk+ka [14].

Table 1 – Representative abbreviations from the compounds present in cochineal species [13,14].

Abreviations	Extended designations
dcII	Dactylopius coccus II
ppI	Porphyrophora polonica I
ppII	Porphyrophora polonica II
CA	Carminic acid
dcIV	Dactylopius coccus IV
dcVII	Dactylopius coccus VII
fk+ka	Flavokermesic + Kermesic acids

[2] The structure of these markers' chromophores and respective UV spectra can be found in Appendix 3.

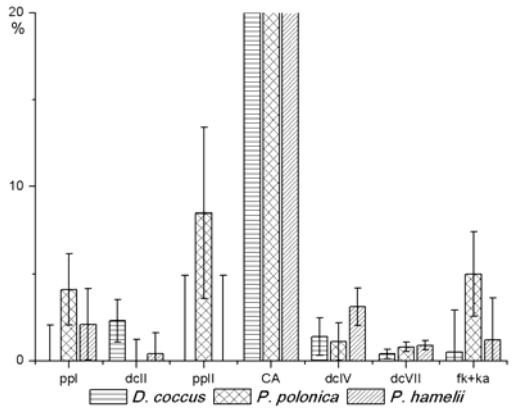

Figure 1 – Graphical representation from markers' relative percentages and respective standard deviations of cochineal insects aqueous extracts, calculated at 275 nm [13,14].

The dye extraction with HCl [12, 20, 23, 25] can be problematic for anthraquinone red dyes as shown in previous work [26]. With the application of mild reagents, like formic acid, oxalic acid or TFA, more information about the dye source can be obtained, mainly for the yellow dyes [27, 28]. Furthermore, cochineal chromatograms of dyed textiles display poor resolution, and, as a consequence, the quantification of the minor markers is difficult [15]. For instance, in recent work, the dcII compound was co-eluted together with carminic acid, compromising the identification of American cochineal [10,17,29].

3. Proposed study on cochineal characterization

High-resolution chromatograms are fundamental for markers identification, as well as Principal Component Analysis (PCA). Multivariate analysis is required in order to analyze and obtain a more accurate distinction of the cochineal species than the markers method quantification. Previous work has used PCA analysis to distinguish *D. coccus* of different geographic origins [30]. In this work, PCA analysis is used for the first time to distinguish six cochineal species (*D. coccus, D. opuntiae, D. confusus, D. ceylonicus, P. polonica* and *P. hamelii*), and identify the red dye source used in a group of eight historical textiles. In a first phase, different solvent preparation tests and soft extraction methods were performed on *D. coccus* dye-samples and cochineal-dyed textile reproductions, respectively, in order to optimize the chromatograms resolution of the red-dyed historical textiles and to ease direct comparison with insect specimens, through PCA analysis. In a second phase, 100 cochineal specimens from six known species (reference library specimens) were analyzed by HPLC/DAD and submitted to PCA, which successfully distinguished them by species. Afterwards, 94 insect specimens of unidentified species were also analyzed and submitted to PCA analysis in order to determine their species. In a third phase, 25 related red samples from historical silk textiles of different dates (15th to 17th centuries) and provenances (Turkey, Italy, Iran and India), were analyzed and compared with the previous results. Finally, to ascertain the presence of the minor markers in the results, HPLC/DAD/MS^n analyses were accomplished on historical textiles samples. In this manner, it was possible to characterize the historical textiles, and, in

conjunction with historical documentation, to establish possible centers and dates of production, as well as the trade routes of these important dyestuffs [18].

Experimental

1. Materials and Solvents

1.1 Chemicals

Water from Millipore Simplicity Simpak 2, *R* = 18.2MΩ cm, USA, methanol, 99,9%, from Panreac (Barcelona, Spain) and perchloric acid from Riedel-de-Haën (Seelze, Germany) were used in all extractions and mobile phase preparations, in dyes analyses with HPLC/DAD. Acetone, C_3H_6O, from Aga (Prior Velho, Portugal), formic acid from Riedel-de-Haën (Seelze, Germany), hydrochloric acid from Panreac (Barcelona, Spain); oxalic acid from BDH (Poole, England) and TFA from Riedel-de-Haën (Seelze, Germany) were applied to the red fibres' extraction solutions.

1.2 Cochineal samples

Analyses were conducted on identified cochineal insect species, Appendix 4: **(a)** 33 samples of *Dactylopius* species (*D. coccus*, *D. ceylonicus*, *D. confusus*, *D. opuntiae* and *D. tomentosus*), from the 17th and early 20th centuries, from different sources (Canary Islands, Madeira, USA, Ceylon, Argentina, and Mexico) provided by Douglas Miller; **(b)** 30 samples of *D. coccus* and 3 samples of *D. opuntiae* from Mexico, Peru, Chile, and the Canary Islands, given by Liberato Portillo [4] and Mónica González [30]; **(c)** 9 samples of *D. coccus* purchased from Dott. Alessandro Bizarri (Florence), Zecchi (Florence, Italy) and Kremer (Aichstetten, Germany); **(d)** 9 samples of *Porphyrophora hamelii* and 3 samples of *P. medicaginis* species, collected in Iran, and obtained from Hassan-Ali Vahedi [5]; **(e)** 20 samples of *P. polonica* from Hungary and Poland, obtained from Ferenc Kozár, Katarzyna Golan and Ewa Simon.

Further analyses were carried out on unidentified insect species, Appendix 5: **(a)** 70 samples from different places and mainly collected through the 19th century, were provided by the Royal Botanic Garden at Kew (London, UK); **(b)** 12 samples, supplied by Dominique Cardon [4]; **(c)** and 12 samples offered by Piero Tiano (ICVBC-CNR, Florence), and Jenny Balfour (Exeter University).

1.3 Red silk fibres

Initial tests to evaluate the extraction method and samples preparation were undertaken on 24 cochineal-dyed silk fibre references, 0,2 mg each. These samples were taken from a piece of silk cloth, previously dyed with *D. coccus* from Kremer (Aichstetten, Germany). Mordanting and dyeing procedures were adapted from Cardon [4] and Golikov [31] cochineal dyeing recipes: 1g silk cloth was pre-mordanted with 0,03M alum and tartar cream, and dyed with 0,03M Kremer cochineal, at pH=7 during 1h, with T=80ºC and constant mechanical agitation.

HPLC-DAD analyses were conducted on 25 red silk fibres, circa 0,2 mg each, from six historical velvets in the Calouste Gulbenkian Museum (MCG) distinguished by different styles of decoration, but sharing a similar strong crimson colour. Five velvets are from Turkey (MCG 1388A, probably Bursa, 16th/17th century), Iran (MCG 1446 probably Isfahan, 17th century, and MCG 1513, 16th century) and India (MCG 1449 and MCG 1422, probably Mughal India, both 17th century), while the sixth one, MCG 245, is European, and attributed to 16th-century Italy, probably Genoa. In addition, a mid-16th-century "Small Silk Kashan" carpet, MCG T100, from Iran was analyzed, along with an important Chasuble, MNAA 1616Tec, in the Museu Nacional de Arte Antiga (Lisbon, late 15th-century Italian velvet), Appendix 6.

2. Samples preparation

2.1 Insect dye-extraction

Extraction of dye from the cochineal insect specimens was adapted from [32]: after being finely powdered, three samples from the same insect with circa 0,2-0,3 mg were extracted using 200µL water in 1,5mL eppendorfs for 10min in a 60ºC water bath, with constant mechanical agitation. Following the procedure of [14], a dilapidation with methanol/chloroform (2/1, v/v) was performed for one *Porphyrophora polonica* sample, prior to its dye extraction. The resulting dye extract solutions were filtered and diluted in water, when necessary (1:5, v/v). Prior to HPLC/DAD analysis of the cochineal extract solution, several solvent proportions in the final dye extract were tested: **(a)** Aqueous dye extract solution 100%; **(b)** Aqueous dye extract solution: CH_4O (50:50, v/v); **(c)** H_2O: CH_4O: $H_2O/HClO_4$ (50:20:30, v/v/v). The best result acquired was applied to all the insect and textile samples.

2.2 Dyed-fibre extraction methods

Four different extraction solutions were undertaken on the cochineal-dyed silk fibre references, to optimize the best extraction method for the cochineal dyestuff: **(a)** Formic acid method – CO_2H_2: CH_4O (5: 95, v/v) [33]; **(b)** HCl method - HCl 37%: CH_4O: H_2O (2:1:1, v/v/v) [34]; **(c)** Oxalic acid method - $C_2O_4H_2$ (0,2M): C_3H_6O: CH_4O: H_2O (0,1: 3: 3: 4, v/v/v/v) [34]; and **(d)** TFA method **-** TFA 2M [28]. The analyses were performed in six replicates, for each extraction method. Fibre' samples, with circa 0,2-0,3 mg, collected from the red cochineal-dyed silk fibre references were extracted in 200 µL extraction solution, at 60ºC for 30 min, with constant mechanical agitation. After extraction, each extract was dried in a vacuum system, and the resulting dry residues were reconstituted with H_2O: CH_3OH: $H_2O/HClO_4$ (50:20:30, v/v/v).

3.HPLC/DAD/UV

The dye analyses were carried out in a *Thermofinnigan Surveyor* HPLC-DAD system with a *Thermofinnigan Surveyor PDA 5* diode-array detector (Thermofinnigan, USA), an autosampler and a gradient pump. The samples' separations were performed in a reversed-phase column, Eclipse Plus C18 with 100Å - 5µm particle size and 150 x 2,1 mm dimensions, with a flow rate

of 0,5 mL/min at 35ºC constant temperature, and were injected onto the column by a Rheodyne injector with 25μL loop. A solvent gradient of A-pure methanol and B-0,3% (v/v) aqueous perchloric acid (v/v) adapted from [32] was applied to the insect extracts and textiles: 0-2 min 7A:93B isocratic, 8 min 15A:85B linear, 25 min 75A:25B linear, 27 min 80A:20B linear, 29min 95A:5B linear, and 33-40min 7A:93B isocratic.

4. MS (Mass Spectrometry)

The characterization of the cochineal minor markers was based in the retention time and mass spectrometry. Optimal ESI-MS^n conditions were established for a standard solution of carminic acid prepared in aqueous methanol (H_2O: MeOH, 20:80 (v/v)) analyzed by direct injection. The LC-ESI-MS analysis were performed with a ProStar 410 autosampler, two 210-LC chromatograph pumps, a ProStar 335 diode array detector and a 500-MS ion trap mass spectrometer with an electrospray ionisation (ESI) ion source (Varian Inc., Palo Alto, CA, USA). Data acquisition and processing were performed using Varian MS Control 6.9 software. Separations were carried out using a Polaris (Varian) C18-A (150 mm × 2 mm I.D., 5 μm of particle size), with controlled temperature (35 ◦C). The samples were injected onto the column *via* a Rheodyne injector with a 20 μL loop. The mobile phase was delivered at a flow rate of 200 μL/min, using a 2-min isocratic elution, with 5% acetonitrile in 0,1% aqueous formic acid, followed by a 30-min linear gradient from 5-60% acetonitrile, a 5-min linear gradient to 100% acetonitrile. The mass spectrometer was operated in negative ESI mode; the optimized operating parameters were: ion spray voltage, -5.2 kV; capillary voltage, 60 V; and RF loading, 80%. Nitrogen was used as nebulising and drying gas, at pressures of 50 and 30 psi, respectively; the drying gas temperature was 350°C. The multistage MS (MS*n*) spectra were obtained with an isolation window of 2.0 Da, excitation energy values between 1.2 and 1.7 V and an excitation time of 10ms (collision induced dissociation (CID) experiments up to MS^3).

5. PCA (Principal Components Analysis)

Given the multivariate nature of the cochineal samples' chromatograms, multivariate data analysis was required in order to analyse the samples. Principal components analysis (PCA) was selected to perform a similarity analysis [35]. PCA results were analysed on the basis of the principal components retaining the major part of the original chromatogram data variance. Since principal components represent the original chromatograms in a smaller dimension, space scatter plots can be used to visualize the original data. PCA calculations were carried out using Matlab version 7.4 release 2007a (MathWorks, Natick, MA) and the PLS toolbox version 4.2.1 (Eigenvector Research, Wenatchee, WA). The algorithm for PCA was written using the method described in Naes et al. [35]. It is based on the singular value decomposition of the chromatographic data covariance matrix. Each row in the chromatographic data matrix corresponds to a chromatogram of a cochineal sample (signal intensity over time, which means, the chromatograms' peaks areas). Model scores and loadings were obtained from the covariance matrix eigenvectors.

Similarity between the cochineal samples was assessed with the chromatogram data. A preliminary analysis was made for samples belonging to the same species and it was found that retention times were consistent (slight retention time shifts were observed). Therefore, no retention time correction was adopted as a pre-processing step.

The PCA models were estimated using the chromatographic data (absorbance at 275 nm) obtained from 15 to 25 min (retention time) since all peaks were found to be within this region. For each chromatogram, 600 points were available for the selected retention time region (1 second intervals). Prior to PCA modelling, all chromatograms were pre-processed using the standard normal variate method (SNV) and mean centering. The consistence between replicates and adjustment of analyzed samples was assessed and guaranteed through the analysis of scores and Hotelling T2/residuals statistics [36].

The major peak at 19 min (CA) was excluded from the analysis since it provides no differentiation between samples. Depending on the analysis purpose different chromatographic regions were selected. The differentiation between *Dactylopius* and *Porphyrophora* species was optimally observed considering the chromatogram regions 17,5 - 18.8 min. Distinction between different species of *Porphyrophora* was performed using the regions 15 - 18,8 min and 19,7 – 25,0 min. Distinction between different species of *Dactylopius* was performed considering the regions 19,6 - 21,3 min and 23,3 - 25 min.

The analysis of unidentified historical specimens and red textiles samples was performed by projecting the correspondent chromatograms onto PCA models developed using known origin samples of *Dactylopius* and *Porphyrophora* species. Therefore, textile samples on score plots were never used to calibrate the model. The matching of these samples to the calibration samples (known origin) was assessed by evaluating the Hotellin T2/residuals statistics. These statistics for the textile samples must be below the confidence level obtained for the calibration samples, in order to validate the projection. The extraction methods produced additional peaks on the chromatograms. This was circumvented by restricting the PCA analysis to elution time regions where chromatograms are consistent.

Results and Discussion

1. Characterization of cochineal

As reported in the literature [14] all the cochineal species are composed by carminic acid (m/z=491), the major red chromophore, and several minor markers, which diverge as well as their concentration according with the cochineal species, figure 2[3]. The results obtained were in agreement with the literature [13, 14], however, the markers dcII, reported in *D. coccus*, and ppII and ppIII from P*. polonica* [12, 14] were not found in the analyzed insect specimens.

[3] The structure of these markers' chromophores and respective UV spectra can be found in Appendix 3. The representative chromatograms for the other five characterized cochineal species are found in Appendix 7.

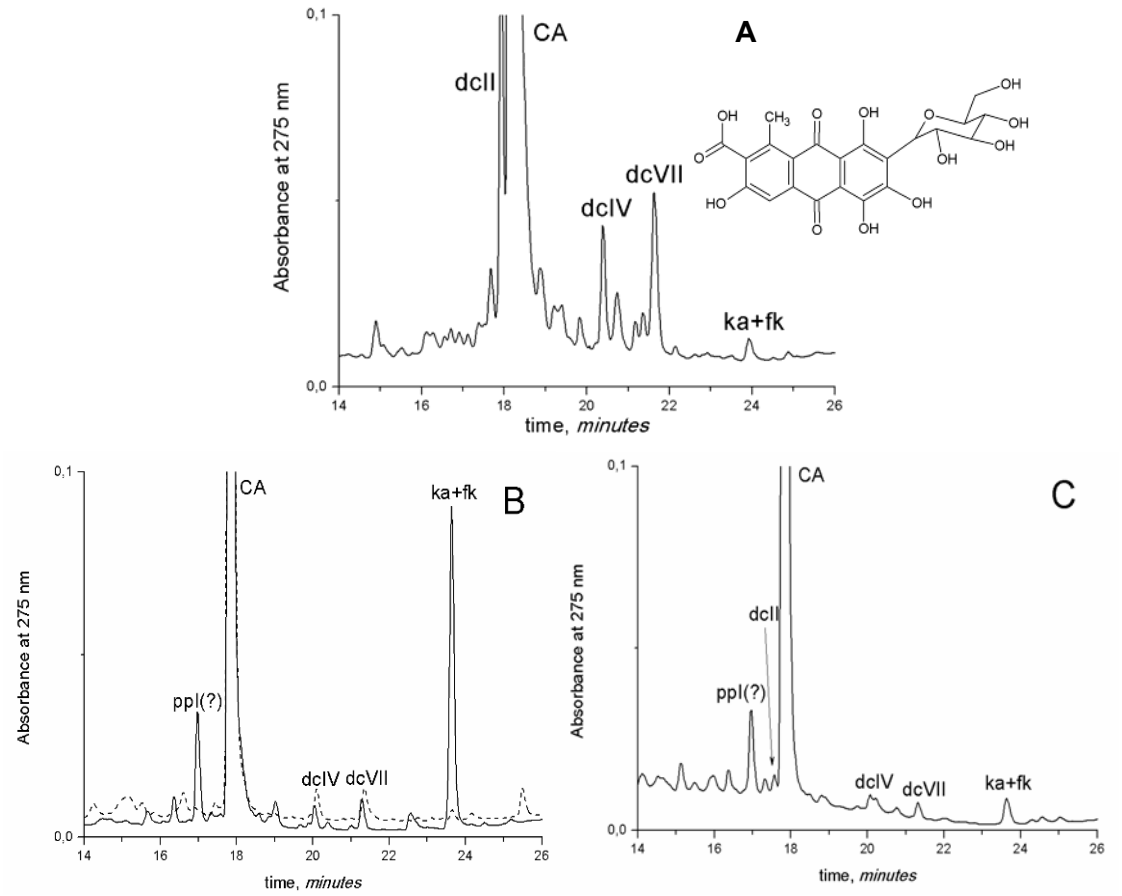

Figure 2 – Representative chromatograms (monitored at 275 nm) of cochineal species **A -** *D. coccus*, with CA structure; **B -** *P. Polonica* female (solid line) and *P. polonica* male (traced line) and **C -** *P. hamelii*, with respective markers. The peaks of ppI and fk+ka in *P. polonica* insects can vary significantly: in the female insect (solid line) the ppI and fk+ka are very intense, while in the male insect these peaks are practically absent and with a concentration comparable to *P. hamelii* insect (for more details see next sections).

HPLC/DAD/MS^n analysis of American cochineal aqueous extracts confirmed the presence of the minor markers, in the following order of elution: dcII, dcIV, dcVII and ka+fk, table 2 and figure 3. In the *Porphyrophora* insects it was also detected the presence of another minor compound, eluted before dcII and with a λ_{max}= 342 nm. The negative ion ES-MS spectrum of this compound exhibited an ion at m/z 475. According to the literature, this compound can be the ppI marker, which after hydrolysis is converted into fk [14]. Indeed, the tandem mass spectrometric (MS/MS) experiments of both ppI(?) and fk compounds exhibited the ion at m/z 269, pointing for a common molecule core.

Table 2 – Retention times and MS data of the main anthraquinone components identified in extracts of several cochineal insects by HPLC/DAD/ESI/MS^n. The product ion spectra were acquired in negative ionisation mode [16,37].

Peak	Rt (min)	UV-Vis λ max (nm)	$[M-H]^-$	MS^n fragments *m/z* (% relative abundance) (neutral fragment loss, possible species)
ppI (?)	17,16	282, **342**	475	**MS^2** **475** → **431** (100); **269** (45)
dcII	17,86	284, **440**	475	**MS^2** **475** → **431** (100) (- 44, CO_2); **413** (15) (- 62, CO_2+H_2O) ; **383** (60) (- 90, $C_3H_6O_3$); **341** (55) (- 120, $C_4H_8O_4$); **311** (30) (-164, $C_6H_{12}O_5$) **MS^3** **475, 431** → **413** (50) (-18, H2O); (**341** (100) (- 90, $C_3H_6O_3$); **311** (60) (- 120, $C_4H_8O_4$)
Carminic Acid	18,00	274, **494**	491	**MS^2** **491** → **447** (100) (-44, CO_2); **429** (15) (- 62, CO_2+H_2O); **357** (30) (-134, CO_2+ $C_3H_6O_3$); **327** (10) (-164, $C_6H_{12}O_5$) **MS^3** **475, 447** → **429** (25) (-18, H2O); **357** (100) (-90, $C_3H_6O_3$); **327** (60) (-120, $C_4H_8O_4$)
dcIV	20,38	276, **498**	491	**MS^2** 491 → 447 (100) (-44, CO_2); **357** (10) (-134, CO_2+ $C_3H_6O_3$); **327** (4) (-164, $C_6H_{12}O_5$) **MS^3** **475, 447** → **357** (100) (-90, $C_3H_6O_3$); **327** (80) (-120, $C_4H_8O_4$)
dcVII	21,58	276, **496**	491	**MS^2** **491** → **447** (100) (-44, CO_2); **429** (15) (- 62, CO_2+H_2O); **357** (15) (-134, CO_2+ $C_3H_6O_3$) **MS^3** **475, 447** → **429** (10) (-18, H2O); **357** (100) (-90, $C_3H_6O_3$); **327** (15) (-120, $C_4H_8O_4$)
ka	23,93	271, **492**	329	**MS^2** **329** → **285** (-44, CO_2) → **257** (-28) → **213** (-44, CO_2) → **185** (-28)
fk	23,65	276, **448**	313	**MS^2** **313** → **269** (100); **270** (18)

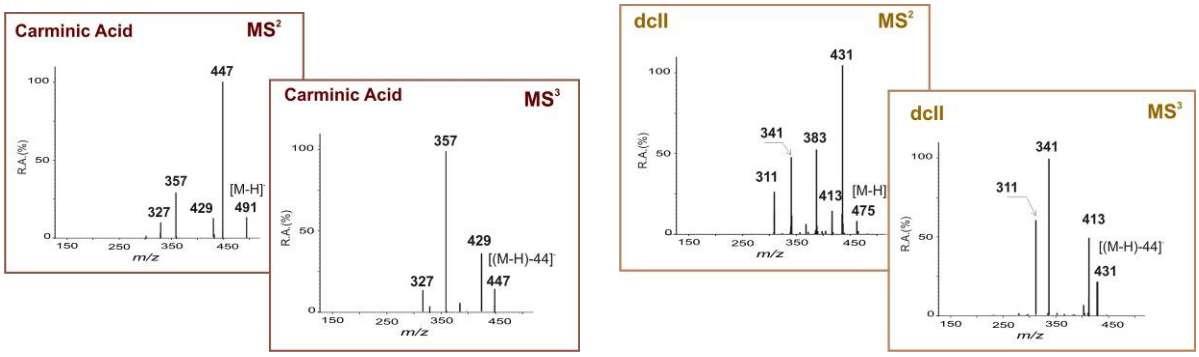

Figure 3 – MSN spectrums from carminic acid and dcII obtained in negative mode.

2. Samples preparation

2.1 Insect dye extracts

All the cochineal insect species were extracted in 100% H_2O, simulating old dyeing recipes [4], in order to avoid the higher formation of fk+ka, produced by strong acidic conditions, and allowing the comparison with soft extraction methods applied in textiles cochineal recovery. The dilapidation of *Porphyrophora* specimens prior to dyes extraction [14] did not improve significantly the amount of dyestuff extracted and the *Porphyrophora* specimens were extracted with the same procedure as *Dactylopius* species.

Better HPLC separations in insect extracts were achieved with H_2O: CH_4O: H_2O/ $HClO_4$, (50:20:30, v/v/v) in the final dye extract than using only H_2O, or H_2O: CH_4O (50:50) [15]. Also, the chromatographic separation of cochineal extracts from dyed textiles improves significantly

when the final extract is reconstituted with H_2O: CH_4O: H_2O/ $HClO_4$ (50:20:30, v/v/v). When the reconstituted extract, from dyed textiles samples, was not acidified, the chromatograms obtained displayed very poor resolution, comparable to chromatograms reported in literature [17,29] and the dcII peak was co-eluted with carminic acid peak, figure 4a. Better results were obtained by acidifying the extracts, and so the dcII and the carminic acid eluted at distinct retention times, figures 4b and 4c. Therefore, the elution profiles in figure 3 shows that much more information can be obtained with the slight acidification of the reconstituted extract, being this result applied in all the insect and textile specimens' preparations.

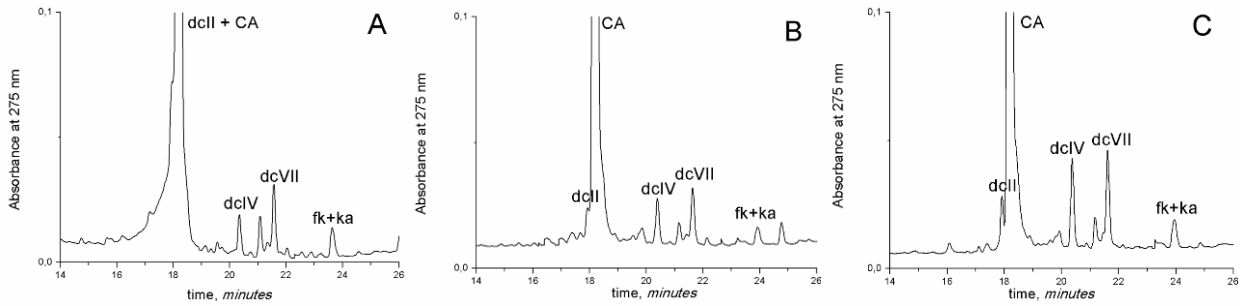

Figure 4 – HPLC/DAD chromatograms (monitored at 275 nm) of dyed cochineal textiles with different solvent proportions in the final dye extract: **A –** Dye-sample from textile fibre extracted with TFA method, not acidified, and comparable to chromatograms in [17,29]; **B -** Dye-sample from textile fibre extracted with TFA method, and reconstituted with H_2O: CH_3OH: $H_2O/HClO_4$ (50:20:30, v/v/v); and **C -** Dye-sample from textile fibre extracted with oxalic acid method, and reconstituted with H_2O: CH_3OH: $H_2O/HClO_4$ (50:20:30, v/v/v). All the tests were performed in three replicates.

2.2 Dyed-fibre extraction methods

As reported in the literature, the maximum amount of carminic acid extracted from American cochineal dyed fibres was obtained with oxalic acid [28]. In the performed tests, oxalic acid extracted almost the same amount of carminic acid as TFA solution in agreement with [28], figure 5a. The oxalic acid method showed better chromatograms' resolution than extraction with other methods, figure 4c, and, consequently, it was selected for all the fibres' extractions.

PCA scores showed a high accordance between the samples of dyeing solution (open symbols), red-dyed textile references (solid black symbols) and *D. coccus* specimens (solid red symbols), figure 5b. Only the textile references extracted with TFA method (solid blue symbols) were deviated significantly from the *Dactylopius* cluster to the *Porphyrophora* insect' extracts. Thus, it was confirmed that the extraction with oxalic acid method and reconstruction of the dye-extract with H_2O: CH_3OH: $H_2O/HClO_4$ (50:20:30, v/v/v), did not promote significant alterations in the cochineal dye elution profile. Therefore, cochineal extracts from dyed textiles could be compared directly with cochineal insect extracts.

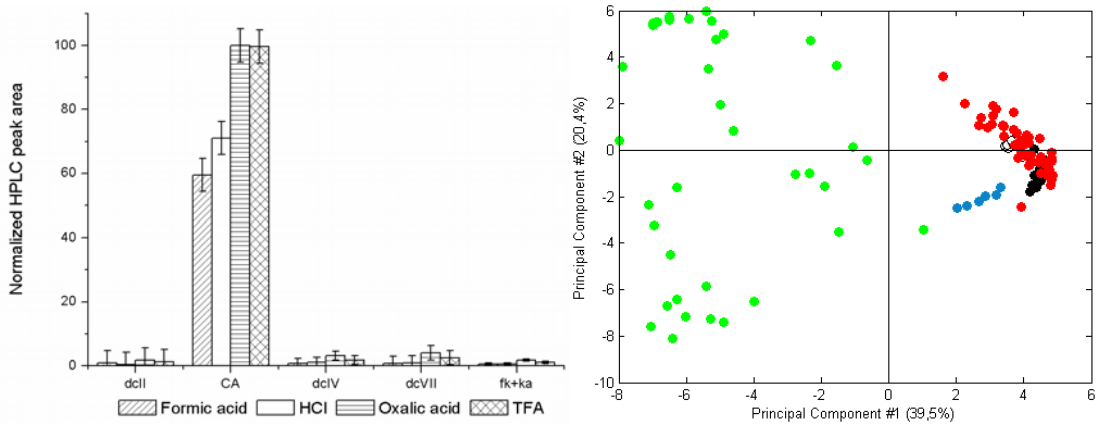

Figure 5 – Results obtained with the extraction methods: **A –** HPLC peak areas for cochineal chromophores extracted from dyed fibres with formic acid, HCL, oxalic acid and TFA method. **B -** PCA scores obtained from mean centered cochineal chromatograms (17,5 – 18,8 min.), acquired at 275nm: cochineal extracts from *D. coccus* dyed textiles (solid black symbols) are comparable with *D. coccus* insect' extracts (solid red symbols), as well as with the dyeing solution (open symbols). The TFA method (solid blue symbols) deviates the extracts from dyed fibres from the *D. coccus* cluster to the *Porphyrophora* insect' extracts (solid green symbols).

3. Insect samples

3.1 Reference cochineal specimens identified by entomologists

As reported in literature [12,14] *D. coccus* is distinguished from *Porphyrophora* species, due to the higher amount of dcII compound and minor amount of fk+ka in *D. coccus* than in *Porphyrophora* species, figures 1 and 2, and table 3. However, it is difficult to distinguish *P. hamelli* from *P. polonica* due to the similar content of fk+ka. Also, it should be noticed that the standard deviations presented by table 3, either for literature or for the results obtained in this work, are very high, and careful attention should be given when using the marker's relative percentage for cochineal species recognition. For instance, in *P. polonica*, the relative percentage of fk+ka has an error of circa 90%. This high value can be explained by the variations in the insect composition according to its development stage [12,14].

Table 3 – Markers' relative percentages from cochineal insects aqueous extracts calculated at 275nm.

	Literature[1]	**Obtained Results**[2]
D. coccus	2,3±1,0% dcII, 95,2±1,3% CA, 1,4±0,4 % dcIV, 0,4±0,2% dcVII, 0,5±0,3% fk + ka (8 specimens analyzed)	2,4±0,9% dcII, 95,3±1,3% CA, 1,0±0,4% dcIV, 1,0±0,4% dcVII, 0,2±0,1% fk + ka (39 specimens analyzed)
P. polonica	4,5±0,1% ppI, 87,8±5,5% CA, 1,2±0,4% dcIV, 0,8±0,1% dcVII, 5,7±5,8% fk+ka (6 specimens analyzed)	2,2±1,7% ppI (?),~0% dcII, 92,4±3,6% CA, 0,6±0,4% dcIV, 1,4±0,8% dcVII, 3,5±3,1% fk+ka (20 specimens analyzed)
P. hamelli	2,1±1,0% ppI, 0,4±0,1% dcII,92,6±0,6% CA, 3,1±0,4% dcIV, 0,9±0,2% dcVII, 1,2±0,5% fk + ka (4 specimens analyzed)	1,4±1,3% ppI, 0,5±0,3% dcII,96,3±1,3% CA, 0,6±0,2% dcIV,0,6±0,1% dcVII, 1,4±0,8% fk + ka (9 specimens analyzed)

[1] The average values and standard deviation of the cochineal insects relative peak areas were calculated from data presented in [13,14].

[2] The relative peak areas were calculated with the chromatographic program ChromQuest 4.1 at the maximum wavelength absorption of 275 nm (Appendix 4).

The differentiation of the species can be more reliable if obtained with PCA, considering the chromatographic data between 15 and 25 min., and not only the minor markers relative peak areas. In figure 6a, *Dactylopius* family is grouped in a distinctive cluster, from *Porphyrophora* family. Therefore, PCA is able to distinguish between both cochineal families. With PCA it is also possible to distinguish *P. hamelii* and *P. polonica*, inside the *Porphyrophora* family, figure 6b. *P. polonica* samples gather in two distinct regions, due to the differences shown by the stage development of the insect, already mentioned before. Thus, samples joined in the upper left quadrant of figure 6a and in the upper right quadrant of figure 6b correspond to females with eggs, which chromatogram have shown a higher content of fk+ka [14]. Also, with PCA analysis it is possible to distinguish *D. coccus* (solid red symbols) and the wild *Dactylopius* species, figure 6c. The wild species groups showed some similarity between each other, and with the PLS model it was possible to distinguish the species with circa 80% success.

Due to the few number of *D. tomentosus* and *P. medicaginis* specimens analyzed, PCA analyzes could not be accomplished. However, its representative chromatograms can be observed in Appendix 7, along with the other representative chromatograms for each identified species.

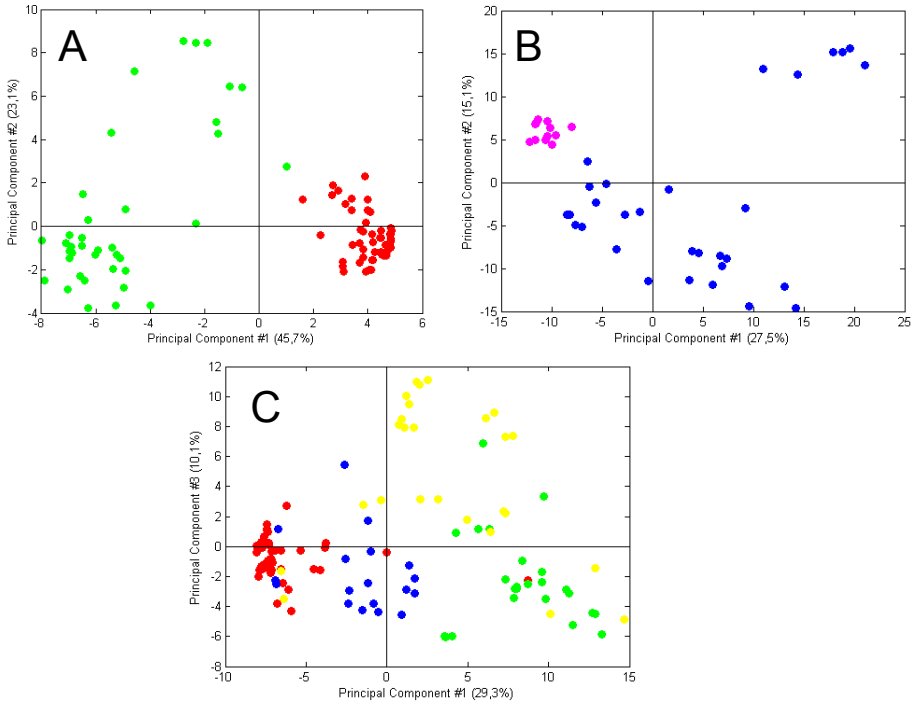

Figure 6 – PCA scores obtained from mean centered chromatograms acquired at 275 nm, where it is possible to distinguish *Dactylopius* and *Porphyrophora* and the species inside the both families: **A** - *Dactylopius* (solid red symbols) and *Porphyrophora* (solid green symbols) families, obtained for 17.5-18.8 min.; **B –** *P. polonica* (solid blue symbols) and *P. hamelii* (solid pink symbols) species, acquired in the region between 15.0-18.8 and 19.7-25.0 min; and **C –** *D. coccus* (solid red symbols) and the wild *Dactylopius* species, *D. confusus* (solid green symbols), *D. ceylonicus* (solid blue symbols) and *D. opuntiae* (solid yellow symbols), obtained in 19,6-21,3 and 23,3-25 min.

3.2 Unidentified cochineal insect species

From the 94 specimens of unidentified insect species (historical collection from Royal Botanic Garden, at Kew, and insects supplied by Cardon, Balfour and Tiano, table 4 and Appendix 5), the presence of carminic acid and several minor cochineal markers were detected in 87 specimens. In the remaining seven specimens from Kew (circa 4% of the collection), yellow unidentified compounds were detected in six dye-samples (55338 and 55340, Appendix 8), and one insect sample produced a colorless extraction (73057), excluding these insects as cochineal, table 4.

Table 4 – Unidentified specimens' labelled as cochineal with respective species attribution, identified by HPLC/DAD, marker's relative percentages and PCA analysis.

Classification[1]	Collection Date	Source, donor	Observations	Species attribution (markers' relative percentages) [2]
Royal Botanic Garden, at Kew				
54387	1856	Índia, Madras, from James A. Mann	Dry, brown, medium dimensions, 10,4±3,0 mg	***D. coccus*** (3,3±0,5% dcII, 94,6±0,6% CA, 1,0±0,1% dcIV, 0,9±0,1% dcVII, 0,3±0,1% fk + ka)
54388	1918	Peru, Callas, from London Drug Market	Dry, black, medium dimensions, 14,2±2,8 mg	***D. coccus*** (3,4±0,9% dcII, 94,7±1,2% CA, 0,8±0,1% dcIV, 0,8±0,2% dcVII, 0,3±0,1% fk + ka)
54389	1899	Ecuador, Chimborazo Province, Guana, from Edward Whimper	Dark dry cake mixture of cochineal and other ingredients, 1,0±0,2 mg	***Dactylopius sp.*** (1,2±0,3% dcII, 96,7±0,2% CA, 0,6±0,1% dcIV, 0,2±0,1% dcVII, 1,3±0,6% fk + ka)
54390	Before 1879	Indonesia, Java, Buitenzorg, Tyikoppo, donated by India Museum	Dry, dark brown, medium dimensions, 14±2 mg	***D. coccus*** (2,7±0,3% dcII, 95,4±0,8% CA, 0,9±0,3% dcIV, 0,8±0,1% dcVII, 0,3±0,1% fk + ka)
54391	Before 1879	Indonesia, Java, Buitenzorg, Bandok, donated by India Museum	Dry, dark brown, medium dimensions, 16,2±1,9 mg	***D. coccus*** (2,3±0,3% dcII, 96,0±0,5% CA, 0,8±0,3% dcIV, 0,6±0,0% dcVII, 0,3±0,1% fk + ka)
54392	Late 19th century	[4], A.S. Hill & Son, London	Dry, dark brown, medium dimensions, 21,7±5,2 mg	***D. coccus*** (2,6±0,2% dcII, 95,1±0,5% CA, 0,8±0,1% dcIV, 1,2±0,5% dcVII, 0,4±0,1% fk + ka)
54393	Before 1879	Índia, Andhra Pradesh, Scinde, Hyderabad, donated by India Museum	Dry, shiny red, medium dimensions, 2,8±1,1 mg	***D. coccus*** (2,5±0,9% dcII, 95,6±1,8% CA, 0,8±0,3% dcIV, 0,9±0,4% dcVII, 0,2±0,3% fk + ka)
54394	1867	India, Calcutta, from International exhibition, Paris	Dry, dark brown, medium dimensions, 11±2,8 mg	***D. coccus*** (2,6±0,2% dcII, 95,6±0,7% CA, 0,6±0,4% dcIV, 0,8±0,3% dcVII, 0,4±0,1% fk + ka)
54402	Late 19th century	Honduras and Vera Cruz	Dry, light brown, little dimensions, 1,6±0,4 mg	***D. coccus*** (2,7±0,5% dcII, 94,4±1,2% CA, 1,4±0,2% dcIV, 1,3±0,4% dcVII, 0,3±0,2% fk + ka)
54403	Before 1879	India, Punjab, donated by India Museum	Dry, dark brown, medium dimensions, 11±4,7 mg	***D. coccus*** (2,9±0,7% dcII, 95,1±0,4% CA, 0,9±0,3% dcIV, 0,8±0,2% dcVII, 0,3±0,1% fk + ka)
54404	Probably 1851	Mexico, Oaxaca, from J. Sadler, probably International exhibition, London	Dry, dark brown, medium dimensions, 10,8±1,7 mg	***D. coccus*** (4,0±2,1% dcII, 93,9±1,7% CA, 0,9±0,1% dcIV, 0,9±0,3% dcVII, 0,3±0,2% fk + ka)
54410	1977	Madeira, from Jane Stubbs	Dry, greyish brown, medium dimensions, 7,8±1,4 mg	***D. coccus*** (3,0±0,8% dcII, 95,1±1,2% CA, 0,8±0,2% dcIV, 0,9±0,2% dcVII, 0,3±0,1% fk + ka)
55338	1855	Australia (NSW), from International exhibition, Paris	Dried naturally, rusted red colour, little dimensions, 2,1±0,3 mg	Not cochineal insect, unidentified yellow compounds (Main peak: rt= 19,86 min, λ_{max}= 421 nm)
55340	1862	Australia (Victoria), from International exhibition, London	Dried naturally, rusted yellow colour, little dimensions, 1,8±0,7 mg	Similar to 55338
58236.1	Late 19th century	[4], Ripley, Roberts & Co. 3. Mincing lane	Dry, dark shiny red, medium dimensions, 20,7±1,7 mg	***D. coccus*** (2,5±0,4% dcII, 95,9±0,4% CA, 0,6±0,1% dcIV, 0,7±0,2% dcVII, 0,2±0,1% fk + ka)
58236.2	Late 19th century	[4], Ripley, Roberts & Co. 3. Mincing lane	Dry, dark shiny red, medium dimensions, 15,9±2,0 mg	***D. coccus*** (3,0±0,9% dcII, 94,5±0,3% CA, 0,7±0,1% dcIV, 1,2±0,8% dcVII, 0,6±0,3% fk + ka)
58236.3	Late 19th century	[4], Beazley & Co. Dunster House, Mincing Lane	Dry, dark shiny red, medium dimensions,	***D. coccus*** (2,6±0,2% dcII, 95,8±0,1% CA, 0,6±0,0% dcIV,

			18,7±2,5 mg	0,9±0,2% dcVII, 0,3±0,1% fk + ka)
58236.4	Late 19th century	[4], Beazley & Co. Dunster House, Mincing Lane	Dry, black, medium dimensions, 16,3±6,9 mg	***D. coccus*** (2,5±1,5% dcII, 95,2±2,0% CA, 0,1±0,2% dcIV, 0,9±0,2% dcVII, 0,5±0,4% fk + ka)
58236.5	Late 19th century	[4], Ripley, Roberts & Co. 3. Mincing lane	Dry, salmon light colour, medium dimensions, 19,5±4,7 mg	***D. coccus*** (2,9±1,2% dcII, 95,4±1,0% CA, 0,7±0,3% dcIV, 0,8±0,2% dcVII, 0,3±0,1% fk + ka)
58236.6	Late 19th century	[4], Ripley, Roberts & Co. 3. Mincing lane	Dry, black, little dimensions, 0,7±0,3 mg	***D. coccus*** (2,0±1,5% dcII, 95,5±1,4% CA, 1,1±0,1% dcIV, 1,0±0,2% dcVII, 0,3±0,0% fk + ka)
58236.7	Late 19th century	[4], Ripley, Roberts & Co. 3. Mincing lane	Dry, dark brown, medium dimensions, 10,6±3,5 mg	***D. coccus*** (2,5±0,4% dcII, 95,9±0,8% CA, 0,6±0,3% dcIV, 0,7±0,3% dcVII, 0,2±0,0% fk + ka)
58236.8	Late 19th century	[4], Beazley & Co. Dunster House, Mincing Lane	Dry, dark red, medium dimensions, 5,0±1,0 mg	***D. coccus*** (2,3±0,5% dcII, 96,1±0,9% CA, 0,7±0,3% dcIV, 0,7±0,1% dcVII, 0,2±0,0% fk + ka)
73057	1800-1857	[4], Royal Pharmaceutical Society of Great Britain (Museum)	Naturally dried, orange colour, big dimensions, 7,20 mg	Not cochineal insect. No coloured compounds.
73237	1851	Mexico, Oaxaca, from J. Sadler, probably International exhibition, London	Dry, black, medium dimensions, 11,6±2,4 mg	***D. coccus*** (2,3±0,8 dcII, 96,1±1,0% CA, 0,7±%0,3 dcIV, 0,6±0,1% dcVII, 0,2±0,0% fk + ka)
Dominique Cardon unidentified specimens				
Armenian cochineal	[3]	[4], Dominique Cardon	Naturally dried, dark brown, medium dimensions, 10,8±2,4 mg	***D. coccus*** (3,2±0,5 dcII, 94,5±0,8% CA, 0,4±%0,1 dcIV, 0,4±0,1% dcVII, 0,6±0,1% fk + ka)
American cochineal	[3]	[4], Dominique Cardon	Naturally dried, white dusty, medium dimensions, 12,1±4,1 mg	***Porphyrophora sp.*** (1,5±0,2 ppI, 0,5±0,2 dcII(?), 95,5±0,3% CA, 0,6±%0,2 dcIV, 0,8±0,0% dcVII, 0,7±0,1% fk + ka)
"Kermes noir"	16/10/2002	Market in Athens, Dominique Cardon	Dry, dark brown, medium dimensions, 10,4±2,8 mg	***D. coccus*** (1,4±1,1 dcII, 96,4±1,0% CA, 0,5±%0,2 dcIV, 0,6±0,2% dcVII, 1,2±1,0% fk + ka)
D. coccus	[3]	[4], Dominique Cardon	Dry, dark brown, medium dimensions, 10,9±6,0 mg	***D. coccus*** (2,7±1,3 dcII, 96,0±0,9% CA, 0,5±%0,1 dcIV, 0,6±0,3% dcVII, 0,3±0,1% fk + ka)
Piero Tiano unidentified specimens				
Tiano-Fi	[3]	[4], Piero Tiano	Dry, dark brown, medium dimensions, 10,7±3,6 mg	***Dactylopius sp.*** (4,5±1,8 dcII, 93,9±2,0% CA, 0,6±%0,1 dcIV, 0,7±0,1% dcVII, 0,4±0,2% fk + ka)
Tiano-Brx	[3]	[4], Piero Tiano	Dry, dark red, medium dimensions, 12,9±5,8 mg	***Dactylopius sp.*** (2,5±1,0 dcII, 96,6±0,6% CA, 0,7±%0,3 dcIV, 0,6±0,2% dcVII, 0,6±0,1% fk + ka)
Jenny Balfour unidentified specimens				
Cochineal I	[3]	[4], Dyes in History and Archaeology, France, 2004, from Jenny Balfour	Dried naturally, pink colour, hairy, medium dimensions, 11,6±9,4 mg	***P. hamelii*** (2,8±0,5 ppI, 0,8±0,2 dcII, 95,4±1,0% CA, 0,5±%1,0 dcIV, 0,5±0,1% dcVII, 1,1±0,3% fk + ka)
Cochineal II	[3]	[4], Dyes in History and Archaeology, France, 2004, from Jenny Balfour	Dried naturally, red colour, scarce hairy, medium dimensions, 19,0±1,1 mg	***P. hamelii*** (2,4±0,5 ppI, 0,4±0,1 dcII, 95,4±0,8% CA, 0,3±%0,1 dcIV, 0,4±0,0% dcVII, 1,1±0,4% fk + ka)

[1] Classification of the insects, given by the donor.

[2] The relative peak areas were calculated with the chromatographic program ChromQuest 4.1 at the maximum wavelength absorption of 275 nm.

[3] Donation date unrecorded.

[4] Source unidentified.

The chromatographic PCA data obtained for the 63 cochineal specimens from Kew, figure 6a, held consistent results to identify the majority of the insects (solid blue triangles) as *D. coccus* species. These results seem to be conclusive with the specimens source and macroscopic appearance, namely their medium dimensions, rounded shape and evidence of treatment (wrinkled and without hair, legs and claws), Appendix 5 [4]. Also, they show consistency with the homogeneous relative percentages among the insects' markers, table 4.

Although, there seems to be an exception with the insects of 54359 samples (solid black triangles), which correspond to the cake compounded by a mixture of cochineal (probably from wild origin) and other unknown components, Appendix 5. Marker's relative percentages (table 4) cannot set a clear distinction between this sample and the others.

The 58236 samples belong to a box which includes eight varieties of cochineal from the late 19th and early 20th centuries, and that were used for dyeing purposes, Appendix 5. Their appearance points to different varieties of cochineal, and, as PCA analysis have shown that they are possibly *D. coccus* species, it may be likely that the same specie was subject to different methods of killing and preparation, Appendix 1 [1,4].

Relatively to the results obtained for the other unknown samples, PCA analysis indicated that Balfour's specimens are likely *Porphyrophora* species; as well the "American cochineal" samples of Cardon, which were thought to be wrongly labelled in the past, due to their physical appearance, figure 7b and Appendix 5. This situation occurred also with the "Armenian cochineal" samples from Cardon, which had a dubious appearance and which were pointed as *D. coccus* species by PCA analysis. Also from Cardon, the samples labelled as "Kermes noir" and "*D. coccus*" were recognized as *D. coccus* species, according with their resembled appearance. At last, Tiano's samples seem to deviate slightly from the *D. coccus* cluster to the *Porphyrophora* samples' concentration. These results seem to be in accordance with the respective relative percentages in table 4, which show homogeneity if compared with the relative percentages from *Porphyrophora* and *D. coccus* insects, Appendix 4. Yet, by comparison with literature [14], it was possible to verify the UV spectra of the insects' respective minor markers.

Porphyrophora samples from Balfour and Cardon were projected onto a model calibrated with *P. polonica* and *P. hamelii* species, figure 7c. Balfour samples are clearly gathered on the cluster of *P. hamelii,* though Cardon samples deviate to concentration zone of *P. polonica.* However, due to the differences seen on results of *P. polonica* specimens, more studies and analysis on this specie would be needed to ensure a better identification of these unidentified *Porphyrophora* specimens from Cardon.

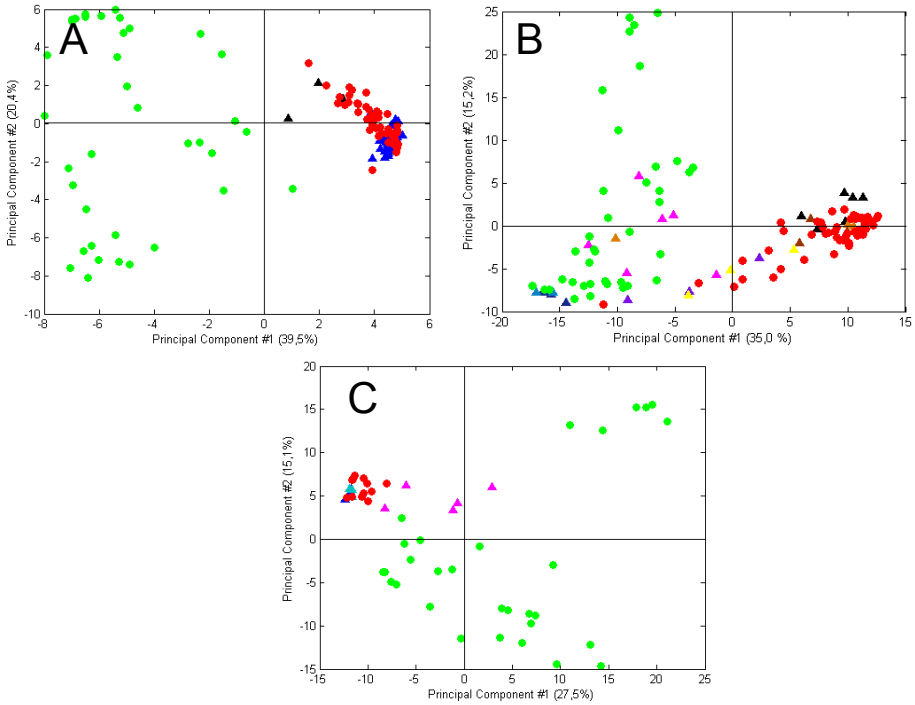

Figure 7 – PCA scores for the 87 unknown cochineal specimens projected onto reference cochineal samples *D. coccus* (solid red circles) and *Porphyrophora* (solid green circles): **A –** Royal Botanic Garden (solid triangle symbols) obtained for 17,5-18,8 min.; **B –** Cardon samples (Armenian cochineal – solid black triangles; American cochineal – solid pink triangles; "Kermes noir" – solid brown triangles; and *D. coccus* – solid orange triangles), Tiano samples (Fi – solid yellow triangles and Brx – solid purple triangles) and Balfour samples (I - solid dark blue triangles; II – solid light blue triangles), acquired for 15,0-18,80 and 19,7-25,0 min.; and **C -** unknown cochineal specimens of Cardon (solid pink triangles) and Balfour (I - solid dark blue triangles; II – solid light blue triangles) samples, projected on *P. hamelii* (solid red symbols) and *P. polonica* (solid green symbols), obtained for 15,0-18,80 min. and 19,7-25,0.

4. Historical textile dye identification

The chromatograms obtained for the historical red-dyed textiles are homogeneous (Appendix 6) and it is possible to observe the presence of carminic acid and minor markers. Nevertheless, the elution profile of the historical red-dyed textiles is slightly different from the insect specimens in the HPLC-DAD library, figure 8. For instance, in the historical red-dyed textile samples it was difficult to identify with certainty the presence of dcII due to its low content and the presence of other small peaks eluted near dcII. When possible, its presence was confirmed with MS analysis. Furthermore, new peaks that were not present in the insect specimens were identified in the historical red-dyed textiles. For instance, it was detected a peak around 19,46 min., with a λ_{max}=370 nm, Appendix 8. Analyses using HPLC/DAD/MS^n show that this unknown compound corresponds to ellagic acid, as the negative ion ES-MS spectrum of this compound exhibits an ion at *m/z* 301 [38]. The presence of ellagic acid (EA) in historical textiles dyed with cochineal was already reported in literature [16,17,25]. This compound indicates the presence of tannins, which were used in the past for the dyeing process, providing a bluish-black colour in the presence of iron, and making the tissues heavier

[16,17]. It can probably be related to the use of plant species belonging to dicotyledonous families, or as a result of the photo- or auto-oxidation of gallotanins, in alkaline environment [4].

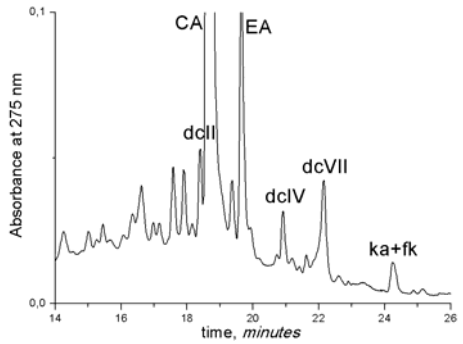

Figure 8 – Representative chromatogram of the historical red-dyed textile sample CGM 1449 velvet, identified as *D. coccus*, monitored at 275 nm.

Table 5 provides the origins and dates attributed to the textiles, along with the relative average percentage calculated for their chromatograms. Given the significant relative percentage of dcII present in the samples acquired from CGM 1388A and CGM 1449, and dcX presence in the other samples[4], it was thought that they could belong to *D. coccus*, or *P. hamelii*. However, this data must be considered with care as the standard deviation is very high in these samples.

Table 5 – Historical textile samples, with relative percentages, obtained by HPLC/DAD analysis.

Classification[1]	Date	Provenance	Expected specie[2]	Markers' relative percentages[3]
Calouste Gulbenkian Museum				
CGM 1388A Velvet	16th/17th century	Turkey, Bursa	*D. coccus*	***P. Polonica (?)*** (2,8±3,4% dcII, 92,9±7,0% CA, 1,1±0,2% dcIV, 2,2±2,8% dcVII, 1,2±1,0% fk + ka)
CGM 245 Velvet	16th century	Italy, Genoa	*D. coccus*	***P. Polonica (?)*** (1,8±0,0% dcX, 94,4±0,0% CA, 1,3±0,0% dcIV, 1,9±0,0% dcVII, 0,7±0,0% fk + ka)
CGM 1446 Velvet	17th century	Iran, Yazd (?)	*Porphyrophora* sp.	***P. Polonica (?)*** (0,4±0,0% dcX, 96,3±0,3% CA, 1,8±0,4% dcIV, 1,2±0,1% dcVII, 0,31±0,02% fk + ka)
CGM 1513 Velvet	17th century	Iran	*Porphyrophora* sp.	***P. Polonica (?)*** (0,6±0,5% dcX, 95,7±1,3% CA, 1,6±0,4% dcIV, 1,7±0,5% dcVII, 0,4±0,0% fk + ka)
CGM 1449 Velvet	17th century	India	*D. coccus*	***D. coccus*** (1,7±0,2% dcII, 94,4±1,0% CA, 1,3±0,1% dcIV, 1,7±0,8% dcVII, 0,8±0,0% fk + ka)
CGM 1422 Velvet	17th century	India	*Porphyrophora* sp.	***P. Polonica (?)*** (0,4±0,0% dcX, 95,2±1,1% CA, 2,4±1,5% dcIV, 1,6±0,7% dcVII, 0,5±0,1% fk + ka)
CGM T100 Carpet	16th century	Iran	*P. hamelii*	***P. Polonica (?)*** (1,1±0,5% dcX, 96,0±0,7% CA, 1,1±0,2% dcIV, 1,4±0,3% dcVII, 0,4±0,1% fk + ka)
Museu Nacional de Arte Antiga				
MNAA 1616Tec Chasuble	15th century	Italy, Florence (?)	*P. polonica*	***P. Polonica or mixture (?)*** (0,8±0,1% dcX, 90,0±2,7% CA, 1,3±0,2% dcIV, 1,8±0,3% dcVII, 6,2±2,1% fk + ka)

[4] When the peak eluting at 17,86 min., corresponding to dcII compound was confirmed with MS analysis, the peak was labelled as dcII. When its molecular structure was not confirmed with MS analysis, this peak could be another compound, and so it was labelled as dcX. For instance, it was possible to verify that dcII was totally absent in CGM 1422 velvet with MS^n analysis and another compound, with $[M-H]^-$ *m/z*=505, was initially misidentified as dcII in this textile.

[1] Inventory numbers of the historical textiles, given by the institutions.

[2] Expected species by comparison with results given by previous publications.

[3] The relative peak areas were calculated with the chromatographic program ChromQuest 4.1 at the maximum wavelength absorption of 275 nm.

In order to perform PCA analysis with direct comparison with the insect specimens from the cochineal HPLC/DAD library, it was necessary to restrict the PCA analysis to elution time regions where chromatograms are consistent, namely between 17,5-18,8 min, figure 9a, and between 15,0-18,8 and 19,7-24,7 min, figure 9b.

With PCA analysis, the majority of the historical red-dyed textiles samples coincide with the distribution of *Porphyrophora* specimens, figure 9a. However, samples taken from CGM 1449 appear in close proximity with *D. coccus* cluster. Analysis by HPLC/DAD/MS^n confirmed the presence of dcII, and a vestigial presence of ka, thus these samples are probably *D. coccus*. As this is a 17th-century Indian velvet, this is an important result as it confirms the adoption of American cochineal shortly after the establishment of the English textile factory in Surat in 1612, and well before it came to dominate Indian dyeing practices in the 19th-century [10].

The remaining historical samples cluster occupied the region of the reference samples of *Porphyrophora* species, figure 9a. A better distinction of these samples is provided by PCA analysis in another chromatographic region, where these samples are projected over *P. hamelii* and *P. polonica* reference samples, figure 9b. In this figure, it is possible to observe a clear distinction between MNAA 1616Tec and the other historical samples. The former occurs in the region of *P. polonica* reference samples, which are richer in fk+ka, table 5. Hence, it is likely that these results point to the presence of *P. polonica* specie, as previously observed (figure 2b), or to a mixture of *Porphyrophora* specie and kermes, which has two main component peaks (ka+fk) [4]. These results also appear to be in accordance with the attributed place and date of production of this 15th-century Italian velvet. On the one hand, *D. coccus* insects were not traded to Europe before the 16th century, and its use in Italy was only sanctioned in the middle of the century [10], on the other hand, Italy was a major centre for the international trade in dyestuffs and, for this reason, a wide variety of *Porphyrophora* species would have been available to Italian dyers [7].

As for the remaining historical samples, they gather in a homogeneous cluster as shown in figure 9b, and can be associated with the same *Porphyrophora* species. Although figure 9b shows these samples in the region of distribution of *P. polonica* references, great care should be taken with this identification, as the results for *P. polonica* insects are highly influenced by the insect's stage of development [14]. The correct identification of this insect species is only possible with a larger number of analyses and more in-depth study of *Porphyrophora* specimens and their interaction with textiles during the dyeing process. Today, there are 47 known species of *Porphyrophora* [5] and it is important to acknowledge that insects gathered in the past could reflect species other than *P. polonica* or *P. hamelii*, especially due to the strong resemblance between different species co-habiting the same geographical space [4]. This problem needs to be explored with further analyses as the historical sources indicate that either

of the two species could have been present in Ottoman markets at this time, as American cochineal had begun arriving in Venice by 1543, and thus could easily have been exported eastwards shortly after this date (Appendix 1) [7,9].

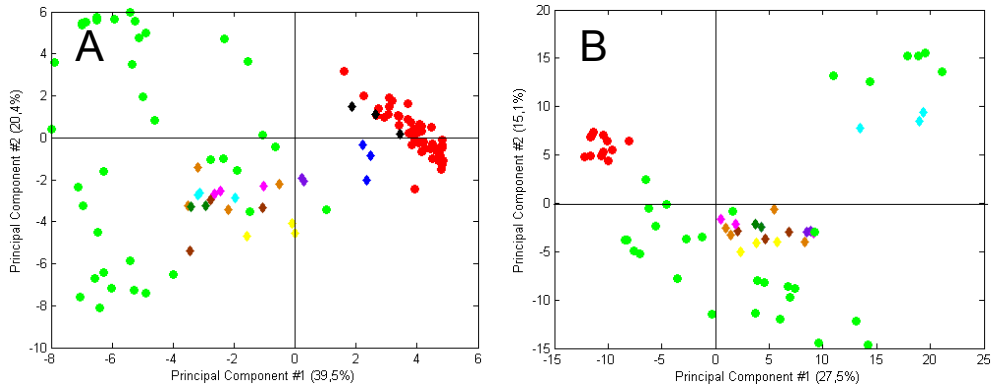

Figure 9 – PCA scores, for the historical red-dyed textiles (solid lozenge symbols) - CGM 1388A velvet (solid yellow lozenges), CGM 245 velvet (solid purple lozenges), CGM 1446 velvet (solid dark green lozenges), CGM 1513 velvet (solid orange lozenges), CGM T100 carpet (solid pink lozenges), CGM 1449 velvet (solid dark blue lozenges), CGM 1422 velvet (solid brown lozenges), MNAA 1616Tec chasuble (solid light blue lozenges) and CGM T100 and MNAA 1616Tec samples extracted by HCl method (solid dark lozenges): **A -** projected onto reference cochineal samples *Dactylopius coccus* (solid red symbols) and *Porphyrophora* (solid green symbols) obtained at 17,5 – 18,8 min.; and **B -** projected onto *P. hamelii* (solid red symbols) and *P. polonica* (solid green symbols) samples, obtained for 15-18,8 and 19,7-24,7 min..

Additionally, two other textiles samples were analysed, one belonging to a velvet (CGM 1513), which had an orange colour, and another belonging to a silk carpet (CGM T100), with a yellow colour. The former was identified as a species of madder, owing to the presence of alizarin, although the presence of purpurin is almost residual [25,39]. Other yellow compounds were also detected and hence it is probably a mixture of madder and another yellow dyestuff, Appendix 8. Analyses of the latter fibre indicate the presence of yellow compounds, namely rutin-based compounds, with a very similar elution profile to a yellow silk fibre sample from a related “Small Silk Kashan” rug in the Museu Nacional de Machado de Castro (MNMC T744) [39], Appendix 8. These yellow compounds do not match the most common sources of yellow, such as *Reseda luteola*, and, indeed, point to the use of a specific dyestuff by the workshop that produced this group of Persian carpets. Future research on the precise species of this yellow dye may aid in identifying the geographical location of this workshop in Iran.

Analyses of historical textile samples extracted with oxalic acid yield good results, and, moreover, PCA analysis demonstrates that extraction with HCl [14] causes a serious deviation of these samples from the *Porphyrophora* concentration region, in the direction of the *D. coccus* cluster, figure 9a. However, results for textile reference samples dyed with *D. coccus* show that extraction with HCl is not responsible for this deviation, figure 5b, and hence, this occurrence is probably due to the hydrolysis of the precursors present in the *Porphyrophora* species.

All the results obtained in this study and the data available in the literature [13,14] were represented graphically to distinguish the *D. coccus* samples from *P. hamelii* and *P. Polonica,*

based on the relative percentages of dcII and fk+ka, as shown in previous publications [14], figure 10a. According to the resulting diagram, the samples from the Persian silk carpet (T100 - 1) and the Italian and Turkish velvets (CGM 245 - 2, CGM 1388A - 3), attributed to a *Dactylopius coccus* insect, might not actually have the dcII marker but probably the unknown compound dcX, due to the considerable amount of "dcII" compound; hence, in this case, it could reflect the presence of a *Porphyrophora* species. Although, the presence of dcII marker was confirmed for the Turkish velvet (GCM 1388A) by MS^n, it was not sufficient to confirm this sample as a *D. coccus* species, owing to the chromatogram similarity with *Porphyrophora* species as shown by PCA analysis. For rigorous results, all samples of historical textiles and other existent *Porphyrophora* species should be analyzed simultaneously by PCA and MS^n. Although the red dye in the Indian velvet (CGM 1449 - 4) is considered to be *D. coccus,* the samples from the other velvets (CGM 1513 – 3, CGM 1422 – 5 and CGM 1446 - 6) did not present dcII marker, and this was corroborated by MS^n analysis of the CGM 1422 sample. Consequently, it is possible that these samples might be *P. polonica*. Finally, the Italian chasuble (MNAA 1616Tec - 8) is confirmed as *P. polonica* species or a mixture of this species with kermes [15] which is also in accordance with the PCA models described above. Special care should be taken when using this quantification system of graphic representation with other cochineal species. For example, it was observed that wild *Dactylopius* show some inconsistent results in this system. In addition, PCA models of all the minor markers quantification, using the same average relative percentages as the prior type of graphic representation, show the same trend towards non-distinguishable clusters, owing to the high deviation standards resulting from this method, figures 10b [12,13], Tables 3, 4 and 5, and Appendix 4.

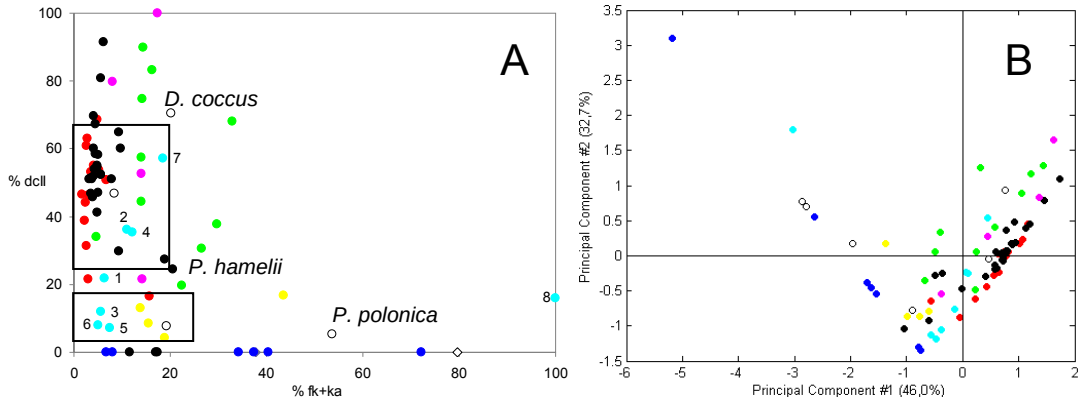

Figure 10 – Two different graphic systems made with the relative percentages of dcII and fk+ka, calculated at 275 nm, for all the analysis accomplished in this study and found in the literature [14]: *D. coccus* (solid red symbols), *Dactylopius* wild species (solid green symbols), *P. polonica* (solid blue symbols), P. hamelii (solid yellow symbols), Kew Garden samples (solid black symbols), extraction methods (solid pink symbols), historical samples (solid cyan symbols) and literature data (open symbols): **A –** quantification system to distinguish between *D. coccus*, *P. hamelii* and *P. Polonica* [14]; and **B -** PCA scores with the same data.

Conclusion

This study aimed to highlight the importance of cochineal species characterization for understanding the application and dissemination of this red dyestuff in textile production in Europe and Asia from 15th to 17th centuries. This interdisciplinary approach offers the opportunity to look more widely at the history of dye technology and trade routes, and, consequently to characterize textiles in terms of their provenance and date. However, this study also recognizes, for the first time, the limitations of prior cochineal species identification and emphasizes the necessity of an approach which combines entomological studies with chemical analysis of dyes and historical textiles.

It has revealed that a reliable system of HPLC/DAD, allied with PCA analysis and MS^n, can satisfactorily differentiate between six cochineal insect species, and make a valuable contribution to the characterization and identification of historical insect species and red-dyed textiles. In this way, 60 cochineal samples belonging to Royal Botanical Garden, at Kew, were confirmed as *D. coccus* species, while seven samples were considered to be incorrectly labeled, as they exhibited no characteristics of cochineal species.

The combination of HPLC/DAD and PCA analysis was only successful after optimization of the separation parameters, which was an important step to obtain improved results in comparison to previous studies. Slight acidification of cochineal dye samples improved the resolution, permitting a good overview of the minor markers identification and a satisfactory correlation between the peak areas in the PCA analysis, for species differentiation. Oxalic acid was determined to be a less harmful extracting solution for historical textile fibres, and presented better resolution than other extraction solutions. The majority of the red-dyed samples from the historical textiles, dating from 15th to 17th centuries, were identified as *Porphyrophora* species, and more scientific research is needed for accurate identification of the precise species. However, samples taken from the 17th-century Indian velvet (CGM 1449) indicate the presence of *D. coccus*, while samples from the Italian chasuble (MNAA 1616Tec) point to *P. polonica* or a mixture of a *Porhyrophora* species and kermes. From this small study, it would appear that the adoption of American cochineal might have been slower than has been postulated in the historical literature, which seems to have over-emphasized the rapid adoption of American cochineal after its documented arrival in 1521 in Spain, 1543 in Italy (and the Ottoman Empire), 1614 in Iran and ca. 1612 in India [10]. Thus, it is absolutely essential that a wider range of well-dated textiles, especially of Asian origin, are examined to look at the speed and scope of adoption of this dyestuff and the relationship between dyeing practices in major textiles manufacturing centres, such as Venice, Bursa, Isfahan, Kashan and Agra, which were involved in international trade and those of more remote regions.

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Appendixes

Appendix 1: Cochineal, the dye and its textile history

1.1 The Colour Red

Colours have been intrinsic to human culture, since early times. Essentially considered a social language, colours are charged with emotions and symbolism, and can vary between cultures and religions, and across time [1,2]. This is why so many interpretations of the colour red are possible. For instance, in West Asia, in Islamic times, under the Ottoman and Safavid dynasties, red was considered a warm colour associated with love and desire, as well as matrimony and procreation. Red could also be connected with violence, danger, war, and even death. In other words, in these cultures, red was viewed as the colour of the life cycle [2,3,4]. However, the colour red also signified power, majesty, and hierarchy in various parts of Europe [1,5]. This symbolism seems to be intrinsically linked with the enormous difficulties associated with acquiring this vivid colour.

Dyeing procedures require specialized artisanal skills, and although there was an endless number of available dyestuffs, the richest, most-enduring, and brightest reds were achieved by dyeing textiles of animal origin (silk and wool) with scale insects [5,6]. However, the difficult process of gathering and preparing the large quantities of these rare insects necessary to prepare a satisfactory dye, in turn, made red textiles extremely expensive. Hence, the dyeing process could substantially raise the final price of a woven fabric, so that it was only affordable to members of the wealthy elite [5].

1.2 A Red Source

All scale insects belong to the superfamily Coccoidea. Within this superfamily, only a small number of insects are known to be capable of supplying high-quality red dyes, namely kermes, lac dye, and cochineal [6,7]. Cochineal species belong to two families from the Coccoidea superfamily, Margarodidae and Dactylopiidae [7,8]. Currently, Margarodidae family includes, among others, *Porphyrophora* genus (47 species) [9], and Dactylopiidae family is composed of the *Dactylopius* genus (10 species) [10]. It is not certain if all these species were used to dye textiles in the past, even though, if collected in sufficient quantities, they had the capacity to yield a potential red dye [8,9].

Today, there is general agreement, on the basis of several studies and historical documentation, that at least two species of *Porphyrophora*, *P. polonica* and *P. hamelii*, and one from *Dactylopius*, the domesticated *D. coccus*, were applied to dyeing textiles. However, it must be emphasized that, from an entomological perspective, insects from the same family are relatively similar to each other, with only slight taxonomical differences observable, except in specific cases, such as *D. confusus* species which is notably different from the other *Dactylopius* species, Table I [8,9]. This apparent similarity between insects needs to be considered more closely for two reasons: firstly, some *Porphyrophora* species are found

together in the same geographical location, and hence, it is possible that, in the past, a certain number of slightly different species were collected and named under the same designation; and secondly, it was very common for inferior grades of cochineal to be fraudulently traded for the price of the superior quality one, which could also lead to misidentification, especially in the case of *Dactylopius* species [1]. Consequently, when identifying red cochineal-dyes in historical textiles, great care must be taken when affirming that a dye was derived from *P. polonica, P. hamelii* or *D. coccus* species, as not all the species have accurately been identified.

Table I - Some cochineal insects which are supposed to have been used for textiles dyeing [8].

Insect Specie	Morphology	Characteristics
P. polonica (Linnaeus, 1758), *P. crithmi* (Goux, 1938) – Polish cochineal	Adult Female Dimensions: 1,5 - 6,5 mm long Shape: small elongated to oval-rounded shape Dark red to purplish, dense short hair, membranous and soft skin, short and solid forelegs, no mouthpart, very mobile Adult Male Dimensions: 2,3 – 3,5 mm long Dark purplish-red, long antennae, extended and transparent filaments, transparent wings	Very similar morphologically, although *P. crithmi* female has 9-segmented antenna. Host plant: in the underground of the base of stems or roots like *Sleranthus perennis* L. (Caryophyllaceae), and *Potentilla bifurcata* L. (*P. polonica*); and *Crithmum maritimum* L. (*P. crithmi*)
P. hamelii (Brandt, 1835) or P. cynodontis (Archangelskaja, 1935) (?) – Armenian cochineal	Adult Female Dimensions: 10 mm long and 7 mm wide Shape: oval-shaped Dark purplish-red, slightly different from *P. polonica* specie by the hairs density and the segments number	Host plant: in the underground of roots, rhizomes and culm base of the grasses (Graminae) *Aeluropus littoralis* (Gouan) Paul and *Phragmites communis* Trin
P. sophorae (Archangelskaja, 1935)	Adult Female Dimensions: 8 mm long, 7 mm wide Shape: oval-shaped Dark purplish-red, differs from *P. hamelii* by the sparser hairs and the number of antenna segments Adult Male Oblong in shape, smaller, outnumbers the female in quantity	Wild cochineal from Central Asia, very similar to *P. hamelii* and *P. polonica* species. Host plant: Leguminosae, Papilionoidae *Sophora alopecuroides* L., *Glycyrrhiza glabra* L.
P. hirsutissima (Hall, 1924)	Adult Female Dimensions: 8 - 10 mm long Dense coat of long hairs, one of the largest of the genus	Host Plant: *Imperata cylindrica* Beauv. (Gramineae)
D. coccus (O. Costa, 1835)	Adult Female Dimensions: 3 - 6 mm long, 2,5 - 4,5 mm wide, 4,2 mm high Weight: up to 46 mg, when full of eggs - if dried, it can be reduced by 70% Shape: elliptical shape, plain abdomen Purplish colour, fine whitish waxy coating, marked segments, short antenna, tiny legs with a claw and star-shaped pores Adult Male Dimensions: 1,3 - 2,5 mm long Characteristics: red, colourless wings covered with a fine whitish waxy coating, no mouthparts (it lives for 2 days)	Domestic and vulnerable insect dependent to the human care; longer life cycle than other *Dactylopius* species - 2/3 harvests per year. Host plant: surface from Opuntia cactus species *(Cactaceae, Opuntioideae)*
D. ceylonicus (Green, 1986), ***D. confusus*** (Cockerell, 1893), ***D. opuntiae*** (Cockerell, 1896), ***D. tomentosus*** (Lamark), ***D. austrinus*** (De Lotto), ***D. confertus*** (De Lotto), ***D. salmianus*** (De Lotto), ***D. zimmermanni*** (De Lotto), ***D. bassi*** (Bendov & Marotta, 2001)	Adult Female Dimensions: 2,3 - 3,3 mm long, 1,5 - 2,5mm wide Shape: almost spherical Strong white mealy wax, short and robust legs, short antenna, six/seven segments, variable dorsal spines in size, narrow-rimmed pores	Wild and strong insects with higher climatic tolerance; smaller life cycle than *D. coccus*, but it can be harvested throughout the year. *D. confusus* are usually smaller and produce more thickly white secretions than the other wild species. Host plant: surface from Opuntia cactus species *(Cactaceae, Opuntioideae)*

Geographically, *Porphyrophora* species are found throughout the Palearctic Region, particularly in Central and Eastern Europe, Asia, and North Africa [9]. These species are collected, especially the female insects, when they are full of eggs, as they are richer in

colorant, and then applied to dyeing textiles by the indigenous populations of these diverse regions, or even traded throughout countries, as mentioned by historical documentation [6,8].

Insects of *P. polonica*, figure 1, are mostly found in Central and Eastern Europe, but also in Central and East Asia, reaching Mongolia. Historically, this insect was extensively collected in Poland, hence its name. It can live on the roots and lower stems of several kinds of plants, in contrast to the related *P. crithmi*, which has been recorded in France, on the roots of only one kind of plant, Table I [8,11]. The insect is collected around the summer solstice, when the females are full of eggs, by uprooting the host plant from the ground, removing the insects with a trowel and then replacing the plant again. The harvested insects are then placed in vinegar or very cold water, and finally dried in a warm oven or under the sun [6,8].

P. hamelii species, figure 2, grow on the roots and rhizomes of Graminae grasses (Table I) found in sandy saline soils near seas or rivers. Widely distributed between Central and East Asia, these insects are widely found, along with other *Porphyrophora* species, in dry steppes or semi-desert regions [8]. These insects were commonly found in the Arak river plain, near Mount Ararat, in Armenia, which is why the insect has long been designated as Armenian cochineal. It may be gathered between mid-July and mid-September, very early in the morning, when the females emerge at the ground surface to mate with winged-males. This method is easier than for collecting *P. polonica*, and involves a similar killing method [6,8]. *P. hamelii* was always cheaper than *P. polonica*, in spite of its increased dimensions. Nevertheless, Polish cochineal was always considered more profitable, as dyeing with the Armenian variety, owing to its high fatty content, required twice as many insects, and thus was never cost-competitive [8]. Both insects yielded the same crimson to dark reddish-purple colours. However, despite the challenges of dyeing with *P. hamelii* insects, these were appreciated for providing more vibrant and brighter colours than *P. polonica*, but the latter was always highly esteemed [6,8].

Figures 1 and 2 - Winged-male and female of *P. polonica* specie, and female of *P. hamelii* specie [8].

It is important to note that *P. hamelii* might not have been properly identified in the past, especially in zones where this species was cohabiting with other very similar *Porphyrophora* species. In fact, Vahedi [9] identifies *P. cynodontis* as a synonym of *P. hamelii*, and this may be in fact be the case, as analyses carried out in this study presented here have revealed similar characteristics to *P. hamelii*. Hence, the collection and preparation methods used for other *Porphyrophora* species are poorly recorded, although it is possible to conjecture, for example, that, given the similarities between *P. sophorae* and *P. hamelii*, the former would have been gathered and prepared in a similar way to the latter [8].

Until the 16th century, *Porphyrophora* cochineal species was as popular in the dye trade of West Asia and Europe as kermes. However, both would eventually be replaced by a cochineal species imported from the other side of the world.

When the Spanish conquerors arrived in Mexico by 1512, they saw the Aztec Indians dyeing and painting with a strong and vivid red colour, which was prepared from “little grains” sold in the local market [1,12]. Knowledge of this superior red dyestuff from the Americas is first recorded in a Spanish report dating from the end of 1523, which refers to the presence of cochineal in colonial Mexico and the arrival of shipments of it in Spain. This report confirms the high interest of the Spanish Crown, as, shortly after the conquest of Mexico in 1521, as requests for information and predictions about its future cultivation were urged to be sent to Spain [1,5,6]. It is possible that samples of cochineal and cochineal-dyed fabrics may have reached Spain around 1518-20 with the arrival of the first cargoes, laden with Mexican products. Among these new products, King Charles I (r. 1519-1558) recognized the presence of a profitable commodity which would contribute significantly to the Spanish economy [1,5,12]. Thus, soon after 1530, cochineal was included in the royal tribute system and with increasing demand, tons of cochineal started to be sent to Europe annually [1,6].

The American cochineal brought commercially to Europe, from the 16th century onwards, consisted essentially of the domesticated species *Dactylopius coccus*. Like the wild species *Dactylopius*, *D. coccus* is indigenous to the Americas. While the origins of *D. austrinus, D. confertus, D. salmianus* and *D. zimmermanni* are attributed to South America (Argentina, Bolivia, Peru, and Paraguay), *D. opuntiae, D. bassi, D. confusus, D. tomentosus and D. coccus* are considered primitive species from North America (Mexico, New Mexico, Texas and Arizona). *D. ceylonicus* is attributed to both American regions [10]. These insects live on the surface of several species of cactus of the *Opuntia* genus, but there is a clear distinction between the domesticated and wild cochineal, Table I. The domestic species *D. coccus* is the result of optimal breeding of the wild species by the pre-Colombians, which is why the insects exhibit larger dimensions, longer lifespan, and are also more vulnerable than the wild insects [1,8]. However, the most important advantage of *D. coccus* is its brilliant and enduring purplish crimson dye, which cannot be obtained from other species, and its high dye content [8].

Figure 3 - Adult *D. coccus* females [8].

The cactus plantations were generally of small dimensions and maintained by the indigenous population, since cochineal needs frequent care and assiduous management. Like *Porphyropora* insects, the females full of eggs yield larger quantities of colorant, and so harvesting occurs immediately before they lay the eggs [5,8]. The preparation would then involve several different procedures, which could influence the quality of the dyeing. The most common and best procedure was to spread the insects on mats under the hot sun, which resulted in the so-called “silver cochineal”. However, other

methods considered to produce insects of an inferior quality involved, for example, immersing or steaming the insects in boiling water and then drying them under the sun (brown, dull red or rosy black cochineal), baking the insects in hot pans or metal sheets (black cochineal), or in moderate warm ovens (ash-grey cochineal). Another method which was less practical for trade and transport was that used commonly by the Indians in which the insects were transformed into tablets or cakes, by mixing them with other materials like alum, resulting in a substantial increase of weight but without any financial gain upon resale in Europe, Appendix 4 [1].

Soon after the first shipments of cochineal began arriving in Spain at the beginning of the 16th century, news of the existence of an insect which could provide a brilliant and vibrant red swiftly spread across Europe, as far as West Asia. This insect was immediately recognized as representing a revolution in the art of dyeing. *D. coccus* not only surpassed the wild version of *Dactylopius* species, but also all other coccid insect dyes, that had been used in Europe and Asia until this time. The rapid triumph of American cochineal over these other species was due to two main factors: firstly, American cochineal could bring significant savings, as only 7% of the weight of the cloth in American cochineal was required to obtain the same scarlet shade as was obtained with, at least, 71,5% of the weight of the same cloth using kermes insect; and secondly, it facilitated the creation of more than twenty shades of colours when combined with other dyes, although crimson silk was always the most highly esteemed product [8]. Hence, American cochineal brought new possibilities for dyers and merchants, allowing scarlet, crimson, and an infinite number of other colours to be made available in an increasing variety of textiles, and, moreover, to larger numbers of people. This dyestuff naturally attracted increasing demand in the principal luxury textile manufacturing centres in Europe and West Asia, and, in fact, it would become one of the most sought-after and expensive luxury dyestuffs in Europe [5]. However, it is important to emphasize that the other red dyestuffs (*Porphyrophora* and kermes insects) used previously in Europe and Asia would continue to be exploited long after the arrival of American cochineal, especially in regions more distant from international trade, until well into the 19th century [1,6].

With the Spanish occupation of Central and most of South America, it was possible to create a strict and successful monopoly, in which, the secrecy of production and even the true identity of domestic cochineal would remain within the frontiers of the Spanish empire until the 18th century. When the dried insects arrived in Europe, they were thought to be seeds, not just because of the commercial name “grana fina” (fine grain), but also because of its “grained” appearance, which also resembled a berry [1,13]. In the colonies, the Spanish created a complex political structure to encourage the Indians to grow cochineal, as well as laws and hard punishments for those who attempted to acquire the secrets of domestic cochineal production, or to smuggle the insect out of the Spanish colonies. In this manner, cochineal became, after silver, the most important item in Spanish trade for over three centuries, while the rest of Europe, unable to compete, contributed to the high profits involved which went directly to the Spanish treasury [1,5,13].

Mexico was the main producer of cochineal during the colonial period, especially in the regions of Oaxaca, Tlaxcala, Yucatan and Mixteca, where the dried insects of cochineal were packed (1 kilo would correspond to approximately 130.000 insects [2]) and sent to the main ports of the colony, Vera Cruz and Acapulco. From Acapulco, shipments departed to Asia, via the Philippines (China, Cambodia, Siam, Cochin China, Bangal and Madras), and from Vera Cruz, consignments were loaded for Spain (Cadiz or Seville), figure 4.

Peru also produced domestic cochineal since pre-Colombian times, although it was not prominent in the Spanish trade. This is probably due to the fact that Mexico represented a geographical connection between the sea trade of Europe and Asia. Nicaragua, Honduras, Salvador and Guatemala also contributed to the wild cochineal trade in the colonial period, but with only a limited trade in domestic cochineal [1,6].

Once the cochineal had arrived in Spain, it was re-exported across Europe: Ancona, Venice, Mila, Netherlands (Antwerp), and England. In Venice, cochineal also entered trade with West Asia, travelling by the Venice-Levant route directly to Iran and from there to India, or by the Venice-Constantinople route and the ports of the Black Sea to Turkey and the Caspian region, figure 4.

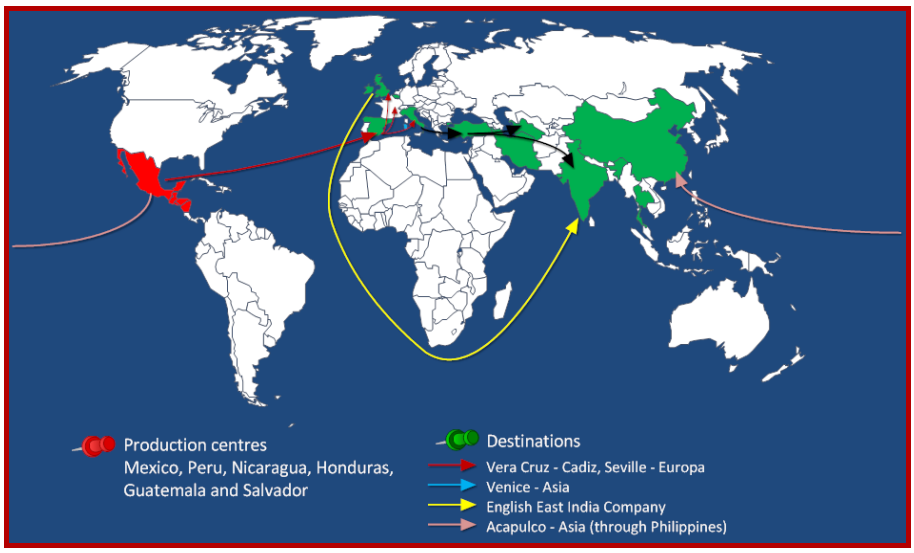


Figure 4 - Trade routes map of cochineal during the colonial epoch [8].

American cochineal was reported in Venice by 1543 [6,10], and in a letter dated from 1586, an English merchant living in Istanbul, mentions that the product stored in Pera would be shipped to Cairo and Aleppo [6,14]. The earliest record of further east is a diplomatic mission sent in 1614 by the king of Spain, Phillip III (r. 1598 - 1621), to the Persian shah ‘Abbas I (r. 1587 - 1629), which delivered a large quantity of valuable gifts, among the most expensive of which were five barrels full of this scale insect [3]. However, it has long been presumed that American cochineal was probably already in use in Iran by this time [14], something which was not confirmed by this study. *Porphyrophora* and kermes found a ready market in luxury textile manufacturing centres, such as Bursa, and at court workshops in places such as Istanbul and Isfahan [4,6,15], and it remains to be determined the extent of the impact of American

cochineal. The Persian cities of Khorasan and Kirman are known to have appreciated this new dyestuff, and to have acted as a link in trade with India. In this way, American cochineal could have reached Sind, before the establishment of the English textile factory there, in Surat, in 1612. [4,6,14]. However, the re-exportation of cochineal from England to India, by the English East India Company, does not appear to have been particularly successful as the demand for cochineal was initially limited, and also purchased with cheaper prices from merchants coming from the Silk Road [1,6,8].

Throughout the 18th and the first half of the 19th centuries, a series of events would overturn the important role of Spain in the global trade of cochineal. The incessant exterior attempts to smuggle and breed American cochineal outside the Spanish colonies would finally be successful. Several species of *Dactylopius* and the respective host cactuses were introduced and reared experimentally across the world, sometimes with consequent failures, other times with great success [8,13]. In 1821, the Mexican War of Independence, which ended Spanish control of the colony, resulted in decay and decline of the cochineal trade. However, at the same time, other countries such as Guatemala, Peru, and the new place of Spanish production, Canary Islands, achieved successful results and consequently became leading export centres during the first half of the 19th century. On the other hand, the increase in breeding locations for cochineal consequently led to a dramatic decrease in the insects' price. The luxury dyestuff was finally eclipsed by the development of the chemical dye industries in Germany, in the second half of the century. The new synthetic dyes would practically substitute natural dyes until the second half of the 20th century, when a new interest would flourish, owing to the harmful consequences of synthetic dyes to the human body [5,8].

Today, *Dactylopius* is found not just in the countries where it originated, but also in Indonesia, India, the lands around the Mediterranean coast (Spain, France, Italy), Canary Islands, Algeria, Senegal, Bourbon, Southern Africa, Cape region, Australia, Madeira, Botswana, Nepal and Sri Lanka [1]. Peru is now the main producer of this insect, followed by Chile, Canary Islands and Bolivia. Cochineal is almost entirely used as a food, pharmaceutical and cosmetic colorant, although in occasional cases, it is still used as a dyestuff for textiles [1,8].

1.3 Analysing Red Dyes

It is currently possible to identify red dyes using High-Performance Liquid Chromatography. This identification method was developed relatively recently, in the 1980's, by Jan Wouters and Andre Verhecken [16]. It overtook other methods of identification used previously, such as microchemical and TLC (Thin-Layer Chromatography) analysis, and is now regarded to be the most reliable method for identifying dyestuffs, especially if coupled with additional analytical instrumentation, such as Diode-Array Detector and Mass Spectrometry [2,17]. With these analyses, it is possible to obtain profile chromatograms for colouring compounds, and thus contribute to studies about centres of production of dyes and textiles, trade routes for dyestuffs, and even the application, function, and appreciation of dyes in different parts of the world.

Up until now, analyses of red cochineal-dyed textiles frequently identified the presence of American and Armenian cochineal in European and West Asian textiles, based on the determinant presence of minor markers [15-18]. These studies, however, cannot be considered complete owing to the need of references that can ensure a reliable species identification. Since the pioneering work of Wouters [17], little progress has been made towards achieving correct identifications of the cochineal species used in historical red-dyed textiles. A major, in-depth study on all the *Dactylopius* and *Porphyrophora* species is required, which will look at the insect at different stages of growth, followed by the correct identification and differentiation of each species from an entomological point of view, and by accurate assessment of the characteristic proportions and minor markers present in each [8]. Finally, with this information it will be possible to identify the correct species in an analyzed red-dye, whether it is a historical textile or a pigment.

1.4 Red Islamic and European Textiles

In this study, red-dyed fibres collected from a group of six Islamic and two Italian textiles, from 15th to 17th centuries, and belonging to Portuguese collections, are analyzed to determine the possible source of the red dyestuff present. The Islamic textiles selected (five velvets and one silk carpet) originated from diverse places in West Asia (Turkey, Iran and India), and are contemporary with the Italian textiles (two velvets) (Appendix 2). The defining feature that connects all of these objects is the presence of red-dyed silk fibres, which exhibit a strong vivid crimson colour, typically used for large areas of the background, and occasionally, for smaller decorative motifs. All of the textiles were manufactured around the same time as the arrival of American cochineal in these regions, and hence the following study represents a first step towards developing a major comparative study which will look at the dissemination and adoption of this insect in relation to European and Islamic dyeing practices.

Made exclusively or almost entirely of silk, the velvets have elaborate designs and complex weaving structures, and are often decorated with gilt-metal and silver threads, and dyed with the finest available dyestuffs. The silk pile is created from secondary warp threads that, at intervals in the weaving, are carried over thin rods to create loops which are cut when the rods are removed [2,19].

Pile carpets are made by two proceses: knotting and weaving. Short pieces of yarn are wrapped around pairs of warps to create “knots”, and then a fixing weft is added to secure the knots in place. The free ends of the “knots” form the pile on the surface of the carpet, and the pattern results from the different colours of the yarn chosen for the knots [2].

I - European textiles

The Italian city states had strong commercial connections with the Turkish Empire from early on, and served as the gates of Europe for the trade in Asian products, brought by merchants along the Silk Road. Such products included luxury dyestuffs and silk, which were not obtainable in Europe. Products of European origin were also negotiated in Italy with West Asian

merchants who would transport them to Turkey and beyond. Textiles produced in Italy were traded to other countries, owing to their high quality, characterized by the combination of luxurious materials and complex weaving structures. As they were usually created according to the instructions of the client, the motifs often reflect foreign styles, but the woven structure remains characteristically Italian, and thus the Italian copies can be distinguished from other velvets with similar decorative motifs, such as related Ottoman textiles [6,15].

Ottoman fabrics enjoyed a high quality design and manufacturing throughout the 15th century, however Italian textiles became appreciated by the Ottoman court in the late 15th and early 16th centuries. A large number of documentary sources confirm this interest, and an extraordinary similarity is found between textiles from both places, especially the use of crimson grounds and gold brocading, as well as shared compositions and motifs [15]. However, Italy had a better and more well-established silk-weaving industry, especially in the cities of Florence, Venice and Genoa, which were internationally renowned for manufacturing the richest and most elaborate silk velvets. By comparison, Turkish velvets were not as luxurious as Italian products, and even the simpler Italian velvets were worn by Ottoman sovereigns. Hence, Italian silk-weaving workshops produced extensively Ottoman-style fabrics for the Ottoman market, which, in turn, resulted in the introduction of Italian artistic influences in the pattern layouts and artistic motifs of Turkish textiles [4,15].

The Italian textiles studied here comprise a 15th-century Christian chasuble, with large motifs in red velvet, and a 16th-century brocaded velvet textile, in which the entire background as well as some of the motifs are in cut red velvet (Appendix 2). The presence of pomegranates in the decoration of the former is very characteristic of Italian art at its time, but the latter resembles the decoration of Ottoman textiles. Analyses carried out on the red fibres from both velvets indicate the presence of cochineal. The cochineal used in the velvet of the chasuble appears to be *P. polonica*, or a mixture of *Porphyrophora* and kermes, and the cochineal in the other textile velvet is pointed as *Porphyrophora* specie, probably *P. polonica*.

II - Islamic textiles

Turkey, Iran, and India were connected historically throughout the period under study, and also shared a similar high esteem for the textile arts, which achieved their highest expression in court-sponsored production and consumption. Textiles were a reflection of the authority and prosperity of the Ottoman, Safavid and Mughal dynasties, and were often offered as diplomatic gifts [4,15]. Among the many rich and complex textiles produced during this period, velvets were considered to be one of the most prestigious and luxurious cloths available [15].

II - a) Ottoman textiles

The Ottoman Empire has its origins in 13th century, when the Turks embarked on an ambitious expansionist policy. The resulting tributes and taxes from the newly-captured territories, as well as their raw materials and manufactured articles, spurred the Ottoman economy. Owing to its geographical location, the Ottoman Empire served as the main

commercial connection between Europe and Asia. However, it was only in the 15th century that the arts began to flourish. The city of Bursa became the main entrepôt for the silk trade, and a major centre of textile manufacturing, producing silks for the Ottoman court as well as for the international export market [4,15].

Silk imported from Asia was extensively used as a raw material in luxury textile manufacture, and played a prominent role in the Ottoman economy as a highly profitable trade commodity. Although the Ottomans were extremely rich, they were also involved in continuous wars with Iran, from the 16th century onwards, which made trade difficult, and often halted the sale of silk and other raw materials between Asia and Europe. As a result, at the beginning of 16th century, the Ottomans started to grow mulberry trees to produce their own silkworms, which became a successful and lucrative industry [4, 15].

With a strong emphasis on authority and economic power, Ottoman textiles from this period are a mirror of the wealth enjoyed by the elite. These textiles are a rich form of artistic expression and were seen as ideological vehicles for transmitting power and order across the Empire and beyond [15]. Characterized by simple patterns, Ottoman textiles feature strong designs repeated across the textile's width and length, which are then enriched with vivid ground colours and luxurious quantities of metal thread applied over the motifs. Human figures and animal motifs are noticeably absent, and the designs are chiefly based on geometric and floral patterns, such as *çintamani,* star-and-cross or *saz* (water reed) patterns. The flower motifs include peonies, lotus, pomegranates, prunus, carnations or dianthus, roses, bluebells and tulips, often framed in *saz* and acanthus leaves [4,15]. In all of these luxurious textiles, the bluish-red crimson colour was achieved with scale insects, such as cochineal and kermes, and was applied mainly to large areas of the ground in both velvets and brocades [15]. Analyses of the red fibres belonging to the Ottoman brocaded velvet indicate the presence of cochineal, probably a *Porphyrophora* polonica species.

II – b) Safavid textiles

The Safavid dynasty reigned from 1501 to 1723. The first Safavid ruler, Shah Isma'il (r. 1502 - 1524) conquered the whole of Iran and established the capital at Tabriz, which became a major centre for artistic production, especially under his son, Shah Tahmasp (r. 1525 - 1576), who encouraged the migration of artisans from Herat to Tabriz, resulting in a fusion of the artistic styles of the Timurids and Turkmen, respectively. Here, textile production received a major impetus with the development of the court library which also produced designs for the loom, both for magnificent woven fabrics and carpets. Other important centres for weaving were Kirman and Kashan, as well as Isfahan, later under Tahmasp's grandson, Shah Abbas I (r. 1587 - 1629) [4,20], who moved the capital there, in 1598. This was the same shah who received the fine gift of five barrels of American cochineal from Phillip III [3,20].

The Safavid economy was highly influenced by the trade in raw silk. Silkworm production was extensively developed throughout Iran, and, in spite of almost constant war with Turkey throughout the 16th century, the Ottomans were important players in the Iranian economy, not

just as important silk consumers, but also because they controlled the main gates to the European market [4,15]. Kashan was an important place for silk cultivation, and a centre for the production of silk pile carpets in the 17th century [4,20] However, there is no direct evidence for attributing the so-called “Small Silk Kashan’ carpet in this study to this city and this group of carpets could have been made anywhere in Iran.

Persian luxury textiles are intrinsically linked to the power and opulence of the Safavid court, and were always considered very desirable commodities by merchants and travellers. Although textile production was connected with the court, the designs are not embedded with the strong symbolism of order and power witnessed in Ottoman textiles. Persian fabrics exploit a more subtle visual language, with a curvilinear style and a wider range of colours and techniques, including both human and animals figures, as well as birds and flower motifs, which are strongly outlined and fill the entire background; in stark contrast to the large areas occupied by a single colour in Ottoman velvets (Appendix 2) [4,15]. The patterns were designed to minimize vertical and horizontal order, by reversing the motifs in alternate units and rows. These fabrics were of equivalent quality to Ottoman ones, although the colours of many Safavid silks have faded over time, in contrast to Ottoman textiles. Indeed, it is known that, on certain occasions, some of these silks were sent as diplomatic gifts to the Ottoman sultans [4].

The Iranian textiles analysed in this study are characterized by compositions in which horizontal or vertical alignment is absent, and the decorative motifs are usually organized in structured vines, almost completely covering the background. Analyses of their red fibres confirm the presence of cochineal, probably *P. polonica* species, for both the velvets and the carpet.

II – c) Mughal textiles

The first Mughal Emperor, Babur, invaded India in 1526 and founded the Mughal dynasty, but it was only under the Emperor Akbar (r. 1556 - 1605) and his cosmopolitan court that the art of Mughal India achieved great artistic development [21]. The Mughal style is a synthesis of the indigenous Indian artistic tradition, characterized by subtlety, delicacy and elegance, and the great Timurid aesthetic (the illustrious 15th-century ancestors of the Mughal dynasty) which emphasized a preference for geometric designs and elegant arabesque motifs [22].

After the Mughal Emperor Humayun had contact with the Safavid court in 1544, he brought Persian artists to India, and the arts became strongly influenced by Safavid models [15,22]. Mughal textiles are characterized essentially by patterns in rich colours, in which yellow was prominent, with naturalistic flowers, displayed in bunches or vines, leaves and blossoms, on a plain background, and combined with gilt-metal and silver threads [15,23]. The most common motifs are large single leaves-and-blossoms or flowers-in-vase on a red field [20,22].

The most important court palaces in Delhi, Lahore and Agra were filled with vividly patterned textiles. These expensive textiles were commonly made in silk, such as velvets, brocades and satins, in provincial textile centres such as Ahmedabad, Cambay, Banaras and Dacca. These textiles, along with the famous carpets and rugs, constituted a significant component of the

lucrative textile trade, conducted by agents of the Mughal emperors, with Southeast Asia and later Europe, especially in the 17th and 18th centuries [20,22].

The Indian textiles under study here are essentially composed of flower motifs in a structure reminiscent of the Iranian textiles also analyzed here (Appendix 2). The red fibres taken from both of the Indian velvets were identified as having been dyed with cochineal. The dyestuff present in one velvet with a resembling European decoration, due to the presence of *saz* leaves (CGM 1449), was determined to be *D. coccus* specie, while the other red velvet (CGM 1422) was dyed probably with a *Porphyrophora polonica* specie.

1.5 - Interpretation

The results of this study reveal a more complex picture of dyeing practices in Europe and the Islamic world in the 16th and 17th centuries than hypothesized. While the red areas of the 15th-century chasuble were probably dyed with a mixture of *Porphyrophora* species (possibly in conjunction with kermes), as expected, the red velvet imitating Ottoman Turkish designs from the following century was also identified as *Porphyrophora* species. The absence of *D. coccus* was also registered in the Ottoman velvet, which calls into question previous research on these textiles which has presumed the presence of Mexican cochineal [14,15]. Further research of Ottoman textiles is imperative to determine the extent to which *D. coccus* was adopted in Turkish dyeing practices.

All of the Iranian silk textiles analysed, including the important 'Small Silk Kashan' carpet, revealed the presence of *Poprhyrophora* species, namely *P. polonica*, emphasizing the importance of overland trade for the importation of dyestuffs into the Iranian Empire in the 16th century. In addition, the yellow dye used in the Gulbenkian 'Kashan' rug was related with another yellow dye found for the Machado de Castro carpet analysed in 2007 (MNMC T744), confirming a shared provenance for these two carpets.

The results for the Mughal textiles, by contrast, were not as homogeneous. One of the velvets with red floral motifs was identified as *Poprhyrophora polonica*, while the other, with a strong red background, points to the presence of *D. coccus*. This is an important result as it offers the first scientific confirmation of the use of this dye in 17th-century Indian textiles, something which is recorded in historical sources.

This small study has demonstrated the importance of a species-oriented approach for examining the application and diffusion of dyestuffs in the context of pre-Modern globalization. It opens a window onto the wider world of dye technology and emphasizes the necessity of further scientific research to develop a more nuanced picture of dye traditions and trade routes than offered, until now, from the historical sources. It would appear from these results that the adoption of cochineal was more gradual and possibly site-specific, and it is fundamental to broaden the range of textiles to look at the relationship between dyeing practices in major centres of production, such as Venice, Bursa, Isfahan, Kashan and Agra, in comparison to more remote regions.

Appendix 2: Textile Catalogue

2.1 Velvet (CGM 1388A)

Turkey, Bursa, 16th/17th century

Cut, voided and brocaded velvet

Satin ground, silk warp and cotton weft, with pile pattern of silk, brocaded with gilt silver metal-wrapped wefts

172 x 64 cm

Prov.: Count Vitali Collection, Paris, 1925

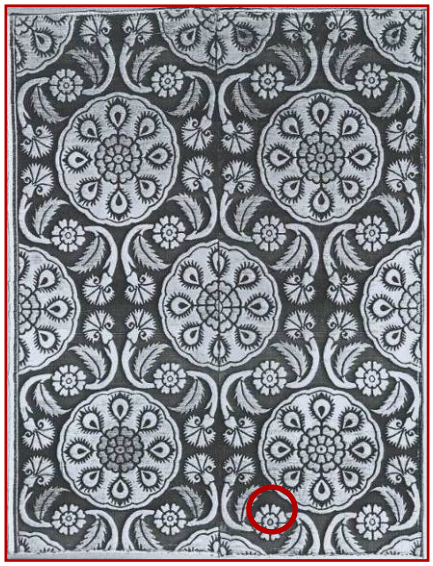
Lisbon, Calouste Gulbenkian Museum

1388A

Ottoman classic style composed by a dark red ground, covered by a pattern of large circles parallel repeated and vertically organized, and containing crescent-like *çintamani* roundels surrounded by sprays of tulips, hyacinths and carnations [4].

The deep dark red homogeneous background (L* 12,86±0,28, a* 22,18±0,26, b* 7,85±0,10[5]) was identified as *P. polonica* cochineal.

Note: It resembles a fragment of a cover, also with *çintamani* roundels in staggered rows, dated from the second half of the 16th century, belonging to the National Museum of Cracow [15].



2.2 Velvet (CGM 245)

Italy, Genoa, 16th century

Cut and brocaded velvet

Satin ground, silk warp and cotton weft, with pile pattern of silk, brocaded with metal-wrapped wefts

165 x 123 cm

Prov.: Abernon Collection, 1929

Lisbon, Calouste Gulbenkian Museum

245

Vertical point repeat of roundels with a central rosette and radiating peacock feathers, surrounded by *saz* leaves, carnations and small rosettes, on a deep red background [24]. The red colour was identified as *P. polonica* cochineal and it possesses a homogeneous, lighter shade (L* 23,99±0,70, a* 31,97±0,01, b* 8,55±0,02[5]).

[5] The color coordinate measurement (L * a * b *, CIELAB) were performed in the areas with an open circle. It was performed three analysis in each area.

2.3 Velvet (CGM 1446)

Iran, Yazd (?), 17th century
Cut and voided velvet
Satin ground, silk warp and cotton weft, with pile pattern of silk
70 x 49 cm
Prov.: Beselièvre collection, Paris, 1914
Lisbon, Calouste Gulbenkian Museum
1446

Above a red ground, a rich, symmetric and sophisticated pattern of ogival lattices appear to form a composition of large, yellow-and-blue-lobed medallions filled with stylised floral motifs that are connected through a complex system of widely varied scrolls and leaves. These alternate with large palmettes and pairs of birds (phoenix and peacock) that are either facing or back-to-back. This type of decoration combining floral and zoomorphic motifs was very popular with Safavid court for decorating tents and palaces [25].
The red colour is obtained from cochineal dye *P. polonica*. It shows a very homogeneous and vivid shade (L* 28,79±0,09, a* 20,03±0,17, b* 8,89±0,01[5]).
Note: Another part of this silk was sold at Sotheby's London [26].

2.4 Velvet panel (CGM 1513)

Iran, 17th century
Cut velvet
Satin ground, silk warp and cotton weft, with pile of silk
104 x 84 cm
Prov.: London, 1921
Lisbon, Calouste Gulbenkian Museum
1513

This velvet panel is decorated with continuous network of polychrome scrolls and flowers, framed by a border with floral motifs connected by scrolls and leaves. The scrolls are made by leaves connected by rosettes, lotus and blossoms to create an organized panel whose function is evidently decorative [25].
The red colour was identified as cochineal dye *P. polonica*, and it presents a homogenous and red deep shade (L* 16,22±0,85, a* 23,68±0,43, b* 6,77±0,08[5]).
Note: A Farahan carpet exemplar, from west Persia, with an analogous flower composition, and dated from 1854-5 AD, can be found in [27].

2.5 Velvet (CGM 1449)

India, 17th century

Velvet

Satin ground, silk warp and cotton weft, with pile of silk

113 x 75 cm

Prov.: London, 1921

Lisbon, Calouste Gulbenkian Museum

1449

Vine scroll pattern organized above a red ground and carrying a polychrome pattern of lotus flowers, blossoms, rosettes and sickle-shaped leaves, with large rosettes in the voids [28]. The red background was identified as cochineal-dyed fibres, with *D. coccus.* This colour exhibits a slightly darker and homogeneous shade (L* 18,20±0,01, a* 19,47±0,02, b*7,52±0,01[5]).

2.6 Velvet (CGM 1422)

India (?), 17th century

Cut velvet

Satin ground, silk warp and cotton weft, with pile of silk

127 x 162cm

Prov.: London, 1921

Lisbon, Gulbenkian Museum

1422

Large velvet with an organized latticework comprising a vine network pattern of opulent flowers, such as roses, lotus and rosettes, linked by serrated leaves and stems [19][28]. The red colour was identified as cochineal dye *P. polonica,* and it possesses a homogeneous lighter shade (L* 29,52±0,05, a* 25,70±0,01, b* 3,88±0,02[5]).

Note: Another part of this velvet belongs to The David Collection, Copenhagen [19].

2.7 "Small Silk Kashan" carpet (CGM T100)

Iran, Kashan, mid-16th century
Pile Carpet
Silk foundation (warp and weft) and silk pile
230 x 180 cm
Prov.: Amsterdam, 1936
Lisbon, Calouste Gulbenkian Museum
T100

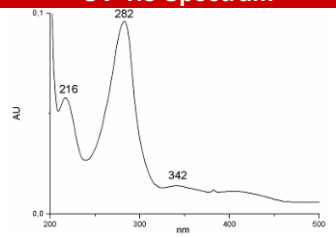
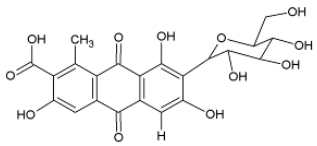
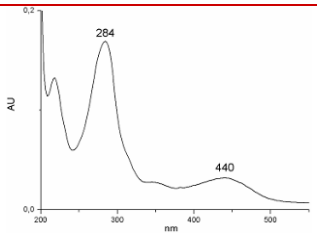
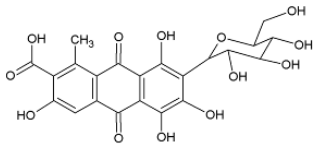
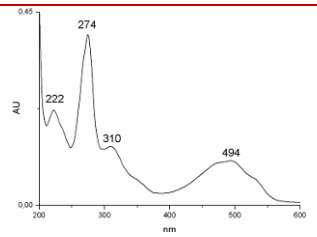
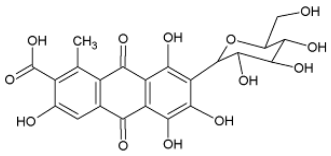
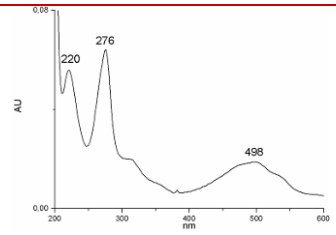
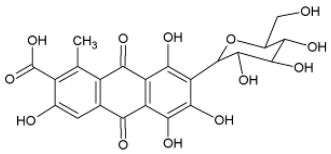
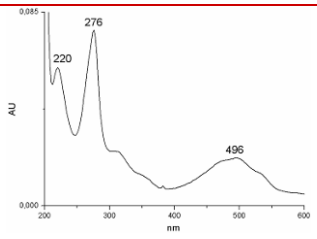
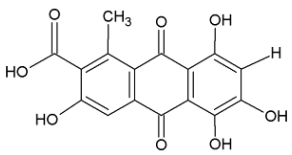
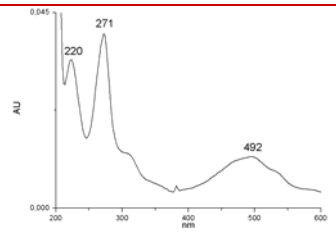
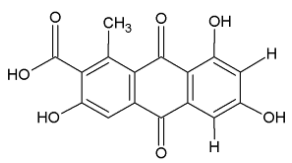
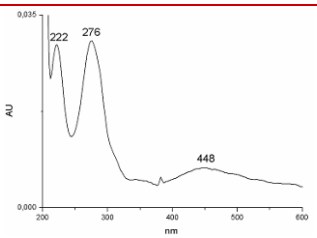
Complex and rich composition organized above a red ground surrounded by a green border. In the centre, there is a large quatrefoil medallion with a blue ground with two pairs of phoenix and two large palmettes. Surrounding it, there are several species of animals fighting, such as tigers, panthers, lions, antelopes, deers and bovines, as well as lotus, rosettes and small blossoms. In the corners of the field are yellow quarter-medallions decorated with flowers and birds. The major border carries palmettes alternating with pheasants. The interior minor border shows a meander with rosettes and "tchi" shape clouds, above a light blue ground, while the exterior minor border is filled with a different meander with several types of flowers on a red background [29]. The red colour of the central field was identified to be cochineal dye *P. polonica,* and it exhibits a homogeneous deep red shade (the colour coordinate measurement could not be accomplished on this piece, as it was on exhibition).

2.8 Portuguese chasuble (MNAA 1616 Tec)

Italy, probably Florence, 15th century (velvet), 16th century (embroidery)
Cut, voided velvet, brocaded with metal thread and small rings scattered throughout (*allucciolato*)
119 x 71 cm
Prov.: Acquisition, 1915
Lisbon, Museu Nacional de Arte Antiga
1616 Tec

The Portuguese chasuble cloth has large motifs in velvet, with large twisted leaves covered with artichokes with little pomegranates and simple flowers brocaded in metal thread [30][31]. The red velvet motifs were identified as cochineal-dyed fibres *P. polonica* or a mixture of *Porphyrophora* with kermes species, and they possess a deep shade (L* 14,51±0,31, a* 25,21±0,18, b* 5,02±0,10[5]).

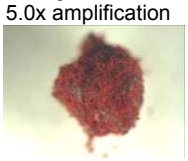
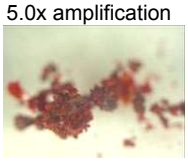
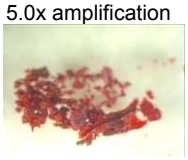
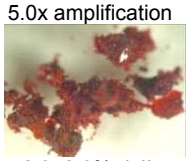
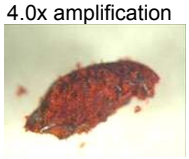
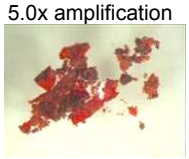
Appendix 3: Cochineal Markers Database (monitored at 275 nm)

Compound	t_R (min.)	λ_{max} (nm)	UV-vis Spectrum
PpI(?) (unknown yellow compound)	17,16	216 282 342	
DcII (Yellow compound) 	17,86	284 440	
Carminic Acid (Red compound) 	18,00	222 274 310 494	
DcIV (Red compound) 	20,38	220 276 498	
DcVII (Red compound) 	21,58	220 276 496	
Kermesic Acid (Red compound) 	23,93	220 271 492	
Flavokermesic Acid (Yellow compound) 	23,65	222 276 448	

Appendix 4: Reference cochineal specimens identified by entomologists

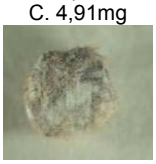
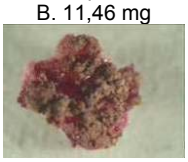
Description	Insect Samples[6]			Observations
***Dactylopius* specimens from D. Miller – Systematic Entomology Laboratory, Maryland, U.S.A.**				
2 *D. opuntiae* **Collection date:** 1918 **Local:** California	2.5x amplification A. 2,70mg 	2.5x amplification B. 2,48mg 	3.2x amplification C.0,69mg 	Red pinkish, wrinkled, with white, grey and yellow spots. 2,8±0,1% dcII, 93,1±1,1% CA, 1,3±0,6% dcIV, 1,9±0,5% dcVII, 0,9±0,1% fk + ka
9 *D. opuntiae* **Collection date:** 1947 **Local:** Mexico	4.0x amplification A.1,31mg 	4.0x amplification B. 0,95mg 	5.0x amplification A.0,62mg 	Little dimensions, wrinkled, orange pinkish, with pink hair. 1,9±0,2% dcII, 93,3±0,3% CA, 2,1±0,2% dcIV, 0,8±0,1% dcVII, 1,8±0,1% fk + ka
10 *D. opuntiae* **Collection date:** 1925 **Local:** Mexico	3.2x amplification A.1,69mg 	4.0x amplification B. 0,62mg 	5.0x amplification C. 0,06mg 	Reddish, with white hair. Little dimensions. 2,2±0,1% dcII, 94,8±0,1% CA, 1,2±0,0% dcIV, 0,9±0,0% dcVII, 0,9±0,0% fk + ka
3 *D. confusus* **Collection date:** 1933 **Local:** Texas	3.2x amplification A.4,21mg 	2.0x amplification B. 7,40mg 	2.0x amplification C. 7,25mg 	Reddish with a white dust, striped. 1,5±0,1% dcII, 94,8±0,9% CA, 0,9±0,3% dcIV, 1,2±0,6% dcVII, 1,7±0,4% fk + ka
11 *D. confusus* **Collection date:** 1925 **Local:** Kansas	4.0x amplification A. 0,60mg 	3.2x amplification B. 0,98mg 	3.2x amplification C. 0,43mg 	Little dimensions, with a white dust on a dark red, wrinkled body. 3,7±0,1% dcII, 78,4±0,2% CA, 11,2±0,1% dcIV, 5,8±0,1% dcVII, 0,9±0,0% fk + ka
1. *D. confusus*	5.0x amplification A. 0,66mg 	5.0x amplification B. 0,93mg 	5.0x amplification C. 0,840mg 	Little dimensions, white dusty with red veins. 4,4±1,3% dcII, 89,9±2,5% CA, 2,5±1,0% dcIV, 2,3±0,5% dcVII, 0,9±0,7% fk + ka
6 *D. ceylonicus* **Collection date:** 1716 **Local:** Ceylon	3.2x amplification A. 4,26mg 	3.2x amplification B. 1,76mg 	3.2x amplification C. 2,25mg 	Rounded shape, with white hair and a brown body. 4,1±0,8% dcII, 93,3±1,1% CA, 0,8±0,4% dcIV, 0,8±0,1% dcVII, 1,0±0,4% fk + ka
7 *D. ceylonicus* **Collection date:** 1945 **Local:** Argentina	2.0x amplification A. 2,38mg 	4.0x amplification B. 0,50mg 	3.2x amplification C. 4,87mg 	Pinkish with white brownish hair or black spots. 3,4±1,2% dcII, 90,8±0,9% CA, 1,9±0,9% dcIV, 1,9±0,4% dcVII, 2,0±1,0% fk + ka
5 *D. coccus* **Local:** Canary Islands	2.5x amplification A. 6,16mg 	2.5x amplification B. 5,47mg 	2.5x amplification C. 5,26mg 	Shiny red, wrinkled with stripes. 0,8±0,0% dcII, 96,6±1,9% CA, 0,6±0,3% dcIV, 1,0±0,6% dcVII, 1,0±0,9% fk + ka
8 *D. tomentosus* **Collection date:** 1924	3.2x amplification A. 0,22mg	4.0x amplification B. 0,18mg	4.0x amplification C. 0,10mg	Little dimensions, with a red striped belly and white hair on the back. 1,0±0,3% dcII, 90,5±4,5% CA,

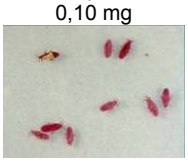
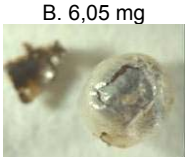
Local: New Mexico				2,7±1,4% dclV, 4,5±2,4% dcVII, 1,4±0,5% fk + ka
D. coccus **Collection date:** 1921 **Local:** Funchal, Madeira	2.5x amplification A. 9,90mg 	2.0x amplification B. 18,35mg 	2.0x amplification C. 12,36mg 	Dark red, with stripes, orange and yellow spots on the belly, and wrinkled. 3,4±0,1% dcII, 92,6±0,7% CA, 1,6±0,3% dclV, 2,1±0,3% dcVII, 0,3±0,0% fk + ka

***D. coccus* specimens from M. González - Instituto Canario de Investigaciones, Tenerife**					
1. Ayres, Chile 5.0x amplification  2,5±0,3% dcII, 94,5±0,1% CA, 1,3±0,2% dclV, 1,2±0,3% dcVII, 0,4±0,3% fk + ka	**2. Peru** 5.0x amplification  3,1±0,3% dcII, 94,3±0,2% CA, 1,3±0,1 dclV, 1,1±0,2% dcVII, 0,2±0,0% fk + ka	**3. Jalisco, Mexico** 5.0x amplification  2,2±0,7% dcII, 95,7±1,0% CA, 1,1±0,2 dclV, 0,9±0,1% dcVII, 0,2±0,0% fk + ka	**4. Hidalgo, Mexico** 5.0x amplification  2,3±2,0% dcII, 94,0±0,6% CA, 1,3±0,3 dclV, 0,9±0,0% dcVII, 0,2±0,1% fk + ka	**5. Tenerife, Canary Islands, Spain** 4.0x amplification  2,3±0,1% dcII, 95,6±0,3% CA, 1,3±0,0 dclV, 0,8±0,3% dcVII, 0,1±0,0% fk + ka	**6. Lanzarote, Canary Islands, Spain** 5.0x amplification  3,0±0,2% dcII, 95,0±0,5% CA, 0,8±0,1 dclV, 1,0±0,2% dcVII, 0,2±0,0% fk + ka

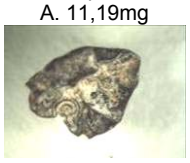
***Dactylopius* specimens from L. Portillo – University of Guadalajara**				
D. coccus, Zapopan **Collection date:** 11/2008 **Local:** Jalisco, Mexico	3.2x amplification A. 1,95mg 	3.2x amplification B. 3,45mg 	3.2x amplification C. 4,52mg 	Grainy, dark brown body with dusty white spots between the stripes. 1,1±0,2% dcII, 97,0±0,4% CA, 0,8±0,1 dclV, 1,0±0,2% dcVII, 0,2±0,1% fk + ka
D. coccus, Coquo **Collection date:** 11/2008 **Local:** Jalisco, Mexico	3.2x amplification A. 5,80mg 	2.5x amplification B. 7,33mg 	2.5x amplification C. 8,42mg 	Dark brown with some yellow wax containing and white spots between the stripes. 1,9±0,6% dcII, 95,7±0,7% CA, 1,3±0,2 dclV, 1,0±0,0% dcVII, 0,1±0,0% fk + ka
D. coccus, Nopalte pec **Collection date:** 11/2008 **Local:** Edo. Mexico, Mexico	2.5x amplification A. 6,21mg 	2.0x amplification B. 12,54mg 	2.0x amplification C. 13,91mg 	Dark brown with high proportion of white spots and less stripes and some reddish spots. 1,5 ±0,2% dcII, 96,2±0,4% CA, 1,0±0,2 dclV, 1,0±0,2% dcVII, 0,2±0,0% fk + ka
D. coccus, A. Requipo **Collection date:** 11/2008 **Local:** Peru	3.2x amplification A. 5,38mg 	2.5x amplification B. 9,29mg 	2.5x amplification C. 7,60mg 	Brown and grainy, with stripes, and some white, yellow and red spots. 2,6±0,3% dcII, 95,2±0,2% CA, 1,2±0,2 dclV, 0,8±0,0% dcVII, 0,2±0,1% fk + ka
D. opuntiae (cockerell) **Plant:** Opuntia Ficus-Indica (L.) Miller **Collection date:** Feb. - March 2009 **Local:** Zapopan, Jallisco, México	2.5x amplification A. 5,43mg 	2.5x amplification B. 5,60mg 	2.5x amplification C. 13,10mg 	Dark brown with pinkish and whitish spots and some yellowish wax content. Hairy with cotton content. Stripped. 1,7±0,8% dcII, 95,4±2,6% CA, 0,8±0,0% dclV, 1,8±1,5% dcVII, 0,3±0,2% fk + ka

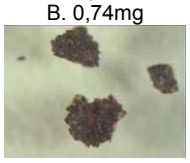
***D. coccus* from Zecchi, Kremer and Dott. Alessandro Bizarri**				
Zecchi Cochineal (coccus cacti)	2.5x amplification A. 14,22mg	2.5x amplification B. 5,60mg	2.5x amplification C. 11,84mg	Dark brown with reddish and white spots, striped.

Source: Florence, Italy				2,7±0,3% dcII, 95,5±0,6% CA, 0,7±0,2 dcIV, 0,8±0,3% dcVII, 0,3±0,2% fk + ka
Kremer Cochineal (coccus cacti) **Source:** Aichstetten, Germany - Materials house for Conservation and Restoration and Fine Arts	3.2x amplification A. 4,05mg 	2.5x amplification B. 9,87mg 	2.5x amplification C. 13,03g 	Reddish dark, stripped and wrinkled. 2,3±0,8% dcII, 96,4±0,9% CA, 0,5±0,1 dcIV, 0,6±0,0% dcVII, 0,2±0,0% fk + ka
Drugstore Cochineal **Source:** Dott. Alessandro Bizarri, Florence	2.5x amplification A. 10,65mg 	2.5x amplification B. 10,85mg 	2.5x amplification C. 8,18mg 	Back brown, wrinkled with white and reddish stripes. 2,6±1,0% dcII, 95,9±1,3% CA, 0,6±0,2 dcIV, 0,6±0,1% dcVII, 0,3±0,1% fk + ka
***Porphyrophora* specimens from Hassan-Ali Vahedi - Razi University, Kermanshah**				
P.Cynodontis **Plant:** on roots of *Cynodon Dactylon* **Local:** Iran, Mezraella, Songhor, Kermanshah	2.5x amplification A. 7,58mg 	2.0x amplification B. 5,54mg 	2.0x amplification C. 8,28mg 	Preserved material dried in alcohol 15%. Rounded shape, reddish hair, with some stripes. It presents two rounded cramps. 2,2±0,7% ppl, 0,4±0,1% dcII, 95,4±1,1% CA, 0,5±0,1 dcIV, 0,5±0,0% dcVII, 1,0±0,2% fk+ka
***P. Cynodontis* (with egg sack)** **Plant:** on roots of *Cynodon Dactylon* **Local:** Iran, Mezraella, Songhor, Kermanshah	2.5x amplification A. 3,20 mg 	2.0x amplification B. 8,36 mg 	2.0x amplification C. 4,91mg 	Dried naturally after oviposition. Rounded shape with a big density of white yellowish hair and a cramp. 2,5±1,5% ppl, 0,6±0,3% dcII, 97,3±0,4% CA, 0,3±0,2 dcIV, 0,5±0,1% dcVII, 0,9±0,5% fk+ka
***P. Cynodontis* (cyst)** **Plant:** on roots of *Cynodon Dactylon* **Local:** Iran, Mezraella, Songhor, Kermanshah	2.5x amplification A. 7,71 mg 	2.0x amplification B. 11,46 mg 	2.0x amplification C. 9,97 mg 	It seems a rounded pinkish and waxy crust. 0% ppl, 0,8±0,4% dcII, 95,1±0,8% CA, 0,9±0,2 dcIV, 0,5±0,1% dcVII, 2,7±0,3% fk+ka
P. Medicaginis **Plant:** on roots of *Mesticago sativa* **Collection date**: 30.VIII.1990 **Local:** Iran, Mezraella, Songhor Kermanshah	5.0x amplification A. 0,76mg 	4.0x amplification B. 0,46 mg 	4.0x amplification C. 0,45 mg 	Little dimensions, with two rounded cramps, some brownish hair. 0,7±0,1% ppl, 0,2±0,1% dcII, 97,4±0,2% CA, 0,5±0,0 dcIV, 0,7±0,0% dcVII, 1,2±0,1% fk+ka
***P. polonica* specimens from Ferenc Kozár - Hungarian Academy of Sciences, Budapest**				
***P. polonica* male** **Plant:** Gypsophila **Collection date :** 14/07/2004 **Local:** Orgovány, Hungary	4.0x amplification A. 0,17 mg 	4.0x amplification B. 0,18 mg 	5.0x amplification C. 0,12 mg 	Samples preserved in 96% ethanol. They exhibit pinkish and reddish colours; probably due to the preserving conditions. 2,4±1,1% ppl, 95,0 ±0,9% CA, 1,0±0,1 dcIV, 1,2±0,1% dcVII, 0,4±0,0% fk+ka
***P. polonica* female** **Plant:** Gypsophila **Collection date:** 22/09/2004 **Local:** Orgovány, Hungary		5.0x amplification 0,11 mg 		Sample preserved in 96% ethanol. Its appearance and little dimensions seem to be due to a significant shrinkage, probably due to the preserving conditions.

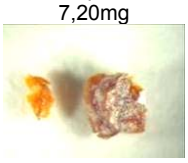
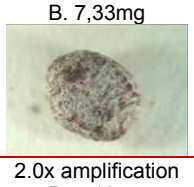
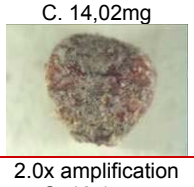
				3,1% ppI, 94,1% CA, 0,9% dcIV, 1,5% dcVII, 0,5% fk+ka
P. polonica nymphs **Plant:** Gypsophila **Collection date :** 22/09/2004 **Local:** Orgovány, Hungary		3.2x amplification 0,10 mg 		Samples preserved in 96% ethanol. The nymphs exhibit pinkish colours; probably due to the preserving conditions they were submitted. 4,7% ppI, 88,1% CA, 1,0% dcIV, 4,1% dcVII, 2,1% fk+ka
***P. polonica* cysts** **Plant:** *Umbelliferae* (*Seseli* genus) **Collection date :** 18/July/1987 **Local:** Hungary	2.0x amplification A. 0,27 mg 	1.6x amplification B. 0,20 mg 	1.6x amplification C. 0,29 mg 	The cysts were found attached to the plant roots. 2,7±1,3% ppI, 92,5±2,6% CA, 0,9±0,8 dcIV, 1,6±0,2% dcVII, 2,3±1,0% fk+ka
***P. polonica* specimens from Katarzyna Golan - University of Life Sciences, Lublin**				
***P. polonica* female** **Plant:** *Scleranthus perennis* **Local:** Roztocze, Poland	3.2x amplification A. 4,05 mg 	3.2x amplification B. 5,17 mg 	3.2x amplification C. 3,68 mg 	Dried samples. Large dimensions, with a brown reddish colour. Red tiny legs. 1,9±3,0% ppI, 94,0±2,2% CA, 0,7±0,2 dcIV, 0,8±0,3% dcVII, 2,5±2,3% fk+ka
***P. polonica* cysts** **Plant:** *Scleranthus perennis* **Local:** Roztocze,Poland	3.2x amplification A. 2,58 mg 	2.5x amplification B. 6,05 mg 	2.5x amplification C. 4,47 mg 	Dried samples compounded by the white cyst and some fine brownish and yellow crusts. 1,0±0,5% ppI, 93,9±1,9% CA, 0,3±0,1 dcIV, 2,4±1,2% dcVII, 2,3±1,2% fk+ka
***P. polonica* specimens from Ewa Simon - University of Silesia, Katowice**				
***P. polonica* female**	2.5x amplification A. 1,89 mg 	3.2x amplification B. 3,60 mg 	3.2x amplification C. 3,70 mg 	Samples preserved in alcohol. Slightly red pinkish insects, with little dimensions, probably due to the preserving conditions. 1,7±1,1% ppI, 92,5±0,7% CA, 0,4±0,2 dcIV, 1,0±0,1% dcVII, 4,5±1,6% fk+ka
***P. polonica* female**	3.2x amplification A. 5,15 mg 	3.2x amplification B. 4,78 mg 	2.5x amplification C. 2,87 mg 	Naturally dried samples. Bigger dimensions and more brownish shade. The hair is still remaining. 4,0±1,6% ppI, 84,7±3,8% CA, 0,5±0,1 dcIV, 0,9±0,0% dcVII, 9,9±2,2% fk+ka

Appendix 5: Unidentified cochineal insect species

Cochineal insects from Royal Botanic Kew Garden, London				
54387 - Cochineal farmed **Plant:** *Opuntia* **Collection date:** 1856 **Source:** James A. Mann (Botanist) **Local:** India (Madras)	2.5x amplification A. 10,05mg 	2.5x amplification B. 7,65mg 	2.5x amplification C. 13,63mg 	Brown with white stripes and reddish spots. Wrinkled, furrowed and ellipsoidal shape.
54388 - Cochineal **Plant:** *Opuntia* **Collection date:** 1918 **Source:** London Drug Market (Trade) **Local:** Peru (Callas)	2.0x amplification A. 11,19mg 	2.0x amplification B. 14,62mg 	2.0x amplification C. 16,74mg 	Black red and shiny, or brown. Wrinkled, with white spots. **Notes:** 43 bags Peruvian cochineal at per lb 28 bags each about 1.0.25 cwt lbs, 15 bags each about 1.0.13 - 43 Dalton & Young 38 Fenchurch street; E.C. 8.v.1918

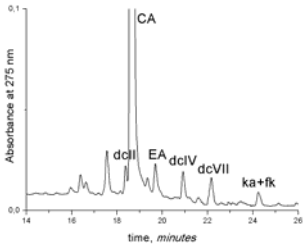
54389 - Prepared cochineal **Plant:** *Opuntia* **Collection date:** 1899 **Source:** Edward Whymper (Explorer) **Local:** Ecuador (Chimborazo Province, Guana)	4.0x amplification A. 1,22mg 	4.0x amplification B. 0,74mg 	4.0x amplification C. 0,93mg 	Dark reddish brown samples belonging to a compact cake, mixture of cochineal ad other components. 
54390 - Cochineal **Plant:** *Opuntia* **Collection date:** Before 1879 **Source:** India Museum **Local:** Indonesia (Java, Buitenzorg, Tyikoppo)	2.0x amplification A. 12,51mg 	2.0x amplification B. 13,19mg 	2.0x amplification C. 16,34mg 	Dark brown with stripes and white and yellow/orange spots. Wrinkled. **Notes:** Class IV, B.5. Lacs, Pigments and Dyes Cochineal (Coccus cacti) from the plantation of Dr Steenstra Touissant
54391 - Cochineal **Plant:** *Opuntia* **Collection date:** Before 1879 **Source:** India Museum **Local:** Indonesia (Java, Buitenzorg, Bandok)	2.0x amplification A. 17,80mg 	2.0x amplification B.14,15mg 	2.0x amplification C.16,61mg 	Dark brown with white spots, that seem wax, and yellow grooves. **Notes:** Class IV, B.5. Lacs, Pigments and Dyes. Cochineal (Cocus cacti) from the plantation at Pondok (Bandok on other label)
54392 - Fine cochineal **Plant:** *Opuntia* **Collection date:** Late 19th century **Source:** A.S. Hill & Son, London (Trade)	2.5x amplification A.21,52mg 	2.5x amplification B.16,64mg 	2.0x amplification C. 26,97g 	Dark brown with white and yellow spots that seem to be wax content. **Notes:** known in commerce as 'Silver Grain'
54393 - Cochineal **Plant:** *Opuntia* **Collection date:** Before 1879 **Source:** India Museum **Local:** India (Andhra Pradesh, Scinde, Hyderabad)	3.2x amplification A.1,67mg 	3.2x amplification B. 2,75mg 	3.2x amplification C. 3,94mg 	Deep and shiny red, or just brown, with grooves and a wax content in the abdomen region.
54394 - Cochineal **Plant:** *Opuntia* **Collection date:** 1867 **Source:** Exposition Universelle (International exhibition, Paris, 1867) **Local:** India (Calcutta)	2.5x amplification A.9,87mg 	2.5x amplification B.8,88mg 	2.5x amplification C.14,20mg 	Rough appearance, striped, dark brown with white spots. Plain abdomen.
54402 - "Granilla" – inferior cochineal **Plant:** *Opuntia* **Collection date:** Late 19th century **Local:** Honduras and Vera Cruz	4.0x amplification A. 1,73mg 	4.0x amplification B. 1,96mg 	4.0x amplification C. 1,18mg 	Wrinkled brown appearance, with white spots. **Notes:** an inferior cochineal imported from Vera Cruz and Honduras.
54403 - Cochineal **Plant:** *Opuntia* **Collection date:** Before 1879 **Source:** India Museum (London) **Local:** India (Punjab)	2.0x amplification A. 16,22mg 	2.0x amplification B. 9,61mg 	2.0x amplification C. 7,08mg 	Very dark brown, with white and red spots. Wrinkled. **Notes:** Animal products
54404 - White cochineal **Plant:** *Ópuntia* **Collection date:** Probably 1851 **Source:** J. Sadler; Probably	2.0x amplification A. 10,22mg	2.0x amplification B. 12,73mg	2.0x amplification C. 9,40mg	Dark brown with orange spots and grooves. **Notes:** Class 4, item 76a in *Official Catalogue*

The Great Exhibition (London, 1851): **Local:** Mexico (Oaxaca)				(International exhibition)
54410 - Stem with Coccus cacti L. (Cochineal insect) **Plant:** *Opuntia* **Collection date:** 1977 **Source:** Jane Stubbs (Botanist) **Local:** Madeira, near Funchal	2.5x amplification A. 6,21mg 	2.5x amplification B. 8,853mg 	2.5x amplification C. 8,40mg 	Dark brown with high white wax content (yellow stains on the white stripes). **Notes:** Section of stem with the scale insect Coccus cacti L.
55338 - Cochineal **Plant:** Eucalyptus **Collection date:** 1855 **Source:** Exposition Universelle (International exhibition, Paris, 1855) **Local:** Australia (NSW)	2.5x amplification A. 1,88mg 	2.5x amplification B. 2,41mg 	2.5x amplification C. 2,04mg 	Rusted colour, with yellow and orange spots. Little dimensions.
55340 - Cochineal **Plant:** Eucalyptus sp. twig **Collection date:** 1862 **Source:** International Exhibition, London (1862) **Local:** Australia (Victoria)	2.5x amplification A. 2,30mg 	2.5x amplification B. 2,19mg 	2.5x amplification C. 1,03mg 	Rusted yellowish colour, with undefined shape.
58236 - 8 varieties of cochineal **Plant:** Opuntia **Collection date:** Late 19th century **Source:** Ripley, Roberts & Co., Indigo and colonial brokers, Mincing Lane, London; Beazley & Co., Dunster House, Mincing Lane, London (Trade)				
58236.1 - Rosy black **From:** Ripley, Roberts & Co. 3. Mincing lane	2.0x amplification A. 20,35mg 	2.0x amplification B. 18,48mg 	2.0x amplification C. 21,92mg 	Dark red, shiny, with light red spots.
58236.2 - Rosy **From:** Ripley, Roberts & Co. 3. Mincing lane	2.0x amplification A. 14,66mg 	2.0x amplification B. 18,25mg 	2.0x amplification C. 14,87mg 	Dark red, shiny, with light red spots. Wrinkled, black stripes.
58236.3 - Dark rosy red, ACW, 6 Bags 1/10, 12/3/81 **From:** Beazley & Co. Dunster House, Mincing Lane	2.0x amplification A. 15,91mg 	2.0x amplification B. 20,63mg 	2.0x amplification C. 19,69mg 	Dark red, shiny, with light red spots. Striped.
58236.4 Weighted 2/5/93 (??) 1316 **From:** Beazley & Co. Dunster House, Mincing Lane	2.5x amplification A. 22,72mg 	2.5x amplification B. 14,91mg 	2.5x amplification C. 11,23mg 	Dark brownish red, shiny, with red sripes. Wrinkled. Apparently an inferior grade than the previously three.
58236.5 - Weighted silver	2.5x amplification A. 18,33mg	2.5x amplification B. 24,71mg	2.0x amplification C. 15,48mg	Salmon colour with dark brown veins. Yellow

From: Ripley, Roberts & Co. 3. Mincing lane				spots.
58236.6 - Granilla wild cochineal **5ptt** **From:** Ripley, Roberts & Co. 3. Mincing lane	5.0x amplification A. 1,04mg 	5.0x amplification B. 0,68mg 	5.0x amplification C. 0,35mg 	Little dimensions, black brownish colour with small particles of white wax, especially in the stripes.
58236.7 - Weighted **From:** Ripley, Roberts & Co. 3. Mincing lane	3.2x amplification A. 12,93mg 	2.5x amplification B. 12,16mg 	2.5x amplification C. 6,56mg 	High white wax content. Wrinkled, dark brown shiny.
58236.8 - Rosy Black **From:** Beazley & Co. Dunster House, Mincing Lane 8-10-92	2.5x amplification A. 5,91mg 	3.2x amplification B. 5,17mg 	3.2x amplification C. 3,97mg 	Dark red shiny colour with bright red spots.
73057 - Cochineal beetles **Plant:** Opuntia **Collection date:** 1800-1857 **Source:** Royal Pharmaceutical Society of Great Britain		2.0x amplification 7,20mg 		Big dimensions orange insect – it does not seem to be cochineal.
73237 - Black cochineal, dried female insects **Plant:** Opuntia **Collection date:** 1851 **Source:** Sadler J, Probably The Great Exhibition (London, 1851) **Local:** Oaxaca	2.0x amplification A. 9,87mg 	2.0x amplification B. 10,58mg 	2.0x amplification C. 14,26mg 	Reddish with orange spots. Wrinkled. **Notes:** Class 4, item 76a in *Official Catalogue* (International exhibition)
Cochineal specimens from Dominique Cardon - CIHAM/UMR, Lyon				
Armenian Cochineal, Porphyrophora hamelii	2.0x amplification A. 13,53mg 	2.0x amplification B. 10,12mg 	2.0x amplification C. 8,83mg 	Dark brown, with stripes. It seems a grain.
American Cochineal, Dactylopius coccus	2.0x amplification A. 14,88mg 	2.0x amplification B. 7,33mg 	2.0x amplification C. 14,02mg 	Reddish brown, oval shape, with a white dust.
Kermes noir **Collection date:** 16/10/2002 **Source:** Athens market	2.0x amplification A. 11,88mg 	2.0x amplification B. 7,10mg 	2.0x amplification C. 12,17mg 	Dark brown, shiny and wrinkled.
Cochineal Dactylopius coccus	2.5x amplification A. 5,70mg	2.5x amplification B. 9,66mg	2.0x amplification C. 17,41mg	Purplish red and brown, shiny, with stripes.

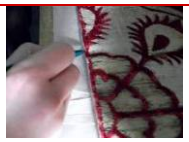
				
Cochineal specimens from Piero Tiano - ICVC-CNR, Florence				
Cocciniglia (Tiano-Fi)	2.5x amplification A. 7,27mg 	2.0x amplification B. 14,42mg 	2.0x amplification C. 10,29mg 	Dark brown and shiny on the back, with white stripes.
Cochenilla (Tiano-Brx)	2.5x amplification A. 9,97mg 	2.5x amplification B. 9,18mg 	2.0x amplification C. 19,64mg 	Grainy red, or shiny, rounded or wrinkled.
Cochineal specimens from Jenny Balfour-Paul – Exeter University				
Cochineal I **Source:** 2004 Conference (France) - Dyes in History and Archaeology	2.0x amplification A. 4,99mg 		2.0x amplification B. 18,28mg 	Reddish pink, hairy, with stripes. Big dimensions.
Cochineal II **Source:** 2004 Conference (France) - Dyes in History and Archaeology	2.0x amplification A.18,86mg 	2.0x amplification B. 18mg 	2.0x amplification C. 20,23mg 	Wrinkled, red colour and scarce hairy.

Appendix 6: Historical Textile Samples

Hstorical Textile	Textile Samples	Chromatograms
1388A, Turkey, 16th/17th centuries 	1.  1.6x amplification 0,85 mg  2.  0.71x amplification 1,52 mg  3.  0.71x amplification 1,35 mg  1. Sample taken on the upper side, from the border, where there is no galloon attached. The sample presents a homogeneous shade. 2. and 3. Samples taken on the back of the piece, both in zones of medallions. They present a homogeneous shade and they were not belonging to the weaving web.	*P. polonica* (?) 

245, Turkey, 16th century


1.

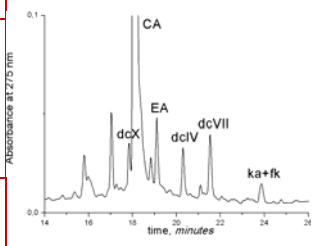

2.

2.5x amplification, 0,24 mg

2.5x amplification, 0,41 mg

Samples taken from the upper side, from the background and from the left border side. They present a homogeneous colour.

P. polonica (?)



1446, Iran, 17th century


1.

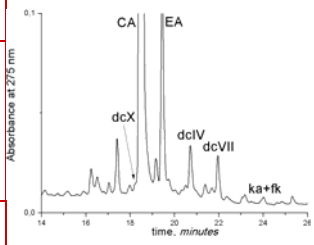

2.

2.0x amplification, 0,74 mg

2.0x amplification, 0,20 mg

Samples taken on the back of the piece, from the protected border, between the textile and the lining. The samples seem to have a homogeneous and lighter shade.

P. polonica (?)



1513, Iran, 16th century

1.

2.

L1.

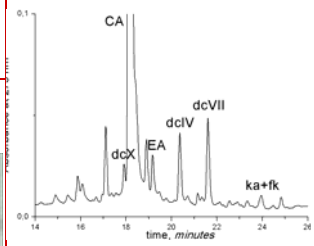
2.0x amplification 0,55 mg

3.2x amplification 0,20 mg

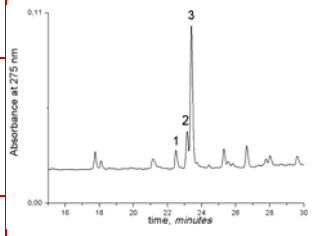
3.2x Amplification 0,10 mg

1. and 2. Samples taken from red flowers, located in two different places of the velvet's skirting. The samples' colour seems to be pinkish and homogeneous.
L1. - Sample taken from a lack zone, on the velvet's skirting. It appears to be a homogeneous yellowish orange.

P polonica (?)



Madder from fibre L1 (Appendix 8)

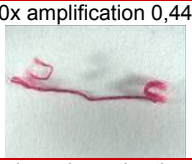



3.


4.

3.2x amplification, 0,22 mg

1.00x amplification 0,44mg



3. Sample taken from the carpet's border, where the threads seem to be unweaved. It pretends to be a deeper red pinkish homogeneous colour.
4. Sample taken from the red flower from the exterior border.

1449, India, 17th century

1. 2. 3.



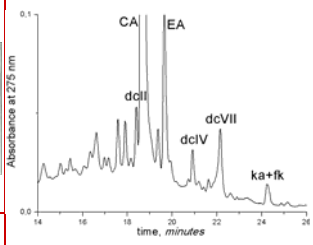
1.6x amplification 0,73 mg

2.0x amplification 0,64 mg

1.6x amplification 0,59 mg

1. Sample taken on the back of the piece, from the protected border, between the textile and the lining. It presents a red and homogeneous shade.
2. Sample taken from the border side. It presents a homogeneous red colour.
3. Taken on the back of the piece, from the protected border, between the textile and the lining. It appears to be homogeneously light reddish.

D. coccus



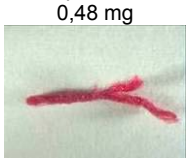
1422, India, 17th century

1. 2. 3.



2.0x amplification 0,74 mg

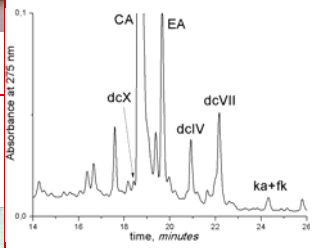
1.6x amplification 0,48 mg



2.0x amplification 0,20 mg

1. and 2. Two unweaved samples taken from the back of the textile. They present a homogeneous red.
3. The sample was taken from a red part of a flower located in the border of the textile. It presents a red homogeneous shade.

P polonica (?)

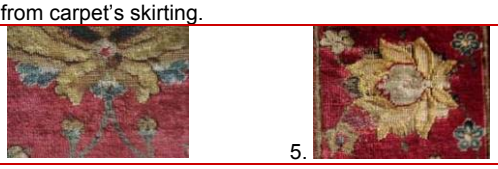


T100, Iran, 16th century

1. 2. 3.

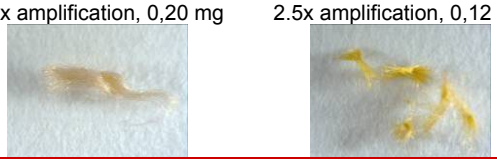
1.25x amplification 3,32 mg | 3.2x Amplification 0,02 mg | 1.6x amplification 1,05 mg

Red pinkish homogeneous samples taken on background areas from carpet's skirting.



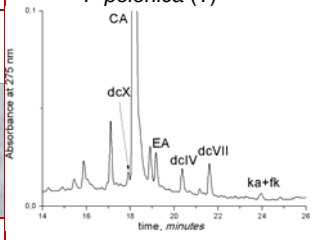
4. 5.

2.0x amplification, 0,20 mg | 2.5x amplification, 0,12 mg

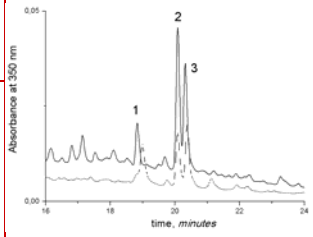


From left to the right, non dyed and yellow samples taken from motif flowers, located in the central composition and in the carpet's skirting, respectively. Obviously, analysis on the non dyed fibre did not identify any colouring compound.

P polonica (?)



Unknown yellow from 5. (Appendix 8)

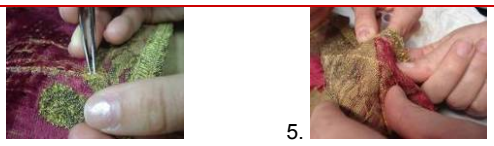


1616 Tec, Italy, 15th century

1. 2. 3.

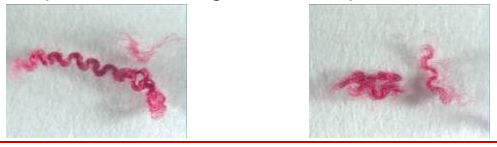
2.0x amplification 0,06 mg | 3.2x amplification 0,08 mg | 2.0x amplification 0,46 mg

Red pinkish homogeneous samples taken from three different flower motifs, located on Portuguese copes' front side.



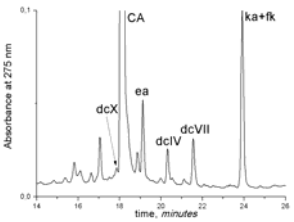
4. 5.

1.6x amplification, 0,40 mg | 2.0x amplification, 0,12 mg



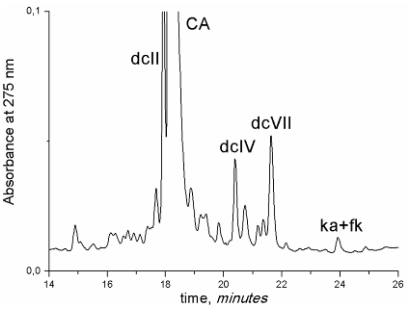
4. Red pinkish homogeneous sample taken from another flower motif, located on Portuguese copes' front side.
5. Homogeneous res pinkish sample obtained from the seam, which attaches the Portuguese copes' front and back sides.

P polonica or mixture with kermes (?)

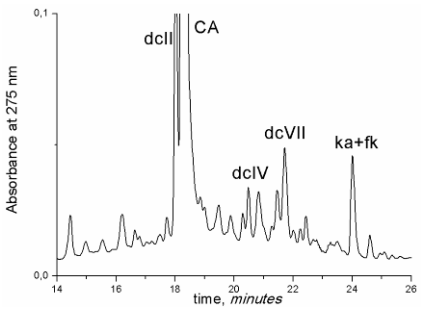


Appendix 7: Cochineal Species Database

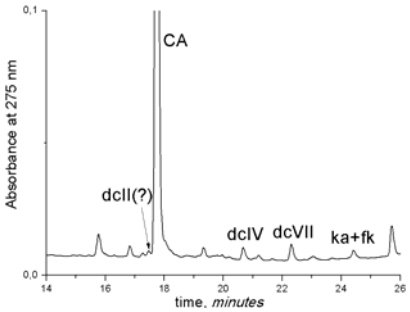
D. coccus



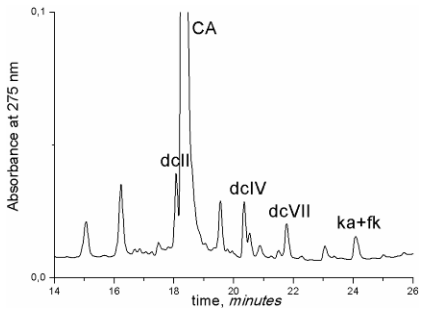
D. confusus



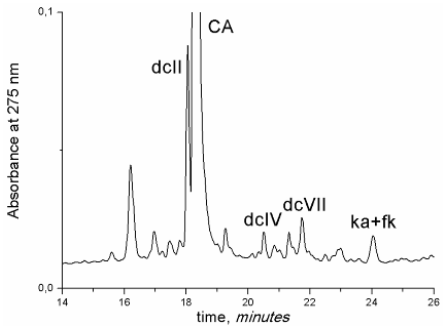
D. tomentosus



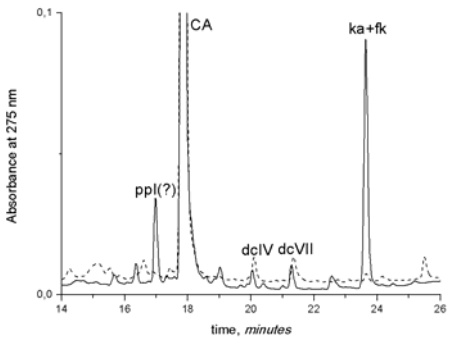
D. opuntiae



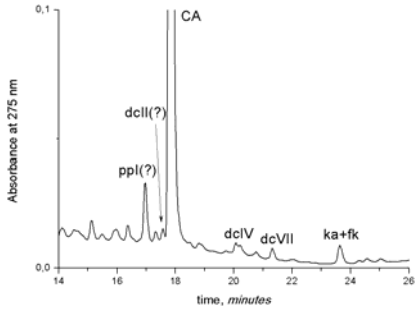
D. ceylonicus



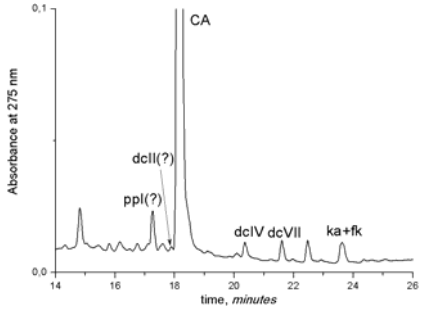
P. polonica



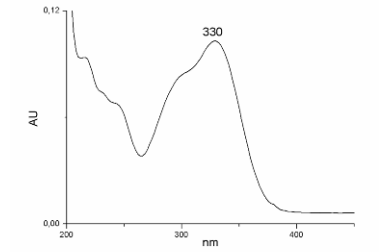
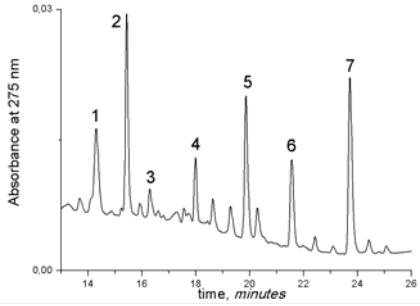
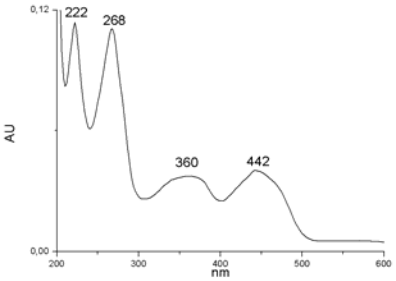
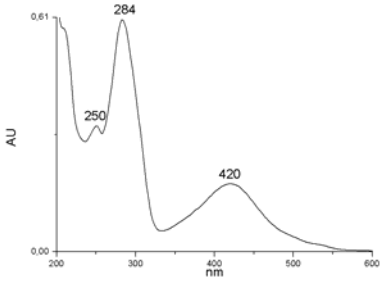
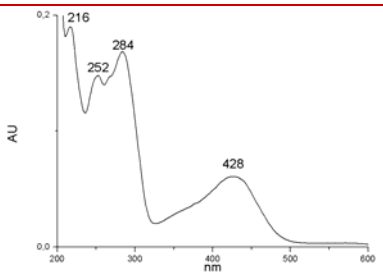
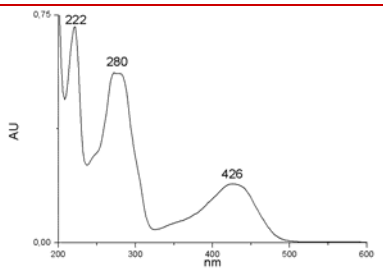
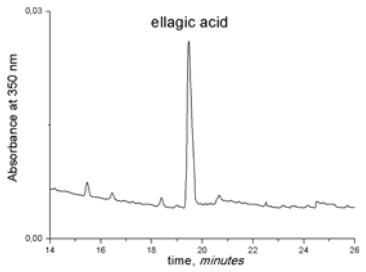
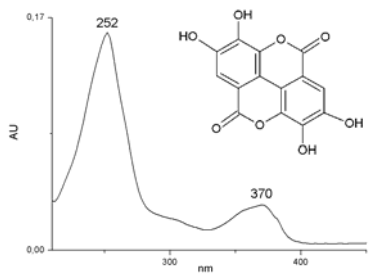
P. hamelii

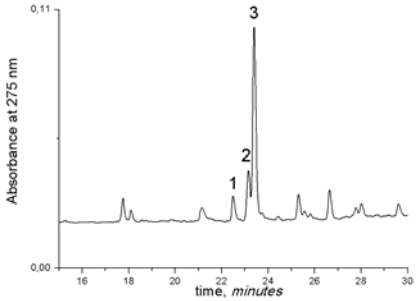
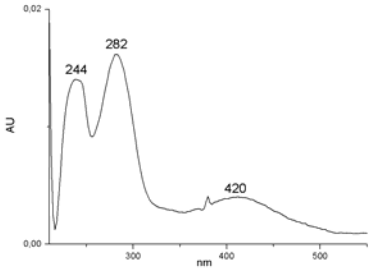
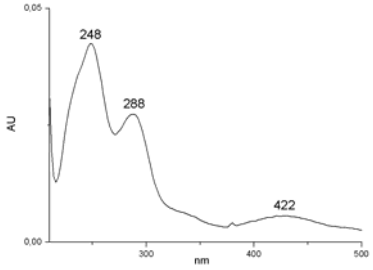
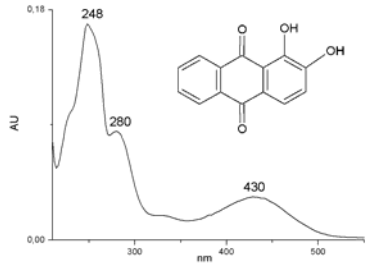
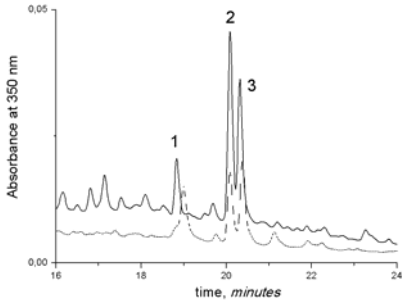
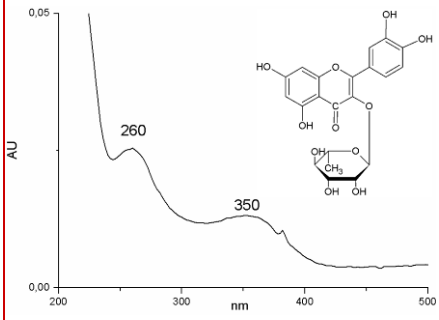
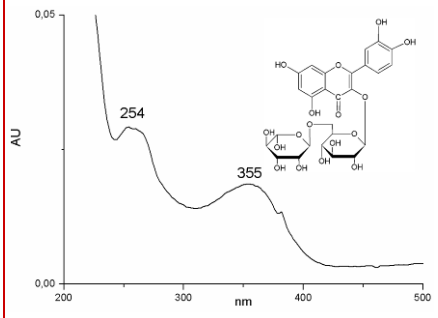


P. medicaginis



Appendix 8: Non-cochineal Samples Database

Representative chromatogram	t_R (min.)	λ_{max} (nm)	UV-vis Spectrum
	1. 14,32 2. 15,42 4. 18,03	330	
Kew Garden yellow (monitored at 350 nm) 	3. 16,30	222 268 360 442	
	5.19,87	250 384 420	
	6. 21,58	216 252 284 428	
	7. 23,75	222 280 426	
Ellagic acid present in historical textiles (monitored at 350 nm) 	19,43	370	

Madder from fibre L1, CGM 1513 velvet (monitored at 275 nm) 	1. 22,48	244 282 412	
	2. 23,13	248 288 422	
	3. 23,40	248 280 430	Alizarin 
Unknown yellow from fibre 5, CGM T100 carpet and MNMC T744 rug (monitored at 350 nm) 	1. 18,90 2. 20,18	260 350	Quercitrin 3-Rhamnoside, 5,7,3',4' - tetrahydrocyflavone (?) 
	3. 22,38	254 355	Rutin, 3-Rutinoside, 5, 7,3', 4'- pentahydroxyflavone (?) 

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