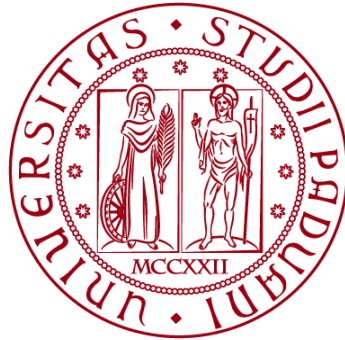


UNIVERSITÀ DEGLI STUDI DI PADOVA

DIPARTIMENTO DI BIOLOGIA

Corso di Laurea in Scienze Naturali



ELABORATO DI LAUREA

**Post-genomic era sheds light on Goethe's Palm
origins (*Chamaerops humilis* L.)**

Tutor: Prof. Francesco Dal Grande
Dipartimento di Biologia

Co-tutor: Dott.ssa Nùria Beltrán-Sanz
Centro di Ateneo Orto Botanico Università di Padova

Laureanda: Agnese Di Marzio

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ABSTRACT

The increasing development of the post-genomic era is becoming more and more reliable among the scientific research field. In particular, reference genomes are acquiring great importance for the conservation of biodiversity. In this regard, one of the aims of this study was to obtain a first draft genome of the non-model palm species *Chamaerops humilis* L. Pursuing this goal drove our research to better understanding the origin of the *C. humilis* specimen present in the Botanical Garden of Padua. This last aspect has been of great importance due to the great cultural and historical significance of this individual, better known as the Goethe's palm. Our results provided us with a fragmented but quite complete genome; moreover, it helped us to understand that Goethe's palm belongs to the western part of the *C. humilis* clusters found among the Mediterranean Basin. In particular, we relied on the information provided by ten microsatellites found across the genome. These have been merged and compared to the Giovino's et al. 2023 dataset. From now on, we only have to wait to see what future studies will reserve for us.

1. INTRODUCTION

After the death of the chaste tree (1984), Goethe's Palm, *Chamaerops humilis* L. - the Mediterranean dwarf palm - became the oldest plant in the Botanical Garden of Padua. Despite the epithet "dwarf", it is known as *Chamaerops humilis* L. var. *arborescens* (Pers) Steud. as its stalks reach 10 metres in height ("Mediterranean palm | OrtoBotanico di Padova," n.d.).

This individual was planted in the Botanical Garden in 1585 ("La gigante nana dell'Orto botanico," 2019). *C. humilis* displays great morphological variability and ecological plasticity (Giovino et al., 2014). It is called Goethe's palm as it inspired the romantic German poet Johann Wolfgang von Goethe to formulate the theory and the relative work "Metamorphosis of plants", based on the observations of the palm's leaves polymorphism where basal-young leaves have whole sheets while higher leaves start splitting, becoming gradually palmate (Figure 1). Despite its cultural value, no information has been retrieved to shed light on its provenance.



Figure 1. *Chamaerops humilis* L. "Tavola VII tratta da Piante del R. Orto botanico di Padova". Century XVIII-XIX, tempera on paper, (525mm x 370mm), Biblioteca Orto Botanico O.f.g. 42. Image taken from <https://phaidra.cab.unipd.it/o:25941>

Chamaerops humilis is one of the two European native palms, together with *Phoenix theophrasti* Greuter (Vardareli et al., 2019), and it is the only native palm in the West Mediterranean region (García-Castaño et al., 2014). *C. humilis* originated in the Western-Central Mediterranean Basin and then separated from *Trycarpus* during the Miocene in a frame of 8.32-5.83 Ma years ago (García-Castaño et al., 2014; Giovino et al., 2020). In particular, data seems to suggest a first biogeographical divergence between East of Spain and Italy. Subsequently,

from Italy populations the palm expanded southward to Sicily, up until Tunisia and Morocco, reaching the High Atlas. Finally, *C. humilis* settled westward and northward returning to Peninsula Iberica (García-Castaño et al., 2014).

This plant belongs to the *Arecaceae* family and it is the only species of the genus *Chamaerops*. It is an evergreen and usually small palm, even though there are many morphological variants, for example with palmated highly-lobed leaves. It is diploid ($2n=36$) and dioecious with rigid panicles constituted by little yellow flowers, usually pollinated by weevil species or coleopterans attracted by the scents released by the leaves (Guzmán et al., 2017; Mai, 1989). Fruits are fleshy reddish-yellow poly drupes (grouped in 1-3 drupes) and the dispersion of seeds is mammal-mediated. (Figure 2) (Guzmán et al., 2017). It is well adapted in arid and semi-arid environments and it can adapt to harsh conditions as it is highly resilient. (Giovino et al., 2020).



Figure 2. *C. humilis* subsp. *humilis* Palm and fruits. Image taken from https://dryades.units.it/asinara/index.php?procedure=taxon_page&id=7844&num=5399

The genetic structure of this species has been recently investigated by Giovino and collaborators (Giovino et al. 2023). Using 10 SSR (Simple Sequence Repeat) loci, the authors found out that populations are distinguished into two main

genetic clusters. In particular, the western cluster groups together populations from Spain, Portugal, Algeria and Morocco, while the eastern one comprises populations from Italy, Tunisia and France. Furthermore, they could observe that spatial proximity is associated with specific genetic structures; in particular, the eastern part of the distribution appears to be characterised by a low polymorphism and genetic variation (Fig. 3). This is probably due to the lower connectivity and geographical proximity among the eastern populations, likely caused by some biogeographical changes, such as the Mediterranean salinity crisis (6.0-5.3 Ma years ago), the Quaternary glaciations (ca 2.5 Ma years ago) and the subsequent weakening of this species diffusion (García-Castaño et al., 2014; Guzmán et al., 2017). On the other hand, the populations from higher northern-Morocco and southern-Spain are connected via a higher gene flow (Giovino et al., 2023; Guzmán et al., 2017).

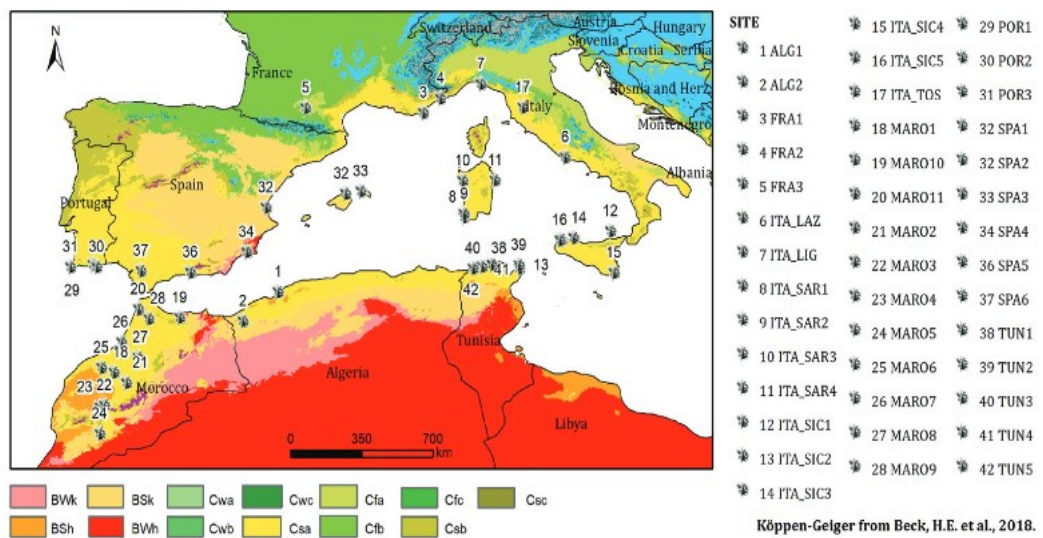


Figure 3. The figure represents the genetic landscape analysis for 42 populations of *Chamaerops humilis*, based on variation for ten microsatellite loci (from Giovino et al. 2023). Red areas represent lower distance by genetic relationship and more genetic variation, compared to green areas. Yellow areas show intermediate relationships.

Chamaerops humilis is widely found across the Mediterranean Basin even though its range appears to be in a continuously declining trend. However, it has been classified by the IUCN red list as *least concerning* species (Errol Vela

(University of Montpellier and Lauren Gardiner (Cambridge University Herbarium, 2016) and it is often present in large populations. The causes of this trend mostly refer to the increasing presence of urban settlements and anthropic disturbance such as fire, trade of invasive plants, which could compete in *C. humilis* natural environment, its plant and seed collection for ornamental use, etc. Concerning this latter aspect, both natural and cultivated specimens are used for ornamental purposes. Indeed, this practice can cause genetic contamination between wild and domesticated individuals (Giovino et al., 2014), as well as fragmentation of natural population and habitats. Overall, since ancient times *C. humilis* has always had a great economic value for local trades and activities such as food sources or handicrafts products (Guzmán et al., 2017; “Palma di S. Pietro | OrtoBotanico di Padova,” n.d.).

Given the constantly decreasing biodiversity and loss of locally adapted populations caused by the accelerating environmental changes, implementing post-genomic technologies is more and more crucial. It is necessary to clarify that post-genomic era is the period after the achievement of the whole human genome sequencing in 2003 (“The Human Genome Project,” n.d.). Indeed, it has been a starting point to implement new biomedical researches and to give access to whole genome sequencing of many other organisms. During the last decades, the post-genomic era developed High-Throughput (HT) sequencing in order to obtain reference genomes. These play an important role in conservation and restoration, as discussed below. The high-throughput sequencing technologies are considered of great importance in acquiring useful genetic information about non-model organisms. HT sequencing allows accurate long and short reads, and in a cost-time efficient way. Compared to DNA barcoding, single or few loci phylogenies and other traditional methods limited in throughput, HT allows researchers to sequence thousands of samples in a single run, instead of reading one stretch of DNA at one time (Kennedy et al., 2020). It also allows to obtain less fragmented sequences. Genome-wide data provided by HT gives access to a significant range of ecological information about adaptation and evolution of natural populations, ecosystems and

genetic diversity of plants. It is useful also to understand more in depth the wide polymorphism and the adaptive plasticity of *Chamaerops humilis*.

As a non-model organism, genetic research about *Chamaerops humilis* is lacking. However, as HT sequencing becomes more accessible, the availability of low coverage Whole-Genome Sequencing (lcWGS) of population samples or Reduced Representation Sequencing (RRS) is increasing. This kind of application provides information about the variability of alleles at the population and landscape level (Christiansen et al., 2021; Ellegren, 2014). Moreover, in this specific case of study it could help to better understand the genetic relationship of *C. humilis* with other *Areaceae* palms and the future conservation of its biodiversity.

A contiguous and accurate assembly of an organism's genome can lead to the creation of a reference genome, which is representative of the genetic structure of its species. In particular, reference genomes can be useful to understand the functioning of a species in relation to its natural ecosystem, with the purpose of preserving biodiversity, protecting and restoring habitats ("A reference standard for genome biology," 2018; Formenti et al., 2022). Concerning this issue, the European Union is funding initiatives such as the European Reference Genome Atlas (ERGA). The main aim of this project is to generate reference genomes for all European eukaryotic species due to the fact that almost one fifth of the ca. 200,000 species are at risk of extinction. A similar organisation has been established globally with the so-called Earth BioGenome Project (EBP). ("The era of reference genomes in conservation genomics - ScienceDirect," n.d.). These new projects and initiatives are crucial to deeply understand the function of biodiversity and to implement new restoration and conservation strategies.

Concerning the introductory aspects illustrated, the objectives of this project are two-fold: 1) to obtain a first draft genome of the *C. humilis*: it will be implemented using Single Molecule Real-Time sequences (SMRT) PacBio sequencing in order to provide highly accurate long-read sequences. Obtaining a draft genome, the prior step to building a reference genome, could possibly lead to significant population-scale information on a non-model species, in a cost and time

efficient way; 2) to shed light on the provenance of the Goethe's palm by comparing its diversity at 10 SSR loci with the dataset of Giovino et al. (2023), implementing DAPC analysis using R software. Furthermore, this last goal may lay the foundation for new historical and scientific research concerning both Goethe's Palm and the Botanical Garden of Padua.

2. MATERIALS AND METHODS

2.1 DNA isolation and long-read Genome sequencing

A fresh young, basal leaf has been collected from the so-called Goethe's Palm, an individual of *Chamaerops humilis* located at the Botanical Garden of Padua and then sent to the LOEWE Translational Biodiversity Genomics centre in Frankfurt, Germany, where its DNA was extracted.

The DNA has been extracted from fresh samples using a modified CTAB (cetyltrimethylammonium bromide base) method (Cubero and Crespo, 2002). This type of DNA isolation helps denaturing or separating polysaccharides and phenols which reduce the DNA purity. Also, this method is the most suitable for maintaining the integrity of the DNA during the extraction.

For sequencing, a long-read approach was used, namely the Pacific Biosciences' single molecule real-time (SMRT) sequencing technology. SMRTbell library preparation has been conducted following the manufacturer's protocol and using a DNA Template Prep Kit. A SMRTbell library, also known as SMRTbell template is a double stranded DNA capped with hairpin adapters at the extremity of the strand (SMRT bell adapters).

Two runs of SMRT sequencing on the Sequel system II were performed. Sequel system II is a type of machine containing many chips called SMRT cells and a DNA-sequencer, constituted by a nanophotonic structure, the Zero-mode waveguide (ZMW). Every SMRT cell contains ca. 150.000 ZMW. At the end of the ZMW there is an immobilised DNA polymerase which adds nucleotides

generating a *de novo* DNA strand. The bases in the nucleotide are labelled, then the incorporation of the nucleotide is reported by the emission of a coloured fluorescent pulse in real time. The different colours for every pulse emitted identify a specific base (Roberts et al., 2013).

After these steps, it is possible to get a circular consensus read (CCS), when the insert size of the sequence is <20 kb, or a continuous long read (CLR) when the insert size is >20kb. In this study a circular consensus read (CCS) has been generated (Fig. 4). The CCS was the result of the alignment of the subread taken from the new DNA strand synthesised and it can generate the so-called HiFi reads. High and accurate long-read sequencing produces the so-called HiFi reads which are a smart combination between the high accuracy of short reads and the bigger picture of the genome given by long reads. This sequencing approach allows us to obtain reads hundreds of times the length of short reads with an accuracy of 99.9% at lower costs and shorter time, compared to other sequencing methods (Blood, 2023; “How HiFi sequencing works,” n.d.).

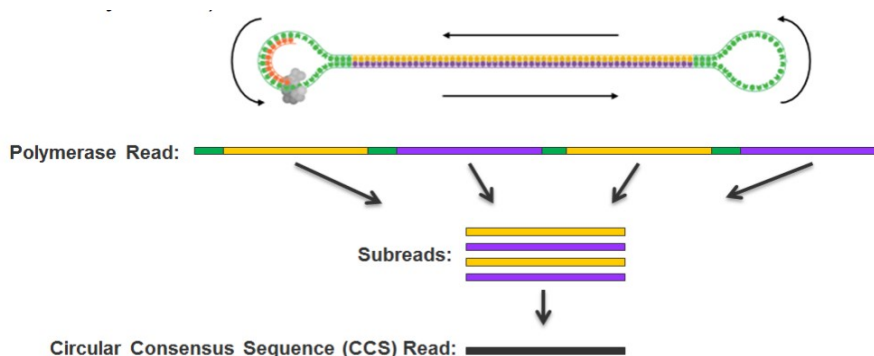


Figure 4. Passages from library preparation to generation of a Circular Consensus Sequence. Image taken from <https://www.pacb.com/wp-content/uploads/2015/09/Pacific-Biosciences-Glossary-of-Terms.pdf>

2.2 *C. humilis* genome assembly

CCS reads were first processed into highly accurate ($> 99.8\%$) consensus sequences. For this purpose, DeepConsensus (Baid et al., 2023) was used. This is an algorithm which helps reduce errors in HiFi sequences, thus resulting in more accurate reads. In particular, subreads obtained from the sequencing have some different, randomly distributed-errors (e.g., insertions, deletions and mismatches) and the main aim is to combine them to get a more correct consensus read. DeepConsensus further uses consensus reads and subreads to generate more accurate reads. To do this it uses a transformer architecture which works with subreads and CCS reads and splits them in 100 bp windows. Each window goes to a transformer model which extracts information about it. Then, every window goes through the DeepConsensus model which is an Encoder-only transformer that polishes the read by combining and stitching all the polished sequences obtained from each window (Baid et al., 2023) (Fig. 5).

Finally, *de novo* assembly of the *C. humilis* genome has been performed using the Hifiasm genome assembler (Cheng et al., 2021, 2022), which is a software specifically developed for HiFi reads that preserves the contiguity of all the haplotypes for the purpose of phasing the genome.

Basic assembly statistics were calculated using Assemblathon 2 (Bradnam et al. 2013). Genome completeness for both haplotypes was estimated using BUSCO v5 (Manni et al., 2021).

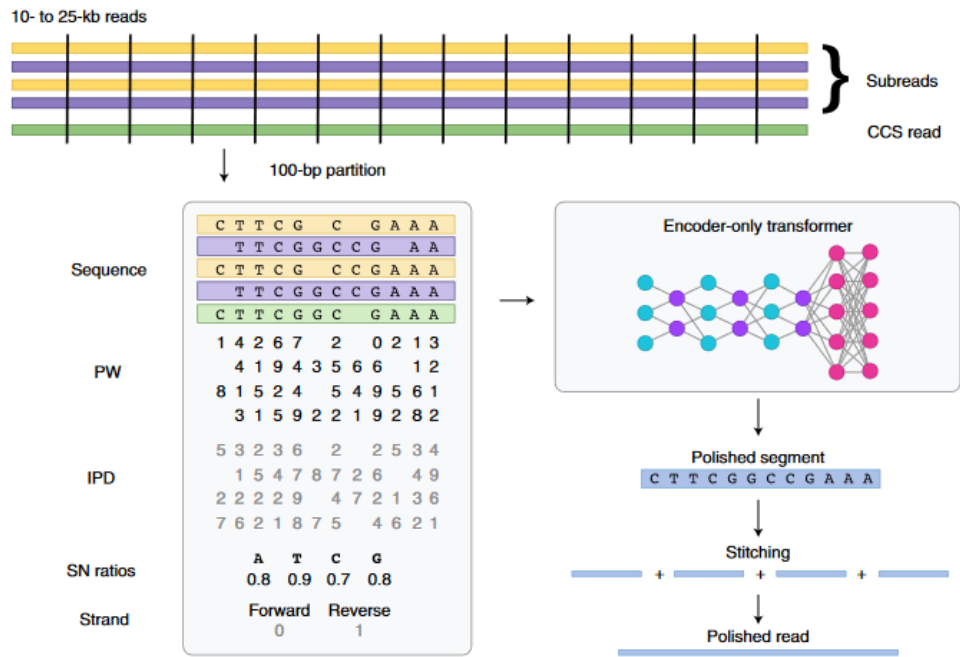


Figure 5. DeepConsensus workflow is illustrated in the image. Subreads of 10 to 25 kb are aligned to get a CCS read, then divided in 100-bp partitions. Each partition (window) goes to a transformer model. DeepConsensus polishes all the segments, then it stitches them to obtain a final polished read. Image taken from Baid et al., 2023.

2.3 Inferring the origin of Goethe’s palm

In order to infer the provenance of the Goethe’s palm, we compared the genetic variability at 10 SSR (Simple Sequence Repeats) loci (Giovino et al., 2023) with the genetic variability of the species across its entire distribution range (Giovino et al., 2023). SSRs of 2-5 bp are neutral markers dispersed throughout the genome that have often been used to study genetic similarity among individuals of a species, or DNA mutation rate, which frequently leads to high polymorphism, in diverse groups of organisms.

To perform this comparison, we first extracted the alleles at the 10 SSR loci from the newly obtained *C. humilis* genome. To do that, we used the Blast nt algorithm (“BLAST: Basic Local Alignment Search Tool,” n.d.) to locate the primer sequences of all SSR loci in the genome of both haplotypes.

Once the loci were extracted, we merged the data with the allele table from Giovino et al. (2023) (see Appendix 1) and performed a Discriminant Analysis of Principal Component (DAPC) using the ADEGENET package 2.1.1.0 (Jombart et al., 2023) in R (Jombart, 2008). ADEGENET is a package with specific functions used to implement multivariate analysis (as DAPC and PCA) in order to store, handle and analyse genetic markers.

DAPC is a multivariate analysis useful to identify and describe clusters of genetically related individuals. DAPC is composed by PCA (Principal Component Analysis) and DA (Discriminant Analysis), and the latter one aims to maximise the differences between-groups instead of within-groups, achieving the discrimination of individuals into groups or clusters defined (Fig. 6) (Jombart et al., 2010). It also provides a probabilistic assignment of individuals to each cluster (see DAPC results). The meta-analysis was performed in RStudio (“RStudio,” 2023).

DAPC was performed using the `dapc` function on the `genind` object obtained by importing the merged allele table.

The script used for the analysis is given in Appendix 2.

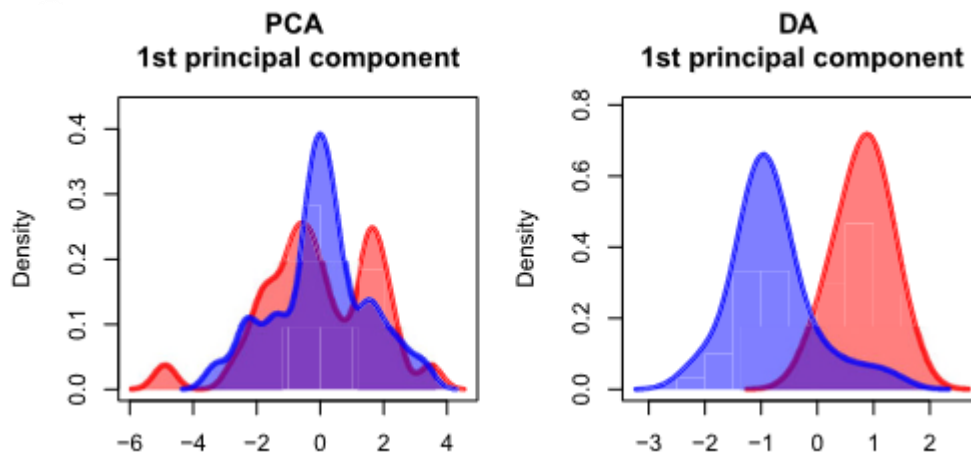


Figure 6. The picture illustrates the difference between PCA and DA analyses. PCA shows the variability among individuals (variation within groups), whereas DA represents groups differences. Image taken from Jombart et al. 2010.

3. RESULTS

3.1 PacBio sequencing and genome assembly

We used two PacBio SMRT sequencing runs to obtain a total of 11,592,180 subreads of 11 kb mean length. After running DeepConsensus, we obtained a total of 5,678,973 HiFi reads. Summary assembly statistics for both haplotypes are given in Table 1.

Table 1. Assembly statistics for the two haplotypes of Chamaerops humilis

Results	Haplotype 1	Haplotype 2
Number of contigs	5193	4833
Total size of contigs (Gb)	4039879672	3921249479
Longest contigs (Mb)	21541770	27303349
Shortest contigs (kb)	3082	8230
Mean contigs size (kb)	777947	811349
Median contigs size (kb)	352136	405490
N50 contigs length (Mb)	1796000	1675054

3.3 Genome completeness

Completeness of the genome has been estimated for both haplotypes using the software Benchmarking Universal Single-Copy Orthologs BUSCO. For haplotype 1 we obtained 406 genes of complete BUSCOs out of 425 genes expected, 6 fragmented and 13 missing genes. While for haplotype 2 we have reached 410 out of 425 genes expected, 3 fragmented and 12 missing genes. (Fig. 7).

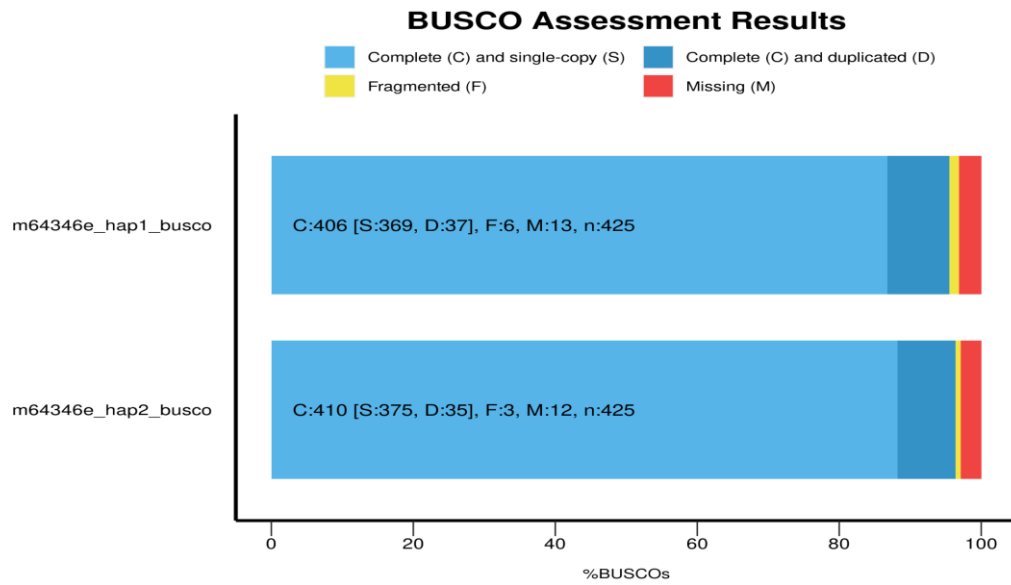


Figure 7. Barplot showing the completeness of the draft genome assembly of *C. humilis* as calculated in BUSCO v5. For both haplotypes we obtained a completeness greater than 95%. Missing genes results are reported in Appendix 3.

3.6 DAPC results

Performing DAPC analysis using the dataset of ten SSRs, our palm grouped within the western genetic cluster of *C. humilis*; in particular, it seems to have similarities with the Spanish and Moroccan populations (Fig. 8). The first PC axis explained 58,8% of the variance, while the second PC axis explained 17,7% of the variance.

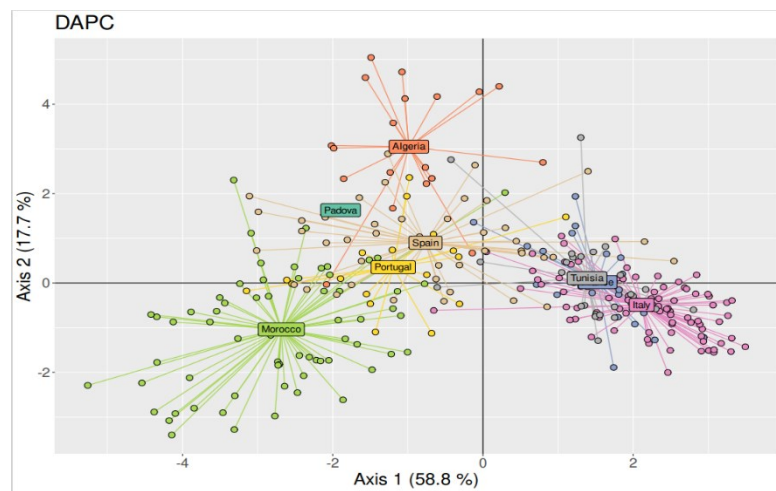


Figure 8. DAPC analysis of the *Chamaerops humilis* population clusters. Axis 1 represents the PC1 (58.8%), Axis 2 represents the PC2 (17.7%).

4. DISCUSSION

4.1 *C. humilis* draft assembly

We obtained the first *C. humilis* draft genome assembly using a PacBio HiFi long-read sequencing approach. The genome length of 4.4 Gb obtained is in line with the expected genome size for palm species, usually between 800 Mb and 3 Gb (Wang et al., 2022).

Table 2. Comparing the genome lengths of eight species of palms belonging to the *Arecaceae* family with the *C. humilis* one.

Palm species (<i>Arecaceae</i> family)	Genome size
<i>Chamaerops humilis</i> L.	4.4 Gb
<i>Areca catechu</i> L.	2.8 Gb
<i>Calamus simplicifolius</i> C.F.Weii	2 Gb
<i>Cocus nucifera</i> L.	2.1 Gb
<i>Elaeis guineensis</i> Jacqu.	1.5 Gb
<i>Elaeis oleifera</i> (Kunt) Còrtes	1.4 Gb
<i>Metroxylon sagu</i> Rottb.	472.4 Mb
<i>Phoenix dactylifera</i> L.	772.3 Mb
<i>Phoenix roebelenii</i> O'Brien	471.7 Mb

Almost eight species of the *Arecaceae* family have been sequenced in the last decade, such as *Phoenix dactylifera* and *P. roebelenii*, *Cocus nucifera* and *Elaeis guineensis* (Table 2) (“Genome,” n.d.). These species in particular, including *C. humilis*, have an important socio-economic value as they have been used principally as palm crops, manufacturing, ornamental exploitation and other human activities.

The first palm genome was the one of *Phoenix dactylifera* L. (Fig. 9), a species of great economic importance, endemic to the Arabian Peninsula and cultivated between the Euphrates and Nile rivers since 3700 BC (Al-Mssallem et al., 2013). The species was sequenced in 2011, with last updated draft in 2019, for a total of 772.3 Mbp in size, 36,764 genes and a contig N50 of 897.2 kb (“Phoenix dactylifera Annotation Report,” n.d.).



Figure 9. *Phoenix dactylifera* L. Image taken from https://dryades.units.it/torlonia/index.php?procedure=taxon_page&id=8572&num=6139

Another palm of economic importance whose genome has been sequenced is the sago palm *Metroxylon sagu* Rottboll (Fig. 10). This is a tropical halophytic palm native to the Southeast Asian countries that produces sago starch. *Metroxylon sagu* genome has been sequenced using Illumina obtaining a low BUSCO genome completeness score (21,5%) for its ~500 Mb size. This has been recently improved using the Oxford Nanopore long-read sequencing technique (Lim et al., 2022). The updated, hybrid genome assembly achieved a genome completeness of 97.9%.



Figure 10. *Metroxylon sagu* Rottboll. Image taken from <https://www.gbif.org/species/2733781>

On the one hand, these reference genomes provide genome-scale data and are useful to build a robust phylogenomic framework which is often linked to the realisation of phylogenetic trees. If in the past phylogenetic trees were mainly used in the systematic field, nowadays they have been implemented to predict gene and genome functions, to study gene family evolution and for phylogenetic comparative researches on monocot group of Palms (order Arecales, family *Areaceae*). Furthermore, detailed and accurate palm phylogenetic trees represent an important tool to understand divergence time estimates, morphology, ecology and genetic macroevolution. Moreover, at the level of palm species phylogenetic trees could achieve a great importance in the economic field of crop plants and for understanding tropical forest paleoecology (Barrett et al., 2016). Lastly, *C. humilis* draft genome represents a first step toward a better understanding of the genetic basis of environmental adaptation of the species. For instance, with the help of the annotated draft, it would be possible to infer if the species' high polymorphism is

due to genetic variation or natural selection, avoiding long time sequencing and high costs.

Concerning *C. humilis*, this is a wide range distributed species with such great diversity. From a study based on 10 SSR markers and comparing 35 genotypes (Giovino et al., 2020), the analysis showed that genetic variation seems to be correlated with the presence of rare alleles; these are restricted to some genotypes belonging to particular environments with associated stress conditions, which also influence morphological traits. In particular, two different subspecies can be identified. Among Moroccan coasts it is possible to find the common *C. humilis* subsp. *humilis*, while harsh conditions and high altitudes on High Atlas (4167 m) and Anti Atlas (3304 m) Mountains (Morocco) led to the isolation and subsequent differentiation of *C. humilis* subsp. *cerifera* Becc. (Fig. 11). The latter is also known as *Chamaerops humilis* var. *argentea*, even though this name seems not to have ever been published (Giovino et al., 2020). This species is characterised by grey-silver leaves, for the great presence of waxes on the surface, covered with scaly hairs and characterised by a compact tuft habit. (Giovino et al., 2020).



Figure 11. *Chamaerops humilis* subsp. *cerifera*. Image taken from <https://www.plantdelights.com/products/chamaerops-humilis-var-argentea>

4.2 Shedding light on Goethe's Palm's origins

Following the approach by Giovino et al. (2023) we extracted allelic information at 10 SSR loci from our newly obtained draft. This clearly indicated that Goethe's palm belongs to the western genetic lineage of the species.

To more accurately pinpoint the origin of Goethe's palm individual, the individuals of this large dataset could be sequenced using a low-coverage individual whole-genome resequencing (lcWGR) method (Fig. 12c). This method aims to obtain a 2-4 x coverage per individual (Lou et al., 2021) to analyse and get an accurate population-scale screening with a high cost-benefit ratio.

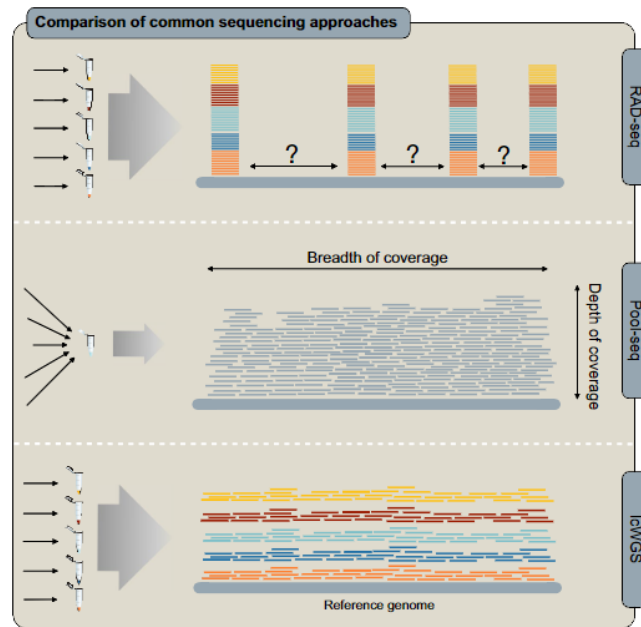


Figure 12. In this image three different sequencing approaches are illustrated. They allow to obtain reference genomes at various depths: **a)** RAD-seq; **b)** Pool-seq; **c)** lcWGS. In 12c many separated barcoded individuals are represented. The sequence data obtained are spread across the genomes in order to maximise the information content. Information about individual genotype is sacrificed for a greater breadth of coverage. Images taken from Lou et al., 2021.

Another example of low-coverage whole genome sequencing implementation for non-model species is the study by Luqman et al. (2023). In this instance, the authors focussed on how climate-change could influence population range shift by directly relating post-Last Glacial Maximum (LGM) environmental change with allelic adaptive responses in *Dianthus sylvestris* Wulfen. Occurrence of adaptive response mechanisms could be observed and analysed by modelling genotype to environmental pressure. In particular, this study showed that this

species comprises three distinct genetic lineages, namely in the Alps, Apennines and Balkans. Divergence occurred during the Penultimate Glacial-Interglacial Period ca 200-150 ky ago. More than 1000 individuals were sampled across these regions from more than 100 populations and sequenced at 2x depth of coverage. Secondly, the phylogenetic trees of these three distinct lineages inferred implementing lcWGS have been useful to get information about the microevolutionary patterns in *D. sylvestris*.

Our results constitute the basis upon which a targeted search of the historical archives could be performed to cross-check science-based inference with historical proof. *Chamaerops humilis* used to have a great socio-economic importance in the Mediterranean trade as its leaf tissues were used for producing brooms and other handicrafts, its sprouts were cooked and its underground parts used to add flavour (García-Castaño et al., 2014; “Palma di S. Pietro | OrtoBotanico di Padova,” n.d.). Nowadays, it is still used by basket manufacturers, for ornamental purposes and it replaces animal hair in industrial usage (Lachkar et al., 2020).

In particular, more in-depth searches, e.g., in the Historical Archive of Venice, should be done on the trade in the 16th century between western Mediterranean localities and the Republic of Venice. Intriguingly, from the XV century onwards, commerce from Maghreb to Venice, passing through Tripoli and Gerba (Tunisia), was flourishing. From these western countries, some of the typical traded products were cotton, oil, dates, peanuts, flavours, a great variety of spices and tissue (Pedani, 1992). Moreover, at the beginning of the XVI century a Moroccan Amir’s commercial Treaty with Venice 913/1508 was signed in Badis (Wansbrough, 1962) which was the only Moroccan port not occupied by the Spanish and the Portuguese. The treaty aimed to regulate trade between Morocco and the Republic of Venice, which had great influence and economic interests in that geographic area. Indeed, in the middle of the XVI century, new commerce was established and other treaties made with Morocco had been signed in 1765 and 1795. (“APPUNTI SUL CONSOLATO VENETO IN MAROCCO NELLA SECONDA METÀ DEL XVIII SECOLO on JSTOR,” n.d.).

The type of products traded, such as oil and flour, and the amount of historical documentation concerning Venice's trades with the West Mediterranean could constitute a starting point and new research hypothesis related to the origin of Goethe's Palm. In this regard, in the future it would be interesting to find the original population of this individual in order to better understand the genetic background of the palm and consequently the distribution of its genetic lineage.

5. CONCLUSIONS

A new era is starting to unfold in the field of biology and genomics with reference genomes. New research and knowledge horizons are giving the opportunity to conserve, restore and monitor the natural world. This is crucial especially for globally endangered species due to climate change and the increasing loss of biodiversity. Furthermore, reference genomes and high-throughput sequencing methods are introducing more and new efficient ways of reconstructing the evolutionary history of species such as finding points of divergence among phylogenetic trees and the relative causes that led to differentiation of lineages.

In our case, we managed to add more information on the *Chamaerops humilis* palm and its distribution. In particular, we tried to understand why it is a species well adapted to many different environments and climatic conditions around the Mediterranean Basin, as it is the only native European fan palm, and to unravel the deep genetic causes of its polymorphism and variability. This represents a starting point to better understand the phylogenetic relationships not just among the *Chamaerops* genus, but around the whole *Arecaceae* family and also what led to the differentiation of these species.

This special palm was the plant which led to Goethe's illuminating theory of the Metamorphosis of Plants, which states that the morphological and structural development of every organ such as the goblet, the fruit and gynoecium and androecium originate from a primary leaf. Furthermore, it is the most ancient specimen of the Botanical Garden and the application of HT sequencing and

microsatellites methods allowed us to find out its western origin. Beyond genetic and naturalistic importance, this palm can be a valuable resource to shed light on the historical aspects of the Botanical Garden of Padua that are still little known.

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APPENDIX

1. SSR Goethe's Palm merged with Giovino's Dataset

sample	location	locus19	locus25	locus27	locus15	locus16	locus23	locus35	locus37	locus44	locus48
Goethe	Padova	177/165	201/0	124/124	176/176	136/136	190/190	0/0	0/158	121/121	0/0
A1_1	Algeria	177/181	227/227	142/142	199/205	154/154	214/214	119/119	155/167	132/140	156/156
A1_10	Algeria	185/185	221/230	142/145	199/205	154/154	214/214	101/121	169/169	132/136	140/140
A1_2	Algeria	185/185	227/233	133/142	199/205	154/154	214/214	101/119	169/175	132/136	136/136
A1_3	Algeria	177/181	224/233	136/136	199/199	154/154	214/214	109/119	153/167	132/136	136/140
A1_4	Algeria	181/185	224/227	136/136	199/205	157/157	211/214	105/121	167/167	132/136	136/142
A1_5	Algeria	181/189	224/227	157/157	199/205	154/154	214/214	101/101	151/167	132/136	140/140
A1_6	Algeria	181/189	236/239	136/145	199/205	154/154	214/214	101/121	167/167	132/136	136/142
A1_7	Algeria	181/189	224/227	136/145	199/199	154/154	214/214	101/101	167/167	132/136	136/136
A1_8	Algeria	181/185	224/224	133/136	199/199	154/157	214/214	101/101	167/167	132/140	140/146
A1_9	Algeria	185/189	224/230	133/157	199/199	154/154	208/214	101/121	169/169	132/136	142/142
A2_1	Algeria	185/185	227/233	136/136	199/199	157/157	211/211	101/121	151/167	132/136	140/140
A2_10	Algeria	185/185	233/236	142/148	199/199	154/154	211/211	101/121	163/185	132/136	136/136
A2_2	Algeria	181/181	218/227	136/136	196/196	154/157	214/214	101/121	167/169	132/136	136/140
A2_3	Algeria	181/185	227/227	136/136	199/199	154/157	211/214	101/121	153/167	132/136	140/142
A2_4	Algeria	185/185	230/239	136/142	199/205	154/154	214/214	101/121	157/157	132/136	136/142
A2_6	Algeria	185/189	236/236	136/136	199/199	157/157	214/214	101/121	167/173	132/136	136/142
A2_7	Algeria	181/185	224/236	142/142	199/199	157/157	214/214	101/121	153/153	132/136	136/140
A2_8	Algeria	181/185	224/233	142/142	199/199	157/157	214/214	101/109	153/153	132/136	136/140
A2_9	Algeria	185/189	224/227	136/136	199/199	154/154	214/214	101/119	167/167	132/136	142/142
F1_10	France	193/193	221/221	142/157	199/199	154/157	214/214	101/121	153/159	132/136	136/146
F1_12	France	185/193	218/224	136/154	199/202	154/154	214/214	101/121	151/151	132/136	140/140
F1_13	France	185/189	221/224	139/142	199/199	154/154	214/214	109/119	153/165	132/136	136/146
F1_14	France	185/193	221/224	142/142	199/199	154/154	214/214	101/121	153/159	132/136	132/136
F1_16	France	193/193	218/224	136/157	199/202	154/154	214/214	101/121	151/159	132/136	136/146
F1_17	France	193/193	221/224	142/142	196/199	154/154	214/214	101/121	153/157	132/136	136/136
F1_18	France	197/197	218/224	136/139	196/199	154/154	214/214	101/121	151/153	132/136	136/140
F1_19	France	193/193	218/224	154/154	199/199	154/157	214/214	109/119	167/173	132/132	136/136
F1_2	France	193/193	221/221	142/142	196/202	154/154	214/214	101/121	151/159	132/136	136/140
F1_3	France	193/193	221/236	142/142	196/199	154/154	214/214	101/121	153/159	132/136	136/136
F1_4	France	189/193	221/221	136/142	196/199	154/154	214/214	101/101	159/167	132/138	136/136
F1_7	France	185/189	221/224	139/142	199/199	154/154	214/214	109/119	159/159	132/136	136/146
F1_8	France	193/193	221/224	142/157	199/202	154/154	211/214	101/121	153/159	132/136	136/146
F1_9	France	197/197	218/224	142/142	199/202	154/154	214/214	101/109	159/159	132/136	146/146
F3_1	France	189/193	224/227	142/142	199/199	157/157	214/214	101/121	149/151	132/136	136/136
F3_3	France	193/193	221/221	142/142	196/199	154/154	214/214	101/121	159/177	132/136	136/146

F3_4	France	193/193	221/221	142/142	196/196	154/157	208/214	101/121	155/159	132/136	142/146
F3_5	France	193/193	227/236	142/142	199/199	154/154	214/214	101/105	155/177	132/136	140/140
F5_1	France	185/185	221/221	145/154	196/199	154/157	214/214	101/119	163/177	132/136	136/136
F5_2	France	185/185	221/224	142/145	199/202	154/157	214/214	101/121	163/177	132/136	136/136
F5_3	France	185/193	218/224	142/145	196/202	154/157	214/214	101/109	159/159	132/136	136/136
LZ1_1	Italy	185/193	221/221	136/154	196/199	154/154	214/214	101/121	159/177	132/136	136/140
LZ1_10	Italy	193/193	221/221	154/154	199/199	154/154	214/214	101/121	159/177	132/138	136/146
LZ1_11	Italy	185/185	227/227	142/154	196/199	154/154	211/211	101/121	165/177	132/132	140/146
LZ1_12	Italy	193/193	221/221	154/154	199/199	154/154	214/214	101/121	159/177	132/132	136/146
LZ1_13	Italy	185/189	215/227	142/142	196/199	154/154	214/214	101/121	159/165	132/138	136/146
LZ1_14	Italy	193/193	221/221	154/154	199/199	154/154	214/214	101/121	159/177	132/138	136/146
LZ1_15	Italy	185/185	227/227	142/154	196/199	154/154	211/211	101/121	165/177	132/138	140/146
LZ1_3	Italy	193/193	227/227	142/154	196/196	154/154	214/214	101/121	159/177	132/136	140/146
LZ1_4	Italy	185/193	215/215	145/145	196/199	154/154	214/214	101/121	159/165	132/132	136/146
LZ1_6	Italy	193/193	221/221	154/154	196/199	154/154	214/214	101/121	159/177	132/138	140/146
LZ1_7	Italy	185/185	227/227	136/142	196/199	154/154	211/211	101/121	165/177	132/138	140/146
LZ1_8	Italy	193/193	215/221	136/145	199/199	154/154	214/214	101/121	159/177	132/138	136/146
LZ1_9	Italy	185/185	221/221	142/154	196/196	154/154	211/211	101/119	165/177	132/136	146/146
LG1_2	Italy	185/193	218/224	142/142	196/196	154/154	214/214	101/121	155/159	132/136	146/146
LG1_3	Italy	185/185	218/224	142/157	199/199	157/160	214/214	101/109	153/155	132/136	140/146
SD1_1	Italy	193/193	227/227	142/154	199/199	154/154	214/214	101/109	159/159	132/136	136/146
SD1_2	Italy	189/189	227/227	157/157	199/199	154/157	214/214	101/109	163/163	132/136	136/142
SD1_3	Italy	185/189	227/227	157/157	199/199	154/154	214/214	101/121	155/179	132/136	138/142
SD1_4	Italy	185/189	221/221	154/154	199/199	157/157	214/214	99/109	147/179	132/136	136/136
SD1_6	Italy	189/189	227/227	157/157	199/199	154/154	214/214	101/109	155/163	132/136	138/146
SD1_8	Italy	189/189	206/227	142/157	199/199	154/154	214/214	101/121	147/155	132/136	136/136
SD2_10	Italy	189/189	227/227	142/157	199/199	154/154	214/214	101/119	179/179	132/136	136/136
SD2_2	Italy	189/189	227/236	157/157	199/199	154/157	214/214	101/109	159/163	132/136	136/142
SD2_3	Italy	189/189	227/233	142/157	199/199	154/154	214/214	101/109	163/179	132/136	136/136
SD2_4	Italy	193/193	206/227	142/157	199/199	154/157	214/214	101/105	153/167	132/136	136/146
SD2_5	Italy	189/189	206/227	154/157	199/202	154/154	214/214	105/109	155/155	132/136	136/146
SD2_6	Italy	189/189	215/227	142/154	199/199	154/154	214/214	101/105	151/151	132/136	136/142
SD2_8	Italy	185/185	227/227	157/157	199/199	154/157	214/214	101/105	159/163	132/136	136/136
SD2_9	Italy	189/189	227/227	157/157	199/199	154/157	214/214	101/109	163/179	132/136	136/136
SD3_3	Italy	185/185	227/227	142/157	199/199	154/154	214/214	121/121	177/177	132/136	142/142
SD3_5	Italy	189/189	227/227	142/154	199/199	154/157	214/214	101/109	153/177	132/136	136/146
SD3_6	Italy	185/185	227/227	142/142	199/199	154/157	214/214	101/121	159/177	132/136	136/142

SD3_7	Italy	185/189	227/227	157/157	199/202	154/157	214/214	101/109	155/159	132/136	142/142
SD4_1	Italy	193/193	221/221	136/154	196/199	154/154	214/214	101/105	159/159	132/136	146/146
SD4_10	Italy	193/193	221/221	142/142	196/196	154/154	214/214	101/121	177/179	132/138	136/136
SD4_2	Italy	193/193	203/212	154/154	196/196	154/154	214/214	109/121	159/159	132/136	136/146
SD4_3	Italy	193/197	221/221	136/142	196/202	154/154	214/214	101/105	159/159	132/136	136/146
SD4_4	Italy	193/193	203/221	136/136	199/199	154/154	214/214	101/121	153/153	132/136	136/146
SD4_5	Italy	193/193	203/221	136/154	199/199	154/154	214/214	101/121	157/177	132/136	136/136
SD4_6	Italy	193/193	221/221	136/142	199/199	154/154	214/214	101/105	159/159	132/136	136/146
SD4_7	Italy	193/193	203/221	136/136	196/196	154/154	214/214	101/105	157/159	132/136	136/136
SD4_8	Italy	193/193	203/212	136/142	196/199	154/154	214/214	101/105	157/177	132/136	136/146
SC1_1	Italy	193/193	212/221	145/145	196/196	154/154	214/214	101/117	155/159	132/136	136/146
SC1_2	Italy	193/193	227/227	142/154	196/199	154/154	214/214	101/107	155/177	132/136	136/136
SC1_3	Italy	193/193	221/221	142/154	199/205	154/154	214/214	101/121	159/159	132/136	136/136
SC1_4	Italy	193/193	221/221	142/142	196/196	154/154	214/214	101/109	155/159	132/138	136/136
SC1_6	Italy	193/193	221/224	145/145	196/199	154/154	214/214	101/121	151/159	132/138	136/136
SC2_1	Italy	193/193	221/221	145/145	199/199	154/154	214/214	101/121	157/159	132/138	136/146
SC2_10	Italy	193/193	221/224	157/157	199/205	154/154	214/214	101/121	153/177	132/136	136/136
SC2_2	Italy	193/193	221/224	157/157	199/202	154/154	214/214	101/121	159/159	132/136	136/136
SC2_3	Italy	193/197	221/221	154/154	196/199	154/154	214/214	101/121	159/177	132/136	146/146
SC2_4	Italy	193/193	221/221	142/154	196/196	154/154	214/214	101/121	177/177	132/136	140/146
SC2_5	Italy	193/193	221/221	142/142	199/205	154/154	214/214	101/121	159/177	132/136	140/146
SC2_6	Italy	193/193	221/221	142/154	199/205	154/154	214/214	101/101	157/159	132/136	136/146
SC2_7	Italy	185/193	221/221	142/142	199/205	154/154	214/214	101/121	159/159	132/136	136/146
SC2_8	Italy	185/189	221/221	154/154	199/199	154/154	214/214	101/121	159/159	132/136	136/146
SC2_9	Italy	193/197	221/221	154/154	199/205	154/154	214/214	101/121	159/159	132/136	136/146
SC3_1	Italy	193/193	221/221	142/154	199/205	154/154	214/214	101/121	153/159	132/136	136/136
SC3_2	Italy	193/197	221/221	154/154	199/202	154/154	214/214	101/119	159/159	132/138	136/146
SC3_3	Italy	193/193	218/224	154/154	199/199	154/154	214/214	101/121	177/177	132/136	136/146
SC3_4	Italy	193/193	221/221	142/142	199/199	154/154	214/214	101/121	159/177	132/136	136/146
SC3_5	Italy	193/193	221/227	142/142	199/199	154/154	214/214	101/121	159/177	132/136	136/150
SC3_6	Italy	193/193	221/221	142/142	199/199	154/154	214/214	101/121	159/159	132/136	136/146
SC3_7	Italy	193/193	221/221	142/142	199/199	154/154	214/214	101/109	151/159	132/138	136/136
SC3_9	Italy	193/197	221/221	142/154	199/205	154/154	214/214	101/121	159/159	132/136	136/136
SC4_1	Italy	197/197	221/224	136/154	199/199	154/154	214/214	101/121	159/165	132/136	140/146
SC4_2	Italy	197/197	221/221	142/142	196/199	154/154	214/214	101/121	155/179	132/136	136/146
SC4_3	Italy	193/193	221/221	142/142	199/199	154/154	214/214	101/121	155/159	132/136	136/146
SC4_4	Italy	197/197	221/221	142/142	199/199	154/154	214/214	101/121	155/159	132/136	136/146

SC4_5	Italy	197/197	221/221	136/154	199/199	154/154	211/214	101/121	159/177	132/136	146/146
SC4_7	Italy	185/197	221/221	142/142	199/205	154/154	214/214	101/121	153/159	132/136	136/146
SC4_8	Italy	197/197	221/221	136/142	199/199	154/154	217/217	101/121	159/177	132/136	136/146
SC4_9	Italy	193/193	221/221	142/142	196/199	154/154	214/214	101/121	155/159	132/136	146/146
SC5_1	Italy	197/197	215/221	142/142	199/199	154/154	214/214	101/121	153/159	132/136	136/136
SC5_10	Italy	185/193	221/221	142/142	196/202	154/154	214/214	101/121	157/177	132/136	136/146
SC5_2	Italy	193/197	221/221	142/142	199/202	154/154	214/214	101/109	159/159	132/136	136/146
SC5_3	Italy	193/193	221/221	142/142	199/205	154/154	214/214	101/121	159/159	132/136	136/146
SC5_4	Italy	193/197	221/221	136/142	196/199	154/154	214/214	101/121	157/159	132/136	136/146
SC5_5	Italy	185/193	221/221	142/154	196/199	154/157	214/214	101/121	159/177	132/136	136/146
SC5_6	Italy	193/197	221/224	142/142	199/205	154/157	214/214	101/121	157/177	132/136	136/146
SC5_7	Italy	197/197	221/221	136/154	196/202	154/154	211/214	101/109	157/159	132/136	136/146
SC5_8	Italy	185/193	221/221	142/142	199/202	154/154	214/214	101/121	157/159	132/136	136/146
SC5_9	Italy	193/197	221/221	154/154	196/196	154/154	211/214	101/121	157/159	132/136	136/136
TS1_1	Italy	185/193	218/224	139/145	196/196	154/154	214/214	101/121	159/159	132/136	136/146
TS1_2	Italy	185/185	218/224	139/145	196/196	154/154	214/214	101/121	151/153	132/138	136/146
TS1_3	Italy	185/185	218/224	139/139	196/199	154/154	214/214	101/121	151/161	132/138	136/140
TS1_4	Italy	185/185	218/224	139/139	196/199	154/154	214/214	101/121	151/159	132/138	136/140
M1_2	Morocco	189/189	221/242	133/136	196/196	154/154	214/214	101/121	151/173	132/144	154/156
M1_3	Morocco	185/185	230/245	127/139	196/199	148/154	211/211	101/121	151/173	132/136	142/144
M10_1	Morocco	185/193	212/224	133/142	199/199	154/154	214/214	101/121	175/177	132/136	142/144
M10_10	Morocco	177/177	206/215	136/136	196/199	154/154	214/214	101/105	175/175	132/142	140/142
M10_2	Morocco	185/193	215/227	136/142	199/202	154/157	211/217	101/121	169/173	132/138	142/150
M10_3	Morocco	185/185	224/224	133/136	199/199	154/154	211/214	109/121	147/177	132/136	142/146
M10_4	Morocco	185/185	212/245	136/136	199/199	157/157	214/214	101/109	157/175	132/136	142/144
M10_5	Morocco	185/193	206/224	142/142	199/199	148/148	214/214	101/121	159/169	132/138	138/142
M10_6	Morocco	189/193	206/224	142/142	199/199	157/157	214/214	101/121	173/175	132/140	142/142
M10_7	Morocco	185/185	269/269	142/142	199/199	154/154	211/214	101/107	151/159	132/136	138/138
M10_8	Morocco	185/185	233/233	133/136	199/199	154/157	214/214	101/121	153/159	132/132	142/142
M10_9	Morocco	185/189	263/263	136/142	199/199	154/157	211/214	101/109	147/173	132/136	142/142
M11_10	Morocco	189/189	224/224	142/142	199/202	157/157	214/214	101/121	151/159	132/136	136/142
M11_3	Morocco	185/189	224/224	142/142	196/199	154/154	214/214	101/119	159/167	132/136	146/158
M11_4	Morocco	185/185	233/242	133/136	199/199	154/157	214/214	101/109	167/175	132/136	142/150
M11_5	Morocco	185/189	224/224	142/142	196/199	154/154	214/214	101/119	159/167	132/136	146/158
M11_6	Morocco	189/189	242/242	136/142	199/199	154/154	214/214	101/119	159/163	132/136	142/146
M11_7	Morocco	185/185	215/224	133/142	196/199	154/154	211/214	101/121	153/153	132/136	142/150
M11_8	Morocco	185/189	221/233	136/136	199/199	148/154	211/211	101/121	159/163	132/136	142/142

M11_9	Morocco	185/185	224/224	142/142	199/199	154/154	214/214	101/117	151/171	132/136	142/150
M11_2	Morocco	185/185	215/242	133/142	196/199	154/154	211/214	101/105	153/153	132/136	142/150
M2_1	Morocco	189/189	224/245	133/136	199/202	148/154	211/211	0/0	151/175	132/136	146/156
M2_10	Morocco	189/189	221/221	142/142	196/202	148/148	211/211	0/0	151/151	132/136	154/156
M2_5	Morocco	185/189	215/215	145/151	199/199	148/154	214/214	101/121	159/181	132/136	144/146
M2_6	Morocco	189/189	218/218	133/133	199/202	148/154	214/214	101/121	169/173	132/136	144/146
M2_7	Morocco	185/189	221/227	136/136	199/202	148/154	211/211	121/121	153/167	132/132	136/156
M2_8	Morocco	185/189	218/218	133/136	196/202	148/154	208/211	101/121	151/169	132/136	144/156
M2_9	Morocco	185/189	242/245	133/133	196/199	148/154	211/214	101/121	173/175	132/136	144/156
M3_1	Morocco	189/189	230/230	136/142	196/199	148/154	211/214	101/121	151/179	132/142	144/144
M3_10	Morocco	185/189	215/215	139/145	196/196	148/148	214/214	105/105	151/157	132/140	142/144
M3_2	Morocco	189/189	230/245	133/136	196/202	148/148	214/214	101/121	151/173	132/140	142/144
M3_6	Morocco	185/189	215/215	139/139	202/202	148/148	214/214	101/121	155/157	132/140	144/144
M3_7	Morocco	185/185	215/215	139/139	199/202	148/148	214/214	101/121	155/155	132/140	132/142
M3_8	Morocco	185/185	215/215	139/139	199/202	148/148	214/214	101/121	147/155	132/140	132/144
M4_1	Morocco	189/189	215/215	133/139	202/202	148/154	214/214	97/101	151/151	132/136	146/146
M4_10	Morocco	185/189	215/215	148/148	196/199	148/157	214/214	99/105	151/183	132/140	142/146
M4_5	Morocco	185/189	215/215	139/139	202/202	148/148	214/214	101/121	147/147	132/136	144/144
M4_6	Morocco	185/185	215/215	139/139	196/202	148/148	214/214	101/121	151/157	132/136	144/144
M4_7	Morocco	189/189	215/227	139/139	199/199	148/148	214/214	101/105	151/153	132/140	142/144
M4_8	Morocco	185/189	215/215	139/145	199/208	154/154	214/214	97/105	151/165	132/136	142/142
M4_9	Morocco	189/189	215/215	148/148	196/202	154/154	214/214	101/121	153/153	132/140	144/146
M5_1	Morocco	185/189	215/215	142/142	199/205	154/154	214/214	101/107	151/153	132/136	144/144
M5_10	Morocco	185/189	215/215	142/142	199/205	154/154	214/214	101/107	151/153	132/136	144/144
M5_4	Morocco	185/189	206/215	136/139	196/196	154/154	205/214	101/101	155/159	132/134	140/144
M5_7	Morocco	185/189	215/215	142/142	199/205	154/154	214/214	101/107	151/153	132/136	144/144
M5_8	Morocco	185/189	215/215	142/142	199/205	154/154	214/214	101/107	151/153	132/136	144/144
M5_9	Morocco	185/189	215/215	142/142	199/205	154/154	214/214	101/107	151/153	132/136	144/144
M6_1	Morocco	0/0	221/221	133/133	196/199	154/157	208/208	101/121	151/163	132/136	142/156
M6_2	Morocco	185/185	221/224	136/142	199/199	148/154	208/208	101/101	161/171	132/136	136/136
M6_3	Morocco	189/189	212/221	136/136	199/199	0/0	208/208	101/121	149/169	132/136	154/156
M7_4	Morocco	185/185	221/248	142/142	196/196	148/154	208/214	101/121	149/153	132/136	154/156
M7_5	Morocco	189/189	221/227	136/136	196/196	148/148	211/214	101/121	153/169	132/144	136/136
M7_6	Morocco	189/189	221/239	136/136	196/202	148/155	214/214	105/121	153/167	132/136	150/156
M7_8	Morocco	189/189	221/239	136/136	196/202	148/154	214/214	105/121	153/167	132/136	150/156
M7_9	Morocco	185/185	221/248	142/142	196/196	148/154	208/214	101/121	149/153	132/136	156/156
M8_1	Morocco	189/189	215/215	145/151	196/199	148/154	211/214	101/121	153/167	132/134	146/146

M8_10	Morocco	189/189	215/215	139/139	202/202	148/157	214/214	101/121	147/165	132/136	148/152
M8_2	Morocco	189/189	233/233	148/148	199/199	148/148	214/214	105/107	153/165	132/138	140/142
M8_3	Morocco	185/189	233/233	145/145	199/201	148/154	214/214	101/121	159/173	132/136	144/148
M8_4	Morocco	189/189	221/221	136/139	199/199	148/148	214/214	99/107	173/179	132/136	142/148
M8_5	Morocco	189/189	221/227	136/139	196/199	148/154	214/214	101/105	147/165	132/140	144/156
M8_7	Morocco	185/189	227/233	136/136	196/208	148/154	214/214	101/121	159/159	132/136	144/150
M8_8	Morocco	189/189	215/215	139/139	202/202	148/148	214/214	101/121	147/165	132/136	148/152
M9_1	Morocco	185/189	233/245	136/136	199/199	148/148	214/214	101/121	151/161	132/136	140/140
M9_10	Morocco	189/189	221/221	130/139	199/202	148/148	214/214	101/131	155/157	132/136	140/140
M9_2	Morocco	185/189	233/245	136/136	199/199	148/148	214/214	101/105	151/161	132/132	140/140
M9_3	Morocco	189/189	221/221	130/139	199/202	148/154	211/214	101/131	155/157	132/136	140/142
M9_4	Morocco	189/193	230/230	130/139	199/199	157/157	214/214	101/129	153/155	132/136	142/144
M9_6	Morocco	189/193	224/242	136/136	199/199	154/154	211/211	101/105	147/153	132/136	150/150
M9_7	Morocco	185/185	227/236	130/139	187/199	157/157	214/214	101/121	155/161	132/136	140/140
M9_8	Morocco	185/185	212/215	139/148	187/199	154/154	211/211	101/105	169/175	132/136	142/142
M9_9	Morocco	193/193	233/233	136/142	199/199	148/148	211/214	101/105	175/177	132/136	150/150
P1_1	Portugal	189/189	227/227	136/136	199/199	154/154	214/214	101/121	167/169	132/136	136/136
P1_2	Portugal	185/185	227/242	136/142	196/199	154/154	214/214	101/121	171/175	132/136	136/142
P1_3	Portugal	185/185	224/242	136/136	196/199	154/154	211/214	101/105	159/175	132/150	142/146
P1_4	Portugal	189/189	227/227	136/136	199/199	154/154	214/214	101/121	167/169	132/136	136/136
P1_5	Portugal	177/185	230/230	139/142	199/199	154/157	214/214	101/121	161/167	132/136	136/142
P1_6	Portugal	185/185	227/227	133/142	199/199	154/154	211/211	101/121	177/177	132/136	136/136
P3_1	Portugal	177/185	242/242	136/136	196/196	154/157	211/211	101/121	151/167	132/136	136/136
P3_10	Portugal	185/193	227/242	136/139	199/199	154/157	211/214	101/121	159/159	132/136	142/142
P3_2	Portugal	177/185	242/242	133/136	196/199	154/154	211/214	101/121	159/173	132/136	136/142
P3_4	Portugal	185/185	227/242	142/142	196/199	154/154	211/214	101/121	165/175	132/136	136/142
P3_5	Portugal	177/189	245/245	133/136	196/199	154/157	211/211	101/121	159/171	132/136	142/142
P3_6	Portugal	189/189	221/221	133/133	199/199	154/154	211/214	101/101	159/167	132/136	142/142
P3_8	Portugal	185/189	230/230	136/139	199/199	154/154	211/214	101/101	159/171	132/136	136/144
P4_1	Portugal	181/185	224/224	136/136	199/199	154/154	211/211	101/121	163/163	132/150	136/142
P4_10	Portugal	185/185	224/227	133/133	199/199	154/154	211/211	101/121	159/179	132/136	142/142
P4_2	Portugal	185/185	224/224	139/148	196/199	148/154	211/211	105/121	181/181	132/150	136/142
P4_3	Portugal	185/189	221/248	136/136	199/199	148/154	214/214	101/121	167/179	132/136	142/142
P4_4	Portugal	189/189	224/227	136/136	196/196	154/154	214/214	101/121	159/171	132/136	142/142
P4_6	Portugal	185/185	224/224	136/136	199/199	154/154	211/211	101/121	163/163	132/150	136/142
P4_7	Portugal	185/185	224/227	133/133	199/199	154/154	211/211	101/121	159/179	132/136	142/142
P4_8	Portugal	185/185	224/227	133/133	199/199	154/154	211/211	105/121	159/179	132/136	142/142

P4_9	Portugal	185/189	221/248	136/136	199/199	148/151	214/214	105/121	167/179	132/136	142/142
S1_1	Spain	185/189	227/239	139/142	199/202	154/154	214/214	101/121	157/157	132/136	142/150
S1_10	Spain	185/185	227/236	136/136	199/202	154/154	214/214	101/121	153/169	132/136	136/142
S1_2	Spain	185/185	236/239	136/142	199/199	154/154	214/214	101/121	151/157	132/136	136/146
S1_3	Spain	185/185	224/224	136/136	199/202	154/154	211/214	101/121	169/171	132/136	136/136
S1_4	Spain	197/197	227/227	142/151	202/202	154/157	214/214	101/101	153/169	132/136	144/144
S1_5	Spain	185/193	212/230	136/142	199/199	154/154	214/214	109/121	151/157	132/136	136/146
S1_6	Spain	185/197	218/227	136/136	199/199	154/157	214/214	109/121	151/167	132/136	136/142
S1_7	Spain	185/197	212/236	142/157	199/202	154/154	214/214	101/121	157/169	132/136	146/146
S1_8	Spain	181/185	227/242	136/136	199/202	154/157	214/214	101/121	161/169	132/136	142/146
S1_9	Spain	181/181	230/239	142/151	199/199	154/157	214/214	101/121	153/157	132/136	136/144
S2_1	Spain	193/193	224/233	154/154	199/199	154/157	214/214	101/121	151/151	132/138	146/146
S2_3	Spain	185/189	227/230	136/142	199/202	154/157	214/214	109/121	151/151	132/140	146/146
S2_4	Spain	181/185	218/224	136/136	199/202	154/154	214/214	109/119	151/159	132/136	146/146
S2_5	Spain	185/193	218/224	139/139	199/202	154/154	214/214	101/105	151/151	132/132	138/156
S2_6	Spain	185/185	227/236	142/154	199/199	154/157	214/214	109/121	151/151	0/0	0/0
S2_7	Spain	181/181	227/227	142/142	199/199	154/154	214/214	109/121	151/153	132/136	146/146
S2_8	Spain	185/185	212/233	142/142	199/199	154/154	214/214	101/121	151/153	132/138	146/146
S3_1	Spain	185/193	227/227	142/142	199/199	154/154	214/214	101/105	161/175	132/136	146/146
S3_10	Spain	185/193	209/227	133/142	199/199	154/157	214/214	101/109	151/153	132/136	136/136
S3_2	Spain	181/189	227/236	142/142	186/199	154/157	214/214	101/105	151/153	132/136	136/136
S3_4	Spain	185/189	227/230	142/154	186/199	154/154	214/214	105/109	161/161	132/136	136/146
S3_6	Spain	193/193	227/227	139/142	199/199	154/154	214/214	109/117	153/177	132/136	136/136
S3_9	Spain	185/185	209/227	136/154	186/199	154/157	214/214	101/121	153/159	132/136	136/138
S4_1	Spain	185/185	206/227	136/142	199/202	154/154	211/214	101/121	157/161	132/136	136/144
S4_3	Spain	185/193	206/215	136/154	196/199	154/157	214/214	115/121	163/169	132/136	146/146
S4_4	Spain	185/189	227/233	154/154	199/199	154/157	214/214	115/121	153/169	132/136	142/144
S4_5	Spain	185/185	227/233	127/136	186/199	148/154	214/214	101/115	153/167	132/136	136/142
S4_6	Spain	185/185	218/224	154/157	199/199	154/157	214/214	101/115	163/169	132/134	144/146
S4_7	Spain	185/185	230/230	133/157	186/199	154/157	211/214	115/121	163/175	132/136	144/146
S4_8	Spain	185/185	227/227	154/157	199/202	154/157	214/214	101/109	157/163	132/136	136/136
S6_1	Spain	185/193	224/227	142/142	199/199	154/154	211/214	101/115	147/157	132/136	138/142
S6_2	Spain	185/185	206/224	142/142	186/199	154/157	211/214	101/109	147/167	132/136	142/146
S6_3	Spain	185/185	224/233	142/142	186/199	154/157	211/211	101/121	169/175	132/136	138/144
S6_4	Spain	185/185	227/230	133/142	186/199	154/154	211/214	101/105	151/169	132/136	144/146
S6_6	Spain	185/193	221/224	142/142	186/199	154/157	211/211	101/121	147/167	132/136	142/142
S6_7	Spain	185/185	215/215	136/142	199/199	154/157	211/211	101/109	157/167	132/136	142/146

S6_8	Spain	185/185	224/242	142/142	186/199	154/157	211/211	101/109	145/169	132/136	142/146
S7_1	Spain	185/189	206/224	136/139	199/199	157/157	214/214	101/121	147/167	132/136	142/144
S7_2	Spain	185/185	215/239	136/136	199/199	157/157	208/214	101/121	161/167	132/136	142/150
S7_3	Spain	185/189	221/227	133/136	199/199	148/154	214/214	101/119	179/179	132/136	144/144
S7_4	Spain	185/185	239/248	136/136	196/199	148/154	211/214	101/121	151/177	132/136	136/142
S7_5	Spain	185/189	212/212	136/142	199/199	154/157	202/214	101/119	151/167	132/136	142/150
S7_6	Spain	185/189	242/242	136/142	199/199	154/154	211/211	101/121	165/171	132/136	142/142
S7_7	Spain	185/189	221/227	133/136	199/199	148/154	214/214	101/119	179/179	132/136	144/144
S7_8	Spain	177/185	230/239	133/139	199/199	148/154	214/214	101/121	159/183	132/136	142/142
S7_9	Spain	193/193	206/227	136/136	199/199	154/154	202/214	101/121	159/165	132/136	142/144
T1_10	Tunisia	185/185	215/221	154/154	196/202	154/154	214/214	101/107	151/159	132/136	136/136
T1_2	Tunisia	185/193	215/221	154/154	196/202	154/154	214/214	109/119	151/153	132/136	136/136
T1_3	Tunisia	193/197	218/227	136/139	196/199	154/154	214/214	101/119	151/153	132/136	136/146
T1_4	Tunisia	185/193	215/221	154/154	199/202	154/154	214/214	101/121	153/159	132/136	136/146
T1_5	Tunisia	193/193	224/224	154/154	196/196	154/154	214/214	101/121	155/159	132/136	136/140
T1_6	Tunisia	185/193	215/221	142/154	196/196	154/154	214/214	101/121	147/153	132/136	136/136
T1_8	Tunisia	193/197	221/221	142/154	199/205	154/154	214/214	101/121	153/159	132/136	146/146
T1_9	Tunisia	197/197	215/215	142/142	196/199	154/154	214/214	101/121	157/159	132/136	136/136
T2_1	Tunisia	189/189	224/227	142/142	196/196	154/154	214/214	101/121	153/155	132/136	136/136
T2_3	Tunisia	0/0	224/227	142/142	196/196	154/154	214/214	101/121	153/155	132/136	136/136
T3_2	Tunisia	181/189	215/221	142/142	196/202	154/157	214/214	101/121	151/153	132/136	136/146
T5_1	Tunisia	185/193	218/221	145/154	199/199	154/160	214/214	101/121	159/159	132/136	136/136
T5_2	Tunisia	185/193	218/224	142/142	199/202	154/154	214/214	101/101	159/159	132/136	156/156
T5_3	Tunisia	181/185	218/221	133/133	199/202	154/154	211/211	101/121	159/167	132/136	136/146
T5_4	Tunisia	193/197	215/221	136/154	199/199	154/154	214/214	101/121	155/159	132/138	136/142
T6_1	Tunisia	185/193	224/224	142/145	199/199	154/154	214/214	101/121	151/159	132/136	136/136
T6_2	Tunisia	193/193	218/227	136/142	202/202	154/154	214/214	101/121	159/175	132/136	136/146
T6_3	Tunisia	185/193	221/224	142/142	202/202	154/154	214/214	101/121	153/155	132/136	136/136
T6_4	Tunisia	181/185	218/227	145/145	202/202	154/154	214/214	101/121	155/159	132/136	136/140
T6_5	Tunisia	193/197	224/224	142/142	196/199	154/154	214/214	101/121	153/155	132/136	136/140
T4_2	Tunisia	185/193	218/227	142/142	199/199	154/154	214/214	101/121	159/159	132/136	146/146
T7_1	Tunisia	193/197	218/224	142/142	196/199	154/154	205/214	101/121	159/159	132/138	140/146
T7_2	Tunisia	193/193	215/221	142/142	196/196	154/154	214/214	101/121	153/159	132/136	140/146
T7_3	Tunisia	193/193	218/224	142/142	196/199	154/154	214/214	101/121	159/161	132/138	146/146
T7_4	Tunisia	193/193	218/224	142/145	199/199	154/154	211/214	101/121	159/159	132/138	136/146
T7_5	Tunisia	185/193	218/224	136/142	196/199	154/157	214/214	101/121	155/155	132/136	136/146
T8_1	Tunisia	185/193	203/212	142/142	196/199	154/154	214/214	101/121	155/161	132/136	136/146

T8_2	Tunisia	185/193	203/212	142/142	199/199	154/154	214/214	101/121	155/159	132/136	140/146
T8_3	Tunisia	185/193	203/212	142/142	199/199	154/154	214/214	101/121	155/161	132/136	140/146
T8_4	Tunisia	185/193	203/212	142/142	199/199	154/154	214/214	101/121	155/161	132/136	140/146
T8_5	Tunisia	193/197	203/212	145/145	196/199	154/154	214/214	101/121	155/155	132/138	136/136
T8_6	Tunisia	193/197	203/212	145/145	196/199	154/154	214/214	101/121	155/155	132/138	136/136
T8_7	Tunisia	185/193	203/212	142/142	199/199	154/154	214/214	101/121	155/161	132/136	140/146
T8_8	Tunisia	193/197	203/212	145/145	196/199	154/154	214/214	101/121	155/155	132/138	136/136

2. Script used for DAPC analysis

```
# 1. Install the package 'adegenet'
# Tools -> Install Packages -> Write down 'adegenet'-> Install

# 2. Define the directory:
setwd("~/Desktop/agnese")

# 3. Change de name of the file (only if it's required).
df <- read_excel("boac053_suppl_supplementary_file_s2.xlsx")

#4. Activate the package we are interested in
library(adegenet)

# 5. Create our data frame
df <- as.data.frame( df )
ind <- as.character( df$Sample )
site <- as.character( df$Pop )
df <- df[, -c(1:2) ]

#6. Change the format of our data frame in order to perform the DAPC
df <- df2genind( df, ploidy = 1, ind.names = ind, pop = site, sep = "" )

#7. DAPC analysis
# Identify the optimal number of PCs
dapc <- dapc( df, df$pop, n.pca = 40, n.da = 3 )
```

```
optim.a.score(dapc)$best
```

```
# Run the DAPC analysis
```

```
#8. Calculus of the genetic variability explained by the analysis
```

```
percent <- dapc$eig/sum (dapc$eig) *100
```

```
percent [ 1:2]
```

```
# 9. Plot the DAPC analysis
```

```
scatter (dapc, scree.da = FALSE)
```

3. Missing results from BUSCO assessment

id	Haplotype	Gene
103382at33090	Hap 1	Prolyl 3-hydroxylase 1
11664at33090	Hap 1	DNA topoisomerase, type IIA, subunit A/C-terminal
128774at33090	Hap 1	Proteasome assembly chaperone 2
145061at33090	Hap 1	protein high chlorophyll fluorescent 107
147262at33090	Hap 1	protein ABCI12, chloroplastic
148236at33090	Hap 1	Uncharacterised protein family UPF0454
177296at33090	Hap 1	methyltransferase-like protein 5
182493at33090	Hap 1	Peptidyl-prolyl cis-trans isomerase
185172at33090	Hap 1	Molybdopterin synthase

		catalytic subunit
196443at33090	Hap 1	thylakoid lumenal 15 kDa protein 1, chloroplastic
225336at33090	Hap 1	predicted protein
228201at33090	Hap 1	predicted protein
75785at33090	Hap 1	P-loop containing nucleoside triphosphate hydrolase
149782at33090	Hap 2	Ribosome recycling factor
158957at33090	Hap 2	HAD superfamily
161309at33090	Hap 2	Calycin
164019at33090	Hap 2	Class IV aminotransferase
181108at33090	Hap 2	probable plastid-lipid-associated protein 8, chloroplastic
193351at33090	Hap 2	DCC family protein At1g52590, chloroplastic
196443at33090	Hap 2	thylakoid lumenal 15 kDa protein 1, chloroplastic
197780at33090	Hap 2	tRNA (Guanosine(18)-2'-O)-methyltransferase
211645at33090	Hap 2	Ribosomal RNA small subunit methyltransferase G
63411at33090	Hap 2	fe-S cluster assembly factor HCF101, chloroplastic
88687at33090	Hap 2	cell cycle checkpoint control protein RAD9A
89796at33090	Hap 2	conserved oligomeric Golgi complex subunit 6