Tulipa: the taxonomy and evolutionary history of the genus and its impact on conservation priorities in Central Asia



Brett Donald Wilson

St John's College

Department of Plant Sciences

University of Cambridge

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Declaration

This thesis is the result of my own work and includes nothing which is the outcome of work done in collaboration except as declared in the Preface and specified in the text. I further state that no substantial part of my thesis has already been submitted, or, is being concurrently submitted for any such degree, diploma or other qualification at the University of Cambridge or any other University or similar institution except as declared in the Preface and specified in the text. It does not exceed the prescribed word limit of 60,000 for the Biology Degree Committee.

Brett Wilson

28th September 2022

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Tulips are one of the most recognisable plants with their current horticultural trade estimated as a billion-euro industry. This trade initially relied heavily on wild specimens but now relies less on natural diversity. Yet, wild tulips are an important genetic reserve for future breeding efforts, especially for disease resistance. They also have significant cultural value, can act as ecological indicators, and support insect populations. Their taxonomy is notoriously complex, complicating their study and conservation. Most taxa grow in Central Asia, which has been proposed as the place of origin of this genus, albeit with limited evidence. Many tulips are declining in this area, but there is no cohesive regional overview of the genus. Here, we address phylogeny and taxonomy within the genus, then using this insight to explore the evolutionary history of *Tulipa* and its potential to inform conservation priorities in Central Asia.

First, we used modern phylogenetic techniques, with extensive sampling of the genus including large amounts of wild material collected during three fieldwork seasons, to generate both a plastome based and 35S rDNA phylogeny. These phylogenies allowed us to make a number of taxonomic decisions with respect to the synonymization and reinstatement of a number of species. We also reorganised the higher-level taxonomic groups of this genus recognising a new subgenus, *Eduardoregelia*, and simplifying the sections of this genus, primarily merging *Tulipa*, *Tulipanum*, *Lanatae*, *Vinistriatae*, and *Spiranthera* into one broader section. Within this work we identified a new species, *Tulipa toktogulica,* which we formally describe within this thesis as well.

Second, we used molecular dating techniques to estimate the ages of nodes on the tulip species phylogeny. With this dated phylogeny we modelled the biogeographical history of the genus, generated a lineage through time plot, and assessed the phylogenetic signal for the trait of genome size, which has commonly been used in the taxonomy of tulips including in the description of new species. We then assimilated dates, biogeography, and geological history to propose how this genus diversified and migrated to its current distribution. We confirmed a broader Central Asian origin of this genus, highlighting the importance of this region for the diversification of this genus throughout its evolutionary history, and linking speciation to aridification, mountain building, and global cooling.

Finally, we modelled the impacts of climate change on tulip species in Central Asia showing the large negative impact this threat will have. This information was then used in the Red Listing of a range of Central Asian tulip species, which was undertaken at a workshop in Bishkek in Spring 2022. Through this process, a large amount of information was collated and many taxa from this region were assigned a threat status. Using data from the Red List assessments we undertook several post-analyses, showing that national assessments often overinflate threat status, as well as calculating EDGE scores in order to stimulate evolutionarily informed conservation efforts. Overall, we have provided a foundation for the development of a regional tulip conservation strategy and improved conservation prioritisation, both of which directly support the work of our iCASE partner Fauna & Flora International.



“A tulip doesn’t strive to impress anyone. It doesn’t struggle to be different than a rose. It doesn’t have to. It is different. And there’s room in the garden for every flower.” **Marianne Williamson**

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"Never doubt that a small group of thoughtful, committed citizens can change the world; indeed, it is the only thing that ever has." **Margaret Mead**

Table of Contents

Chapter 1. The genus *Tulipa*: current and future conservation challenges 1

1.1. Introduction to the Genus *Tulipa* 1

1.2. Life History and Ecology 1

1.2.1. Habitat 1

1.2.2. Initial growth from seed 2

1.2.3. The bulb 2

1.2.4. Stem and leaves 3

1.2.5. Flowering 4

1.2.6. Reproduction 5

1.2.7. Life span 6

1.3. Biogeography 6

1.3.1. Global distribution of Tulipa relatives 6

1.3.2. Global distribution of Tulipa 7

1.3.3. Evolutionary origin and diversity hotspots 7

1.4. Cultural Significance 9

1.4.1. The first cultivated tulips 9

1.4.2. The Ottoman influence 9

1.4.3. Tulips enter Europe 10

1.4.4. A growing influence in Europe 12

1.4.5. Tulip mania 13

1.4.6. Horticultural significance 13

1.4.7. Contemporary culture 14

1.5. Taxonomic History 14

1.5.1. The name Tulipa and the type specimen 14

1.5.2. Subgenera and expansion of the Genus 17

1.5.3. Modern taxonomy 20

1.5.4. Estimated number of species 24

1.6. Species, Population, and Genome Dynamics 29

1.6.1. Hybridisation, karyology, and polyploidy 29

1.6.2. Population genetics 32

1.7. Conservation Perspective 33

1.7.1. Global threat status of tulips 33

1.7.2. Ex-situ collections of tulips 34

1.7.3. Taxonomy underpins tulip conservation 35

1.7.4. Tulip conservation should focus on the centres of diversity 36

1.7.5. The perennial geophytic life history presents some challenges for conservation 39

1.7.6. Impact of hybridisation and polyploidy on tulip conservation 40

1.7.7. The horticultural history of tulips could promote their conservation 40

1.8. Thesis Structure 42

Chapter 2. The evolutionary relationships of wild tulips 45

2.1. Introduction 45

2.2. Materials and Methods 49

2.2.1. Samples and extraction 49

2.2.2. Genome assembly and annotation 51

2.2.3. Phylogenetic analysis 55

2.2.4. Assessment of phylogenies 58

2.3. Results and Discussion 62

2.3.1. Plastome structure 63

2.3.2. Support for phylogenies 72

2.3.3. Taxonomy 80

2.4. Conclusion 91

Chapter 3. *Tulipa toktogulica* (Liliaceae), a new endangered cryptic species from the western Tien-Shan, Kyrgyzstan 95

3.1. Introduction 95

3.2. Material & Methods 96

3.2.1. Plant material 96

3.2.2. DNA extraction and sequencing 98

3.2.3. Plastid genome assembly and annotation 99
3.2.4. 35S rDNA assembly and annotation 99
3.2.5. Phylogenetics 100
3.3. Results 101
3.4. Discussion 101
3.5. Taxonomy 104
3.5.1. Etymology 106
3.5.2. Distribution and habitat 106
3.5.3. Phenology 107
3.5.4. Ecology 107
3.5.5. Conservation status 107
Chapter 4. Divergence times, biogeography, and genome size evolution 109
4.1. Introduction 109
4.2. Materials and Methods 112
4.2.1. Molecular dated phylogeny 112
4.2.2. Biogeography 114
4.2.3. Genome size evolution 115
4.3. Results and Discussion 117
4.3.1. Molecular dated phylogeny 117
4.3.2. Biogeographic models 124
4.3.3. Evolutionary history of the genus Tulipa 124
4.3.4. Genome size is expanding in the genus Tulipa 131
4.4. Conclusion 133
Chapter 5. Central Asian wild tulip conservation requires a regional approach, especially in the face of climate change 135
5.1. Introduction 135
5.2. Methods 138
5.2.1. Study site 138
5.2.2. Species distribution modelling 138

5.3. Results **144**

5.4. Discussion **151**

Chapter 6. Generating an evidence-base to identify conservation priorities **162**

6.1. Introduction **162**

6.2. Materials and Methods **164**

6.2.1. Pre-workshop preparation 164

6.2.2. Workshop 165

6.2.3. Post-workshop analyses 168

6.3. Results **169**

6.3.1. Country level diversity 169

6.3.2. Global Red List results 170

6.3.3. National vs. global status 173

6.3.4. EDGE 175

6.3.5. Regional strategy 179

6.4. Discussion **180**

Chapter 7. Conclusion and future perspectives **188**

Bibliography **192**

List of Figures and Tables

Figure 1.1. The global distribution of the genus *Tulipa* 9

Figure 1.2. Tulips and their cultural history 11

Figure 1.3. Tulip festivals of the world 15

Figure 1.4. Graph showing the accumulation of accepted tulip species over time with respect to the 93 currently recognised tulip species 19

Figure 1.5. The extent of tulip *ex-situ* collections based on BGCI's PlantSearch tool 35

Figure 1.6. Tulip species representation on GBIF 38

Figure 2.1. Plastome gene maps for five tulips, one from each recognised subgenus 71

Figure 2.2. Phylogeny inferred using the partitioned plastome dataset through a maximum likelihood approach 73

Figure 2.3. Phylogeny inferred using the partitioned 35S rDNA dataset through a maximum likelihood approach 75

Figure 2.4. Subgenera of the genus *Tulipa* 78

Figure 2.5. A comparison of plastome and 35S rDNA phylogeny structures 79

Figure 2.6. Genus level tree of the tribe Tulipeae inferred using plastome data 81

Figure 2.7. Plastome based phylogeny of the genus *Tulipa* colour coded by section 83

Figure 2.8. Species tree inferred using plastome data 94

Figure 3.1. The collection location of all specimens used in the phylogenetic analysis of this study excluding *Tulipa iliensis* and *T. altaica*, which both lacked GPS data 98

Figure 3.2. Phylogeny of the new species and its close relatives 102

Figure 3.3. The new species and its habitat 105

Figure 3.4. Photos showing the broader stamens in *Tulipa toktogulica* compared to *T. talassica* 106

Figure 4.1. Geographic distribution of GBIF data for *Tulipa* 116

Figure 4.2. Molecular dated phylogeny of tulip species, including Duc van Tol horticultural variety 119

Figure 4.3. Dated phylogeny of the major taxonomic groups of the genus *Tulipa* with a basic timeline of geological timeline of geological events occurring in Central Asia during this period 122

Figure 4.4. Lineage through time results 123

Figure 4.5. Maps of selected regions for the five area and and six area BioGeoBEARS analyses and corresponding results 125

Figure 4.6. A map of the two dispersal routes out of Central Asia 129

Figure 4.7. Genome size values (1C) for each species mapped to the dated phylogeny of *Tulipa* 132

Figure 4.8. The area that should be considered within the Central Asian tulip diversity hotspot 134

Figure 5.1. Maps showing distribution data used in modelling 139
Figure 5.2. Species distribution models produced in MaxEnt without a bias file 146
Figure 5.3. Bar chart showing the percentage of a species high and very high native suitable habitat area captured within protected areas across present day, 2050, and 2070 150
Figure 5.4. Climate modelling outputs showing suitable habitat areas for the four species with predicted future habitat 153
Figure 5.5. Bar chart showing, for nine modelled species, the percentage of present-day suitable habitat area and that projected to remain in 2050 and 2070 155
Figure 5.6. Boxplots showing the altitudinal range encompassed in high and very high suitable habitat areas for four species with projected habitat across the present day, 2050, and 2070 156
Figure 6.1. Red List results 171
Figure 6.2. Conservation information collated in Red List process 174
Figure 6.3. The count of Difference in Assessment scores for four different datasets 175
Figure 6.4. EDGE scores calculated using fair proportions method and equal splits method mapped onto copies of the same phylogeny 176
Figure 6.5. The three National sites deemed a priority for tulip conservation by experts, (A) Kazakhstan, (B) Kyrgyzstan, (C) Tajikistan, and (D) Uzbekistan 179
Figure 6.6. Priority sites for tulips across Central Asia as selected by experts 182

Table 1.1. Tulip species described since 2010 with details of their distribution, endemicity, year of description, and relevant citation 8
Table 1.2. Summary of the major taxonomic changes in key publications throughout the scientific history of tulips 16
Table 1.3. All currently recognised species, their subgenus classification, their distribution, and their known ploidy forms 25
Table 1.4. Tulip species that are listed on the global IUCN Red List 34
Table 2.1. The reference sequences used to extract chloroplast and ITS reads from the full read datasets 51
Table 2.2. The reference genomes used in the annotation process of plastomes 52
Table 2.3. A list of the plastome sequences downloaded from Genbank and their NCBI reference sequence 53
Table 2.4. Details of the five 35S rDNA sequences generated through the de-novo method, including the subgenus of each specimen and its sequence length in base pairs 55
Table 2.5. Datasets used in the multi-partition plastome based phylogenetic analyses. 57
Table 2.6. Specimens sequenced that were removed from all phylogenies due to their ambiguous identity 59

Table 2.7. The 80 specimens used to infer the species tree 61

Table 2.8. Details of the number of taxa obtained from each source type and the percentage of misidentified specimens from each source 63

Table 2.9. Details of the 245 specimens successfully sequenced within this project 64

Table 2.10. All genes annotated in the *Tulipa* chloroplast assemblies 70

Table 2.11. A list of the 96 species, and several subspecies, accepted within this work placed within the reorganised subgenera and sections of the genus *Tulipa* 89

Table 3.1. Specimens collected and sequenced for use in the description of the new species 97

Table 3.2. Plastomes downloaded from GenBank for use as references in the assembly process 99

Table 3.3. Morphology of *Tulipa toktogulica* compared to its most similar species 103

Table 4.1. Geological time scale of epochs and ages during the existence of the genus *Tulipa* 110

Table 4.2. Crown ages of all genera, subgenera, and sections in the dated phylogeny 121

Table 4.3. Stem ages of all genera, subgenera, and sections in the dated phylogeny 121

Table 4.4. The Akaike Information Criterion values for our models run in BioGeoBEARS with a dispersal matrix for the five and six area distribution analyses 124

Table 5.1. Details of the ten species of Central Asian tulip included in this analysis 141

Table 5.2. The environmental layers selected, after autocorrelation testing, to be included in the MaxEnt model and their source 145

Table 5.3. The test data AUC values for the present day and each climate scenario for the ten selected species with standard deviation in brackets 148

Table 5.4. The most significant variables that contributed to each species present day model 149

Table 5.5. Percentage declines in high and very high native suitable habitat areas for all species for which climate modelling was undertaken 152

Table 5.6. The statistical results of the TukeyHSD test used to assess the change in mean altitude of species over time 152

Table 6.1. The National Red List status of species occurring across Central Asian countries 166

Table 6.2. The IUCN Red List category assigned to each Central Asian tulip species assessed alongside the IUCN criterion used and reported criteria details 172

Table 6.3. EDGE scores and details of the parameters used to calculate these for species with an assigned IUCN Red List status 177

Table 6.4. The 15 objectives presented to participants at the workshop and corresponding voting results 181

List of Abbreviations

AFLP Amplified fragment length polymorphism

AIC Akaike information criterion

AOO Area of occupancy

AUC Area under the receiver operating characteristic curve

BC Best case scenario

BGI Beijing Genomics Institute

BU Business as usual

CCSM4 GCM Community Climate System Model version 4

CDS A coding sequence

CGE Cambridge University Herbarium

CR Critically endangered

CTAB Cetyltrimethyl ammonium bromide

DD Data deficient

DNA Deoxyribonucleic acid

ED Evolutionarily distinct

EDGE Evolutionarily distinct and globally endangered

EN Endangered

EOO Extent of occurrence

ES Equal splits

EW Extinct in the wild

EX Extinct

FP Fair proportions

FRU Institute for Biology of National Academy of Sciences of the Kyrgyz Republic herbarium

GBIF the Global Biodiversity Information Facility

GE Globally endangered

GPS Global positioning system

HPD Highest posterior density

IR Inverted repeat

ISSR Inter-simple sequence repeats

ITS Internal transcribed spacer

IUCN The International Union for Conservation of Nature

K Royal Botanic Gardens, Kew herbarium

L Naturalis Biodiversity Center herbarium

LC Least concern

LE Komarov Botanical Institute of Russian Academy of Sciences herbarium

LSC Large single copy

LTT Lineage through time

MCCR Monte Carlo constant rates

MCMC Markov chain Monte Carlo

Miroc ESM Model for Interdisciplinary Research on Climate

MRCA Most recent common ancestor

Mya Million years ago

NaCl Sodium chloride

NE Not evaluated

NT Near threatened

PVP Polyvinylpyrrolidone

RAPD Random amplified polymorphic DNA

RASP Reconstruct ancestral state phylogenies

rDNA Ribosomal deoxyribonucleic acid

RE Regionally extinct

RPM Revolutions per minute

rRNA Ribosomal ribonucleic acid

SSC Small single copy

tRNA Transfer ribonucleic acid

VU Vulnerable

Chapter 1.

The genus *Tulipa*: current and future conservation challenges

This chapter summarises the current literature on wild tulips, particularly the ecology of this plant and the taxonomic history of this group. At the end of this chapter there is a section on the conservation of wild tulips where we explore the impact of their life history, ecology, and taxonomy on the research and protection of this plant. Overall, this chapter aims to provide a comprehensive overview of wild tulips establishing a suitable foundation for the rest of the thesis.

1.1. Introduction to the Genus *Tulipa*

The genus *Tulipa* contains a range of terrestrial, perennial, geophytic species. There are currently four subgenera and twelve sections recognised in the genus, which are broadly based on distinct morphological characters. In general, all tulips grow from underground bulb structures composed of fleshy leaf bases or scales and which are covered by a specialised leaf base called a tunic. From the apical bud of the bulb arise flowering structures that are usually simple but can be branching with leaves that are either all basal or spread along the stem and are often undulating. Flowers form at the end of the stem and can occur in a range of colours (Everett, 2013). Some species are multiflorous, whilst most only grow a single flower. Due to its striking flower, the tulip has an extensive cultural history, and centuries of interest have led to a complex taxonomic record (Christenhusz *et al.*, 2013). Crucially, today, many wild species are known to be Threatened and their populations and habitat in decline (Davletkeldiev, 2006; Tojibaev and Beshko, 2015; IUCN, 2022). The genus's current plight and its significant value, especially to horticulture, mean there is an urgent need to assess and collate research focused on this genus. This chapter provides an overview of the biology, geography, evolutionary and cultural history of the genus *Tulipa,* as a foundation for the remainder of the thesis.

1.2. Life History and Ecology

1.2.1. Habitat

Generally, tulips only grow in areas with little summer rain and in soil that drains freely. Some species, such as *Tulipa sylvestris*, have adapted to wetter and cooler conditions, but these are in the minority (Wilford, 2006, 2013). Tulips are known to inhabit a range of soil types, from the slightly acidic to the slightly alkali, and from high organic content to low organic content, although they generally favour sandy soil with a high organic content (Coskuncelebi *et al.*,

2008). Notably, tulips do not grow well in clay soils or any that are highly acidic or alkali (Wilford, 2006). Given this, some species are markedly more difficult to grow in *ex-situ* collections, including those of the *Orithyia* subgenera, but others will thrive if provided with somewhat natural conditions (Wilford, 2006). Tulips require reasonable sunlight to grow and cannot tolerate prolonged shade, whilst good air movement is also important (Wilford, 2013). Wild tulips can be found growing in open areas of meadows, steppes, chaparral, deserts, stony mountainsides and in human influenced landscapes including fields, pastureland, road sides, abandoned gardens, and orchards (Hall, 1940; Christenhusz *et al.*, 2013; Everett, 2013). Many endemic species have a small distribution often in isolated gorges or on specific hill sides (Millaku and Elezaj, 2015; De Groot and Tojibaev, 2017). Importantly, across this diversity of habitat areas tulips can act as a useful indicator of broader ecosystem health with declines in tulip populations associated with broader declines in habitat quality (Pocock, 2019).

1.2.2. Initial growth from seed

A tulip seed established in soil, in suitable growing conditions, will develop a single leaf during the late winter or spring months. This will enable first-year growth of the plant as well as the extension of a main root into the soil. Seedlings will not flower in the first or second year and most will take between four to ten years of bulb development to store enough energy for flowering (Hall, 1940; Van Eijk *et al.*, 1991). After the first year of growth the main root is lost and new adventitious roots are developed annually which lack root hairs, are not branched, and cannot be replaced if removed (Botschantzeva, 1982). During years of non-flowering, the plants above ground structures remain relatively modest and therefore can be hard to locate.

1.2.3. The bulb

The bulb is made up of a basal plate and one to six fleshy leaf bases and is covered by a tunic. Variation in these traits can be helpful in species delimitation (Botschantzeva, 1982), but requires destructive digging up of specimens. In all species, the bulb is regrown each year leading to the accumulation of multiple layers across the tunic and fleshy leaf layers. Moreover, if the growing season is productive, multiple bulblets can form from the basal plate of the parent bulb, and in some species new bulblets can occur as droppers or stolons (Wilford, 2013). Stoloniferous species such as *Tulipa saxatilis* have been known to grow bulbs half a yard away from the parent plant enabling relatively quick spread of the population (Hall, 1940). Dropper bulbs descend further into the soil and in many cases the lower bulb will replace the original parent plant in the following growing season (Wilford, 2006). This is most observed in seedlings that need protection from the heat of the summer.

The bulb itself contains concentrated amounts of alkaloid and glycoside compounds, which are found in lower concentrations throughout the plant, except for the petals where they are absent. These compounds ensure the plant, and especially the bulb, is poisonous to humans and livestock, however the direct impact of consumption remains relatively understudied (Knight, 2006) and the consumption of this organ seems to even be common in some regions (Pieroni *et al.*, 2019). The bulb's fleshy scales act as a food and moisture reserve for the plant. Two fleshy scales present in the same bulb broadly act as an indicator that the tulip will flower in the coming spring. Adults can develop up to around six fleshy scales during their lifetime. Once ready to flower the bulb will develop as usual in the late winter-spring period with the basal leaf emerging containing a secondary leaf wrapped within. These will provide some energy for both fresh bulb development and above ground structure growth, however, most energy for flowering is stored in the bulb.

Growth in the bulb is triggered by cold winter conditions, known as vernalization, however the bulb is primarily an adaptation for surviving summer drought conditions (Hall, 1940; Wilford, 2006, 2013). The tulip bulb therefore provides an energy store that enables the persistence of an individual plant across multiple years, yet it also provides a mechanism for vegetative reproduction as well. All offspring produced through bulblet formation are genetic clones of the parent plant with some species more prone to this form of reproduction due to failings in seed production, for example it is common in triploid plants; nevertheless, some minor variations have been known to occur in asexually produced offspring (Hall, 1940). The renewal of bulbs on a yearly basis mean that disturbance of this structure during the growing season can be extremely detrimental to the plant (Wilford, 2013).

1.2.4. Stem and leaves

The stem length varies greatly between species (approx. 7cm up to 75cm) as well as to a lesser extent within species depending on growing conditions (Orlikowska *et al.*, 2018). The stem is usually stiff and straight, although in some species it droops at the flower end and can be glabrous or hairy. In general, leaf size declines with distance from the bulb and adult tulips will grow between one and twelve leaves, with most species developing between two to five leaves. These leaves may be located near the base of the stem or spaced apart depending on the species. Leaf shapes can be oval, elliptical, equilateral but are always lanceolate to some degree whilst the texture of the leaf can vary greatly between populations. In some species leaves may have dark purple patches, however most leaves are plain grey-green with a waxy layer on top (Botschantzeva, 1982; Orlikowska *et al.*, 2018). Although damage to leaves is often

not fatal, it can inhibit growth and therefore reproduction of the plant, meaning grazing damage is a significant threat to populations (Tojibaev and Beshko, 2015).

1.2.5. Flowering

Like leaf number, flowering time varies between species, with an array of species flowering across the months of March, April, May, and June; the variation in phenology means that not all species flower at the same time even if growing in the same area. All features of the tulip flower are cyclic and are arranged in alternate whorls. The flower is pentacyclic i.e. comprising five whorls, and therefore made up of two sets of three tepals, the inner tepals and the outer tepals, six anthers across two whorls, and a single carpel (Botschantzeva, 1982). The carpel is relatively similar in all species, with a three-lobed stigma positioned on top of the ovary, however a few species have a short style present, which is the defining feature of the *Orithyia* subgenus (Christenhusz *et al.*, 2013).

The stamens can show variation in length and are found in equal numbers to the tepals (Botschantzeva, 1982). A stamen consists of a filament and an anther, with a key characteristic used to differentiate between species, being the presence or absence of hairs on these filaments, and the existence of a boss, or basal swelling at the base of the filaments. In general, the *Eriostemones* subgenus consists of species with hair on the boss at the base of the filament, however, some species in this grouping have wispy hairs along the entire length of the filament and, in several species a boss exists without hair. The other species, which make up the *Tulipa, Orithyia, and Clusianae* subgenera, completely lack both the boss and the hairs on the filaments.

Blotch colour, margin, shape, and basal shading on flowers can vary not only between species or populations, but also from the outer to inner tepals, and even from one side of the tepal to the other (Botschantzeva, 1982). Generally, the outer tepals are thicker than the inner, especially at the base. Tulip flowers are often cup or star shaped and can be an important habitat for insects especially spiders (Su *et al.*, 2020). They range from plain white and pink through to yellow, orange, and red; with a range of shades between these colours known – blue and black do not exist (Wilford, 2013; Orlikowska *et al.*, 2018).

Some tulip species are multiflorous, primarily those of the *Eriostemones* subgenus but also some of the *Orithyia* subgenus, whilst those of the *Tulipa* and *Clusianae* subgenera are mostly uniflorous. Regardless, multiflorous taxa broadly have similar sized flowers to closely related uniflorous plants. If growing conditions are favourable, plants that have developed only a single

flower in previous years can become multiflorous and produce more leaves (Botschantzeva, 1982). Multiflorous species in the other subgenera are also known, but are much less common with single flowers the norm (Zonneveld, 2009). Many flower traits, including colour and number, have often been used to describe species, but are known to be highly plastic even within populations (Everett, 2013).

1.2.6. Reproduction

Tulips generally develop fertile seeds through pollination, however apomixis has been observed in *Tulipa* × *gesneriana* (Kashin, Kritskaya and Schanzer, 2016). Both self-pollination and cross-pollination occurs in tulips meaning offspring can be produced by a single plant or with neighbouring individuals. It is unclear to what extent both these forms of pollination occur and there is limited literature on the pollination of tulips in the wild. Insects such as small flies or bees are thought to be the most common pollinators of tulips; however, the wind and animals are also thought to contribute to the transfer of pollen (Kashin, Kritskaya and Schanzer, 2016). The balance of these pollination mechanisms is unclear, however in general, pollen is usually not carried far from the parent plant (Kashin, Kritskaya and Schanzer, 2016). In some cases, tulips have been known to hybridise between species, but significant pre- and post-fertilisation barriers are present between most interspecific crosses (Van Eijk *et al.*, 1991; Van Raamsdonk and De Vries, 1995; Van Tuyl and van Creij, 2006; Qu *et al.*, 2018). It remains difficult to measure to what extent hybridisation occurs in the wild, although *T.* × *tschimganica* is thought to be the established hybrid of *T. kaufmanniana* and *T. dubia* (Christenhusz *et al.*, 2013), which also both readily hybridise with *T. greigii* (Wilford, 2006; Zonneveld, 2009), and *T. ostrowskiana* and *T. kolpakowskiana* are recognised to have formed multiple natural hybrids (Botschantzeva, 1982).

After flowering the plant becomes desiccated due to the increasingly hot and dry climate. During this period the stem will remain, and in some cases even elongate (Wilford, 2006), whilst the leaves and the flower die back making identification of species exceptionally difficult. Once pollinated the ovary will swell and darken, with the fruit beginning to form from the syncarpous and superior ovary at the head of the stem. This fruit is trilocular due to the three connate carpels. Each of the fruits trivalent seed segments may contain more than 100 seeds (Orlikowska *et al.*, 2018). These rounded triangular seeds are brown and flat. Eventually the fruit containing the seeds will brown and dry out enough to split open leading to seed dispersal, usually via the wind, but sometimes by animals, birds, or water. In general, these seeds travel

only a small distance from the maternal plant (Hall, 1940; Kashin, Kritskaya and Schanzer, 2016).

1.2.7. Life span

Once the tulip has reached flowering maturity, which can take anywhere between four to ten years (T. Freeth & T. Hall 2020, personal communication, 21st March), it does not necessarily flower every year after that (Hall, 1940). The plant will continue to redevelop bulbs and, depending on the growing season, flower where possible. This inconsistency in flowering makes it difficult to relocate individuals from year to year. Eventually the parent plant will die back completely leaving a range of offspring bulbs which have been developed throughout its life. By this point, however, it will also have produced and dispersed seeds across multiple annual cycles leading to seedling growth nearby of genetically unique plants. Some bulbs in *ex-situ* collections have been known to survive for over 50 years, with a minimum of 25 years common (T. Freeth & T. Hall 2020, personal communication, 21st March). The life span of wild specimens remains understudied but is likely to be similar to *ex-situ* collections with a minimum of at least a few decades predicted.

1.3. Biogeography

1.3.1. Global distribution of Tulipa relatives

The *Amana* genus, which forms part of the *Tulipeae* tribe alongside *Tulipa, Erythronium*, and *Gagea (and Lloydia),* has a distribution at the eastern end of the natural tulip distribution covering areas of Japan, the Korean peninsula, and China. This genus, although accepted as a distinct clade shares many traits with *Tulipa* (Christenhusz *et al.*, 2013; Li *et al.*, 2017; Kim and Kim, 2018) and it is still common for *Amana* species to be identified as East Asian Tulips (Li *et al.*, 2017; Xing *et al.*, 2017), especially in Western and Central China where their ranges overlap. In addition, *Amana edulis*, frequently called *Tulipa edulis*, is used in traditional medicine and often closely related species including several tulips that lack the same medicinal properties (Ma *et al.*, 2014), are often misidentified as this species and collected.

Erythronium, the other sister clade to *Tulipa,* has a contrastingly broad distribution that covers the whole northern hemisphere, albeit discontinuously (Clennett *et al.*, 2012). This genus is thought to have originated in North America unlike the other genera of the *Tulipeae* tribe which are predicted to have originated in Asia (Huang *et al.*, 2018; Kim and Kim, 2018). The *Gagea* genus, which is the most distantly related genus to *Tulipa* in the *Tulipeae* tribe, has a very broad distribution, like *Erythronium*, that covers areas of north Africa, North America, and much of

Eurasia (Kim and Kim, 2018). The *Tulipeae* tribe's most recent common ancestor (MRCA) is estimated to have existed 57.63 Mya, whilst *Gagea*'s MRCA existed around 44.24 Mya, *Erythronium*'s around 24.38 Mya, and *Tulipa*'s MRCA is estimated to have existed 20.74 Mya (Kim and Kim, 2018).

1.3.2. Global distribution of Tulipa

Tulips grow across the temperate regions of the Old World, either side of the 40th parallel north in Eurasia (Pavord, 1999), and unlike many Liliaceae do not occur in the New World (Kim and Kim, 2018). This plant can occur from sea level up to 3500-3900 metres (Hall, 1940; Christenhusz *et al.*, 2013; GBIF.org (24 June 2021) GBIF Occurrence Download https://doi.org/10.15468/dl.b94693), yet, most are alpine species occurring in the lower and middle areas of mountain belts (Botschantzeva, 1982). Tulips are known to range from the southern Iberian peninsula, across Morocco and northern Africa (including Egypt and the Levant), are found on the island of Sicily, in Greece including Crete, throughout the southern Balkans and southern Ukraine, up into central Siberia, around the Black sea coast and into Turkey, Iran, Iraq, and the Middle East including northern Saudi Arabia, in the Caucasus, and east into Central Asia, western China, and Mongolia (Figure 1.1; (Christenhusz *et al.*, 2013; Everett, 2013)). They have become naturalised in some areas of North America and across much of Western Europe where their naturalised and natural range overlaps. Here, introgression of naturalised populations with true wild species may pose a threat to wild genetic variation (Christenhusz *et al.*, 2013). Species distributions vary considerably in size with several species spanning a large proportion of the global range of the genus, but most having much narrower ranges. The majority of new species have been described from Central Asia and the Balkans (Table 1.1; (Millaku and Elezaj, 2015; De Groot and Tojibaev, 2020; De Groot and Zonneveld, 2020; Rukšāns and Zubov, 2022)).

1.3.3. Evolutionary origin and diversity hotspots

Even today the evolutionary origin of tulips remains somewhat uncertain. For many centuries Turkey was considered the ancestral home of tulips as most European cultivar ancestors emerged from this region (Hoog, 1973). Yet, in the past 50 or so years, Central Asia has been proposed and favoured as the true origin of the genus because it harbours the greatest diversity of species, more than any other region (Hoog, 1973; Botschantzeva, 1982; Ivashchenko and Belyalov, 2019). Within Central Asia the Pamir-Alay, Tien Shan, and Deserts of Middle Asia have been presented as key diversification centres for the genus (Botschantzeva, 1982). A secondary centre of diversity has also been identified across Turkey, the Caucasus, and Iran,

however it harbours significantly less species than Central Asia (Hoog, 1973; Botschantzeva, 1982), although several new species have recently been described (De Groot and Zonneveld, 2022; Rukšāns and Zubov, 2022). The Mediterranean region has also been noted as a potential further area of diversity due to the many polyploid species found in this area, which may represent a recent radiation event (Botschantzeva, 1982).

Table 1.1. Tulip species described since 2010 with details of their distribution, endemicity, year of description, and relevant citation.

Species	**Distribution**	**Endemic**	**Year**	**Citation**
Tulipa albanica Kit Tan & Shuka	Albania	Yes	2010	(Shuka, Tan and Siljak-Yakovlev, 2010)
Tulipa koyuncui Eker & Babac	Turkey	Yes	2010	(Eker and Tekin Babaç, 2010)
Tulipa kosovarica Kit Tan, Shuka & Krasniqi	Kosovo	Yes	2011	(Shuka, Tan and Krasniqi, 2012)
Tulipa talassica Lazkov	Kyrgyzstan, Uzbekistan	No	2011	(Lazkov and Pashinina, 2011)
Tulipa kolbintsevii Zonn.	Kazakhstan	Yes	2012	(Zonneveld and De Groot, 2012)
Tulipa lemmersii Zonn., Peterse, J.J. deGroot	Kazakhstan	Yes	2012	(Zonneveld, 2009)
Tulipa ivasczenkoae Epiktetov & Belyalov	Kazakhstan	Yes	2013	(Эпиктетов and Белялов, 2013)
Tulipa intermedia Tojibaev & J.deGroot	Uzbekistan	Yes	2014	(Tojibaev, De Groot and Naralieva, 2014)
Tulipa akamasica Chrisdoulou, Hand & Charalambous	Cyprus	Yes	2014	(Christodoulou, Hand and Charalambous, 2014)
Tulipa turgaica Perezhogin	Kazakhstan	Yes	2014	(Perezhogin, 2013)
Tulipa auliekolica Perezhogin	Kazakhstan	Yes	2014	(Perezhogin, 2013)
Tulipa narcissicum N.Y.Stepanova	Russia	Yes	2014	(Stepanova, 2014)
Tulipa jacquesii Zonn.	Kyrgyzstan	Yes	2015	(Zonneveld, 2015)
Tulipa luanica Millaku & Elezaj	Kosovo	Yes	2015	(Millaku and Elezaj, 2015)
Tulipa zonneveldii J.J. de Groot & Tojibaev	Kyrgyzstan	Yes	2017	(De Groot and Tojibaev, 2017)
Tulipa dianaeverettiae J.J. de Groot & Zonn	Kazakhstan, Mongolia	No	2020	(De Groot and Zonneveld, 2020)
Tulipa annae J.J. de Groot & Zonn	Kazakhstan	Yes	2020	(De Groot and Zonneveld, 2020)
Tulipa bactriana J.J. de Groot & K.S. Tojibaev	Uzbekistan	Yes	2020	(De Groot and Tojibaev, 2020)
Tulipa brinkii J.J. de Groot & B.J.M. Zonneveld	Iran	Yes	2022	(De Groot and Zonneveld, 2022)
Tulipa lorestanica Rukšāns & Zubov	Iran	Yes	2022	(Rukšāns and Zubov, 2022)
Tulipa salsola Rukšāns & Zubov	Kazakhstan	Yes	2022	(Rukšāns and Zubov, 2022)

Species status checked May 2022 (POWO, 2022), except for *T. brinkii*, *T. salsola*, and *T. lorestanica* which have not been added to this database yet, but are accepted

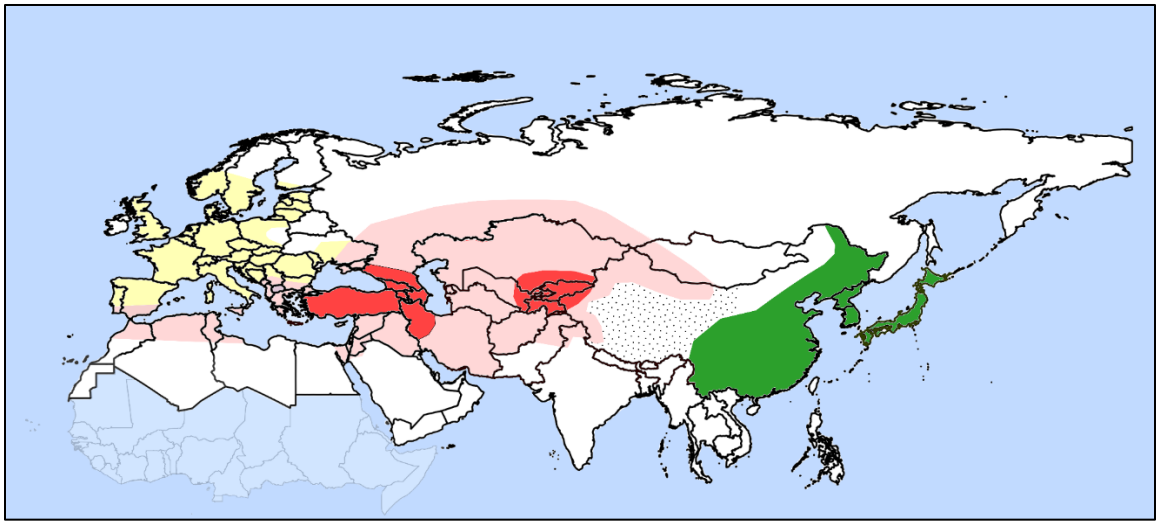


Figure 1.1. The global distribution of the genus *Tulipa*.
The light red area shows the natural distribution of tulips with darker red regions the two centres of diversity for the genus. Yellow indicates areas where tulips have become naturalised. Green represents the distribution of *Amana* with the green dotted area where tulips and *Amana* may overlap.

1.4. Cultural Significance

1.4.1. The first cultivated tulips

Today the tulip is one of the most well-known monocots in the horticultural world. Nevertheless, this flower was once relatively unknown outside its natural range with only a single species of tulip noted in Byzantium until the Seljuk invasion of Baghdad in 1055 (Segal, 1993; Christenhusz *et al.*, 2013). After this invasion, tulips became an increasingly common sight in the gardens of Bactria and Persia (Christenhusz *et al.*, 2013), and also further abroad in India (Pavord, 1999). In 1123 tulips were mentioned for the first time in global literature in a publication by a Russian author (Botschantzeva, 1982), and then in 1190 were included in the prose of the Persian poet Omar Khayyam (Hall, 1940). In the following centuries tulips featured in numerous works including Hafiz's writings in 1390 (Hall, 1940) and in the verses of the mystic poet Celaleddin Rumi in the 13th Century (Segal, 1993). Little information survives about tulips from the Seljuk's homeland in the mountains of Central Asia, barring decorated 15th century Uzbek poetry (Botschantzeva, 1982). Nonetheless it is likely they were well known and a common sight along trade routes of the silk road (Everett, 2013).

1.4.2. The Ottoman influence

Throughout the 13th, 14th, and 15th centuries tulips became an established garden flower in the Middle East, especially in the Ottoman empire, where cultivation of tulips began. New varieties

were bred, which unlike their future European relatives, were selected to have pointed tepals (Hall, 1929). Increasing efforts to collect wild species from neighbouring regions, such as Persia, provided novel material for breeding. Uncertainty remains around which species were used to breed these ancient cultivars, however *Tulipa suaveolens, T. schrenkii,* and *T.* × *gesneriana* are all thought to have contributed (Hall, 1940; Hoog, 1973; Kritskaya *et al.*, 2020). Yet, species such as *T. armena*, *T. agenensis*, *T. lanata*, and potentially others from Central Asia were also likely involved (Christenhusz *et al.*, 2013).

During the Ottoman empire tulips increasingly became a cultural phenomenon. This was most notably the case under Suleyman the Magnificent (c. 1495 – 1566), under whom tulips developed into an important national symbol and were increasingly used as religious and cultural images (Pavord, 1999), yet even following Suleyman's reign, tulips continued to grow in popularity, especially with high-ranking officials and Sultans who could afford these increasingly valuable commodities. Throughout this time patterning with tulips became more frequent on furniture and pottery, especially of the Iznik style, which this plants colouring is thought to have influenced (Figure 1.2; (Christenhusz *et al.*, 2013)). The prominent status of tulips in Ottoman culture reached its pinnacle during the 'tulip era' (1718-1730) under the rule of Ahmed III, who grew vast swathes of tulips in pastureland which were then planted into his palace gardens where he hosted special tulip parties (Pavord, 1999).

1.4.3. Tulips enter Europe

Although there is some evidence to suggest that *Tulipa sylvestris* was recognised in Andalusia somewhere between the end of the 11th Century and beginning of the 12th Century (Bermejo and Sánchez, 2009) tulips were not widely known in Europe before the 16th Century (Hall, 1940; Pavord, 1999; Christenhusz *et al.*, 2013); this is supported by their absence in European or Persian art and the flowery border designs of European medieval manuscripts (Hall, 1940; Pavord, 1999). They were first mentioned in cultivation in Europe in 1530, but this was only on a very small scale in Portugal (Pavord, 1999). The first records of a European trade in tulips, written by Belon (1517 – 1564), describe the import of tulips to the port city of Antwerp in 1562. But, these imported bulbs were not grown correctly and many were mistaken for onions, so were roasted over fires, emphasising their rarity in Europe at this time (Hall, 1929; Pavord, 1999).

A pivotal moment for European tulip horticulture occurred on the trip of Ghiselin de Busbecq (1522 – 1591) in the early 1550s. At the time he was the ambassador of Emperor Ferdinand I to the Court of Suleyman the Magnificent. At this point tulips were known as lale in both Turkish

and Arabic but lacked a Latin name. However, a misunderstanding between de Busbecq and his Ottoman counterparts led de Busbecq to use the name tulipan on his return to Europe. This is believed to have occurred through a mix up between the word for turban, dulband (in Persian) or tulband (in Turkish), and the name of the flower. It is unclear whether this is because the flower was described as looking like a turban, because tulips were worn in turbans, or because of a general misunderstanding of language (Hall, 1940; Pavord, 1999; Christenhusz *et al.*, 2013). Nevertheless, during this trip de Busbecq encountered tulips for the first time and crucially was gifted seeds by his Ottoman counterparts, which he sent back to Vienna and Prague.

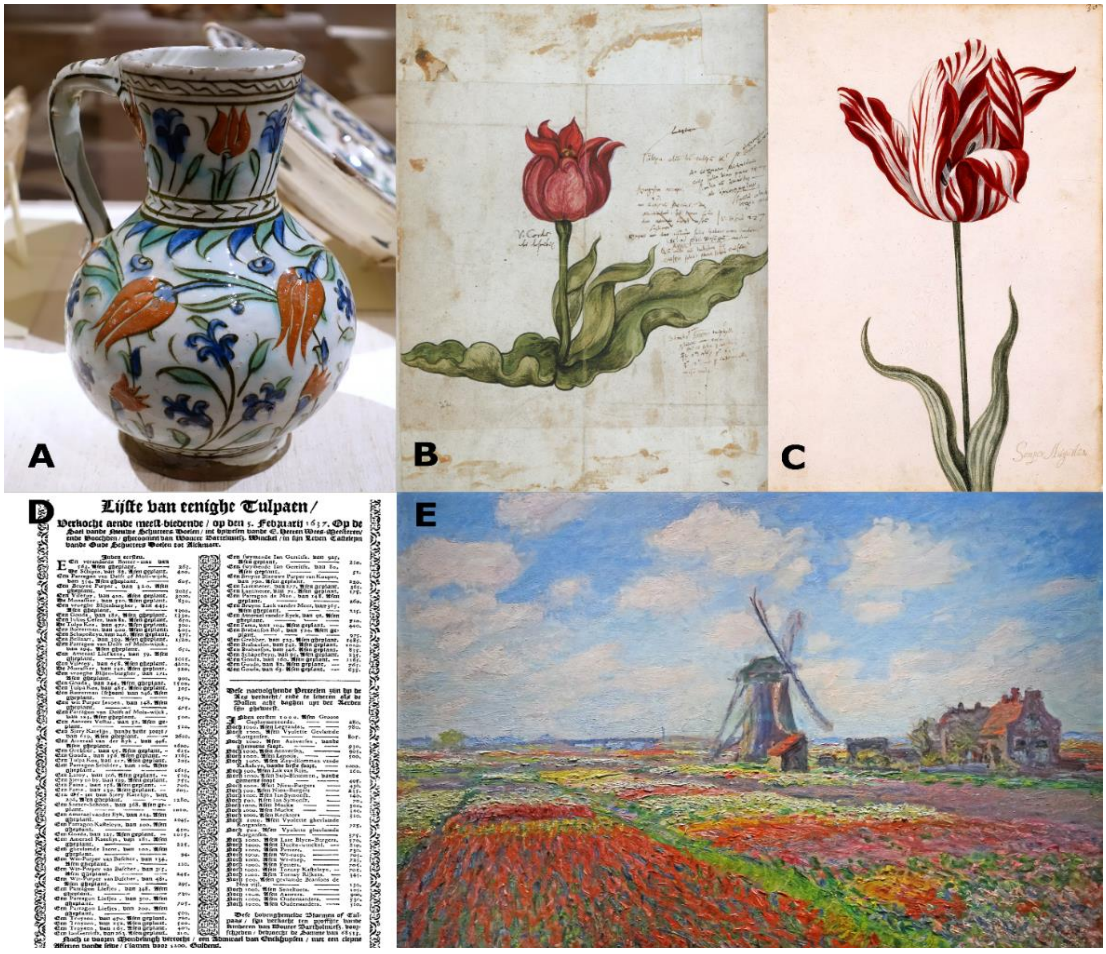


Figure 1.2. Tulips and their cultural history. (a) shows an Ottoman Iznik pottery jug from the 16th Century with tulips portrayed in the design. (b) shows the tulip drawing produced by Conrad Gesner based on a specimen growing in Hewart's garden in 1561 (c) shows a painting from an unknown artist of the Semper Augustus which is thought to be the most expensive tulip sold during tulip mania in the Netherlands in 1637 (d) shows a list of tulips and their prices from 1637 in the Netherlands (e) shows a painting entitled 'English Tulip Fields in Holland' created by Claude Monet in 1886. (All photos from Wikipedia Commons)

Although de Busbecq's seeds are often regarded as the earliest source of tulip material in Europe, this flower is actually thought to have entered the region several times during the 16th Century (Hall, 1940; Pavord, 1999). The botanist Gesner first encountered flowering tulips in Hewart's garden, in Augsburg, in 1559. His illustration of a tulip specimen is believed to be one of the first in Europe (Figure 1.2), and due to the flowering time of the plant must have come from a source other than de Busbecq (Hall, 1940; Christenhusz *et al.*, 2013). Belon may also have acted as another source of tulip material through his work establishing a plant collection near Le Mans in 1540 (Pavord, 1999).

1.4.4. A growing influence in Europe

After the initial introduction of this plant into Europe tulips quickly became an important part of horticultural and scientific collections. In the 1570s, the botanist Carolus Clusius, after becoming the Praefectus of the Imperial Medicinal Garden in Vienna, established his first tulip collection (Clusius, 1576; Hoog, 1973). De Busbecq and Clusius were long term acquaintances, and it is likely that some of the original seeds sent to Vienna in the early 1550s would have been grown by the Praefectus. Further seeds were sent by de Busbecq to Clusius in 1593 to strengthen his now expanding collection (Christenhusz *et al.*, 2013). Yet, Clusius established his most distinguished collection of tulips in the Botanic Gardens at the University of Leiden, after accepting the title of Horti Praefectus in 1593. This collection expanded quickly to become one of the most extensive in Europe and Clusius is believed to have significantly contributed to the popularity of tulips across the European continent through his distribution of bulbs and seeds to other collections (Christenhusz *et al.*, 2013).

Demand for tulips grew rapidly throughout the 16th and 17th centuries across Eurasia, but most intensively in western Europe where, by 1600, tulips were widespread (Hall, 1929). In the 17th century the rounded tepal shape, which is common today, began to be cultivated. Tulips with this trait quickly superseded the Ottoman pointed tepal varieties. Moreover, at this time rare, flamed forms, caused by the then undiscovered tulip breaking virus, began to attract significant attention. Although, scientific collections continued to expand in the early 17th Century, including those of Lobelius and Clusius, it was during these centuries that royalty and the growing middle-classes of the Netherlands, England, France, and Germany began to buy and trade cultivated varieties to show off their nouveau riches (Pavord, 1999; Christenhusz *et al.*, 2013).

This led to a flourishing horticultural trade in France fuelled by Flemish tulip breeders where many new varieties were being bred. Specimens from this region were reportedly sold for thousands of pounds in today's money, traded for horses and carriages, and even exchanged

for an entire brewery (Hall, 1929; Pavord, 1999). Although this trade preceded the more famous 'tulip mania' period in the Netherlands, the French based trade is now thought to have been equally extensive (Pavord, 1999). Nonetheless trade in France would subside and the market would be driven northwards into the Netherlands where these flowering plants would trigger one of the most famous financial speculative bubbles of all time (Hall, 1929; Pavord, 1999).

1.4.5. Tulip mania

The trade in tulips thrived in Holland throughout the 1620s with prices continually rising. The climbing value of tulips led to a period known as 'tulip mania' which began in 1634 and is recognised as one of the first ever financial bubbles (Figure 1.2). At its peak the speculative bubble led to dramatic inflation in the value of tulips, well beyond their true value; some bulbs were sold for more than £5 million in today's money or traded for properties (Pavord, 1999). This financial bubble was driven by increasing wealth in the Netherlands and the gamble that a relatively valueless bulb could develop traits that would make it extremely valuable. Prices in this bubble reached their peak in 1636 before the market collapsed in 1637 leading to financial catastrophe (Pavord, 1999). Increasing demand drove market values up (Hall, 1929), but eventually the bubble burst due to oversupply (Pavord, 1999). Nevertheless, in Europe the horticultural demand for this flower remained after the bubble burst (Christenhusz *et al.*, 2013). Tulip breeding therefore continued to flourish in the following decades and centuries with the constant development of new cultivars.

1.4.6. Horticultural significance

This enduring horticultural demand meant that rare bulbs continued to command relatively large sums from avid collectors; in England in the 1830s, 40s and 50s bulbs were reportedly sold for what would be today between £8000 to £20,000 (Hall, 1929). The constant interest for unique cultivars ensured breeding of new tulips persisted and that tulip fields continued to be a common sight across Europe with Claude Monet even painting this landscape (Figure 1.2). Even today tulips remain extremely popular with around 140-150 new cultivars registered by the Royal General Bulb Growers' Association (KAVB) between 2014 and 2016 (Orlikowska *et al.*, 2018). This has led to the accumulation of around 14,000 named varieties over the horticultural history of tulips (Orlikowska *et al.*, 2018).

Today, cultivated tulips are grown across the temperate regions of both hemispheres, covering 13,000 ha of agricultural land and are at the centre of a trade worth billions of euros (Christenhusz *et al.*, 2013; Orlikowska *et al.*, 2018). Although 88% of land used to cultivate tulips is situated in the Netherlands breeding of new cultivars is a relatively global affair

(Orlikowska *et al.*, 2018). There is now also increasing research efforts focused on crossing wild and cultivated specimens to support the development of more diverse and hardy varieties to meet horticultural demand (Qu *et al.*, 2018; Xing *et al.*, 2020). Essentially, the genetic uniformity of all current cultivars means that any significant horticultural developments are likely to be underpinned by the broader genetic variation harboured in wild species (Orlikowska *et al.*, 2018; Qu *et al.*, 2018)

1.4.7. Contemporary culture

The history of the tulip is intertwined with human civilisation, trade, and empires and therefore they are not only an important garden flower, but also hold considerable cultural value in many regions. Today, tulip festivals are a regular mainstay on the calendars of many countries, including the Netherlands, Canada, the U.S., and Turkey (Figure 1.3 (Roding and Theunissen, 1993)). The tulip has also been adopted as a national symbol in an array of countries where many wild species grow including Turkey, the nations of Central Asia, as well as Iran. Notably, the political uprising of the Kyrgyz public in 2005 was named the 'tulip revolution' because of the importance of this flower to the people of this country (A tulip revolution, 2005). Yet, tulips have also achieved more mainstream fame through their role in books such as 'The Black Tulip' (Dumas, 1850) and 'Tulip fever' (Moggach, 1999) including in the more recent film adaptation (Chadwick, 2017). Today, this once uncommon plant is universally recognised, commonly depicted, and remains a symbol of pride for many nations.

1.5. Taxonomic History

1.5.1. The name Tulipa and the type specimen

In 1601 the botanist Clusius, although much of his collections were focused on cultivated varieties, endeavoured to order the broader Lilionarcissus of which tulips were a part. During this work he accepted the Latin name Gesner had given to the specimens he had described in Hewart's garden, *Tulipa*. Clusius based his classifications on flowering time, and it is likely he included a range of cultivated and naturalised plants, which today are not recognised as true species. Nonetheless this still remains the inaugural attempt to bring order to the tulip group and the first case of a Latin name being used for the scientific grouping of this plant (Christenhusz *et al.*, 2013).

The taxonomic genus was formally created by Carl Linnaeus, who in 1753, following his establishment of the binomial nomenclature system, formally described three species of *Tulipa* in his Species Plantarum (Table 1.2 (Linnaeus, 1753)). The initial specimens were named

Tulipa breyniana, *T. sylvestris*, and *T.* × *gesneriana*. The material used to determine *T. breyniana* was later reidentified as a member of the *Homeria* genus (Lewis, 1914), which has now been synonymised under *Moraea* Mill. (Goldblatt, 1973). The specimen used to describe *T. sylvestris* was also reidentified as a member of the *Liriopogon* Raf. (Rafinesque, 1837), with, the name *T. sylvestris* today associated with a valid but different tulip species. These errors mean that *T.* × *gesneriana* is the lectotype of the genus by exclusion (Zonneveld, 2009; Christenhusz *et al.*, 2013) and that previous conflict over the correct type has now been resolved (Hitchcock and Green, 1929; Van Raamsdonk and De Vries, 1995; Veldkamp and Zonneveld, 2012; Christenhusz *et al.*, 2013). Yet, no populations of wild *T.* × *gesneriana* are known and the plant is now considered a complex garden hybrid and not a species (Fay and Christenhusz, 2013); most mentions of *T.* × *gesneriana* in literature relate to the wild species *T. suaveolens*. It therefore remains the type specimen, but should not be considered a true wild species and the source of the original lectotype specimen remains uncertain (Christenhusz *et al.*, 2013).



Figure 1.3. Tulip festivals of the world. Starting top left and moving clockwise: the tulip festival held in Ottawa, Canada (Saffron Blaze, via http://www.mackenzie.co), the tulip festival of Tesselaar in the Netherlands (Chris Phutully from Australia - 2013 Tesselaar Tulip Festival), and the Skagit valley tulip festival held in the United States (Karyn Sig from Marysville, WA - Washington tulips Uploaded by X-Weinzar). (All photos from Wikipedia Commons).

Table 1.2. Summary of the major taxonomic changes in key publications throughout the scientific history of tulips. The first column lists the researchers who have contributed significant changes to tulip taxonomy with the changes they made detailed under the relevant major grouping heading that have existed during the taxonomic history of tulips detailed in the first row.

	Orithyia (D.Don) Baker, J.	**Clusianae** (Baker) Zonn. & Veldkamp	**Tulipa** (Marais)	**Leiostemones** (Boiss.)	**Eriostemones** (Boiss.) Hall	**Eutulipa** (Baker)	**Other**
(Linnaeus, 1753)	-	-	-	-	-	-	-
(J. Baker, 1874)	Subgenus	-	-	-	-	Subgenus Separated into five sections: Eriobulbi, Gesnerianae, Scabrisscapae, Saxatiles, and Sylvetris	-
(Boissier, 1882)	-	-	-	Section	Section	-	-
(A. I. Vvedensky, 1935a)	Section	-	-	Section	Section	-	Sections: Tulipanum, Spiranthera, and Lophophyllon
(Hall, 1940)	-	-	-	Section Separated into five subsections: Clusianae, Gesnerianae, Oculis-Solis, Eichleres, and Kolpakowsianae	Subgenus Separated into three sections: Australes, Saxatiles, and Biflores	-	Solitary species
(Botschantzeva, 1982)	Section	-	-	Section	Section	-	Sections: Tulipanum, Spiranthera, and Lophophyllon
(Stork, 1984)	Section	-	Section	-	Section	-	-
(Van Raamsdonk and De Vries, 1992, 1995)	-	-	Subgenus Separated into five subsections: Clusianae, Tulipa, Kolpakowskianae, Tulipanum (separated into two series Tulipanum and Aureo-fasciatae), and Eichleres (separated into eight series Eichleres, Vinistriatae, Undulatae, Multiflorae, Spiranthera, Lanatae, Glabrae, and Luteo-apiculatae)	-	Subgenus Separated into three sections: Australes, Biflores, and Saxatiles	-	-

(Zonneveld, 2009; Veldkamp and Zonneveld, 2012)	Subgenus One section: Orithyia	Subgenus One section: Clusianae	Subgenus Separated into seven sections: Kolpakowskianae Multiflorae, Lanatae, Vinistriatae, Spiranthera, Tulipanum, and Tulipa	-	Subgenus Separated into three sections: Sylvestris, Biflores, and Saxatiles	-	-
(Christenhusz *et al.*, 2013)	Subgenus	Subgenus	Subgenus	-	Subgenus	-	-

1.5.2. Subgenera and expansion of the Genus

Before the formal taxonomic nomenclature was introduced a range of tulips were already recognised in the wild (Hall, 1940) consequently many were described shortly after the formation of the genus: *Tulipa sylvestris* was described in 1753, *T. biflora* in 1776, and *T. suaveolens* Roth in 1794; a specimen of *T. biflora* was found in Linnaeus's herbarium from before 1776, but had been misidentified (Hall, 1940). A further two species were described by Redouté during his work on Liliaceaes between 1803-1815, *T. clusiana* and *T. agenensis*. Both species would be re-described due to complications with the use of the name *T. praecox*, which was only resolved in recent decades (Christenhusz, Fay and Govaerts, 2013). These initial species were broadly those with large distributions although further species began to be formally recognised from areas such as Greece, Asia Minor and upper India in the early 19th Century (Hall, 1940).

Around the same time a range of new taxa were described from specimens collected across western Europe, predominantly France, Italy, and Switzerland. These tulips represented varieties that had either escaped or been introduced to the region and had formed naturalised populations. Although naturalised populations were reported as far back as the 16th and 17th centuries, with the famous botanist John Parkinson describing such tulips as the "Red Bolonia" growing in northern Italy (Hall, 1940), it was only in the 1800s that most were formally described. Naturalised populations have led to the description of several accepted species, using specimen material from populations far outside of their native range. For example *Tulipa agenensis* which was described from material collected in western Europe (Christenhusz *et al.*, 2013) yet it is actually native to the Middle East. Historically many of these naturalised species were classified under the grouping neotulips or *Neo-tulipae*, however predominantly scientists now disregard most of the species described from naturalised populations and have removed them from official species lists (Hall, 1940; Fay and Christenhusz, 2013). Most of these varieties

are now thought to be related to cultivated *T.* × *gesneriana* and have therefore been placed within the taxonomic complex Gesneriana which encompasses all naturalised cultivars (Christenhusz *et al.*, 2013).

In 1829 the *Tulipa* genus was described within the broader tree of life for the first time by Roemer and Schultes (1829) who placed it between *Fritillaria* and *Erythronium* (Roemer and Schultes, 1829). This work was followed by descriptions of several new genera based on tulip material. Some from naturalised American specimens, but most notably the description of the *Orithyia* genus based on material of *Tulipa uniflora* (Don, 1836), the first suggestion this group may differ from other tulips. Subdivision of the genus *Tulipa* itself would not occur until Baker (1874), who rejected previous classifications, separated the genus into two subgenera, *Eutulipa* and *Orithyia* (Table 1.2 (J. Baker, 1874)). This split was based on the morphological trait of style length, with *Orithyia* species recognised from their long styles and *Eutulipa* from their rudimentary styles (Christenhusz *et al.*, 2013). In preceding years, the Russian botanist Regel had endeavoured to organise the, then 26, recognised species into groups. He suggested that species within *Eutulipa* could be split further into two sections, but this separation was not suitably classified and his work was widely ignored (Regel, 1873b; Botschantzeva, 1982).

Discovery of new species throughout the first three quarters of the 19th century led to the steady growth of the genus. Then, in the late 19th century, an array of new species were discovered in the, yet unexplored Central Asia region (Figure 1.4). Regel, who was at the time the director of the Imperial Botanical Garden of Saint Petersburg, described most of these species. His descriptions were made using material received from Russian military expeditions into Middle Asia, including from his son Albert Regel who spent time in western China (Regel, 1873b, 1877; Hoog, 1973). The large diversity of unique specimens from this region highlighted the potential diversity still to be described in Central Asia (Hall, 1940; Christenhusz *et al.*, 2013). Several of these new species were brought into cultivation in the 1890s expanding the genetic resources available to horticulturalists (Hall, 1940).

Using this new wealth of taxa, Boisser in 1882, formally described the split of *Eutulipa*, previously suggested by Regel (Table 1.2). He named the two new sections *Eriostemones* and *Leiostemones* (Boissier, 1882). The *Eriostemones* section was characterized by an enlarged boss at the base of the filaments which had hairs growing from it, whereas *Leiostemones* represented species that lacked these hairs. Moreover, at this time the taxonomic position of *Amana,* a genus often recognised as sister to *Tulipa,* and *Orithyia* remained uncertain with

these groupings changing from the status of genera to subgenera depending on the publication (Clennett *et al.*, 2012; Christenhusz *et al.*, 2013; Kim and Kim, 2018).

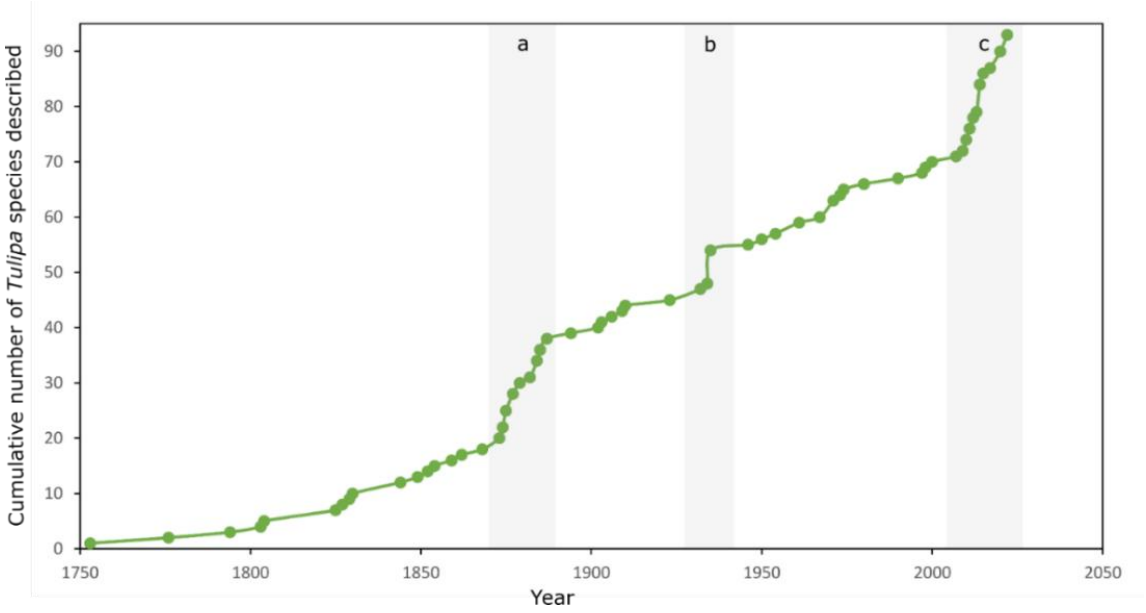


Figure 1.4. Graph showing the accumulation of accepted tulip species over time with respect to the 93 currently recognised tulip species. (a) highlights the species described by Regel as he began his work on Central Asian species, section (b) displays the work of Vvedenskyi as he named many new species from Russia and Central Asia, and section (c) shows the many new species described in recent years.

Boisser's work provided the foundation for numerous advances in tulip taxonomy over the next two centuries. In 1940, the British botanist Alfred Daniel Hall published a comprehensive revision of the genus and raised *Eriostemones* from section to subgenera level; there were numerous traits found in this clade that made it distinct from the rest of tulip diversity (Hall, 1940). Hall, however maintained the *Leiostemones* as a section because it encompassed all species that didn't fit into *Eriostemones* rather than representing a clade with defining features (Hall, 1940). In this revision he further split the *Eriostemones* taxon into three distinct sections: *Australes*, *Saxatiles*, and *Biflores* and the *Leiostemones* clade into five subsections: *Clusianae*, *Gesnerianae*, *Oculis-Solis*, *Eichleres*, and *Kolpakowsianae* (Table 1.2). Similar work at the time investigating chromosomal morphology provided support that the grouping of *Clusianae* was as different from *Leiostemones* as it was from *Eriostemones* (Upcott and La Cour, 1936), although Hall still maintained it as a subsection of the *Leiostemones*. Hall also described three new species based solely on the ploidy characteristic (Hall, 1938), whilst also identifying a few

solitary species that did not fit his framework, such as *Tulipa schmidtii* and *T. sprengeri*, showing that further work was needed to develop a complete classification system (Hall, 1940).

New species continued to be discovered regularly during the early 20th century, with most described from Eastern and Central Asia (Figure 1.4). Whilst Hall was carrying out work at the John Innes Horticultural Institution in the U.K., Alexander Vvedensky was working in Russia and it was he that described many of these new species and using his material added the subsection *Spiranthera* to the *Leiostemones* (A. Vvedensky, 1935). His work greatly increased the number of species known from Central Asia and he provided a range of important specimens to be grown at the Royal Botanic Gardens at Kew (Hoog, 1973). Hall, notably, acknowledged in his work that he lacked material for many of these new species and that the herbarium material he could obtain was of little value as identification of a species was difficult from faded, dried specimens (Hall, 1940). So although these new species were poorly represented in Halls taxonomic assessment of the genus, material from Central Asia was becoming more widely available as botanists, such as Hoog and Vvedensky, obtained and distributed specimens throughout European collections (Stork, 1984).

Vvedensky's work laid the foundation for the Kazakh botanist Zinaida Botschantzeva's comprehensive study of Central Asian wild tulip diversity which she published in 1962. This work was originally published in Russian and was not therefore accessible to the wider scientific community, but in 1982 an English translation became available and after this Botschantzeva's research became an important resource for those working on tulips around the world (Botschantzeva, 1982). Pivotally this publication connected the scientific knowledge collated in the U.S.S.R. to the broader literature. In this work, Botschantzeva generally followed the taxonomic classification system of Vvedensky, splitting the genus into six sections: *Orithyia*, *Leiostemones*, *Eriostemones*, *Tulipanum*, *Spiranthera*, and *Lophophyloon*; although no tulips from the *Orithyia* section were described within her work (Table 1.2). Yet much like Vvedensky's work the taxonomic classification system did not become established with researchers continuing to favour the work of Hall (Hoog, 1973; Van Raamsdonk and De Vries, 1992, 1995). Nevertheless, an array of new species were described through Botschantzevas's extensive expeditions in the region.

1.5.3. Modern taxonomy

Throughout the 20th century, aided by the work of Vvedensky, Hall, and Botschantzeva, understanding of tulip taxonomy greatly increased, yet there remained fundamental issues with the current framework. The accepted lectotype of the genus, *Tulipa* × *gesneriana*, remained

classified within the *Leiostemones* where it had been for over a century, however, in 1984 Marais proposed that the section *Leiostemones* should be renamed to *Tulipa* reflecting its inclusion of the type specimen in accordance with taxonomic regulations. This nomenclature change led to the reclassifying of tulips into one subgenera called *Eriostemones* and another section called *Tulipa* (Table 1.2. (Marais, 1984)). Further support for this long standing split was provided by a pigmentation analysis of tulip flowers; *Eriostemones* species contained delphinidin anthocyanidins whilst *Tulipa* (*Leiostemones*) species contained pelargonidin anthocyanidins and higher levels of carotenoids (Nieuwhof, Van Raamsdonk and Van Eijk, 1990).

In the late 20th century, wild genetic diversity became increasingly important to horticulture with the desire to breed wild traits into cultivars. Projects were undertaken to collect and cross species to introduce new genetic diversity into domesticated tulips (Van Eijk, Garretsen and Eikelboom, 1979; Van Eijk, Eikelboom and Hogenboom, 1986; Van Eijk *et al.*, 1991; Van Raamsdonk, Van Eijk and Eikelboom, 1995; Creij, Kerckhoffs and Tuyl, 1997). As the focus was to introgress these traits into cultivars, *Tulipa* × *gesneriana,* an ancient cultivar, was commonly used as one of the parents in crosses. Nonetheless these experiments also provided an insight into reproductive barriers between several wild taxa. Initial conclusions of crossing work showed that most sections were reproductively isolated, and even within sections such as *Clusianae*, *Tulipanum*, and *Kolpakowskianae* species were intersterile (Creij, Kerckhoffs and Tuyl, 1997). Unsurprisingly, *T.* × *gesneriana* crossed easily with any species within *Gesnerianae* and with some species from the *Eichleres* section, which contain taxa thought to be closely related to this complex hybrid (Van Eijk *et al.*, 1991). Overall however, the use of *T.* × *gesneriana* in most crosses constrained these studies broad impact (Christenhusz *et al.*, 2013) and the *Gesnerianae* subsection would be disbanded after further research highlighted its horticultural origin (Van Raamsdonk and De Vries, 1995).

During this period systematic studies of both subgenera were undertaken (Table 1.2). These experiments used thirty morphological traits within a principal component analysis to group species within the clades of *Eriostemones* and *Tulipa* (Van Raamsdonk and De Vries, 1992, 1995). The *Eriostemones* subgenera was maintained in the three sections originally dictated by Hall (Hall, 1940; Van Raamsdonk and De Vries, 1992), however some species complexes were recognised where clusters of species could not be separated, for example *Tulipa biflora* and *T. sogdiana* became species that represented a range of morphologically similar taxa (Van Raamsdonk and De Vries, 1992). The *Tulipa* section was raised to subgenera level, and, in

turn, previously described subsections were raised to the level of section whilst several new ones were described. Overall, five sections were presented within *Tulipa*: *Clusianae*, *Tulipanum*, *Tulipa*, *Kolpakowskianae*, and *Eichleres*. Furthermore, ten series were described with *Tulipanum* split into two and *Eichleres* consisting of eight; the previous section *Spiranthera* was demoted to a series as it was deemed too closely related to *Eichleres* (Van Raamsdonk, Van Eijk and Eikelboom, 1995). In general however, these analyses remained greatly limited by their patchy sampling which did not include specimens from *Amana*, *Orithyia*, or many other previously identified morphologically isolated species (Christenhusz *et al.*, 2013).

In 2001 the first phylogenetic analysis of the *Tulipa* genus was undertaken, which used five plastid regions and showed the genus as monophyletic and distinct from closely related *Erythronium* and *Amana* (Fay *et al.*, 2001). Furthermore, both the *Eriostemones* and *Tulipa* subgenera were found to be monophyletic providing significant support for this century old division. In this work it was additionally noted that two other monophyletic groups, which today are recognised as the *Clusianae* and *Orithyia* subgenera, could potentially be distinguished and described as new clades, however this study could not provide the conclusive evidence required to do so.

This previous molecular and morphological work provided a foundation for the comprehensive study of the genus embarked upon by Zonneveld and Veldkamp. Who, combining newly obtained cytological data with previous data on geographic distributions, morphological variation, crossability, and molecular data, and with relatively comprehensive species representation, formally reorganised the genus (Zonneveld, 2009; Veldkamp and Zonneveld, 2012). Importantly, the authors included an array of specimens not included in previous taxonomic work as well as specimens representing all recognised sections. This work showed that although most species have 24 chromosomes the 2C-value of species, representing their relative genome size, varied greatly, ranging from 32 to 69pg. They used this variable trait to designate the two new subgenera, *Clusianae* and *Orithyia* (Table 1.2). The cytological data from this study also led to reclassifications at lower taxonomic levels, with a broader range of sections recognised removing the requirement for series (Van Raamsdonk and De Vries, 1995). The newly described *Clusianae* and *Orithyia* subgenera contained only a single section which, following systematic rules, carried the same name as the subgenus. The *Tulipa* clade was split into seven sections *Kolpakowskianae*, *Multiflorae*, *Lanatae*, *Vinistriatae*, *Spiranthera*, *Tulipanum*, and *Tulipa* whilst the *Eriostemones* subgenera remained split into the three sections introduced by Hall in 1940 (Hall, 1940). Within these new classifications there still remained

groupings of species that could not be separated, which the authors chose to synonymize under the oldest taxon name in the group.

In 2013, the most comprehensive DNA-based phylogenetic study of tulips was undertaken (Christenhusz *et al.*, 2013), even so, this study only included around one third of known species. Yet, importantly, it contributed to the production and publication of an up to date monograph of the genus, which is an important resource for conservationists (Everett, 2013), and provided a much needed insight into the molecular relationships between species. The study used six molecular markers, five from the plastid genome and the Internal Transcribed Spacer from the nuclear genome, to resolve the relationship between 25 tulip taxa (Christenhusz *et al.*, 2013). Both Maximum Parsimony and Bayesian analysis supported the monophyletic nature of the genus and its division into four subgenera. Importantly, the research highlighted the subgenera *Orithyia* as sister to the rest of tulip diversity. *Tulipa, Clusianae*, and *Eriostemones* all formed monophyletic groups with *Clusianae* and *Eriostemones* most closely related of the three (Table 1.2). Moreover, this publication showed that several sectional level groupings were not supported (Zonneveld, 2009; Veldkamp and Zonneveld, 2012), and therefore the authors concluded that sectional level groupings should be avoided and only reviewed when more comprehensive molecular data becomes available (Christenhusz *et al.*, 2013). Importantly within this publication a complete review of the typification of species was undertaken. Historically the genus had an array of typification errors leading to taxonomic confusion. Multiple new neotypes and lectotypes were assigned to overcome this, whilst many similar taxa were placed into synonymisation. The outcome of this work was an exhaustive species list (Christenhusz *et al.*, 2013), nevertheless this publication led to taxonomic disagreements, leading many recent tulip publications to reference previous species classifications (Eker, Babaç and Koyuncu, 2014; Tojibaev and Beshko, 2015; Xing *et al.*, 2017; Jalilian, Assadi and Nemati, 2019) and to the continued use of different names for the same species by different communities.

Since 2013 no study has investigated the systematics of the *Tulipa* genus as a whole, nonetheless many new species have been described (Figure 1.4; Table 1.1). Recently a number of studies have provided broader taxonomic understanding of the genus with *Tulipa* now securely placed sister to a clade containing both *Erythronium* and *Amana* within the Liliaceae (Givnish *et al.*, 2016; Huang *et al.*, 2018; Kim and Kim, 2018). In addition, a range of country wide studies have been carried out; Uzbekistan (Tojibaev and Beshko, 2015), Turkey (Turktas *et al.*, 2013; Eker, Babaç and Koyuncu, 2014), Iran (Kiani, Memariani and Zarghami, 2012; Abedi, Babaei and Karimzadeh, 2015; Khaleghi, Khadivi and Zonneveld, 2018; Jalilian,

Assadi and Nemati, 2019), Kosovo (Hajdari *et al.*, 2021), and China (Xing *et al.*, 2017; Li *et al.*, 2021) as well as some more regional work on Central Asian tulips (Dekhkonov *et al.*, 2022). These studies provide an abundance of national level information including geographic distributions, morphological datasets, karyotype analyses, and molecular systematic analyses, including the first ever plastome based phylogeny, yet due to their constrained geographic focus, limited sampling extent, inconsistent use of taxonomic nomenclature, and poor resolution they provide limited progress towards a clear genus wide taxonomic framework. Nonetheless they highlight the need for an integrated taxonomic approach that uses genetic, morphological, and biogeographical data.

1.5.4. Estimated number of species

Historically species delimitation has been exceptionally difficult, and uncertainty in the number of species and their relationships has troubled botanists for centuries. Limited understanding of natural variation within this taxon, species described from cultivation and naturalised populations, and a lack of typification for many species has led to over 300 *Tulipa* species being described since the genus was formally created (Christenhusz *et al.*, 2013; POWO, 2022; WCVP, 2022). Estimates of the true number of species at any one time has ranged from between 40 and 55 (Hall, 1929; Stork, 1984; Van Raamsdonk *et al.*, 1997), up to 100 or more species (Hall, 1940). Most recent works suggest there are between 75 and 90 species (Zonneveld, 2009; Veldkamp and Zonneveld, 2012; Christenhusz *et al.*, 2013; Fay and Christenhusz, 2013) with 76 species formally typified by Christenhusz (Christenhusz *et al.*, 2013).

Even so there remains considerable ambiguity surrounding many accepted species and synonymization is still commonplace (Jalilian, Assadi and Nemati, 2019). Furthermore, new species continue to be described, especially from Central Asia, Iran, and the Balkans, yet many need additional work to justify their status as species and not as synonyms (Hajdari *et al.*, 2021). So whilst understanding surrounding naturalised populations, ploidy, and the natural variation of tulips has greatly increased throughout the history of tulip taxonomy and this increased understanding has enabled a more reliable species delineation, conflict remains common between the use of nomenclature in literature (Zonneveld, 2009; Christenhusz *et al.*, 2013; Tojibaev and Beshko, 2015). In this thesis we initially recognise 93 species split into four subgenera (Table 1.3), whilst we agree with Christenhusz et al (2013) that existing sectional level groupings are premature (Christenhusz *et al.*, 2013).

Table 1.3. All currently recognised species, their subgenus classification, their distribution, and their known ploidy forms. The number of chromosomes for ploidy forms is stated in brackets and unknown ploidy stated where this trait has not been measured.

Species	**Subgenus**	**Natural distribution**	**Ploidy forms known**
Tulipa agenensis Redouté	Tulipa	Israel, Lebanon, Northwest Iran, Palestine, Saudi Arabia, Syria, Turkey	Diploid (24) Triploid (36)
Tulipa akamasica Chrisdoulou, Hand & Charalambous	Eriostemones	Cyprus	Diploid (24)
Tulipa albanica Kit Tan & Shuka	Tulipa	Northeast Albania	Diploid (24)
Tulipa alberti Regel	Tulipa	Kazakhstan, Kyrgyzstan	Diploid (24)
Tulipa aleppensis Boiss.	Tulipa	Syria, southern Turkey	Diploid (24) Triploid (36)
Tulipa altaica Pall.	Tulipa	Kazakhstan, Russia (Altai), northwestern China	Diploid (24) Tetraploid (48)
Tulipa anisophylla Vved.	Tulipa	Tajikistan, Uzbekistan	Diploid (24)
Tulipa annae J.deGroot & Zonn.	Tulipa	Kazakhstan	Diploid (24)
Tulipa armena Boiss.	Tulipa	Northwestern Iran, Transcaucasus (Georgia, Armenia, Azerbaijan), north-eastern Turkey	Diploid (24) Triploid (36)
Tulipa auliekolica Perezhogin	Eriostemones	Kazakhstan	Unknown
Tulipa bactriana J.J. De Groot & K.S. Tokibaev	Tulipa	Uzbekistan	Diploid (24)
Tulipa banuensis Grey-Wilson	Tulipa	Afghanistan	Diploid (24)
Tulipa biflora Pall.	Eriostemones	Afghanistan, Egypt, Iran, Iraq, Kazakhstan, Ukraine, Crimea, Lebanon, Syria, Pakistan, Israel, Palestine, Saudi Arabia, southern Russia, Tajikistan, Transcaucasus (Georgia, Armenia, Azerbaijan), Turkey, Turkmenistan, Uzbekistan, northwestern China, Greece, Former Yugoslavia (Bosnia and Herzegovnia, Serbia, Croatia, Montenegro, Slovenia, North Macedonia, Kosovo)	Diploid (24) Triploid (36) Tetraploid (48)
Tulipa bifloriformis Vved.	Eriostemones	Kazakhstan, Kyrgyzstan, Tajikistan, Uzbekistan	Diploid (24) Triploid (36) Tetraploid (48)
Tulipa boettgeri Regel	Tulipa	Tajikistan	Unknown
Tulipa borszczowii Regel	Tulipa	Kazakhstan, Uzbekistan	Diploid (24)
Tulipa botschantzevae S.N.Abramova & Zakal.	Tulipa	Iran, Turkmenistan	Unknown
Tulipa brinkii J.J. de Groot & B.J.M. Zonneveld	Tulipa	Iran	Diploid (24)

Tulipa butkovii Botschantz.	Tulipa	Uzbekistan	Diploid (24)
Tulipa carinata Vved.	Tulipa	Afghanistan, Tajikistan, Uzbekistan	Diploid (24)
Tulipa cinnabarina K.Perss.	Eriostemones	Turkey	Diploid (24)
Tulipa clusiana Redoute	Clusianae	Afghanistan, Iran, Iraq, northern Pakistan, India	Diploid (24) Triploid (36) Tetraploid (48) Pentaploid (60)
Tulipa cretica Boiss. & Heldr.	Eriostemones	Crete	Diploid (24)
Tulipa cypria Stapf	Tulipa	Cyprus	Triploid (36)
Tulipa dasystemon Regel	Eriostemones	Kazakhstan, Kyrgyzstan, Tajikistan, Uzbekistan, northwestern China	Diploid (24) Tetraploid (48)
Tulipa dianaeverettiae Zonn & J.deGroot	Eriostemones	Kazakhstan, Mongolia	Tetraploid (48)
Tulipa dubia Vved.	Tulipa	Kazakhstan, Kyrgyzstan, Uzbekistan	Diploid (24)
Tulipa faribae Ghahr.	Tulipa	Iran	Unknown
Tulipa ferganica Vved.	Tulipa	Kyrgyzstan, Uzbekistan	Diploid (24)
Tulipa foliosa Stapf	Tulipa	Turkey	Unknown
Tulipa fosteriana W.Irving	Tulipa	Afghanistan, Kyrgyzstan, Tajikistan, Uzbekistan	Diploid (24)
Tulipa greigii Regel	Tulipa	Iran, Kazakhstan, Kyrgyzstan, Tajikistan, Uzbekistan	Diploid (24)
Tulipa harazensis Rech.f.	Clusianae	Iran	Unknown
Tulipa heteropetala Ledeb.	Orithyia	Kazakhstan, Russia (Altai), northwestern China	Diploid (24)
Tulipa heterophylla (Regel) Baker	Orithyia	Southern Kazakhstan, Kyrgyzstan, northwestern China	Diploid (24)
Tulipa heweri Raamsd.	Tulipa	Afghanistan	Diploid (24)
Tulipa hissarica Popov & Vved.	Tulipa	Tajikistan, Uzbekistan	Diploid (24)
Tulipa hoogiana B.Fedtsch.	Tulipa	Northern Iran, southern Turkmenistan	Diploid (24) Triploid (36)
Tulipa humilis Herb.	Eriostemones	Afghanistan, Iran, Iraq, Lebanon, Syria, Transcaucasus (Georgia, Armenia, Azerbaijan), Turkey, Russia	Diploid (24)
Tulipa hungarica Borbas	Tulipa	Bulgaria, Romania	Diploid (24)
Tulipa iliensis Regel	Tulipa	Kazakhstan, Kyrgyzstan, northwestern China	Diploid (24) Triploid (36)
Tulipa ingens Hoog	Tulipa	Tajikistan, Uzbekistan	Diploid (24)
Tulipa intermedia Tojibaev & J.deGroot	Tulipa	Uzbekistan	Unknown

Tulipa ivasczenkoae Epiktetov & Belyalov	Tulipa	Kazakhstan	Unknown
Tulipa jacquesii Zonn.	Eriostemones	Kyrgyzstan	Diploid (24)
Tulipa julia K.Koch	Tulipa	Iran, Lebanon, Syria, Transcaucasus (Georgia, Armenia, Azerbaijan), Turkey	Diploid (24)
Tulipa kaufmanniana Regel	Tulipa	Kazakhstan, Kyrgyzstan, Tajikistan, Uzbekistan	Diploid (24) Triploid (36)
Tulipa kolbintsevii Zonn.	Eriostemones	Kazakhstan	Diploid (24)
Tulipa kolpakowskiana Regel	Tulipa	Afghanistan, Kazakhstan, Kyrgyzstan, northwestern China	Diploid (24) Tetraploid (48)
Tulipa korolkowii Regel	Tulipa	Kazakhstan, Kyrgyzstan, Tajikistan, Uzbekistan	Diploid (24)
Tulipa kosovarica Kit Tan, Shuka & Krasniqi	Tulipa	Kosovo	Unknown
Tulipa koyuncui Eker & Babac	Eriostemones	Turkey	Diploid (24)
Tulipa kuschkensis B.Fedtsch.	Tulipa	Afghanistan, Iran, Turkmenistan	Diploid (24)
Tulipa lanata Regel	Tulipa	Afghanistan, northern Pakistan, Tajikistan, Uzbekistan, northern India	Diploid (24) Triploid (36)
Tulipa lehmanniana Merckl.	Tulipa	Afghanistan, Iran, Kazakhstan, Kyrgyzstan, Tajikistan, Turkmenistan, Uzbekistan	Diploid (24) Triploid (36)
Tulipa lemmersii Zonn., Peterse, J.deGroot	Tulipa	Kazakhstan	Diploid (24)
Tulipa linifolia Regel	Clusianae	Afghanistan, Iran, Tajikistan, Uzbekistan	Diploid (24)
Tulipa lorestanica Rukšāns & Zubov	Eriostemones	Iran	Unknown
Tulipa luanica Millaku & Elezaj		Kosovo	Diploid (24)
Tulipa montana Lindl.	Clusianae	Iran, Turkmenistan	Diploid (24)
Tulipa narcissicum N.Y.Stepanova	Eriostemones	Russia	Unknown
Tulipa orithyioides Vved.	Eriostemones	Kyrgyzstan, Tajikistan, Uzbekistan	Diploid (24)
Tulipa orphanidea Boiss.	Eriostemones	Bulgaria, Greece, East Aegean islands, Turkey	Diploid (24) Triploid (36) Tetraploid (48)
Tulipa ostrowskiana Regel	Tulipa	Kazakhstan, Kyrgyzstan	Tetraploid (48)
Tulipa persica (Lindl.) Sweet	Tulipa	Iran	Diploid (24)
Tulipa platystemon Vved.	Tulipa	Kyrgyzstan	Unknown

Tulipa praestans H.B.May	Tulipa	Tajikistan	Diploid (24)
Tulipa regelii Krassn.	Eriostemones	Kazakhstan	Diploid (24)
Tulipa salsola Rukšāns & Zubov	Eriostemones	Kazakhstan	Unknown
Tulipa saxatilis Sieber ex Spreng.	Eriostemones	Turkey, South Aegean Islands, Crete	Diploid (24) Triploid (36)
Tulipa scardica Bornm.	Tulipa	Greece, Former Yugoslavia (Bosnia and Herzegovnia, Serbia, Croatia, Montenegro, Slovenia, North Macedonia, Kosovo)	Diploid (24)
Tulipa scharipovii Tojibaev	Tulipa	Kyrgyzstan, Uzbekistan	Unknown
Tulipa schmidtii Fomin	Tulipa	Iran, Azerbaijan	Diploid (24)
Tulipa serbica Tatic & Krivosej	Tulipa	Kosovo, Serbia	Unknown
Tulipa sinkiangensis Z.M.Mao	Orithyia	China	Diploid (24)
Tulipa sosnowskyi Achv. & Mirzoeva	Tulipa	Armenia, Azerbaijan	Diploid (24)
Tulipa sprengeri Baker	Eriostemones	Turkey	Diploid (24)
Tulipa suaveolens Roth	Tulipa	Iran, Kazakhstan, Ukraine (Crimea), Transcaucasus (Georgia, Armenia, Azerbaijan), Turkey	Diploid (24)
Tulipa subquinquefolia Vved.	Tulipa	Tajikistan, Uzbekistan	Unknown
Tulipa sylvestris L.	Eriostemones	Albania, Algeria, Russia, Belarus, Bulgaria, France, Greece, Italy, Kazakhstan, Ukraine, Libya, Morocco, Caucasus, Portugal, Romania, Spain, Switzerland, Tunisia, Turkey, China, Greece, Former Yugoslavia (Bosnia and Herzegovnia, Serbia, Croatia, Montenegro, Slovenia, North Macedonia, Kosovo)	Diploid (24) Triploid (36) Tetraploid (48)
Tulipa systola Stapf	Tulipa	Iran, Iraq, Lebanon, Syria, Palestine, Turkey, Egypt	Diploid (24)
Tulipa talassica Lazkov	Tulipa	Kyrgyzstan, Uzbekistan	Unknown
Tulipa tetraphylla Reel	Tulipa	Kazakhstan, Kyrgyzstan, China	Diploid (24) Tetraploid (48)
Tulipa turgaica Perezhogin	Eriostemones	Kazakhstan	Unknown
Tulipa turkestanica Regel	Eriostemones	Kyrgyzstan, Tajikistan, Uzbekistan, China	Diploid (24) Tetraploid (48)
Tulipa ulophylla Wendelbo	Tulipa	Iran	Diploid (24)
Tulipa undulatifolia Boiss.	Tulipa	Greece, Iran, Tajikistan, Tanscaucasus (Georgia, Armenia, Azerbaijan), Turkey, Turkmenistan, Uzbekistan, Greece, Former Yugoslavia (Bosnia and Herzegovnia, Serbia,	Diploid (24) Triploid (36)

		Croatia, Montenegro, Slovenia, North Macedonia, Kosovo)	
Tulipa uniflora (L.) Besser	Orithyia	China, Mongolia, Kazakhstan, Russia	Diploid (24)
Tulipa urumiensis Stapf	Eriostemones	Iran, Kazakhstan, Kyrgyzstan	Diploid (24)
Tulipa uzbekistanica Botschanz & Scharipov	Tulipa	Uzbekistan	Unknown
Tulipa vvedenskyi Botschanz.	Tulipa	Tajikistan, Uzbekistan	Diploid (24) Triploid (36)
Tulipa × *tschimganica*	Tulipa	Uzbekistan, Kyrgyzstan	Diploid (24)
Tulipa zonneveldii J. de Groot & Tojibaev	Tulipa	Kyrgyzstan	Unknown

In this list we hesitantly opt to retain *Tulipa faribae* as a species, although there is evidence to suggest it is a synonym of either *T. armena* (Jalilian, Assadi and Nemati, 2019) or *T. systola* (Christenhusz *et al.*, 2013). We also cautiously maintain *T. biflora, T. orphanidea,* and *T. sylvestris* as single species, these complexes still require significant focused work to determine the best taxonomic structure of these groupings. This work will include assessing newly described species that are clearly closely related to *T. biflora* (Rukšāns and Zubov, 2022). Moreover, we include *T. luanica* and *T. kosovarica* as species, although there is molecular data to suggest these should be treated as synonyms of *T. serbica* (Hajdari *et al.*, 2021). Many of the new species of recent years are here accepted but will require assessment of their genetic distinctiveness (Table 1.1).

1.6. Species, Population, and Genome Dynamics

1.6.1. Hybridisation, karyology, and polyploidy

In 1900 relatively simple techniques revealed that tulips have 24 chromosomes in their diploid form (Guignard, 1900) and subsequent research in the following decades enabled researchers to explore genome size of specimens (Newton and Darlington, 1927, 1929). These cytological studies provided the first broad insight into the molecular environment highlighting that genome size varied greatly between species (Hall, 1940) and that polyploid tulips existed (Hall, 1938). At this time polyploidy was a relatively new concept and polyploid individuals were often used to describe new species even given their morphological similarity to diploid forms. In addition, the lack of natural aneuploids, which have an abnormal number of chromosomes in the haploid state and which were expected outcomes from sexual reproduction, was thought to imply that

polyploids reproduced only clonally in the wild and therefore infrequently formed stable populations (Upcott and La Cour, 1936).

Research published later in the 20th century highlighted the relatively frequent occurrence of polyploidy (Botschantzeva, 1982; Kroon and Jongerius, 1986), yet it was estimated that 70% of tulip diversity was diploid with tetraploid (19%), triploid (6%), and pentaploid (3%) tulips far less common. Furthermore, these polyploids usually occurred in species where a diploid individual was also known (Kroon and Jongerius, 1986). In these works, it was also noted that polyploidy occurred mostly on the edges of tulip species distributions, in cultivation, or in high mountain areas where tulips are rare; specifically work highlighted that many polyploid forms of Central Asian tulips evolved in cultivation (Botschantzeva, 1982). In general, therefore most polyploids were found in either the far west or far east of the Eurasian continent, and were observed more commonly in higher altitudinal ranges above and away from the middle and lower mountain belts where the richest variety of tulips, and centre of species formation, were reported (Botschantzeva, 1982). Even so, odd species were identified that had stabilised polyploid forms, such as *Tulipa ostrowskiana*, an allotetraploid species, and *T. tetraphylla* that is most commonly found in autotetraploid form, although a diploid form exists (Botschantzeva, 1982).

A range of basic chromosomal number changes within specimens were also noted when investigating polyploidy, but were so infrequently found that they were not considered an important molecular trait of the genus (Botschantzeva, 1982). Exceptionally few tulips showed an increased basic chromosome number, nonetheless a tetraploid individual was collected in Iran which had 52 chromosomes (Botschantzeva, 1982). On the other hand, there were several examples of tulips that had a reduced basic chromosome number, usually these specimens contained only 22 chromosomes. Many of these abnormal specimens were also reported to have shorter chromosomes with near terminal centromeres (Botschantzeva, 1982).

Breeding and molecular work showed hybridisation could occur in the genus. Horticultural focused research showed that certain closely related tulip species can hybridise, primarily through crosses between cultivars and wild taxa, however significant pre- and post-fertilisation barriers were present between many interspecific pairs (Van Eijk *et al.*, 1991; Van Raamsdonk, Van Eijk and Eikelboom, 1995; Creij, Kerckhoffs and Tuyl, 1997). Work on wild tulips has identified several occurrences of natural hybridisation: *Tulipa ostrowskiana* can form multiple natural hybrids with *T. kolpakowskiana* and *Tulipa korolkowii* (Botschantzeva, 1982; Christenhusz *et al.*, 2013), moreover *T. greigii*, *T. kaufmanniana*, and *T. dubia* form a species complex where hybridisation is common and has led to the existence of a stable hybrid species

T. × *tschimganica,* thought to be the offspring of a cross between *T. dubia* and *T. kaufmanniana* (Botschantzeva, 1982). Nonetheless, recent work supports the view that there are significant barriers to crossing between most wild species with poor rates of fruit-setting reported and failure to obtain offspring in many crossing attempts (Van Tuyl and van Creij, 2006; Qu *et al.*, 2018).

In 2009, polyploidy in the genus was revisited in a comprehensive analysis of genome sizes (Zonneveld, 2009). Within this investigation, Zonneveld confirmed that most tulips are diploid (2n=24), however triploids, tetraploids, pentaploids, and hexaploids exist, with a similar pattern observed in cultivated tulips a few years later (Table 1.3 (Marasek-Ciolakowska *et al.*, 2012)). Notably, this work revealed that many ploidy forms can occur within a single species (Zonneveld, 2009), which has been further corroborated (Orlikowska *et al.*, 2018). Given this, it is now broadly accepted that many ploidy forms, especially triploid forms that cannot form natural populations by sexual reproduction (Fay and Christenhusz, 2013), do not represent distinct species, ultimately leading to their synonymization under their related diploid forms (Christenhusz *et al.*, 2013). Nonetheless, recent research suggests that polyploidy in general may play a role in colonisation and speciation in plants, so these forms should not be completely disregarded. Polyploids are relatively effective at colonising new areas, due to genetic based shifts in ecological tolerances (Otto and Whitton, 2000; Suda *et al.*, 2007), and because they mostly reproduce by asexual seed production and therefore don't rely on the presence of other individuals to produce offspring (Dynesius and Jansson, 2000). Although infrequent, polyploid tulips may therefore indicate recent inhabitation of new areas and speciation events (Botschantzeva, 1982; Zonneveld, 2009), which may be of considerable interest, especially in the Mediterranean where many polyploid tulips are located.

In the past decade an array of research has focused on tulip chromosomal structure and number (Abedi, Babaei and Karimzadeh, 2015; Kiran, Dogan and Demirkan, 2016; Lan *et al.*, 2018; Qu *et al.*, 2018). Several of these studies have contributed to an increased understanding of tulip chromosome lengths, number, and molecular weights, especially of the species of Iran (Masoud *et al.*, 2002; Abedi, Babaei and Karimzadeh, 2015). The results of these separate works have highlighted that genome size, chromosome volume and chromosome length varied considerably, but most importantly that these traits had greater intra-specific variation than interspecific. This discovery provides some evidence that karyological data may be limited in its use in distinguishing between species. Similar work on Chinese tulip species (Qu *et al.*, 2018),

which verified the karyotype and chromosome number of eight wild species, identified all as diploid and highlighted broad variation in intra-species chromosome length as well.

In this later work by Qu et al 2018 interspecific hybridisation of Chinese species was also explored, which showed that parents with equal ploidy levels have a greater crossing efficiency (Qu *et al.*, 2018). This work has been followed up in a very similar study, that also investigated the compatibility of wild and cultivated crosses in Chinese tulips (Xing *et al.*, 2020). Xing et al (2020) showed that offspring germination rates varied across crosses between wild species and cultivars, again suggesting significant reproductive barriers between species. Yet, much like the majority of recent studies, this work is limited primarily by its use of cultivars in all crosses, as well as by its geographical scope, species coverage, and outdated systematics.

1.6.2. Population genetics

Many species of tulips show high plasticity in lots of traits and so in the past few decades, several studies have used modern genetic techniques to investigate closely related taxa and population level diversity. In 2001, amplified fragment length polymorphisms (AFLPs) were used for the first time to explore variation within *ex-situ* specimens of *Tulipa sprengeri* and showed that collections of this, (extinct in the wild) species, likely all stemmed from the same bulb grown at the Royal Botanic Gardens Kew (Maunder *et al.*, 2001). In 2013, AFLPs were used to investigate variation between four species in Russia with results showing that AFLP variation within species was greater than between species (Kutlunina, Polezhaeva and Permyakova, 2013), however these results must be critically interpreted as they used out-dated species concepts with many of the taxa they recognised synonyms of *T. sylvestris* (Christenhusz *et al.*, 2013). Similar research undertaken on Iranian tulips, again using AFLPs, showed significant genetic variation between taxa and the authors hypothesised that *T. biebersteiniana*, currently a synonym of *T. sylvestris* (Christenhusz *et al.*, 2013), should form its own subgenus based on results from a hierarchical cluster analysis of the taxa's genetic profiles (Asgari *et al.*, 2020). This idea contradicts previous literature and certainly needs more comprehensive evidence before being considered further. Recently, a study was published on Iranian tulips which used Conserved DNA-derived polymorphisms (CDDP) to explore genetic diversity, which again showed through a molecular variance analysis that within population genetic variation was generally larger than between species (Haerinasab *et al.*, 2021). Overall, these works highlight that polymorphisms may be useful in understanding intraspecific genetic variation both in wild populations as well as across *ex-situ* collections even if there are limitations in taxonomic implications.

Inter-Simple Sequence Repeats (ISSR) have also been considered for examining genetic variation with studies conducted on Iranian tulips (Kiani, Memariani and Zarghami, 2012), as well as within the species *Tulipa suaveolens* (Kritskaya *et al.*, 2020, 2021) and the complex hybrid *T.* × *gesneriana* (Kashin, Kritskaya and Schanzer, 2016). These studies show ISSR to be a relatively effective method to both investigate closely related species and population level variation and, in some cases, explore historical population biogeography due to the fairly rapid rate at which these repeats are evolving. In 2008, random amplified polymorphic DNA (RAPD) was used for the first time to investigate genetic polymorphisms in four wild species from China and ten cultivars (Luan *et al.*, 2008). This work showed that wild species were genetically distant from cultivars and that they had a much higher rate of genetic polymorphism. In 2018, researchers used microsatellites to assess the genetic diversity of 280 individuals from 36 wild and cultivated accessions obtained from the countries of Iran and the Netherlands (Pourkhaloee *et al.*, 2018). This work again provided evidence that wild species generally have a higher genetic variability than cultivated species. In this work it was also proposed that *T. systola* and *T. micheliana* could potentially be the ancestors of *T.* × *gesneriana,* although further work is needed, especially given that *T. micheliana* is an accepted synonym of *T. undulatifolia* (POWO, 2022). Overall, it seems that microsatellites, like ISSR and RAPD, may provide evolutionary insights into tulips especially regarding population level genetics and polymorphisms, but may be limited in their use for identifying higher taxonomic groupings due to their rapid rate of evolution leading to large amounts of intraspecific variation.

1.7. Conservation Perspective

1.7.1. Global threat status of tulips

There are eight tulip taxa on the IUCN Red List (IUCN, 2022), however, of these eight, three are recognised synonyms (POWO, 2022), meaning only five true species have been assessed (Table 1.4). Within these five, three are Threatened, one is Near Threatened, and one is Least Concern. Overall, therefore less than 6% of tulip species are globally assessed. To exacerbate matters, the five assessed species all occur within the Balkans, Crete, or Cyprus drawing attention to the lack of formal assessment of any species from the tulips most diverse regions, most notably Central Asia. Yet, many countries record wild tulips within their national flora lists (Ivaschenko, 2005; Gabrielian and Fragman-Sapir, 2008), and there are a few large-scale conservation focused surveys of national tulip diversity. These efforts have largely occurred in China, Kazakhstan, Kyrgyzstan, Iran, Tajikistan, Turkey, and Uzbekistan (Davletkeldiev, 2006; Eker, Babaç and Koyuncu, 2014; Tojibaev and Beshko, 2015; Trias-Blasi, Gücel and Özden,

2017; Xing *et al.*, 2017; Nowak *et al.*, 2020). From these reports it is clear that many wild tulips are Threatened, however information from these reports is limited as data is often outdated and species nomenclature inaccurately used.

Table 1.4. Tulip species that are listed on the global IUCN Red List. Alongside the species name is listed the distribution of the species, its IUCN Red List status, whether it is a synonym of another species and if this is the case the true species name under which it falls.

Species	**Distribution**	**IUCN Red List status**	**Synonym**	**True species**
Tulipa albanica	Albania	Critically Endangered	No	
Tulipa akamasica	Cyprus	Critically Endangered	Potentially	Unknown (Most likely *Tulipa orphanidea*)
Tulipa cretica	Greece (Crete only)	Least Concern	No	
Tulipa cypria	Cyprus	Endangered	No	
Tulipa gumusanica	Turkey	Critically Endangered	Yes	*Tulipa armena*
Tulipa hageri	Greece	Data Deficient	Yes	*Tulipa orphanidea*
Tulipa hungarica	Bulgaria, Greece, Romania (introduced)	Near Threatened	No	
Tulipa lownei	Israel, Lebanon, Syrian Arab Republic	Least Concern	Yes	*Tulipa humilis*

1.7.2. Ex-situ collections of tulips

The *ex-situ* collections of botanic gardens remain a critical tool in the conservation of species (Mounce, Smith and Brockington, 2017). *Ex-situ* collection records of the genus *Tulipa* from BGCI's PlantSearch tool (BGCI, 2022) show that of the 93 species recognised at the start of this project (Table 1.3) 67 are protected in one or more botanic garden collection (Figure 1.5). This means 26 species, or 28% of tulip diversity, is not protected in any *ex-situ* collections. Many of these are new species that have only recently been described, which suggests they have a high probability of being threatened (Liu *et al.*, 2022). The existence of multiple *ex-situ* collections of a species is important as it safeguards populations from sporadic events such as disease outbreaks as well as promotes the preservation of greater genetic diversity (Cibrian-jaramillo *et al.*, 2013). Of the 67 species known from botanic garden collections 60 of these can be found in two or more. Notably, 41 of the 56 species from the Central Asian diversity hotspot are found in *ex-situ* collections with only five of these known from only a single botanic garden (Figure 1.5).

Whilst commonly there still remains limited communication between gardens leading to plant collections not always being complementary (Cibrian-jaramillo *et al.*, 2013), in general tulip collections are relatively comprehensive. Through our brief assessment of collections we can report that 72% of global tulip diversity and 73% of Central Asian tulip diversity is recorded in

ex-situ collections. Furthermore, the number of tulips reported in collections here is likely an underestimate of the true number. This is because not all botanic gardens have carefully curated species lists that have then been uploaded to the BGCI PlantSearch database, the PlantSearch database is not completely up to date with new collections, and many tulips grow in Russian speaking regions where data are often not uploaded to western scientific databases. Markedly, understanding of how to propagate this plant and the required growing conditions for different tulip species has been greatly expanded during the long horticultural history of this flower (Wilford, 2006), and this continues to be strengthened for wild species through specialised research efforts targeted at improving *ex-situ* practices (Yurievna and Vladimirovna, 2019) and expanding *ex-situ* collections (Pechenitsyn *et al.*, 2020).

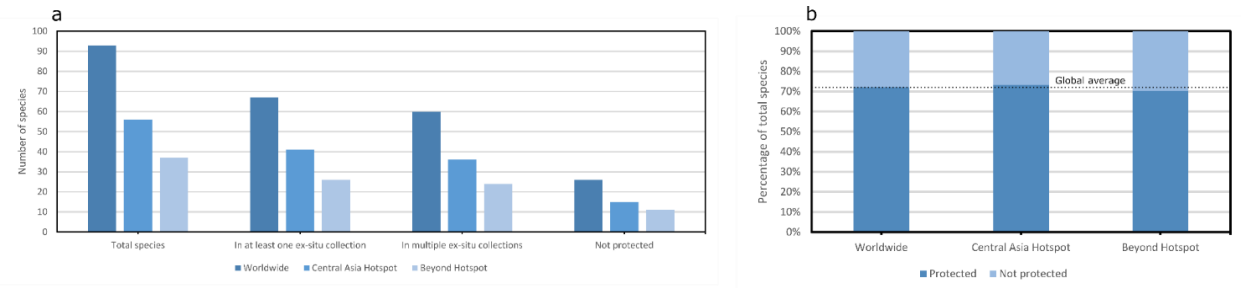


Figure 1.5. The extent of tulip *ex-situ* collections based on BGCI's PlantSearch tool. (a) shows a breakdown of the number of species recorded in collections with the total number of accepted species shown first for comparison and panel (b) shows the percentage of tulips protected in collections with the global average for tulips shown. Both graphs are broken down into three separate data categories consisting of worldwide which includes all accepted species; Central Asia Hotspot which includes all species that are known to occur within, but not necessarily exclusively, the countries of China, Kazakhstan, Kyrgyzstan, Tajikistan, and Uzbekistan; Beyond Hotspot which covers all species. that have no part of their range within these countries.

1.7.3. Taxonomy underpins tulip conservation

Conservation is a primarily species driven discipline (Godfray, Knapp and Mace, 2004) and therefore a strong taxonomic framework is essential (Garnett and Christidis, 2017). Databases such as the IUCN Red List and BGCI's PlantSearch tool all rely on species level information. The historical taxonomic complexities associated with *Tulipa* and limitations of recent work, including low molecular resolution and poor sampling, mean there remains a significant need for a genus-wide phylogenetic evaluation of tulips. Currently, the evolutionary relationships of many

species remain ambiguous and species names are inconsistently used within the literature. Although some research has made taxonomic progress, with *Tulipa faribae* highlighted as a synonym of *T. armena* (Jalilian, Assadi and Nemati, 2019) and *T. kosovarica* and *T. luanica* presented as synonyms of *T. serbica* (Hajdari *et al.*, 2021), it is on a limited scale. Overall, these issues have made publications incomparable and their results difficult to apply outside of their narrow context, especially for conservation.

A broad analysis of the genus needs to be complemented with focused research on more ambiguous areas of the current taxonomic framework including species complexes surrounding *Tulipa biflora*, *T. orphanidea*, *T. sylvestris*, and *T. suaveolens*, which currently encompass a long list of varieties and synonyms, as well as poorly studied species such as *T. undulatifolia*. In addition, the description of any new taxon must be carried out carefully to prevent the reoccurrence of historical typification errors and the unnecessary over splitting of species. Without this fundamental taxonomic structure in place, that provides clear and broadly accepted species concepts, it will be very difficult to effectively carry out any future targeted conservation work on the genus. It is therefore vital that research now builds upon a stable framework like that set out by Christenhusz et al (2013). This work underpins the tulips on the Plants of the World online database and the published monograph of *Tulipa* (Everett, 2013), both of which are also excellent taxonomic tools for this genus. We have used the taxonomic understanding published by Christenhusz et al (2013) as the foundation for our up-to-date species list published here (Table 1.3), although we recognise that there are still significant areas of this framework that need to be reassessed.

1.7.4. Tulip conservation should focus on the centres of diversity

Today two tulip diversity hotspots are widely recognised (Figure 1.1): the primary centre located in the Tien Shan and Pamir-Alay mountain ranges in Central Asia where over half of all species are known to occur and which is often cited as the evolutionary origin of the genus, and the secondary centre located across the Caucasus, Iran, and Turkey (Hoog, 1973; Botschantzeva, 1982; Zonneveld, 2009). Both areas contain a disproportionately large number of tulip taxa and therefore should be the focus of tulip conservation efforts. Yet this has not been the case, as highlighted by the previously discussed biases of the IUCN Red List (IUCN, 2022). Importantly, in the Central Asian region there remains a pressing need to understand species diversification dynamics, concepts, and distributions. It is also vital to ensure classifications of *Amana* and *Tulipa* genera are consistently used, especially in western China.

Although less pressing, targeted research is also required to unravel the species complexes surrounding several widespread tulip taxa, such as *Tulipa sylvestris*, *T. biflora*, and *T. suaveolens*. These species have broad distributions covering much of Eurasia and are associated with long lists of corresponding synonyms that are often treated as true species in different regions. This greatly impacts conservation priorities in these regions, as conservation is often focused on preventing recognised species from going extinct (Garnett and Christidis, 2017). Work is also still needed to investigate how Mediterranean diversity fits into broader biogeography, especially given the Threatened status of some species in this region and significant uncertainty around taxonomic concepts (Christenhusz *et al.*, 2013; Trias-Blasi, Gücel and Özden, 2017). A similar focused effort is needed for the neighbouring Balkans region where a number of new species have recently been described and assessed as Threatened (Shuka, Tan and Siljak-Yakovlev, 2010; Shuka, Tan and Krasniqi, 2012; Millaku and Elezaj, 2015), although some molecular work suggests the over splitting of taxa in this region (Hajdari *et al.*, 2021).

Distribution data limitations for tulips

Although, there is an increasing array of localised mapping efforts especially in Uzbekistan (Tojibaev and Beshko, 2015; Abduraimov *et al.*, 2020; Dekhkonov *et al.*, 2021), Iran (Jalilian *et al.*, 2021), and across the Balkans region (Millaku, Elezaj and Berisha, 2018) much of this data is only present as figures in scientific papers and so is not readily available to conservation practitioners. Many of these localised research efforts do not share data on open access databases like the Global Biodiversity Information Facility (GBIF). In a quick analysis of tulip species' data we downloaded GBIF data for the genus Tulipa L., only including points from countries in the known natural range of the genus (GBIF.org (10 August 2021) GBIF Occurrence Download https://doi.org/10.15468/dl.dht7nu), cleaned it using the R package 'CoordinateCleaner' (Zizka *et al.*, 2019) before further filtering the data through a stringent process to remove unreliable points. This analysis excluded all species named after the date the data was downloaded as these species do not occur on the GBIF database, meaning 90 species are recognised and 55 of these occur in Central Asia. This data showed that only 61% of tulip diversity has recorded coordinate data. Moreover, of the recorded species only 20 have 10 or more GPS points, only seven have more than 25 points, and only three of these have over 100 GPS points demonstrating the exceptionally limited population data available for many species (Figure 1.6). Around 70% of Central Asian species are represented in this database yet around 84% of locations are linked to only five species, of which four are known to occur in this region.

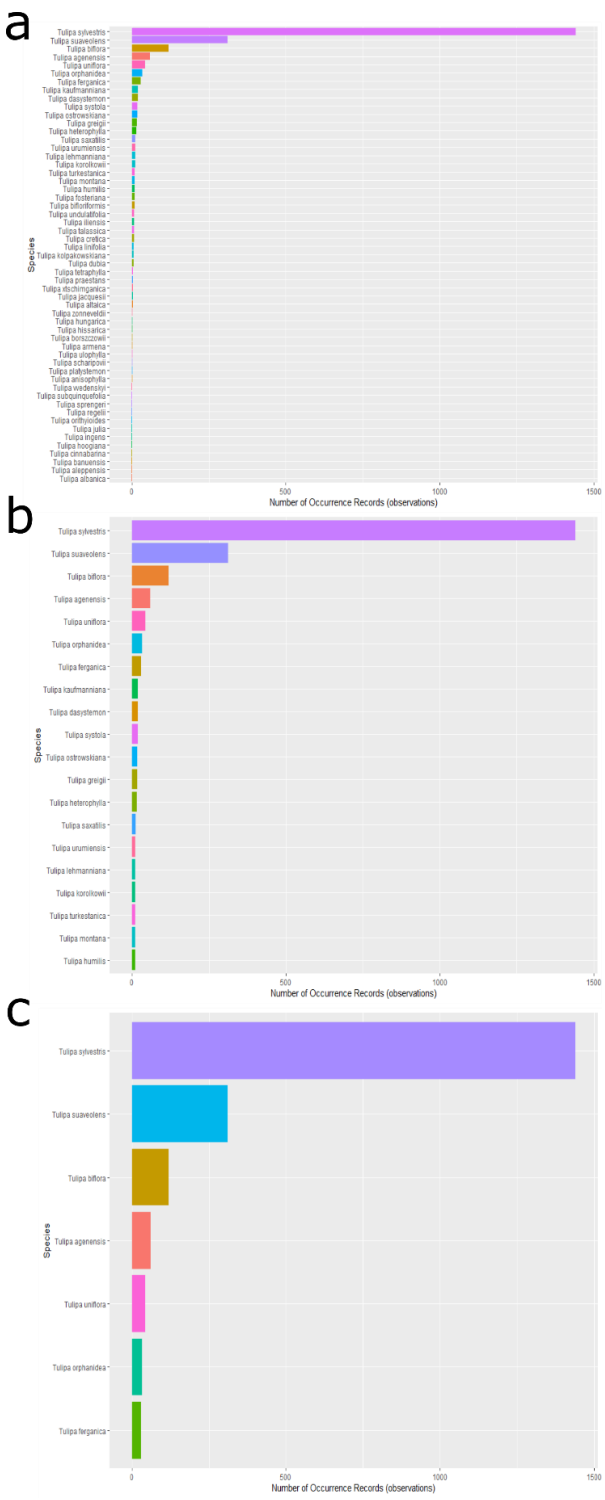


Figure 1.6. Tulip species representation on GBIF. The number of occurrences for a species on this database is shown in descending order. (a) shows all the accepted species which have GPS data in their natural range, (b) shows only species with ten or more locations points, and (c) shows only species with more than 25 location points.

Notably, 61% of all location points are the species *Tulipa sylvestris;* a species with a broad distribution covering much of the global tulip range and a taxon with a high degree of taxonomic uncertainty. *T. suaveolens*, a species known mainly from Russia, Ukraine, and Kazakhstan has the second largest number of points, yet this is mostly due to extensive recording efforts for this species. Whilst *T. biflora* is the third most common species again likely due to its broad distribution across much of the global tulip range. These three species represent over three quarters of the tulip GPS data available on the GBIF database. Most data therefore falls not in the most diverse regions, but in areas where extensive research efforts have taken place and where specific overly represented species grow highlighting the extremely limited applicability of the GBIF database for understanding distributions. Furthermore, many datapoints are recorded under the names of recognised synonyms making the database somewhat disorientating. Curiously, over 8945 GPS points exist for the taxon *T.* × *gesneriana*, which is now not an accepted species (Christenhusz *et al.*, 2013). This may somewhat be explained by many naturalised populations in Europe and the United States, as well as the incorrect use of this name for wild *T. suaveolens* specimens but highlights the taxonomic inconsistency underlying this database and, in the genus generally.

1.7.5. The perennial geophytic life history presents some challenges for conservation

Tulips provide valuable ecological services such as supporting pollinator and insect populations making their protection important for the ecosystem. Yet, their geophytic nature presents numerous challenges to conservation as plants can only be located during their brief growing season, seeds and bulbs can only be collected during a short period once the plant has dried and died back, and their inconsistent flowering pattern makes monitoring difficult. In addition, the extent of natural variation within species makes identification complicated and this process often requires destruction of the plant. To exacerbate matters many populations are located in remote and inaccessible mountainous areas and populations are often relatively small and well camouflaged unless flowering.

The perennial nature of the tulip means an individual can survive for many years, but because of the long generation length of the tulip, colonisation or repopulation of an area can be slow. Vegetative reproduction can allow few individuals to persist and repopulate an area but may leave a genetically uniform population that could be susceptible to disease. In sexual reproduction pollen and seeds are not dispersed far from the parent plant meaning that the migration potential of tulips is extremely limited. This in turn makes them vulnerable to localised

threats such as grazing as well as broader shifts in climate. Nonetheless their longevity, remote habitat, ability to reproduce both sexually and vegetatively, and short growing season may also limit the impact of some threats and therefore aid their survival.

1.7.6. Impact of hybridisation and polyploidy on tulip conservation

Although understanding of the hybridisation and polyploidy of tulips has increased in recent years there remains significant uncertainty of their importance in species and population dynamics. There is still a need to analyse natural hybridisation and the threat of hybridisation between naturalised and wild taxa. Introgression between species and cultivars may pose a threat to wild genetic variation where populations of tulips overlap and this is especially important to consider in *ex-situ* collections where hybridisation can occur relatively easily due to the proximity of specimens (Christenhusz *et al.*, 2013). Furthermore, work is needed to assess the distribution and evolutionary importance of polyploidy tulips, as well as explore tools to better assess both species and population level genetic diversity, especially in the face of climate change (Wilson *et al.*, 2021).

Currently there exists only a limited range of resources available for use in analysing the genetic properties of tulips, however this is beginning to expand. There are now a broader range of DNA markers and genetic maps (Shahin *et al.*, 2012; Tang *et al.*, 2015), publication of a high quality transcriptome (Moreno-Pachon *et al.*, 2016), a large database of species genome sizes (Leitch *et al.*, 2019), and the sequencing of several complete chloroplast genomes (Zhou *et al.*, 2019; Do *et al.*, 2020; Ju, Shi, *et al.*, 2020; Ju, Tang, *et al.*, 2020; Ju *et al.*, 2021; Li *et al.*, 2021). Recently, the first complete genome of tulips, *Tulipa* × *gesneriana*, was sequenced and still remains one of the world's largest sequenced genomes (ISAAA, 2017). With the growing availability of genetic tools and techniques, as well as the growing literature on the molecular level of tulips, there is increasing potential to conserve genetic variation and evolutionary processes both in the wild as well as in *ex-situ* collections.

1.7.7. The horticultural history of tulips could promote their conservation

Historically many wild tulip species have been used to breed new cultivars (Hall, 1940; Marasek, Mizuochi and Okazaki, 2006; Christenhusz *et al.*, 2013; Kritskaya *et al.*, 2020), even today wild tulip diversity is an important resource for the horticultural trade (Orlikowska *et al.*, 2018; Pourkhaloee *et al.*, 2018). Wild diversity harbours broader genetic diversity than that of their more uniform cultivar cousins, particularly showing higher potential adaptability to climate change and enhanced disease resistance especially for diseases such as tulip breaking virus and tulip fire which can greatly impact annual yields (Van Eijk, Garretsen and Eikelboom, 1979;

Van Eijk, Eikelboom and Hogenboom, 1986; Marasek-Ciolakowska *et al.*, 2012; Orlikowska *et al.*, 2018; Pourkhaloee *et al.*, 2018). Specifically there are no known cases of tulip breaking virus in wild tulips and several taxa, including *Tulipa tarda*, show full resistance to the fungus that causes tulip fire, which can spread rapidly through the soil and can wipe out whole fields of tulips. Given the value of this wild genetic diversity it is highly likely that the horticultural trade will still rely heavily on wild tulips in the future and could provide a significant source of funding and support for conservation efforts.

Moreover, given the cultural history of this genus and the widespread recognition of this plant, tulips are in a relatively unique position. Plant blindless is a common problem in the field of conservation with threatened flora often overlooked (Balding and Williams, 2016), yet wild tulips present a rare opportunity for a plant group to be treated as a flagship genus (Simberloff, 1998). This is especially significant in the tulip's most diverse region, Central Asia, where the mountains of Central Asia are recognised as a biodiversity hotspot (Critical Ecosystem Partnership Fund, 2016) but currently conservation efforts are heavily dominated by megafauna (WWF, 2017; Leonardo Dicaprio Foundation, 2018). Furthermore, in this region community-based conservation initiatives have already been recognised as vital (Berkes, 2007; Xenarios *et al.*, 2019). Future conservation efforts could therefore utilise the iconic status of this plant to highlight the need to protect the broader community in which this flower grows and to engage communities in conservation initiatives including in potential citizen science initiatives.

1.8. Thesis Structure

The primary purpose of this thesis is to provide an evidence-base to support future conservation efforts of the genus *Tulipa*. The research in this thesis was undertaken to fulfil the requirements for a Natural Environment Research Council iCASE doctoral training partnership PhD, where the industry partner was Fauna & Flora International, a conservation non-governmental organisation. This work was also conducted under the activities of a parallel Department for Environment, Food & Rural Affairs Darwin Initiative grant entitled "Securing wild tulips and the pastoral communities in the Kyrgyz mountains" led by Fauna & Flora International with Cambridge University Botanic Gardens a formal partner alongside: Bioresurs, the National Academy of Science of the Kyrgyz Republic, the Association of Forest Users and Land Users of Kyrgyzstan (AFLUK), National Pasture Users Association of Kyrgyzstan Kyrgyz Jayity, and Gareev Botanical Garden.

The PhD had a relatively broad scope exploring the genus as a whole, which led to collaborations with researchers in Kosovo, Turkey, Uzbekistan, and Tajikistan as well as in Kyrgyzstan. The Darwin Initiative project work mostly focused on the tulip diversity of Kyrgyzstan especially on pastureland management, however by the end of this project we began to think about how Kyrgyz tulips fitted into Central Asian tulip diversity as a whole. Importantly, within the Darwin Initiative project there were several partners in Kyrgyzstan who undertook community engagement projects, outreach campaigns, and on the ground conservation action, which was not in the scope of the PhD. Yet, the intertwined nature of the PhD and Darwin Initiative project meant that our research was often driven by the needs of these conservation organisations, and we designed our work to help inform both the scientific community as well as conservation practitioners.

The overall achievements of these endeavours were far greater than the research element of these projects and are too numerous to include in this thesis. In this thesis therefore we display the major scientific outputs from our work, placing it within the scientific literature as well as a practical conservation context. The thesis is structured into five data chapters that target three main objectives. Below we present these objectives, introduce the methods we chose to achieve these objectives, and highlight how this work connects with the needs of the conservation sector:

I. **Reassess the taxonomy of the genus *Tulipa* (Chapter two and three)**

We aim to build on previous taxonomic work (Zonneveld, 2009; Veldkamp and Zonneveld, 2012; Christenhusz *et al.*, 2013) by developing a robust phylogenetic backbone for *Tulipa*. We will infer phylogenies using sequence data of complete plastomes as well as the 35S rDNA region of the nuclear genus. This genetic data will be by far the largest molecular dataset used to investigate evolutionary relationships within the genus *Tulipa*. In addition, we will endeavour to use material representing the majority of currently accepted species, many of which have never been included in phylogenetic studies before. An up-to-date robust taxonomic framework, will underpin the rest of the work in this thesis and crucially inform species-focused conservation efforts.

II. Explore the evolutionary history of wild tulips (Chapter four)

We will apply molecular dating techniques to our phylogeny to explore the origin and diversification of this genus. This will provide evidence as to the origin of tulips as well as enable us to explore how and why tulips diversified to their current distribution linking historical geological events to the diversification of this genus. In this work we will also assess the evolutionary informativeness of genome size, which has so often been used in taxonomic decision making (Zonneveld, 2009). An improved evolutionary understanding of wild tulips will highlight key areas of diversification for conservation action especially showcasing how the Mountains of Central Asia Biodiversity Hotspot (Critical Ecosystem Partnership Fund, 2016) and surrounding areas fit into this evolutionary history.

III. Generate an evidence-base for Central Asian conservation decisions

a) Assess the threat of climate change to Central Asian wild tulips (Chapter five)

We will use species distribution modelling techniques, specifically MaxEnt software, to explore how distributions traverse borders in this political tense region especially with regards to how climate change may impact the extent of suitable tulip habitat. Overall, this will enable us to assess the impact of climate change as a threat to tulips and more broadly the flora of this region.

b) Formally Red List and explore conservation priorities for Central Asian wild tulips (Chapter six)

We will collate information from the literature, our own research, and from Central Asian tulip experts to prepare Red List reports for a range of Central Asian tulip species. The collated information and the assigned threat categories will be used in a number of analyses to inform conservation prioritisation including EDGE. This work will help identify key species for conservation and raise awareness of the conservation needs of this genus. In addition, this work will provide a foundation for the development of a regional conservation strategy for Central Asian tulips.

Chapter 2.

The evolutionary relationships of wild tulips

In this chapter we undertake the largest phylogenetic analysis of the genus *Tulipa* using new sequence data obtained from an array of samples collected during expeditions, from living collections and herbaria, and downloaded from GenBank. In this work we explore the implications of our phylogeny for the taxonomy of wild tulips specifically the genus, subgenera, sections, and species. In this process we also assess the frequency of misidentifications, the resolution of different DNA barcodes, and highlight areas where taxonomic ambiguities remain.

2.1. Introduction

Tulips remain one of the most recognisable geophytes in the world, supporting a billion euro flower industry (Christenhusz *et al.*, 2013), yet the taxonomy of wild tulips has proven to be notoriously difficult (Zonneveld, 2009; Veldkamp and Zonneveld, 2012; Christenhusz *et al.*, 2013). In 1753 the first tulip species was described (Linnaeus, 1753) and since, in the following four centuries, a huge variety of new species have been discovered by numerous different botanists, from a variety of backgrounds, institutes, and countries, leading to a complex entanglement of ideas and names. Throughout this period the fields of both systematics and botany have also developed significantly. Understanding of the natural variation within tulip species has increased, especially with regards to the impermanence of traits over generations and plasticity within species. In addition, the extent and importance of ploidy in tulips, a trait historically used to describe species (Hall, 1938), has been somewhat resolved. Plus, collections of wild tulips in botanic gardens have grown dramatically increasing the availability of samples for research (Christenhusz *et al.*, 2013). However most notably, methods in phylogenetics have developed and the phylogenetic species concept is now commonly applied in taxonomy (Mishler and Brandon, 1987; Nixon and Wheeler, 1990; Isaac, Mallet and Mace, 2004).

All these developments have not only led to the description of a range of new species in both past decades and more recent years (Table 1.1), but equally importantly have also led to a range of species previously described becoming recognised as synonyms, cultivated varieties, or naturalised species (Hall, 1940; Zonneveld, 2009; Veldkamp and Zonneveld, 2012; Christenhusz *et al.*, 2013). Many historical errors are only now being corrected (Christenhusz, Fay and Govaerts, 2013; Christenhusz and Wilson, 2022), with the type specimen of the genus, *Tulipa* × *gesneriana,* not long ago recognised as a complex hybrid from cultivated origin rather

than a true species (Christenhusz *et al.*, 2013). Today, around 93 species are accepted (Table 1.3), but since the genus was described over 300 species have been named (WCVP, 2022). Even those recognised today are still burdened with taxonomic uncertainty associated with the history of this clade. An array of research encompassing morphology, genetics, cross-ability analyses, and ecology have been used in an attempt to create order within the genus *Tulipa* and this has led to the establishment of four accepted subgenera (Hall, 1940; Botschantzeva, 1982; Van Raamsdonk *et al.*, 1997; Zonneveld, 2009; Veldkamp and Zonneveld, 2012; Christenhusz *et al.*, 2013). These four subgenera are now supported through multiple lines of evidence, but even here uncertainty lingers, with the monophyletic nature of the *Orithyia* subgenus still be to be thoroughly tested (Christenhusz *et al.*, 2013). Moreover, relationships within these subgenera remain ambiguous and many lower level taxonomic sections have been described based on genome size and morphology only (Zonneveld, 2009; Veldkamp and Zonneveld, 2012) with no support from sequence data (Christenhusz *et al.*, 2013). As new species are described the poor phylogenetic backbone of this genus is becoming increasingly apparent.

Up to now, all phylogenetic work on tulips has focused on the use of either a small range of plastid and nuclear markers or specific repetitive regions within the genome (Kiani, Memariani and Zarghami, 2012; Christenhusz *et al.*, 2013; Turktas *et al.*, 2013; Pourkhaloee *et al.*, 2018; Hajdari *et al.*, 2021). This research has resolved some of the evolutionary questions surrounding the genus; however, resolution limitations have left many outstanding issues (Christenhusz *et al.*, 2013). Furthermore, to exacerbate issues there has consistently been poor taxon representation within these works meaning many species have never been considered in a phylogenetic context. Overall, therefore current phylogenetic understanding is far behind morphological and cytological species classifications, even though today sequence data is considered a fundamental taxonomic tool (Baker *et al.*, 2022). The most complete tulip phylogeny contains only 24 species, or 26% of tulip diversity, with 14 new species having been described since its publication. In addition, this phylogeny lacks the resolution to determine the majority of relationships between the few taxa that are represented (M. J. M. Christenhusz et al., 2013). With new species being described frequently and the historical issues surrounding this clade it is becoming increasingly desirable to generate a reliable taxonomic framework for this genus through modern molecular methods.

Today's phylogenetic techniques far outperform even those of a few years ago and have been crucial in improving understanding of evolutionary relationships within many clades of animals

and plants (Chiari *et al.*, 2012; Sullivan *et al.*, 2017; Leebens-Mack *et al.*, 2019). Next generation sequencing methods are becoming more efficient leading to cheaper and shorter sequencing efforts. This in turn has allowed the generation of vast amounts of sequence data revolutionising modern phylogenetics. To date only a few tulip plastomes have been assembled, all from China (Zhou *et al.*, 2019; Do *et al.*, 2020; Ju, Shi, *et al.*, 2020; Ju, Tang, *et al.*, 2020; Ju *et al.*, 2021; Li *et al.*, 2021) limiting both comparative analysis of chloroplast structure within the genus as well phylogenetic understanding. Nonetheless, evidence from a recent plastome phylogenetic study of the closely related *Amana* genus shows that this type of data can lead to more strongly supported phylogenetic structure than previous work using only several plastid and nuclear markers (Li *et al.*, 2017). This suggests that plastome sequence data has the potential to resolve evolutionary relationships in the *Tulipa* genus providing some much-needed clarity on long-standing taxonomic issues.

The plant chloroplast genome, or plastome, is a relatively large, but now easy to sequence and assemble dataset. Moreover, the diversity of spacers and genes in this genome allow studies from the intraspecific level to higher clade analyses (Gitzendanner *et al.*, 2018). It has therefore become common to use plastome data in plant phylogenetics (Carbonell-Caballero *et al.*, 2015; Lu *et al.*, 2015; Wysocki *et al.*, 2015). The large amounts of sequence data provided by the plastome and the corresponding array of genetic markers have been able to resolve relationships which short sequence data have previously failed to determine (Jansen *et al.*, 2007; Gitzendanner *et al.*, 2018). However, there are some limitations with the use of the chloroplast genome. Although the complete chloroplast contains a large amount of sequence information to inform inference, it can still show incorrect evolutionary relationships due to gene tree conflict (Walker *et al.*, 2019) and may show discord with the species tree for both biological (Sullivan *et al.*, 2017) and systematic reasons (Walker *et al.*, 2019). Furthermore, as a single locus, it only presents a single estimate of evolutionary history, compared to nuclear markers (Doyle, 2022), so must be used carefully. Often plastome sequence data is generated through genome skimming approaches that are also effective at generating complete 35S rDNA nuclear genome data. The 35S rDNA region of the nuclear genome can also aid in taxonomic decision making (Wu *et al.*, 2020) and markedly provides an important resource for understanding cyto-nuclear discordance and therefore for assessing the usefulness of the plastome in inferring evolutionary relationships especially with regards to hybridisation and chloroplast capture (Liu *et al.*, 2020).

DNA barcodes are easy to sequence genetic markers that are useful for identifying between species in phylogenetics. There have been multiple suggestions as to the best DNA barcodes for land plants (CBOL Plant Working Group 1 *et al.*, 2009; Chen *et al.*, 2010; Hollingsworth, Graham and Little, 2011; Kress, 2017). These primarily focus on a select few plastid regions or repetitive nuclear regions, which are able to resolve a large number of accepted species across most clades and are easy to sequence. Specifically, the *matK* and *rbcL* regions of the plastome have been proposed as a 2-locus plant barcode (CBOL Plant Working Group 1 *et al.*, 2009), whilst the Internal Transcribed Spacer (ITS*)* region of the nuclear genome has also been widely considered (Kress *et al.*, 2005; Hollingsworth, Graham and Little, 2011). Nonetheless in groups where species are genetically extremely closely related, such as *Tulipa* (Christenhusz *et al.*, 2013), the performance of these markers is often limited. Both the 35S rDNA region and the plastome have been shown to be effective DNA super-barcodes (Li *et al.*, 2015; Wu *et al.*, 2020) and are becoming much simpler and cheaper to sequence. These have much higher resolution than previous DNA barcodes and can aid in identifying specimens which previous markers could not. The plastome and 35S rDNA regions present opportunities therefore within the genus *Tulipa* to not only reassess species concepts, but also to test these regions as DNA barcodes for this taxonomically messy clade.

The choice of genetic markers is important, however obtaining reliable specimens also remains crucial. Wild collected specimens provide the optimum material, however collecting expeditions are time consuming and resource heavy. They are therefore often part of a broader approach to sampling, which utilises living collections, herbaria, and genetic databanks to ensure high species representation in studies. Both living and herbaria collections of the genus *Tulipa* have greatly expanded in recent years, whilst collaboration between botanic gardens has broadly increased (Krishnan *et al.*, 2019; Pearce *et al.*, 2020; Westwood *et al.*, 2021). This has hugely improved sample availability providing a more robust foundation for taxonomic analysis. These collections are an accumulation of plant material from different expeditions, horticultural sources, and material shared between various institutions over multiple centuries and so although material is extremely useful, it must be assessed carefully for its representativeness of wild diversity. Sequence databanks also have limitations as they rely on the author to have correctly identified the species and appropriately assembled the sequence data, which is not always guaranteed. It is often common to find a number of misidentified specimens within databases and collections (Le *et al.*, 2020) and tulips may be especially prone to this as there are many morphologically similar taxa and older specimens frequently do not reflect the currently accepted taxonomy. As well not all species are adequately represented in collections

either, especially many newly described tulips (BGCI, 2022). The process of working with collections can drive the identification of errors (Ji, Liu, *et al.*, 2019; Wu *et al.*, 2020) and highlight underrepresented taxa leading to an improved understanding of collections and increasing their value. This is of special importance given the use of botanic collections for future conservation work, educational purposes and broader scientific and horticultural research efforts (Heywood, 2011; He and Chen, 2012; Faraji and Karimi, 2020).

A more complete understanding of evolutionary relationships of tulips and a comprehensive phylogeny are urgently needed and will be a critical foundation for understanding the genus, opening the door to analyses of biogeography, speciation, and trait evolution (Donoghue, 2008; M. Wu *et al.*, 2018; Revell, 2018), whilst aiding conservation decision makers (Godfray, Knapp and Mace, 2004). Studies have already shone light on the origin of many Liliaceae genera (Allen, Soltis and Soltis, 2003; Huang *et al.*, 2018; Kim and Kim, 2018) and therefore further specific work on tulips could provide a much broader understanding of the clade and its place in today's tree of life. The primary objective of this chapter was to develop the most comprehensive phylogeny of wild tulips to date. This work has been undertaken using plastome and 35S rDNA sequence data for both accepted species, new and old, as well as a variety of synonyms. Crucially this work has utilised the extensive botanic garden network, herbarium samples, online databases and also new fieldwork efforts to sample more species than any other phylogenetic research effort before. Our work is the most comprehensive study of the genus and most significant advancement of our understanding of tulip taxonomy over its lengthy history.

2.2. Materials and Methods

2.2.1. Samples and extraction

Leaf material for 253 taxa were collected through multiple fieldtrips, from botanic garden collections, or from herbarium specimens throughout the years of 2019, 2020, and 2021. Specifically for this project we undertook three expeditions in Kyrgyzstan in 2019, visiting the Batken, Osh, Jalal-Abad, Talas, and Chuy regions. In 2020 two expeditions in Kyrgyzstan were undertaken focused on obtaining specific species of note, including to the as yet unsampled Issyk-Kul region as well as to the Talas, Jalal-Abad and Chuy regions. An expedition around Tajikistan was also undertaken in 2020 that collected material from the Gorno-Badakhshan Autonomous Region, as well as the Khatlon, Districts of Republican Subordination, and Sughd regions. In 2021, final material was collected in Kyrgyzstan through expeditions to the Jalal-Abad, Osh, Chuy, and Talas regions with a final expedition completed in Tajikistan also, which

visited the Gorno-Badakhshan Autonomous Region, and Khatlon, Districts of Republican Subordination, and Sughd regions. Outside of the project, collaborators undertook expeditions to collect material in Kosovo, Turkey, and Uzbekistan during this time. A voucher specimen for each wild collected individual was stored in either CGE, FRU, TASH, UP, or VANF herbarium and duplicated where possible between local and U.K. based herbaria.

Where living material could be accessed, the leaf material was either frozen using liquid nitrogen or dried using silica gel at the source plant. To extract DNA from leaf samples we used a modified CTAB protocol (Doyle and Doyle, 1987). In brief, approximately 100-200mg of leaf tissues was either ground up by hand in a pestle and mortar or using a TissueLyser II (Qiagen) The ground leaf tissue was then cleaned using a washing buffer that contained 1M HEPES, 0.1% Polyvinylpyrrolidone (PVP), and 4% β-Mecap-2-Mercaptoethanol in MilliQ water. The washing buffer was removed by centrifuging for 2 minutes at 13,500 RPM and then pouring the supernatant away, with the remaining pellet resuspended in 1ml of 2% CTAB buffer (for V_T = 100ml: 1g PVP, 2g hexadecyltrimethylammonium bromide, 10ml of 1M Tris hydrochloride at pH 8, 4ml of 0.5M Ethylenediaminetetraacetic acid, 28ml of 5M NaCl, filled with MilliQ water). Then, 5µl of RNase A (stock 10mg/ml) was added and samples were incubated for 30 minutes at 65°C, mixing by inversion every five minutes. 1ml of chloroform was added and the solution mixed by inversion, before centrifuging at 4°C for 10 minutes. The upper aqueous layer was then pipetted into a fresh 2ml tube and the tube with the bottom layer in disposed of. Approximately 0.7 ml (70% of volume in tube) isopropanol was added to the upper layer, mixed by inversion, and placed in the fridge at 4°C for 20 minutes. The solution was then centrifuged at 8500 RPM at 4°C for 20 minutes, and the supernatant removed by pipetting. The remaining pellet was washed in 1ml of 75% ethanol solution for between 5-15 minutes, and the supernatant removed by pipetting. The tubes were then left with the lids open for the pellet to dry for 5-15 minutes. The pellet was then finally dissolved in 50µl of MilliQ water and allowed to dissolve at 4°C overnight. Extraction quality was assessed using a NanoDrop2000/2000c and through gel-electrophoresis. All samples that showed adequate DNA concentration (≥12.5 ng/µl) and were not extensively degraded shown by a relatively intact band of over 15kb on the gel were frozen and then sent for sequencing at Beijing Genomics Institute (BGI) in Hong Kong. DNBseq Normal DNA libraries or Low input DNA libraries were constructed depending on the quality of the extraction, as assessed by Qubit Fluorometer, NanoDrop, microplate reader and agarose gel-electrophoresis. Libraries were sequenced with DNBseq Paired-End 100 sequencing. Reads were processed through quality control using SOAPnuke (Chen *et al.*, 2018) and data filtered and adaptors trimmed (-n 0.001 -l 10 -q 0.4 –adaMR 0.25 –ada_trim). At least

1.2 Gigabases of clean data were obtained for each sample. All reads were additionally quality checked using FastQC (Andrew, 2018). This process led to 245 usable read datasets.

2.2.2. Genome assembly and annotation

Plastome

An initial single tulip chloroplast genome was assembled de-novo using SPAdes v. 3.15.0 (-k 55,77,99) (Nurk *et al.*, 2013). Then the longest contigs from this assembly which also had the highest SPAdes k-mer coverage, were mapped to the reference genome of *Amana edulis* (NC 034707) (Li *et al.*, 2017) and scaffolded together manually on Molecular Evolutionary Genetics Analysis Version 7.0 (MEGA7) where overlapping regions were over 20 base pairs and identical. This produced a full plastome with a large single-copy region (LSC), a small single-copy region (SSC), and two identical inverted repeats (IR). We then generated the same chloroplast genome through a more streamlined approach using the assembler and SPAdes wrapper Unicycler (Wick *et al.*, 2017), specifically using the script map_bwa_and_assemble.sh (https://github.com/NatJWalker-Hale/sequtils). Briefly, the script extracts only chloroplast reads using six reference genomes (Five *Amana* and one *Erythronium*; Table 2.1) copied and concatenated back to back (Wang *et al.*, 2018) and the Burrows-Wheeler Alignment Tool (Li and Durbin, 2009). The extracted reads were then assembled into contigs using Unicycler with conservative mode selected. Both the streamlined and 'manual' methods generated identical assemblies. We therefore elected to assemble all datasets using Unicycler and map the output contigs to a reference genome as this was much more efficient.

Table 2.1. The reference sequences used to extract chloroplast and ITS reads from the full read datasets. Both the accepted species name and NCBI reference number are reported.

Plastome		**ITS**	
Species	**NCBI Reference Sequence**	**Species**	**NCBI Reference Sequence**
Amana anhuiensis	NC_034706	*Tulipa biflora*	HF952957
Amana edulis	NC_034707	*Tulipa humilis*	HF952962
Amana erythronioides	KY401424	*Tulipa korolkowii*	HF952966
Amana kuocangshanica	NC_034708	*Tulipa clusiana*	EU912093
Amana wanzhensis	NC_034705	*Tulipa systola*	HF952975
Erythronium sibiricum	NC_035681	*Tulipa uniflora*	HF952979

For most samples, the Unicycler assembly produced three contigs representing the LSC region, SSC region and an IR region. These assembled contigs were then scaffolded together by

mapping them to the 'manually,' assembled plastome using the 'map to reference function' on Geneious Prime 2020.2.5 (https://www.geneious.com) ensuring they were all orientated in the same direction. We did this as there can be different structural forms of the chloroplast occurring within cells which vary in the relative orientation of the single copy regions (Wang and Lanfear, 2019). Within the mapping process, for each dataset, the inverted repeat was duplicated, and the two versions orientated appropriately. For 19 out of the 245 assemblies four or more contigs were generated. This most likely occurred because of a repeat region that the Unicycler programme could not bridge. In these cases, we still mapped the reads to a 'manually' assembled plastome using the map to reference function on Geneious Prime and scaffolded them together, however in two cases large gaps remained in the resulting assembly. For these two the initial SPAdes assembled contigs as well as the raw reads that had mapped to the chloroplast, were used to generate a consensus sequence which was used to manually fill these gaps in where coverage of individual bases was greater than three and the consensus had over 95% similarity to the closest assembled relative.

We initially selected a single tulip plastome, Complete_A (*Tulipa biflora binutans*), to annotate de novo using GeSeq (Tillich *et al.*, 2017). We then aligned this newly annotated sequence with five reference genomes from GenBank (Table 2.2) representing five different research studies and therefore a variety of different annotation attempts. Using this alignment, we manually altered incorrectly labelled start and stop regions and named each of the marked de novo genes on Geneious Prime. In this process de novo annotations were used where discrepancies occurred in the reference sequences. The fully annotated plastome was then used as a reference to annotate all other plastomes on Geneious Prime using the 'Annotate from' application with similarity set to 80%. Each subsequent annotated plastome was assessed for inaccurate start and stop codons as well as for any other errors or structural changes that may have occurred. Gene maps were produced using OGDRAW v1.3.1 (Greiner, Lehwark and Bock, 2019).

Table 2.2. The reference genomes used in the annotation process of plastomes. Both the accepted species name and NCBI reference number are reported.

Species	**NCBI Reference Sequence**
Tulipa altaica	NC_044780
Tulipa sylvestris	MT261172
Erythronium sibiricum	NC_035681
Amana erythronioides	KY401424
Amana edulis	NC_034707

Several *Tulipa* plastomes were also downloaded from Genbank and their annotations edited to match that of the assembled plastid genomes (Table 2.3). In addition, plastomes for five *Amana* specimens, two *Erythronium* specimens, and two *Gagea* specimens were also downloaded from Genbank and annotated through the same method as the assembled *Tulipa* plastid genomes (Table 2.3). These non-tulip plastomes were prepared so they could be used to root the phylogenies inferred using plastid data.

Table 2.3. A list of the plastome sequences downloaded from Genbank and their NCBI reference sequence.

Species	**NCBI Reference Sequence**
Tulipa sylvestris	MT261172
Tulipa biflora (*buhseana*)	MT316022
Tulipa iliensis	MT316023
Tulipa iliensis (*thianschanica*)	MT327741
Tulipa iliensis (*thianschanica*)	MW077738
Tulipa iliensis	MW077740
Tulipa sylvestris (*patens*)	MW077739
Tulipa altaica	MW077741
Tulipa altaica	NC_044780
Amana edulis	NC_034707
Amana anhuiensis	NC_034706
Amana kuocangshanica	NC_034708
Amana erythronioides	KY401424
Amana wanzhensis	NC_0347065
Erythronium japonicum	MT261155
Erythronium sibiricum	NC_035681
Gagea triflora	MT261157
Gagea (*Lloydia*) *tibetica**	NC_058295

* *Gagea tibetica* was recorded on Genbank as *Lloydia tibetica*, however the *Lloydia* genus is currently considered a synonym of *Gagea* (POWO, 2022)

Nuclear genome (ITS and 35S rDNA)

In addition to the use of plastome data at the initial stages of the project we isolated the ITS nuclear sequence from the read datasets. The ITS sequence has been previously identified as a useful genetic barcode for plants (Yao *et al.*, 2010; Hollingsworth, Graham and Little, 2011; Li *et al.*, 2011) and would enable us to explore whether there was any cyto-nuclear discordance suggestive of hybridisation or chloroplast capture (Baraket *et al.*, 2011; Huang *et al.*, 2014). We used the Burrows-Wheeler Alignment Tool (Li and Durbin, 2009) and six reference ITS sequences from Genbank representing all four recognised subgenera, with two sequences representing the larger *Eriostemones* and *Tulipa* subgenera, to extract ITS reads from raw read datasets (Table 2.1). In some cases, the SPAdes assembly (-k 21) of the ITS region failed,

which was thought to be due to the high number of reads available. To resolve this issue, in these cases we subsampled 500 reads using seqtk. In all cases a contig was assembled that was at least the length of the expected ITS sequence. If multiple contigs were generated, we selected the longest contig assembled which had the highest SPAdes k-mer coverage to represent an individual's ITS sequence. Genome skimming methods have also been shown to generate enough reads to produce the broader 35S rDNA region, which incorporates both *ITS1* and *ITS2* as well as the *18S, 28S,* and *5.8S* rRNA regions (Wu *et al.*, 2020). This region provides a 5-10 fold larger nuclear sequence region than just the ITS alone and therefore provides more data to assess cyto-nuclear discordance and species concepts. We therefore decided part way through the project that we would assemble the 35S rDNA region for all specimens. The closest related reference 35S rDNA sequence available on Genbank was *Lilium tsingtauense* (KM117263), so we selected this as a reference sequence in this work.

We generated a single specimen's (Complete_1) finalised sequence both through a de-novo assembly method as well as a more streamlined method. We assembled contigs from the raw read dataset using SPAdes before mapping all produced contigs to the *Lilium tsingtauense* reference (KM117263) on Geneious Prime using the map to reference function. Any mapped contigs were then pilon polished (Walker *et al.*, 2014) and remapped to the *L. tsingtauense* reference again on Geneious Prime using the map to reference function. Any contigs with lower SPAdes k-mer coverage than 10 were removed and the remaining mapped contigs were then used to generate a consensus sequence using the generate consensus sequence function on Geneious Prime with the strict-50% threshold selected. We then carried out a similar process to this, however adding an initial step where we used the *L. tsingtauense* reference sequence with the six tulip ITS sequences (Table 2.1), through the Burrows-Wheeler Alignment Tool (Li and Durbin, 2009) to extract reads from the selected specimen's read dataset, which were then used in the assembly. The streamlined and de-novo assembly methods led to extremely similar outputs (98.5% identical bases and 99.2% pairwise identity) although the de-novo method led to longer assembled sequences (7365bp compared to 5978bp).

Given these results we decided that we would therefore assemble specimens from all the recognised subgenera using the de-novo assembly method before using these as references in the streamlined method instead of the more distantly related *Lilium* specimen. This combined method would maximise the length of sequence data available for phylogenetic analyses, whilst also ensuring the pipeline was efficient. We therefore generated five species' 35S rDNA regions representing the four subgenera and with two sequences from the largest subgenus *Tulipa*

(Table 2.4). Through this we created five relatively complete 35S regions, however the assembled genomes had considerable base heterogeneity outside of the 35S rDNA region. To remove this highly variable sequence data at either end of the 35S regions we aligned all the sequences with the *Lilium* reference and transferred the annotations from this reference to our sequences. We then removed regions at either end outside of the annotations leaving only the annotated 35S region.

Table 2.4. Details of the five 35S rDNA sequences generated through the de-novo method, including the subgenus of each specimen and its sequence length in base pairs.

Species	**Subgenus**	**Sequence length (bp)**
Tulipa biflora (binutans)	Eriostemones	5852
Tulipa linifolia	Clusianae	5852
Tulipa korolkowii	Tulipa	5856
Tulipa greigii	Tulipa	5856
Tulipa heterophylla	Orithyia	5858

There were several assembly issues associated with the orientation and repetitiveness of the 35S region which occurred in the larger raw read datasets, and so we decided to use these five tulip sequences as references to extract reads. All 35S sequences for other specimens were assembled with an initial step where reads were extracted using the five *Tulipa* 35S references and the Burrows-Wheeler Alignment Tool (Li and Durbin, 2009). The extracted reads were then assembled into contigs using SPAdes, polished using Pilon, and then mapped to the most closely related reference sequence. At this stage we removed contigs that had a SPAdes k-mer coverage <10 or that clearly diverged considerably from the reference sequence or other contigs mapped to the same region, this being suggestive of sequencing errors. We then generated a consensus sequence from the remaining mapped contigs using the Generate Consensus Sequence tool with the strict setting selected on Geneious Prime. The consensus sequence was annotated using the reference sequences and sequence data from outside the annotated regions removed. The same process was used to generate a 35S rDNA sequence for the species *Amana baohuaensis* using short read archive available on GenBank (SRR12599520).

2.2.3. Phylogenetic analysis

Both plastome and 35S rDNA sequences were used in an array of analyses. We primarily selected to use Maximum Likelihood (ML) methods to infer phylogenies as Bayesian methods are computationally intractable with many tips, especially if the data is also partitioned. Nonetheless, we also ran a Bayesian analysis on a single-partition plastome dataset to assess

the consistency of evolutionary relationships across differing methods. For the ML methods several different datasets were used and we elected to use both a single partition as well as a multi-partition approach (Bull *et al.*, 1993). Model selection software was not used as it has been deemed unnecessary (Abadi *et al.*, 2019) and so a GTR+F+G (Generalised Time Reversible model, empirical state frequencies, and gamma distributed rate heterogeneity with four discrete rate categories) model was used for all analyses. This did not include the commonly used invariable sites parameter (+I) due to potentially pathological numerical behaviour when combined with gamma-distributed rate heterogeneity (Yang, 2014).

Plastome single partition

For the single partition process, we first removed the second IR region of each plastome in Geneious Prime to prevent pseudo replicating phylogenetic signal. The modified plastomes were then aligned using MAFFT (--auto) (Katoh and Standley, 2013) and cleaned to reduce erroneous signal using the phyx function pxclsq (Brown, Walker and Smith, 2017) to trim columns with less than 10% occupancy (-p 0.1). A Maximum Likelihood tree was generated for this dataset using raxml-ng (Kozlov *et al.*, 2019) under the GTR+F+G model and 500 non-parametric bootstrap trees were produced (--bs-trees 500 --model GTR+G+F). This alignment was converted from fasta to nexus format using the pxs2nex tool from the phyx toolbox (Brown, Walker and Smith, 2017) and converted to an interleaved format on Geneious Prime. The alignment was then used to undertake a Bayesian analysis using a heated Markov chain Monte Carlo (MCMC) approach as implemented in MrBayes v3.2.7 (Ronquist *et al.*, 2012). Three separate runs of 20 million generations were undertaken with a default three cold and one heated chain, model GTR+G selected, and with parameters and trees sampled every 1000 generations. Using the sumt function a majority consensus tree was generated (sumt burnin=2500). Convergence was assessed using the Tracer programme, with an ESS>200 for parameters accepted as adequate (Rambaut *et al.*, 2018).

Plastome multiple partitions

Six subsets of annotated plastome regions were used to infer the phylogeny in the multi-partition approach (Table 2.5). These consisted of protein coding sequences only (Dataset 1), protein coding sequences with introns (Dataset 2), all gene regions without introns (Dataset 3), gene regions with introns (Dataset 4), all annotations including spacers (Dataset 5), and introns and spacers (Dataset 6). These annotations were extracted on Geneious Prime, and we manually edited the *trnI* and *trnK* intron annotations so that they exclude bases which genes overlapped with. Each extracted annotated region was then aligned with the corresponding

sequence for all specimens using MAFFT (--auto). These alignments were then merged into a supermatrix using the pxcat tool from the phyx toolbox (Brown, Walker and Smith, 2017). The supermatrix was cleaned using the phyx toolbox tool pxclsq to trim columns with less than 10% occupancy (-p 0.1). Repetitive regions in the tail end of *trnH-psbA* and *rpl22-rps19* intergenic spacers which were found to be difficult to align, were removed. The CDS sequences in the supermatrix were then further partitioned based on codon positions. We chose to use the IQ-TREE ModelFinder software v. 1.6.12 (Kalyaanamoorthy *et al.*, 2017) to partition the supermatrix. We selected to use the relaxed hierarchical clustering command rcluster set to 90 (Lanfear *et al.*, 2014) (-rcluster 90 -pre tulipsGTR.merge -m MFP+MERGE -mset GTR -mfreq F -mrate G) as using the greedy strategy with this number of partitions and specimens proved intractable (Lanfear *et al.*, 2012). This developed a best scheme partition model for each dataset. We then, for each dataset, produced a phylogeny using raxml-ng with each partition assigned the GTR+F+G model with 500 non-parametric bootstrap trees generated. These supermatrix combined analyses are an identified effective method for allowing evolutionary variation in long sequence data encompassing multiple loci (Yang, 2014).

Table 2.5. Datasets used in the multi-partition plastome based phylogenetic analyses. The first six rows of data detail the type of annotated sequences included in each dataset. The penultimate row shows the number of initial partitions in each dataset and the final row specifies the number of partitions after the merging process. CDS partitions are considered as three times the number of CDS sequences as the genetic code was split by codon number.

	Dataset 1	**Dataset 2**	**Dataset 3**	**Dataset 4**	**Dataset 5**	**Dataset 6**
CDS (coding sequence)	78 (x3)	78 (x3)	78 (x3)	78 (x3)	78 (x3)	0
Introns	0	20†	0	20†	20†	20†
Other	0	0	2*	2*	2*	0
rRNA	0	0	4	4	4	0
tRNA	0	0	30	30	30	0
Intergenic spacers	0	0	0	0	108˜	108˜
Number of initial partitions	234	254	270	190	398	128
Number of merged partitions	11	13	9	14	20	14

* The genes ycf15, and ycf68 are pseudogenes

˜ The intergenic spacer between ndhA and ndhH genes was not included as it is only one base pair long and invariable between all sequences.

† Only segments of the introns of trnI-GAU and trnK-UUU genes, that did not overlap with other annotated genes were included to prevent pseudo replication of data.

35S rDNA

We generated two different datasets from the 35S rDNA annotated sequences. We analysed the dataset as a single partition, as well as multiple partitions. We extracted the *5.8S*, *18S*, and *26S* rRNA regions as well as the *ITS1* and *ITS2* regions from our annotated 35S sequences. These independent regions were then aligned using MAFFT (--auto). We then concatenated them to generate a single partition dataset and merged them into a supermatrix using the pxcat tool from the phyx toolbox for the multiple partition dataset. All alignments were cleaned using the phyx toolbox tool pxclsq to trim columns with less than 10% occupancy (-p 0.1). IQ-TREE ModelFinder was then used to develop a best scheme partition model for the multi-partition analysis using the greedy algorithm (Lanfear *et al.*, 2012), leading to two partitions being identified (-m MFP+MERGE -mset GTR -mfreq F -mrate G). We then produced phylogenies for the separate datasets using raxml-ng with partitions assigned the GTR+F+G model where appropriate and with 500 non-parametric bootstrap trees generated.

Barcode datasets

The two plastid coding regions *matK* and *rbcL* were extracted from all assembled plastomes, whilst the *ITS1-5.8S* rRNA-*ITS2* region was extracted from the 35S rDNA dataset. These alignments were cleaned using the phyx toolbox tool pxclsq to trim columns with less than 10% occupancy (-p 0.1). The *matK* and *rbcL* region were merged into a supermatrix using the pxcat tool from the phyx toolbox (Brown, Walker and Smith, 2017) with the sequences further partitioned based on codon positions, whilst the *ITS1-5.8S* rRNA-*ITS2* was concatenated and treated as a single partition as is common (Hollingsworth *et al.*, 2016). We used the IQ-TREE ModelFinder software as previously to choose the best partition scheme for the *matK* + *rbcL* dataset leading to three partitions being identified. We then generated a phylogeny for both barcoding regions using raxml-ng with partitions assigned the GTR+F+G model where appropriate, and 500 non-parametric bootstrap trees generated.

2.2.4. Assessment of phylogenies

Specimen errors

Several specimens included in the analyses had uncertainty around their species-level identification. Before the tree was finalised the preliminary trees were assessed for clear errors in specimen positions compared to their expected positions as well as reviewing specimens that remained unidentifiable. A list of clear misidentifications and unidentifiable specimens was developed, and all preliminary trees were pruned of these tips (Table 2.6; Appendix 1). Misidentification was assessed based on the biogeography of the species, phylogenetic position

relative to expected position and to other specimens under the same taxon name, and where possible morphological assessment of the specimen's voucher or herbarium material. If possible the misidentified species were reassigned to a correct species name and we reported this back to the source of the material. We took the approach that any specimen that had some uncertainty surrounding its given name but was not clearly an error was left in the tree. These uncertain specimens that were not clearly errors were highlighted in the finalised tree.

Table 2.6. Specimens sequenced that were removed from all phylogenies due to their ambiguous identity. Code refers to the name given to material in the lab to link it to a specimen. Original species name is the taxonomic name attached to the material before sequencing. Type of issue details whether the specimen could be confirmed as a misidentification or whether sequencing the specimen did not resolve its unidentifiable status. The likely correct species is the species or complex which the sequence data suggests the material most likely represents.

Code	**Original species name**	**Type of issue**	**Likely correct species**
Edi5	*T. praestans*	Misidentification	*T. greigii*/*T. kaufmanniana* complex
Kew42	*T. suaveolens (schrenkii)*	Misidentification	T. alberti
E2	*T. greigii*	Misidentification	T. fosteriana
Herb7	*T.* × *gesneriana*	Misidentification	T. agenensis
Herb14	*T. harazensis*	Misidentification	*T. systola* complex
Kew36	*T. ingens*	Misidentification	*T. systola* complex
MT316023	*T. iliensis*	Misidentification	*T. altaica*
Kew38	*T. kolpakowskiana*	Misidentification	*T. ferganica*
Kew37	*T. jacquesii*	Misidentification	*T. ferganica*
Uzb16	*T. talassica*	Misidentification	*T. korolkowii*
MW077740	*T. iliensis*	Misidentification	*T. tetraphylla*
Kew17	*T. sylvestris (patens)*	Misidentification	*T. biflora* complex
Kew7	*T. dasystemon (dasystemonoides)*	Misidentification	*T. biflora* complex/*T. jacquesii*
Kew20	*T. turkestanica*	Misidentification	*T. sylvestris*/*T. orphanidea*
Edi6	*T. heterophylla*	Misidentification	*T. dasystemon*
Herb5	*T. kuschkensis*	Misidentification	*T. montana*
Kew21	*T. undulatifolia (micheliana)*	Misidentification	*T. systola* complex
Kyr18	unknown	Unidentifiable	*T. biflora* complex
51	unknown	Unidentifiable	*T. biflora* complex
Kyr15	unknown	Unidentifiable	*T. biflora* complex
59	unknown	Unidentifiable	*T. biflora* complex
Bal8	Potential new species	Unidentifiable	Unknown (need more evidence for new species status)

Assessing monophyly of named species

To assess how many of our accepted species (Table 2.11) are recovered as phylogenetically identifiable in each of the four different datasets used for inferring phylogenies (plastome, 35S

rDNA, *matK* + *rbcL* barcode and ITS barcode) we assessed the occurrence of monophyly for these taxa. We undertook two approaches, a conservative assessment where we only evaluated the monophyly of species with multiple tips in the phylogeny, and a lenient approach where we assessed both multi and single tip species. In the lenient approach we also ignored the uncertain specimens under the names *Tulipa agenensis* and *T. linifolia* (*maximowiczii*), as they are clearly not the species they are named as, but also could not be assigned to any other recognised species either. Specifically, we assessed a multi-tip species as monophyletic if all the specimens under the same taxon name, including synonyms, formed a clade with a bootstrap support of 70 or more. In the lenient approach we assessed single tip species as distinguishable if they occurred in the tree in the expected region and were not embedded within another species.

Cytonuclear discordance

To assess the reliability that the plastome inferred tree is an accurate inference of the relationships of this genus, and not presenting misleading phylogenetic positions due to plastid capture or hybridisation, we compared the plastome tree structure to that of the nuclear marker inferred 35S rDNA tree. This was done using the cophylo function in the R package 'phytools' (Revell, 2012). Both trees were pruned of tips that we recognised as misidentified specimens, as previously described. Mismatches between the 35S rDNA and plastome phylogeny were then individually assessed.

Generation of final species tree

A phylogenetic tree with only a single tip representing each species, was created using a filtered version of Dataset 5 (Table 2.5). The specimens selected to be removed from the overall dataset were decided upon using information from the 35S rDNA and plastome tree, through the cytonuclear discordance assessment using the cophylo tool on phytools (Revell, 2012), as well as biogeographical understanding, previous hybridisation knowledge, and taxonomic expertise (Christenhusz *et al.*, 2013; Everett, 2013). A list of duplicate specimens and unreliable specimens was developed. Then, the specimens in all alignments were initially renamed using the pxrls function in the phyx toolbox, before the identified specimens to be removed were cut from the alignments using the created list and the phyx tool pxrms (Brown, Walker and Smith, 2017). This left all alignments with 80 selected tulip sequences which were then used to infer the species tree (Table 2.7). These alignments were realigned to account for the loss of a range of specimens using MAFFT (--auto) and then cleaned using the pxclsq tool in phyx, trimming columns with less than 10% occupancy. A supermatrix was then created from the alignments

using pxcat from the phyx package. All CDS markers were partitioned further to represent each codon position. The best scheme partition for this supermatrix was inferred using IQ-TREE ModelFinder as before for multi-partition datasets with a phylogeny inferred using raxml-ng with settings as before.

Table 2.7. The 80 specimens used to infer the species tree. Code refers to the name given to material in the lab to link it to a specimen. The original species name shows the taxonomic name attached to the material when sampled. The tip on the species tree shows which of the accepted species names recognised in Table 2.11 the specimen represented in the species tree.

Code	**Original species name**	**Tip on species tree**
D	*T. saxatilis*	*T. saxatilis*
F	*T. montana*	*T. montana*
O	*T. undulatifolia*	*T. undulatifolia*
R	*T. orphanidea*	*T. orphanidea*
T	*T. clusiana (cashmeriana)*	*T. cashmeriana*
W	*T. lemmersii*	*T. lemmersii*
X	Duc van Tol	Duc van Tol
Edi1	*T. agenensis*	*T. agenensis*
Edi2	*T. cretica*	*T. cretica*
Edi7	*T. sprengeri*	*T. sprengeri*
Kew1	*T. foliosa*	*T. foliosa*
Kew4	*T. carinata*	*T. carinata*
Kew5	*T. clusiana*	*T. clusiana*
Kew11	*T. orphanidea* (*hageri*)	*T. hageri*
Kew18	*T. systola*	*T. systola*
Kew22	*T. borszczowii*	*T. borszczowii*
Kew23	*T. hungarica*	*T. hungarica*
Kew28	*T. alberti*	*T. alberti*
Kew29	*T. altaica*	*T. altaica*
Kew31	*T. cinnabarina*	*T. cinnabarina*
Kew34	*T. hoogiana*	*T. hoogiana*
Kew39	*T. undulatifolia* (*micheliana*)	*T. micheliana*
Kew43	*T. suaveolens* (*schrenkii*)	*T. suaveolens*
Kew44	*T. sosnowskyi*	*T. sosnowskyi*
Kew47	*T. ulophylla*	*T. ulophylla*
2	*T. lehmanniana* (*zenaidae*)	*T. zenaidae*
3	*T. ferganica*	*T. ferganica*
4	*T. talassica*	*T. toktogulica*
5	*T. tetraphylla*	*T. tetraphylla*
6	*T. turkestanica*	*T. turkestanica*
8	*T. korolkowii*	*T. korolkowii*
9	*T. dasystemon*	*T. dasystemon*
10	*T. fosteriana* (*affinis*)	*T. affinis*
17	*T. platystemon*	*T. platystemon*
22	*T. urumiensis* (*tarda*)	*T. urumiensis*
23	Sp. unknown	*T. brachystemon*
27	*T. ostrowskiana*	*T. ostrowskiana*
35	*T. greigii*	*T. greigii*
40	*T. dasystemon* (*dasystemonoides*)	*T. neustruevae*
42	*T.* × *tschimganica* (*anadroma*)	*T. anadroma*
43	*T. zonneveldii*	*T. zonneveldii*

48	*T. dubia*	*T. dubia*
49	*T. bifloriformis*	*T. bifloriformis*
50	*T. kaufmanniana*	*T. kaufmanniana*
Jac	*T. jacquesii*	*T. jacquesii*
Tjk2	*T. linifolia*	*T. linifolia*
Tjk4	*T. subquinquefolia*	*T. subquinquefolia*
Tjk5	*T. anisophylla*	*T. anisophylla*
Tjk7	*T. vvedenskyi*	*T. vvedenskyi*
Tjk9	*T. praestans*	*T. praestans*
Tjk10	*T. hissarica*	*T. hissarica*
Tjk12	*T. lanata*	*T. lanata*
Bal1	*T. albanica*	*T. albanica*
Bal13	*T. scardica*	*T. scardica*
Bal15	*T. serbica*	*T. serbica*
Bal19	*T. sylvestris* (ssp. *sylvestris*)	*T. sylvestris*
Kyr1	*T. heterophylla*	*T. heterophylla*
Kyr5	*T. kolpakowskiana*	*T. kolpakowskiana*
Kyr6	*T. talassica*	*T. talassica*
Tur1	*T. koyuncui*	*T. biflora*
Tur6	*T. humilis*	*T. humilis*
Zonn4	*T. kolbintsevii*	*T. kolbintsevii*
Zonn8	*T. ivasczenkoae*	*T. ivasczenkoae*
Zonn9	*T. regelii*	*T. regelii*
Zonn11	*T. heteropetala*	*T. heteropetala*
Zonn14	*T. dianaeverettiae*	*T. dianaeverettiae*
Got2	*T. julia*	*T. julia*
Got6	*T. uniflora*	*T. uniflora*
Herb16	*T. kuschkensis*	*T. kuschkensis*
Herb20	*T. armena*	*T. armena*
MT327741	*T. iliensis* (*thianschanica*)	*T. iliensis*
MW077739	*T. sylvestris* (*patens*)	*T. patens*
Uzb1	*T. uzbekistanica*	*T. uzbekistanica*
Uzb2	*T.* × *tschimganica*	*T.* × *tschimganica*
Uzb3	*T. orithyioides*	*T. orithyioides*
Uzb7	*T. fosteriana*	*T. fosteriana*
Uzb10	*T. ingens*	*T. ingens*
Uzb15	*T. lehmanniana*	*T. lehmanniana*
Uzb17	*T. scharipovii*	*T. scharipovii*
Uzb20	*T. butkovii*	*T. butkovii*

2.3. Results and Discussion

The long horticultural and taxonomic history of the genus *Tulipa* has led to many ambiguities in nomenclature and understanding of natural diversity, many of which still remain to be solved (Christenhusz *et al.*, 2013), while the importance of wild tulip populations is once again growing, as recognition of the need for greater genetic diversity and new traits within horticulture increases (Orlikowska *et al.*, 2018). Tulip species are also increasingly recognised as Threatened (Tojibaev and Beshko, 2015) meaning there is both an urgent need to understand tulip taxonomy for conservation purposes (Godfray, Knapp and Mace, 2004) as well as

economic reasons. The number of wild tulip species remains unclear and the taxonomy of the genus, although recently greatly improved (Zonneveld, 2009; Christenhusz *et al.*, 2013), is still hampering conservation (Garnett and Christidis, 2017). Here we have used next-generation sequencing methods and modern phylogenetics to unravel the complex evolutionary relationships of this genus providing the most comprehensive tulip phylogeny to date. This phylogeny and corresponding taxonomic inferences provide a much needed and timely step forward in our understanding of wild tulip diversity.

2.3.1. Plastome structure

In this work a total of 254 tulip plastomes were used representing ~86% of accepted species in the genus. An annotated chloroplast genome was assembled for 245 tulips from wild collected material, living collections, or herbaria (Table 2.8) with nine obtained from GenBank (Table 2.3). Our assembled plastomes range from 151,059-152,675 bp in size with all having a total of 136 genes (Table 2.9; Table 2.10). Of these 136 genes 114 are unique coding regions with 22 duplicate genes mapped. These 114 genes consist of 78 CDS sequences, four rRNA sequences, 30 tRNA sequences, and two pseudogenes with no clear function or were only present as partial sequences (Table 2.10; Figure 2.1). The GenBank specimens were generally similar although a few gaps were noted in the plastome construction of several. This was likely due to assembly and sequencing issues rather than the outcome of any evolutionary process given the consistency of our much broader plastome dataset. Across all the chloroplasts there is no clear loss of specific genes from the closely related genera of *Amana* and *Erythronium* (Li *et al.*, 2017). The *infA* gene region contained a number of premature stop codons and therefore our work supports previous research that suggests it has been partially deleted in *Tulipa* (Do *et al.*, 2020).

Table 2.8. Details of the number of taxa obtained from each source type and the percentage of misidentified specimens from each source.

Source	**Number of taxa**	**Percentage of taxonomic misidentifications**
Wild collected	124	0.8
Living collections	104	10.6
Herbaria	17	17.6
Genbank	9	22.2

Table 2.9. Details of the 245 specimens successfully sequenced within this project. Both accepted taxon name based on Table 1.3 and synonym names given under named as where relevant. Code refers to the name given to material in the lab to link it to a specimen. Country, given as the ISO alpha-3 country code or GO if garden origin, refers to where material was originally sourced. Sources are wild, given as an herbarium acronym, or a living collection acronym (CUBG – Cambridge University Botanic Garden, RBK – Royal Botanic Gardens Kew, RBE – Royal Botanic Gardens Edinburgh, GBG – Gothenburg Botanical Garden, and PC – private collection). Size refers to the overall plastome assembly length in base pairs, with GC% given and length of large single-copy region (LSC), inverted repeat (IR), and small single-copy region (SSC) also reported in base pairs.

Accepted Taxon	Named as	Code	Country	Source	Voucher or accession no.	Size (bp)	GC%	LSC (bp)	IR (bp)	SSC (bp)
Tulipa biflora	binutans	1	KGZ	Wild	BW001	152,016	36.6	82,153	26,332	17,199
Tulipa lehmanniana	zenaidae	2	KGZ	Wild	BW003	152,023	36.6	82,192	26,334	17,163
Tulipaferganica	-	3	KGZ	Wild	BW005	152,135	36.7	82,212	26,372	17,179
Tulipa talassica	-	4	KGZ	Wild	BW007	152,215	36.6	82,372	26,344	17,155
Tulipa tetraphylla	-	5	KGZ	Wild	BW009	152,072	36.6	82,224	26,342	17,164
Tulipa turkestanica	-	6	KGZ	Wild	BW011	152,040	36.6	82,199	26,331	17,179
Tulipa korolkowii	rosea	7	KGZ	Wild	BW013	152,090	36.6	82,256	26,339	17,156
Tulipa korolkowii	-	8	KGZ	Wild	BW015	152,071	36.6	82,236	26,339	17,157
Tulipa dasystemon	-	9	KGZ	Wild	BW017	152,097	36.6	82,240	26,336	17,185
Tulipa fosteriana	affinis	10	KGZ	Wild	BW019	152,119	36.6	82,171	26,369	17,210
Tulipa turkestanica	-	13	KGZ	Wild	BW025	152,024	36.6	82,162	26,332	17,198
Tulipa fosteriana	affinis	14	KGZ	Wild	BW027	152,152	36.6	82,203	26,369	17,211
Tulipa ferganica	-	16	KGZ	Wild	BW031	152,145	36.7	82,225	26,372	17,176
Tulipa platystemon	-	17	KGZ	Wild	BW033	152,114	36.6	82,305	26,326	17,157
Tulipa ferganica	-	18	KGZ	Wild	BW035	152,140	36.7	82,227	26,372	17,169
Tulipa urumiensis*	tarda	22	KGZ	Wild	BW042	152,015	36.6	82,138	26,335	17,207
Sp. Unknown (Chuy)	-	23	KGZ	Wild	BW044	152,090	36.6	82,281	26,326	17,157
Tulipa ostrowskiana	-	27	KGZ	Wild	BW052	152,098	36.6	82,290	26,326	17,156
Tulipa dasystemon	-	31	KGZ	Wild	BW060	152,118	36.6	82,258	26,336	17,188
Tulipa lehmanniana	zenaidae	34	KGZ	Wild	BW065	152,018	36.7	82,199	26,334	17,151
Tulipa greigii	-	35	KGZ	Wild	BW067	152,248	36.6	82,259	26,371	17,247
Tulipa tetraphylla	-	39	KGZ	Wild	BW076	152,057	36.6	82,209	26,342	17,164
Tulipa dasystemon	dasystemonoides	40	KGZ	Wild	BW078	152,072	36.6	82,161	26,346	17,219
Tulipa x tschimganica	anadroma	41	KGZ	Wild	BW080	152,458	36.6	82,528	26,359	17,212
Tulipa x tschimganica	anadroma	42	KGZ	Wild	BW082	152,333	36.6	82,436	26,359	17,179
Tulipa zonneveldii	-	43	KGZ	Wild	BW084	152,455	36.6	82,529	26,359	17,208
Tulipa zonneveldii	-	44	KGZ	Wild	BW086	152,354	36.6	82,438	26,358	17,200
Sp. Nov.	-	45	KGZ	Wild	BW088	152,156	36.7	82,212	26,372	17,200
Tulipa dubia	-	48	KGZ	Wild	BW092	152,171	36.6	82,259	26,365	17,182
Tulipa bifloriformis	-	49	KGZ	Wild	BW094	152,042	36.6	82,171	26,330	17,211
Tulipa kaufmanniana	-	50	KGZ	Wild	BW096	152,228	36.6	82,241	26,371	17,245
Sp. Unknown (Chatkal)	-	51	KGZ	Wild	BW098	152,004	36.6	82,164	26,331	17,178
Tulipa kaufmanniana	-	52	KGZ	Wild	BW100	152,226	36.6	82,239	26,371	17,245

Tulipa bifloriformis	-	57	KGZ	Wild	BW110	151,981	36.6	82,112	26,328	17,213
Tulipa greigii	-	58	KGZ	Wild	BW112	152,239	36.6	82,251	26,373	17,242
Sp. Unknown (Talas)	-	59	KGZ	Wild	BW114	152,029	36.6	82,208	26,330	17,161
Tulipa ostrowskiana	-	60	KGZ	Wild	BW116	152,095	36.6	82,287	26,326	17,156
Tulipa greigii	-	62	KGZ	Wild	BW118	152,245	36.6	82,257	26,371	17,246
Tulpa jacquesii	-	Jac	KGZ	Wild		151,751	36.6	81,893	26,337	17,184
Tulipa biflora	-	A	KAZ	CUBG	2008-0427	152,036	36.6	82,174	26,332	17,198
Tulipa bifloriformis	-	B	KAZ	CUBG	2008-0426	151,973	36.6	82,134	26,331	17,177
Tulipa lehmanniana	-	C	KAZ	CUBG	2008-0406	151,137	36.7	81,340	26,353	17,091
Tulipa saxatilis	-	D	GRC	CUBG	1971-0212	152,057	36.7	82,179	26,335	17,208
Tulipa saxatilis	-	D2	GRC	CUBG	1974-0176	152,062	36.6	82,184	26,334	17,210
Tulipa greigii	-	E	KAZ	CUBG	2008-0416	152,246	36.6	82,244	26,373	17,256
Tulipa greigii	-	E2	UZB	RBK	1984-1535	152,556	36.6	82,533	26,363	17,297
Tulipa montana	-	F	IRN	CUBG	2014-0503	151,589	36.7	81,599	26,391	17,208
Tulipa kaufmanniana	-	G	KAZ	CUBG	2008-0417	152,243	36.6	82,245	26,373	17,252
Tulipa tetraphylla	-	H	KAZ	CUBG	2008-0725	152,067	36.6	82,218	26,342	17,165
Tulipa turkestanica	-	I	KAZ	CUBG	2008-0414	152,031	36.6	82,195	26,330	17,176
Tulipa dasystemon	-	J	KAZ	CUBG	2008-0423	152,120	36.6	82,263	26,336	17,185
Tulipa dasystemon	-	K	KAZ	CUBG	2008-0418	152,094	36.6	82,239	26,336	17,183
Tulipa vvedenskyi	-	L	UZB	RBK	1998-2799	152,226	36.6	82,241	26,372	17,241
Tulipa vvedenskyi	-	L2	UZB	RBK	2007-2212	152,246	36.6	82,260	26,371	17,244
Tulipa x tschimganica	-	M	UZB	RBK	1984-1528 BRMW 10655A	152,255	36.6	82,269	26,372	17,242
Tulipa ingens	tubergeniana	N	GO	RBK	2004-3736	152,366	36.6	82,421	26,357	17,231
Tulipa undulatifolia	-	O	GO	RBK	2015-0133	152,410	36.6	82,417	26,363	17,267
Tulipa ostrowskiana	-	P	KAZ	RBK	1995-4406 DVNA955	152,099	36.6	82,291	26,326	17,156
Tulipa biflora	sogdiana	Q	GO	RBK	2004-3641	152,016	36.6	82,173	26,331	17,181
Tulipa orphanidea	-	R	TUR	RBK	19753003	151,850	36.6	82,243	26,197	17,213
Tulipa humilis	-	S	TUR	RBK	1978-3514 BAYT	152,054	36.6	82,174	26,333	17,214
Tulipa clusiana (f. cashmeriana)	-	T	GO	RBK	2004-3703	151,649	36.7	81,696	26,370	17,213
Tulipa urumiensis	tarda	U	KAZ	RBK	1983-3941	152,001	36.6	82,140	26,335	17,191
Tulipa butkovii	-	V	UZB	RBK	1978-838 NYRK	152,242	36.6	82,254	26,371	17,246
Tulipa lemmersii	-	W	KAZ	CUBG		152,100	36.6	82,299	26,334	17,133
Duc van Tol	-	X	GO	CUBG		151,997	36.6	82,175	26,351	17,120
Tulipa foliosa	armena var lycica	Kew1	TUR	RBK	20071840	152,429	36.5	82,372	26,389	17,279
Tulipa biflora	binutans	Kew2	KAZ	RBK	20102251	151,899	36.6	82,150	26,332	17,085
Tulipa carinata	-	Kew4	UZB	RBK	20043220	152,425	36.6	82,469	26,366	17,224
Tulipa clusiana	-	Kew5	IRN	RBK	200670	151,583	36.7	81,642	26,370	17,201
Tulipa clusiana	aitchisonii	Kew6	AFG	RBK	19884160	151,535	36.7	81,594	26,350	17,241
Tulipa dasystemon	dasystemonoides	Kew7	UZB	RBK	20043221	152,152	36.6	82,311	26,331	17,179
Tulipa kaufmanniana	-	Kew8	KAZ	RBK	20083068	152,244	36.6	82,258	26,371	17,244
Tulipa montana	chrysantha	Kew9	IRN	RBK	19782434	151,569	36.7	81,612	26,370	17,217
Tulipa orithyioides	-	Kew10	UZB	RBK	20072478	152,059	36.6	82,203	26,332	17,192
Tulipa orphanidea	hageri	Kew11	TUR	RBK	19893055	152,091	36.6	82,210	26,333	17,215
Tulipa orphanidea	theophrastii	Kew12	GRC	RBK	19893058	151,894	36.6	82,244	26,197	17,256
Tulipa praestans		Kew13	TJK	RBK	199224	152,211	36.6	82,279	26,368	17,196
Tulipa saxatilis	bakeri	Kew14	GRC	RBK	19771713	152,068	36.6	82,194	26,333	17,208
Sp. Unknown (Varzob)	-	Kew15	TJK	RBK	20142333	152,171	36.6	82,227	26,367	17,210
Tulipa sylvestris	biebersteiniana	Kew16	IRN	RBK	200696	151,857	36.6	82,238	26,197	17,225

Tulipa sylvestris	patens	Kew17	KAZ	RBK	20043226	151,997	36.6	82,140	26,332	17,193
Tulipa systola*	-	Kew18	IRN	RBK	19762914	152,349	36.6	82,339	26,371	17,268
Tulipa x tschimganica	-	Kew19	UZB	RBK	19801917	152,232	36.6	82,245	26,372	17,243
Tulipa turkestanica	-	Kew20	KAZ	RBK	19962763	151,886	36.6	82,237	26,197	17,255
Tulipa undulatifolia	micheliana	Kew21	IRN	RBK	19782016	152,308	36.6	82,341	26,371	17,225
Tulipa borszczowii	-	Kew22	KAZ	RBK	20191973	151,059	36.7	81,321	26,353	17,032
Tulipa hungarica	-	Kew23	BGR	RBK	20191962	152,057	36.6	82,228	26,351	17,127
Tulipa lemmersii	-	Kew24	KAZ	RBK	2020530	152,088	36.6	82,287	26,334	17,133
Tulipa hungarica	rhodopea	Kew25	BGR	RBK	20191975	152,090	36.6	82,257	26,352	17,129
Tulipa talassica	-	Kew26	KYZ	RBK	20191976	152,078	36.6	82,272	26,334	17,138
Tulipa agenensis	-	Kew27	ISR	RBK	19922451	152,281	36.6	82,338	26,383	17,177
Tulipa alberti	-	Kew28	KAZ	RBK	2017391	152,374	36.6	82,377	26,372	17,253
Tulipa altaica	-	Kew29	KAZ	RBK	2017288	151,633	36.7	81,698	26,352	17,231
Tulipa cinnabarina	-	Kew31	TUR	RBK	20093202*1	151,892	36.6	82,183	26,386	17,369
Tulipa cretica	-	Kew32	GRC	RBK	19782398	152,028	36.6	82,152	26,334	17,208
Tulipa systola	florenskyi	Kew33	ARM	RBK	20101890	152,675	36.5	82,620	26,385	17,285
Tulipa hoogiana	-	Kew34	IRN	RBK	196459315*1	152,313	36.6	82,346	26,371	17,225
Tulipa hoogiana	-	Kew35	IRN	RBK	19743463	152,309	36.6	82,341	26,371	17,226
Tulipa ingens	-	Kew36	TUR	RBK	19743419	152,329	36.6	82,344	26,371	17,243
Tulipa jacquesii	-	Kew37	KGZ	RBK	2017290	152,145	36.6	82,254	26,369	17,153
Tulipa kolpakowskiana	-	Kew38	KAZ	RBK	19888654*1	152,149	36.7	82,228	26,373	17,175
Tulipa undulatifolia	micheliana	Kew39	IRN	RBK	200675	152,338	36.6	82,395	26,373	17,197
Tulipa montana	-	Kew40	IRN	RBK	200685	151,583	36.7	81,594	26,391	17,207
Tulipa suaveolens	schrenkii	Kew42	KAZ	RBK	2009209	152,371	36.6	82,379	26,373	17,246
Tulipa suaveolens	schrenkii	Kew43	RUS	RBK	19891110	152,090	36.6	82,261	26,352	17,125
Tulipa sosnowskyi	-	Kew44	ARM	RBK	20101889	152,674	36.5	82,620	26,385	17,284
Tulipa systola	stapfi	Kew45	IRN	RBK	19891948	152,304	36.6	82,336	26,371	17,226
Tulipa sylvestris	australis talijevii	Kew46	KAZ	RBK	20131298	152,041	36.6	82,180	26,333	17,195
Tulipa ulophylla	-	Kew47	IRN	RBK	20162430	152,308	36.6	82,340	26,371	17,226
Tulipa uniflora	-	Kew48	RUS	RBK	20042147	152,120	36.6	82,266	26,355	17,144
Tulipa dasystemon	neustruevae	Kew49	UZB	RBK	19853764*1	152,162	36.6	82,264	26,343	17,212
Tulipa albanica	-	Herb1	ALB	C	C10014169	152,162	36.6	82,252	26,350	17,210
Tulipa agenensis	-	Edi1	GO	RBE	20071838	152,391	36.6	82,353	26,370	17,298
Tulipa cretica	-	Edi2	GO	RBE	19973517	152,073	36.6	82,195	26,334	17,210
Tulipa gesneriana	-	Edi3	RUS	RBE	20141033	152,127	36.6	82,217	26,354	17,202
Tulipa lanata	-	Edi4	TJK	RBE	19890304	152,416	36.6	82,388	26,357	17,314
Tulipa praestans	-	Edi5	TJK	RBE	20151040	152,231	36.6	82,241	26,372	17,246
Tulipa heterophylla	-	Edi6	KGZ	RBE	20130643	152,134	36.6	82,276	26,336	17,186
Tulipa sprengeri	-	Edi7	GO	RBE	19871972	152,362	36.5	82,474	26,354	17,180
Tulipa praestans	-	Tjk1	TJK	Wild	BM1	152,216	36.6	82,283	26,368	17,197
Tulipa linifolia	-	Tjk2	TJK	Wild	BM3	151,477	36.7	81,575	26,350	17,202
Tulipa linifolia	-	Tjk3	TJK	Wild	BM5	151,477	36.7	81,575	26,350	17,202
Tulipa subquinquefolia	-	Tjk4	TJK	Wild	BM7	152,412	36.6	82,384	26,357	17,314
Tulipa anisophylla	-	Tjk5	TJK	Wild	BM9	152,094	36.6	82,202	26,357	17,178
Tulipa praestans	subpraestans	Tjk6	TJK	Wild	BM11	152,097	36.6	82,179	26,361	17,196
Tulipa vvedenskyi	-	Tjk7	TJK	Wild	BM13	152,552	36.6	82,537	26,379	17,257
Tulipa ingens	-	Tjk8	TJK	Wild	BM15	152,427	36.6	82,398	26,364	17,301

Tulipa praestans	-	Tjk9	TJK	Wild	BM17	152,166	36.6	82,223	26,367	17,209
Tulipa hissarica	-	Tjk10	TJK	Wild	BM19	152,450	36.6	82,463	26,359	17,269
Tulipa anisophylla	korshinskyi	Tjk11	TJK	Wild	BM21	152,090	36.6	82,207	26,357	17,169
Tulipa scardica	-	Herb2	MKD	B	B101068479	151,658	36.6	81,838	26,349	17,137
Tulipa albanica	-	Bal1	ALB	Wild	'00000158'	152,167	36.6	82,257	26,350	17,210
Tulipa albanica	-	Bal2	ALB	Wild	'00000157'	152,252	36.6	82,435	26,258	17,210
Tulipa albanica	-	Bal3	ALB	Wild	'00000156'	152,170	36.6	82,259	26,350	17,211
Tulipa kosovarica	-	Bal4	XKX	Wild	'00000155'	152,410	36.6	82,248	26,350	17,209
Tulipa kosovarica	-	Bal5	XKX	Wild	'00000154'	152,158	36.6	82,247	26,350	17,211
Tulipa kosovarica	-	Bal6	XKX	Wild	'00000153'	152,154	36.6	82,245	26,350	17,209
Tulipa kosovarica	-	Bal7	XKX	Wild	'00000152'	152,151	36.6	82,242	26,350	17,209
Sp. Nov.	-	Bal8	XKX	Wild	'00000150'	152,231	36.6	82,263	26,349	17,270
Tulipa luanica	-	Bal9	XKX	Wild	'00000149'	152,147	36.6	82,242	26,349	17,207
Tulipa luanica	-	Bal10	XKX	Wild	'00000146'	152,147	36.6	82,242	26,349	17,207
Tulipa luanica	-	Bal11	XKX	Wild	'00000148'	152,140	36.6	82,236	26,349	17,206
Tulipa luanica	-	Bal12	XKX	Wild	'00000145'	152,156	36.6	82,247	26,350	17,209
Tulipa scardica	-	Bal13	XKX	Wild	'00000167'	152,101	36.6	82,260	26,350	17,141
Tulipa scardica	-	Bal14	XKX	Wild	'00000166'	152,100	36.6	82,259	26,350	17,141
Tulipa serbica	-	Bal15	XKX	Wild	'00000164'	152,149	36.6	82,239	26,350	17,210
Tulipa serbica	-	Bal16	XKX	Wild	'00000163'	152,149	36.6	82,239	26,350	17,210
Tulipa sylvestris	Ssp. Sylvestris	Bal17	XKX	Wild	'00000161'	151,924	36.6	82,207	26,230	17,257
Tulipa sylvestris	Ssp. Australis	Bal18	XKX	Wild	'00000160'	151,887	36.6	82,235	26,197	17,258
Tulipa sylvestris	Ssp. Sylvestris	Bal19	XKX	Wild	'00000159'	151,879	36.6	82,228	26,197	17,257
Tulipa heterophylla	-	Kyr1	KGZ	Wild		151,833	36.7	81,822	26,419	17,173
Tulipa heterophylla	-	Kyr2	KGZ	Wild		151,852	36.7	81,836	26,418	17,180
Tulipa heterophylla	-	Kyr3	KGZ	Wild		151,836	36.7	81,852	26,404	17,176
Tulipa heterophylla	-	Kyr4	KGZ	Wild		151,867	36.7	81,832	26,429	17,177
Tulipa kolpakowskiana	-	Kyr5	KGZ	Wild		152,101	36.6	82,293	26,326	17,156
Tulipa talassica	-	Kyr6	KGZ	Wild		152,078	36.6	82,273	26,334	17,137
Tulipa jacquesii	-	Kyr7	KGZ	Wild		151,751	36.6	81,914	26,337	17,163
Tulipa koyuncui	-	Tur1	TUR	Wild	MF35497	152,040	36.6	82,194	26,333	17,180
Tulipa biflora	-	Tur2	TUR	Wild	MF35480	152,039	36.6	82,194	26,333	17,179
Tulipa biflora	-	Tur3	TUR	Wild	MF35494	152,047	36.6	82,201	26,333	17,180
Sp. Unknown	-	Tur4	TUR	Wild	MF35484	152,039	36.6	82,194	26,333	17,179
Tulipa koyuncui	-	Tur5	TUR	Wild	MF35488	152.049	36.6	82,202	26,333	17,181
Tulipa humilis	-	Tur6	TUR	Wild	MF35500	151,781	36.7	82,170	26,199	17,213
Tulipa biflora	-	Tur7	TUR	Wild	MF35481	152.039	36.6	82,194	26,333	17,179
Tulipa biflora	-	Tur8	TUR	Wild	MF35482	152,039	36.6	82,194	26,333	17,179
Sp. Unknown	-	Tur9	TUR	Wild	MF35485	152,041	36.6	82,195	26,333	17,180
Sp. Unknown	-	Tur10	TUR	Wild	MF35486	152,039	36.6	82,194	26,333	17,179
Tulipa koyuncui	-	Tur11	TUR	Wild	MF35489	152,049	36.6	82,202	26,333	17,181
Tulipa koyuncui	-	Tur12	TUR	Wild	MF35490	152,039	36.6	82,194	26,333	17,179
Tulipa lemmersii	-	Zonn3	KAZ	PC	L 0822655	152,100	36.6	82,299	26,334	17,133
Tulipa kolbintsevii	-	Zonn4	KAZ	PC	L 0821329	152,067	36.6	82,221	26,323	17,200
Tulipa zonneveldii	-	Zonn5	KGZ	PC	L 3971774	152,389	36.6	82,456	26,358	17,217
Tulipa jacquesii	-	Zonn6	KGZ	PC		151,737	36.6	81,898	26,337	17,165
Tulipa annae	-	Zonn7	KAZ	PC	L 3986814	151,718	36.6	81,799	26,354	17,211
Tulipa ivasczenkoae	-	Zonn8	KAZ	PC	G 014-1, adm nr. 4317ha	152,454	36.6	82,428	26,363	17,300
Tulipa regelii	-	Zonn9	KAZ	PC	G 03-1, adm. Nr 6007ea	152,114	36.6	82,202	26,337	17,238
Tulipa uzbekistanica	-	Zonn10	UZB	PC	G 012-9, adm. Nr 4347na	152,387	36.6	82,342	26,362	17,321
Tulipa heteropetala	-	Zonn11	KAZ	PC	G05alt5, adm. Nr 5107ce	152,361	36.6	82,405	26,359	17,238
Tulipa alberti	-	Zonn12	KAZ	PC	G 05-1, adm. Nr 4347ga	152,379	36.6	82,378	26,372	17,257

Tulipa alberti	-	Zonn13	KAZ	PC	G 05-18, adm. Nr 4347md	152,391	36.6	82,384	26,367	17,273
Tulipa dianaeverettiae	-	Zonn14	KAZ	PC	L 3986813	152,005	36.6	82,149	26,332	17,192
Tulipa heweri	-	Chr 1	GO	PC	M.J.M. Christenhusz no 9119	152,291	36.6	82,358	26,363	17,207
Tulipa julia	-	Herb4	TUR	E	EOO332503 2275*4	152,526	36.6	82,555	26,371	17,229
Tulipa kuschkensis	-	Herb5	IRN	E	EOO332529 2275*7	151,505	36.7	81,613	26,343	17,206
Tulipa lehmanniana	-	Herb6	AFG	E	EOO332532 2275*9	151,121	36.7	81,328	26,351	17,091
Tulipa gesneriana	-	Herb7	IRN	E	EOO329775 2275*11	152,428	36.6	82,387	26,393	17,255
Tulipa banuensis	-	Herb8	AFG	E	EOO329548 2275*22	152,284	36.6	82,356	26,363	17,202
Tulipa lehmanniana	-	Herb9	AFG	E	EOO332534 2275*10	151,139	36.7	81,336	26,353	17,097
Tulipa sosnowskyi	-	Herb10	ARM	E	EOO329791 2275*27	152,673	36.5	82,619	26,385	17,284
Tulipa banuensis	-	Herb11	AFG	E	EOO373862 2275*23	152,285	36.6	82,358	26,363	17,201
Tulipa harazensis	-	Herb14	IRN	E	EOO329590 2275*13	152,350	36.6	82,340	26,371	17,268
Tulipa hoogiana	-	Herb15	IRN	E	EOO329594 2275*14	152,283	36.6	82,315	26,371	17,226
Tulipa kuschkensis	-	Herb16	AFG	E	EOO332528 2275*8	152,419	36.6	82,412	26,357	17,293
Tulipa agenensis	-	Herb17	TUR	E	EOO329512 2275*16	152,200	36.6	82,256	26,351	17,242
Tulipa armena	-	Herb18	TUR	E	EOO329523 2275*21	152,437	36.6	82,503	26,373	17,188
Tulipa sosnowskyi	-	Herb19	ARM/AZE	E	EOO329790 2275*28	152,674	36.5	82,620	26,385	17,284
Tulipa armena	-	Herb20	TUR	E	EOO329519 2275*20	152,554	36.6	82,583	26,371	17,229
Tulipa julia		Got2	TUR	GBG	1990-2510	152,503	36.6	82,568	26,373	17,189
Tulipa suaveolens	schrenkii	Got3	RUS	GBG	2004-2595	152,144	36.6	82,305	26,353	17,133
Tulipa armena	-	Got4	TUR	GBG	2006-0170	152,323	36.6	82,429	26,343	17,208
Tulipa altaica	-	Got5	KAZ	GBG	2007-2169	151,560	36.7	81,647	26,298	17,317
Tulipa uniflora	-	Got6	RUS	GBG	2010-0908	152,297	36.5	82,349	26,356	17,236
Tulipa regelii	-	Got7	KAZ	GBG	2010-1981	152,105	36.6	82,195	26,337	17,236
Tulipa heteropetala	-	Got9	KAZ	GBG	2012-1506	152,423	36.6	82,469	26,359	17,236
Tulipa heteropetala	-	Got10	KAZ	GBG	2012-1510	152,272	36.6	82,316	26,359	17,238
Tulipa regelii	-	Got11	KAZ	GBG	2013-0505	152,117	36.6	82,206	26,337	17,237
Tulipa uzbekistanica	-	Uzb1	UZB	Wild	DD01042021002	152,359	36.6	82,334	26,362	17,301
Tulipa x tschimganica	-	Uzb2	UZB	Wild	DE12052021007	152,166	36.6	82,247	26,372	17,175
Tulipa orithyioides	-	Uzb3	UZB	Wild	OA11062021003	152,067	36.6	82,231	26,332	17,172
Tulipa greigii	-	Uzb4	UZB	Wild	DZD30042021006	152,225	36.6	82,239	26,371	17,244
Tulipa vvedenskyi	-	Uzb5	UZB	Wild	DD02042021001-2	152,198	36.6	82,210	26,372	17,244
Tulipa carinata	-	Uzb6	UZB	Wild	OO31032021023-1	152,453	36.6	82,461	26,363	17,266
Tulipa fosteriana	-	Uzb7	UZB	Wild	UK16042021005	152,457	36.6	82,432	26,363	17,299
Tulipa korolkowii	-	Uzb8	UZB	Wild	DD01042021004-1	152,071	36.6	82,250	26,339	17,143
Tulipa lanata	-	Uzb9	UZB	Wild	DD525032021022	152,318	36.6	82,374	26,357	17,230
Tulipa ingens	-	Uzb10	UZB	Wild	DD31032021027-1	152,465	36.6	82,468	26,363	17,271
Tulipa biflora	-	Uzb11	UZB	Wild	53-1	151,982	36.6	82,144	26,334	17,170
Tulipa kaufmanniana	-	Uzb12	UZB	Wild	DZD30042021004	152,167	36.6	82,135	26,373	17,286
Tulipa dubia	-	Uzb13	UZB	Wild	1606202103	152,290	36.6	82,305	26,371	17,243
Tulipa turkestanica	-	Uzb14	UZB	Wild	220320204	152,027	36.6	82,186	26,330	17,181

Tulipa lehmanniana	-	Uzb15	UZB	Wild	DD15042021011	151,169	36.7	81,346	26,354	17,115
Tulipa talassica	-	Uzb16	UZB	Wild	DD19042021002	152,067	36.6	82,246	26,339	17,143
Tulipa scharipovii	-	Uzb17	UZB	Wild	20032020044	151,989	36.7	82,110	26,362	17,155
Tulipa intermedia	-	Uzb18	UZB	Wild	2003202047-5	152,141	36.6	82,275	26,339	17,188
Tulipa ferganica	-	Uzb19	UZB	Wild	220320202-2	152,123	36.7	82,202	26,372	17,177
Tulipa butkovii	-	Uzb20	UZB	Wild	DZD30042021001	152,167	36.6	82,135	26,373	17,286
Tulipa bifloriformis	-	Uzb21	UZB	Wild	422003	152,038	36.6	82,171	26,332	17,203
Tulipa cinnabarina	-	Got1	TUR	GBG	1990-1696	151,112	36.6	82,232	26,333	17,214
Tulipa kolbintsevii	-	Got8	KAZ	GBG	2012-1501	152,060	36.7	82,216	26,323	17,198
Tulipa lanata	-	Tjk12	TJK	Wild	BM23	152,420	36.6	82,417	26,357	17,289
Tulipa korolkowii	nitida	Tjk13	TJK	Wild	BM25	152,002	36.6	82,183	26,339	17,141
Tulipa linifolia	maximowiczii	Tjk14	TJK	Wild	BM27	152,020	36.6	82,099	26,373	17,175
Tulipa korolkowii	rosea	Tjk15	TJK	Wild	BM29	152,061	36.6	82,225	26,339	17,158
Tulipa platystemon	-	Kyr8	KGZ	Wild		152,118	36.6	82,309	26,326	17,157
Tulipa dubia	-	Kyr9	KGZ	Wild		152,281	36.6	82,370	26,365	17,181
Tulipa korolkowii	-	Kyr10	KGZ	Wild		152,137	36.6	82,276	26,339	17,183
Tulipa tetraphylla	brachystemon	Kyr11	KGZ	Wild		152,101	36.6	82,292	26,326	17,157
Tulipa lehmanniana	zenaidae	Kyr12	KGZ	Wild		151,974	36.7	82,165	26,326	17,157
Tulipa talassica	-	Kyr13	KGZ	Wild		152,076	36.6	82,270	26,334	17,138
Tulipa talassica	-	Kyr14	KGZ	Wild		152,239	36.6	82,376	26,343	17,177
Sp. Unknown	-	Kyr15	KGZ	Wild		152,001	36.6	82,161	26,331	17,178
Tulipa dasystemon	-	Kyr16	KGZ	Wild		152,048	36.6	82,191	26,336	17,185
Tulipa dasystemon	dasystemonoides	Kyr17	KGZ	Wild		152,068	36.6	82,158	26,346	17,218
Sp. Unknown	-	Kyr18	KGZ	Wild		152,045	36.6	82,175	26,331	17,208

Table 2.10. All genes annotated in the *Tulipa* chloroplast assemblies. The first column lists the groups of different types of gene present in the plastome and the right column details the individual genes present in the assembled plastomes with relation to which group they fall in.

Type of gene	**Name of gene**
Ribosomal RNAs	*rrn16* (×2), *rrn23* (×2), *rrn4.5* (×2), *rrn5* (×2)
Transfer RNAs	*trnA-UGC** (×2), *trnC-GCA*, *trnD-GUC, trnE-UUC, trnF-GAA, trnfM-CAU, trnG-GCC**, *trnG-UCC, trnH-GUG* (×2), *trnI-CAU* (×2), *trnI-GAU** (×2), *trnK-UUU**, *trnL-CAA* (×2), *trnL-UAA**, *trnL-UAG, trnM-CAU, trnN-GUU* (×2), *trnP-UGG, trnQ-UUG, trnR-ACG* (×2), *trnR-UCU, trnS-GCU, trnS-GGA, trnS-UGA, trnT-GGU, trnT-UGU, trnV-GAC* (×2), *trnV-UAC*, trnW-CCA, trnY-GUA*
Photosystem I	*psaA, psaB, psaC, psaI, psaJ*
Photosystem II	*psbA, psbB, psbC, psbD, psbE, psbF, psbH, psbI, psbJ, psbK, psbL, psbM, psbN, psbT, psbZ*
Cytochrome	*petA, petB*, petD*, petG, petL, petN*
ATP synthase	*atpA, atpB, atpE, atpF*, atpH, atpI*
Rubisco	*rbcL*
NADH dehydrogenase	*ndhA*, ndhB** (×2), *ndhC, ndhD, ndhE, ndhF, ndhG, ndhH, ndhI, ndhJ, ndhK*
ATP-dependent protease subunit P	clpP†
Chloroplast envelope membrane protein	*cemA*
Large units	*rpl2** (×2), *rpl14, rpl16*, rpl20, rpl22, rpl23* (×2), *rpl32, rpl33, rpl36*
Small units	*rps2, rps3, rps4, rps7* (×2), *rps8, rps11, rps12˜* (×2), *rps14, rps15, rps16*, rps18, rps19* (x2 - one copy is #)
RNA polymerase	*rpoA, rpoB, rpoC1*, rpoC2*
Miscellaneous proteins	*matK, accD, ccsA*
Hypothetical proteins & Conserved reading frame	*ycf1* (x2 - one copy is #), *ycf2* (×2), *ycf3†, ycf4, ycf15#* (×2), *ycf68#* (×2)

* A gene containing a single intron
† A gene containing two introns
# Pseudogenes (genes with internal stop codons and unknown functions)
˜ Truncated gene
(×2) A gene duplicated in the IR regions

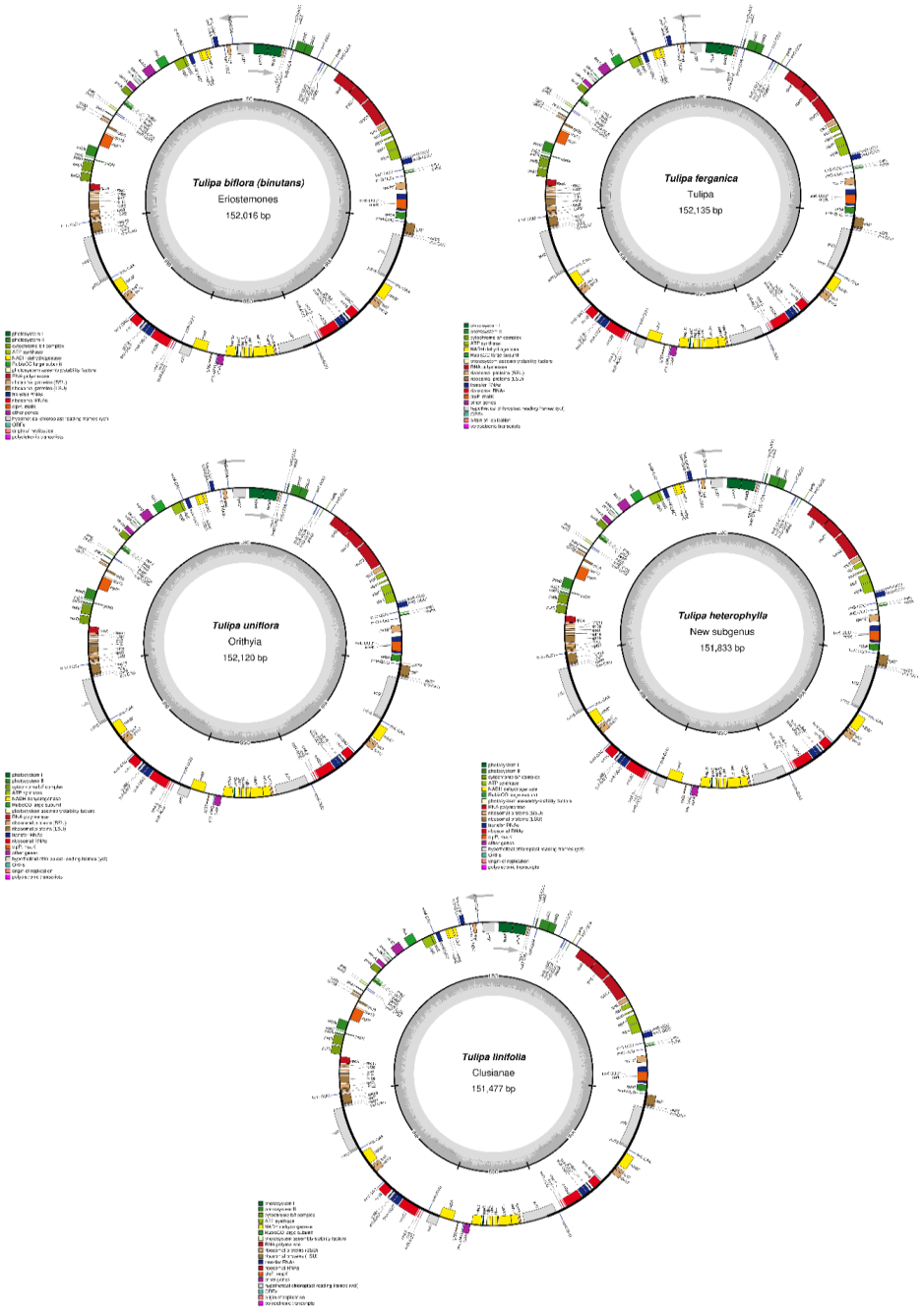


Figure 2.1. Plastome gene maps for five tulips, one from each recognised subgenus. Genes on the outside are transcribed anti-clockwise and genes on the inside are transcribed clockwise. Different gene groups are colour coded and the darker grey regions on the inner graph show the GC content. The species and subgenus of each plastome is detailed in the centre of the map.

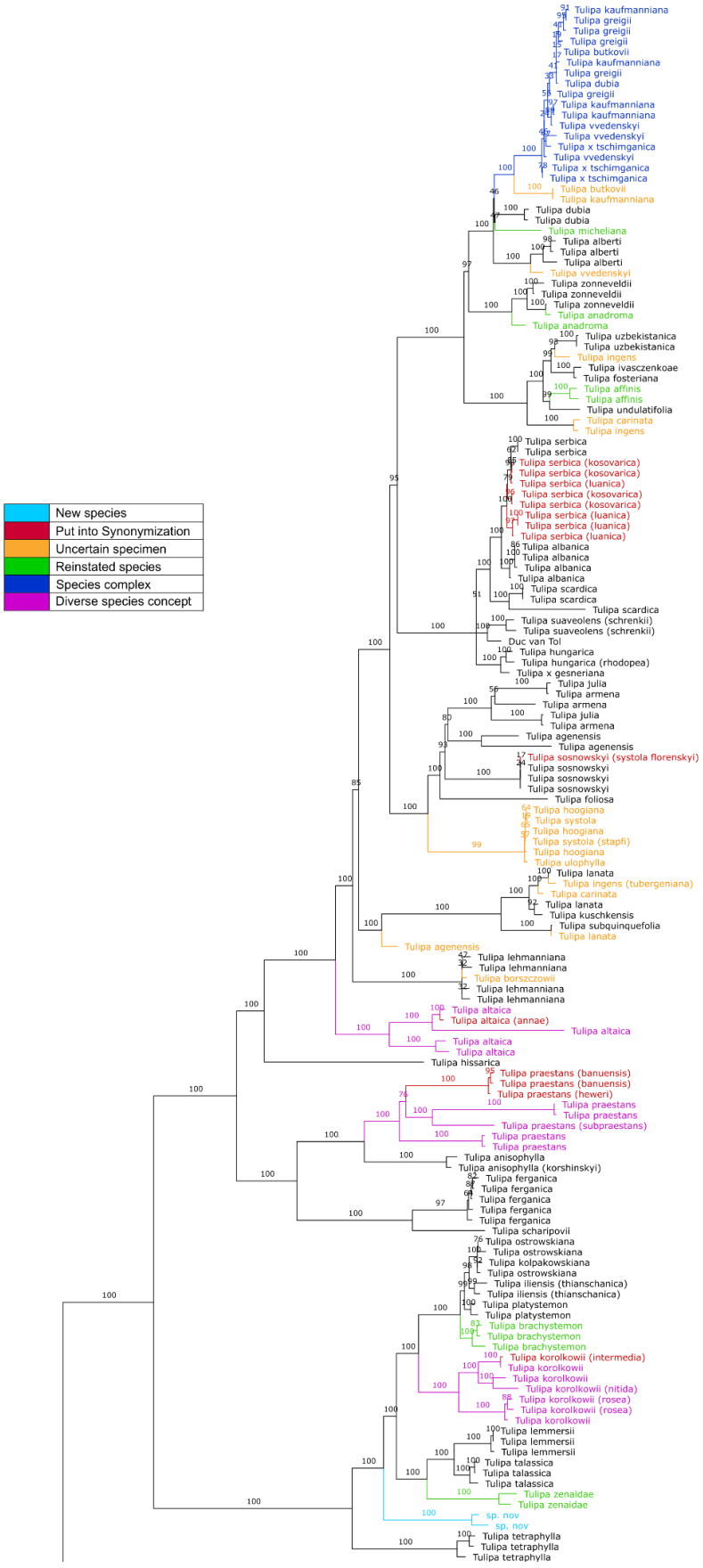
2.3.2. Support for phylogenies

Plastome

The phylogeny inferred using plastome sequence data in a single partition had the highest support values, as expected. Nonetheless the multi-partitioned trees all showed extremely similar tree topologies to both the single partition maximum likelihood and Bayesian inferred trees (Appendix 2; Appendix 3) as well as to each other (Figure 2.2; Appendix 4), with only marginal tip movement in areas where support values were generally low across all trees. Specifically, the differences between the phylogenies occurred in species complexes where it seems that species are closely related and the plastome dataset lacked resolution. The phylogenies inferred with fewer genetic markers showed lower bootstrap support values, nevertheless the topology remained similar showing that phylogenetic signal is consistent across multiple datasets. Overall, the phylogenetic trees structure and resolution enabled the identification of subgenera, sections, and a large range of species. We selected to use the multi-partitioned plastome dataset (Figure 2.2) for most inferences as this phylogeny showed high levels of resolution with stronger bootstrap support for nodes than trees inferred with fewer markers, whilst its underlying model more likely reflects the true evolutionary process than the single partition dataset. This plastome based phylogeny provided resolution to investigate subgenera, sections, and a range of species relationships, although there were limitations in resolution in parts of the tree.

35S rDNA

The phylogeny inferred from the single partition 35S rDNA data generally had higher bootstrap support than the phylogeny inferred using the multi-partition model, whilst the phylogeny inferred using the shorter barcoding dataset was the least resolved. Again, we selected to use the phylogeny inferred using the multi-partition model (Figure 2.3) when making inferences from this nuclear dataset as it better captures the variability of substitution rates across different markers, whilst also having good resolution. This phylogeny was able to resolve subgenera, but was of limited use for assessing sections, although some were somewhat resolved. Notably, the 35S rDNA inferred phylogeny lacked the resolution to resolve many species relationships with known closely related species especially inscrutable. The ITS barcode dataset could resolve subgenera, but lacked broad structure with limited resolution below this taxonomic level.


New species
Put into Synonymization
Uncertain specimen
Reinstated species
Species complex
Diverse species concept
Tulipa kaufmanniana
Tulipa greigii
Tulipa greigii
Tulipa greigii
Tulipa butkovii
Tulipa kaufmanniana
Tulipa greigii
Tulipa dubia
Tulipa greigii
Tulipa kaufmanniana
Tulipa kaufmanniana
Tulipa vvedenskyi
Tulipa vvedenskyi
Tulipa x tschimganica
Tulipa vvedenskyi
Tulipa x tschimganica
Tulipa x tschimganica
Tulipa butkovii
Tulipa kaufmanniana
Tulipa dubia
Tulipa dubia
Tulipa micheliana
Tulipa alberti
Tulipa alberti
Tulipa alberti
Tulipa vvedenskyi
Tulipa zonneveldii
Tulipa zonneveldii
Tulipa zonneveldii
Tulipa anadroma
Tulipa anadroma
Tulipa uzbekistanica
Tulipa uzbekistanica
Tulipa ingens
Tulipa ivasczenkoae
Tulipa fosteriana
Tulipa affinis
Tulipa affinis
Tulipa undulatifolia
Tulipa carinata
Tulipa ingens
Tulipa serbica
Tulipa serbica
Tulipa serbica (kosovarica)
Tulipa serbica (kosovarica)
Tulipa serbica (luanica)
Tulipa serbica (kosovarica)
Tulipa serbica (kosovarica)
Tulipa serbica (luanica)
Tulipa serbica (luanica)
Tulipa serbica (luanica)
Tulipa albanica
Tulipa albanica
Tulipa albanica
Tulipa albanica
Tulipa scardica
Tulipa scardica
Tulipa scardica
Tulipa suaveolens (schrenkii)
Tulipa suaveolens (schrenkii)
Duc van Tol
Tulipa hungarica
Tulipa hungarica (rhodopea)
Tulipa x gesneriana
Tulipa julia
Tulipa armena
Tulipa armena
Tulipa julia
Tulipa armena
Tulipa agenensis
Tulipa agenensis
Tulipa sosnowskyi (systola florenskyi)
Tulipa sosnowskyi
Tulipa sosnowskyi
Tulipa sosnowskyi
Tulipa foliosa
Tulipa hoogiana
Tulipa systola
Tulipa hoogiana
Tulipa systola (stapfi)
Tulipa hoogiana
Tulipa ulophylla
Tulipa lanata
Tulipa ingens (tubergeniana)
Tulipa carinata
Tulipa lanata
Tulipa kuschkensis
Tulipa subquinquefolia
Tulipa lanata
Tulipa agenensis
Tulipa lehmanniana
Tulipa lehmanniana
Tulipa borszczowii
Tulipa lehmanniana
Tulipa lehmanniana
Tulipa altaica
Tulipa altaica (annae)
Tulipa altaica
Tulipa altaica
Tulipa altaica
Tulipa hissarica
Tulipa praestans (banuensis)
Tulipa praestans (banuensis)
Tulipa praestans (hewer)
Tulipa praestans
Tulipa praestans
Tulipa praestans (subpraestans)
Tulipa praestans
Tulipa praestans
Tulipa anisophylla
Tulipa anisophylla (korshinskyi)
Tulipa ferganica
Tulipa ferganica
Tulipa ferganica
Tulipa ferganica
Tulipa ferganica
Tulipa scharipovii
Tulipa ostrowskiana
Tulipa ostrowskiana
Tulipa kolpakowskiana
Tulipa ostrowskiana
Tulipa iliensis (thianschanica)
Tulipa iliensis (thianschanica)
Tulipa platystemon
Tulipa platystemon
Tulipa brachystemon
Tulipa brachystemon
Tulipa brachystemon
Tulipa korolkowii (intermedia)
Tulipa korolkowii
Tulipa korolkowii
Tulipa korolkowii (nitida)
Tulipa korolkowii (rosea)
Tulipa korolkowii (rosea)
Tulipa korolkowii
Tulipa lemmersii
Tulipa lemmersii
Tulipa lemmersii
Tulipa talassica
Tulipa talassica
Tulipa talassica
Tulipa zenaidae
Tulipa zenaidae
sp. nov
sp. nov
Tulipa tetraphylla
Tulipa tetraphylla
Tulipa tetraphylla

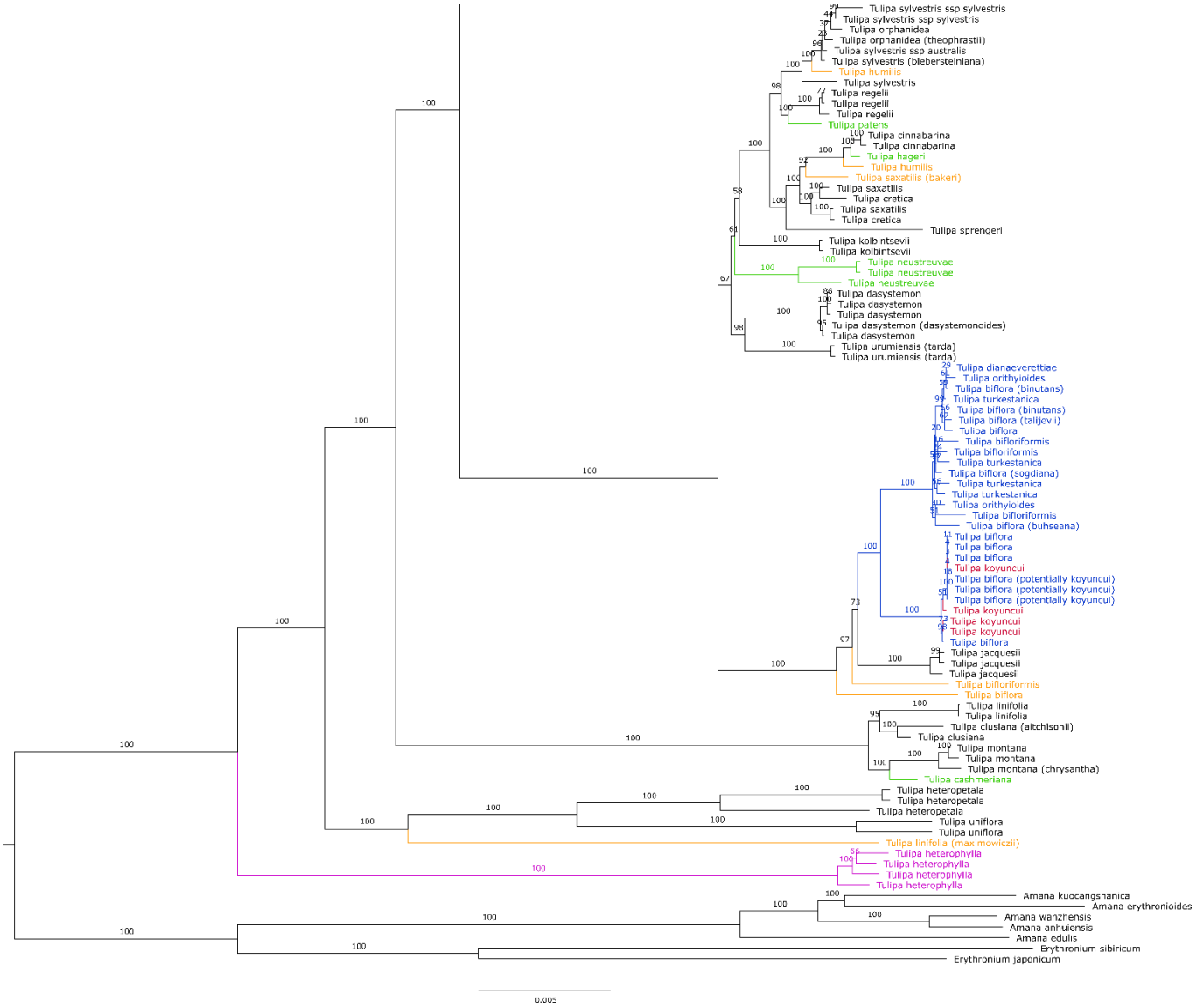
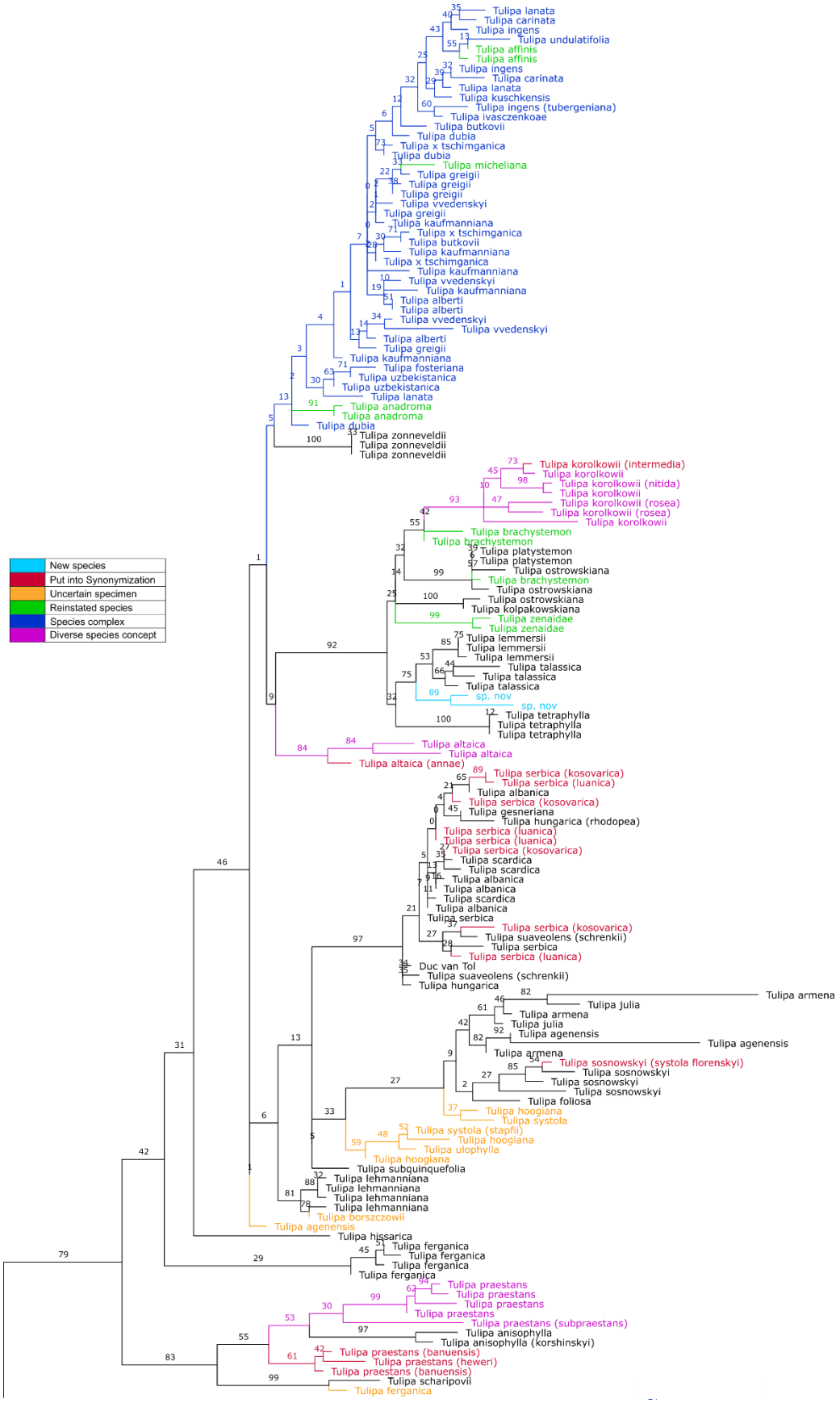


Figure 2.2. Phylogeny inferred using the partitioned plastome dataset through a maximum likelihood approach. Tips which were believed to be errors were pruned and the tree was rooted using *Amana* and *Erythronium* specimens. Non-parametric bootstrap values are shown for branches. Tips are colour coded based on certain taxonomic processes or decisions linked to these specimens.


New species
Put into Synonymization
Uncertain specimen
Reinstated species
Species complex
Diverse species concept
Tulipa lanata
Tulipa carinata
Tulipa ingens
Tulipa undulatifolia
Tulipa affinis
Tulipa affinis
Tulipa ingens
Tulipa carinata
Tulipa lanata
Tulipa kuschkensis
Tulipa ingens (tubergeniana)
Tulipa ivasczenkoae
Tulipa butkovii
Tulipa dubia
Tulipa x tschimganica
Tulipa dubia
Tulipa micheliana
Tulipa greigii
Tulipa greigii
Tulipa greigii
Tulipa vvedenskyi
Tulipa greigii
Tulipa kaufmanniana
Tulipa x tschimganica
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Tulipa kaufmanniana
Tulipa vvedenskyi
Tulipa kaufmanniana
Tulipa alberti
Tulipa alberti
Tulipa vvedenskyi
Tulipa vvedenskyi
Tulipa alberti
Tulipa greigii
Tulipa kaufmanniana
Tulipa fosteriana
Tulipa uzbekistanica
Tulipa uzbekistanica
Tulipa lanata
Tulipa anadroma
Tulipa anadroma
Tulipa dubia
Tulipa zonneveldii
Tulipa zonneveldii
Tulipa zonneveldii
Tulipa korolkowii (intermedia)
Tulipa korolkowii
Tulipa korolkowii (nitida)
Tulipa korolkowii
Tulipa korolkowii (rosea)
Tulipa korolkowii (rosea)
Tulipa korolkowii
Tulipa brachystemon
Tulipa brachystemon
Tulipa platystemon
Tulipa platystemon
Tulipa ostrowskiana
Tulipa brachystemon
Tulipa ostrowskiana
Tulipa ostrowskiana
Tulipa kolpakowskiana
Tulipa zenaidae
Tulipa zenaidae
Tulipa lemmersii
Tulipa lemmersii
Tulipa lemmersii
Tulipa talassica
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Tulipa talassica
sp. nov
sp. nov
Tulipa tetraphylla
Tulipa tetraphylla
Tulipa tetraphylla
Tulipa altaica
Tulipa altaica
Tulipa altaica (annae)
Tulipa serbica (kosovarica)
Tulipa serbica (luanica)
Tulipa albanica
Tulipa serbica (kosovarica)
Tulipa gesneriana
Tulipa hungarica (rhodopea)
Tulipa serbica (luanica)
Tulipa serbica (luanica)
Tulipa serbica (kosovarica)
Tulipa scardica
Tulipa scardica
Tulipa albanica
Tulipa albanica
Tulipa scardica
Tulipa albanica
Tulipa serbica
Tulipa serbica (kosovarica)
Tulipa suaveolens (schrenkii)
Tulipa serbica
Tulipa serbica (luanica)
Duc van Tol
Tulipa suaveolens (schrenkii)
Tulipa hungarica
Tulipa armena
Tulipa julia
Tulipa armena
Tulipa julia
Tulipa agenensis
Tulipa agenensis
Tulipa armena
Tulipa sosnowskyi (systola florenskyi)
Tulipa sosnowskyi
Tulipa sosnowskyi
Tulipa sosnowskyi
Tulipa foliosa
Tulipa hoogiana
Tulipa systola
Tulipa systola (stapfii)
Tulipa hoogiana
Tulipa ulophylla
Tulipa hoogiana
Tulipa subquinquefolia
Tulipa lehmanniana
Tulipa lehmanniana
Tulipa lehmanniana
Tulipa lehmanniana
Tulipa borszczowii
Tulipa agenensis
Tulipa hissarica
Tulipa ferganica
Tulipa ferganica
Tulipa ferganica
Tulipa ferganica
Tulipa praestans
Tulipa praestans
Tulipa praestans
Tulipa praestans
Tulipa praestans (subpraestans)
Tulipa anisophylla
Tulipa anisophylla (korshinskyi)
Tulipa praestans (banuensis)
Tulipa praestans (heweri)
Tulipa praestans (banuensis)
Tulipa scharipovii
Tulipa ferganica

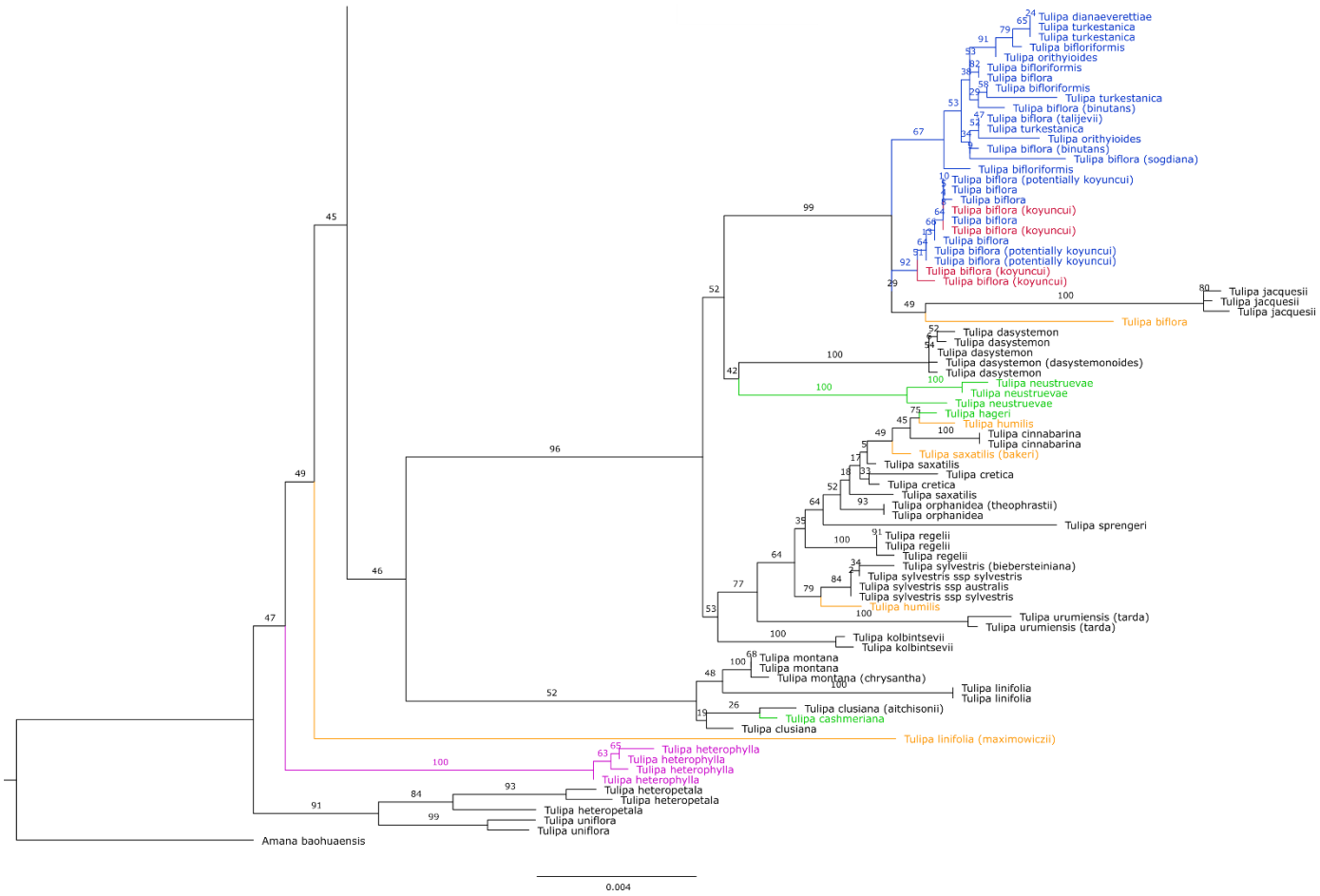


Figure 2.3. Phylogeny inferred using the partitioned 35S rDNA dataset through a maximum likelihood approach. Tips which were believed to be errors were pruned and the tree was rooted using a single specimen from *Amana*. Non-parametric bootstrap values are shown for branches. Tips are colour coded based on certain taxonomic processes or decisions linked to these specimens.

Cytonuclear discordance

There were several structural conflicts when the topology of the 35S rDNA phylogeny was compared to the plastome phylogeny. There was notably discord between the evolutionary relationships of the subgenera between the phylogenies inferred from these two datasets (Figure 2.4). In all phylogenies inferred from plastome data the earliest diverging subgenus is newly recognised *Eduardoregelia* (see 2.3.3) with the second diverging subgenus *Orithyia*. These positions are supported by bootstrap values of 100. In the 35S rDNA tree the *Orithyia* subgenus is the earliest diverging clade with *Eduardoregelia* the second diverging branch. The positioning of these branches has low bootstrap support of only 47. The reason for this conflict at deep internal nodes is unknown, but may have some underlying biological cause or could perhaps be due to the differing outgroups used to root the phylogenies. Given the larger amount of sequence data used to infer the plastome based phylogeny this tree is theoretically more likely to provide an accurate estimate of the unknown true phylogeny and therefore the phylogenetic position of this new subgenus. Nonetheless further nuclear markers will be needed to confirm the order of these basal subgenera.

Further conflict between tree topologies inferred from nuclear data and plastome data is observed between the relationship of the other three subgenera. The plastome based phylogeny supports the closer relationship of *Eriostemones* and *Tulipa* with the *Clusianae* subgenus sister to these. This ordering has bootstrap values of 100 at all relevant nodes. The 35S rDNA inferred topology has *Clusianae* positioned more closely to *Eriostemones*, with the *Tulipa* subgenus sister to these. This ordering is weakly supported with a bootstrap value of only 45 at the node where the *Tulipa* subgenus diverges from these other two. Given the much lower bootstrap support of the 35S rDNA inferred phylogeny and the significantly larger sequence dataset used to infer the plastome phylogeny it seems more probable that *Tulipa* and *Eriostemones* are sister with *Clusianae* an earlier diverging branch. This is further supported by the low bootstrap value for the monophyletic nature of *Clusianae* of only 52, in the 35S rDNA inferred phylogeny, highlighting the limited divergence of this nuclear region between subgenera.

In general, there were very few tips that changed position across the phylogenies and on closer inspection of the sequences it seemed likely that the tips that moved were primarily due to a few variant sites in the nuclear sequences (Figure 2.5). These variant sites often only led to topological changes within a relatively poorly supported clade, and it is likely that sequencing

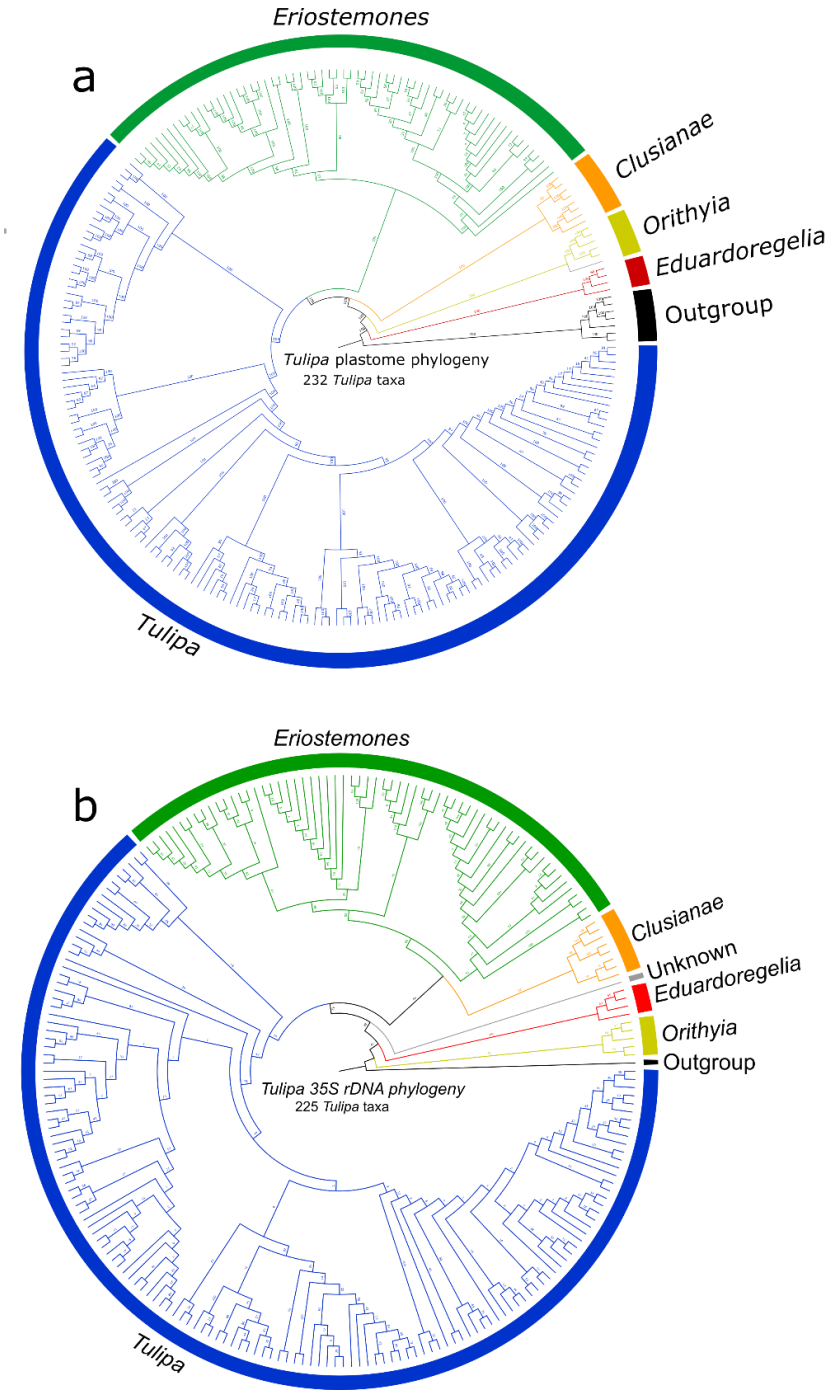


Figure 2.4. Subgenera of the genus *Tulipa*. (a) Plastome inferred phylogeny with subgenera labelled for the genus *Tulipa.* The tree was rooted using both *Amana* and *Erythronium* specimens with non-parametric bootstrap values are shown along the branches. (b) 35S rDNA inferred phylogeny with subgenera labelled for the genus *Tulipa.* Rooted using *Amana* with non-parametric bootstrap values shown along the branches.

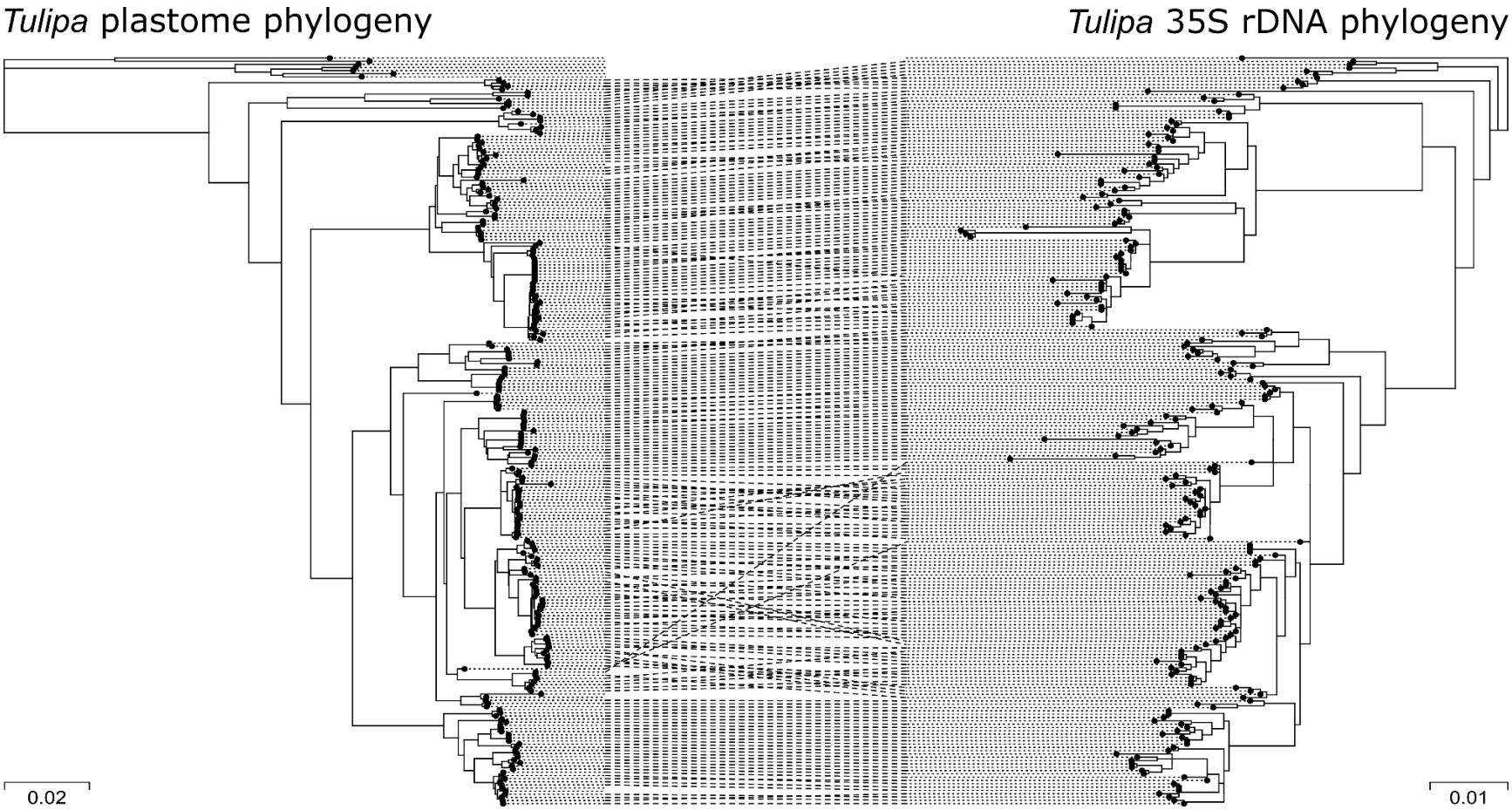


Figure 2.5. A comparison of plastome (left) and 35S rDNA (right) phylogeny structures. The position of the same specimens on each tree are joined by dotted lines.

errors rather than evolutionarily significant changes could be causing the conflicts. The 35S rDNA region is approximately 4% the length of the plastome and therefore there is considerably less sequence data available for resolving taxonomic relationships. Even so, the majority of tips did not alter in position between the two trees suggesting that hybridisation and chloroplast capture may not be commonly occurring processes in this genus supporting the plastome as an informative sequence region.

Nonetheless there were several instances where there were signs of potential hybridisation or chloroplast capture. In the 35S rDNA tree both the species *Tulipa zonneveldii* and *T. anadroma* were inferred to be monophyletic with strong bootstrap support. Yet, in the plastome phylogeny these are intertwined and polyphyletic. These species occur in a small area of Kyrgyzstan, primarily in the Sary-Chelek Biosphere Reserve and whilst these species are morphologically distinguishable, their populations are geographically close enough to interact. In addition, the nuclear data inferred the monophyly of both *T. orphanidea* and *T. sylvestris*, which the plastome data did not, again implying there may be chloroplast capture or hybridisation occurring between these closely related species that do overlap biogeographically. Moreover, *T. scharipovii* was inferred sister to multiple *T. ferganica* specimens in the plastome tree, whilst in the 35S rDNA tree it was linked to a single specimen collected in Sary-Chelek Biosphere Reserve believed to be *T. ferganica,* but with only two leaves. This *T. ferganica* specimen fell separately from the other *T. ferganica* specimens in the 35S rDNA phylogeny, potentially showing some complex biological process may be impacting these taxa, although their currently accepted ranges are not known to overlap.

2.3.3. Taxonomy

Tulipa is monophyletic and sister to a clade containing both Amana and Erythronium

Our plastome phylogeny confirms the monophyletic status of the genus *Tulipa* (Christenhusz *et al.*, 2013) and shows that the *Amana* and *Erythronium* genera are more closely related to each other than either is to *Tulipa* (Figure 2.6). This means that a clade containing both *Amana* and *Erythronium* is sister to *Tulipa*. The genus *Gagea* is the most distantly related genus to *Tulipa* in the Tulipeae tribe as expected. Importantly the genetic variation within the genus *Tulipa*, and between *Tulipa, Amana*, and *Erythronium*, is relatively small compared to the genetic distance between *Gagea* and any of the other genera. This shows that the species, subgenera, and genera relationships within the *Amana-Erythronium-Tulipa* clade are based on relatively limited genetic variation and that these clades are evolutionarily closely related and could have been

treated as a single genus if it were not for the wide usage of their names in horticulture (Christenhusz *et al.*, 2013).

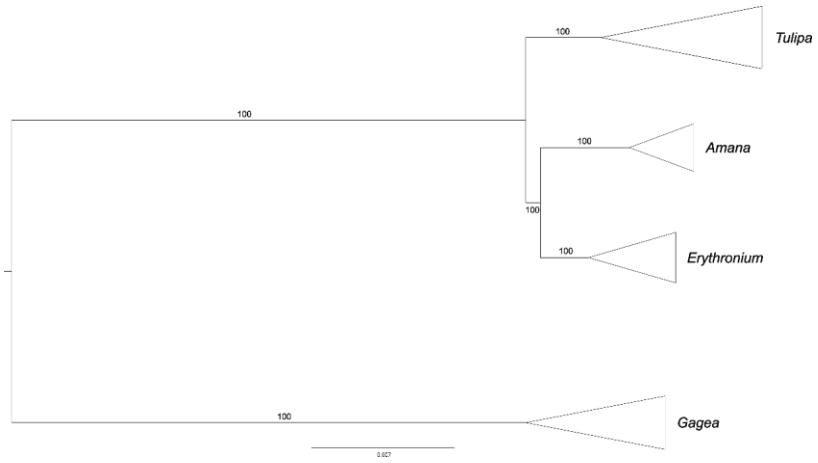


Figure 2.6. Genus level tree of the tribe Tulipeae inferred using plastome data. Triangles show that multiple tips are present in each group, but are not scaled to the number of tips. The tree is rooted using *Gagea* with non-parametric bootstrap values shown for the branches.

A new subgenus within Tulipa: Eduardoregelia

Previously the genus *Tulipa* has been split into four subgenera based on a phylogeny inferred from five plastid markers (Fay *et al.*, 2001) and genome size measurements (Zonneveld, 2009; Veldkamp and Zonneveld, 2012) and later supported by more comprehensive molecular data (Christenhusz *et al.*, 2013). Both our plastome and 35S rDNA confirm the existence of these four subgenera, but also indicate that a fifth subgenus should now be recognised (Figure 2.4). The new subgenus contains only one species, *Tulipa heterophylla,* which was previously included in the *Orithyia* subgenus alongside *T. uniflora*, *T. heteropetala*, and *T. sinkiangensis.* The *Orithyia* subgenus should therefore now only be considered to contain these three species. *T. heterophylla* was originally placed in *Orithyia* as it has a style on top of the ovary, produces only two leaves, and has a yellow flower, which are characteristic of this grouping (Fay and Christenhusz, 2013). However, *T. heterophylla* has a unique drooping flower which is a unique morphological trait of this now genetically supported subgenus. This new subgenus should be called *Eduardoregelia* as it contains only a single species which was originally described as *Eduardoregelia heterophylla* (Regel) Popov and so this name ensures taxonomic consistency.

Sections are convoluted and need to be simplified

When this analysis was undertaken there were 12 accepted sections in the genus. Each subgenus consisting of at least one section, with the *Eriostemones* subgenus separated into three sections: *Biflores, Saxatiles,* and *Sylvestres,* and the *Tulipa* subgenus separated into seven sections: *Kolpakowskianae, Multiflorae, Lanatae, Vinistriatae, Spiranthera, Tulipanum*, and *Tulipa* (Zonneveld, 2009). The plastome inferred phylogeny showed that only *Clusianae* and *Multiflorae* are monophyletic (Figure 2.7) supporting previous research suggesting most sections are not taxonomically informative (Christenhusz *et al.*, 2013). The *Orithyia* section is non-monophyletic with *Tulipa heterophylla* specimens forming a unique clade. As previously stated, the positioning of this unique clade leads to the recognition of a new subgenus and corresponding section *Eduardoregelia*. In the subgenus *Eriostemones* the three recognised sections of *Biflores, Sylvestres*, and *Saxatiles* have some topological support, however the positioning of a few species leads to all these sections not being truly monophyletic. The sections of the subgenus *Tulipa* are distinguishable based on genome size (Zonneveld, 2009), yet only the *Multiflorae* section can be resolved based on sequence data and even this is itself embedded within the *Kolpakowskianae* section.

In the *Eriostemones* subgenus the sections *Saxatiles* and *Sylvestres* are monophyletic when the suggested movement of several taxa between groups is undertaken. There is clear evidence that *Tulipa regelii* should be recognised as part of the *Sylvestres* section rather than the *Biflores* section. The reinstatement of *T. hageri* as an accepted species leads to its treatment as part of the *Saxatiles* section, with its previously parental species, *T. orphanidea*, remaining part of the *Sylvestres* section. There is some conflict in the positioning of two specimens of *T. humilis* within the phylogeny. These specimens are polyphyletic with one specimen falling within the *Sylvestres* section and the other in the *Saxatiles* section. There is no clear evidence to refute the reliability of either of these specimens and so the true sectional position of *T. humilis* cannot be assessed and it is hence left in the *Saxatiles* section. The section *Biflores* is not monophyletic across most of our phylogenies with the species *T. kolbintsevii*, *T. neustruevae*, *T. urumiensis*, and *T. dasystemon* falling unexpectedly on early diverging branches of a broader clade that contains the *Saxatiles* and *Sylvestres* sections, although with weak bootstrap support. Nonetheless, on the single partition plastome phylogeny both inferred using maximum likelihood and Bayesian approaches these species are basal in the *Biflores* group as expected, albeit again with weak bootstrap support in the maximum likelihood approach. We suggest they should remain in the *Biflores* group, until further, more comprehensive work can be done. Overall, we therefore retain all three sections of this subgenus.

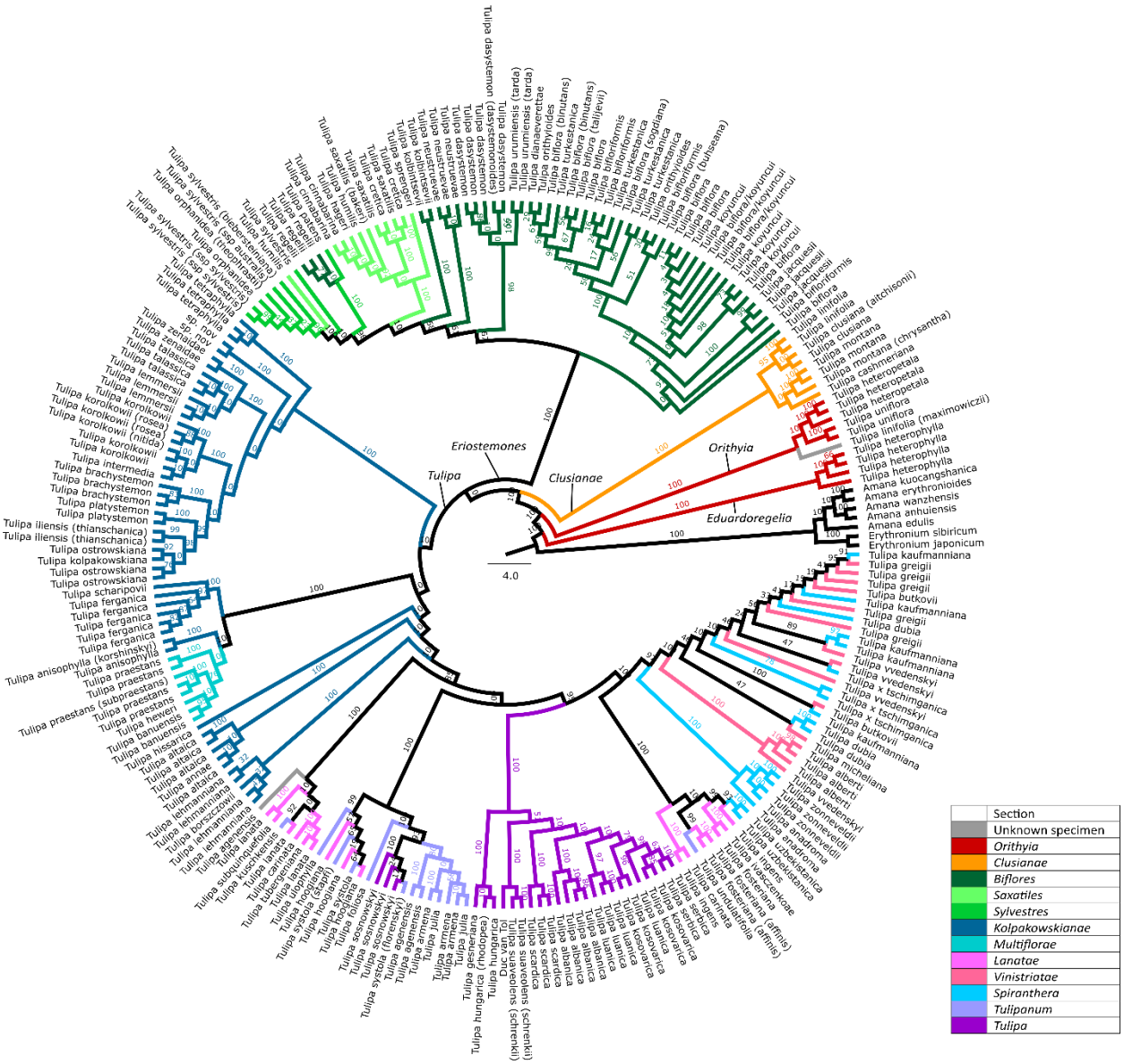


Figure 2.7. Plastome based phylogeny of the genus *Tulipa* colour coded by section. The 12 sections designated in Zonneveld (2009) are labelled: *Orithyia, Clusianae*, and within *Eriostemones; Biflores*, *Saxatiles*, and *Sylvestres*, and within *Tulipa; Kolpakowskianae, Multiflorae, Lanatae, Vinistriatae, Spiranthera, Tulipanum*, and *Tulipa*. The five subgenera are labelled at the base of the corresponding branches. Tree rooted using the outgroup genera *Amana* and *Erythronium* and non-parametric bootstrap values are shown along the branches.

In the *Tulipa* subgenus only *Multiflorae* is monophyletic, although part of the *Kolpakowskianae* section also groups together. However, *Tulipa ferganica, T. scharipovii, T. anisophylla, T. hissarica, T. altaica (T. annae)*, *T. lehmanniana*, and *T. borszczowii* all fall outside this core group. We propose shifting both *T. ferganica* and *T. scharipovii* to the *Multiflorae* section based

on their close relationship with species from this section and treating the monophyletic core clade of *Kolpakowskianae* as a section. The remaining five sections are clearly not monophyletic and shifting only a few taxa from section to section does not resolve this issue. The *Lanatae* section is polyphyletic, with *T. hoogiana*, *T. affinis*, *T. fosteriana, T. ingens, T. ivasczenkoae*, and a specimen of *T. carinata* falling outside of the expected clade. There is also a specimen under the name *T. kuschkensis* embedded within the core *Lanatae* section, although there is some uncertainty surrounding this specimen. The *Tulipanum* section has some structure, however specimens of *T. hoogiana* and *T. sosnowskyi*, which don't belong in this section, fall within the group making it non-monophyletic. The *Tulipa* section is also somewhat identifiable, apart from several *T. sosnowksyi* specimens that don't fall within this section as expected. The specimens of the *Vinistriatae* and *Spiranthera* sections are broadly intertwined. We show that these five sections are not currently informative and suggest they should be merged into a single section called *Tulipa*, which will simplify the sectional taxonomy of this subgenus greatly.

Misidentification and collection errors are common

The identification of wild tulip species is often difficult due to the broad variation of morphology even within species leaving few taxonomically informative traits (Zonneveld, 2009). We recorded 17 specimens as clear errors from the tulip taxa investigated in this work (Table 2.6; Appendix 1). We sampled 254 specimens within this project meaning there is an error rate of ~7% across the sampled specimens. There was an especially high error rate with plastomes uploaded to Genbank with 2 out of the 9, or 22%, misidentified (Table 2.8). This was driven by specimens uploaded under the name *Tulipa iliensis.* Both specimens under this name were originally wild collected in China before being sequenced and uploaded to Genbank under the sequence numbers MT316023 and MW077740. The specimen sequenced under MT316023 appears to be *T. altaica*, whilst that under MW077740 is a specimen of *T. tetraphylla*. Error rates across living collections and herbaria were estimated at 11% and 18% respectively, whilst only a single wild collected specimen was designated a clear misidentification (Table 2.8). There are also a range of specimens, primarily from the wild and living collections, that fell in the tree in unexpected places which could represent further cases of misidentification, but there was not enough evidence to confirm these. These were hesitantly maintained in the tree but marked (Figure 2.2; Figure 2.3).

Our phylogenies also provided evidence of inconsistent identification of specimens by differing parties. There are several cases where specimens under the same species name, but from

different countries, fall in very different clades, whilst there is evidence of a similar pattern even within countries where different people have collected specimens. An example of this can be seen in the *Tulipa talassica* specimens collected separately from the wild in Uzbekistan and Kyrgyzstan. The Kyrgyz specimen was collected by the researcher who described the species, whilst the species from Uzbekistan was collected by a researcher from Tashkent. The Uzbek specimen was embedded within the species *T. korolkowii*, far from the true position of *T. talassica.* A similar pattern can be seen for specimens under *T. dubia* and *T. vvedenskyi*. In some cases there is clearly an issue with in-country identification for example specimens collected in Turkey under *T. julia* and *T. armena* are polyphyletic with specimens of each species more closely related to a specimen of the other species than to other specimens of the same species. A similar pattern is observed for *T. cretica* and *T. saxatilis* where all specimens were collected from Crete, Greece.

There are also cases in the phylogeny where specimens under the same species name are distantly positioned from each other, not only making them polyphyletic but also related to a range of different species. The primary example of this in our tree is for specimens of *Tulipa carinata*, which were both collected from Uzbekistan, but one falls near to *T. lanata*, whilst the other is more closely related to *T. ingens.* Historically *T. carinata* is part of the *Lanatae* section, and therefore should be more closely related to *T. lanata* suggesting that the other specimen may be a misidentification. However, there is further evidence of confusion within multiple species concepts in this area of the tree, which leads to two distinct clades with multiple cases of the same species being represented in both. These cases provide further evidence that there are some species that are difficult to identify consistently.

Overall, we found that misidentification of tulip species is relatively common especially in living collections, herbaria, and online databases and it is clear that some taxa are more commonly mistaken than others. Across our work we found examples where specimens were clearly misidentified in collection records, where material received did not correspond to the species name it was under, cases where there was a geographical bias to relationships between collected specimens, and even a specimen that was named but did not correspond to any known taxon. In this project we also obtained a range of specimens that could not be assessed as misidentifications but did not fall where expected in the phylogeny. It is therefore important that detailed collection data is recorded, good voucher specimens are maintained, identification guides are used where possible, and when mistakes are uncovered these are corrected or uncertainty recorded. Crucially, our work emphasises the importance of the engagement of

experts in the identification process, although even these can make mistakes (Culverhouse *et al.*, 2003).

Species complexes still need to be resolved

There are several parts of the phylogenies inferred using nuclear and plastome data where specimens of the same species are polyphyletic and occur on very short poorly supported branches (Figure 2.2; Figure 2.3). One complex is centred around *Tulipa biflora*, a small white flowered species in the *Biflores* section of the *Eriostemones* subgenus. Currently the widespread *T. biflora* has a large range of synonyms as well as having similar morphology to many range-restricted species including *T. bifloriformis*, *T. turkestanica*, *T. orithyioides* and *T. dianaeverettiae*. In our phylogenies a range of synonyms and accepted species from the *Biflores* section were represented. Yet, neither the 35S rDNA nor plastome inferred phylogenies could resolve species surrounding *T. biflora*. Another unresolvable species complex was noted centred around a group of Central Asian species in the section *Tulipa* of the *Tulipa* subgenus. Many of these species are known to be closely related with some hybridisation events reported (Wilford, 2006; Zonneveld, 2009). They are all primarily large red flowering species, with the clade in our tree including *T. greigii*, *T. kaufmanniana*, *T. butkovii*, and *T. x tschimganica* as well as some specimens of *T. dubia* and *T. vvedenskyi.* In both complexes the limited tree topology would support the lumping of these species together, however other sources of data suggest differences in these taxa. We didn't feel our evidence was enough to alter the taxonomy and suggest further work is needed to provide clarity in these groups.

The plastome is a reasonable barcode region

Both the plastome and 35S rDNA have been recognised as useful 'super' barcodes for identifying plant species (Wu *et al.*, 2020), with the ITS region and combined *matK* and *rbcL* plastid markers often used as simpler barcodes (Yao *et al.*, 2010; Hollingsworth, Graham and Little, 2011; Li *et al.*, 2011). Overall, there was limited genetic variation between species across *Tulipa.* Nonetheless here we note that all regions were able to resolve the subgenera of this genus, except the *matK* and *rbcL* barcode which could not separate the *Clusianae* subgenus from the *Eriostemones*. We report that the plastome resolves species relationships much better for tulips than the 35S rDNA region, and both provide significantly more information than the commonly used ITS region or *matK* and *rbcL* 2-locus barcode. The ITS region is known to struggle in some plant groups due to problems with paralogy and complex concerted evolution (Chase *et al.*, 2005), whilst the *matK* + *rbcL* barcode effectiveness is known to vary greatly depending on the plant group (Hollingsworth, Graham and Little, 2011). Specifically, the

plastome dataset detected the monophyly of 34 out of the 62 species in the conservative approach and 46 of the 79 species in the more lenient approach. Whilst the 35S rDNA resolved only 22 species out of 61 in the conservative approach with 25 of the 78 accepted species (*Tulipa iliensis* was not represented in the 35S rDNA tree) identifiable in the lenient approach. The ITS barcode dataset resolved only 19 species out of 61 monophyletic in the conservative approach and only 20 of the 78 accepted species grouping in the lenient approach with the *matK* + *rbcL* barcode performing worst resolving only 11 species of the 62 in the conservative approach and 13 of 79 in the lenient approach. Based on the more reliable conservative approach output we estimate that the plastome can resolve around 55% of species within the genus whilst the more constrained 35S rDNA resolves around 22%, and the ITS barcode and *matK* + *rbcL* barcode resolve only 19% and 18% respectively.

In general, the plastome resolved a much more structured phylogeny than the 35S rDNA marker, which in turn inferred a phylogeny with many fewer low bootstrap support nodes than either the ITS region or *matK* + *rbcL* barcode. Given the limitations of the 35S rDNA, ITS, and *matK* +*rbcL* inferred phylogenies in resolving species we used the multi-partition plastome inferred phylogeny for the majority of our taxonomic inferences. Nonetheless the nuclear marker phylogenies provided support for the acceptance of the species *Tulipa zonneveldii*, which the plastome dataset did not. Its separation from *T. anadroma* was not inferred based on plastome data, however given the separation of *T. anadroma* and *T. zonneveldii* in the nuclear based phylogeny and the large morphological differences between these species there is significant evidence for accepting these taxa. In addition, the ITS barcode and 35S rDNA phylogeny provided some informative structure over the plastome phylogeny for specimens of *T. sylvestris and T. orphanidea*. Both these species clustered together on the nuclear based phylogenies, whereas these were polyphyletic on the plastome phylogeny as they were entangled together. There was very weak support for both these clades on the ITS phylogeny, however bootstrap support for this clustering on the 35S rDNA phylogeny was somewhat stronger at 84 for the *T. sylvestris* clade and 93 for the *T. orphanidea* clade highlighting some significant evolutionary grouping.

Notably, much of the literature inferring *Tulipa* phylogenies through genetic sequence data have used the ITS alone or with a limited selection of plastid markers (Christenhusz *et al.*, 2013; Turktas *et al.*, 2013; Hajdari *et al.*, 2021; Nikitina *et al.*, 2021). Although there are considerable limitations in our assessment of these sequence regions especially the inadequate lack of sampling of some species, uncertainty in the identity of some sequenced specimens, and the

complete discounting of biological processes for the absence of monophyly, we highlight the limited genetic variation between all species in the genus even across the entire plastid genome, but especially across the nuclear ITS marker and *matK* + *rbcL* plastid markers. It is clear therefore that any phylogenies inferred using only a few genetic markers will be severely limited especially with respect to resolving species level relationships. In some cases, erroneous relationships may be inferred between species when only a few select genetic markers are used due to the enhanced impact of sequencing errors and gene tree conflict. Markedly, the ability to resolve species within tulips is considerably lower than most plant groups (Hollingsworth, Graham and Little, 2011) highlighting both the remaining taxonomic uncertainty in the group as well as the genetic similarity between many of the taxa in this genus. We therefore recommend that either a large selection of nuclear markers or where resources are more limited the plastome, which can be obtained through increasingly cheaper genome skimming methods, be used to resolve relationships between tulip species.

Reorganisation of species

Our work enabled the production of an updated species list containing 96 accepted species (Table 2.11), with several subspecies recognised, improving upon the previous recent estimates of 76 (Christenhusz *et al.*, 2013) and 87 species (Zonneveld, 2009). This increase in species numbers is partly due to an influx of new species described since previous work was undertaken (Table 1.1), with our work highlighting a further new species to be described. In addition, there was considerable evidence to reinstate a number of species based on their separation from specimens of taxa that they were synonymised under. The species to be reinstated are *Tulipa micheliana*, *T. anadroma*, *T. affinis*, *T. brachystemon*, *T. zenaidae*, *T. patens*, *T. hageri*, *T. neustruevae* and *T. cashmeriana*. We also found evidence to synonymise a selection of species with considerable support for the placing of *T. kosovarica* and *T. luanica* under the species *T. serbica*, for *T. heweri* and *T. banuensis* to be treated under *T. praestans,* as well as to synonymise *T. intermedia* under *T. korolkowii, T. annae* to be placed under *T. altaica,* and *T. koyuncui* to be considered as *T. biflora*. We also note that the variety of *T. systola* called *florenskyi* that should be in the *T. systola* complex could be *T. sosnowskyi* given its position as sister to this species, but there is not enough evidence to formalise this synonymisation here.

In general, we expect that the total number of species within this genus is likely to change again in the near future, as new species continue to be described (De Groot and Zonneveld, 2022; Rukšāns and Zubov, 2022) and our phylogeny highlights several areas where further analyses

Table 2.11. A list of the 96 species, and several subspecies, accepted within this work placed within the reorganised subgenera and sections of the genus *Tulipa.* Both the accepted taxonomic classifications are listed alongside the authors who described them.

Tulipa* subgenus *Eduardoregelia (Popov) B.D.Wilson & Christenh., ined.
Section Eduardoregelia B.D.Wilson & Christenh., ined.
Tulipa heterophylla (Regel) Baker

Tulipa* subgenus *Orithyia (D.Don) Baker J. Linn. Soc. Bot. 14: 277. (1874)
Section *Orithyia* (D.Don) Vved.
Tulipa heteropetala Ledeb.
Tulipa sinkiangensis Z.M.Mao*
Tulipa uniflora (L.) Besser

Tulipa* subgenus *Clusianae (Baker) Zonn. & Veldkamp, Pl. Syst. Evol. 298: 89. (2012)
Section *Clusianae* Baker
Tulipa cashmeriana (A. D. Hall) Raamsd.
Tulipa clusiana Redoute
Tulipa harazensis Rech.f.*
Tulipa linifolia Regel
Tulipa montana Lindl.

Tulipa* subgenus *Eriostemones (Boiss.) Hall, Book of the Tulip: 60 (1929), as *Eriostemon*.
Section *Sylvestres* (Baker) Baker
Tulipa akamasica Chrisdoulou, Hand & Charalamb.*
Tulipa narcissicum N.Y.Stepanova*
Tulipa orphanidea Boiss.
Tulipa patens C.Agardh ex Schult&Schult. f
Tulipa regelii Krassn.*
Tulipa sylvestris L. subsp. *australis* (Link) Pamp.
Tulipa sylvestris L. subsp. *primulina* (Baker) Maire & Weiller
Tulipa sylvestris L. subsp. *sylvestris* (type)
Tulipa turgaica Perezhogin*
Section *Biflores* A.D.Hall ex Zonn. & Veldk.
Tulipa auliekolica Perezhogin.*
Tulipa biflora Pall.
Tulipa bifloriformis Vved.
Tulipa dasystemon (Regel) Regel
Tulipa dianaeverettiae J.de Groot & Zonn.*
Tulipa jacquesii Zonn.*
Tulipa kolbintsevii Zonn.*
Tulipa lorestanica Rukšāns & Zubov*
Tulipa neustruevae Pobed.*
Tulipa orithyioides Vved.
Tulipa salsola Rukšāns & Zubov*
Tulipa turkestanica Regel
Tulipa urumiensis Stapf
Section *Saxatiles* (Baker) Baker
Tulipa cinnabarina subsp. *cinnabarina* K.Perss.*
Tulipa cinnabarina subsp*. toprakii* Yildrim & Eker*
Tulipa cretica Boiss. & Heldr.*
Tulipa hageri Heldr.
Tulipa humilis Herb.
Tulipa saxatilis Sieber ex Spreng.
Tulipa sprengeri Baker*

Tulipa* subgenus *Tulipa
Section *Kolpakowskianae* Van Raamsd. Ex Zonn.& Veldk.
*Sp. nov**
Tulipa brachystemon Regel
Tulipa iliensis Regel
Tulipa kolpakowskiana Regel
Tulipa korolkowii Hoog
Tulipa lemmersii Zonn., Peterse, J.de Groot*
Tulipa ostrowskiana Regel
Tulipa platystemon Vved.*
Tulipa talassica Lazkov*
Tulipa tetraphylla Regel
Tulipa zenaidae Vved.
.
Section *Multiflorae* (Van Raamsd.) Zonn.
Tulipa anisophylla Vved.
Tulipa boettgeri Regel*
Tulipa ferganica Vved.
Tulipa scharipovii Tojibaev*
Tulipa praestans H.B.May*

Section *Tulipa* (Van Raamsd.) Zonn.

Tulipa affinis Botschantz.
Tulipa agenensis Redouté
Tulipa albanica Kit Tan & Shuka*
Tulipa alberti Regel*
Tulipa aleppensis Boiss.
Tulipa altaica Pall.
Tulipa anadroma Botschantz.*
Tulipa armena Boiss.
Tulipa bactriana J.de Groot & Tojibaev*
Tulipa borszczowii Regel
Tulipa botschantzevae S.N.Abramova & Zakal.
Tulipa brinkii J.de Groot & Zonn.*
Tulipa butkovii Botschantz.*
Tulipa carinata Vved.
Tulipa cypria Stapf
Tulipa dubia Vved.
Tulipa faribae Ghahr., Attar & Ghahrem-Nejad*
Tulipa foliosa Stapf*
Tulipa fosteriana W.Irving
Tulipa greigii Regel
Tulipa hissarica Popov & Vved.
Tulipa hoogiana B.Fedtsch
Tulipa hungarica Borbás
Tulipa ingens Hoog
Tulipa ivasczenkoae Epiktetov & Belyalov*
Tulipa julia K.Koch
Tulipa kaufmanniana Regel
Tulipa kuschkensis B.Fedtsch.
Tulipa lanata Regel
Tulipa lehmanniana Merckl.
Tulipa micheliana Hoog
Tulipa persica (Lindl.) Sweet*
Tulipa scardica Bornm.
Tulipa schmidtii Fomin
Tulipa serbica Tatic & Krivošej
Tulipa sosnowskyi Achv. & Mirzoeva*
Tulipa suaveolens Roth
Tulipa subquinquefolia Vved.*
Tulipa systola Stapf
Tulipa × *tschimganica* Botschantz.*
Tulipa ulophylla Wendelbo*
Tulipa undulatifolia Boiss.
Tulipa uzbekistanica Botschanz. & Scharipov*
Tulipa vvedenskyi Botschanz.
Tulipa zonneveldii J.de Groot & Tojibaev*

* endemic species

are needed that could lead to further taxonomic changes. These primarily correspond to species complexes, genetically diverse species, as well as several specimens either under synonym names or accepted species that did not fall in the expected area of the tree. We note specifically that several species are polyphyletic and group around *Tulipa systola,* around *T. gregii, and T. biflora* on very short branches all warranting further detailed analysis. In addition, there is considerable taxonomic uncertainty around specific species. An example of this is *T. undulatifolia* where we sampled a specimen under the name *T. undulatifolia (micheliana)* which fell within the *T. systola* complex, whilst we sampled another specimen under *T. undulatifolia* which sits near to *T. fosteriana.* We hesitantly accepted the second specimen as the true position of this species, however the uncertainty around both these specimens mean the true position cannot be assessed reliably and further work is needed.

The presence of species complexes limited the taxonomic understanding for some species, whilst there was also a range of specimens that fell in unexpected places, but due to uncertainty in their origin and identification, could not be used to make any conclusive taxonomic statements. Nonetheless given the unique nature of some of these specimens, such as that of

Tulipa linifolia (maximowiczii) or *T. humilis*, they certainly warrant further analysis. In addition, several extremely diverse species were also identified. These diverse species were represented by multiple specimens in the tree that were monophyletic but had long branches between them. These identified diverse species centred around *T. heterophylla*, *T. praestans*, *T. korolkowii*, and *T. altaica*. The species of *T. agenensis* also showed relatively high intraspecific diversity although only two true specimens were present in the phylogeny. These diverse taxa were hesitantly maintained as single species, but more focused work is needed to understand their genetic diversity. There are also a range of species and synonyms that were not sampled in this project that will need to be assessed (Appendix 5).

The ancestors of cultivars

Our phylogeny highlights several notable results that may contribute to broader understanding of the tulips used in the initial breeding of cultivars. Our tree shows that one of the oldest cultivars known, Duc van Tol, is closely related to *Tulipa suaveolens* supporting the theory that this species may have been used in the initial breeding of cultivated tulips (Kritskaya *et al.*, 2020). In addition, we note that a specimen of *T. x gesneriana* which is thought to be an old hybrid cultivar (Christenhusz *et al*. 2013), is closely related to *T. hungarica.* This could suggest that *T. hungarica* may have played a role in the history of horticultural tulips, or that this species is an escaped cultivar that has naturalised in this region. Another specimen of *T.* × *gesneriana,* recognised in this work as a likely misidentification due to its collection in Iran where this taxon is not known, is closely related to specimens of *T. agenensis*, a species known to be widely naturalised in Europe. Although likely not a true representative of *T. x gesneriana* we do not rule out *T. agenensis* as another potential contributor to cultivar diversity. There is still much ambiguity around which species contributed to the original cultivars, especially with regard to the role of Central Asian species (Christenhusz *et al*. 2013). However, research is now beginning to highlight a few candidate species as the most important contributors to ancient cultivars, with our work adding another layer of information to resolve this mystery.

2.4. Conclusion

Using phylogenies inferred from our large sequence datasets, we were able to investigate plastome structure in tulips, explore misidentification, and most importantly reassess the taxonomy of the genus both at higher taxonomic levels as well as broadly at the species level. Here we have shown that plastome structure is consistent across the genus, and that the sequencing of this genome is of significant use for identifying species especially when compared to other commonly used DNA barcodes. Although misidentification is somewhat

common in the genus, we were able to make a number of taxonomic discoveries and decisions. We note a new subgenus is required for the genetically distinct species *Tulipa heterophylla*. This new subgenus, *Eduardoregelia,* contains only this single species. In addition, the sections of this genus were reorganised based on our results improving their informativeness and simplicity. The genus now has ten sections: one for each of the subgenera *Eduardoregelia*, *Orithyia*, and *Clusianae*, and three each for the *Tulipa* and *Eriostemones* subgenera. The historical sections of the *Eriostemones* have been maintained (Zonneveld, 2009), whilst the *Multiflorae*, *Tulipa*, and *Kolpakowskianae* sections of the *Tulipa* subgenus have been modified to represent monophyletic groups, with the *Tulipa* section encompassing the vast majority of species in this subgenus. After accounting for synonymisation, reinstation, and new species we recognise 96 species of tulip (Table 2.11).

Our project is timely given the need for an updated taxonomic framework for the genus, especially as new species continue to be described frequently complicating matters (Perezhogin, 2013; Stepanova, 2014; Zonneveld, 2015; De Groot and Tojibaev, 2017, 2020; De Groot and Zonneveld, 2020). Here we have provided an important step forward in developing this framework, which other projects can now build upon. Within this project we have also identified a new species, which we will describe in the next chapter. Given the discovery of novel taxa in this project it seems likely that new species of tulips are still to be discovered. We advise that description of these new species be done carefully when comprehensive evidence has been obtained given the occurrence of misidentification and over splitting (Hajdari *et al.*, 2021) in this genus. Properties used to describe species must be consistent across populations in the wild and be of evolutionary significance in this genus. Species should not be described from single specimens grown *ex-situ* or from highly variable intra-specific traits. It is also important that seeds of new species should be distributed to multiple botanic gardens and herbarium specimens to several herbaria worldwide so that material for new species is accessible.

There is still significant work to be undertaken within this genus especially surrounding species complexes and diverse species. These areas highlighted in our discussion will require extensive sampling as well as the use of large nuclear marker datasets to better understand evolutionary relationships and resolve remaining ambiguities in species concepts. This is likely the next major step to be taken in ordering the taxonomy of this genus. Notably in this project we have shown that the ancestral cultivar Duc van Tol is closely related to *Tulipa suaveolens* supporting the theory that this species was used in the initial breeding of cultivated tulips (Kritskaya *et al.*,

2020), with a specimen of *T. x gesneriana* closely related to *T. hungarica* promoting additional investigation of this species as well in the history of horticultural tulips. Further work will also be needed to assess some of the species and synonyms for which we lacked material and for those specimens for which we had material but could not make concrete taxonomic decisions (Appendix 5). In addition, newly described species need to be sequenced as soon as possible, especially given the quick rate in which they are being described (De Groot and Zonneveld, 2022; Rukšāns and Zubov, 2022). Our phylogeny represents ~86% of all accepted species at the time of the analysis and provides a robust taxonomic backbone for future work related to wild tulips be it genetic, ecological, or conservation (Figure 2.8). However, we emphasise that extensive collaborative efforts will be needed to keep the taxonomy of *Tulipa* up to date.

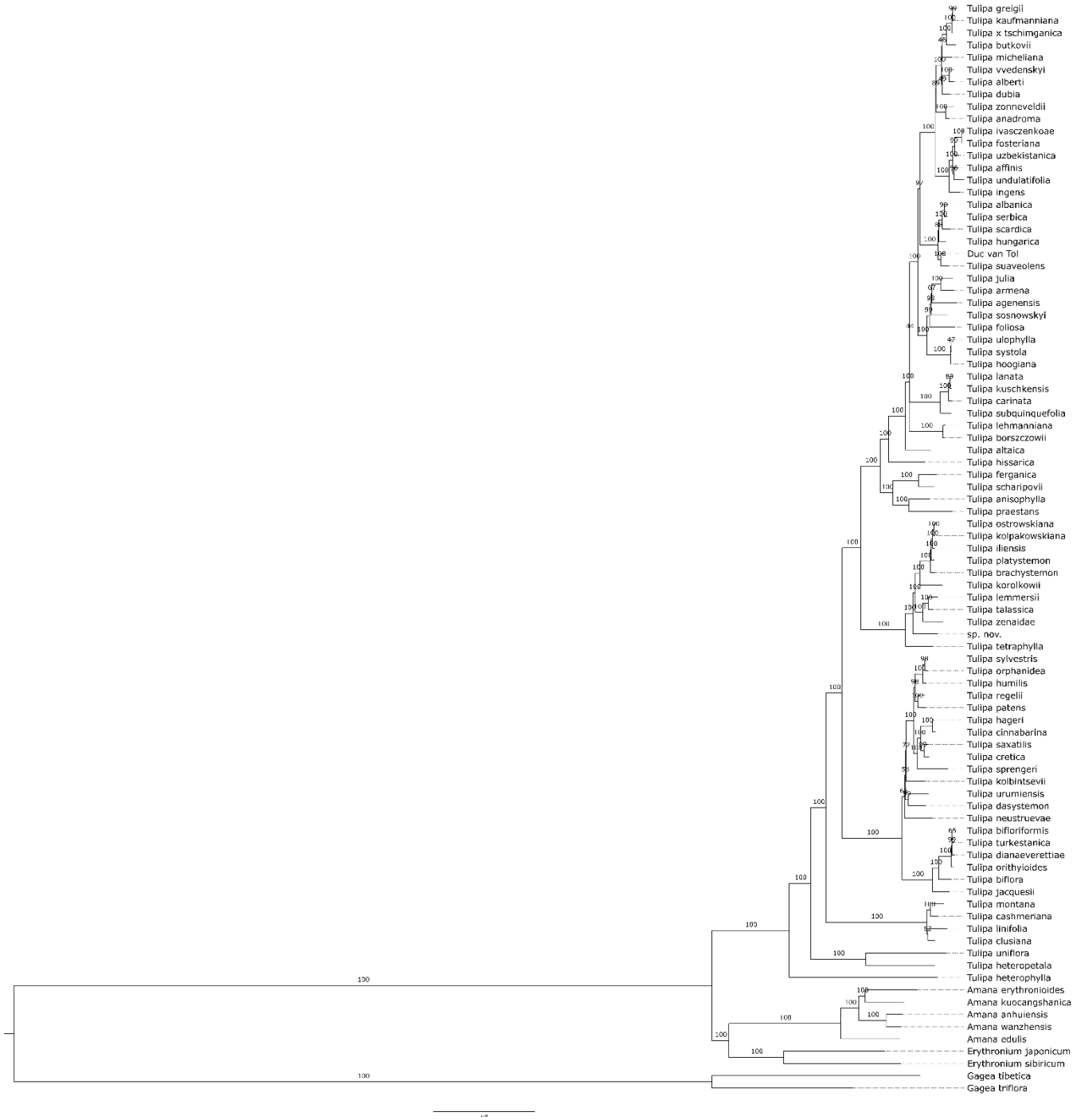


Figure 2.8. Species tree inferred using plastome data. This phylogeny was developed using only a single selected specimen for each accepted species. Rooted using *Amana*, *Erythronium* and *Gagea* specimens, with non-parametric bootstrap support values shown for branches.

Chapter 3.

Tulipa toktogulica (Liliaceae), a new endangered cryptic species from the western Tien-Shan, Kyrgyzstan

The basis of this chapter was published in the peer reviewed journal Phytotaxa: Wilson, B. *et al.* (2022) '*Tulipa toktogulica* (Liliaceae)' a new endangered cryptic species from the western Tien-Shan, Kyrgyzstan, *Phytotaxa*. https://doi.org/10.11646/phytotaxa.00.0.0

In this chapter we formally describe the new species *Tulipa toktogulica*, which was identified as a potentially new species in our broader phylogenetic analysis in chapter two. Here we infer a phylogeny focused specifically around this species and other closely related taxa. We use this phylogenetic evidence alongside morphological and biogeographical information also gathered in this project to highlight the novelty of this tulip. Within this process we assessed the extinction risk of this plant as well.

3.1. Introduction

The description of new species is not uncommon in the genus Tulipa (Table 1.1). Today, *Tulipa* Linnaeus (1753: 305) is estimated to consist of 70–100 species depending on species concepts (Zonneveld 2009, Christenhusz *et al.* 2013, POWO 2022, WCVP 2022), but this number is ever-changing as new species are recognised. This flower group has been historically regarded as a complex genus with many taxonomic alterations already made over the several centuries since it was originally recognised (Christenhusz *et al*. 2013). The complexity surrounding tulip species concepts is mainly due to the overlap in morphological characteristics across species and reliance on multiple often cryptic traits to identify species from one another (Zonneveld, 2015). This is further complicated by the historical use of traits that are highly variable even within species, such as flower colour or genome size (Zonneveld 2009; see comments on species concepts in Christenhusz *et al*. 2013). In general, therefore, an integrated taxonomic approach would be preferred but this is rarely undertaken for recently discovered taxa.

Currently, the genus comprises four subgenera, *Clusianae* (Baker 1883: 626) Zonneveld & Veldkamp (2012: 89), *Eriostemones* (Boissier 1882:191) Hall (1929: 60), *Orithyia* (Don 1836: 336) Baker (1874: 277) and *Tulipa* (Christenhusz *et al.*, 2013) with twelve sections proposed based on morphology, biogeography and genome size (Zonneveld, 2009). However, we have already shown that new phylogenetic data provides evidence for considerable changes to this framework. In addition, there is already evidence of some cases of over-splitting of recently described species (Hajdari *et al.*, 2021), and many previously recognised species have since

had to be synonymised (Christenhusz *et al.*, 2013). Therefore, there are still many considerable taxonomic challenges remaining in the genus, and there is a need to be careful and integrative when describing new species (Dayrat, 2005), especially when assigning them to respective subgenera and sections.

The primary centre of diversity for the genus is Central Asia (Botschantzeva 1982, Hoog 1973), which is estimated to encompass approximately 55% of all species. Several new species have been described during the last decade, primarily from this region and based exclusively on morphology (De Groot & Tojibaev 2020, De Groot & Zonneveld 2020, Rukšāns & Zubov 2022) with many species endemic and occurring in small gorges, specific pastureland and more remote areas (Zonneveld 2009, 2015, Zonneveld & de Groot 2012). In addition, the conservation status of many new species has not been assessed (IUCN, 2022). Even though national level assessments suggest a large proportion of tulip diversity may be threatened (Davletkeldiev 2006 ,Baitulin 2014, Tojibaev & Beshko 2015, Nowak *et al.* 2020), and new species may be particularly prone to extinction (Liu *et al.*, 2022).

Here, we present a cryptic new species that is morphologically similar to *Tulipa talassica* Lazkov (2011: 11), a relatively recently described species from the Talas Region of Kyrgyzstan (Lazkov and Pashinina, 2011). However this new taxon is genetically distinct from any previously described species and is in urgent need of conservation intervention. This is the first case where phylogenetic data, alongside morphological and geographical data, has been used to describe a new taxon within this genus and highlights the value of this data type for understanding the diversity of the genus *Tulipa*.

3.2. Material & Methods

3.2.1. Plant material

Plant material for all Kyrgyz specimens was collected primarily by Brett Wilson, Kaiyrkul Shalpykov and Georgy Lazkov in springs of 2019–2022. This includes *Tulipa biflora* Pallas (1776: 727) *s.l.*, *T. greigii* Regel (1873a: 290) and *T. heterophylla* (Regel 1868: 440) Baker (1874: 295) specimens used as references (Table 3.1). Specifically, material of the new taxon was collected from four populations in the Toktogul area, Jalal-Abad Region, with material sequenced from population one and two in this project (Figure 3.1). The *T. linifolia* Regel (1884: 648) reference specimen was collected in Tajikistan in 2020 by Mariyo Boboev of the Kulob Botanic Garden, and the *T. undulatifolia* Boissier (1844: 57), *T. talassica* and *T. altaica* Pallas in Sprengel (1825: 63) specimens were obtained from the Royal Botanic Gardens, Kew. The *T.*

talassica specimen was collected by GL in Kyrgyzstan and the *T. altaica* in Kazakhstan. All leaf material collected in silica gel. A voucher specimen was also collected from each sampled population and deposited at FRU or CGE. A *T. lemmersii* Zonneveld, Peterse & de Groot (2012: 91) specimen was obtained from Ben Zonneveld at the Naturalis Biodiversity Center, Leiden,

Table 3.1. Specimens collected and sequenced for use in the description of the new species. The source of the material is given including the herbarium location of the voucher specimen and the country where the sample was collected.

Species	**Source**	**Country**
Tulipa korolkowii	Between Baul and Korgon villages, Batken Region, 14/04/2019, Lazkov and Wilson, BW015 (CGE).	Kyrgyzstan
Tulipa ostrowskiana	Eastern part Kyrgyz Mt. R., near Djil-Aryk village, Chuy Region, 26/04/2019, Lazkov and Wilson, BW052 (CGE).	Kyrgyzstan
Tulipa kolpakowskiana	Chuy valley, near Leninskoe village, Chuy Region, 27/03/2020, Lazkov and Shalpykov, without number (FRU)	Kyrgyzstan
Tulipa iliensis (= *T. thianschanica*)	Genbank (MT327741) – Western China.	China
Tulipa platystemon	Inter Fergana and Alai Mt. R., Kara-Kulja river basin, near Sary-Kamysh village, Osh Region, 18/04/2019, Lazkov and Wilson, BW033 (CGE)	Kyrgyzstan
Tulipa talassica 1	Kyrgyz Mt. R., southern slope, opposite of Talas city, Talas Region, 27/04/2020, Lazkov and Shalpykov, without number (FRU)	Kyrgyzstan
Tulipa talassica 2	Wild collected – Talas Mt. R., northern slope, Urmaral riv. Gorge, Talas Region, 08/05/2021, Lazkov and Shalpykov, without number (FRU)	Kyrgyzstan
Tulipa talassica 3	Royal Botanic Gardens, Kew, accession number 2019-1976*1 – Kyrgyz Mt. R., southern slope, opposite of Talas city, Talas Region, 27/04/2011, Lazkov, BLCKg-981, (FRU).	Kyrgyzstan
Tulipa lemmersii	Obtained from J. J. de Groot, De Zilk in the Netherlands, de Groot, 0822655, (L)	Kazakhstan
Tulipa zenaidae	Wild collected – Kyrgyz Mt. R., northern slope, Kara Balta river gorge, Chuy Region, 12/04/2019, Lazkov and Wilson, BW003 (CGE).	Kyrgyzstan
Tulipa toktogulica 1	Sussamyr Mt. R., southern slope, Zagyra mountains, Toktogul distr., Jalal-Abad Region, 12/04/2019, Lazkov and Wilson, BW007 (CGE).	Kyrgyzstan
Tulipa toktogulica 2	Sussamyr Mt. R., south-eastern slope, to the north of Bel-Aldy and Sary-Seget villages, Toktogul, Jalal-Abad Region, 04/05/2021, Lazkov and Shalpykov, without number (FRU)	Kyrgyzstan
Tulipa tetraphylla	Fergana Mt. R., northern slope, Jalal-Abad Region, 13/04/2019, Lazkov and Wilson, BW009 (CGE).	Kyrgyzstan
Tulipa brachystemon	Kastek ridge, southern slope, gorge near the Beisheke village Chuy Region, 14/05/2021, Lazkov and Shalpykov, without number (FRU)	Kyrgyzstan
Tulipa altaica	Royal Botanic Gardens, Kew, accession number 2017-288*1, Kolbintsev, VK36, (K)	Kazakhstan

who in turn obtained it from de Groot and de Zilk in the Netherlands. The original parent plant of this specimen was collected at the type location of the species in Kazakhstan. Finally, we included a *T. iliensis* Regel (1879a: 162) [= *T. thianschanica* Regel (1879b: 508)] specimen that was collected in western China, where this species is native, and its plastome was sequenced and uploaded to Genbank (MT327741). The original collection location is not available. The putative new taxon varies significantly in size in the wild, much like *T. talassica,* so measurements of morphological attributes are estimates based on five specimens, but this will likely not reflect the entire diversity within this species. Flower size is generally similar across this species so only a single flower was dissected and measured.

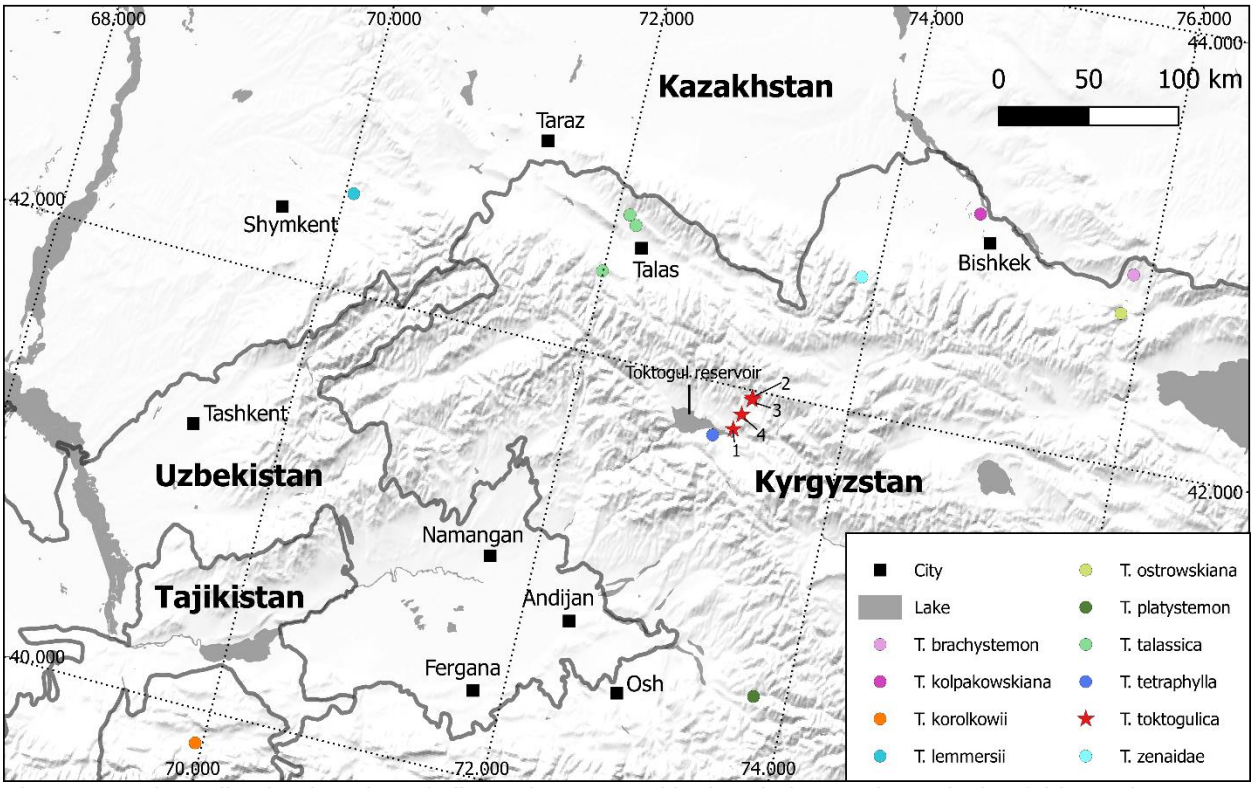


Figure 3.1. The collection location of all specimens used in the phylogenetic analysis of this study excluding *Tulipa iliensis* and *T. altaica*, which both lacked GPS data. Populations of the new species *T. toktogulica* are labelled in order of discovery.

3.2.2. DNA extraction and sequencing

DNA was extracted from silica-gel dried leaf material using a modified CTAB protocol (Doyle and Doyle, 1987). All samples were sequenced at Beijing Genomics Institute, Hong Kong (BGI). DNBseq normal DNA libraries or low input DNA libraries were constructed depending on the quality of the extraction assessed using SOAPnuke (Chen *et al.*, 2018). The libraries were then processed through DNBseq Paired-End 100 sequencing. A minimum of 1.2 Gigabases of clean data was generated for each specimen sequenced.

3.2.3. Plastid genome assembly and annotation

For generation of the plastid genomes the following process was followed with the raw reads, except for *Tulipa iliensis* (MT327741, as *T. thianschanica*) because this was downloaded as a complete plastome. The raw reads were filtered using a range of plastome references downloaded from GenBank (Table 3.2) using the Burrows-Wheeler Alignment Tool (Li and Durbin, 2009). The filtered reads were then assembled into contigs through the SPAdes (Nurk *et al.*, 2013) wrapper Unicycler (Wick *et al.*, 2017). This generated three contigs per specimen representing the large single-copy (LSC), small single-copy (SSC) and a single inverted repeat (IR). The contigs were scaffolded to a *T. undulatifolia* reference genome, assembled in a broader taxonomic project, using the map to reference function in Geneious Prime 2020.2.5 (Kearse *et al.*, 2012). In this step the IR region was duplicated ensuring the full plastome was assembled. Each chloroplast sequence, including *T. iliensis*, was annotated using a *T. biflora* reference genome, again assembled and annotated in a broader taxonomic project, using the live annotate and predict function in Geneious Prime with similarity set to 80%. Each sequence was then manually inspected, and annotations edited where there was a clear error. A copy of the IR was removed from each sequence before analysis to prevent double weighting of the IR.

Table 3.2. Plastomes downloaded from GenBank for use as references in the assembly process. Both the accepted species name and NCBI reference number are reported.

Species	**NCBI Reference Sequence**
Amana anhuiensis	NC_034706
Amana edulis	NC_034707
Amana erythronioides	KY401424
Amana kuocangshanica	NC_034708
Amana wanzhensis	NC_034705
Erythronium sibiricum	NC_035681

3.2.4. 35S rDNA assembly and annotation

Using the same genome skimming datasets, the 35S rDNA region of each specimen was also generated. Initially we assembled the raw reads using SPAdes 3.15.0 of specimens of *Tulipa biflora*, *T. greigii*, *T. heterophylla*, *T. korolkowii* Regel (1875: 295), and *T. linifolia* representing the four subgenera of the genus with two specimens representing the largest subgenus *Tulipa*. The assembled contigs were mapped to a *Lilium tsingtauense* Gilg (1904: 24) sequence

(KM117263) downloaded from GenBank using the map to reference function on Geneious Prime. Mapped contigs for each specimen were then pilon polished (Walker *et al.*, 2014) and remapped to the reference sequence. Any contig with coverage of less than ten was removed and the remaining contigs were used to generate a consensus sequence using the generate consensus function on Geneious Prime with the strict-50% threshold selected. We then generated 35S rDNA sequences of all other specimens in this work using the most closely related specimen, *T. korolkowii*, as a reference. We also added an initial step where we used the 35S rDNA sequences of *T. biflora*, *T. greigii*, *T. heterophylla*, *T. korolkowii* and *T. linifolia* and the Burrows-Wheeler Alignment Tool (Li and Durbin, 2009) to extract relevant reads from the genome skimming data of all specimens before processing. All sequences were annotated using the *L. tsingtauense* as a reference with the regions at either end trimmed to the extent of the *L. tsingtauense* sequence. The *Amana baohuaensis* Han, Wang & Lu. in Wang *et al*. (2019: 45) sequence, used as the outgroup for the 35S rDNA analysis, was generated in the same way as the reference specimens using publicly available data on the short-read archive (SRR12599520). The previously published sequence data for *T. iliensis* (as *T. thianschanica*) did not include a corresponding 35S rDNA sequence.

3.2.5. Phylogenetics

Four datasets were prepared. The 35S ribosomal cistron was used to infer a single tree using a multi-partition approach. Three plastome datasets were used: the full plastome treated as a single partition, the full plastome treated as multiple partitions based on annotated regions and the coding sequence (CDS) regions of the plastome used in a multi-partition approach. For the multi-partition analyses, the annotated regions were extracted with Geneious Prime and then aligned using MAFFT (--auto) (Katoh and Standley, 2013). These alignments were then cleaned using the pxclsq in the phyx package (Brown, Walker and Smith, 2017) with only columns where at least 10% of specimens contained base data retained and the specimens then renamed using the pxrls function. These separate alignments were then used to create a supermatrix using the pxcat function of the phyx package (Brown, Walker and Smith, 2017). In addition to each annotation being treated as a single partition each codon position of each CDS region was also separated into unique partitions. The greedy algorithm (Lanfear *et al.*, 2012) of IQTree ModelFinder software (Kalyaanamoorthy *et al.*, 2017) was then used to generate a best scheme for partitions. This led to the 35S rDNA being separated into two partitions, the CDS only data into nine partitions, and the full plastome data into 14 partitions. For the single partition plastome data, the sequences were aligned using MAFFT (--auto) (Katoh and Standley, 2013).

A maximum likelihood approach was used to infer trees using the software RAxML (Stamatakis, 2014) with 1000 bootstrap trees run for each dataset and partitions specified where appropriate. The 35S rDNA tree was rooted with *Amana baohuaensis*, whereas the plastome-based trees were rooted using plastome data downloaded from GenBank for *A. wanzhensis* Huang, Han & Zhang in Zhang & Huang (2014: 120) (NC_034705), *A. edulis* (Miquel, (1867: 158) Honda (1935: 20) (NC_034707), *Erythronium japonicum* Decaisne (1854: 284) (MT261155) and *E. sibiricum* (Fischer & Meyer, (1841: 47) Krylov (1929: 641) (NC_035681) specimens, which were annotated and processed through the same process as the specimens sequenced in this project. The trees were viewed and relabelled in FigTree (http://tree.bio.ed.ac.uk/) with tree figures produced using INKSCAPE 1.1. (Inkscape Project, 2022).

3.3. Results

Although there is incongruence between the rDNA and plastid trees, which may be due to ancient hybridisation or introgression, this will not be addressed here because across all datasets the new species forms a unique cluster in a position distinct from other clearly-named species (Figure 3.2), with the three plastid trees identical in topology (Appendix 6). This unique cluster is also distinctly recognisable from the most morphologically similar species *Tulipa talassica* across all inferred trees. Clearly the new species belongs to *T.* subgenus *Tulipa* and probably *T.* section *Kolpakowskianae*. The positions of species in the plastid trees are better supported compared to those in the rDNA tree, so we primarily base the discussion on this result. Specifically, the new species is a member of a clade comprising *T. iliensis*, *T. brachystemon* Regel (1882: 323), *T. lemmersii*, *T. kolpakowskiana* Regel (1877: 266), *T. korolkowii*, *T. ostrowskiana* Regel (1884: 34), *T. platystemon* Vvedensky (1935: 150), *T. talassica* and *T*. *zenaidae* Vvedensky (1935: 150). The topology of the tree is such that these other species are all more closely related to one another than to this new species supporting its description as a novel taxon. Explicitly, this new species diverges from the common ancestor of the *Kolpakowskianae* clade after *T. tetraphylla* Regel (1875: 296), but before these other listed species (Figure 3.2).

3.4. Discussion

Tulipa toktogulica is most morphologically similar to *T. talassica* but has a unique combination of traits that supports its evolutionary separation from this taxon as well as from other biogeographically related taxa (Table 3.3). Specifically, it has a prolonged tunic, broad stamens and a slightly fragrant flower. Importantly, this new species is also clearly genetically and geographically separated from morphologically similar species. *T. talassica* is genetically more

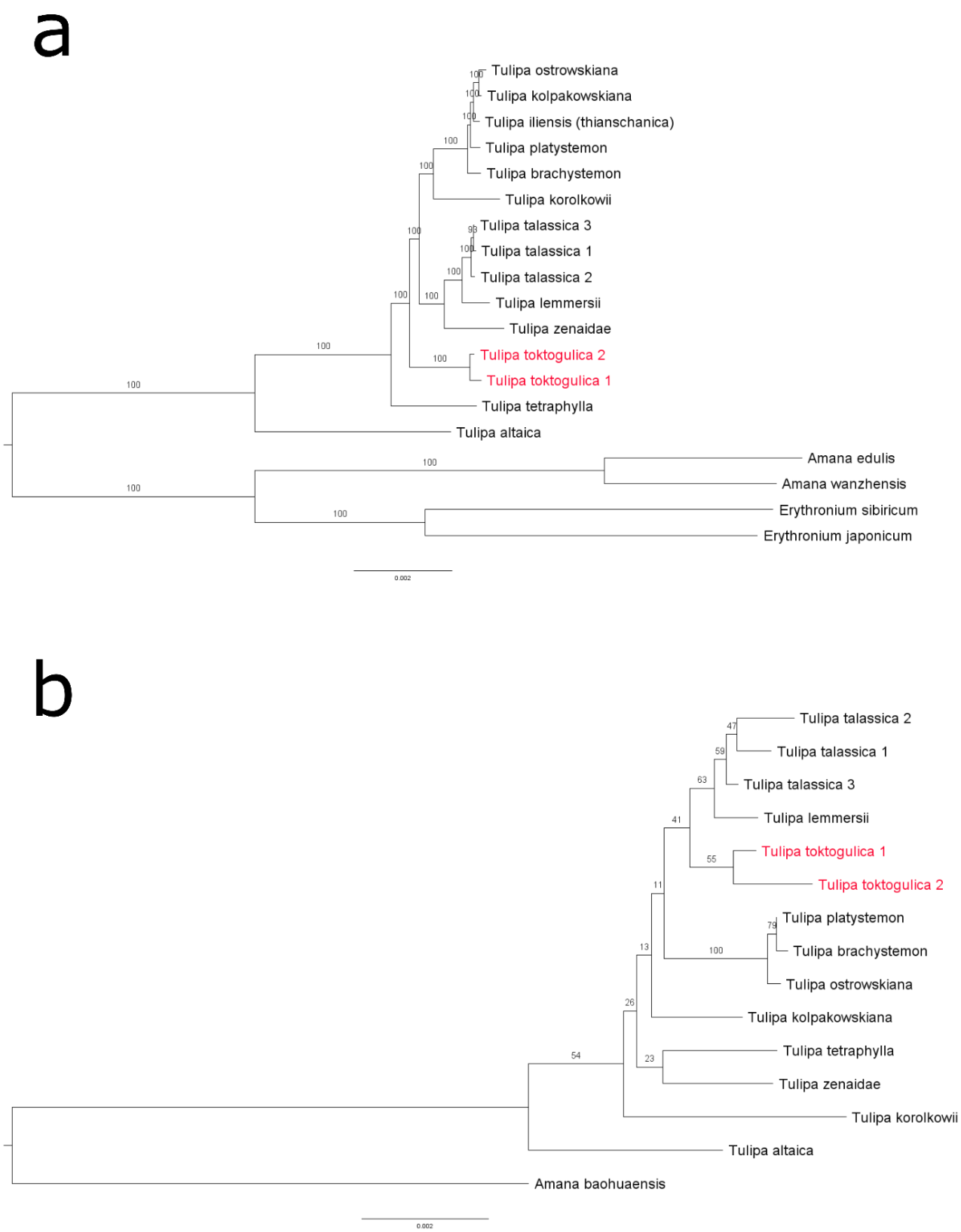


Figure 3.2. Phylogeny of the new species and its close relatives. (a) Multi-partition plastome tree rooted using *Erythronium* and *Amana*. (b) 35S rDNA tree rooted using *Amana*. New species shown in red on both trees and non-parametric bootstrap values shown on branches.

Table 3.3. Morphology of *Tulipa toktogulica* compared to its most similar species. Morphological traits assessed are listed in column one with the morphological traits of *T. toktogulica* and three other closely related tulip species detailed in the other columns.

	T. toktogulica	***T. talassica***	***T. ferganica***	***T. tetraphylla***
Usual leaf number	3	3	3	4-5 (occasionally 3)
Leaf position	Leaves spaced on stem	Leaves spaced on stem	Leaves spaced on stem	All growing from the base
Leaf properties	Mostly glabrous, but sometimes hair on leaf edge	Mostly glabrous, but sometimes hair on leaf edge	Hair on leaf edge or across leaf surface	Mostly glabrous, but sometimes hair on leaf edge
Basal leaf properties	Linear	Lanceolate	Lanceolate	Linear
Stem properties	Glabrous	Glabrous	Short hairs on stem	Glabrous
Flower number	1	1	1	1
Flower colour	Yellow	Yellow	Yellow	Yellow
Filament properties	Conical and conical-ovate	Tubular	Tubular	Conical and conical-ovate
Outer tepal colour	Yellow inside with mostly red exterior with small yellow margin and with central green-yellow teardrop shaped blotch	Yellow inside with mostly red exterior with yellow margin, has a faded yellow teardrop shaped blotch in centre.	Yellow inside with mostly red exterior with small yellow margin and with central green-yellow teardrop shaped blotch	Yellow inside with mostly red exterior with small yellow margin and with central green-yellow teardrop shaped blotch
Scent	Slight scent	No scent	No scent	No scent
Bulb tunic	Prolonged, slightly leathery, with small adpressed hairs on the top and bottom	Prolonged, slightly leathery, with small adpressed hairs on the top and bottom	Not prolonged, hard and brittle tunic with small adpressed hairs on the top and bottom	Not prolonged, hard and brittle tunic with small adpressed hairs on the top and bottom
Habitat	Stony soil in pastureland and foothills surrounding the north-east end of the Toktogul reservoir	Stony soil in the foothills of the Talas mountains	Stony slopes in the foothills of the Tien Shan mountains surrounding the Fergana valley and Toktogul depression	Stony slopes and pastureland in the Tien Shan Mountain range
Country	Kyrgyzstan	Kyrgyzstan	Kyrgyzstan, Uzbekistan	China, Kazakhstan, Kyrgyzstan

closely related to *T. lemmersii* across all trees, with the plastid phylogeny also showing support for *T. talassica* being more closely related to *T. zenaidae, T. ostrowskiana*, *T. platystemon*, *T. korolkowii*, *T. kolpakowskiana*, *T. brachystemon* and *T. iliensis* than to *T. toktogulica*. Moreover, this new species is genetically distinct from closely related *T. tetraphylla,* diverging later in the *Kolpakowskianae*, and also from *T. ferganica* Vvedensky (1935: 148), both of which occur in the same area (Figure 2.2). Notably, it is geographically separated from *T. talassica* by the Talas Ala-Too Mountains, whereas both *T. lemmersii* and *T. zenaidae* are also separated from *T. toktogulica* by large mountain ranges to the north and west. Overall, this new taxon could be considered a cryptic species because it is morphologically similar to several other species (but genetically distinguishable) and geographically separated from closely related taxa. This new description adds to the increasing number of endemic species described from around the Toktogul area, including *Polygonum (Atraphaxis) toktogulicum*, emphasising the importance of this region for biodiversity. In addition, this area falls within the Mountains of Central Asia Biodiversity Hotspot, which is of particular conservation interest (Critical Ecosystem Partnership Fund, 2016). This new species is considered endangered and warrants urgent conservation attention.

3.5. Taxonomy

Tulipa toktogulica B.D.Wilson & Lazkov, ***sp. nov.*** (Figure 3.3)

Type:—KYRGYZSTAN. Jalal-Abad Province: Toktogul distr., Sussamyr Mt. R., south-east facing slope, north of Sary-Seget and Bel-Aldy villages, steppe vegetation with occasional shrubs, pastureland, 41.95854N, 73.28587E, 1670 m, 15 April 2022, *Lazkov, Shalpykov, Wilson 135* (holotype: FRU; isotypes: CGE, FRU, K, LE).

This species is most similar to *Tulipa talassica* due to its prolonged tunic but the new species has broad stamens like those of *T. tetraphylla*, but it has only three leaves, unlike *T. tetraphylla* which usually has four or more leaves. Flowers of the new species have a faint scent, which is not present in these other Central Asian species except *T. kolbintsevii* Zonneveld in Zonneveld & de Groot (2012: 1294).

Perennial geophytes with ovoid bulbs, 15–25 mm in diameter, tunics light brown, prolonged, soft, papery and adpressed hairs at base and beak of inside of bulb tunic. Leaves three, greyish green with red edges near end of leaf, linear, narrow, lanceolate. Bottom two leaves similar length with basal leaf wider, upper leaf narrowest and shortest. Lower leaf 125 ×15 mm (85–193 × 10–20 mm), second leaf 122 × 8 mm (83–195 × 4–11 mm), upper leaf 99 × 5 mm (62–153 ×

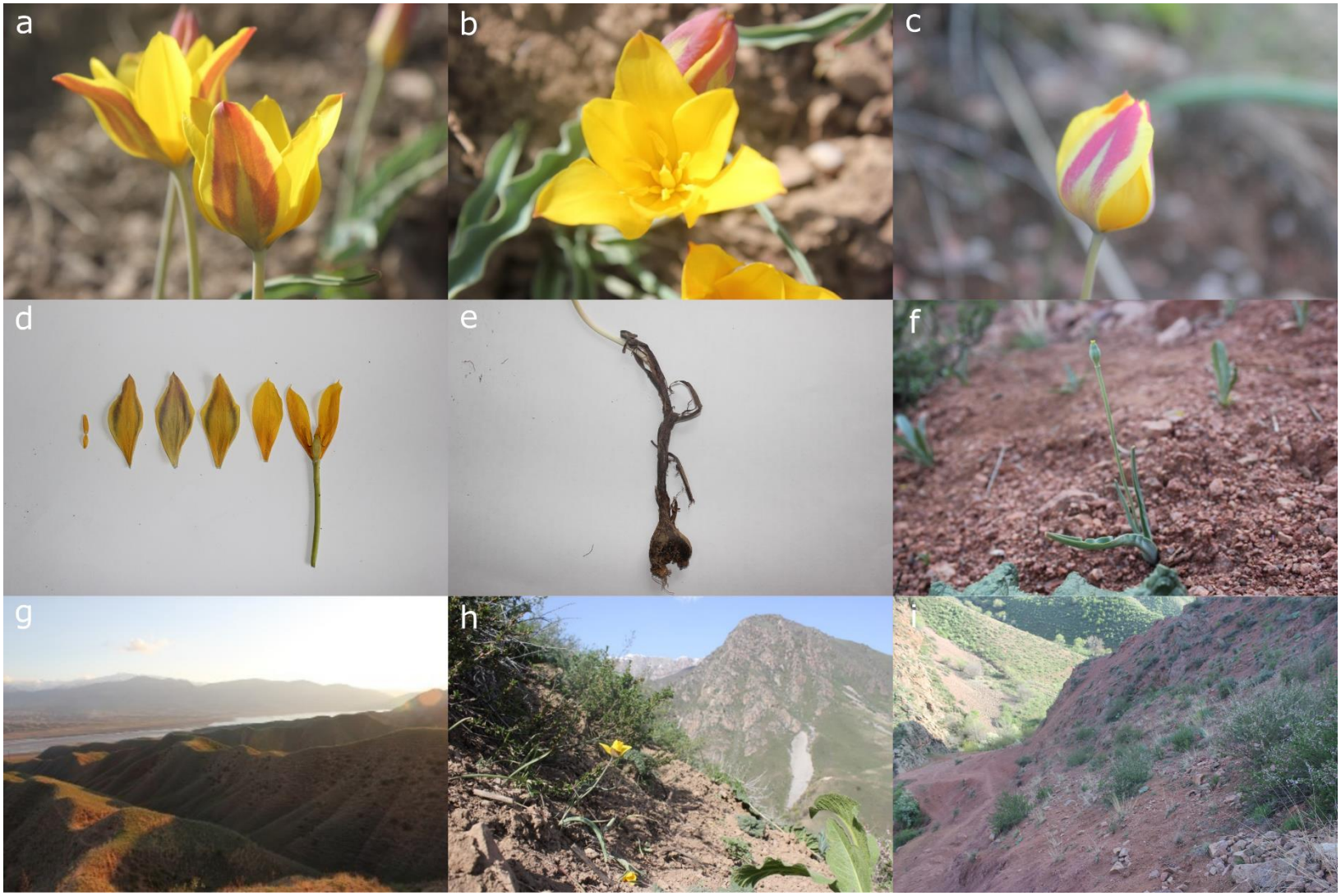


Figure 3.3. The new species and its habitat. (a) Side view of a flower. (b) Flower from above. (c) Side view of a closed flower. (d) Inner and outer tepals, stamen and ovary. (e) Prolonged tunic on bulb. (f) Seed pod. (g) Habitat at population one. (h) Habitat at population two and three. (i) Habitat at population four. Photos by Brett Wilson.

2–8 mm). Plant 133 mm tall (106–191 mm), stem glabrous, 102 mm long (81–155 mm), flower 31 mm long (25–36 mm). Solitary flower, slightly fragrant. Inner tepals less open than outer tepals causing it to be bucket shaped. Inner tepal approximately 35 × 14 mm, outer tepal 38 × 17 mm. Inner tepal oblong-obovate, tapering to point. Outer tepals rhombic, narrowing to point. Tepals primarily yellow, outwardly mostly red with small yellow margin and yellowish green tear-drop shaped central blotch. Stamens around 12 mm, approximately a third the length of the inner tepal. Filament 5 mm long, broad (Figure 3.4), conical-ovate. Anther 7 mm long, oblong-elongate, with ridges. Anther and filament both yellow, glabrous, and of similar width. Ovary usually green but sometimes yellowish with a short sessile yellow stigma. Ovary 10 mm long, longer than filament but shorter than stamen. Seed capsule is triangular in cross section with a small middle ridge on each side and a short yellow pistil.

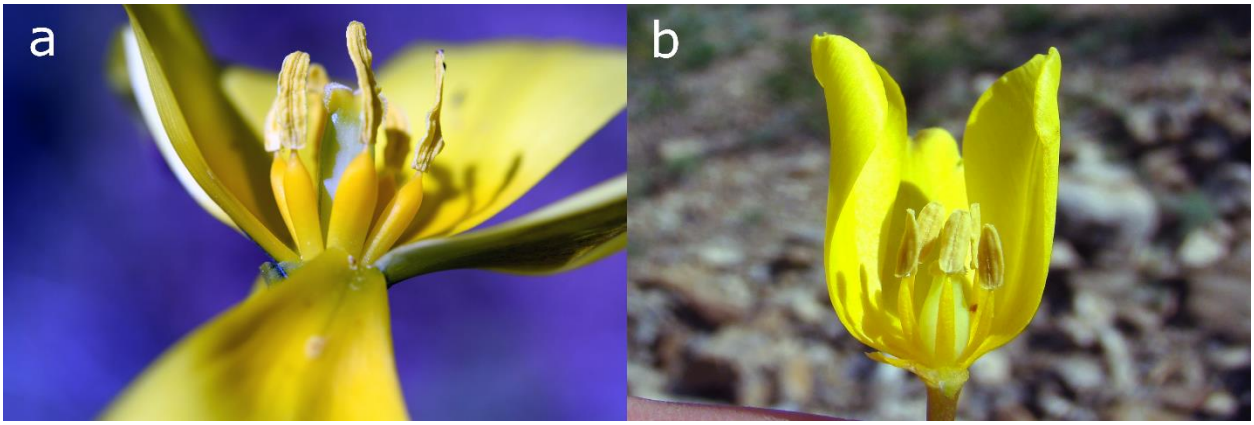


Figure 3.4. Photos showing the broader stamens in *Tulipa toktogulica* compared to *T. talassica*. (a) *Tulipa toktogulica*. (b) *Tulipa talassica*. Photos by Brett Wilson and Georgy Lazkov.

3.5.1. Etymology

Named after the Toktogul region. We hope that naming it after this area will improve awareness of the diversity of the flora of this region and hence its conservation.

3.5.2. Distribution and habitat

Known thus far from four populations occurring to the north-east of the Toktogul Reservoir. One population occurs in pastureland of the Zagyra Mountains to the south-east of Torkent (population one), several populations are known from just north of Sary-Seget and Bel-Aldy villages (populations two and three) and a fourth near where the Bordoo-Kia River joins the larger Torkent River. Specimens were collected from all these populations, but the type

specimen was collected from population three, and only sterile specimens were obtained from population four. The populations found growing north of Sary-Seget and Bel-Aldy villages, including the type location, were located on south-east facing slopes not far from the dusty track leading up the valley at 1670 m, where there was clear evidence of some livestock grazing. The population growing in the Zagyra Mountains was found growing on relatively bare slopes in brown, clayey soil at 1077 m. There was evidence of heavy grazing in the area by cows, sheep and goats, with much of the vegetation damaged. The population found growing near the convergence of the Bordoo-Kia River with the Torkent was found only in fruit, growing on a steep south-east facing slope in the valley of the Bordoo-Kia River, 1138 m elev.

3.5.3. Phenology

Flowering in early to mid-April, around the same time as *Tulipa tetraphylla.* Lower-elevation populations of this species usually flower in early April and release seeds when populations at higher elevations are in flower.

3.5.4. Ecology

In savannah-like vegetation, primarily in shrubland on stony-gravelly soil. It grows near populations of *Tulipa ferganica*, *T. greigii* and *T. tetraphylla.* The last two are often in flower at the same time as this species. At the type locality, it is found with *Alcea nudiflora*, *Carex turkestanica*, *Euphorbia virgata*, *Ferula kuhistanica*, *Festuca valesiaca*, *Lathyrus pratensis*, *Nepeta cataria*, *Origanum vulgare* subsp. *gracile*, *Prangos pabularia*, *Rheum wittrockii*, *Rosa kokanica*, *Spiraea hypericifolia* and *Verbascum songaricum*.

3.5.5. Conservation status

Occurring at three locations consisting of four populations (Figure 3.1). The conservation status of a new species is often precarious (Liu *et al.*, 2022), and this species follows this trend. The area of occupancy (AOO = 12 km^2) and the extent of occurrence (EOO = 12 km^2) are extremely small. There is no clear estimate of how large these populations are, but they are likely below 1000 individuals based on our field observations. Crucially, the species is not known to occur in any protected area and has only recently been added to two *ex-situ* collections explicitly the Cambridge University Botanic Garden in the U.K. and Gareev Botanical Garden in Bishkek, Kyrgyzstan. However, it is believed to be widespread in the Toktogul area with unrecorded populations likely to be discovered soon. *Tulipa toktogulica* is assessed as endangered B1ab(iii) + B2ab(iii) due to the extremely small estimated AOO and EOO; it is only known from three locations and habitat quality is thought to be in decline in areas of the distribution of this species

due to ongoing threats from livestock overgrazing and climate change. Focused efforts to record and monitor more populations of this species are needed, especially to assess whether it occurs in any protected areas, as well as collection of bulbs and seeds to ensure this species is protected in *ex-situ* collections both nationally and internationally. The status of this species may change in the future due to the discovery of new populations.

Population one was found growing in heavily grazed pastureland near the Toktogul reservoir where habitat degradation is clearly an issue (Figure 3.3). Populations two, three and four were found on steep slopes at higher elevation where grazing pressure is less but still present. Several other *Tulipa* species occur in the same area, such as *T. tetraphylla, T. greigii* and *T. ferganica*, which are already recognised as threatened in the Kyrgyz Red Book (Davletkeldiev, 2006). Several of these are morphologically similar and could be easily mistaken for this species, so assessment of populations needs to be undertaken carefully.

Populations of these other species have been reported to be under threat from livestock overgrazing across this area. This new species is also threatened by climate change that is predicted to lead to significant loss of tulip habitat across Central Asia through changes in rainfall and temperature patterns (Wilson *et al.*, 2021). Finally, opportunistic collection of wild tulips has also been observed in the Toktogul area, which may lead to diminishing wild populations. However, collecting occurs only at a small scale only near settlements, and there is no established trade driving extreme specimen removal. It is therefore likely only a minor threat for this species.

Chapter 4.

Divergence times, biogeography, and genome size evolution

In this chapter we use molecular dating techniques to map our phylogeny to evolutionary time. This dated tree is then used in further analyses to explore the historical biogeography of wild tulips as well as assess the taxonomic informativeness of the trait genome size, which has often been used in taxonomic decisions in this genus. Using these outputs we piece together the likely evolutionary history of this genus, specifically assessing the probable origin of tulips and exploring how the geological events of Central Asia have impacted the diversification of this flower.

4.1. Introduction

Tulips are a group of perennial geophytes that have become known worldwide. The economic home of tulips is the Netherlands with around 88% of worldwide land used to grow cultivated varieties found in this country (Orlikowska *et al.*, 2018). Wild tulips can be found across much of Eurasia with the primary centre of diversity in Central Asia and a secondary centre of diversity across Turkey, Iran, and the Caucasus (Hoog, 1973; Botschantzeva, 1982). We currently estimate there are 96 species in this genus (Table 2.11), with other recent estimates varying between 76-86 species (Zonneveld, 2009; Christenhusz *et al.*, 2013). Notably, there have been a number of new species recently described (De Groot and Zonneveld, 2022; Rukšāns and Zubov, 2022) and so understanding of diversity in this genus is constantly changing. Historically, Central Asia has been hypothesised as the origin of tulip diversity due to the enormous variety of species found there (Hoog, 1973; Botschantzeva, 1982), with an estimated 62 tulips found in the region. There currently remains no robust evidence to support this theory, but with wild tulips of increasing interest in the horticultural world, primarily due to their potential genetic resources for future breeding (Orlikowska *et al.*, 2018), there is an increasing need to understand their evolutionary history, including their origin and what drove this genus to diversify and disperse to encompass its current range.

Already some broader studies of evolutionary history have included a subset of tulip species (Givnish *et al.*, 2016; Liu *et al.*, 2017; Kim and Kim, 2018) and the diversification of closely related clades have been explored in some detail (Allen, Soltis and Soltis, 2003; Huang *et al.*, 2018; Kim and Kim, 2018). This literature estimates that the ancestral tulip arose between the end of the Oligocene period and the early Miocene (Table 4.1), specifically around 20.74 million years ago (Mya), somewhere in Eurasia (Kim and Kim, 2018), most likely East Asia (Givnish *et*

al., 2016). The most recent common ancestor (MRCA) of the *Amana* genus, which forms a sister clade to the *Tulipa* with *Erythronium,* likely existed somewhere within eastern Eurasia also (Kim and Kim, 2018), whilst *Erythronium* is thought to have originated 24.38 Mya and have shared a common ancestor with tulips and the rest of the tribe Tulipeae in Asia around 57.63 Mya. However, current evidence suggests that the ancestor of the *Erythronium* clade itself evolved in North America supporting the view that this clade shifted and established itself on a new continent (Kim and Kim, 2018). So, although the broader evolutionary history of the genus *Tulipa* is still to be uncovered, previous work shows it is likely therefore that tulips, much like many closely related genera, and unlike Erythronium, originated and diversified somewhere in Asia, with Central Asia a likely candidate.

Table 4.1. Geological time scale of epochs and ages during the existence of the genus *Tulipa.* The Epoch, name of each each age and the beginning of each age in million years ago is detailed.

Epoch	**Age**	**Began (Mya)**
Holocene	Meghalayan	0.0042
	Northgrippian	0.0082
	Greenlandian	0.0117
Pleistocene	Late Tarantian	0.129
	Chibanian	0.774
	Calabrian	1.8
	Gelasian	2.58
Pliocene	Piacenzian	3.6
	Zanclean	5.333
Miocene	Messinian	7.246
	Tortonian	11.63
	Serravallian	13.82
	Langhian	15.97
	Burdigalian	20.44
	Aquitanian	23.03
Oligocene	Chattian	28.1
	Rupelian	33.9
Eocene	Priabonian	37.8
	Bartonian	41.2
	Lutetian	47.8
	Ypresian	56.0

Central Asia is dominated by the mountain systems that traverse the region, and the corresponding valleys and steppe land. The mountains of Central Asia are a biodiversity hotspot (Critical Ecosystem Partnership Fund, 2016) where over 5000 species of vascular plant occur, one quarter of which are endemic to the region, and where an array of crop-wild relatives can

also be found. The process of mountain building has been proposed as an important driver of diversification (Antonelli *et al.*, 2018) and so the orographic history and current landscape of Central Asia may have been an important factor in speciation and the evolution of new groups (Muellner-Riehl, 2019). In addition, the regression of the Paratethys sea in this region has led to an ever changing landscape of habitat areas (Li *et al.*, 2020). A number of plant groups have their origin in Central Asia with one of the most notable being wild apples (Cornille *et al.*, 2014). Broadly, the historical climatic changes in the region in the last thirty or so million years have shown a trend towards increased aridification and a cooler climate (Lu, Wang and Li, 2010; Tang *et al.*, 2011) as the Paratethys sea receded (Ramstein *et al.*, 1997), orographic changes occurred (Manabe and Broccoli, 1990), and uplift of the Qinghai-Tibetan plateau took place (Miao *et al.*, 2012). In this region there has long been a mosaic of landscapes including large expanses of open grassland and steppe partly maintained by large grazers (Hui *et al.*, 2011; Miao *et al.*, 2012), while deserts (Graham *et al.*, 2019) and the Paratethys sea have created geographical barriers (Li *et al.*, 2020). The climate and open habitat areas favour a geophytic life history, which is associated with seasonal habitats and arid, cool conditions (Howard *et al.*, 2019; Tribble *et al.*, 2021) and therefore unsurprisingly Central Asia has a large range of geophytes, including many endemics (Tojibaev *et al.*, 2018).

Geophytes are known to have large genome sizes (Veselý *et al.*, 2012) with these larger genome sizes associated with fast development and early flowering once dormancy is over, especially useful in seasonal environments (Veselý *et al.*, 2012). Recent work has led to the sequencing of an entire tulip genome (ISAAA, 2017), whilst an array of genome size measurements have been taken for many different species in this genus (Zonneveld, 2009). This work highlights that tulips, like most geophytes, also have large genome sizes with the nuclear genome estimated at 34Gb in size; ten times bigger than the human genome (ISAAA, 2017). Although the evolutionary purpose of this trait remains tenuous (Beaulieu, Leitch and Knight, 2007) it has been commonly used to determine unique evolutionary groupings within the genus (Zonneveld, 2009). A range of higher level taxonomic decisions have been made based primarily on this trait, whilst it has also been used to determine a range of species (Zonneveld, 2009). In addition, it has been, and continues to be, used frequently in the description of new species (Zonneveld and De Groot, 2012; Zonneveld, 2015; De Groot and Zonneveld, 2022). Yet, there remains a significant lack of evidence as to whether this trait has the evolutionary signal to support these decisions. An assessment of its phylogenetic signal is urgently needed to assess this and the corresponding taxonomic groupings it has impacted.

With the advancement of genetic methods, new ways to explore evolutionary history have arisen with divergence time dating and biogeographical analyses based on phylogenetic relationships becoming increasingly robust (Cardillo *et al.*, 2017; Noben *et al.*, 2017; Seidl *et al.*, 2020), and the diversification of clades connected with geological events (Zhang and Fritsch, 2010; Kim and Kim, 2018; Ji, Yang, *et al.*, 2019). Moreover, phylogenetic comparative methods can be used to model trait evolution, which can enable the inference of evolutionary processes (Revell, 2013) and assess traits for their phylogenetic signal and therefore taxonomic usefulness. The evolutionary history of tulips has long been shrouded in mystery with Central Asia often cited as the origin of the genus based on little evidence. Now with modern phylogenetic methods and a relatively complete species phylogeny available, it is possible to resolve long-standing issues in diversification history, biogeography, and trait evolution in the genus *Tulipa*. In this chapter, we undertake the first ever analysis of how tulip phylogenetics can inform us about where, when, and why tulips evolved. Piecing together an evolutionary story that will not only inform academic understanding about tulips, but could also influence conservation priorities (Williams *et al.*, 2017; Fu *et al.*, 2022).

4.2. Materials and Methods

4.2.1. Molecular dated phylogeny

Given the similarity in topology between the CDS only inferred tree (Appendix 4) and the tree inferred using the full plastome dataset (Figure 2.2), and the computationally-intensive nature of Bayesian analyses, we decided to proceed using only CDS data for molecular dating. Initially we reprocessed the 78 CDS alignments already developed for chapter two, removing duplicate tips so that each species was represented only once (Table 2.7). To do this we initially relabelled tips using the pxrls tool from phyx (Brown, Walker and Smith, 2017), before removing duplicates of species from the alignments using the pxrms tool from phyx. In cases where a species was non-monophyletic the removal of tips was based on the best estimate of the true position of each species relying on previous taxonomic literature (Zonneveld, 2009; Christenhusz *et al.*, 2013) alongside an assessment of the reliability and representativeness of each sampled specimen. The CDS regions were then realigned using MAFFT (--auto) (Katoh and Standley, 2013) and the alignments cleaned using pxclsq from phyx, retaining columns with at least 10% occupancy (-p 0.1). The 78 CDS regions were then concatenated to produce a supermatrix using the phyx tool pxcat (Brown, Walker and Smith, 2017).

For this element of the project an initial species phylogeny was also required to act as a starting tree for the dating process. We chose to use the species phylogeny inferred using plastome

data and through a multi-partition approach generated in chapter two (Figure 2.8). To serve as a starting tree concordant with node constraints, this phylogeny was adapted to make branch lengths proportional to time, enforcing the exact constraint age at two calibrated nodes taken from Kim and Kim (2018); with the root of the tree (node 1) aged at 36 Mya (95% highest posterior density (HPD) = ~23.0-47.0) and the *Tulipa* genus crown node (node 2) aged at 20.74 Mya (HPD = 10.2-23.99). This was done using penalised likelihood in the chronos function in ape v5.0 (Paradis and Schliep, 2019) implemented in R version 4.2 (R Core Team, 2022), with a correlated clock model and lambda = 0.1.

Initially we chose to implement a multi-partition approach with each CDS position treated as a separate partition, and further partitioning based on codon position implemented leaving 234 separate partitions. We developed a best scheme partition model for the prepared supermatrix using the IQ-TREE ModelFinder software (Kalyaanamoorthy *et al.*, 2017), specifically the relaxed hierarchical clustering algorithm with rcluster set to 90 (Lanfear *et al.*, 2014), and identified the best evolutionary models for each partition. This software clustered the initial 234 partitions into only 11 separate partitions. The supermatrix was then converted to a nexus file using the Phyx tool pxs2nex (Brown, Walker and Smith, 2017) with 11 partitions specified. The nexus file was then processed in BEAUti to generate an XML file with the substitution model for each partition set to the GTR+F+G model, a relaxed lognormal clock model selected, and a Yule speciation process implemented for the tree, whilst the prepared species phylogeny was added to fix the topology in the dating process. An initial run of this XML file was implemented in BEAST v2.6.2 (Bouckaert *et al.*, 2019), however, the multi-partition approach proved to be computationally intractable, likely due to model complexity and the number of tips in the dataset. Furthermore, and more importantly, there is limited understanding of how partitioning interacts with calibration points in Bayesian molecular dating and so best practice when using these approaches has not been established (Angelis *et al.*, 2018). In light of both these constraints we decided to use our sequence data as a single partition, redoing the analysis with all other settings kept the same.

A finalised dated phylogeny was generated using secondary calibration information from the dated phylogeny of the Liliaceae in Kim and Kim (2018) as fossil evidence is not available for the tribe Tulipeae. We set the root of the tree (node 1) to 36 Mya (95% highest posterior density (HPD) = ~23.0-47.0) and the *Tulipa* genus crown node (node 2) at 20.74 Mya (HPD = 10.2-23.99). These calibration points were assigned as normally distributed priors with the mean set to their mean age and with as much of the 95% HPD captured in this distribution as possible.

This meant that node 1 had a sigma of 4.0 and node 2 of 3.5. A Bayesian analysis using a Markov chain Monte Carlo (MCMC) approach was implemented in BEAST v2.6.2. (Bouckaert *et al.*, 2019). Three separate runs of 500 million generations with parameters and trees being sampled every 1000 generations were implemented. The output logfiles were analysed separately and combined in Tracer v1.7.1 (Rambaut *et al.*, 2018) to assess convergence of parameters using the estimated sample size (ESS) of parameters, with over 200 considered adequate. The ESS of all parameters across all three runs of the BEAST programme, as well as when logs were combined, were all much larger than 200. Due to the excellent mixing of all three runs and the extremely high ESS of all parameters we combined runs into a single dataset. These separate chains were combined in LogCombiner v2.6.2, with a burnin of 25%. A consensus maximum clade credibility tree was generated using a subsample of 100,000 random post-burn-in trees in TreeAnnotator v2.6.2 and visualised in FigTree v1.4.4 (http://tree.bio.ed.ac.uk/software/figtree/).

A Lineage-Through Time analysis was undertaken using the ltt function in the R package Phytools (Revell, 2012). The rate of species diversification was assessed using a simple gamma statistic built into this function (Pybus and Harvey, 2000) with a Monte Carlo constant rates (MCCR) test approach used to account for incomplete lineage representation using 10,000 simulations with Rho set to 79/96 (the fraction of species represented from all those known) and the gtt function used to assesses γ across multiple time segments.

4.2.2. Biogeography

To assess the biogeographical dynamics of the genus *Tulipa* initially a number of subregions of the global distribution had to be selected. To gather information on how best to do this we downloaded all *Tulipa* coordinate points from the Global Information Biodiversity Facility (GBIF.org (10 August 2021) GBIF Occurrence Download https://doi.org/10.15468/dl.dht7nu). We then stringently cleaned these using the R package CoordinateCleaner (Zizka *et al.*, 2019) and manually removing datapoints that we deemed to be unreliable. Specifically, we filtered *Tulipa* distribution data by countries which are known to be a part of the natural distribution of the genus (Christenhusz *et al.*, 2013; POWO, 2021), then used CoordinateCleaner to remove points that fall in cities, institutes or in the ocean. We then removed points with an individual count < 0 and 100 <, points from before the year 1945 when GPS was not broadly in use, those without species names, and duplicate records. We then specifically removed data for *Tulipa* × *gesneriana* and *T. praecox* as these names have historically been used incorrectly (Christenhusz *et al.*, 2013; Christenhusz, Fay and Govaerts, 2013) as well as records of *T.*

agenensis and *T. clusiana* in Europe where they are only known to be naturalised. We finally removed tulips from Spain and Portugal above 38 degrees latitude as this is approximately where the natural distribution ends as well as above 56 degrees latitude in Russia and around the Moscow region as these are not part of the natural distribution also. This dataset was then used to assess if the natural distribution could be effectively split up using the software Infomap Bioregions (Edler *et al.*, 2017). Unfortunately, due to the lack of data for many species and the heavy bias towards one or two commonly recorded species in Europe and the Ukraine/Russia the Infomap Bioregions software did not adequately identify relevant regions for analysis (Figure 4.1). We were therefore forced to split the natural distribution up manually, based on the natural distribution and the identified biodiversity hotspots (Christenhusz *et al.*, 2013), creating two maps limiting the number of areas to five (5A) or six (6A) (Figure 4.5) due to computational power limitations, especially given some tulip species occur across all designated regions (Matzke, 2018).

For biogeographical analysis we tested two different software packages, the R package BioGeoBEARS v1.1.2 (Matzke, 2014, 2018), and the Reconstruct Ancestral State in Phylogenies software, RASP 4 (Yu, Blair and He, 2020); RASP also runs BioGeoBEARS as well as a number of other methods for ancestral state reconstruction. As inputs for both software we used the dated phylogeny generated using BEAST, which had subsequently been cleaned of the Duc van Tol tip as this is a horticultural variety using pxrmt from phyx (Brown, Walker and Smith, 2017). A geographic file was developed detailing the occurrence of species across our chosen regions, with a maximum number of regions in which a species could occur set to five or six for the 5A and 6A models respectively. In addition, we created a dispersal matrix that specified that tulips could only migrate to regions that are connected via direct land borders given tulips limited ability to dispel pollen or seeds far (Kashin, Kritskaya and Schanzer, 2016). Models were run with and without this dispersal matrix. The models were assessed using their Akaike information criterion (AIC) value. We tested DIVALIKE, DEC, and BAYAREALIKE models as well as these models with an added +J function, although this extra parameter is sometimes considered inappropriate (Ree and Sanmartín, 2018). Finalised analyses were undertaken in the BioGeoBEARS package on R, as this allowed for easier comparison of models.

4.2.3. Genome size evolution

A database of the genome size of tulip species was developed collating data from the literature (Zonneveld, 2009, 2015; Shuka, Tan and Krasniqi, 2012; Zonneveld and De Groot, 2012;

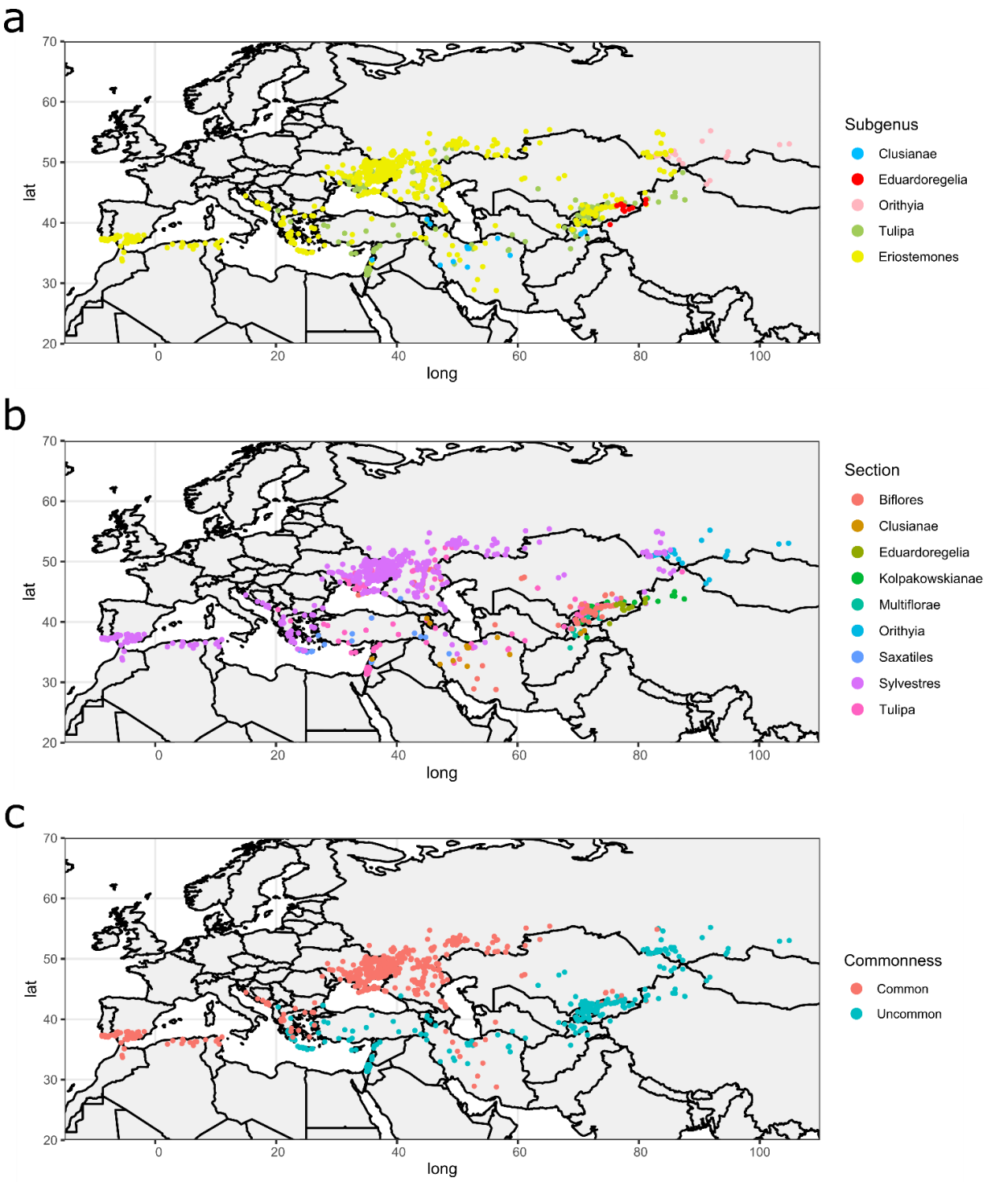


Figure 4.1. Geographic distribution of GBIF data for *Tulipa.* (a) – split by subgenus, (b) split by section, (c) split to show the data for the three most 'common' species and all other species here designated 'uncommon'.

Everett, 2013; Tojibaev, De Groot and Naralieva, 2014; De Groot and Tojibaev, 2017; De Groot and Zonneveld, 2020). To create this database we recorded the measured size of a single set of chromosomes for each species, specifically dividing its 2C value by its number of chromosome sets; for *Tulipa ostrowskiana,* which is a tetraploid, we therefore divided its 2C value by four. We did not include species present on the phylogeny in the database if there was no data available for their genome size. The 1C value of each species was then mapped to our phylogeny and ancestral states estimated using the contMap tool of the Phytools R package (Revell, 2012). Specifically, we used the anc.ML method, which imputes the trait value for those species in the phylogeny that are missing data. The genome size data was tested for normality using the Shapiro-Wilk test of normality (Shapiro and Wilk, 1965) and then assessed for phylogenetic signal using both Blomberg's K (Blomberg, Garland JR. and Ives, 2003) and Pagel's λ (Pagel, 1999) statistics in the Phylosig function of the Phytools package (Revell, 2012).

4.3. Results and Discussion

Central Asia has long been proposed as the birthplace of tulips (Hoog, 1973; Botschantzeva, 1982) alongside other plants such as apples (Cornille *et al.*, 2014). The majority of wild tulip species can be found in this region, and historically this area had climatic and orographic conditions that could have favoured the diversification of this geophytic lineage (Howard *et al.*, 2019, 2020). Yet, no formal evidence has previously existed to support an origin in Central Asia or link tulip diversification to the geological events that have occurred in this region. Using modern phylogenetic techniques we have created a dated phylogeny for tulips, which has allowed us to explore the evolutionary history of this plant. Here we provide the first significant evidence for biogeographic diversification of this genus across its evolutionary history linking it to geological events that have occurred in Central Asia during this time. In addition, we have explored how genome size relates to the evolutionary history of this genus and assessed its phylogenetic signal so as to validate its use in taxonomic decision making (Zonneveld, 2009; Veldkamp and Zonneveld, 2012). Overall, we present the first ever phylogenetic based insight into the origin, evolution, and diversification of wild tulips.

4.3.1. Molecular dated phylogeny

Here we must emphasise that molecular dating relies on an array of assumptions, which mean that any dated phylogenies must be carefully interpreted especially with respect to the selected models of evolution and priors (Bromham *et al*., 2018; Bromham, 2019). There is specifically a need for the molecular data to add an informative layer to the dating process so that it is not only the priors leading to the derivation of dates (Brown and Smith, 2018). In addition, fossil

evidence is often lacking for many lineages, with this the case for *Tulipa*. We have therefore, like many before us, been forced to use secondary calibration points in our molecular dating methods, despite their sole use having been criticised (Schenk, 2016). We have based our calibrations on the molecular dated phylogeny of Kim and Kim (2018), which did utilise fossil calibrations, yet even when fossil evidence is available molecular dates can be vague due to the uncertainty of the age of fossils (dos Reis *et al*., 2015). This means that secondary calibration inferred phylogenies, including ours, are already built on a foundation of ambiguous data. The use of more accurate evolutionary models and partitioned datasets is thought to minimise uncertainty in the dating process, but this relies on the prior assumptions being correct. Specifically partitioning datasets allows for different evolutionary rates across different sections of data. However, with current methods this process is often arbitrary with little biological relevance, so although it can lead to smaller confidence intervals these are not necessarily more informative (Angelis *et al.*, 2018). Molecular clock assumptions are also commonly violated, which in turn also imposes limits on precision (dos Reis *et al*., 2015). Together these constraints can mean that there is sometimes not enough resolution from current methods to construct reliable evolutionary narratives (dos Reis *et al*., 2015) and results can vary greatly due to the selected evolutionary model (Bromham *et al.*, 2018; Guindon, 2020).

Here we have generated a dated phylogeny using secondary calibrations of a single partition plastome dataset (Figure 4.2), which we acknowledge carries uncertainty, yet it is the best effort that could be made with current information. Molecular dating is primarily limited by our understanding of evolutionary processes and we recognise that even with the large amounts of data used here this fundamental understanding may still lead to inaccurate modelling (Bromham, 2019).This uncertainty carries forward to any post-analyses carried out based on our initial phylogeny, whilst some of these analyses also carry other elements of ambiguity. Specifically we note that the LTT analysis provides limited biotic scope (McCarthy, McGlone and Heenan, 2021) and that the biogeography analysis is constrained by assumptions of dispersal and extinction rates which may not be entirely accurate. We therefore have been extremely careful when interpreting the outputs of this chapter and advise that anybody reading this chapter follow suit. Specifically, we frequently present the mean dates of clades, however these dates often have broad confidence intervals (Table 4.2; Table 4.3) and so we urge the reader to bear this in mind and like our dated nodes, geological events also have ambiguity with regards to their timing and this is another layer of complexity to consider. Saying that, our work is based on a foundation of good data processed in a practical way to provide the first genetic based understanding of the evolutionary history of the genus *Tulipa*.

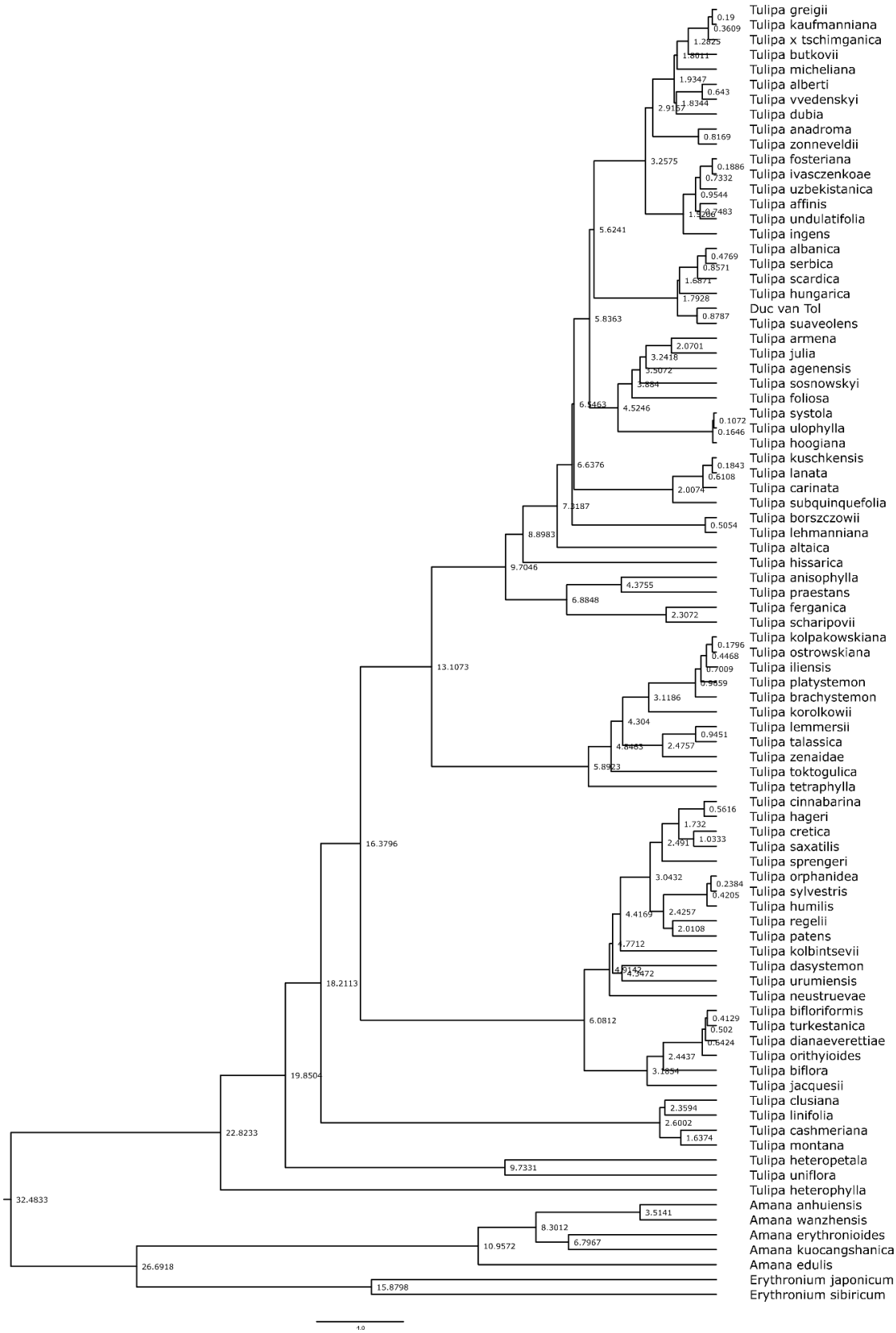


Figure 4.2. Molecular dated phylogeny of tulip species, including Duc van Tol horticultural variety. The mean age of each node on the tree is shown measured in millions of years ago.

The maximum clade credibility phylogeny shows that the most recent common ancestor (MRCA) of tulips is estimated to have existed around 22.82 Mya (Table 4.2), whilst tulips separated from their nearest relatives around 32.48 Mya (Figure 4.2; Table 4.3). The clade *Eduardoregelia* is the oldest subgenus of tulip (Stem age = 22.82 Mya), with the *Orithyia* the next subgenus to diverge (Stem age = 19.85 Mya), then *Clusianae* (Stem age = 18.21 Mya), before finally the *Tulipa* subgenus diverged from the *Eriostemones* (Stem age = 16.37 Mya) (Figure 4.3; Table 4.3). The latter two subgenera would then diversify to become the most species rich, whilst the *Eduardoregelia* remained a single species, the *Orithyia* diverged into only three species, and the *Clusianae* into five species. The section *Biflores* diverges in the subgenus *Eriostemones* around 6.08 Mya with the *Saxatiles*, and *Sylvestres* sections appearing around 3.04 Mya. The earliest section to diverge in the subgenus *Tulipa* is the *Kolpakowskianae* section around 13.11 Mya, with the sections *Multiflorae* and *Tulipa* coming into existence 9.71 Mya.

The dated phylogeny shows that speciation events leading to extant species were infrequent within the first 15 million years of the genus, however in the past ~7 million years a rapid increase in species has been observed across all areas of the tree except the *Orithyia* and *Eduardoregelia* subgenera. Explicitly, the lineage-through time plot and the calculation of the gamma statistic suggest it is appropriate to reject the hypothesis of a constant diversification rate across lineages ($\gamma = 2.720253$, $p = 0.0065$). An assessment of the γ statistic through time, showed that when the time period of existence of the genus was split into ten sections (n=10), there was clearly an initial period where more species were accumulating, before a period of speciation inertia, with a final period of rapid accumulation of species close to the present time across multiple subgenera (Figure 4.4). However, it is difficult to determine whether this is due to increased speciation events or decreasing extinction events (Fordyce, 2010). The MCCR test, which accounts for incomplete lineage representation, showed that diversification rates were not constant even when this was accounted for ($\gamma = 2.720251$ and $p = 6e\text{-}04$). This recent increase in species is primarily due to rapid diversification in the *Eriostemones* and *Tulipa* subgenera. Although the MRCA of *Tulipa* is 13.11 Mya its diversification has mostly taken place in the last 9 million years with the crown ages of the sections of *Kolpakowskianae*, *Multiflorae*, and *Tulipa*, the most diverse section, 5.89, 6.89, and 8.90 Mya respectively. The MRCA of the Eriostemones is even younger at 6.08 Mya with the sections *Biflores*, *Saxatiles*, and *Sylvestres* MRCAs diverging 4.91, 2.49, and 2.43 Mya respectively. Despite *Clusianae* being relatively species poor its diversification has also contributed to the growing number of species in recent times with its MRCA occurring only 2.60 Mya. Overall, we observe 73 of the 79

Table 4.2. Crown ages of all genera, subgenera, and sections in the dated phylogeny. Both the mean age and 95% highest posterior density are shown for each taxonomic group.

Taxonomic group	**Mean age (Mya)**	**95% Highest posterior density**
Genus		
Tulipa	22.82	17.99-27.69
Amana	10.96	7.45-14.76
Erythronium	15.88	10.07-22.59
Subgenus		
Eduardoregelia	22.82	17.99-27.69
Orithyia	9.73	5.86-13.74
Clusianae	2.60	1.57-3.75
Tulipa	13.11	9.89-16.47
Eriostemones	6.08	4.20-7.92
Section		
Sylvestres	2.43	1.47-3.36
Biflores	4.91	3.44-6.42
Saxatiles	2.49	1.69-3.30
Kolpakowskianae	5.89	4.08-7.92
Multiflorae	6.89	4.78-9.23
Tulipa	8.90	6.65-11.37

Table 4.3. Stem ages of all genera, subgenera, and sections in the dated phylogeny. Both the mean age and 95% highest posterior density are shown for each taxonomic group.

Taxonomic group	**Mean age (Mya)**	**95% Highest posterior density**
Genus		
Tulipa	32.48	26.14-39.33
Amana	26.69	19.38-34.22
Erythronium	26.69	19.38-34.22
Subgenus		
Eduardoregelia	22.82	17.99-27.69
Orithyia	19.85	15.51-24.36
Clusianae	18.21	14.18-22.47
Tulipa	16.378	12.52-20.22
Eriostemones	16.37	12.52-20.22
Section		
Sylvestres	3.04	2.12-4.03
Biflores	6.08	4.20-7.92
Saxatiles	3.04	2.12-4.03
Kolpakowskianae	13.11	9.89-16.47
Multiflorae	9.71	7.16-12.21
Tulipa	9.71	7.16-12.21

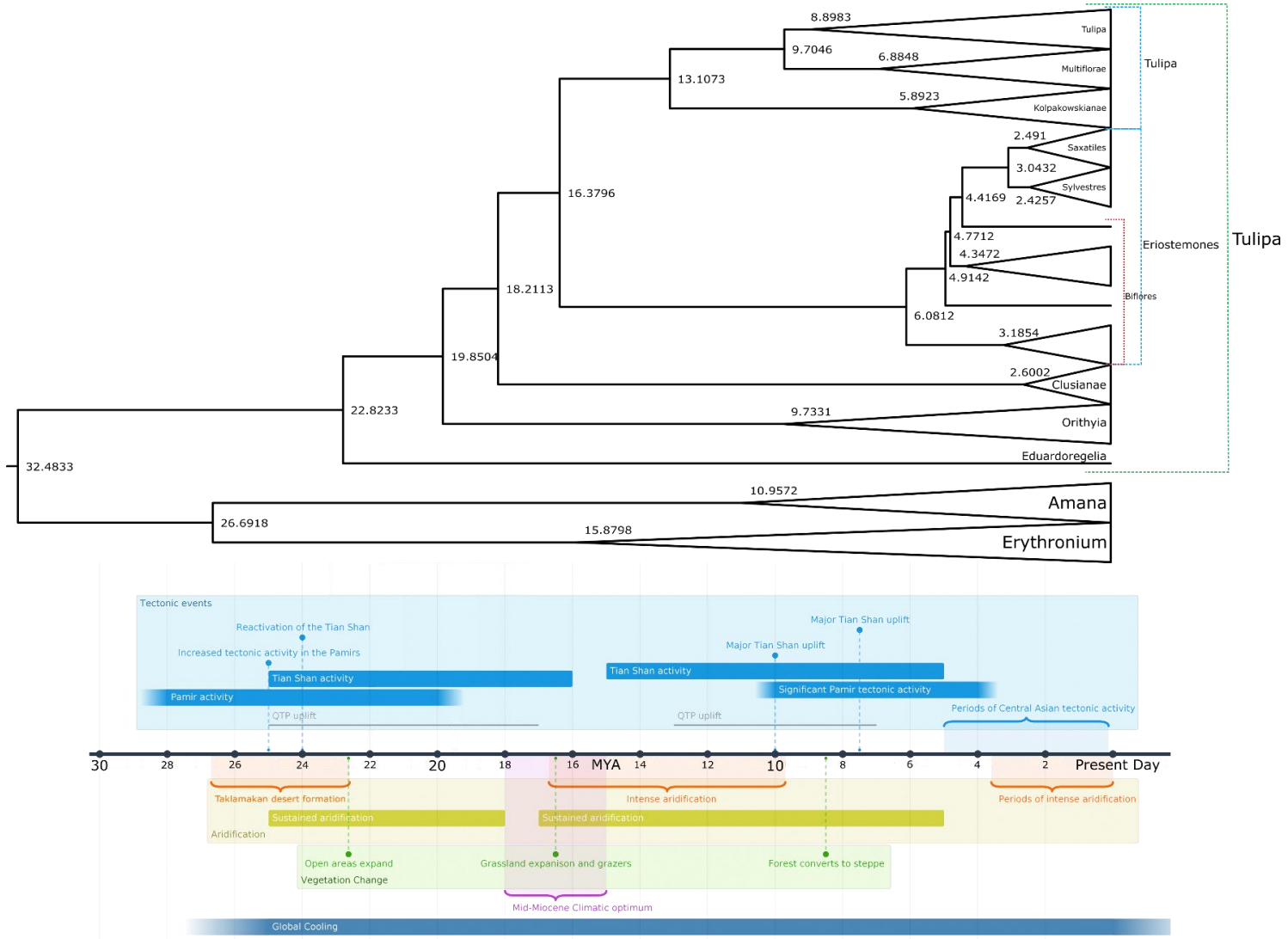


Figure 4.3. Dated phylogeny of the major taxonomic groups of the genus *Tulipa* with a basic timeline of geological events occurring in Central Asia during this period. The mean age of each node on the tree is shown measured in millions of years ago. Triangles at the tips show multiple species with single tips representing only a single species. The timeline represents a summary of the geological events reported in scientific literature broadly grouped by type of event.

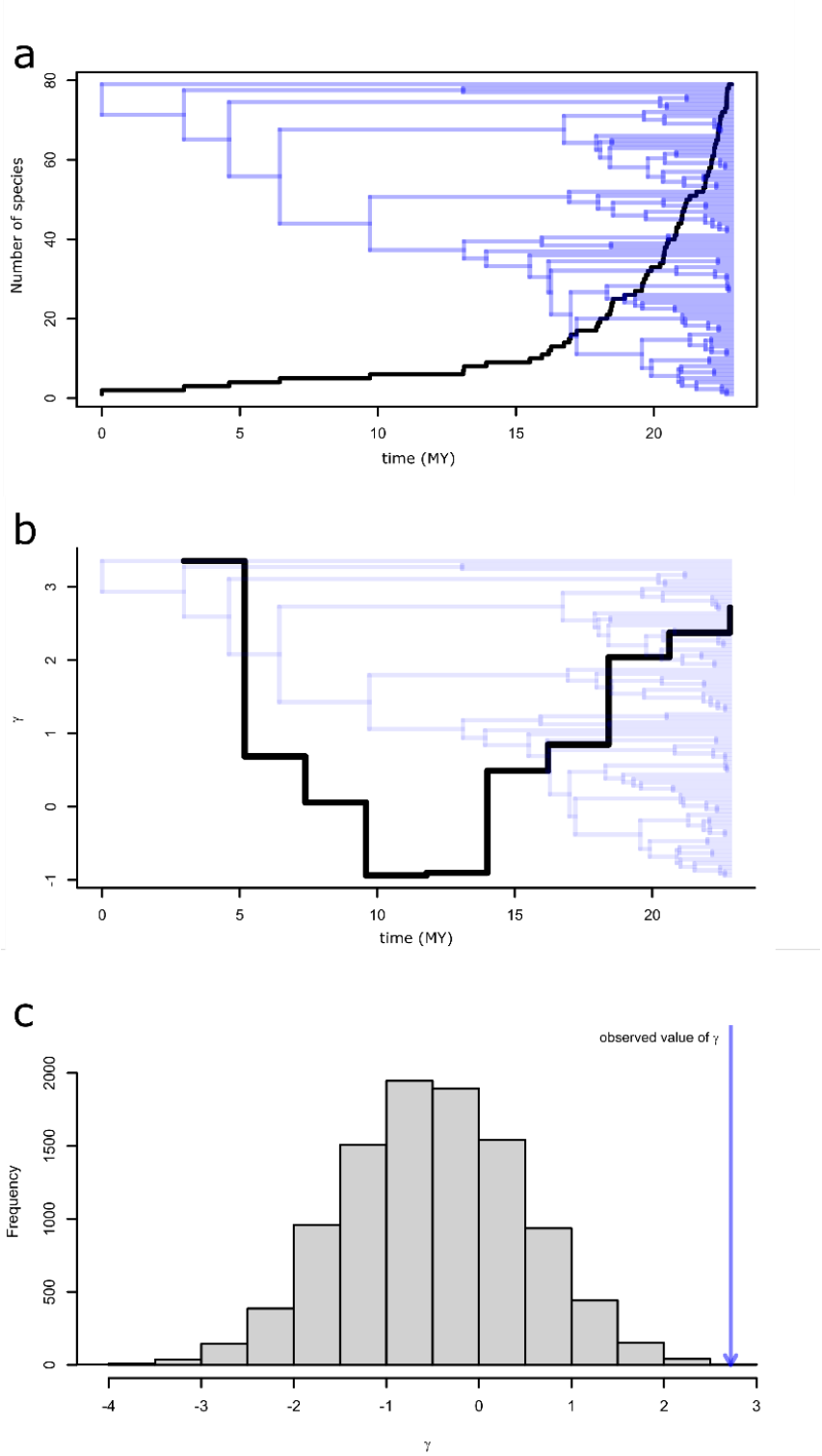


Figure 4.4. Lineage through time results. (a) Lineage through time plot for *Tulipa* with phylogeny overlaid and time measured in millions of years from origin of the genus, (b) Gamma statistic calculated across ten sections of the *Tulipa* genus tree which is shown in background, and (c) MCCR test for Tulipa with calculated gamma value highlighted.

species in our tree diverging from their sister taxa in the last five million years, showing the recent radiation of this genus across multiple parts of the phylogeny.

4.3.2. Biogeographic models

The AIC values of the biogeography models show that the 5A DEC model was the best fitting of all our models including for both biogeographical area runs (Table 4.4) as well as for models both with and without a dispersal matrix. Notably, the DEC model performed better without the jump dispersal function (+J), with the +J function converging on zero in the 5A DEC +J model suggesting that jump dispersal events are not broadly important in the evolutionary history of the genus *Tulipa*. In contrast the DEC +J model was the better fitting model for the 6A run, albeit with marginal difference in AIC between DEC and DEC +J models. In addition, the AIC of models inferred with a user-specified dispersal matrix were lower than those inferred without, showing that range expansion to neighbouring areas primarily accounts for the dispersal of different lineages over time. Moreover, the 5A biogeographical model had a lower AIC than that of the 6A model showing that this analysis resolved ancestral states better, so here we focus mainly on the results of 5A DEC model, with some comparison to the 6A DEC +J model especially with respect to the MRCAs of the subgenera (Figure 4.5).

Table 4.4. The Akaike Information Criterion values for our models run in BioGeoBEARS with a dispersal matrix for the five and six area distribution analyses.

	Five areas	**Six areas**
DEC	316.3	406.4
DEC +J	318.1	406.2
BAYAREALIKE	375.5	450.9
BAYAREALIKE +J	341.1	428.2
DIVALIKE	345.5	442.6
DIVALIKE +J	347.1	438.8

4.3.3. Evolutionary history of the genus Tulipa

Our dated phylogeny suggests that the ancestor of the *Tulipa* genus diverged from a clade that would go on to diversify into the *Amana* and *Erythronium* genera about 32.48 Mya. Previous dated trees have *Erythronium* as sister to *Tulipa*, but show that this broader split likely occurred in eastern Asia (Kim and Kim, 2018). Our phylogeny shows that *Amana* and *Erythronium* form a monophyletic clade and should both together be treated as sister to *Tulipa* meaning that our dating of this node is the first ever assessment of the age of this separation. This initial split occurred approximately around the Eocene-Oligocene transition when there was regional aridification, decreases in temperatures, and pronounced changes in vegetation types

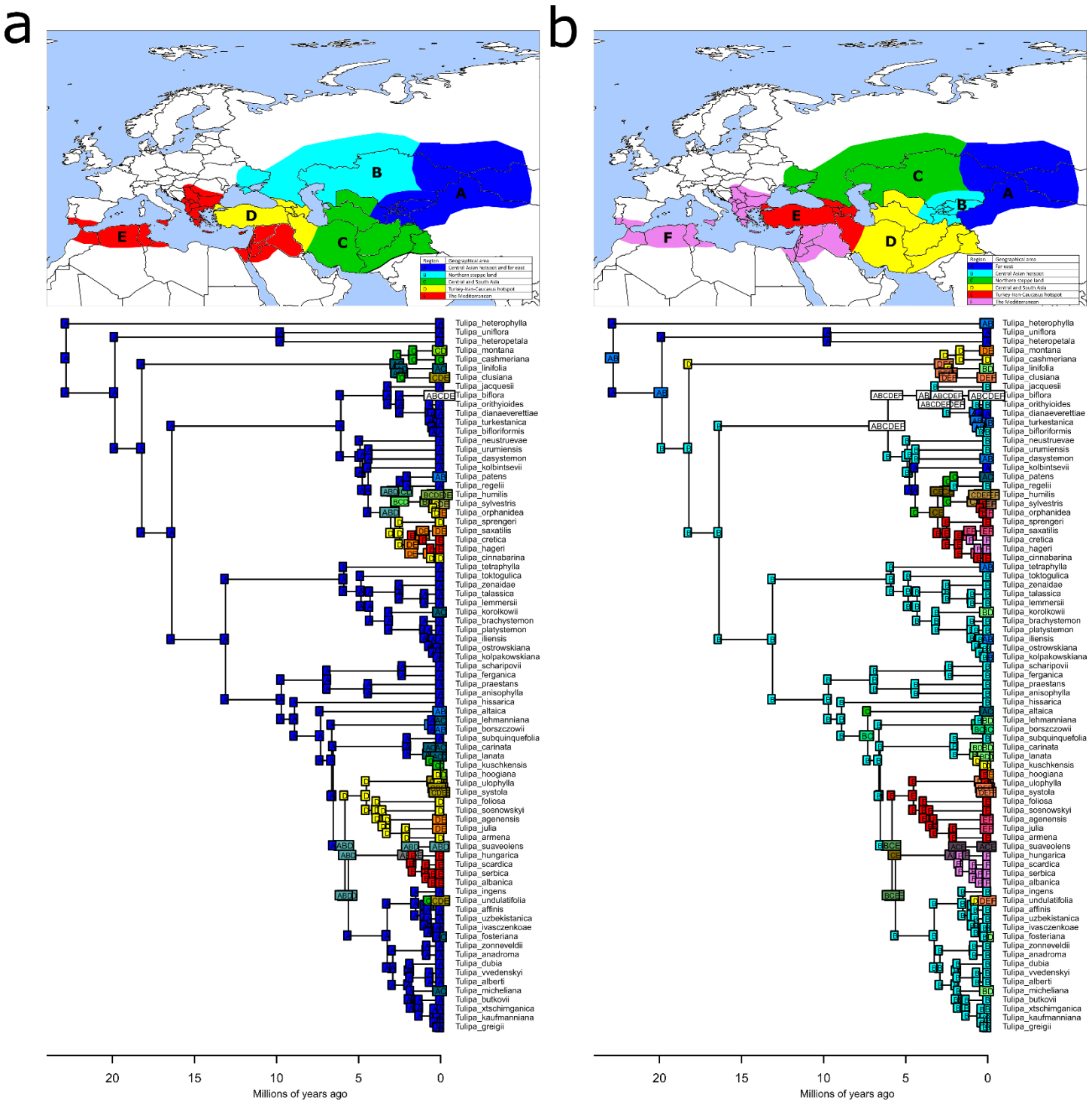


Figure 4.5. Maps of selected regions for the five area and and six area BioGeoBEARS analyses and corresponding results. (a) DEC model with tulip distibution split into five areas as shown in corresponding map with the geographic distribution of ancestral nodes and tips shown on the phylogeny and (b) DEC +J model with tulip distribution split into six areas again shown in corresponding map with the geographic distribution of ancestral nodes and tips shown on the phylogeny.

(Barbolini *et al.*, 2022). Primarily global cooling events and regression of the Paratethys sea have been linked to a significantly cooler and more arid continental Asian climate (Dupont-Nivet *et al.*, 2007; Sun *et al.*, 2022), climatic conditions commonly linked to the evolution of geophyte lineages (Howard *et al.*, 2019). Whilst, there was potentially also a shift to more open seasonal environments in this transition period with steppe like ecosystems already established (Barbolini *et al.*, 2022).

Based on the our biogeographical models we are also able to show that the MRCA of *Tulipa* resided within the Central Asia hotspot and far east region, supporting the historical idea that this region is the centre of origin of the genus (Hoog, 1973; Botschantzeva, 1982), although the continued existence of the Paratethys sea across parts of this area mean at this time the region likely had a very different geography compared to today (Li *et al.*, 2020). We note that the origin of this genus cannot be restricted to single country with our 6A model, which separated area A of the 5A biogeography map regions into two separate parts, unable to resolve the ancestral area of the MRCA further than that of the 5A model. Given that limitations in evolutionary reconstructions are unlikely to be overcome (Bromham, 2019) we suggest that a regional origin viewpoint be taken supporting more regional cooperation (Wilson *et al.*, 2021). The MRCA of the genus *Tulipa* has previously been estimated to have existed around 20.74 Mya (Kim and Kim, 2018), whilst here we support a slightly older origin of around 22.82 Mya.

The MRCA of tulips appears to have existed in a period of significant aridification in the broader Central Asia area (Zachos *et al.*, 2001; Tang *et al.*, 2011), when large arid areas are thought to have already existed (Guo *et al.*, 2002; Sun *et al.*, 2010) and the nearby Taklimakan desert was forming (Zheng *et al.*, 2015). This coincides with a period of global cooling and the retreat of the Paratethys sea which may have contributed to this drying process across Asia (Ramstein *et al.*, 1997; Lu, Wang and Li, 2010; Miao *et al.*, 2012). In this period significant tectonic activity including mountain formation in the Tien Shan is proposed to have been occurring (Hendrix, Dumitru and Graham, 1994; Macaulay *et al.*, 2014, 2016), some activity in the Pamir region (X. Wang *et al.*, 2019), and uplift of the Qinghai-Tibetan plateau (Kutzbach, Prell and Ruddiman, 1993) all of which may have affected rainfall patterns and further promoted aridification (Manabe and Broccoli, 1990; Hellwig *et al.*, 2018). This tectonic activity left a mosaic landscape with some widely spaced mountain ranges and large intermontane basins (Macaulay *et al.*, 2014), whilst large areas of the region were likely still covered by the Paratethys sea limiting available habitat (Li *et al.*, 2020). It appears therefore a combination of environmental factors in Central Asia may have played a role in driving early speciation in the *Tulipa* genus. This aligns well with

evidence of increased species richness in mountain areas, where there is interplay between surface uplift, climate change, and atmospheric circulation (Antonelli *et al.*, 2018), with mountain building often linked to a “species pump” effect through repeated rounds of connection and isolation of habitat areas (Muellner-Riehl, 2019).

The Central Asian hotspot and far east region were crucial to initial speciation events in this genus, with all MRCAs of the subgenera evolving within this region between 23-16 Mya. Our dated phylogeny shows that the *Eduardoregelia* subgenus was the first clade to diverge from the MRCA around 22.8 Mya, with the *Orithyia* subgenera second diverging around 3 million years later, with the biogeographic models projecting both having MRCAs that inhabited the far east zone. While the MRCA of all other subgenera is projected to have an origin in the Central Asian hotspot, with the *Clusianae* clade emerging 1.5 million years after *Orithyia* diverged, and finally the *Eriostemones* and *Tulipa* clades separating around 16.38 Mya. Across this broad Central Asia region there was a general pattern of continued aridification in this period (Tang *et al.*, 2011), increasing variation in precipitation patterns created a mosaic of arid and less arid areas, and climate patterns became increasingly seasonal (Miao *et al.*, 2012; Hellwig *et al.*, 2018). In addition, sustained mountain building activity in the region, especially in an out-of-sequence deformation process, may have provided increasing geographical barriers between populations and diversified habitat areas (Macaulay *et al.*, 2014), with some large grazers and expanses of grassland thought to exist in the region in the latter stages of this period (Atamuradov, 1994; Y. Wu *et al.*, 2018). Like many geophytes, tulip’s bulb structure allows them to inhabit areas where climate varies dramatically over a year, primarily areas where there is a summer drought season (Hall, 1940; Wilford, 2013; Tribble *et al.*, 2021). This is often in opens areas that may be maintained by grazers, and geophyte diversity is generally higher under grazing conditions, although species specific effects can vary (Noy-Meir and Oron, 2001).

Notably, diversification of the genus appears to slow dramatically at approximately 16 Mya, around the time of the Mid-Miocene Climatic optimum and when the step by step regression of the Paratethys sea ceased (Dercourt, Ricou and Vrielynck, 1993; Rögl, 1997; Zhongshi *et al.*, 2007). In this period warming of the planet occurred (Flower and Kennett, 1994), which led to changes in ecosystem structure with pollen records of Central Asia showing an increase in more thermophilous species (Sun and Zhang, 2008) and the expansion of grassland areas (Barbolini *et al.*, 2022). In this period tectonic activity generally slowed and the warmer climate appears to coincide with increased precipitation rates in some areas of Central Asia until 13 Mya (Miao *et al.*, 2012). Precipitation levels remained fairly stable in Central Asia until the late Miocene (Miao

et al., 2012) before increasing aridification began occurring in the region again associated with renewed orographic activity (Lu, Wang and Li, 2010; Tang *et al.*, 2011). Speciation events seem to have rapidly increased around 10-9 Mya, especially within the *Tulipa* subgenus in the Central Asian hotspot, associated with uplift in the region and consequential changes in climate. This included further aridification (Zhisheng *et al.*, 2001; Lu, Wang and Li, 2010), with large areas of forest being replaced by steppe land (Hui *et al.*, 2011). Mountain building increased, with a number of new mountain ranges emerging, which led to a decrease in intermontane basin size and further splitting of habitat areas (Macaulay *et al.*, 2014). Notably, the majority of the Tien Shan are thought to have been constructed within the last 10 million years (Abdrakhmatov *et al.*, 1996). This continually changing landscape, the further increase in grasslands in the region linked to global increases in grasses (Barbolini *et al.*, 2022), and the occurrence of multiple significant drying events in the last six million years (Sun and Zhang, 2008; Lu, Wang and Li, 2010) mean that Central Asia continued to become drier with a more complex orographic landscape. During this period significant further diversification occurred with large radiations occurring in the *Tulipa* and *Eriostemones* subgenera, with smaller diversification in the *Clusianae* subgenus also occurring. In addition, in this time many new sections and species appear in Central Asia, whilst diversification was also occurring more broadly across the Eurasian range of the genus.

The dispersal of tulips outside of the ancestral Central Asian area appears to have occurred several times between 3-7 Mya with clear clades within the subgenera *Clusianae*, *Tulipa* and *Eriostemones* all showing signs of migration. Notably, there appears to be two distinct pathways out of this region (Figure 4.6). Specifically, the *Clusianae* subgenera and a small clade from the *Tulipa* subgenera appear to have dispersed south from the Central Asia hotspot into the Central and South Asia region where they diversified, albeit leading to only a few species in both cases. This route may have low levels of migration as although large regression in the Paratethys sea occurred through the Oligocene to Miocene revealing more land across this part of Eurasia (Dercourt, Ricou and Vrielynck, 1993; Rögl, 1997; Zhongshi *et al.*, 2007; Popov *et al.*, 2010), expansion events of the Caspian sea in the late Pliocene-early Pleistocene period, especially the Akchagylian transgression, may have presented a significant geographic barrier (Van Baak *et al.*, 2013; Lazarev *et al.*, 2021). Whilst both the Karakum and Kyzylkum deserts are thought to have originated in the late Pliocene-early Pleistocene era (~4-5 Mya) developing in areas where the Paratethys sea had retreated (Atamuradov, 1994; Rustamov, 1994), again presenting a significant geographic barrier to dispersal into Iran and the Middle-East. The *Clusianae* subgenus and *Tulipa* clade, may have overcome these barriers by migrating into the Middle

East from a Central Asia origin via a circuitous route around the Karakum desert through the mountains of Afghanistan, which is encompassed in the range of several current species.

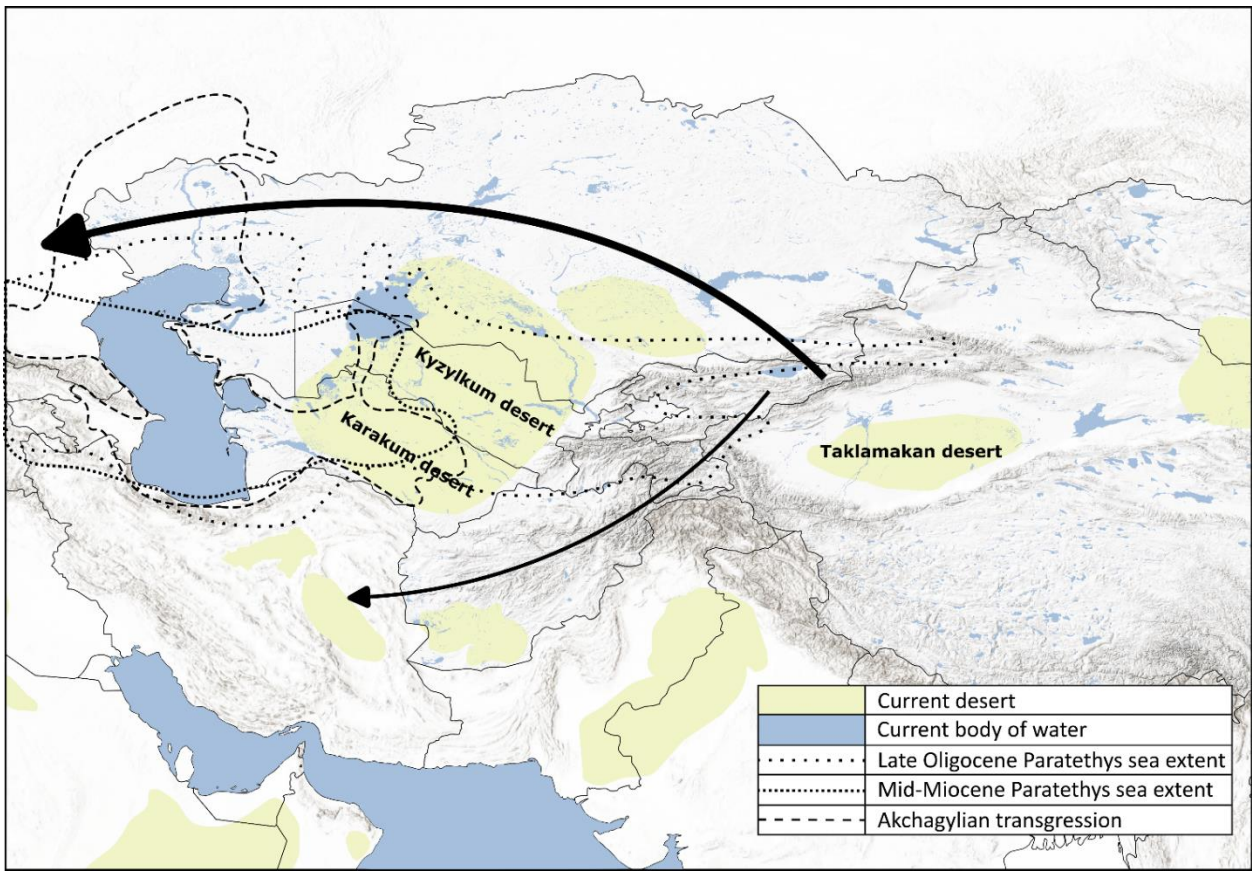


Figure 4.6. A map of the two dispersal routes out of Central Asia. Current day deserts are shown (https://earthworks.stanford.edu/catalog/stanford-bh326sc0899) alongside current bodies of water and mountain ranges.The estimated extent of the Parathys sea in the Oligocene (Li *et al.,* 2020), the approximate extent of the Paratethys sea in the mid-Miocene (Rögl, 1997), and the predicted extent of the Caspian sea expansion during the Akchagylian transgression (Van Baak *et al.,* 2013) are also shown.

Large groups in both the subgenera of *Eriostemones* and *Tulipa* have dispersed to occupy the Turkey-Iran-Caucasus hotspot, the Mediterranean, and the Central and South Asia region in clearly separate events but following a similar trajectory. Both these subgenera appear to have an MRCA that inhabited the Central Asia hotspot, with events leading to the expansion of these groups north and west into the steppe land of southern Russia and western Kazakhstan, which was greatly expanding in the late Miocene and early Pliocene period (Hui *et al.*, 2011; Hurka *et al.*, 2019) increasing dispersal opportunities for tulips (Wesche *et al.*, 2016). This trajectory was likely influenced by the geographic barriers preventing dispersal south stated previously (Atamuradov, 1994; Rustamov, 1994; Van Baak *et al.*, 2013; Lazarev *et al.*, 2021). Having

established across the northern steppe region it appears that both subgenera then diversified southward into the Turkey-Iran-Caucasus hotspot, where they radiated creating the secondary diversity centre of this genus. The *Tulipa* subgenus began diversifying in this region around 6 Mya with the *Eriostemones* radiation beginning around 4 Mya.

In the *Tulipa* subgenus separate clades diversified in the Turkey-Iran-Caucasus hotspot and the Mediterranean, but these likely share a common ancestor that had a broad distribution covering parts of Central Asia, the northern steppe land and the Turkey-Iran-Caucasus hotspot. The diversification of species in the Turkey-Iran-Caucasus hotspot also led to some species migrating eastwards into Iran where they now overlap with some species from the *Clusianae* subgenus. In the *Eriostemones* there is only a single clade that links to the diversification of species westwards from Central Asia, which then led to multiple migrations of taxa between the secondary diversity hotspot and the Mediterranean, with some species expanding their ranges eastwards into Iran. The recent radiation of tulips in the Mediterranean has sometimes been considered as part of a new recent speciation centre due to the high number of polyploid tulips found there (Botschantzeva, 1982) with our work providing some evidence to support multiple recent migrations and speciation events in the region. Overall, tulip dispersals into the west are centred around the sections of *Saxatiles* and *Sylvestres* in the *Eriostemones* subgenus and the *Tulipa* section of the *Tulipa* subgenus, with several species from these clades notably occupying extremely broad distributions, which may be remnants of broader ancestral taxa that bridged these regions.

Generally, the species of the *Eduardoregelia* and *Orithyia* subgenera and the *Kolpakowskianae*, *Multiflorae*, and *Biflores* sections, as well as many species of the *Tulipa* section remain only native in the ancestral area. Nonetheless, several individual species in this genus also show range expansions into additional areas. Of note in the *Biflores* section of the *Eriostemones* is *Tulipa biflora* which has dispersed out of Central Asia into all known regions within the last few million years, whilst most of its sister species are range restricted and remain native only to the ancestral region. Furthermore, in the genus *Tulipa* the evolution of several semi-desert dwelling species has occurred, which mostly occupy Central Asia, but their distributions sometimes extend into northern Turkmenistan and Afghanistan; rarely a species has spread beyond the Karakum desert, such as T. *lehmanniana*. Whilst *T. undulatifolia* also shows extensive migration in our models, we note that its taxonomic position and distributional understanding are tenuous due to uncertainty in the sampled specimens (see Chapter two), historical taxonomic issues, and poor records of occurrence. No biogeographical inferences have therefore been made

based on our results for this species and it is here highlighted as needing extensive further work.

4.3.4. Genome size is expanding in the genus Tulipa

Up to now there have not been any focused studies on the evolutionary pattern of genome size in tulips, here we have used our dated phylogeny to assess this pattern and the taxonomic relevance of this trait for the first time. In general genome size was smallest in the earliest diverging subgenera of *Eduardoregelia*, *Orithyia*, and *Clusianae*, with *Clusianae* having the lowest overall genome sizes in the genus. Across the *Tulipa* and *Eriostemones* subgenera there appeared to be evolution towards larger genome sizes (Figure 4.7). Specifically in the subgenus *Tulipa* the smallest genomes were observed in the earliest diverging section *Kolpakowskianae*, although these were still larger than those of the *Clusianae*, *Orithyia*, and *Eduardoregelia* subgenera. However, a stable polyploid, *Tulipa ostrowskiana,* has arisen in this group, which has the largest overall genome size of all specimens in this study, yet the size of a single set of its chromosomes is similar to the species it is most closely related to. The *Multiflorae,* in general, had larger genomes and in the *Tulipa* section there was clear genome size expansion with eastern European and Caucasian species showing the largest genomes. In the *Eriostemones* there is a broadly similar pattern with the earlier diverging section *Biflores* having the smallest genomes of the group, but again larger than those of the earlier diverging subgenera. Both the more recent diverging *Saxatiles* and *Sylvestres* sections, which occur mainly in the Mediterranean and Turkey, show the largest genome sizes in the *Eriostemones*.

Whether large genomes are adaptive is often debated (Petrov, 2001), but in general the genomes of geophytes, such as tulips, are known to be extensive (Zonneveld, 2009; Veselý, Bureš and Šmarda, 2013), with some evidence to suggest that larger genome sizes have positive ecological and phenological impacts, explicitly enabling early flowering and fast seasonal development (Veselý *et al.*, 2012). Specifically it has been hypothesised that the winter dormancy period of many geophytes, including tulips, presents adequate time to account for the slower mitosis associated with large genomes, decoupling cell division from growth (Grime and Mowforth, 1982). It has also been suggested that the bigger cells caused by large genomes can be expanded quickly through the movement of water even during low temperatures ensuring rapid growth and early flowering in Spring (Grime and Mowforth, 1982). However, why this taxonomic and biogeographical pattern exists within this genus still remains somewhat unclear.

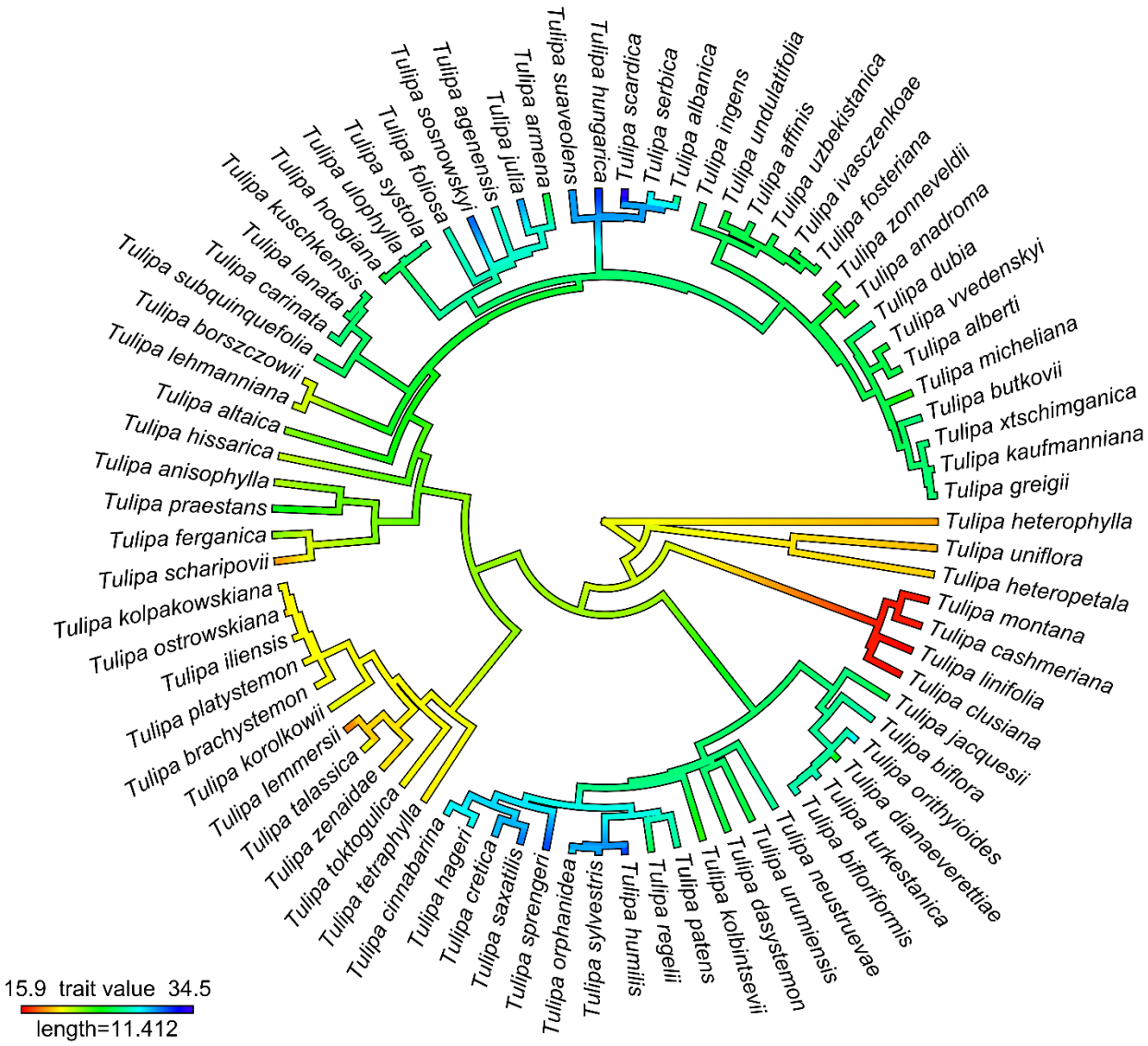


Figure 4.7. Genome size values (1C) for each species mapped to the dated phylogeny of *Tulipa.* The value of genome size is displayed on a colour gradient. Genome size for species lacking data has been estimated in the model.

The diversification of tulip species has frequently been associated with a clear intraspecific difference in genome size (Zonneveld, 2009) with tulip genome size a common trait used to identify taxonomic groupings and frequently used in the discovery and description of new species (Zonneveld and De Groot, 2012; Zonneveld, 2015). However, the phylogenetic signal, and therefore underlying evolutionary significance, of this trait within *Tulipa* has never been previously tested. Here our genome size data did not breach the statistical boundaries of normality W = 0.97108, p-value = 0. 1202 often assumed when using the Brownian motion model of evolution and so we showed that the trait of genome size had significant phylogenetic

signal both when measured using lamda, λ = 0.932185, log(λ) =-157.761, Likelihood Ratio (λ=0) = 73.3354, p = 1.09388e-17, as well as with the K statistic, K = 0.87444, p = 0.001. This significance shows that species with similar genome sizes are generally clumped together on the phylogeny, with closely related species with significantly similar genome sizes (Figure 4.7). This importantly supports previous work that has used this trait to make taxonomic decisions, especially higher-level taxonomic groupings (Zonneveld, 2009; Veldkamp and Zonneveld, 2012). Yet, for closely related species genome size will likely not provide the resolution to substantiate taxonomic decisions meaning that sectional level and within sectional level decisions based solely on this trait may be compromised and a broader integrative taxonomy is favoured (Dayrat, 2005).

4.4. Conclusion

Today the distribution of tulips covers large areas of Eurasia from north Africa and southern Spain across to western China (Everett, 2013), whilst the habitat areas of this genus are varied consisting of meadows, steppes, chaparral, deserts, stony mountainsides as well as fields, pastureland, road sides, abandoned gardens, and orchards (Hall, 1940; Christenhusz *et al.*, 2013; Everett, 2013). Yet, markedly there remains one extremely significant diversity hotspot for tulips, Central Asia, where tulips can be found across the alpine meadows, subalpine forests, alpine steppe and desert steppe of the region (Figure 4.8). Although a secondary diversity hotspot across Turkey-Caucasus-Iran also exists, we have provided evidence to show that broader Central Asia is the ancestral home of tulips and has been the most important region in the evolutionary history of this genus, with the Central Asian hotspot crucial in the diversification of most subgenera. The climate and landscape of Central Asia has undergone many changes over the history of this genus with major orographic changes, persistent aridification, global cooling, and increasing areas of grazed grassland and steppe. These conditions appear to have favoured geophytic tulips (Howard *et al.*, 2019; Tribble *et al.*, 2021) leading to rapid diversification (Howard *et al.*, 2020). Over the evolutionary history of the genus multiple dispersal events out of Central Asia have occurred potentially linked to expansions in genome size, yet today the mosaic of precipitation patterns (Sun and Zhang, 2008), strong seasonality (Miao *et al.*, 2012), and diverse mountainous habitats of this region mean that this still remains the most active region for speciation and the discovery of new species (De Groot and Zonneveld, 2020; Rukšāns and Zubov, 2022). Here we highlight the importance of Central Asia in the evolutionary history of wild tulips, emphasising the significance of the Mountains of Central Asia Biodiversity Hotspot for global plant diversity (Critical Ecosystem Partnership Fund,

2016). Now this rugged landscape that harbours an array of wild fruits and nuts (Cantarello *et al.*, 2014; Orozumbekov, Cantarello and Newton, 2015; Wilson *et al.*, 2019b), as well as iconic megafauna such as snow leopards (Leonardo Dicaprio Foundation, 2018) can reliably be called the birthplace of tulips, with the conservation of its diverse ecosystems potentially promoted under this flagship flower (Pany and Heidinger, 2017).

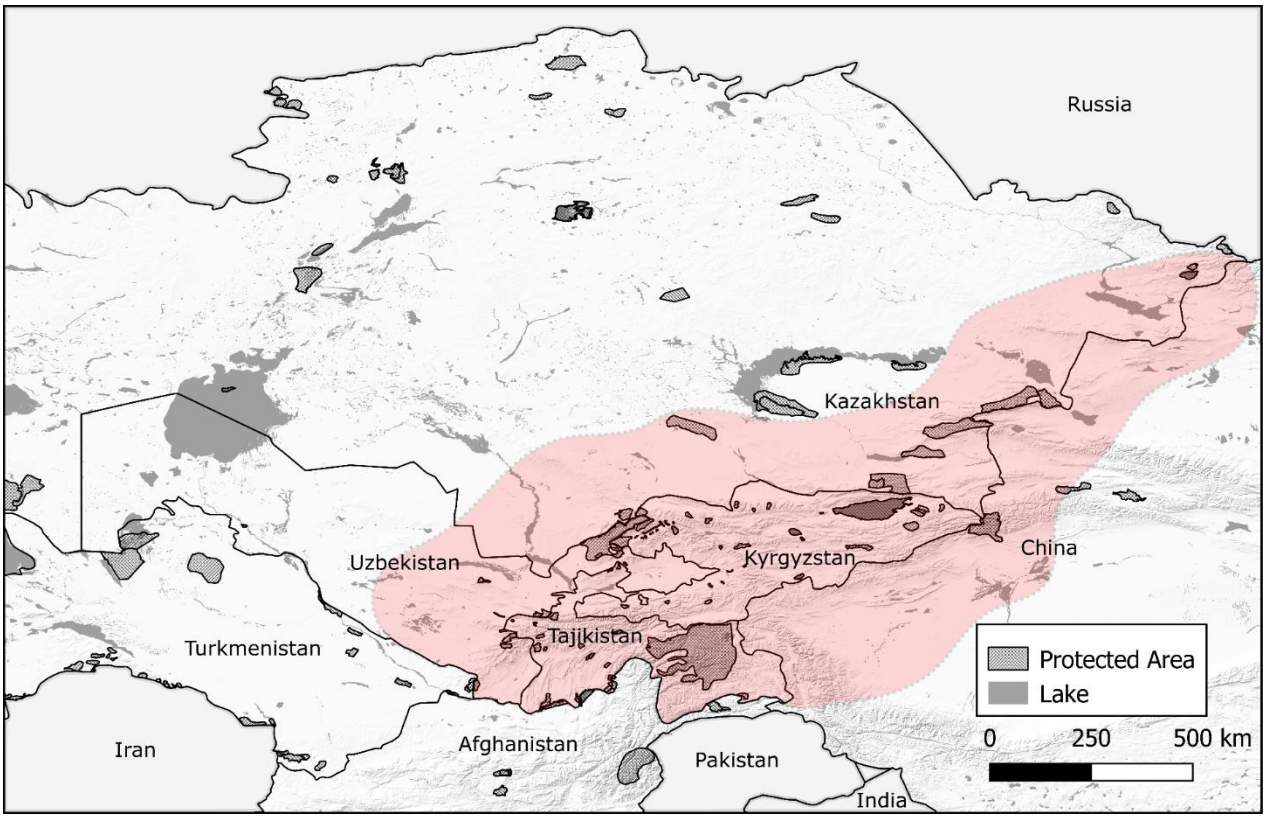


Figure 4.8. The area that should be considered within the Central Asian tulip diversity hotspot. This area was selected based on evidence from the results of our biogeography analyses and known species distributions.

Chapter 5.

Central Asian wild tulip conservation requires a regional approach, especially in the face of climate change

The basis of this chapter was published in the peer reviewed journal Biodiversity and Conservation: Wilson, B. *et al.* (2021) 'Central Asian wild tulip conservation requires a regional approach, especially in the face of climate change', *Biodiversity and Conservation*. doi: 10.1007/s10531-021-02165-z.

In this chapter we use location data collected on our expeditions and data available on GBIF to model the distributions of ten Central Asian species. These species distribution models were used to understand how the national red list status of a tulip interacts with its global range. In addition, these present day models with projected environmental scenarios, were used to infer how the suitable habitat area of these species will change under climate change, including with respect to protected area coverage and altitude. Overall, allowing us to assess the threat of climate change to Central Asian tulips.

5.1. Introduction

Conservation, including conservation assessments such as the global IUCN Red List (IUCN, 2020), are underpinned by an array of data. Primarily, they rely on knowledge of a species' distribution and threats to its survival (IUCN, 2012). For many species, global IUCN Red Listing has not been undertaken, and this is often due to a lack of information to support these reports (Rodrigues *et al.*, 2006). Local experts can provide a valuable insight into threats to species (Keppel *et al.*, 2015), but commonly only country-level efforts have been undertaken to assess the status of flora, and many National Red Lists are therefore more comprehensive than global ones (Tojibaev and Beshko, 2015). This is exacerbated by plants being globally and nationally less commonly assessed than animals (Nic Lughadha *et al.*, 2020), in part due to plant-blindness (Balding and Williams, 2016), whilst crucially, many are known to be declining towards extinction and require urgent conservation action (Nic Lughadha *et al.*, 2020). Therefore, there is a need for more information to support plant conservation assessments, especially across the full geographic range of a species. Species distribution modelling presents a method through which to improve understanding of the suitable habitat area of a species (Phillips *et al.*, 2017), developing knowledge of its extinction risk, and promoting population monitoring, management, and related policy, especially across multiple countries (Pearson *et al.*, 2014; Wilson *et al.*, 2019b).

Tulips are a group of perennial geophytic monocots (Everett, 2013). They are widely recognised for their horticultural varieties which support a billion Euro industry (Christenhusz *et al.*, 2013), but also for their wild species which form a genus that is estimated to comprise between 76 and 90 species (Everett, 2013). This array of wild taxa has historically underpinned the large horticultural trade, something that has significantly complicated the taxonomy of this clade (Christenhusz *et al.*, 2013). Wild species remain a significant genetic resource for horticultural breeders, have considerable cultural value, and play an important role in ecosystems, especially for pollinators and insects (Kashin, Kritskaya and Schanzer, 2016; Su *et al.*, 2020). Wild tulips grow in the temperate regions of the northern hemisphere. Their distribution covers much of Eurasia, extending from western China across to western Europe, whilst a single species occurs on the Mediterranean coast of Africa. Nonetheless most species distributions centre around Central Asia (Botschantzeva, 1982), and specifically the Mountains of Central Asia Biodiversity Hotspot (Critical Ecosystem Partnership Fund, 2016).

Currently, there are only five tulip species published on the global IUCN Red List although none of these are native to the Central Asian centre of diversity (IUCN, 2020). For Central Asian tulips an assortment of national level assessments have been published (Davletkeldiev, 2006; Khassanov and Prastov, 2009; Baitulin, 2014; Tojibaev and Beshko, 2015; Nowak *et al.*, 2020), but these focus on country-wide distributions, not accounting for the fact that many species' distributions cross national borders. Poor representation is a common problem for Central Asian species, with data often lacking (Yesson *et al.*, 2007; Paton *et al.*, 2020), and international evaluation rare due to geopolitical tensions between neighbouring countries (Nowak *et al.*, 2020). Regardless, national conservation assessments undertaken in this region indicate that a range of tulip species are threatened (Davletkeldiev, 2006; Khassanov and Prastov, 2009; Baitulin, 2014; Tojibaev and Beshko, 2015; Nowak *et al.*, 2020). Threats previously recorded for these species include wild collection and trade, livestock overgrazing, and climate change (Nowak *et al.*, 2020), although there remains limited literature and research focused on understanding their impacts. Nonetheless research suggests that the life history, ecology, and cultural value of tulips makes them exceptionally vulnerable to disturbance and consequently to extinction (Tojibaev and Beshko, 2015; Nowak *et al.*, 2020).

Most tulips grow in the lower and middle elevations of mountain belts (Botschantzeva, 1982). These alpine regions are thought to be extremely sensitive to climate change (Rangwala and Miller, 2012) and therefore tulips may be especially vulnerable to this increasing threat (Nowak *et al.*, 2020). Nonetheless the lower semi-desert and steppe areas of Central Asia, where fewer

tulip species grow, are also reportedly fragile to a changing climate with many areas predicted to becoming increasingly arid in future years (Lioubimtseva and Henebry, 2009; Chen *et al.*, 2019). The impacts of climate change in these areas could be exacerbated by tulips' geophytic growth habit, relying on bulb-driven rapid spring growth to survive summer drought conditions common in the temperate latitudes where these species grow (Botschantzeva, 1982). However, tulip bulbs also rely on a cold winter period as a trigger for initial growth, a process known as vernalisation. Furthermore, tulips require dry and freely draining soil conditions, with dampness often leading to rot and disease (Wilford, 2013). Thus, tulip distributions are tightly linked to seasonal triggers, and both temperature and rainfall patterns, meaning changes in these may lead to declines in population numbers and even local extinctions. Moreover, climate change may cause shifts in suitable habitat and therefore there may be an increasing need for species to rapidly adapt or relocate to survive.

Initial flowering of tulips will not occur until there is a large energy store in the bulb, which can take several growing seasons. In addition, if damage occurs to the bulbs or leaves during the short growing season this can greatly weaken the plant, limit growth and reproduction, and sometimes even lead to death (Wilford, 2013). Tulips relatively long reproductive cycle and vulnerability to damage means that colonisation and re-population of areas is slow, exacerbated by their limited ability to disperse pollen and seeds (Kashin, Kritskaya and Schanzer, 2016). Given this and the widespread nature of livestock grazing across the grasslands, pasturelands, shrublands, steppes, and semi-deserts in which this plant commonly grows and the opportunistic collection of flowers by communities, tulips may be at increased risk from disturbance and may be unable to migrate to compensate for climate change (Davletkeldiev, 2006; Tojibaev and Beshko, 2015; Nowak *et al.*, 2020). Additionally, many species are thought to have small, restricted distributions, especially many described endemics that are often only known from a specific hillside or gorge (Millaku and Elezaj, 2015; De Groot and Tojibaev, 2017), a trait widely associated with a heightened risk of extinction (Pearson *et al.*, 2014).

Overall, Central Asian tulips are likely broadly threatened, but the extent and shape of this threat is significantly underreported, especially with regards to the impact of climate change. Strikingly, most species span the borders of the mountains of Central Asia, an ecosystem thought to be particularly vulnerable to the complex impacts of a changing climate (Xenarios *et al.*, 2019; Nowak *et al.*, 2020), and it is of increasing importance to expand upon national efforts to provide a regional perspective on the distribution and threats to wild tulips. This is especially crucial given that new species are frequently described in the region which often have extremely small

distributions and may therefore have an immediate risk of extinction (De Groot and Tojibaev, 2017; De Groot and Zonneveld, 2020). Strikingly the genus *Tulipa* is only one of many geophytic clades that has a large diversity of species in the region including *Amaryllidaceae*, the broader *Liliaceae* of which *Tulipa* is only a small section, *Iridaceae*, and *Asphodelaceae* (Tojibaev *et al.*, 2018). There are also a number of plant communities unique to the region including the walnut-fruit forest (Wilson *et al.*, 2019b) and the mountain grasslands (Borchardt *et al.*, 2011). It is therefore likely that the issues surrounding tulips and the corresponding threats are not exclusive to this plant group and are broadly indicative of the state of flora in this remote corner of the world.

Here we use species distribution modelling with MaxEnt to examine the range of ten Central Asian tulip species and the predicted changes in habitat suitability linked to climate change. Crucially, this work provides the first regional approach to tulip conservation in Central Asia, showcasing how such a practice can provide a more robust evidence base for conservation decision making in this region.

5.2. Methods

5.2.1. Study site

Fieldwork for this study was undertaken in the Republic of Kyrgyzstan in the spring of 2019 and 2020. We performed several field surveys: covering south-western Kyrgyzstan specifically the Batken and Osh regions; western Kyrgyzstan specifically the Jalal-Abad and Talas regions; and northern Kyrgyzstan, explicitly the Chuy and Issyk-Kul regions (Appendix 7). This fieldwork recorded 85 new location points for the ten species of focus (Table 5.1). The broader area of Central Asia, specifically the countries of Afghanistan, China, Kazakhstan, Kyrgyzstan, Mongolia, Tajikistan, Turkmenistan, and Uzbekistan, were included in species distribution modelling (Figure 5.1).

5.2.2. Species distribution modelling

MaxEnt v3.4.0, based on the maximum-entropy approach, is a widely used modelling technique, especially in conservation (Trisurat *et al.*, 2013; Liang, Kang and Pettorelli, 2017; Wilson *et al.*, 2019b). This software is open source, can model past, present and future species distributions given suitable environmental layers, and relies only on presence data (Phillips, Anderson and Schapire, 2006). Specifically, the model uses location data and habitat vectors to predict the probability of presence of a species across a selected area. MaxEnt has been used frequently in conservation to model species distributions primarily because it is highly accurate with small

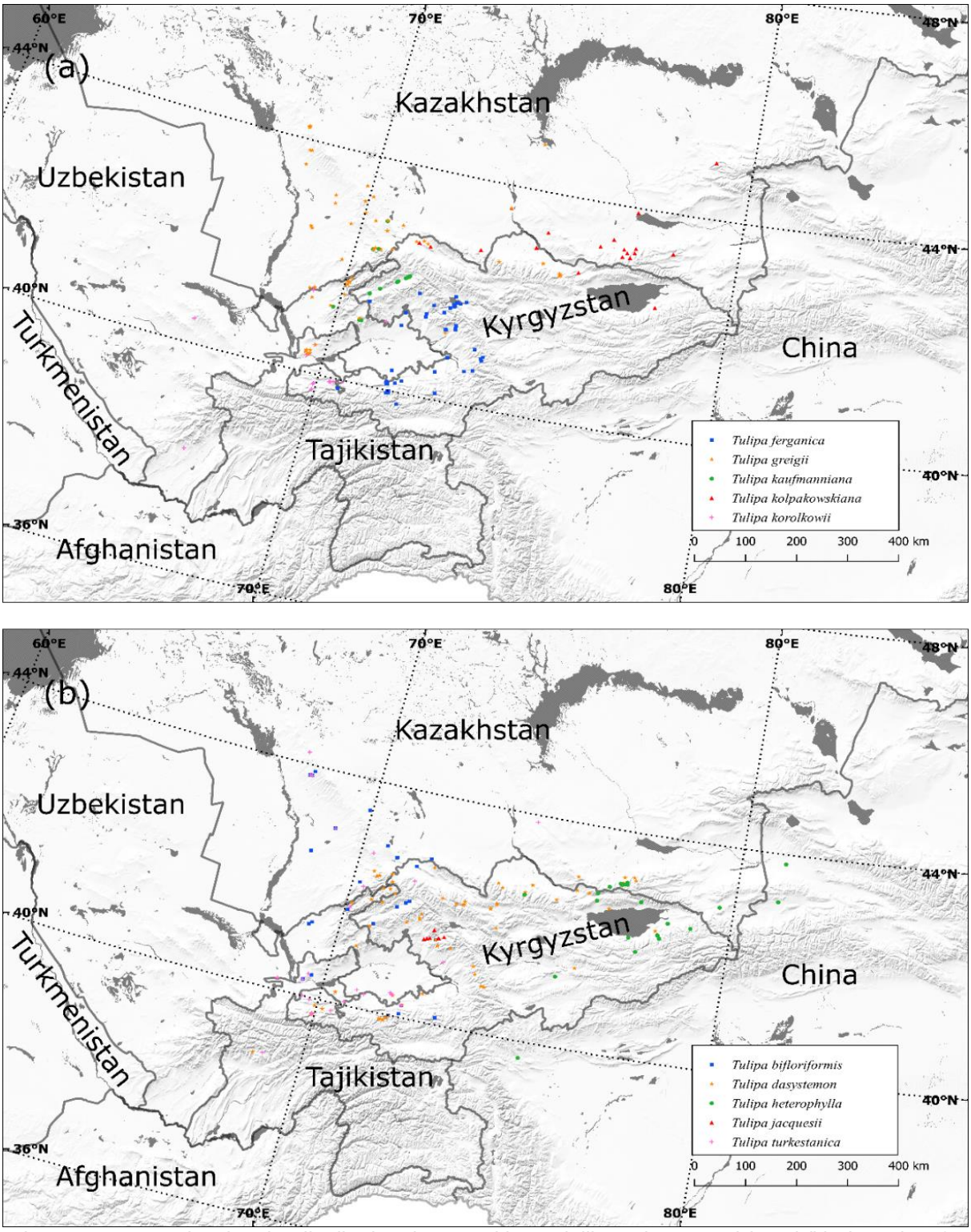


Figure 5.1. Maps showing the distribution data used in modelling. The maps (a) and (b) show data for five species each. Both maps are projected in Asia North Albers Equal Area Conic coordinate reference system (ESPG 102025).

sample sizes (Elith *et al.*, 2011; Qin *et al.*, 2017), characteristic of Threatened species. This software also models distributions under future climate change scenarios, allowing this threat to be assessed (Qin *et al.*, 2017; Hof and Allen, 2019). MaxEnt's inferences are correlative, with the software using a regression framework to produce predictions of occurrence. This method of climate change modelling often does not provide the same resolution as both mechanistic and trait-based approaches. Yet, these other approaches rely on detailed information of taxon-specific parameters, population sizes, interspecific relationships, and well-defined species distributions (Pacifici *et al.*, 2015). In most cases this type of data is just not available for tulips, or not available in sufficient detail. This is especially the case in Central Asia, which is a relatively data deficient region (Pearson *et al.*, 2014). So, although correlative approaches do not provide the same resolution, they require fewer initial data and therefore can be exceptionally useful for not only modelling present distributions but also future habitat in data poor areas.

In this study we focused on ten *Tulipa* species representing a range of habitats, distributions, and threat levels. We primarily selected species that had over twenty datapoints available for modelling to ensure there was enough GPS points present to establish a significant relationship with environmental variables. For comparison, we also selected to model *Tulipa jacquesii* (Zonneveld, 2015) as a representative of the recently described endemic species in the region that is relatively data deficient (Table 5.1). Location data for each species was downloaded from the Global Biodiversity Information Facility (GBIF; GBIF.org 2020) database through the GeoCAT tool (Bachman *et al.*, 2011) and combined with data gathered in our field surveys (Figure 5.1). We selected 23 environmental variable layers to be used as inputs for the MaxEnt programme. These consisted of 19 bioclimatic layers from the WorldClim2 database with a resolution of 30 seconds or ~1km² at the equator (Fick and Hijmans, 2017), high resolution altitude data from the Shutter Radar Topography Mission (SRTM), slope and aspect layers generated from the altitude data using the *QGIS 2.14* terrain analysis tool (QGIS Development Team, 2009), and the land cover data GlobCover2009 (Arino *et al.*, 2012). These layers have previously been effective in determining the distribution of Threatened species where data are limited, including in Central Asia (Kumar and Stohlgren, 2009; Wilson *et al.*, 2019b). All layers were checked for multicollinearity using the ENMTools R package (Warren *et al.*, 2019). A single variable from a group of highly correlated variables was chosen for modelling using a threshold ($r>0.85$) commonly applied in MaxEnt work (Syfert, Smith and Coomes, 2013; Wilson *et al.*, 2019b). We selected the variable from within this group which showed the average

Table 5.1. Details of the ten species of Central Asian tulip included in this analysis. The number of location points for each species is detailed including those collected during our fieldwork, and the number of points used for training and testing the model are stated. The subgenus, habitat, and distribution of each species is also reported alongside the National Red List status of each species (NE – Not Evaluated, LC – Least Concern, NT – Near Threatened, VU - Vulnerable, EN - Endangered, and CR – Critically Endangered and when a species does not occur in that country a - is shown. The Kazakhstan Red Book only reports species as Threatened without further categorisation and so this is reported where appropriate).

		Tulipa bifloriformis	***Tulipa dasystemon***	***Tulipa ferganica***	***Tulipa greigii***	***Tulipa heterophylla***	***Tulipa jacquesii***	***Tulipa kaufmanniana***	***Tulipa kolpakowskiana***	***Tulipa korolkowii***	***Tulipa turkestanica***
Location points added by fieldwork		6	11	22	7	5	4	12	1	10	7
Total number of location points		33	109	58	81	33	6	21	22	22	30
Training location points		22	57	42	47	19	4	14	16	15	20
Test location points		6	15	11	12	5	2	4	4	4	6
Subgenus		Eriostemones	Eriostemones	Tulipa	Tulipa	Orithyia	Eriostemones	Tulipa	Tulipa	Tulipa	Eriostemones
Habitat		Stony slopes in the foothills of mountains	Alpine grasslands	Stony slopes in the foothills of mountains	Shrubland in the foothills of mountains	Alpine grasslands and stony slopes in the middle mountain belt	Stony bare slopes	Grasslands in the foothills of mountains	Stony hillsides and semi-desert areas	Semi deserts and scree slopes in steppe	Clayey and stony slopes in the foothills of mountains
Distribution		Kazakhstan, Kyrgyzstan, Tajikistan, Uzbekistan	Western China, Kazakhstan, Kyrgyzstan, Tajikistan, Uzbekistan	Kyrgyzstan, Uzbekistan	Kazakhstan, Kyrgyzstan, Tajikistan, Uzbekistan	Western China, Kazakhstan, Kyrgyzstan	Kyrgyzstan	Kazakhstan, Kyrgyzstan, Tajikistan, Uzbekistan	Western China, Kazakhstan, Kyrgyzstan	Kazakhstan, Kyrgyzstan, Tajikistan, Uzbekistan	Western China, Kyrgyzstan, Tajikistan, Uzbekistan
National Red List status	*China*	-	LC	-	-	LC	-	-	NT	-	NE
	Kazakhstan	NE	NE	-	Threatened	NE	-	Threatened	Threatened	Threatened	-
	Kyrgyzstan	NE	NE	NE	EN	NE	NE	VU	VU	VU	NE
	Tajikistan	EN	NE	-	VU	-	-	CR	-	EN	NE
	Uzbekistan	NT	VU	VU	VU	-	-	NT	-	NT	LC

highest correlation to all other variables in the group; within this we avoided selecting altitude because this data would be uninformative in any climate modelling as linking a species presence to altitude would not allow for migration of a species.

Multiple location points of a species in the same grid cell were deemed duplicates and were treated as a single point. This reduced the available data for training and testing the model (Table 5.1). Given the limited data of some species, different modelling features were applied. For species with under ten points we used only linear features, for species with <25 points we used linear and quadratic features, whilst for those with ≥25 datapoints linear, quadratic, and hinge features were used in modelling (Elith *et al.*, 2011). K-fold cross-validation (K=5) was used to generate an average present-day model for each species, as this has been empirically shown to neither be affected by excessively high bias nor very high variance (James *et al.*, 2013) and reported as better than simple thresholding (Merow, Smith and Silander Jr, 2013). The model's accuracy was determined by the test data area under the receiver operating characteristic curve (AUC) value with a value of >0.9 representing a very good model, a value between 0.7–0.9 showing a good model, and anything with <0.7 deemed uninformative (Swets, 1988; Baldwin, 2009). We assessed a range of regularization multipliers for several species deeming the default (1.0) to be the most effective modelling parameter based on both TestAUC (Appendix 8) and current understanding of taxa distributions (Everett, 2013), and so this was used in all models. We also generated a dataset of locational data for Liliaceae across Central Asian using GBIF, which we used to perform background manipulation to account for sampling bias (Kramer-Schadt *et al.*, 2013; Syfert, Smith and Coomes, 2013). This generally helps to ameliorate overfitting, however, in our case it distorted distributions somewhat, both broadly lowering the TestAUC value (Appendix 9) and making predictions of suitable habitat in areas where the species is known not to occur (Everett, 2013). We display only present-day modelling efforts and final climate models produced without a bias file. All outputs were presented using a five tier classification system and using the protected area data from Protected Planet (UNEP-WCMC and IUCN, 2020).

Climate models were produced for nine of the ten species; *Tulipa jacquesii* was excluded from this analysis due to its extremely limited location data. Two climate models were selected for comparison, Community Climate System Model version 4 (CCSM4 GCM) and Model for Interdisciplinary Research on Climate (Miroc ESM), to allow us to assess the reliability of our results across models. We selected these as they have both been used in previous studies to assess changes in habitat suitability linked to climate change (Rej and Joyner, 2018; Hof and

Allen, 2019). We also selected two climate change scenarios to model, RCP 2.6 which represents a best-case scenario (BC) where emissions peak in 2020, and RCP 8.5 which is a worst-case scenario and similar to business as usual (BU). We selected to use data from both the years 2050 and 2070 so as to investigate the change in species distribution across multiple future periods. Given the number of models that needed to be generated we trained climate models using 75% of location data and tested them using the remaining 25% avoiding time consuming five-fold cross-validation (Wilson *et al.*, 2019b). All climate models were assessed using the same TestAUC classifications as the present-day models and presented in the same format.

Areas of habitat suitability were calculated for native and non-native sections of the models produced. The native area of a species was estimated based on the model output, location data, and literature (Bachman *et al.*, 2011; Everett, 2013; POWO, 2021). To capture the native area, we created polygon layers on QGIS covering all location data and connected areas deemed suitable in habitat by the MaxEnt outputs and within or closely adjoining the previous estimated range of the species. We maintained a lenient approach to ensure we did not exclude important parts of the natural distribution from calculations and so likely captured some areas outside of the true distribution of the species. Non-native areas were therefore those outside the range of this polygon. Using these polygons, we extracted the number of cells in each of the five suitable habitat categories for each species. In addition, we used the estimated native areas to extract altitudinal information and calculate protected area coverage. To do this we selected cells from our models with a 0.5 or greater habitat suitability within the predefined natural distribution of the species, and specifically for protected area calculations those that overlapped with recorded protected areas from Protected Planet (UNEP-WCMC and IUCN, 2020). We selected this threshold value as it has been previously used in research to represent a presence-absence separation (Carrasco *et al.*, 2020) and would capture areas of habitat deemed to be highly suitable or very highly suitable, which we think represent the most important areas of the species range. To statistically compare altitudinal values from different years, we conducted an analysis of variance (ANOVA), which if significant ($p < 0.05$) was followed by a Tukey's HSD test. We used the area function of the raster 3.1.5 package (Hijmans, 2014) to calculate mean cell size in km^2 across the modelled region. We then used this value, 0.648km^2, both for general area calculations as well as alongside the calculated number of cells meeting the threshold criteria to estimate habitat within protected areas. For both altitudinal and area computation raster layers were manipulated on QGIS v2.18.25 (QGIS

Development Team, 2009) and all calculations were carried out on R v3.4.0 (R Core Team, 2022).

5.3. Results

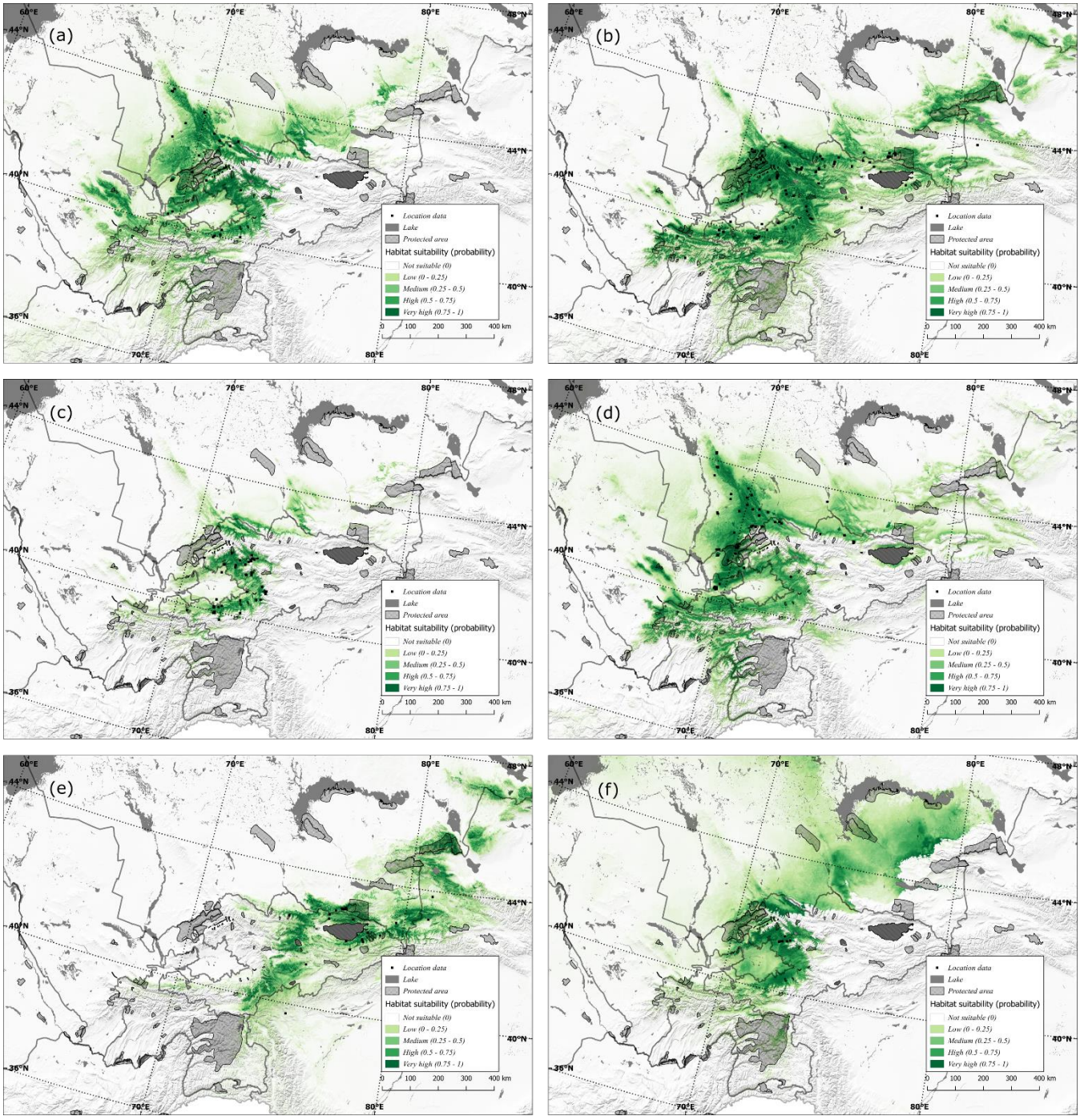
After assessing spatial autocorrelation, we selected to use 14 layers for present-day modelling, whilst landcover was excluded from any climate modelling as future land cover predictions were not available leaving 13 layers for this aspect (Table 5.2). All present-day models had TestAUC values larger than 0.9 and were therefore classified as very good (Figure 5.2; Table 5.3). Yet, we report that the distribution of *Tulipa jacquesii* be used cautiously as only four location points were available to train the model. The most important environmental predictors for every species varied greatly, however, each were linked to precipitation or temperature patterns in the winter or summer months or the seasonality of climate in general (Table 5.4). Models broadly highlighted areas of mountain ranges in the Tien Shan as suitable habitat, although a few species were more closely linked to semi-desert areas. Some species had relatively extensive distributions across the region, occurring in several mountain ranges, yet there was also several taxa which were much more spatially restricted and occurred in single mountain ranges, valleys, or steppe areas. Our models also show that tulip species are often not spatially separated, with considerable overlap between many species' ranges. All species modelled, barring *T. jacquesii* as its model was deemed unreliable, were predicted to occur in at least one protected area. Nonetheless five species had less than five percent of their range in protected areas and both *T. korolokowii* and *T. ferganica* had two percent or less captured in the protected area network (Figure 5.3).

For climate modelling, all models had TestAUC values greater than 0.9 and were deemed 'very good' providing informative results (Table 5.3). We present only the results from the CCSM4 GCM modelling (Figure 5.4) as the results of Miroc ESM were consistently similar to CCSM4 GCM models not only across climate scenarios, but also years, and taxa (Appendix 10). There were two species reported that appeared to retain or expand in suitable habitat area, *Tulipa bifloriformis* and *T. kaufmanniana,* however, much of the future suitable habitat area for these species occurred in the Pamir mountains of Tajikistan, an area hundreds of kilometres from their native range. Given this information all models consistently showed a considerable reduction in the size of suitable habitat for tulip species in their natural distribution (Table 5.5; Figure 5.5; Appendix 11). The suitable habitat area in the native species range of *T. bifloriformis*, *T. dasystemon, T. greigii,* and *T. kaufmanniana* declines in a stepwise manner from present-day to

Table 5.2. The environmental layers selected, after autocorrelation testing, to be included in the MaxEnt model and their source.

Environmental variable	**Source**
Aspect	Generated in GIS from SRTM Altitude data
Slope	Generated in GIS from SRTM Altitude data
Mean diurnal range	WorldClim2 (Fick and Hijmans, 2017)
Isothermality	WorldClim2 (Fick and Hijmans, 2017)
Precipitation of coldest quarter	WorldClim2 (Fick and Hijmans, 2017)
Temperature seasonality	WorldClim2 (Fick and Hijmans, 2017)
Precipitation seasonality	WorldClim2 (Fick and Hijmans, 2017)
Precipitation of driest month	WorldClim2 (Fick and Hijmans, 2017)
Precipitation of wettest month	WorldClim2 (Fick and Hijmans, 2017)
Mean temperature of wettest quarter	WorldClim2 (Fick and Hijmans, 2017)
Mean temperature of driest quarter	WorldClim2 (Fick and Hijmans, 2017)
Mean temperature of coldest quarter	WorldClim2 (Fick and Hijmans, 2017)
Mean temperature of warmest quarter	WorldClim2 (Fick and Hijmans, 2017)
Land cover[a]	GlobCover 2009 (Arino *et al.*, 2012)

[a]Not included in climate change modelling


(a)
(b)
(c)
(d)
(e)
(f)
60°E
70°E
80°E
48°N
44°N
40°N
36°N
Location data
Lake
Protected area
Habitat suitability (probability)
Not suitable (0)
Low (0 - 0.25)
Medium (0.25 - 0.5)
High (0.5 - 0.75)
Very high (0.75 - 1)
0 100 200 300 400 km

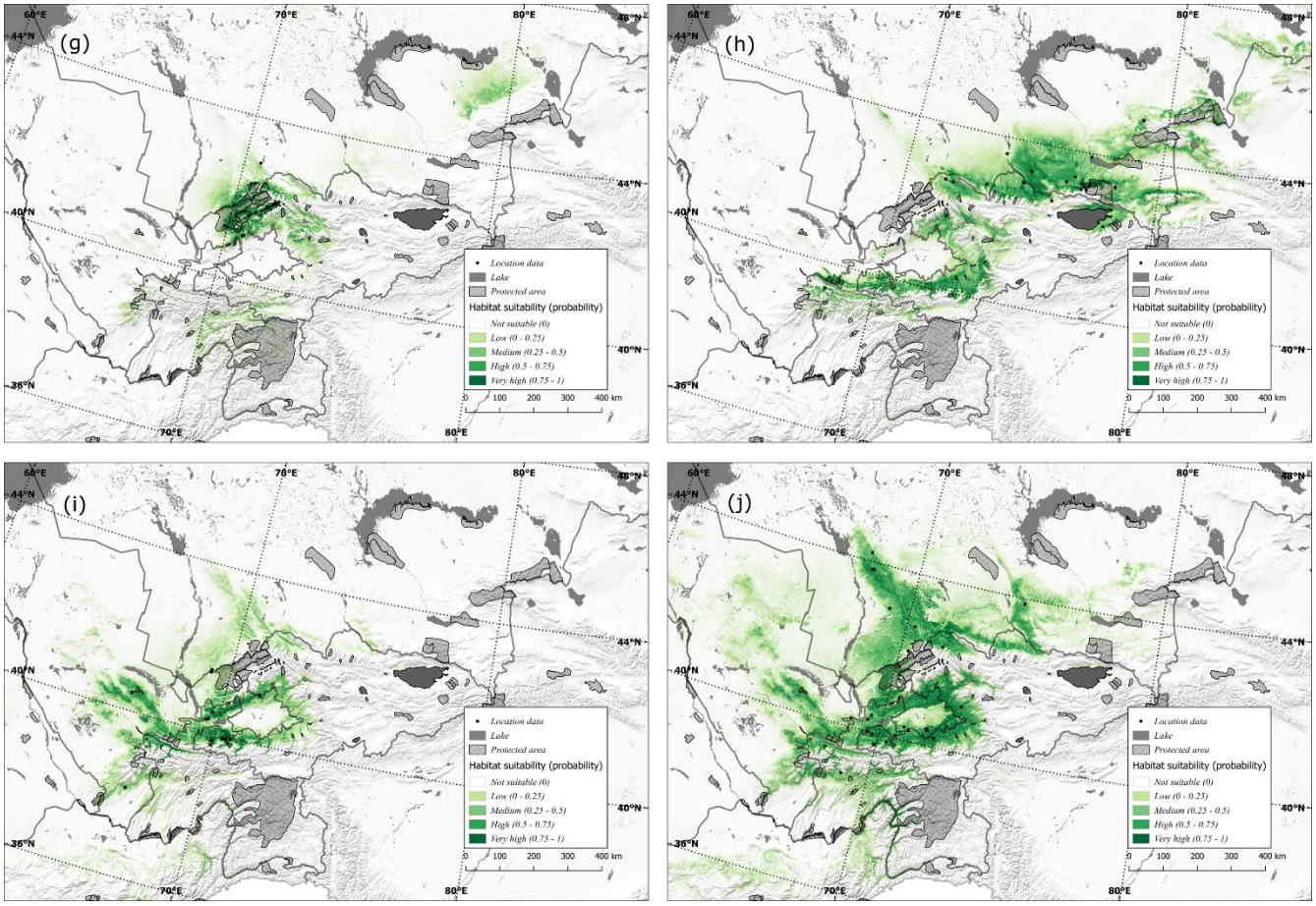


Figure 5.2. Species distribution models produced in MaxEnt without a bias file. Maps are projected in Asia North Albers Equal Area Conic coordinate reference system (ESPG 102025) with cell size of ~0.65km². Panels correspond to different species: (a) – *T. bifloriformis*, (b) - *T. dasystemon*, (c) – *T. ferganica*, (d) - *T. greigii*, (e) – *T. heterophylla*, (f) - *T. jacquesii*, (g) – *T. kaufmanniana*, (h) – *T. kolpakowskiana*, (i) – *T. korolkowii,* and (j) – *T. turkestanica.* The habitat suitability is shown through a colour gradient, with known locations of populations, lakes, and protected areas also shown on each map.

Table 5.3. The test data AUC values for the present day and each climate scenario for the ten selected species with standard deviation in brackets.

	T. bifloriformis	*T. dasystemon*	*T. ferganica*	*T. greigii*	*T. heterophylla*	*T. jacquesii*	*T. kaufmanniana*	*T. kolpakowskiana*	*T. korolkowii*	*T. turkestanica*
Present day (bias file)	0.983 (0.008)	0.976 (0.007)	0.991 (0.007)	0.985 (0.005)	0.976 (0.018)	0.992 (0.006)	0.996 (0.005)	0.989 (0.008)	0.993 (0.004)	0.978 (0.008)
Present day (no bias file)	0.990 (0.003)	0.980 (0.007)	0.991 (0.008)	0.989 (0.005)	0.979 (0.026)	0.995 (0.002)	0.996 (0.005)	0.993 (0.005)	0.994 (0.003)	0.985 (0.006)
CC-BC-2050	0.992 (0.002)	0.974 (0.009)	0.991 (0.004)	0.993 (0.002)	0.998 (0.001)	-	0.999 (0.000)	0.989 (0.005)	0.992 (0.004)	0.984 (0.007)
CC-BA-2050	0.992 (0.002)	0.969 (0.016)	0.991 (0.004)	0.992 (0.002)	0.998 (0.001)	-	0.999 (0.000)	0.989 (0.005)	0.999 (0.000)	0.984 (0.007)
CC-BC-2070	0.992 (0.002)	0.974 (0.009)	0.991 (0.004)	0.993 (0.002)	0.998 (0.001)	-	0.998 (0.001)	0.989 (0.005)	0.995 (0.003)	0.984 (0.007)
CC-BA-2070	0.992 (0.002)	0.974 (0.009)	0.991 (0.004)	0.993 (0.002)	0.998 (0.001)	-	0.999 (0.000)	0.989 (0.005)	0.999 (0.000)	0.984 (0.007)
MR-BC-2050	0.992 (0.002)	0.961 (0.018)	0.991 (0.004)	0.991 (0.002)	0.998 (0.001)	-	0.998 (0.001)	0.989 (0.005)	0.996 (0.002)	0.984 (0.007)
MR-BA-2050	0.992 (0.002)	0.966 (0.014)	0.991 (0.004)	0.989 (0.002)	0.998 (0.001)	-	0.999 (0.000)	0.988 (0.005)	0.996 (0.002)	0.984 (0.007)
MR-BC-2070	0.992 (0.002)	0.982 (0.006)	0.991 (0.004)	0.985 (0.004)	0.998 (0.001)	-	0.999 (0.000)	0.989 (0.005)	0.994 (0.004)	0.984 (0.007)
MR-BA-2070	0.992 (0.002)	0.980 (0.005)	0.991 (0.004)	0.989 (0.003)	0.998 (0.001)	-	0.985 (0.012)	0.988 (0.005)	0.996 (0.002)	0.984 (0.007)

Table 5.4. The most significant variables that contributed to each species present day model. Reported are those that had the greatest percent contribution shown in the first row for each species and permutation importance shown in the second row for each species. If the second row is empty the variable reported in the first row had the largest percent contribution and permutation importance.

	Variable	**Percent Contribution**	**Permutation importance**
T. bifloriformis	Precipitation of Coldest Quarter	38.6	5.3
	Precipitation Seasonality	2.5	60.3
T. dasystemon	Precipitation of Driest Month	27	22.4
	Precipitation of Wettest Month	4.5	50.7
T. ferganica	Precipitation of Coldest Quarter	22.1	0.2
	Precipitation Seasonality	6.5	66.8
T. greigii	Precipitation of Coldest Quarter	22.1	0.7
	Precipitation Seasonality	10.2	44.5
T. heterophylla	Precipitation of Driest Month	23.2	31.4
	Precipitation of Wettest Month	5.7	38.3
T. jacquesii	Precipitation Seasonality	39.9	62.9
	-	-	-
T. kaufmanniana	Precipitation of Coldest Quarter	32.7	13
	Precipitation Seasonality	10.5	60.4
T. kolpakowskiana	Precipitation of Driest Month	25.9	2.4
	Mean Temperature of Coldest Quarter	6.9	39.3
T. korolkowii	Mean Temperature of Driest Quarter	26.8	8.1
	Mean Temperature of Coldest Quarter	11.8	68.6
T. turkestanica	Mean Temperature of Driest Quarter	33	2.3
	Mean Temperature of Coldest Quarter	9	55.1

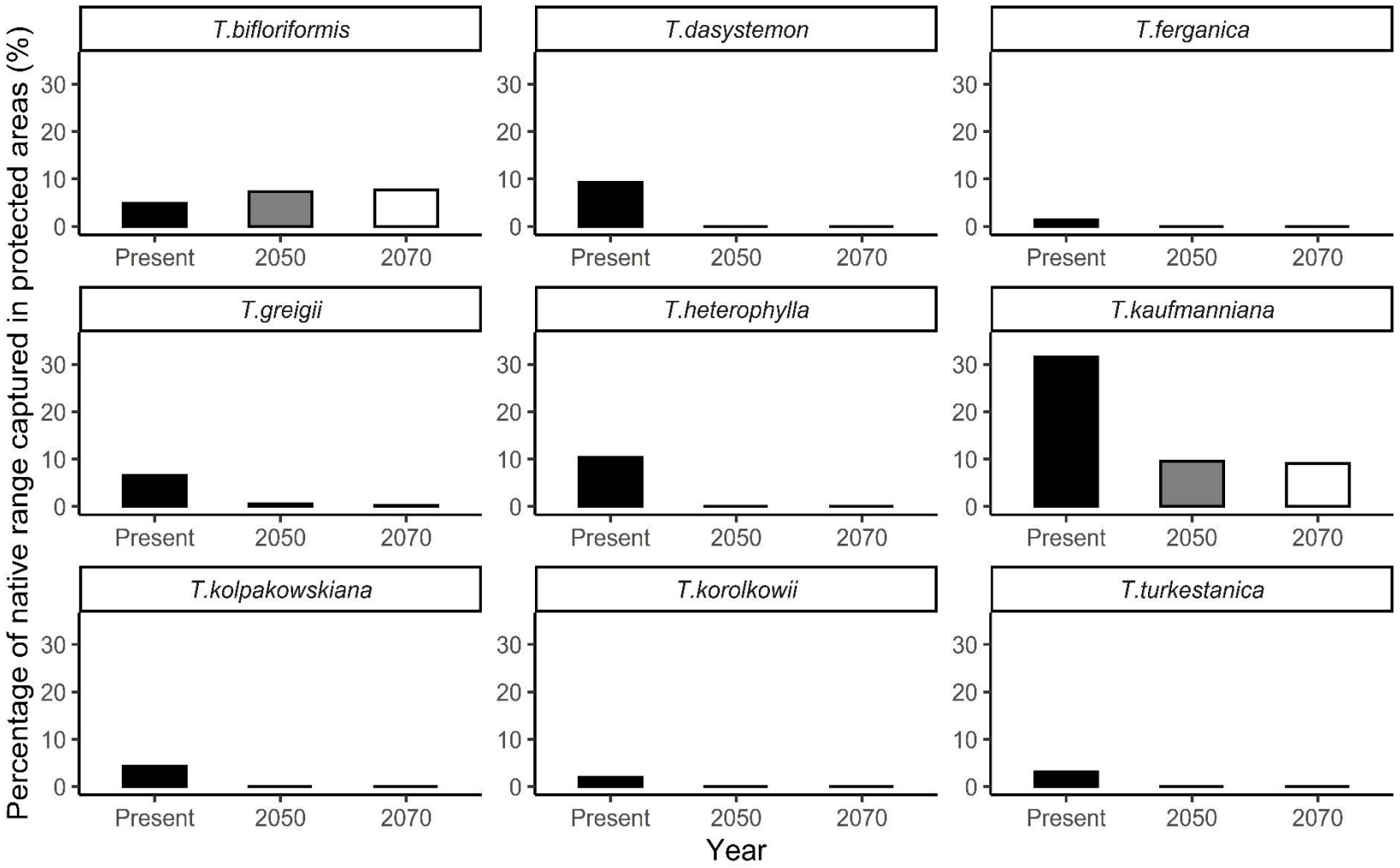


Figure 5.3. Bar chart showing the percentage of a species high and very high native suitable habitat area captured within protected areas across present day, 2050, and 2070.

2050 to 2070, with on average 78% of high and very high suitable habitat areas lost by 2050 and 83% lost by 2070. Whilst suitable habitat for the semi-desert dwelling species, *T. korolkowii* and *T. kolpakowskiana* and the alpine species of *T. turkestanica, T. heterophylla,* and *T. ferganica* is predicted to disappear completely. Overall, BC and BU scenarios were broadly similar however generally BU scenarios showed marginally less suitable habitat than the BC scenarios (Figure 5.4).

Under future climate scenarios protected area coverage in the species native range decreased for all species except *Tulipa bifloriformis*. For seven out of the nine modelled species coverage dropped to below one percent by 2050 and for six no suitable habitat was protected (Figure 5.3; Appendix 12). Our analyses also revealed that there was a significant difference between the mean altitude (metres) between the present day, 2050 and 2070 for *T. bifloriformis* [$F(2, 296712) = 224119$, $p < 2.2\times10^{-16}$], *T. dasystemon* [$F(1, 226007) = 16.098$, $p = 6\times10^{-5}$], *T. greigii* [$F(2, 145406) = 2280.6$, $p < 2.2\times10^{-16}$], and *T. kaufmanniana* [$F(2, 36941) = 29635$, $p < 2.2\times10^{-16}$] (Figure 5.6; Appendix 13). More specifically, the altitudinal range significantly narrowed in future years for all species, whilst the suitable habitat areas for *T. bifloriformis*, *T. dasystemon*, and *T. kaufmanniana* were predicted to shift to higher altitudes. Surprisingly and unlike these other species the suitable habitat for *T. greigii* in 2050 was predicted to occur on average at lower altitudes than the present day, specifically at the base of previously more broadly suitable mountains. Nonetheless, the area of suitable habitat in 2070 was then predicted to shift back to near the present day mean altitude. All comparisons between years for these four species were deemed significant through a post-hoc TukeyHSD test (Table 5.6).

5.4. Discussion

Central Asia is the primary diversity hotspot for wild tulips and many species in this region have an elevated risk of extinction. Several new species have recently been described with highly restricted ranges. However, distributional understanding of tulips across this area is often poor and threats are inadequately reported. This study is the first to take a regional level approach to model current distributions of *Tulipa*, including one newly described endemic species, and assess how habitat suitability may change under different climate scenarios. Our models highlight a range of important results both for present day modelling as well as under future climate scenarios that allows us to draw a number of conclusions, primarily about tulips, with implications for the wider plant community and conservation. We recognise the limitation of modelling approaches to current and future species distributions, nonetheless our models

Table 5.5. Percentage declines in high and very high native suitable habitat areas for all species for which climate modelling was undertaken.

	Year	*T. bifloriformis*	*T. dasystemon*	*T. ferganica*	*T. greigii*	*T. heterophylla*
Decline in habitat from present day (%)	2050	60	100	100	85	100
	2070	61	100	100	97	100
		T. kaufmanniana	***T. kolpakowskiana***	***T. korolkowii***	***T. turkestanica***	
Decline in habitat from present day (%)	2050	65	100	100	100	
	2070	73	100	100	100	

Table 5.6. The statistical results of the TukeyHSD test used to assess the change in mean altitude of species over time. The altitudinal range of highly suitable habitat areas was measured across the present day, 2050, and 2070 under the CCSM4 GCM model. For each species the comparison of the estimated mean altitude between years is shown, alongside the estimated difference in mean altitude, the upper and lower bounds of the 95% confidence interval of the mean, and the significance of the difference recorded using a p value.

	Year comparison	**Estimated difference (m)**	**95% confidence interval for mean lower bound (m)**	**95% confidence interval for mean upper bound (m)**	**p value**
T. bifloriformis	2020 - 2050	2003	1994	2011	<0.0005
	2020 - 2070	2033	2024	2042	<0.0005
	2050 - 2070	30	20	41	<0.0005
T. dasystemon	2020 - 2050	721	369	1074	<0.0005
T. greigii	2020 - 2050	-251	-260	-242	<0.0005
	2020 - 2070	-74	-95	-53	<0.0005
	2050 - 2070	177	155	199	<0.0005
T. kaufmanniana	2020 - 2050	1020	1008	1031	<0.0005
	2020 - 2070	999	986	1013	<0.0005
	2050 - 2070	-20	-36	-5	<0.01

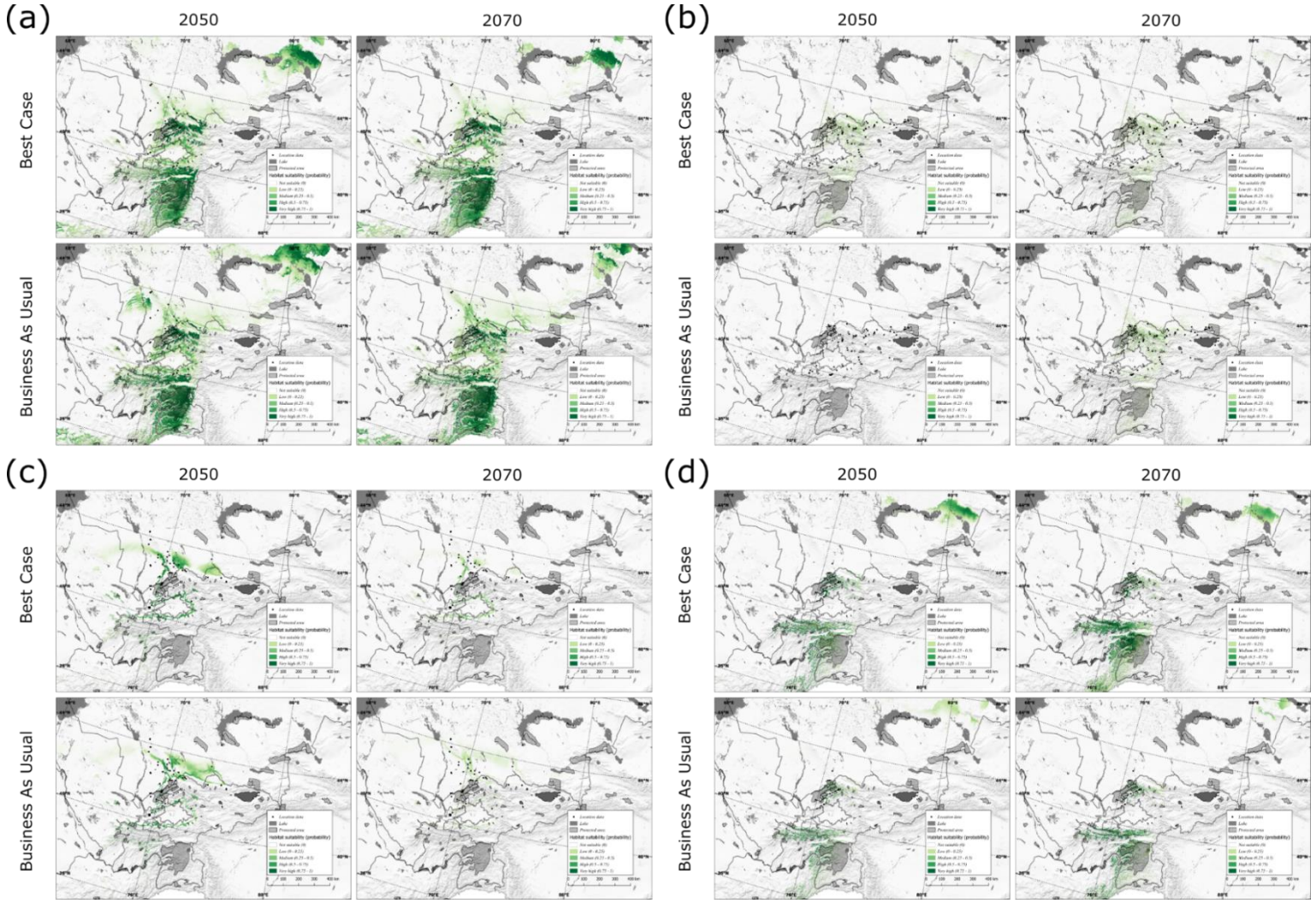


Figure 5.4. Climate modelling outputs showing suitable habitat areas for the four species with predicted future habitat. The maps correspond to (a) – *T. bifloriformis*, (b) – *T. dasystemon*, (c) – *T. greigii*, and (d) – *T. kaufmanniana.* Projections for best case and business as usual climate scenarios produced using the CCSM4 GCM model are shown projected in Asia North Albers Equal Area Conic coordinate reference system (ESPG 102025) with cell size of ~0.65km^2. The habitat suitability is shown through a colour gradient, with known locations of populations, lakes, and protected areas also shown on each map.

provide an important resource, especially to aid future Red Listing efforts for the *Tulipa* genus, as well as to guide appropriate conservation interventions.

First, our models showcase the tight link between most Central Asian tulip's distributions and the mountain ranges of this region in both current and future climate scenarios. Our work underscores the importance of mountains in the niche occupancy of tulips in line with previous studies (Botschantzeva, 1982). Far fewer species inhabit the lowland steppes and semi-desert areas of the region (Everett, 2013), and so although these may still be of importance for conservation efforts, targeting mountainous areas is more urgent given the limited resources available to conservation practitioners (Bottrill *et al.*, 2008). Moreover, our models highlight that many species distributions overlap across these alpine areas. This includes a number that are superficially similar showcasing the taxonomic difficulties of this genus, which are so often reported (Zonneveld, 2009; Christenhusz *et al.*, 2013). Given this, we urge researchers to be cautious when using tulip location data, especially where not supported by herbarium specimens, as well as our models and to critically assess these based on the current known natural distributions. This identification problem is exacerbated by the inconsistent use of the taxonomy of tulips (Christenhusz *et al.*, 2013). We currently recommend basing species concepts off Christenhusz et al. (2013) so as to ensure consistent use of names across the scientific community, until further taxonomic work can update species concepts, which we note is urgently needed.

Many of the transnational species modelled in this project are reported Threatened across parts of their range. Yet, our results highlight that, frequently, the countries in which they are reportedly most Threatened often harbour only a small proportion of the overall distribution, and potential distributions under climate change. This is especially apparent for *Tulipa bifloriformis*, *T. korolkowii* and *T. kaufmanniana* which are recorded in Tajikistan as Endangered, Endangered, and Critically Endangered respectively, yet they are recorded in an exceptionally small area of northern Sughd region, which may represent the extremity of their range. Nonetheless, this is also the case for other species such as *T. greigii* and *T. dasystemon.* This trend highlights that relying on national assessments for an understanding of the extinction risk of the whole species may be misleading and that global assessments provide a much more informative and reliable approach. Nevertheless, remote regions of a species' distribution must still be considered in conservation planning as they potentially represent important sites of local adaptation and therefore genetic novelty (Flanagan *et al.*, 2018). We also note through our work that several species are not evaluated in countries where they are reported as native, for

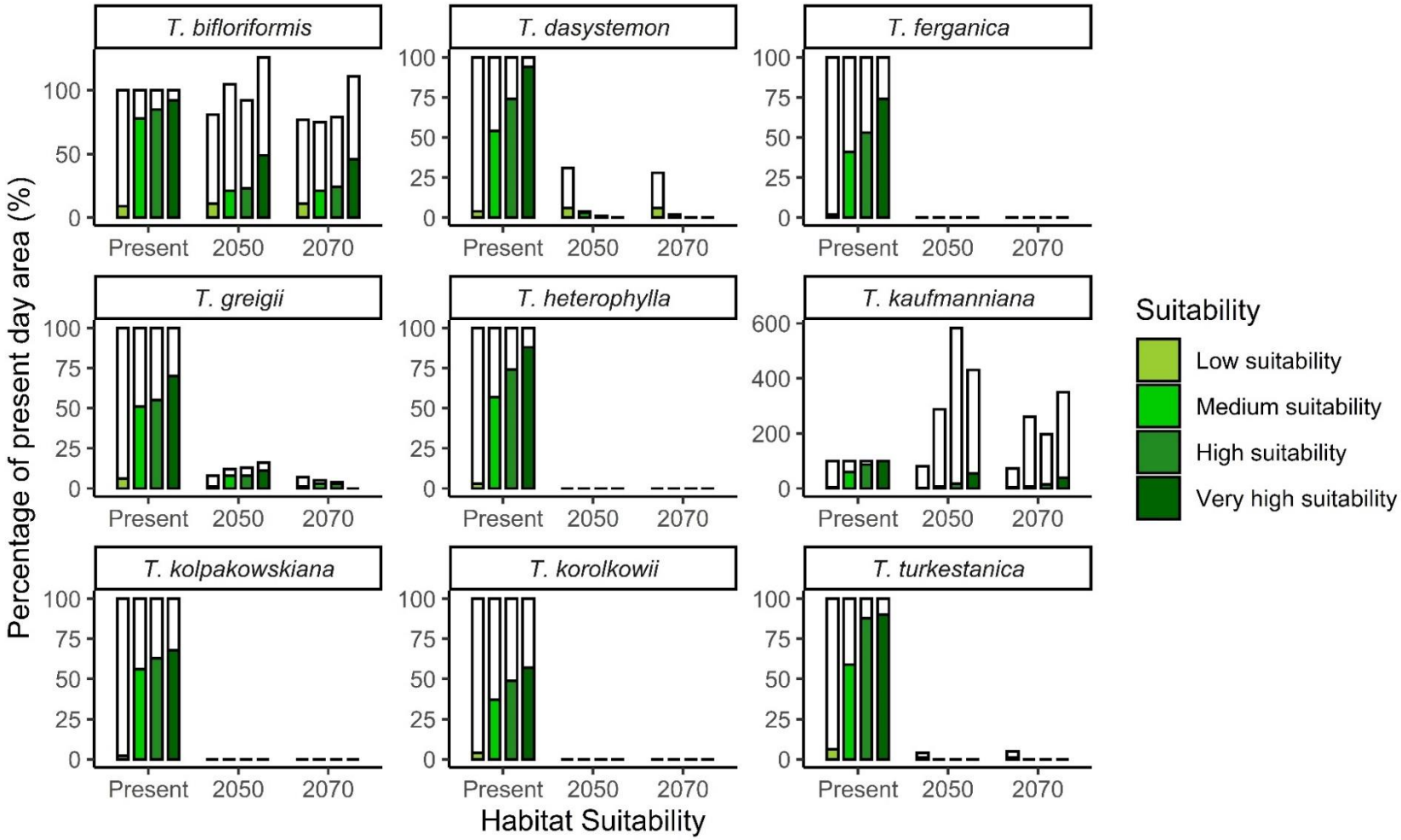


Figure 5.5. Bar chart showing, for nine modelled species, the percentage of present-day suitable habitat area and that projected to remain in 2050 and 2070. White bars represent non-native areas of suitable habitat and coloured bars represent native areas. Habitat suitability is separated into four categories low, medium, high, and very high with different shades of green representing degrees of habitat suitability. N.B. *T. kaufmanniana* graph has a y-axis that extends to 600% because large areas outside of the natural habitat became suitable under future climate scenarios.

example *T. dasystemon* in Kyrgyzstan. This is often because the species is widespread and national documents only focus on Threatened species (Davletkeldiev, 2006). However, many of these taxa are considered Threatened elsewhere in their range e.g., *T. dasystemon* is Vulnerable in Uzbekistan. National level assessments may therefore present species as Threatened and in need of urgent action when across their broader range they could be considered relatively secure. Our work reinforces that although national level information remains an important resource, it needs to be critically assessed and considered in a broader context for use in directing conservation actions for non-endemic species. Furthermore, our climate models show that suitable habitat in future scenarios will remain trans-national and so international efforts will be crucial for tackling the impacts of climate change in Central Asia.

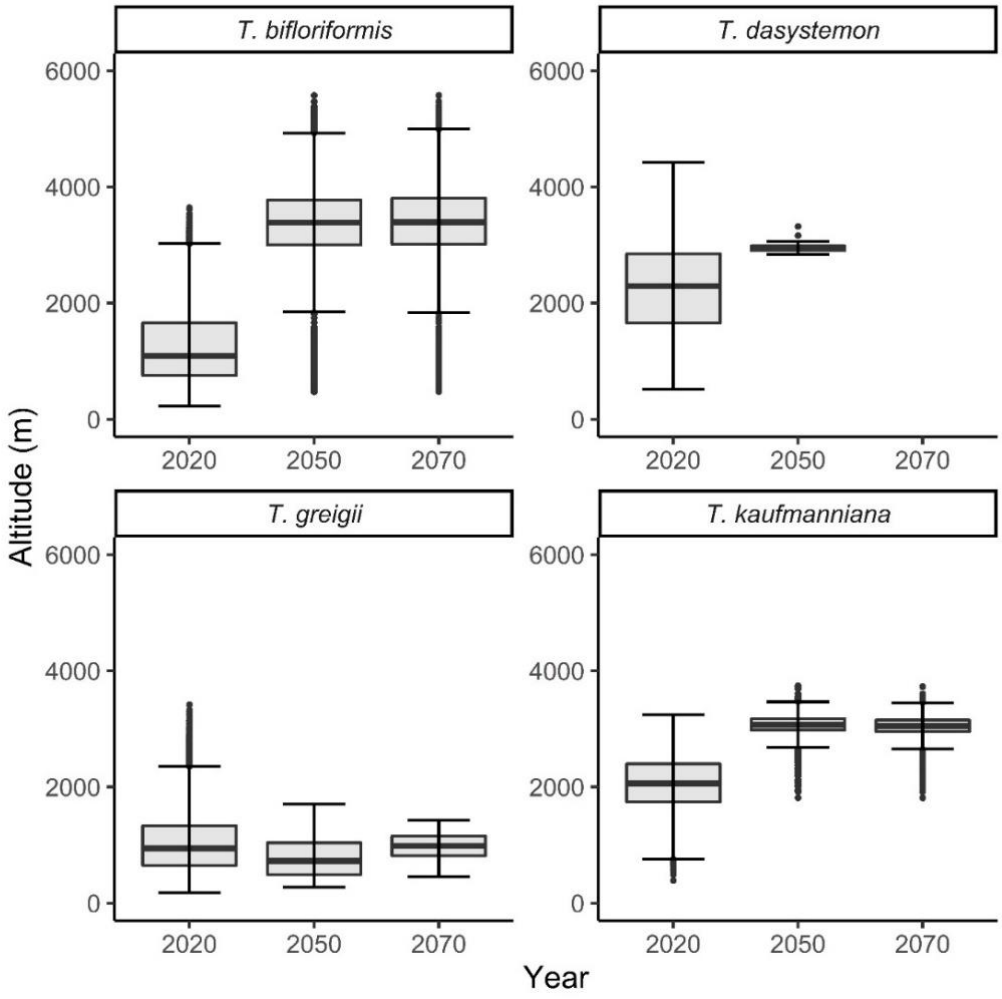


Figure 5.6. Boxplots showing the altitudinal range encompassed in high and very high suitable habitat areas for four species with projected habitat across the present day, 2050, and 2070.

Due to its recent description, the endemic *Tulipa jacquesii,* unlike the other species modelled here, had very limited location data available. We decided to model this species even given its limited GPS data to present an understanding of the challenges associated with a newly described taxon, as in the past decade a number of new tulip species have been described representing a considerable degree of newly discovered diversity (Tojibaev, De Groot and Naralieva, 2014; De Groot and Tojibaev, 2017; De Groot and Zonneveld, 2020). Currently, like *T. jacquesii*, these taxa generally lack location information and conservation assessments. Our modelling of *T. jacquesii* supports previous research suggesting that predicting distributions with an extremely low number of points is highly constrained (Pearson *et al.*, 2007); in our case the predicted range was much larger than expected. Our work therefore importantly highlights the need for efforts to explore distributions for recently described tulip species to enable more accurate modelling and assessment of true distribution size. This forms part of a broader need for more information about these species to facilitate reliable assessment of their conservation status. Our *T. jacquesii* model provides a resource to aid in the search for new populations of this endemic species (Fois *et al.*, 2018), albeit lacking significant resolution.

Protected areas remain essential to conservation efforts globally (Naidoo *et al.*, 2019), as reflected in policy in the Aichi Biodiversity Targets (Venter *et al.*, 2014), and present a useful tool for safeguarding tulip populations. Using our models, we explored the overlap between predicted distributions and coverage of protected areas. Overall, this work emphasises the poor coverage of the protected area network of Central Asia in capturing tulip diversity. In general, most species have only been reported in one protected area (GBIF.org, 2020) and our models support the view that only small parts of most species' distributions are captured in this network. Nonetheless our models do highlight that most species likely occur in several protected areas, but not always with confirmatory location data. Further efforts are needed to document the presence of species in many protected areas across the region. For example, our models of the species *Tulipa korolkowii and T. ferganica* underline the restricted representation of these taxa in protected areas. Current knowledge also suggests that *T. jacquesii* does not occur in any protected areas, yet our model lacks the resolution to confidently assess this. Given the importance of protected areas for plant conservation (Chape, Spalding and Jenkins, 2008; Souza and Prevedello, 2020), the limited coverage provided for Threatened tulips needs to be addressed. Here, our models together with previous work (Botschantzeva, 1982; Everett, 2013) show that large areas of suitable habitat for these species are situated away from settlements in remote mountainous areas where increased protected area coverage may be feasible (Venter *et al.*, 2018). These remote areas form part of the broader Mountains of Central Asia

Biodiversity Hotspot (Critical Ecosystem Partnership Fund, 2016) and so protection of these habitats may improve the survival chances of an array of Threatened species (Nowak *et al.*, 2020). Even so, it is important to recognise that protection of lowland areas, especially semi-desert areas, will clearly also be essential for conserving species such as *T. korolkowii,* that are currently overly exposed to extinction due to the significant underrepresentation of their habitat in the Central Asian protected area network. Importantly, protected areas will not offer a silver bullet, with populations known to have declined in some strictly protected reserves (Krasovskaya and Levichev, 1986), therefore a combination of conservation actions will need to be put in place alongside a strengthened protected area network. Regular monitoring of populations and stronger enforcement of environmental laws will also be critical components of successful implementation.

We undertook climate change modelling of this region to offer the first ever perspective on how this threat may impact future tulip habitat suitability. Across all models, seasonality, and precipitation or temperature patterns in the winter or summer months were deemed important predictors of distribution and this emphasises the importance of seasonal triggers in the life cycle of tulips (Botschantzeva, 1982). Broadly, our models show that areas of habitat suitability will decline in all species including even the widely distributed and relatively common species, such as *Tulipa dasystemon*, which exhibit a significant loss of suitable habitat in their native range. The severity of these declines is captured most clearly for *T. bifloriformis* which showed the lowest recorded loss of native habitat under future climate scenarios, yet even in this species only 40% of the present-day area of ‘high’ and ‘very high’ suitable habitat areas was predicted to remain in 2050. Clearly, climate change poses a significant threat to all tulip diversity in this region, mirroring the situation of many plants worldwide (Parmesan and Hanley, 2015). We note that BU models were broadly worse than BC and so climate change mitigation may play a role in tulip conservation, but our models emphasise the severe plight of tulips even under best case climate scenarios. Although there is uncertainty surrounding our models, they reveal that distinct tulip habitats vary in vulnerability to climatic shifts. For example, our work shows that all semi-desert and steppe dwelling species are predicted to see a complete loss of suitable habitats by 2050, whereas only some alpine species show this. This is likely due to changes in rainfall patterns across these areas with aridity predicted to increase (Lioubimtseva and Henebry, 2009). Given this information there is an urgent need to better protect populations of these semi-arid species now to allow genetic diversity to develop that may enable better resilience to climate change impacts in the future (Jump and Peñuelas, 2005). Suitable habitat for some alpine species undergoes observable shifts to higher altitudes and declines in a

stepwise manner as time progresses. This shift in altitude has been previously observed in different plant groups (Lenoir *et al.*, 2008), but we provide supporting evidence that some tulip species may also show similar migratory trends. Our models suggest that this will also increase fragmentation of alpine refuges, leading to reduced gene flow between populations and an increased risk of extinction (Halloy and Mark, 2003) escalating the need for more targeted conservation actions.

Protected areas and their expansion would likely play a significant role in the conservation of some species under future climate scenarios. Mountainous areas, including the mountains of Central Asia, are predicted to be extremely sensitive to the impacts of climate change (Rangwala and Miller, 2012), yet across Central Asia there are a number of protected areas that encompass high altitudinal habitat, which notably are already connected to landscapes where tulips grow. Broadly our modelling shows that protected area coverage of species will decrease under future climate scenarios yet, they also suggest that several protected areas already encompass suitable habitat into which tulips may eventually migrate and so could be of increasing importance to populations as climate patterns begin to change. As an exception to this rule, *Tulipa bifloriformis* appears to have more suitable habitat in protected areas in future climate scenarios. Even so, given the poor dispersal range of tulips (Kashin, Kritskaya and Schanzer, 2016), a trait deemed important for survival in alpine areas (Rumpf *et al.*, 2019), migration may be slow and could prevent species from reaching suitable habitat areas before dying out. There is already significant evidence that extinction debts and colonisation credits will be widespread in future climate change scenarios in mountainous regions (Rumpf *et al.*, 2019). So, although several alpine tulips are predicted to have suitable habitat at higher altitudes, including within protected area, their survival may still rely on human intervention. Interestingly, suitable areas way outside of several species natural ranges were highlighted in our modelling. This was especially apparent for *T. kaufmanniana* and *T. bifloriformis*, whose native range currently encompasses the mountains around the Fergana valley, but where large parts of Tajikistan's more southerly Pamir mountains became suitable in 2050 and 2070 (Figure 5.2; Figure 5.5). We therefore suggest that future species translocation initiatives (Berger-Tal, Blumstein and Swaisgood, 2020) may be necessary, although considerable further work is needed to determine the effectiveness and ecological safety of such an action.

We note here that our models do not account for a range of factors. Genetic variation and species adaptability to climate change has not been incorporated, but can be important for persistence in areas deemed unsuitable (Graae *et al.*, 2018; Razgour *et al.*, 2019). Moreover,

previous research has shown tulips populations can actively populate and survive in highly disturbed landscapes including agricultural land (Krasovskaya and Levichev, 1986; Pratov *et al.*, 2006) and therefore may survive better in a changing landscape than our models predict. Although changes in climate may decouple seasonal triggers such as flowering time (Wadgymar *et al.*, 2018) which could be exceptionally damaging for tulips and similar plants that rely heavily on these for the timing of their short growing season. Furthermore, alpine habitats encompass a range of microclimatic niches which broad scale modelling overlook as potential refuge areas (Scherrer and Körner, 2011). Some areas deemed unsuitable may therefore in fact present adequate microclimatic conditions for the survival of local populations. The structural composition of communities, which is especially important to tulips due to their requirement for direct sunlight for growth, may mean that areas within predicted suitable habitat cannot in fact support populations (Vittoz *et al.*, 2009). We therefore acknowledge these limitations and accept that some taxa may be more resilient than suggested by our models. Nonetheless, we suggest climate change will be an important threat to tulip populations, and highlight that there are other factors we have not examined, such as the shifting of invasive species into mountainous areas, that could exacerbate impacts further (Petitpierre *et al.*, 2016).

Climate change is not the only threat posed to wild tulip species. Poorly managed livestock can cause significant damage to ecosystems (Wilson *et al.*, 2019b) and livestock overgrazing continues to degrade habitat across much of Central Asia (Tojibaev and Beshko, 2015; Nowak *et al.*, 2020). Given that livestock populations are thought to be on the increase across Central Asia, overgrazing appears to pose a growing threat to tulips. Furthermore, although many settlements are in rural areas (Djanibekov *et al.*, 2016) urbanisation also poses a threat to tulips. Many of the major cities in Central Asia are situated close to mountainous tulip habitat, including Bishkek, Almaty, Dushanbe, and Samarkand. Given the rapid development of these cities and the corresponding loss of habitat, urbanisation needs to be urgently considered as part of any tulip conservation activities in the region; a similar but more localised threat is presented by mining activities. Finally, the horticultural history of the genus and the demand for tulips worldwide has meant that wild collection and trade has been reported as a threat and is believed to have led to previous extinctions and populations declines (Maunder *et al.*, 2001; Menteli *et al.*, 2019). Central Asian tulips have been an important part of tulip horticulture throughout the existence of this trade (Christenhusz *et al.*, 2013) and now many Threatened tulip species are protected by law (Davletkeldiev, 2006). Yet, opportunistic collection continues, and this may exacerbate the impact of other threats including climate change.

Overall, here we have shown that climate change will pose a significant threat to wild tulips, whilst current distributions of most species are tightly linked to the mountains of the broader Central Asia region and are poorly captured in protected areas. This leaves many populations already declining, spanning borders that scientific research and conservation collaboration has not yet bridged, and increasingly exposed to an array of threats and their interactions. Whilst our work has focused on the genus *Tulipa*, and specifically Central Asian species, we recognise that many plant groups require similar focused attention and so, although we advocate for urgent efforts to protect wild tulips from growing threats, we also suggest that efforts are made to carefully assess and use available data, including national level assessments, to improve conservation of plants across broader Central Asia. Yet, most importantly in this paper we have shown that a regional approach is essential for an accurate understanding of a species' risk of extinction, especially with respect to the growing impacts of climate change. Given this, now is the time for the broader conservation community to work together to ensure a more aligned regional approach in Central Asia.

Chapter 6.

Generating an evidence-base to identify conservation priorities

In this chapter we use the outputs from all the previous chapters and the scientific literature to collate information on 53 Central Asian tulip species allowing us to produce Red List reports for these taxa. We then analyse the information in these reports to gain an overview of the status of Central Asian wild tulip diversity. In addition to this, we use the assigned Red List categories for species alongside our phylogeny to calculate EDGE scores for the genus *Tulipa* enabling another form of species prioritisation. Finally, we begin to think about key sites, objectives and activities that could form the foundation of a regional tulip conservation strategy.

6.1. Introduction

Biodiversity is in crisis (Soule, 1985) with continued declines in many species across all kingdoms of life (Pimm *et al.*, 2014). The loss of biodiversity threatens both the functions and services provided by ecosystems (Oliver *et al.*, 2015). Plants are often less well studied than animals (Balding and Williams, 2016), but they are extremely important for supporting the functioning of ecosystems in various ways including through maintaining soil health, regulating climate, and purifying water and air (Cardinale *et al.*, 2011). Plants are also essential to humans providing construction material, food, fuel, and medicine. However, two out of every five plant species are estimated to be threatened with extinction (Antonelli *et al.*, 2020; Nic Lughadha *et al.*, 2020). This is due to an array of threats including, but not exclusively, agriculture, climate change, invasive species, and over-exploitation (Murphy and Romanuk, 2014). Plant conservation is therefore extremely important, so much so that a Global Strategy for Plant Conservation (GSPC) was established in 2002 to directly halt the decline in plant species and to promote more sustainable future use of plants (Wyse Jackson and Kennedy, 2009).

The mountains of Central Asia are a recognised biodiversity hotspot partly due to the diversity of plants growing there, with around 5000 vascular plants known, as well as the large number of crop-wild relatives found in the region (Critical Ecosystem Partnership Fund, 2016). The species of Central Asia are often underrepresented in collections and databases (Yesson *et al.*, 2007; Paton *et al.*, 2020) and due to the history and politics of the region it remains somewhat disconnected from broader conservation movements (Nowak *et al.*, 2020), although in recent years this has begun to shift, exemplified by the fact that Uzbekistan has recently joined the International Union for Conservation (IUCN, 2021). Nonetheless, conservation in this region is still constrained due to funding limitations, language barriers, political instability, poor

infrastructure, and a lack of equipment (European Commission and Directorate-General for International Cooperation and Development, 2019). To exacerbate matters, rural poverty is relatively high in Central Asia (Seitz, 2019), and this continues to drive environmental damage (Fisher and Christopher, 2007). There is therefore an increasing need to raise awareness of the biodiversity of this region and promote development alongside conservation (Adams *et al.*, 2004; Redford *et al.*, 2008).

Central Asia is well-known as the centre of diversity for wild tulips (Hoog, 1973; Botschantzeva, 1982), which are one of the most well-known geophytes worldwide. These plants are not only important indicators of ecosystem health, especially for pastureland, but they also support pollinator and insect populations (Kashin, Kritskaya and Schanzer, 2016; Su *et al.*, 2020). Wild tulips also have broad cultural value (Pavord, 1999), especially in the countries of Central Asia where they are commonly the national flower. This means they could function as a flagship plant for conservation in this region (Pany and Heidinger, 2017). In addition, wild tulips from this region have historically been used in breeding of horticultural varieties (Orlikowska *et al.*, 2018). Increasingly attention is returning to wild diversity in plants, including tulips, as they represent important genetic reserves for future breeding efforts especially surrounding disease resistance and climate change adaptability (Orlikowska *et al.*, 2018).

Many tulips in Central Asia have already been identified as threatened, with multiple national assessments undertaken (Davletkeldiev, 2006; Baitulin, 2014; Tojibaev and Beshko, 2015; Hisoriev *et al.*, 2016) highlighting the need for conservation action. However, there still remains a distinctive lack of regional cohesion in conservation action across Central Asia (Wilson *et al.*, 2021) and this is highlighted by the lack of any Central Asian tulips on the global IUCN Red List (IUCN, 2022). The global IUCN Red List is extremely important in supporting conservation action (Betts *et al.*, 2020) and the lack of tulips on this database is likely greatly hindering their conservation. Without identification of threatened species: funding opportunities are often limited, species are overlooked in policy making, and conservation prioritisation is difficult. In addition further analyses of the broader taxonomic groups are difficult such as: EDGE analyses (Isaac *et al.*, 2007) or the production of IUCN reports (Wilson *et al.*, 2019a). Although national Red books provide important information for conservation, they often misconstrue the extinction risk of species (Mounce *et al.*, 2018), and they lack the scale and influence of global assessments, which are recognised by the international conservation community and governments.

Conservation relies heavily on species concepts and taxonomy (Godfray, Knapp and Mace, 2004), but taxonomy is often messy (Garnett and Christidis, 2017) and uncertainty in species concepts can hinder conservation efforts (Hey *et al.*, 2003). A reliable taxonomic framework is therefore crucial for: conservation, especially for Red Listing, developing species action plans, and obtaining funding. The work presented thus far in this thesis means that this species framework is now in place for tulips, opening the door to a range of opportunities to improve the conservation of this genus. Here we use our new species list, developed through a large phylogenetic analysis of the genus, to drive the Red Listing process for Central Asian tulip species. Using the information gathered in this process and the outputs we then perform further analyses to promote a more evidence-based decision making process (Sutherland *et al.*, 2004; Bottrill *et al.*, 2008) for conservation prioritisation of tulip species, beginning the procedure of developing a regional tulip conservation strategy for Central Asia.

6.2. Materials and Methods

6.2.1. Pre-workshop preparation

A large literature review was undertaken at the start of this project, which provided an important perspective as to which key areas of the ecology and taxonomy of the genus *Tulipa* required more information, especially with respect to improving conservation effectiveness. We then proceeded to resolve elements of the taxonomy to establish a much needed species framework for conservation (Godfray, Knapp and Mace, 2004). This included both the description of a newly discovered species (Wilson *et al.*, 2022) as well as a short proposal to formally change the name of *Tulipa urumiensis* to *T. tarda* based on current usage by the science community (Christenhusz and Wilson, 2022). An updated species list was developed that built primarily upon the work of Christenhusz et al, 2013 (Christenhusz *et al.*, 2013), but also included new species described in recent years, and the outputs of our taxonomic efforts (Table 2.11).

In addition, as this project is focused primarily on the Central Asia region, we began a process of collating specific information for the tulip species in this region. We created species lists for the countries of Kazakhstan, Kyrgyzstan, Tajikistan, and Uzbekistan, as well as a list of all species that occur across these four countries (Appendix 14). We then collated geographical information for each species using the Global Biodiversity Information Facility (GBIF) through the GeoCAT programme (Bachman *et al.*, 2011). These datasets were then manually cleaned based on information provided in books (Everett, 2013), literature (Christenhusz *et al.*, 2013), online databases (POWO, 2022), and personal communications. Location data from expeditions undertaken during this PhD and a broader DEFRA Darwin funded project were added where

relevant. These datasets provided preliminary distribution information for the Red Listing process.

Using the new species list for Central Asia we initially created a database that showed the national Red List status of all 61 species that occur in the four countries of interest (Davletkeldiev, 2006; Baitulin, 2014; Tojibaev and Beshko, 2015; Hisoriev *et al.*, 2016; Nowak *et al.*, 2020) providing a preliminary understanding of the threat status of species across the region (Table 6.1). We then selected 51 species from the list, including the species to be described, for which we would produce draft Red List reports. These species were selected as they had no major taxonomic uncertainty, the majority of their range was in Kazakhstan, Kyrgyzstan, Tajikistan, and Uzbekistan, and finally that they were described before 2022. This excluded ten species from the Red Listing process. We then collated information on the biogeography, populations, habitat and ecology, threats, and use and trade of each species using a variety of sources. This included highlighting the threat of climate change, which some of our previous work has shown will cause large impacts on tulip habitat range and quality in the region (Wilson *et al.*, 2021). These draft Red List reports were entered into the SIS database where a preliminary threatened status was assigned and from where they could be edited during the workshop.

6.2.2. Workshop

A Central Asian tulip workshop was organised in collaboration with Fauna & Flora International with the aim of formally Red Listing a range of tulip species and beginning to develop a regional conservation strategy for the genus. This workshop consisted of one day of IUCN Red List training, two days of Red List assessment of tulip species, and a final strategy day (Appendix 15). Attending participants came from a range of institutions from across Central Asia and the U.K., notably there were leading tulip experts from the core countries of the Central Asian tulip diversity hotspot: Kazakhstan, Kyrgyzstan, Tajikistan, and Uzbekistan (Appendix 16). To formally Red List tulip species, we used the drafted Red List assessments prepared for the previously selected species. Participants were then able to add information to relevant sections. Distribution maps for species were developed using draft maps, which were then manually edited in the GeoCAT programme (Bachman *et al.*, 2011) to correct errors and add known location points that were missing. The IUCN categories and criteria were then applied based on the given information to select a suitable threat status for each species (Appendix 17; IUCN, 2012). During this process the taxonomy of some species were discussed with both *Tulipa*

Table 6.1. The National Red List status of species occurring across Central Asian countries. (NE – Not Evaluated, DD – Data Deficient, LC – Least Concern, NT – Near Threatened, VU – Vulnerable, EN – Endangered, CR – Critically Endangered, and when a species that does not occur in that country a – is shown. The Kazakhstan Red Book only reports species as Threatened (TH) without further categorisation and so this is reported where appropriate).

Species	Kazakhstan	Kyrgyzstan	Tajikistan	Uzbekistan
Tulipa affinis	-	VU	EN	VU
*Tulipa alberti**	TH	-	-	-
Tulipa altaica	NE	-	-	-
*Tulipa anadroma**	-	EN	-	-
Tulipa anisophylla	-	-	CR	NE
*Tulipa auliekolica**	NE	-	-	-
*Tulipa bactriana**	-	-	-	NE
Tulipa biflora	TH	NE	NE	DD
Tulipa bifloriformis	NE	NE	EN	NT
*Tulipa boettgeri**	-	-	NE	-
Tulipa borszczowii	TH	-	-	DD
Tulipa brachystemon	TH	NE	-	-
*Tulipa butkovii**	-	-	-	EN
Tulipa carinata	-	-	NE	VU
Tulipa dasystemon	NE	NE	NE	VU
*Tulipa dianaeverettiae**	NE	-	-	-
Tulipa dubia	NE	NE	NE	VU
Tulipa ferganica	-	NE	-	VU
Tulipa fosteriana	-	-	CR	EN
Tulipa greigii	TH	EN	VU	VU
Tulipa heteropetala	TH	-	-	-
Tulipa heterophylla	NE	NE	-	-
Tulipa hissarica	-	-	NE	DD
Tulipa iliensis	NE	-	-	-
Tulipa ingens	-	-	EN	VU
*Tulipa ivasczenkoae**	NE	-	-	-
*Tulipa jacquesii**	-	NE	-	-
Tulipa kaufmanniana	TH	VU	CR	NT
*Tulipa kolbintsevii**	NE	-	-	-
Tulipa kolpakowskiana	TH	VU	-	-
Tulipa korolkowii	TH	VU	EN	NT
Tulipa lanata	-	-	VU	VU
Tulipa lehmanniana	TH	-	EN	NT
*Tulipa lemmersii**	NE	-	-	-
Tulipa linifolia	-	-	VU	-
Tulipa micheliana	-	-	VU	NT

*Tulipa neustruevae**	-	NE	-	-
Tulipa orithyioides	-	NE	NE	EN
*Tulipa orthopoda**	NE	-	-	-
Tulipa ostrowskiana	TH	VU	-	-
Tulipa patens	TH	-	-	-
*Tulipa platystemon**	-	VU	-	-
*Tulipa praestans**	-	-	CR	-
*Tulipa regelii**	TH	-	-	-
*Tulipa salsola**	NE	-	-	-
*Tulipa scharipovii**	-	NE	-	EN
Tulipa suaveolens	NE	-	-	-
*Tulipa subquinquefolia**	-	-	EN	-
Tulipa sylvestris	NE	-	-	-
*Tulipa talassica**	-	NE	-	-
Tulipa tetraphylla	NE	VU	-	-
*Tulipa toktogulica**	-	NE	-	-
*Tulipa x tschimganica**	-	-	-	EN
*Tulipa turgaica**	NE	-	-	-
Tulipa tubergeniana	-	-	LC	EN
Tulipa turkestanica	NE	NE	NE	LC
Tulipa undulatifolia	-	-	NE	NE
Tulipa uniflora	TH	-	-	-
Tulipa urumiensis (tarda)	TH	NE	-	-
*Tulipa uzbekistanica**	-	-	-	EN
Tulipa vvedenskyi	-	-	NE	EN
Tulipa zenaidae	TH	VU	-	-
*Tulipa zonneveldii**	-	NE	-	-

*endemic species

orthopoda and *T. tubergeniana* proposed to be reinstated. These were therefore included in the Red Listing process making the overall number of Central Asian tulip species 63.

On the final day participants took part in a number of activities that supported the selection of key regional sites for tulip conservation, priority objectives, and began to propose activities to achieve these objectives. To select regional priority areas national groups highlighted important areas for tulips in their own country before the broader group of participants worked together to identify regional priority areas based on these initial national maps. Next, a list of fifteen pre-selected objectives were discussed and edited where participants thought necessary. Participants then each voted for the top three objectives they thought needed to be tackled based on feasibility, extent, and irreversibility, with three votes for each category. Objectives

were then ordered based on a total score across these three criteria. The top six priority objectives were discussed with regards to what activities could be undertaken in each country to address these.

6.2.3. Post-workshop analyses

EDGE

Using the dated phylogeny generated in chapter four a measure of evolutionary distinctiveness (ED) for each species was calculated using both an equal splits (Redding and Mooers, 2006) and fair proportions (Isaac *et al.*, 2007) method in R version v 4.1.2 (R Core Team, 2022) using the evol.distinct function in the picante package v1.8.2. (Kembel *et al.*, 2010). The corresponding value for globally endangered (GE) status for each species with a formal IUCN Red List assessment was recorded. Finally, an EDGE score was calculated for those species with both an ED and GE value based on the previously established equation $EDGE = \ln(1 + ED) + GE \times \ln(2)$ (Isaac *et al.*, 2007). The EDGE scores generated using the equal splits and fair proportions methods were compared, with the more modern fair proportions approach favoured.

Red List analyses

The data from the Red List reports on the SIS database was downloaded through the IUCN SIS Connect programme. This data was then processed on Microsoft Excel version 2208, filtering for an array of data categories. An assessment of: threats, use and trade, protected area coverage, and presence in ex-situ collections for the genus were undertaken. In addition, a comparison between national level threat status and the globally assigned threat category was carried out. For this we used previously collated national status (Table 6.1) and combined this with the preliminary global level status assigned in the workshop based on the IUCN categories: Data Deficient (DD), Least Concern (LC), Near Threatened (NT), Vulnerable (VU), Endangered (EN), Critically Endangered (CR), Regionally Extinct (RE), Extinct in the Wild (EW), and Extinct (EX) (IUCN, 2012). Multiple rows were created for species that had several different national assessments. A score was assigned to the threat category of the species on both the national and global level: LC = 7, NT = 6, VU = 5, EN = 4, CR = 3, RE = 2, EW = 1, EX = 0, with DD species excluded. A difference in assessment score was then calculated by subtracting the national score from the global score (Mounce *et al.*, 2018). As Kazakhstan's Red Book only assigns a status of either Threatened or Not threatened to species, an average score of threatened categories, 4, was assigned to Kazakh species reported as Threatened. In addition, species that were not evaluated (NE) across these four countries are often not assessed as

they are common, so each was individually investigated and either assigned a score for LC or judged to be DD and ignored. Four datasets were presented: All data, data excluding that from Kazakhstan, data excluding NE species, and data excluding both Kazakhstan and NE species. The skew of the distribution of dataset away from a normal distribution was calculated using the SKEW function on Microsoft Excel.

6.3. Results

6.3.1. Country level diversity

Kazakhstan is the most diverse country for tulip diversity in the world containing 33 species with nine endemics. Whilst Kyrgyzstan has 25 species with seven endemics meaning it has the highest percentage of endemic species (28%). Tajikistan has 25 species with three endemics and Uzbekistan has 28 species with five endemics making them both also particularly species rich parts of the global range of tulips.

The diversity of Kazakhstan is likely partly due to the county's large size, it being the ninth largest country in the world covering ~ 2,725,000 km², which means it encompasses a range of different geographical areas that relate to different ecotypes. Kazakhstan contains species from four out of the five subgenera, with only the *Clusianae* subgenus not represented. It has a particularly high abundance of tulips from the *Orithyia* and *Kolpakowskianae* sections, whilst it also harbours the single species of the subgenus *Eduardoregelia*, newly identified in this thesis, and is the only country in the Central Asian hotpot to have species from the *Sylvestres* section. Several new species have been described from this country in the last decade, most of which are endemics and occur in remote mountainous areas or gorges as well as the morphologically distinct *Tulipa regelii*.

Kyrgyzstan is a relatively small country, but encompasses semi-desert areas surrounding the Fergana valley, large areas of mountainous meadows, as well as woodland in the foothills of the Tien Shan. Although a small nation there are representatives of three subgenera in this territory and a high number of endemics, seven. This includes a new species described within this work from the section *Kolpakowskianae,* with two other endemics from this group also known. The country harbours a significant number of species from the sections *Kolpakowskianae* and *Biflores*, and importantly accounts for several populations of *Tulipa heterophylla* from the *Eduardoregelia* section. The species *T. ferganica* from the section *Multiflorae* and *T. zonneveldii* and *T. anadroma* from the *Tulipa* section are also important tulip species that are either endemic or near endemics of this country.

Tajikistan is the most southern country within the Central Asian tulip diversity hotspot. Many species that occur in Tajikistan also occur within Uzbekistan and Kyrgyzstan due to the interlapping borders that don't directly correspond to ecoregions, although due to its southern latitudes this country harbours a range of species that do not occur in its more northern counterparts. Within the country of Tajikistan there are tulips from three different subgenera, and crucially this is the only country in the Central Asian tulip hotspot that contains a species in the *Clusianae* subgenus, while most of the *Clusianae* species occur in the Middle East to the south and west of Tajikistan. In addition, Tajikistan also harbours a large range of species from the *Tulipa* section, the *Biflores* section, and the *Multiflorae* section, with many of these primarily found only in Tajikistan, like *Tulipa praestans* and *T. anisophylla*.

Uzbekistan is an extremely diverse country for tulips given its area. The eastern half of the country is the primary area for tulips and five endemics are known in this territory with several new species having been described recently, including the range restricted *Tulipa bactriana* and *T. scharipovii*. Although Uzbekistan contains a high diversity of species there are only two subgenera represented and four sections. The primary sections are that of *Tulipa* and *Multiflorae.* This territory harbours the largest number of species from the section *Tulipa,* and this considerable diversity is centred around the Fergana valley, the foothills of the Tien Shan, and the semi-desert areas in the centre and south of the country.

6.3.2. Global Red List results

During the workshop 52 species were assessed with *Tulipa toktogulica*, still awaiting to be formally described, assessed outside of this workshop. Overall, therefore 53 IUCN Red List reports were produced representing 84% of all Central Asian tulip species (Figure 6.1, Table 6.2). In global terms this now means 59 species of tulip have Red List reports, which is around 60% of the genus, if species to be reinstated are accounted for. Around 51% of the Central Asian species we assessed were Threatened, with 15 species classified as Vulnerable, 6 Endangered, and 6 Critically Endangered. These were primarily assessed under criterion B meaning they are particularly range restricted, but some were assessed under A, C, and D highlighting that several taxa have exceptionally small populations or there are past, present, or future declines in populations reported. Around 16% of Central Asian tulip diversity remains to be assessed, but most of these species are taxonomically complicated or much of their range falls outside of this area.

The primary threat to Threatened tulip species is livestock overgrazing, but climate change, urban development, agriculture, collection of specimens, and mining are all significant threats

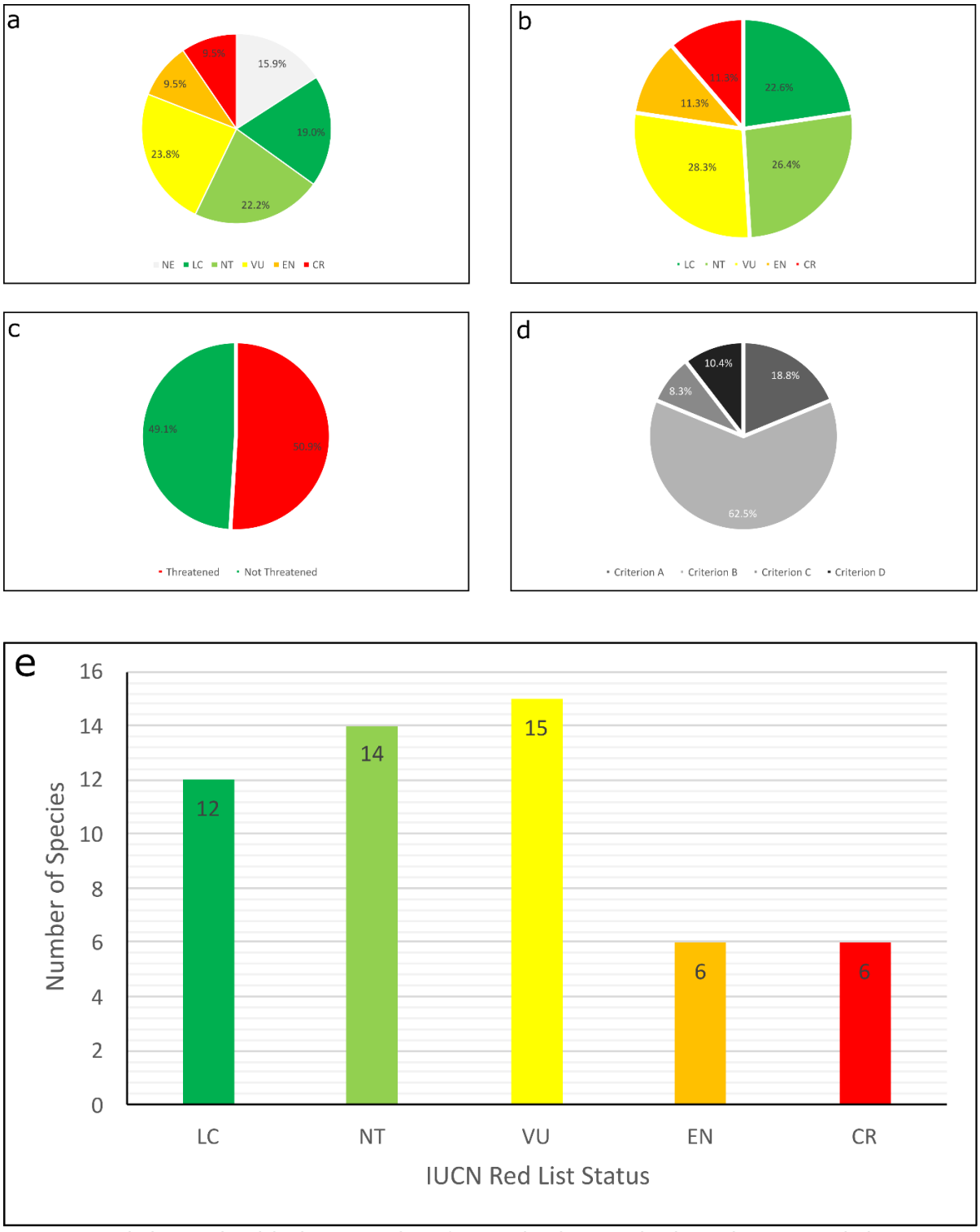


Figure 6.1. Red List results. (a) The proportion of Central Asian species in each IUCN Red List category including Not Evaluated species, (b) the proportion of Central Asian species in each IUCN Red List category excluding Not Evaluated species, (c) proportion of assessed species that are Threatened in Central Asia, (d) the IUCN criterion used to assess Threatened species, (e) the number of Central Asian species in each IUCN Red List category. (NE – Not Evaluated, LC – Least Concern, NT – Near Threatened, VU – Vulnerable, EN – Endangered, and CR – Critically Endangered)

Table 6.2. The IUCN Red List category assigned to each Central Asian tulip species assessed alongside the IUCN criterion used and reported criteria details. (NE – Not Evaluated, DD – Data Deficient, LC – Least Concern, NT – Near Threatened, VU – Vulnerable, EN – Endangered, and CR – Critically Endangered).

Species	**Red List Category**	**Red List Criteria**	**Criteria Details**
Tulipa affinis	VU	A	A4ac
Tulipa alberti	NT	B	B2b(iii)
Tulipa altaica	LC		
*Tulipa anadroma**	VU	D	D2
Tulipa anisophylla	VU	B,C	B2ab(ii,iii,v); C1+2a(i)
*Tulipa bactriana**	CR	B	B1ab(iii)+2ab(iii)
Tulipa bifloriformis	LC		
*Tulipa boettgeri**	CR	B	B1ab(iii)+2ab(iii)
Tulipa borszczowii	NT	B,C	B2b(ii,iii,iv,v); C2a(i)
Tulipa brachystemon	LC		
*Tulipa butkovii**	VU	B	B1ab(i,ii,iii,v)+2ab(i,ii,iii,v)
Tulipa carinata	VU	A	A4ac
Tulipa dasystemon	LC		
*Tulipa dianaeverettiae**	CR	B	B2ab(iii)
Tulipa dubia	NT	B	B1b(ii,iii,v)+2b(ii,iii,v)
Tulipa ferganica	LC		
Tulipa fosteriana	VU	A	A3cd
Tulipa greigii	LC		
Tulipa heteropetala	LC		
Tulipa heterophylla	LC		
Tulipa hissarica	LC		
Tulipa iliensis	NT	B	B2ab(iii)
Tulipa ingens	NT	B	B1b(ii,iii,iv,v)+2b(ii,iii,iv,v)
*Tulipa ivasczenkoae**	CR	D	D
*Tulipa jacquesii**	VU	B,C	B1ab(i,ii,iii,iv,v)+2ab(i,ii,iii,iv,v); C2a(i)
Tulipa kaufmanniana	NT	B	B2b(i,ii,iii,v)
*Tulipa kolbintsevii**	CR	B	B1ab(iii)+2ab(iii)
Tulipa kolpakowskiana	NT	B	B2b(i,ii,iii,iv,v)
Tulipa korolkowii	NT	B	B2b(i,ii,iii,iv,v)
Tulipa lanata	NT	B	B2b(ii,iii,iv,v)
Tulipa lehmanniana	NT	A,B	A3c; B2b(ii,iii,v)
*Tulipa lemmersii**	VU	D	D2
Tulipa micheliana	VU	A	A4ac
Tulipa neustreuvae	EN	B	B1ab(iii)+2ab(iii)
Tulipa orithyioides	EN	B	B2ab(iii,v)
Tulipa orthopoda	VU	B	B1ab(iii)+2ab(iii)
Tulipa ostrowskiana	NT	A,B	A2acd; B2b(ii,iii,v)
*Tulipa platystemon**	VU	D	D2

*Tulipa praestans**	VU	A	A4acd
*Tulipa regelii**	EN	A	A3cd
*Tulipa scharipovii**	EN	B	B1ab(i,ii,iii,iv,v)+2ab(i,ii,iii,iv,v)
*Tulipa subquinquefolia**	VU	B,C	B1ab(iii,v)+2ab(iii,v); C1
*Tulipa talassica**	EN	B	B1ab(iii,v)+2ab(iii,v)
Tulipa tetraphylla	LC		
Tulipa toktogulica	EN	B	B1ab(iii)+2ab(iii)
Tulipa tubergeniana	NT	B	B2b(iii,v)
Tulipa turkestanica	LC		
Tulipa uniflora	NT	B	B2b(iii)
Tulipa urumiensis (tarda)	LC		
*Tulipa uzbekistanica**	CR	B	B1ab(iii)
Tulipa vvedenskyi	NT	A,B	A4ac; B1b(ii,iii,iv,v)+2b(ii,iii,iv,v)
Tulipa zenaidae	VU	B	B1ab(ii,iii)+2ab(ii,iii)
*Tulipa zonneveldii**	VU	D	D2

(Figure 6.2). The broadest threat to all species comes from climate change, although the direct impact on many species remains uncertain. Of the species assessed 19 were known to have specimens collected from wild subpopulations, whilst 11 had local commercial value and were used in some form of trade (Figure 6.2). All species were noted as having potential national and international commercial value due to the large horticultural trade in tulips that exists and the potential for future breeding efforts to utilise wild genetic diversity. Non-Threatened species were well represented in protected areas and ex-situ collections, whereas there were significant gaps in the representation of Threatened species in protected areas or ex-situ collections (Figure 6.2). Specifically, of the six Critically Endangered species only two of these were recorded in ex-situ collections and only one was known to be in a protected area.

6.3.3. National vs. global status

Comparing national threat status and global threat status there was a broad trend that the majority of species status were the same on both levels. However, the mean Difference in Assessments showed that national assessments often have higher threat status than the global Red List with the most common distance of one IUCN category level (Figure 6.3). In addition, normally distributed data has a skew of 0 but in most datasets, with the exception of the dataset excluding only NE species, there was a positive skew away from normality showing in general

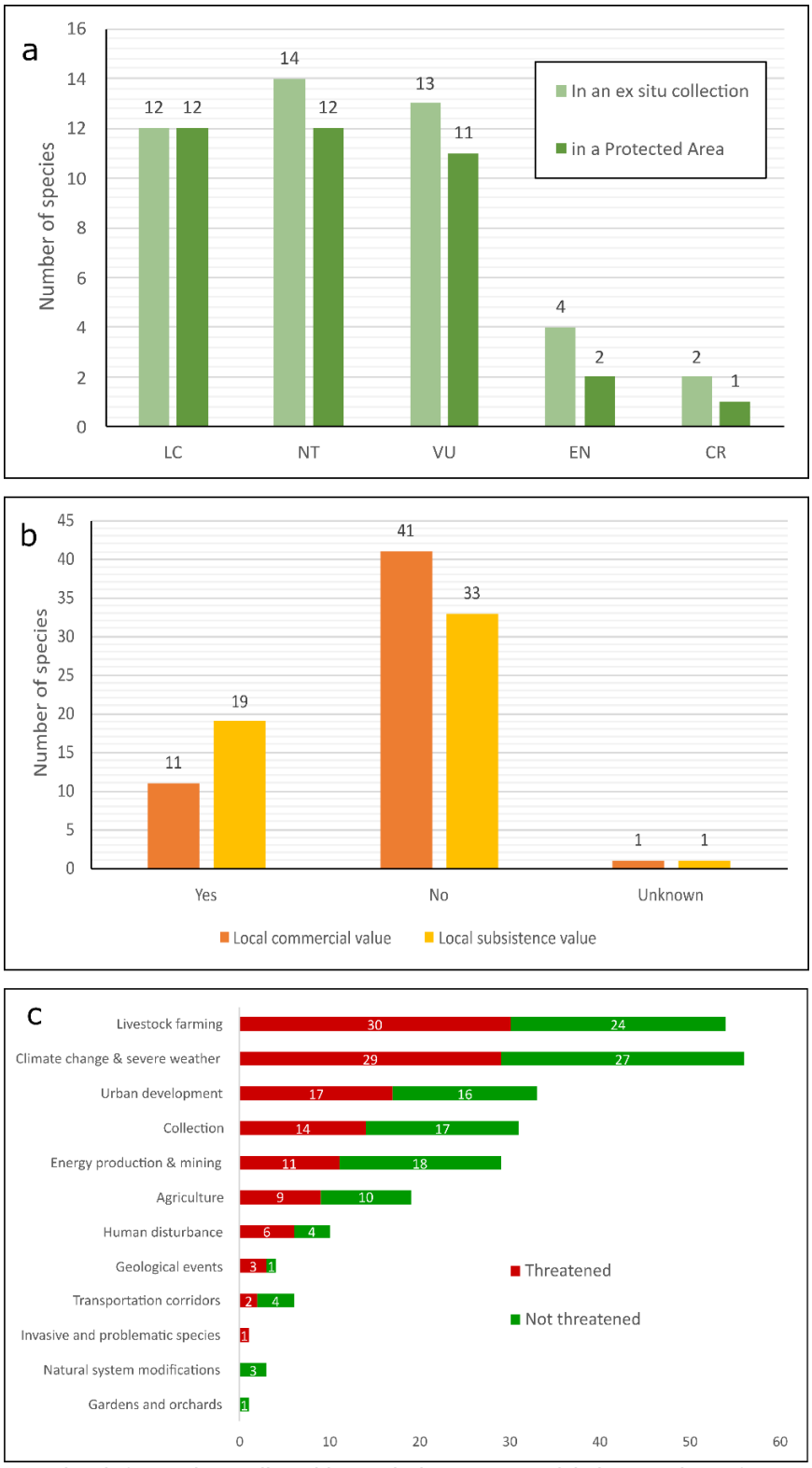


Figure 6.2. Conservation information collated in Red List process. (a) the number of assessed species in protected areas and ex-situ collections based on IUCN categories. (b) the number of assessed species that have local commercial and/or local subsistence value. (c) Threats to species separated by Threatened and Not Threatened taxa.

there were more cases above the mean Difference in Assessment value than below. Specifically, the mean Difference in Assessments for all data was + 1.07 with a skew away from normality of 0.24, whilst the mean Difference in Assessments for datasets without Kazakh data, without NE data, and without Kazakh and NE data were + 0.93 with a skew of 0.36, + 1.34 with a skew of - 0.21, and + 1.11 with a skew of 0.02 respectively. There were only two cases where a negative Difference in Assessment was calculated; one case was in an Uzbek endemic species which is continuing to decline and was assessed only in 2015, whilst the other is a recently reinstated species where available data may have changed for large parts of its range and was also only assessed in 2015.

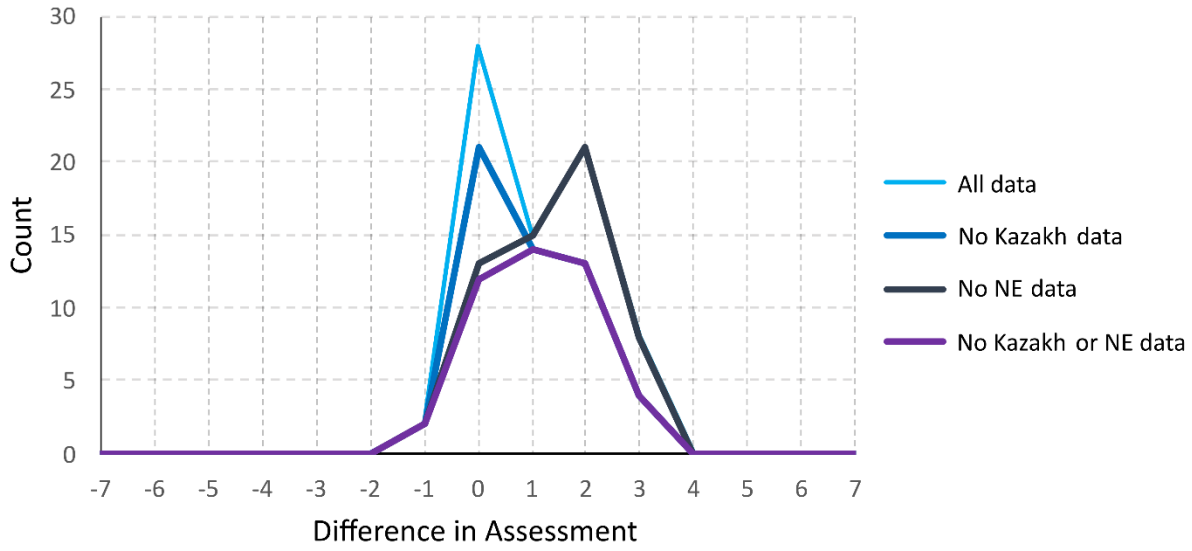


Figure 6.3. The count of Difference in Assessment scores for four different datasets. The score for each species is calculated by subtracting the assigned national Red List status value from the global Red List status value. Different datasets are colour coded and consist of a dataset containing all data, one with Kazakh data removed, one with Not Evaluated (NE) species data removed, and one where both Kazakh and NE data have been removed.

6.3.4. EDGE

Both the fair proportions (FP) and equal splits (ES) method of calculating ED generally highlighted the same species as priorities when the overall EDGE score was calculated (Figure 6.4). However, two species within the top ten of the FP list did not occur in the top ten of the ES list, whilst the positioning of a number of the top ten species on the lists did not correspond (Table 6.3). Broadly the majority of species with the highest EDGE scores were those with high

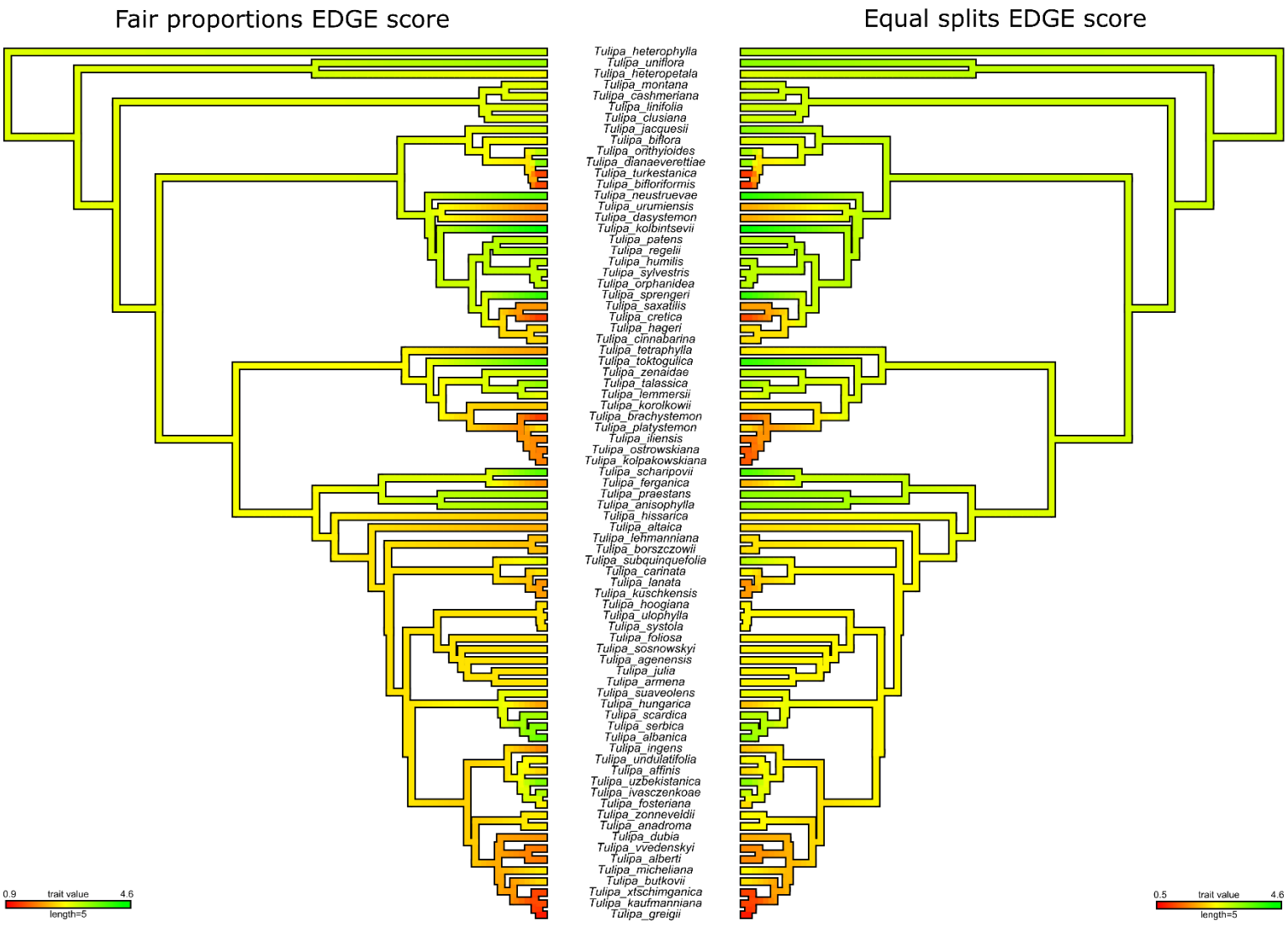


Figure 6.4. EDGE scores calculated using fair proportions method (left) and equal splits method (right) mapped onto copies of the same phylogeny. The EDGE scores are represented by the colour gradient on the phylogenies.

Table 6.3. EDGE scores and details of the parameters used to calculate these for species with an assigned IUCN Red List status. Specifically the evolutionary distinctiveness (ED) measures for species calculated using both the equal splits and fair proportions methods are reported, the assigned score for the Globally Endangered (GE) status of a species is recorded, and both EDGE scores calculated using the equal splits and fair proportions methods are reported.

Species	**Evolutionary distinctiveness (Equal splits)**	**Evolutionary distinctiveness (Fair proportions)**	**Globally Endangered**	**EDGE (Equal splits)**	**EDGE (Fair proportions)**
Tulipa kolbintsevii	5.525426	5.143543	4	4.648295	4.58799
Tulipa sprengeri	3.387661	3.46542	4	4.251385	4.268952
Tulipa toktogulica	7.794273	5.754899	3	4.253542	3.98971
Tulipa neustruevae	8.49661	5.597575	3	4.330376	3.966144
Tulipa scharipovii	6.03686	5.531965	3	4.030604	3.956149
Tulipa dianaeverettiae	1.671071	2.230138	4	3.755068	3.945114
Tulipa albanica	1.155012	2.089869	4	3.540385	3.900717
Tulipa uzbekistanica	1.378193	1.711067	4	3.63893	3.769931
Tulipa ivasczenkoae	0.78341	1.438796	4	3.351116	3.664093
Tulipa talassica	2.603779	3.288674	3	3.361425	3.535419
Tulipa regelii	2.682906	3.205732	3	3.383144	3.51589
Tulipa uniflora	15.535	14.82988	1	3.498626	3.455046
Tulipa anisophylla	7.071012	6.566117	2	3.474573	3.409974
Tulipa praestans	7.071012	6.566117	2	3.474573	3.409974
Tulipa orithyioides	2.840143	2.323704	3	3.424951	3.280521
Tulipa heterophylla	22.82335	22.82335	0	3.170666	3.170666
Tulipa jacquesii	7.632213	4.268061	2	3.541795	3.047957
Tulipa zenaidae	4.262439	4.053973	2	3.046889	3.006469
Tulipa subquinquefolia	4.600702	3.461031	2	3.109186	2.881674
Tulipa lemmersii	2.603779	3.288674	2	2.668277	2.842272
Tulipa heteropetala	15.535	14.82988	0	2.805479	2.761899
Tulipa carinata	2.60574	2.52998	2	2.668821	2.647587
Tulipa micheliana	2.331749	2.486071	2	2.589792	2.63507
Tulipa anadroma	2.303491	2.402297	2	2.581274	2.610745
Tulipa zonneveldii	2.303491	2.402297	2	2.581274	2.610745
Tulipa platystemon	1.737889	2.364053	2	2.393481	2.599441
Tulipa butkovii	1.80712	2.097131	2	2.418453	2.516771

Tulipa affinis	1.385746	1.755486	2	2.255806	2.399888
Tulipa korolkowii	4.583902	4.285023	1	2.413035	2.358024
Tulipa hissarica	10.77333	9.151154	0	2.465837	2.317587
Tulipa borszczowii	4.17359	3.887646	1	2.336714	2.279858
Tulipa lehmanniana	4.17359	3.887646	1	2.336714	2.279858
Tulipa fosteriana	0.78341	1.438796	2	1.964822	2.277799
Tulipa altaica	9.046038	7.615459	0	2.307178	2.153558
Tulipa tetraphylla	10.74221	6.696277	0	2.46319	2.040737
Tulipa hungarica	2.809169	2.833322	1	2.030558	2.036879
Tulipa dubia	2.348431	2.526134	1	1.901639	1.953349
Tulipa lanata	1.395044	2.316764	1	1.566549	1.892137
Tulipa ingens	3.092073	2.311524	1	2.102199	1.890556
Tulipa ferganica	6.03686	5.531965	0	1.951162	1.876708
Tulipa iliensis	1.092348	2.194682	1	1.431434	1.854635
Tulipa dasystemon	5.490546	5.253599	0	1.870347	1.833157
Tulipa urumiensis	5.490546	5.253599	0	1.870347	1.833157
Tulipa kolpakowskiana	0.63596	2.061064	1	1.185377	1.81191
Tulipa ostrowskiana	0.63596	2.061064	1	1.185377	1.81191
Tulipa alberti	1.495708	1.930406	1	1.60772	1.768288
Tulipa vvedenskyi	1.495708	1.930406	1	1.60772	1.768288
Tulipa kaufmanniana	0.636991	1.397274	1	1.186007	1.56748
Tulipa brachystemon	2.774912	2.562848	0	1.328377	1.27056
Tulipa cretica	1.796556	2.546813	0	1.028389	1.26605
Tulipa bifloriformis	1.042004	2.185606	0	0.713932	1.158643
Tulipa turkestanica	1.042004	2.185606	0	0.713932	1.158643
Tulipa greigii	0.636991	1.397274	0	0.49286	0.874332

GE scores, but there were several species with lower GE scores that had high ED scores and were hence highlighted as priorities. Specifically, *Tulipa uniflora*, *T. anispohylla* and *T. praestans* were the highest EDGE scoring species that were recognised as Vulnerable or Near Threatened, with everything above them on the priority list Endangered or Critically Endangered. The highest priority species from both EDGE calculations was *T. kolbintsevii*., with *T. sprengeri*, *T. toktogulica*, *T. neustruevae*, and *T. scharipovii* making up the top five on each list.

6.3.5. Regional strategy

During the strategy meeting national tulip priority areas were mapped (Figure 6.5) as well as key regional sites (Figure 6.6). A range of objectives were ranked in order of extent, feasibility, and irreversibility which led to a scored list of objectives (Table 6.4). The highest priority objective related to assessing and managing the impact of climate change, with minimising threat of livestock overgrazing voted as the second most important and reducing mining activities the third most voted for objective. The most feasible objective to achieve was that of gathering information to assess the extent of threat to tulips in the region. Understanding and managing the impacts of climate change was the objective that scored highest on the irreversibility scale. Whilst the broadest objective to address was reducing the impact of livestock overgrazing to tulip habitat across Central Asia. A number of activities were discussed to address the top six objectives some of which were on a broad scale and others were site specific (Appendix 18). These are preliminary foundations for the development of a Central Asian tulip strategy but require considerable work to refine.

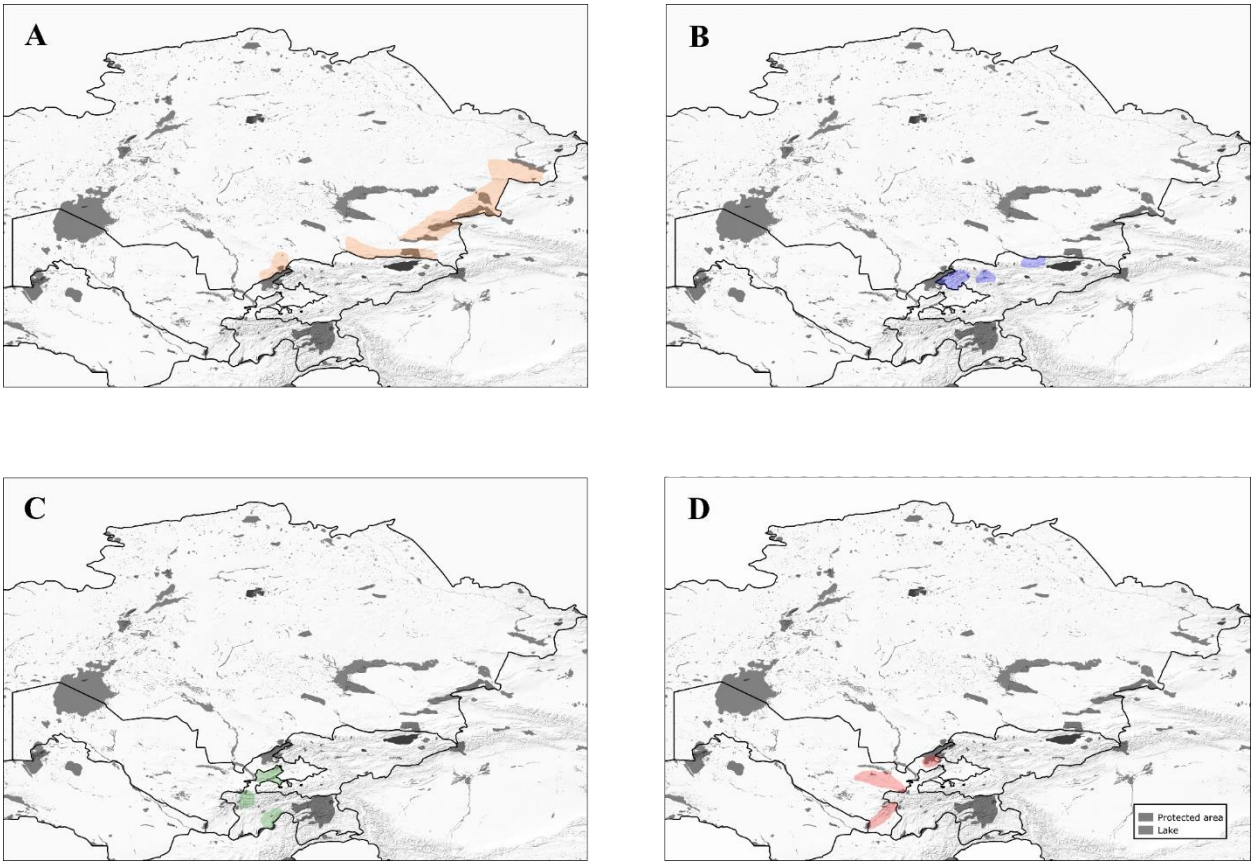


Figure 6.5. The three National sites deemed a priority for tulip conservation by experts, (A) Kazakhstan, (B) Kyrgyzstan, (C) Tajikistan, and (D) Uzbekistan. Protected areas and lakes are also shown on these maps.

6.4. Discussion

Conservation relies heavily on systematics, primarily the concept of species (Godfray, Knapp and Mace, 2004). Having a stable taxonomic framework promotes more effective conservation and decision making (Garnett and Christidis, 2017). In the genus *Tulipa* this framework has only recently been developed (see chapter two). Here we have built upon this recent progression in taxonomic understanding to improve information around the conservation status of species. Specifically preparing 53 reports for Central Asian tulips species to be added to the IUCN Red List, filling the void that currently exists for this group on this database (IUCN, 2022). This will greatly improve awareness of the need for conservation of many species in this genus, including many newly described species, which will require urgent attention (Liu *et al.*, 2022). After collating information and assigning threat status during the Red Listing process we were able to carry out a number of further analyses providing crucial information for conservation prioritisation. This work provides a foundation for the development of a regional tulip conservation strategy in the next few years.

The IUCN Red List is an extremely important resource for conservationists with species represented on this database more likely to be effectively conserved than those absent (Betts *et al.*, 2020). Before our work there were only eight *Tulipa* taxa Red List reports, with multiple of these synonyms. With the addition of the 53 species reports drafted within this project, now around 60% of the accepted tulip species will be represented on the Red List, with just over 80% of all Central Asian species assessed. This is a significant step forward in raising awareness of both the biodiversity of Central Asia, as well as highlighting the urgent need for the conservation of wild tulips. It is clear now that around 51% of all assessed Central Asian tulip species are threatened with extinction, with 28% Vulnerable, 11% Endangered, and 11% Critically Endangered. These species were primarily assessed under criterion B showing many species are range restricted, although estimated population declines, and extremely small population sizes were acknowledged in a range of species also. Notably, 14 species, or 26% of those assessed, are NT and if these continue to slide towards extinction this could mean that in the next few decades over 75% of all tulips in the region could be threatened with extinction.

Tulips are an important horticultural plant with significant cultural value (Orlikowska *et al.*, 2018). Nonetheless, the collection of wild tulips is not reported as being driven by international trade, but some species of wild tulip are collected by local people or those travelling through areas where subpopulations grow, and in some cases a local trade in these flowering plants has been

Table 6.4. The 15 objectives presented to participants at the workshop and corresponding voting results. Each participant was allowed to vote for three objectives based on the scale of the issue, the irreversibility of the impact, the feasibility of achieving the objective. The total votes for each objective across all categories is given in the total score column.

Objectives	**Scale**	**Irreversibility**	**Feasibility**	**Total Score**
Climate change will mean that tulip species will likely decrease in abundance and may be forced to migrate to track shifting habitats, increasing extinction risk	1	9	1	11
Unsustainable grazing in low altitude pastures is causing large-scale degradation of tulip habitat and significant reduction of tulip abundance in these areas	8	1	0	9
Mining of natural resources is leading to damage of areas where important tulip populations grow	3	3	3	9
Direct loss of tulip habitats	6	1	1	8
Wild collection of tulips in areas near human settlements	3	1	4	8
Urbanisation does not account for importance of rare or threatened tulip species	2	6	0	8
Limited information about the extent of all threats to tulip populations	0	0	8	8
Rural communities who live in areas of tulip habitat do not value or understand the need to protect wild tulips	2	0	5	7
Limited available funding from the state for conservation work leads to a reliance on funds from international bodies, which are difficult to obtain without support from organisations with a track record of grant proposals	2	3	0	5
Limited information on populations, threats and trends of all tulip species in Central Asia	1	0	4	5
Livestock rearing is the prominent livelihood for many people living in rural areas, and is often the main income source	3	0	0	3
Legislation and enforcement are insufficient to counter threat from over-collection	0	2	0	2
Limited sharing of tulip material between gardens leading to few comprehensive collections and poor knowledge of species occurring outside of own country	0	0	0	0
Limited access to western scientific literature due to pay walls and language barriers meaning understanding of tulip taxonomy is not universal	0	0	0	0

Local research institutes do not have access to necessary infrastructure, equipment, and resources to answer all relevant research questions	0	0	0	0

observed in the spring months. Of those species assessed in the workshop around 36% of the species were reported as being collected from wild subpopulations, with 11 species or 21% of all assessed diversity traded in local markets or by roadsides. There were particularly significant trades in species that occurred in heavily travelled areas such as *Tulipa praestans*, *T. fosteriana*, and *T. vvedenskyi*, which saw subpopulations in significant decline due to localised trade. Although wild tulip species are currently not internationally traded like other flowering plants such as orchids, which have seen demand drive a large illegal trade and unsustainable collection from the wild (Hinsley *et al.*, 2017), there is potential for collection to increase in the future due to growing demand from the horticultural world for increased diversity (Orlikowska *et al.*, 2018). Whilst current local trade needs to be acknowledged as a significant threat to a variety of tulip species in Central Asia and appropriate policy put in place to control collection as well as improved enforcement of legislation.

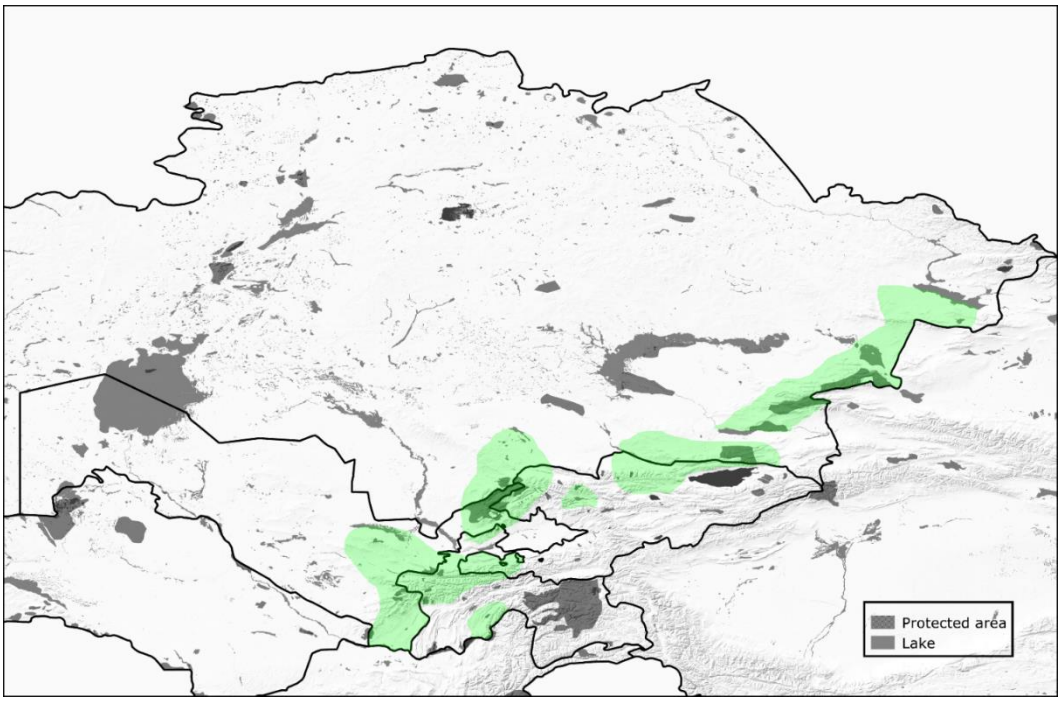


Figure 6.6. Priority sites for tulips across Central Asia as selected by experts. Both protected areas and lakes are also shown on the map.

Both in-situ and ex-situ methods need to be considered for the conservation of tulips. Given that threats continue to cause declines in many species, ex-situ collections represent important stores of specimens which can be used to restore wild populations (Mounce, Smith and Brockington, 2017), whilst reducing threats in the wild will effectively prevent the loss of large amounts of genetic diversity (Wei and Jiang, 2021). It is clear that the most threatened tulip species are often those that are not in ex-situ collections or protected areas and so there is a real need to drive both policy and action to rebalance this. In our work all non-threatened species were shown to be in ex-situ collections, whilst only 87% of Vulnerable, 67% of Endangered, and 33% of Critically Endangered species were represented in these collections. Moreover, 92% of non-threatened Central Asian tulip species are in protected areas, with only 73% of Vulnerable, 33% of Endangered, and 17% of Critically Endangered species found in these preserved habitat areas. There is clearly a trend of the most threatened species being the most underrepresented in formal protection.

Across the native habitats of wild tulips, a range of threats have been recognised. The most widespread impact on already threatened species comes from unsustainable livestock grazing practices. This threat has been highlighted as an issue for many species occurring in ecosystems in this region of the world (Orozumbekov, Cantarello and Newton, 2015; Wilson *et al.*, 2019b; Nowak *et al.*, 2020) and may be an increasing issue with livestock populations expanding in areas of Central Asia (Borchardt and Dorre, 2012). Poverty rates in rural areas of Central Asia are often high, with livestock often used as an investment (European Commission and Directorate-General for International Cooperation and Development, 2019), therefore this threat must be tackled carefully so as not to cause negative social impacts (Brockington and Schmidt-Soltau, 2004). The broadest threat to tulip diversity in general comes from climate change, with modelling suggesting the loss of large suitable habitat areas for some tulips in future decades (Wilson *et al.*, 2021). The scale of this threat and its interaction with other threats is difficult to assess with predictions carrying a lot of uncertainty, and taxa specific effects poorly studied (Parmesan and Hanley, 2015). Regardless, mountainous areas such as Central Asia are believed to be particularly vulnerable to this threat (Rangwala and Miller, 2012; Cazzolla Gatti *et al.*, 2019) and so the impact of this threat needs to be monitored carefully.

More localised threats from mining, urban development, and crop agriculture are impacting a broad range of tulip species but need to be addressed on a species-by-species basis. Mining in Central Asia is often extremely exploitative with large uranium and gold reserves known in the region, as well as oil and gas which make up a significant proportion of several countries' GDP

(European Commission and Directorate-General for International Cooperation and Development, 2019). Continued expansion of mining sites, and oil and gas drilling sites, as well as corresponding infrastructure is clearly leading to an increasing impact on wild tulip subpopulations, whilst a limited legal framework for mitigation and weak governance may be leading to unnecessary damage and needs to be addressed. Large scale infrastructure development, such as the Belt and Road Initiative or CASA 1000 project, are also directly impacting a range of ecosystems including many in which tulips occur, with limited scope for environmental impact mitigation (CASA-1000 Project, 2021; Foggin *et al.*, 2021). At the same time the expansion of settlements as well as broader infrastructure to support the development and population growth of Central Asian nations is leading to the degradation and loss of tulip habitats. Although population density in this region is generally low, populations are increasing faster than the global average, and, primarily due to climatic and resource conditions of this region, this growth is centred around large urban areas, with rural areas sparsely populated (European Commission and Directorate-General for International Cooperation and Development, 2019). Habitat loss outside of the cities of Bishkek, Dushanbe, Tashkent, and Almaty are of particular significance as the mountains surrounding these cities are important tulip habitat areas. Agricultural crop land covers a much less significant area than that used for grazing, yet reorganisation and expansion of cropland into tulip habitat areas since the breakup of the Soviet Union represents a serious threat to wild subpopulations (Hamidov, Helming and Balla, 2016). Secondary effects from water mismanagement, soil contamination, and destructive practices such as ploughing can lead to further degradation of environmental conditions for tulips (Hamidov, Helming and Balla, 2016; European Commission and Directorate-General for International Cooperation and Development, 2019). Careful management of agricultural land is therefore needed and the expansion into important tulip subpopulation areas must be halted.

A greater understanding of threats, and formalised threat status for species enables better conservation prioritisation (Betts *et al.*, 2020). Yet, there are a number of factors that can be considered when deciding which species are most in need of conservation action (Cadotte and Tucker, 2018; Mazel *et al.*, 2018; Kosman *et al.*, 2019; McGowan *et al.*, 2020). Recently, the inclusion of phylogenetic diversity as a tool to select species for conservation has become of increasing interest (Vane-Wright, Humphries and Williams, 1991). This proposal has driven the development of objective methods that combine evolutionary distinctiveness (ED) and globally endangered (GE) status to calculate EDGE scores allowing for priority species to be identified (Isaac and Pearse, 2018). Considering phylogenetic diversity has been treated as a mechanism to conserve functional diversity, evolutionary potential and protect evolutionary history (Winter,

Devictor and Schweiger, 2013). Although there remains uncertainty around the link between phylogenetic diversity and these other traits the method can provide a novel perspective that can be integrated into a broader assessment of priority species. We have carried out a basic EDGE analysis for the genus *Tulipa*, which has now only become possible with our dated phylogeny and broad range of Red Listed tulips. Although our phylogeny is not complete the calculation of ED scores are relatively robust to missing species (Weedop *et al.*, 2019), whilst several species in the tree still need to be assessed and so their EDGE score could not be calculated. Regardless this assessment showed that tulip species with the largest EDGE scores are primarily those that are most severely threatened with extinction, mainly small range endemics. Yet notably several particularly evolutionarily distinct species are highlighted as important to conserve when previously they may have been overlooked, exclusively *Tulipa anisophylla*, *T. praestans*, *T. uniflora* and *T. heterophylla*. Whilst species such as *T. greigii* and *T. kaufmanniana*, which have been considered iconic Central Asian tulips and those which are extremely important to conserve, here are shown to have low phylogenetic diversity and not of particular conservation importance based on this parameter. These EDGE scores should not necessarily be considered as more informative than other methods of prioritisation and we recommend using them within a broader framework, but they do add a further layer of information to ensure more effective decision-making (Bottrill *et al.*, 2008).

The process of taking data deficient Central Asian tulips to published on the IUCN Red List has been a steep learning curve. Tulip data is extremely messy with common use of outdated or incorrect taxonomy (Christenhusz *et al.*, 2013), whilst generally there is few data for most species. Threats have often been described (Tojibaev and Beshko, 2015; Nowak *et al.*, 2020), but have not been tied to specific species or locations. Collating information on threats has required the generation of new data, specifically modelling the impact of climate change on tulips (Wilson *et al.*, 2021), as well as engagement of local experts. Cultural differences, language barriers, personal investments in certain species, and political barriers have complicated matters, but we have carefully engaged multiple stakeholders in this process. This has been achieved through formally establishing relationships by signing several memorandums of understanding as well as sharing data and expertise to build trust. The use of professional translators as well as Fauna & Flora International's local teams has enabled us to overcome language issues as well as enable engagement in a way that fits the local context. Our work led to the first ever regional tulip workshop where knowledge could be shared in a fair and equal environment. This has strengthened relationships between researchers within the region developing a local network of collaboration that can drive future work. Local knowledge proved

to be an exceptionally useful source of information within this data driven process (Rodrigues *et al.*, 2006) with many new locations reported that have not been formally recorded before. Where taxonomic discord occurred we aimed to provide significant objective evidence to make decisions negating any personal values, which generally worked although there were moments of tension. Notably, many species that people valued highly were not threatened and the Red Listing process revealed considerable exaggeration of rarity for many species. It is clear that there are many barriers to this process that must be overcome carefully in a creative manner, but our work shows it can be done if funding and support is in place.

Central Asia is troubled by civil unrest and conflict, high rates of rural poverty, and political instability. In addition, in this region populations are growing, there is a high reliance on extractive industries, and weak governance and inadequate laws are common. All these factors contribute to a broad context of environmental degradation (European Commission and Directorate-General for International Cooperation and Development, 2019). However, this region harbours incredible biodiversity, which is often relied upon by the people of this territory. The protection of this biodiversity is therefore crucial for the ecosystem services that it provides, while also for its biological and evolutionary significance. Central Asian tulips have widely been recognised as important in the flora of this region (Ivashchenko and Belyalov, 2019) and are a crucial part of the Mountains of Central Asia Biodiversity Hotspot (Critical Ecosystem Partnership Fund, 2016). Yet their conservation has been hampered by a lack of IUCN Red List status (IUCN, 2022), limited regional collaboration (Wilson *et al.*, 2021), and taxonomic uncertainty (Garnett and Christidis, 2017). National Red books have highlighted many species are in decline (Davletkeldiev, 2006; Baitulin, 2014; Tojibaev and Beshko, 2015; Hisoriev *et al.*, 2016), but we have shown that these national assessments generally overestimate the risk status of species. We have therefore, importantly, provided a robust and influential assessment of the global status of many Central Asian tulips. Through this process we have collated information on distributions, populations, threats, and trade, as well as highlighting which conservation action is appropriate for each assessed species. Not only will this provide an important database for conservationists and researchers in the region but will also be important in policy decision-making and obtaining funding (Betts *et al.*, 2020). In addition, this project has allowed the conservation status of the genus as a whole to be assessed in the region, with over 50% of those assessed recognised as Threatened showing the precarious position of this plant group compared to the global average of 40% (Antonelli *et al.*, 2020). Moreover, it has enabled priority species to be identified both through their threat status as well as EDGE scores. Together this establishes a strong foundation for the conservation of Central Asian tulips, which

alongside initial identification of key regional sites, proposed key objectives, and preliminary conservation activities pave the way for a regional conservation strategy that can ensure the future of this iconic genus in the mountains, steppe, and deserts of Central Asia.

Chapter 7.

Conclusion and future perspectives

Tulips remain one of the most well-known horticultural plants. Their wild ancestors, however, are under significant threat, while taxonomic issues have hindered the conservation and research of this group. Fauna & Flora International and Cambridge University Botanic Gardens both recognised this and set up a PhD position to work towards resolving many of these issues and provide crucial information to guide the conservation of wild tulips in their most diverse region, Central Asia. This PhD was also placed within a broader Darwin Initiative project "Securing wild tulips and pastoral communities in the Kyrgyz mountains" targeted at improving information on Kyrgyz tulips. This thesis represents the output of this work.

Since the start of this PhD an array of new tulip species have been described (De Groot and Tojibaev, 2020; De Groot and Zonneveld, 2020, 2022; Rukšāns and Zubov, 2022), whilst there have been several studies exploring morphology (Dekhkonov *et al.*, 2022), evolutionary relationships (Hajdari *et al.*, 2021), and presenting chloroplast genomes (Zhou *et al.*, 2019; Ju, Shi, *et al.*, 2020; Ju, Tang, *et al.*, 2020; Ju *et al.*, 2021). Some studies have also aimed at providing evidence of the origin of the horticultural tulip (Kritskaya *et al.*, 2020). Overall, therefore information surrounding the genus *Tulipa* has been increasing rapidly and there is clearly continuing interest in understanding the wild ancestors of this common spring garden flower. Yet, there is a lack of a cohesive work on the whole genus, with the last genus wide study occurring in 2013 (Christenhusz *et al.*, 2013). In the last four years, we have brought together a range of collaborators, samples, and ideas that have allowed us to undertake the most comprehensive assessment of the genus to date, greatly improving knowledge on taxonomy, evolutionary history and the conservation of wild tulips. This has been achieved through a number of complementary avenues that each provide an important step forward in our understanding of this genus.

a) Presented a more stable taxonomic framework

   Previous phylogenetic work used only four plastid genetic markers and the ITS region of the nuclear genome to assess evolutionary relationships. Here we have used contemporary genome skimming methods to construct the full chloroplast genome (plastome) of 245 taxa. Combining this dataset with some freely available plastomes from GenBank we were able to construct a phylogeny using a maximum likelihood approach that represented around 86% of all accepted species at the time as well as

many synonyms. The nuclear region of the 35S rDNA was used to make another phylogeny, so as to check for cyto-nuclear discordance. There was limited evidence of this and so the phylogenies, primarily the plastome based phylogeny, were used to make an extensive number of taxonomic changes. Primarily we were able to show the existence of a fifth subgenus*, Eduardoregelia*, reorganise sections to ensure they are monophyletic and simple to use, specifically merging many sections of the *Tulipa* subgenus into one broader section, whilst also reinstating and synonymizing several species. In this work we also formally described a new cryptic species that is endemic to Kyrgyzstan. Crucially this work has produced an updated taxonomic framework for the genus, which can be used in all aspects of work on this clade.

b) Provided an insight into the evolutionary history of *Tulipa*

We created the first ever *Tulipa* specific dated phylogeny using the molecular data from our sequencing efforts and secondary calibration points. This dated phylogeny was used to carry out a biogeographical analysis of the genus as well as assess the phylogenetic signal of genome size, a trait commonly used to make taxonomic decisions within this genus. In this work we were able to provide evidence for a broader Central Asian origin, as well as the likely importance of mountain building, aridification, and global cooling in the diversification of this genus in Central Asia. Notably we were able to determine that there were multiple migrations of tulips out of this region, with multiple clades within the genus migrating across the steppe of Kazakhstan and Russia before radiating in the secondary diversity hotspot and dispersing into the Mediterranean and back towards Central Asia through Iran. Moreover, within this work we showed that tulips have diversified at different rates throughout their evolutionary history with particularly rapid diversification likely occurring soon after the MRCA as well as in the last 9 million years. The final thing we showed in this section was that genome size has good phylogenetic signal with closer related species having similar sized genomes. This means, therefore, that the use of this trait in higher level taxonomic decision making is justified, although it normally lacks the resolution to determine between closely related taxa and needs to be used in an integrated taxonomic approach, especially when used in describing new species.

c) Assessed the threat of climate change to Central Asian tulips

Climate change has often been mentioned as a potential threat to tulip diversity across Central Asia, however, there has never been any assessment of its projected impact. We undertook the first ever modelling of the impact of climate change on ten Central Asian tulip species. Within this work we were able to show that climate change was projected to cause significant declines in suitable habitat for all species modelled, that regional models were much better at providing a comprehensive understanding of threats to species than national reports, and that protected area coverage is fairly limited for several species with coverage of tulips species in protected areas likely to decrease with climate change. We also highlighted the data deficient nature of many newly described species and how this can hinder their research. Broadly, these results could be extrapolated to show that climate change may be one of the biggest threats to tulip species in Central Asia heading into the future and we proposed that a more regional approach will be needed for the future of tulip conservation.

d) Raised the conservation profile of wild tulips

Finally, we used our new species list to underpin the development of Red List reports for around 80% of all Central Asian tulip species. This process required the collation of data on distributions, ecology, threats and trade of species as well as their predicted conservation requirements. These Red List assessments were presented to tulip experts from the region in a workshop held in Bishkek in Spring 2022, who were able to add, edit, or change information where they saw fit. Overall, this has led to 53 reports being published on the IUCN Red List. In this work we have shown that although livestock overgrazing is the largest threat to already threatened tulip species in Central Asia, the broadest threat to tulip diversity comes from climate change. Notably our work has shown that over 50% of assessed species are threatened with extinction. This is a higher proportion than the global plant average and highlights the specific plight of this genus. In the workshop we also undertook a number of activities aimed at developing preliminary information for a regional conservation strategy. We identified key regional sites, ranked objectives, and discussed activities that could achieve these objectives. We also carried out several post Red Listing analyses, which showed that national assessments often overestimate the extinction risk of species, whilst we calculated EDGE scores for a range of species promoting more effective conservation prioritisation.

Overall, this has led to the first real global overview of extinction risk in this genus, critically highlighting the need for immediate conservation action in Central Asia and providing a foundation for the development of a regional tulip conservation strategy.

This work was urgently needed and fills a knowledge gap that has greatly constrained tulip conservation. We hope that this new evidence-base can support and improve the research and conservation of this culturally significant flower and promote the protection of the broader unique ecosystems in which it occurs.

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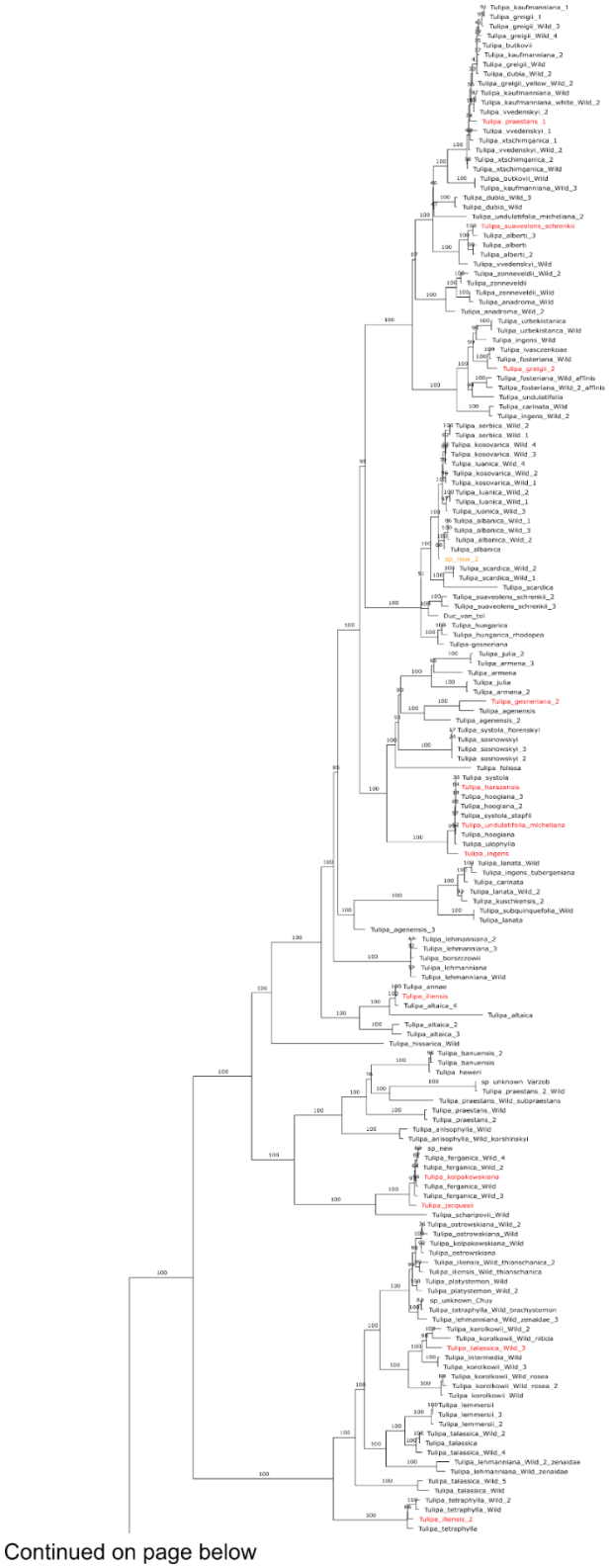
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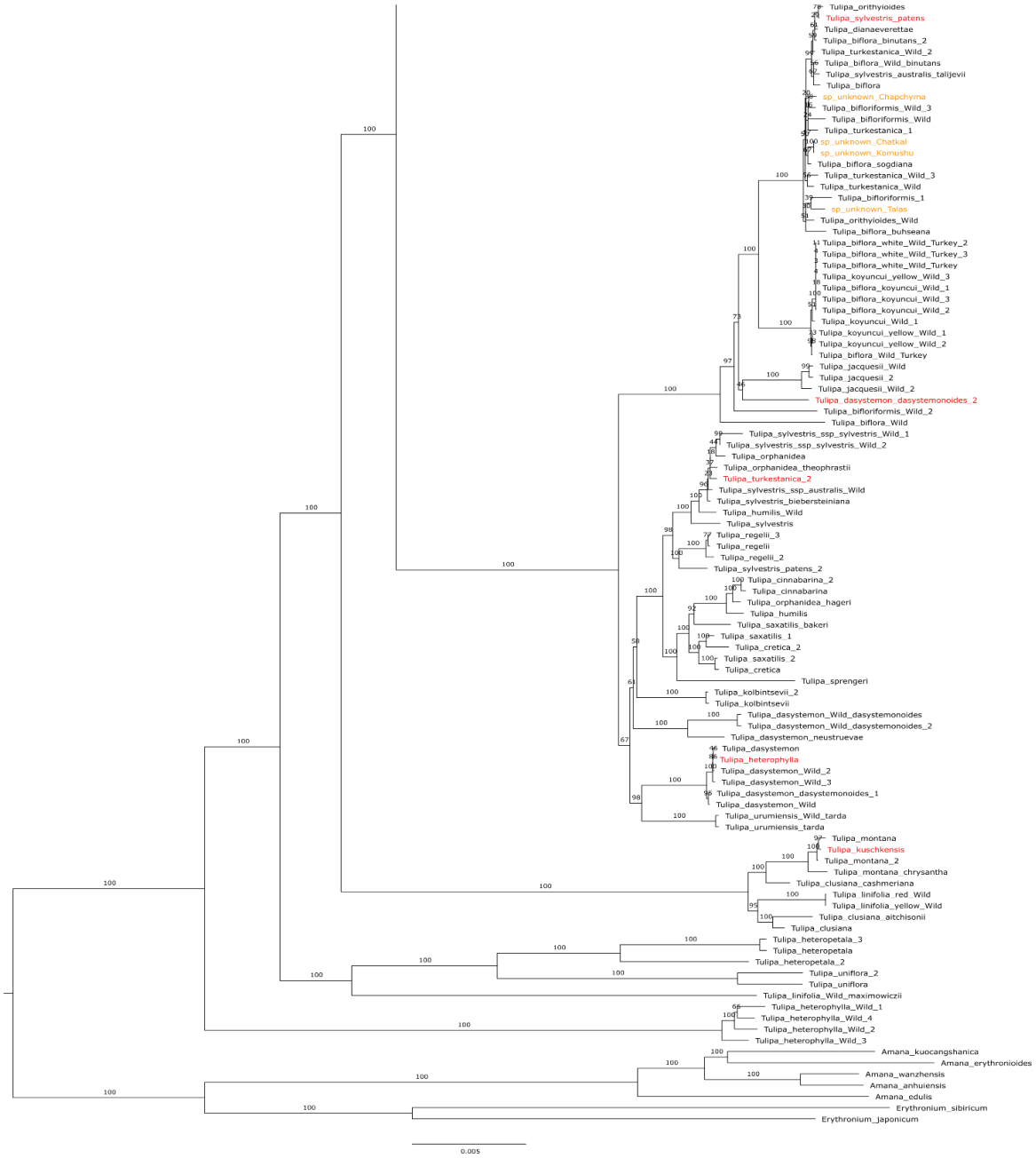
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Appendix 1. Full plastome tree generated using a multi-partitioned approach and Maximum Likelihood methods including identified erroneous specimens.

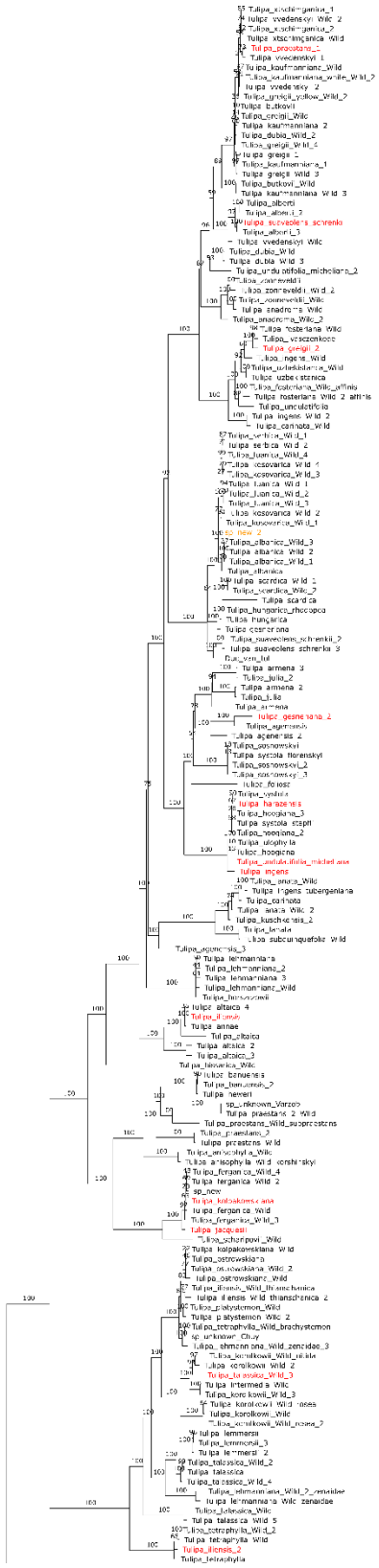


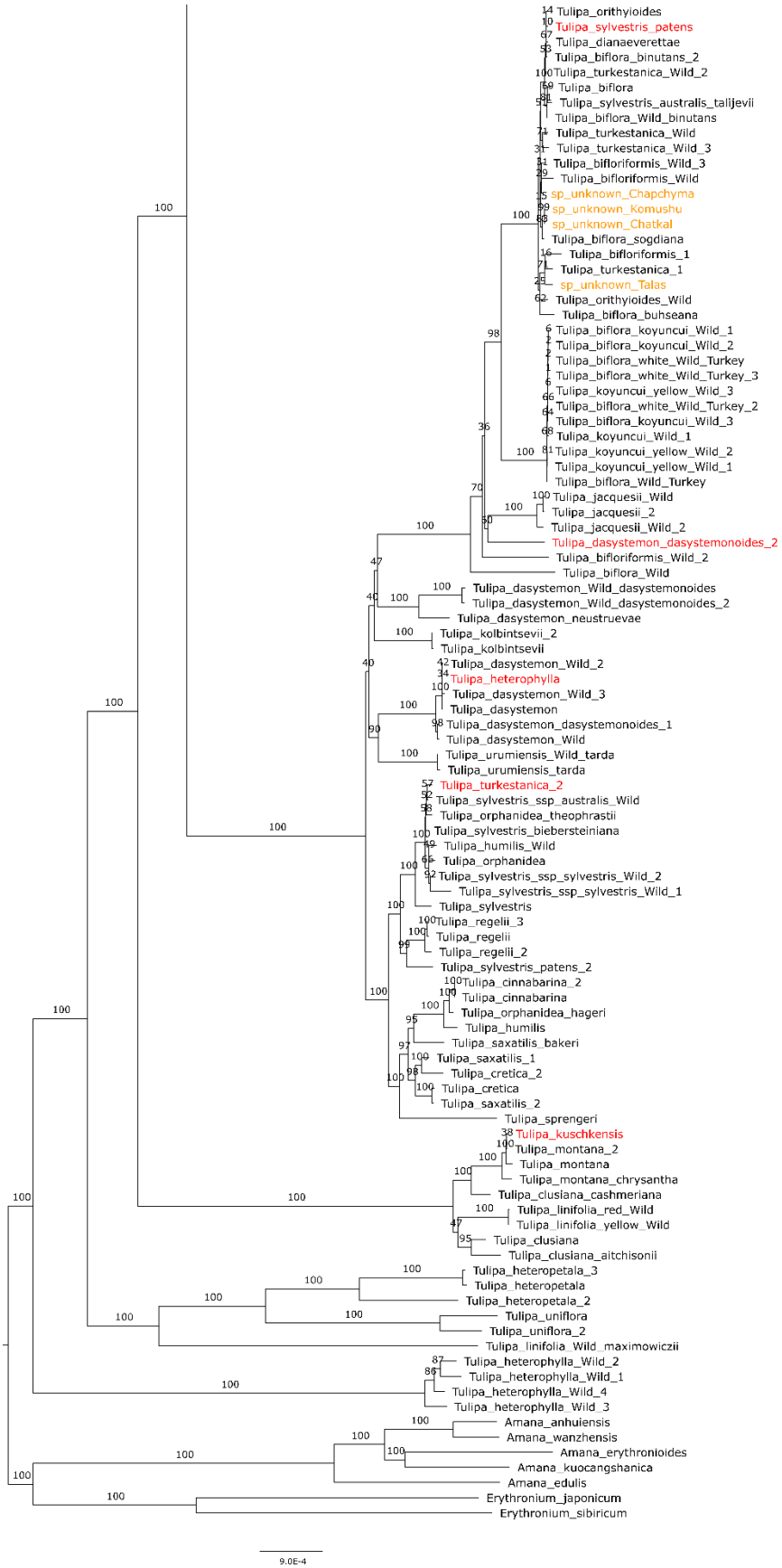
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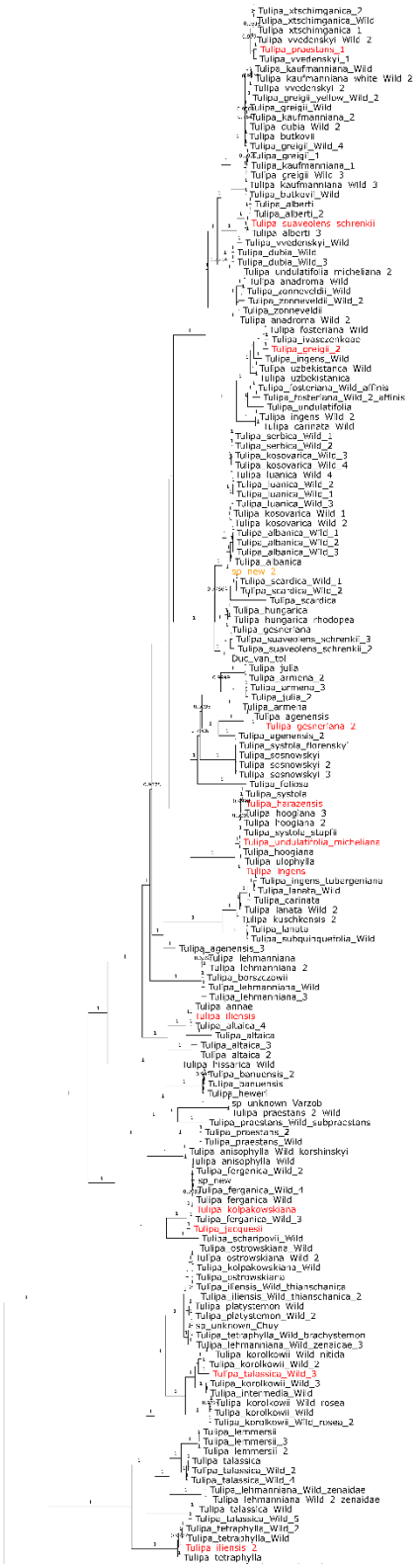


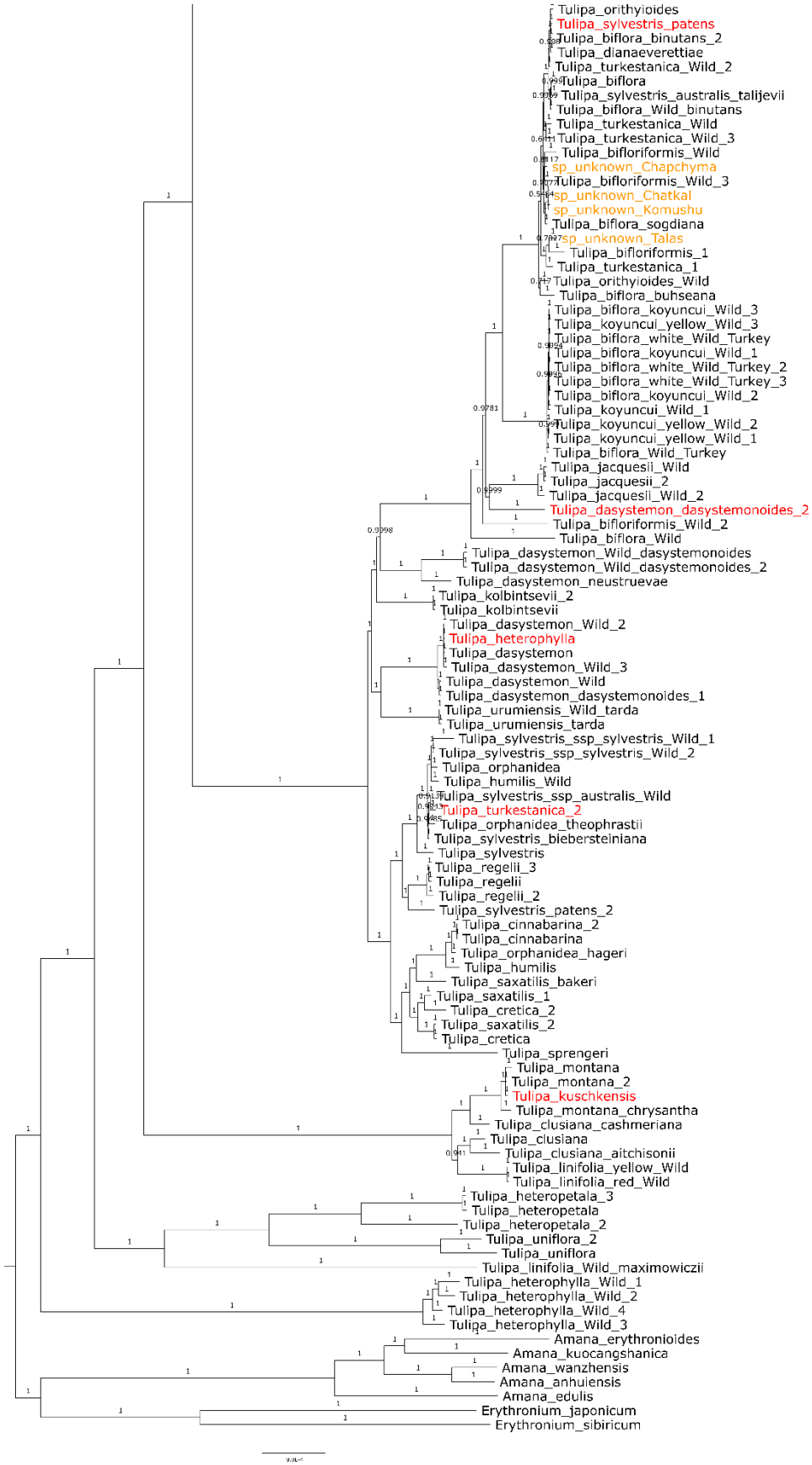
Appendix 2. Full plastome tree generated using a single partition approach and Maximum Likelihood methods including identified erroneous specimens.



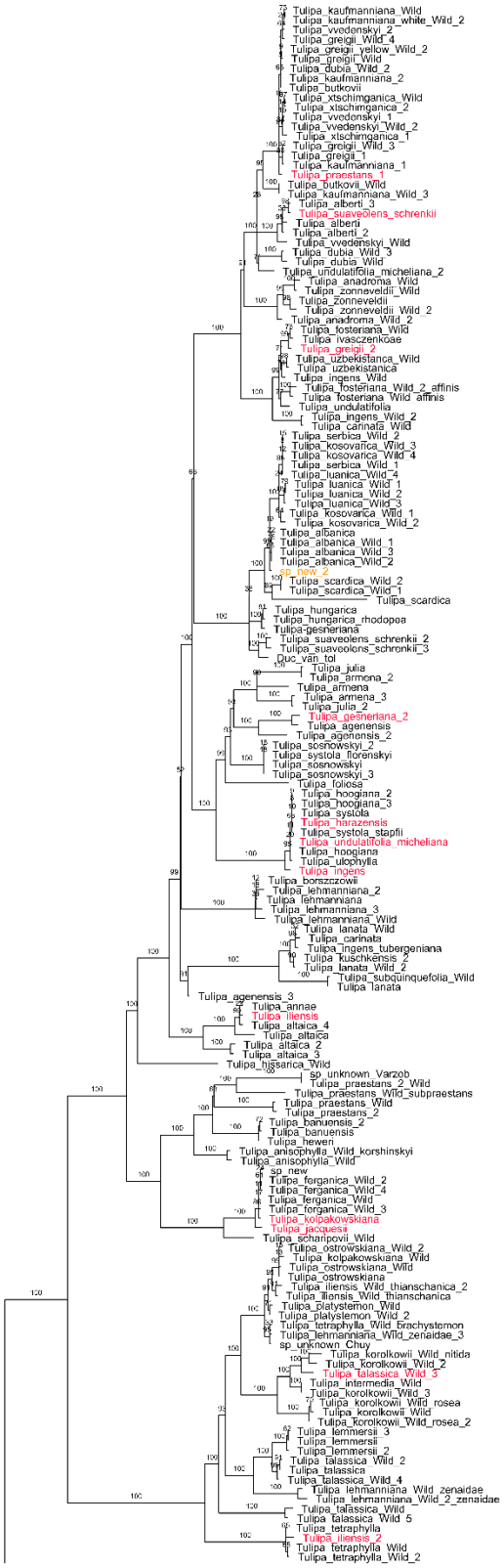

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Tulipa_sylvestris_patens
Tulipa_dianaeverettae
Tulipa_biflora_binutans_2
Tulipa_turkestanica_Wild_2
Tulipa_biflora
Tulipa_sylvestris_australis_talijevii
Tulipa_biflora_Wild_binutans
Tulipa_turkestanica_Wild
Tulipa_turkestanica_Wild_3
Tulipa_biflorifformis_Wild_3
Tulipa_biflorifformis_Wild
sp_unknown_Chapchyma
sp_unknown_Komushu
sp_unknown_Chatkal
Tulipa_biflora_sogdiana
Tulipa_biflorifformis_1
Tulipa_turkestanica_1
sp_unknown_Talas
Tulipa_orithyioides_Wild
Tulipa_biflora_buhseana
Tulipa_biflora_koyuncui_Wild_1
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Tulipa_bifloriformis_Wild_2
Tulipa_biflora_Wild
Tulipa_dasystemon_Wild_dasystemonoides
Tulipa_dasystemon_Wild_dasystemonoides_2
Tulipa_dasystemon_neustruevae
Tulipa_kolbintsevii_2
Tulipa_kolbintsevii
Tulipa_dasystemon_Wild_2
Tulipa_heterophylla
Tulipa_dasystemon_Wild_3
Tulipa_dasystemon
Tulipa_dasystemon_dasystemonoides_1
Tulipa_dasystemon_Wild
Tulipa_urumiensis_Wild_tarda
Tulipa_urumiensis_tarda
Tulipa_turkestanica_2
Tulipa_sylvestris_ssp_australis_Wild
Tulipa_orphanidea_theophrastii
Tulipa_sylvestris_biebersteiniana
Tulipa_humilis_Wild
Tulipa_orphanidea
Tulipa_sylvestris_ssp_sylvestris_Wild_2
Tulipa_sylvestris_ssp_sylvestris_Wild_1
Tulipa_sylvestris
Tulipa_regelii_3
Tulipa_regelii
Tulipa_regelii_2
Tulipa_sylvestris_patens_2
Tulipa_cinnabarina_2
Tulipa_cinnabarina
Tulipa_orphanidea_hageri
Tulipa_humilis
Tulipa_saxatilis_bakeri
Tulipa_saxatilis_1
Tulipa_cretica_2
Tulipa_cretica
Tulipa_saxatilis_2
Tulipa_sprengeri
Tulipa_kuschkensis
Tulipa_montana_2
Tulipa_montana
Tulipa_montana_chrysantha
Tulipa_clusiana_cashmeriana
Tulipa_linifolia_red_Wild
Tulipa_linifolia_yellow_Wild
Tulipa_clusiana
Tulipa_clusiana_aitchisonii
Tulipa_heteropetala_3
Tulipa_heteropetala
Tulipa_heteropetala_2
Tulipa_uniflora
Tulipa_uniflora_2
Tulipa_linifolia_Wild_maximowiczii
Tulipa_heterophylla_Wild_2
Tulipa_heterophylla_Wild_1
Tulipa_heterophylla_Wild_4
Tulipa_heterophylla_Wild_3
Amana_anhuiensis
Amana_wanzhensis
Amana_erythronioides
Amana_kuocangshanica
Amana_edulis
Erythronium_japonicum
Erythronium_sibiricum
9.0E-4

Appendix 3. Full plastome tree generated using Bayesian methods including identified erroneous specimens.




Tulipa_orithyioides
Tulipa_sylvestris_patens
Tulipa_biflora_binutans_2
Tulipa_dianaeverettiae
Tulipa_turkestanica_Wild_2
Tulipa_biflora
Tulipa_sylvestris_australis_talijevii
Tulipa_biflora_Wild_binutans
Tulipa_turkestanica_Wild
Tulipa_turkestanica_Wild_3
Tulipa_bifloriformis_Wild
sp_unknown_Chapchyma
Tulipa_bifloriformis_Wild_3
sp_unknown_Chatkal
sp_unknown_Komushu
Tulipa_biflora_sogdiana
sp_unknown_Talas
Tulipa_bifloriformis_1
Tulipa_turkestanica_1
Tulipa_orithyioides_Wild
Tulipa_biflora_buhseana
Tulipa_biflora_koyuncui_Wild_3
Tulipa_koyuncui_yellow_Wild_3
Tulipa_biflora_white_Wild_Turkey
Tulipa_biflora_koyuncui_Wild_1
Tulipa_biflora_white_Wild_Turkey_2
Tulipa_biflora_white_Wild_Turkey_3
Tulipa_biflora_koyuncui_Wild_2
Tulipa_koyuncui_Wild_1
Tulipa_koyuncui_yellow_Wild_2
Tulipa_koyuncui_yellow_Wild_1
Tulipa_biflora_Wild_Turkey
Tulipa_jacquesii_Wild
Tulipa_jacquesii_2
Tulipa_jacquesii_Wild_2
Tulipa_dasystemon_dasystemonoides_2
Tulipa_bifloriformis_Wild_2
Tulipa_biflora_Wild
Tulipa_dasystemon_Wild_dasystemonoides
Tulipa_dasystemon_Wild_dasystemonoides_2
Tulipa_dasystemon_neustruevae
Tulipa_kolbintsevii_2
Tulipa_kolbintsevii
Tulipa_dasystemon_Wild_2
Tulipa_heterophylla
Tulipa_dasystemon
Tulipa_dasystemon_Wild_3
Tulipa_dasystemon_Wild
Tulipa_dasystemon_dasystemonoides_1
Tulipa_urumiensis_Wild_tarda
Tulipa_urumiensis_tarda
Tulipa_sylvestris_ssp_sylvestris_Wild_1
Tulipa_sylvestris_ssp_sylvestris_Wild_2
Tulipa_orphanidea
Tulipa_humilis_Wild
Tulipa_sylvestris_ssp_australis_Wild
Tulipa_turkestanica_2
Tulipa_orphanidea_theophrastii
Tulipa_sylvestris_biebersteiniana
Tulipa_sylvestris
Tulipa_regelii_3
Tulipa_regelii
Tulipa_regelii_2
Tulipa_sylvestris_patens_2
Tulipa_cinnabarina_2
Tulipa_cinnabarina
Tulipa_orphanidea_hageri
Tulipa_humilis
Tulipa_saxatilis_bakeri
Tulipa_saxatilis_1
Tulipa_cretica_2
Tulipa_saxatilis_2
Tulipa_cretica
Tulipa_sprengeri
Tulipa_montana
Tulipa_montana_2
Tulipa_kuschkensis
Tulipa_montana_chrysantha
Tulipa_clusiana_cashmeriana
Tulipa_clusiana
Tulipa_clusiana_aitchisonii
Tulipa_linifolia_yellow_Wild
Tulipa_linifolia_red_Wild
Tulipa_heteropetala_3
Tulipa_heteropetala
Tulipa_heteropetala_2
Tulipa_uniflora_2
Tulipa_uniflora
Tulipa_linifolia_Wild_maximowiczii
Tulipa_heterophylla_Wild_1
Tulipa_heterophylla_Wild_2
Tulipa_heterophylla_Wild_4
Tulipa_heterophylla_Wild_3
Amana_erythronioides
Amana_kuocangshanica
Amana_wanzhensis
Amana_anhuiensis
Amana_edulis
Erythronium_japonicum
Erythronium_sibiricum

Appendix 4. Plastome tree inferred using only CDS data using Maximum Likelihood methods including identified erroneous specimens.




sp_unknown_Komushu
sp_unknown_Chatkal
Tulipa_biflora_sogdiana
Tulipa_bifloriformis_Wild_3
Tulipa_turkestanica_1
Tulipa_turkestanica_Wild
Tulipa_bifloriformis_Wild
sp_unknown_Chapchyma
Tulipa_sylvestris_patens
Tulipa_dianaeverettiae
Tulipa_orithyioides
Tulipa_biflora_binutans_2
Tulipa_turkestanica_Wild_2
Tulipa_sylvestris_australis_talijevii
Tulipa_biflora_Wild_binutans
Tulipa_biflora
Tulipa_turkestanica_Wild_3
Tulipa_bifloriformis_1
sp_unknown_Talas
Tulipa_biflora_buhseana
Tulipa_orithyioides_Wild
Tulipa_biflora_koyuncui_Wild_1
Tulipa_biflora_white_Wild_Turkey_3
Tulipa_biflora_koyuncui_Wild_2
Tulipa_biflora_white_Wild_Turkey
Tulipa_koyuncui_yellow_Wild_3
Tulipa_biflora_koyuncui_Wild_3
Tulipa_koyuncui_yellow_Wild_1
Tulipa_biflora_white_Wild_Turkey_2
Tulipa_koyuncui_yellow_Wild_2
Tulipa_biflora_Wild_Turkey
Tulipa_koyuncui_Wild_1
Tulipa_bifloriformis_Wild_2
Tulipa_jacquesii_Wild
Tulipa_jacquesii_2
Tulipa_jacquesii_Wild_2
Tulipa_dasystemon_dasystemonoides_2
Tulipa_biflora_Wild
Tulipa_sylvestris_ssp_australis_Wild
Tulipa_turkestanica_2
Tulipa_sylvestris_ssp_sylvestris_Wild_1
Tulipa_sylvestris_ssp_sylvestris_Wild_2
Tulipa_orphanidea_theophrastii
Tulipa_sylvestris_biebersteiniana
Tulipa_orphanidea
Tulipa_humilis_Wild
Tulipa_sylvestris
Tulipa_regelii
Tulipa_regelii_3
Tulipa_regelii_2
Tulipa_sylvestris_patens_2
Tulipa_saxatilis_2
Tulipa_cretica
Tulipa_cretica_2
Tulipa_saxatilis_1
Tulipa_saxatilis_bakeri
Tulipa_cinnabarina
Tulipa_cinnabarina_2
Tulipa_orphanidea_hageri
Tulipa_humilis
Tulipa_sprengeri
Tulipa_kolbintsevii
Tulipa_kolbintsevii_2
Tulipa_dasystemon
Tulipa_dasystemon_Wild_2
Tulipa_heterophylla
Tulipa_dasystemon_Wild_3
Tulipa_dasystemon_dasystemonoides_1
Tulipa_dasystemon_Wild
Tulipa_urumiensis_tarda
Tulipa_urumiensis_Wild_tarda
Tulipa_dasystemon_Wild_dasystemonoides_2
Tulipa_dasystemon_Wild_dasystemonoides
Tulipa_dasystemon_neustruevae
Tulipa_kuschkensis
Tulipa_montana
Tulipa_montana_2
Tulipa_montana_chrysantha
Tulipa_clusiana_cashmeriana
Tulipa_linifolia_yellow_Wild
Tulipa_linifolia_red_Wild
Tulipa_clusiana
Tulipa_clusiana_aitchisonii
Tulipa_heteropetala
Tulipa_heteropetala_3
Tulipa_heteropetala_2
Tulipa_uniflora_2
Tulipa_uniflora
Tulipa_linifolia_Wild_maximowiczii
Tulipa_heterophylla_Wild_2
Tulipa_heterophylla_Wild_4
Tulipa_heterophylla_Wild_1
Tulipa_heterophylla_Wild_3
Amana_erythronioides
Amana_kuocangshanica
Amana_anhuiensis
Amana_wanzhensis
Amana_edulis
Erythronium_sibiricum
Erythronium_japonicum
7.0E-4

Appendix 5. Taxa not sampled in this work

There remains a number of synonyms that could not be assessed in the scope of this project but may require reinstatement if evidence shows their uniqueness. These are primarily Central Asian species that were observed in the wild and appear to have unique characteristics. These include the following:

Potential species

Tulipa biebersteiniana Schult. & Schult.f.
Tulipa binutans Vved.
Tulipa buhseana Boiss.
Tulipa korshinskyi Vved.
Tulipa maximwocizii Regel
Tulipa mogoltavica Popov & Vved.
Tulipa prolongata Vved.
Tulipa sogdiana Bunge
Tulipa tubergeniana Hoog

There are also several species that our work suggests may be synonyms, but due to limited sampling, we didn't feel like we could make any definitive statements on these. These exclude those species in the poorly resolved groups as these pose a different problem. These include the following:

Potential synonyms

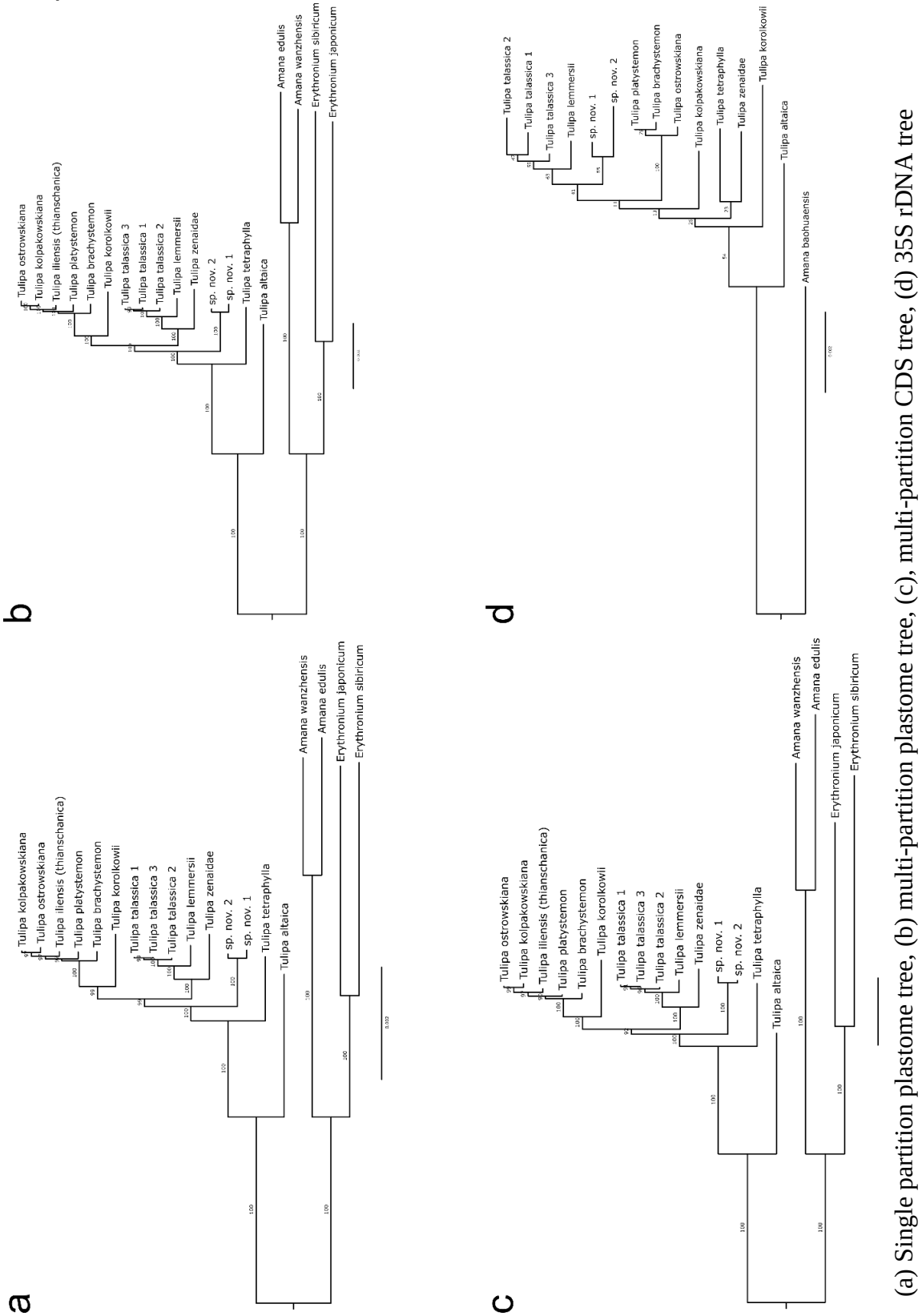
Tulipa borszczowii Regel
Tulipa hoogiana B.Fedtsch
Tulipa ostrowskiana Regel
Tulipa ulophylla Wendelbo

There also remains a range of taxa that are currently recognised as species, but were not obtainable in this project and therefore remain to be tested. These include:

Species to be sampled

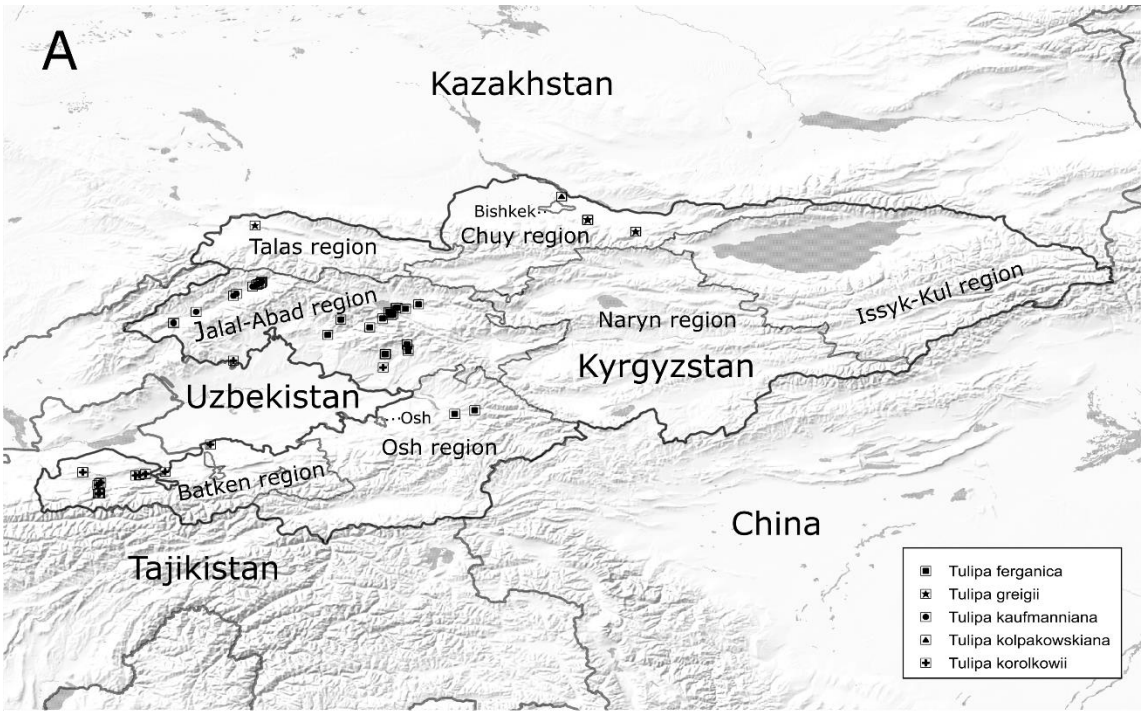
Tulipa akamasica Chrisdoulou, Hand & Charalambous
Tulipa aleppensis Boiss.
Tulipa auliekolica Perezhogin
Tulipa bactriana J.deGroot & Tojibaev
Tulipa. *brinkii J.J. de Groot & B.J.M Zonneveld*
Tulipa botschantzevae S.N.Abramova & Zakal.
Tulipa cypria Stapf
Tulipa faribae Ghahr
Tulipa lorestanica Rukšāns & Zubov.
Tulipa narcissicum N.Y. Stepanova
Tulipa persica (Lindl.) Sweet
Tulipa salsola Rukšāns & Zubov
Tulipa schmidtii Fomin
Tulipa sinkiangensis Z.M.Mao
Tulipa turgaica Perezhogin

Appendix 6. Different dataset inferred phylogenies used to identify new species



(a) Single partition plastome tree, (b) multi-partition plastome tree, (c), multi-partition CDS tree, (d) 35S rDNA tree

Appendix 7. Sample sites across Kyrgyzstan

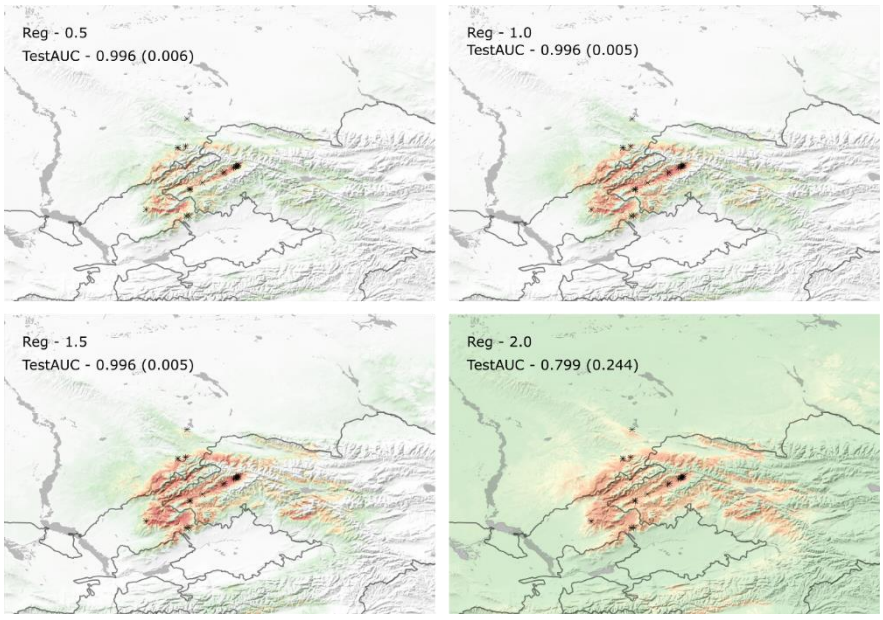




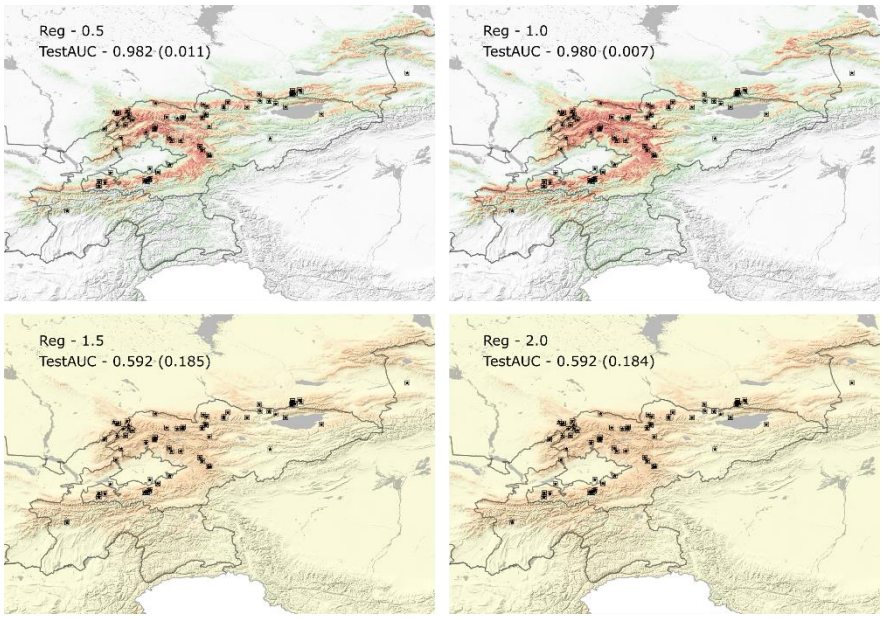
Tulip populations recorded in Kyrgyzstan during field surveys. Presented here in wgs84 ESPG4326.

Appendix 8. Assessment of regularization parameters

T. kaufmanniana



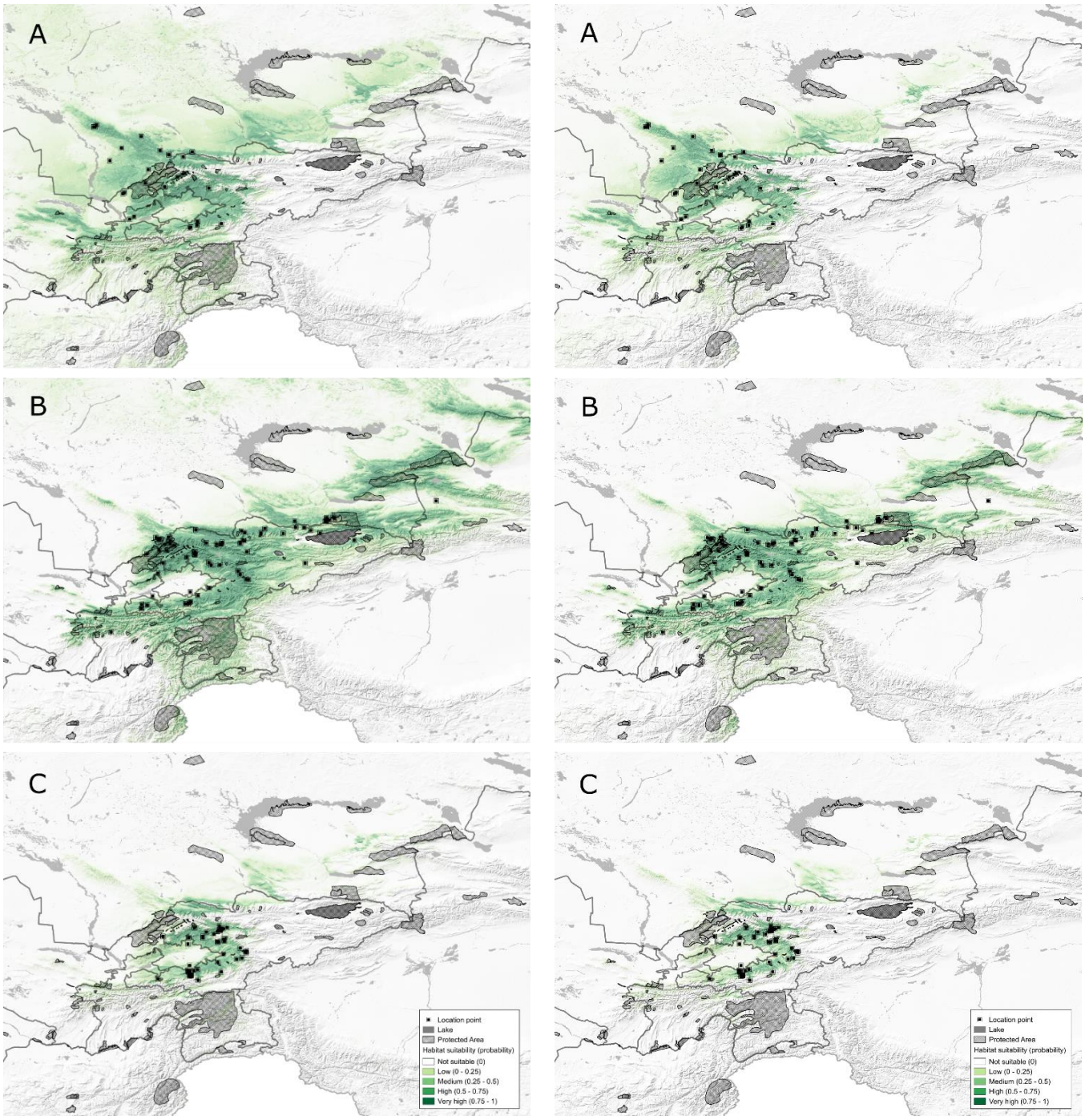
T. dasystemon

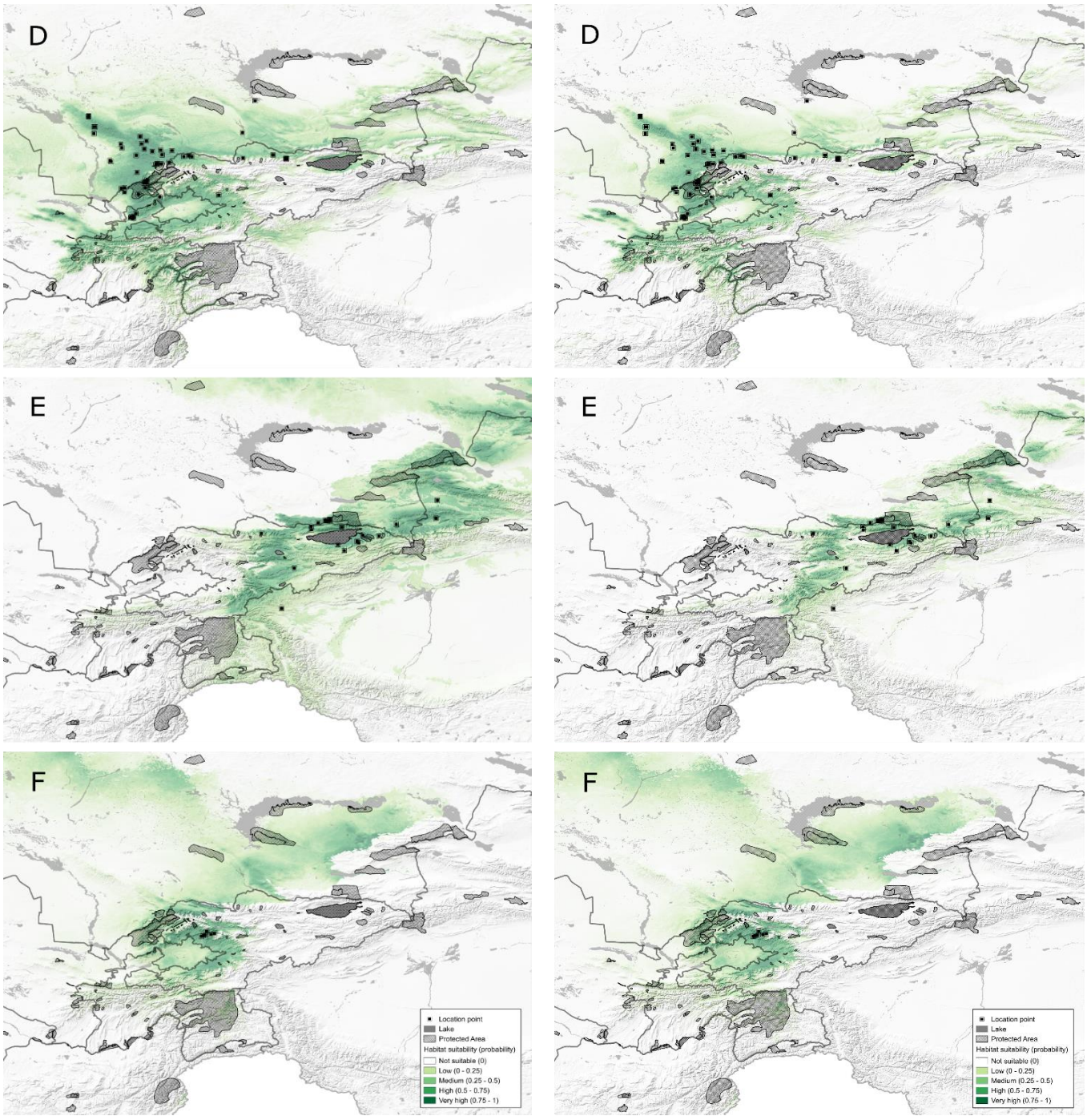


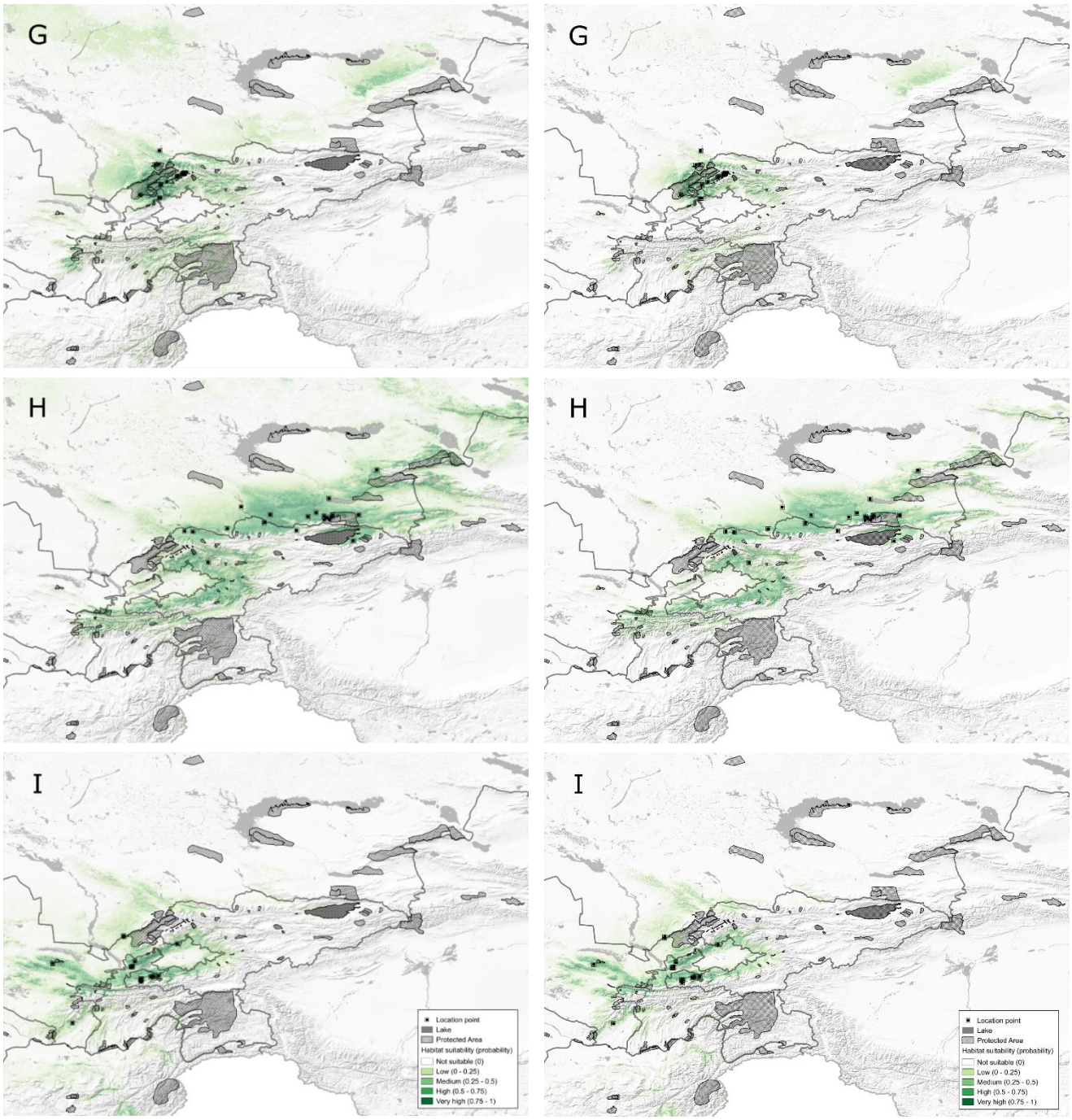
Present day species distribution modelling using a range of regularization factors for *T. kaufmanniana* and *T. dasystemon*.

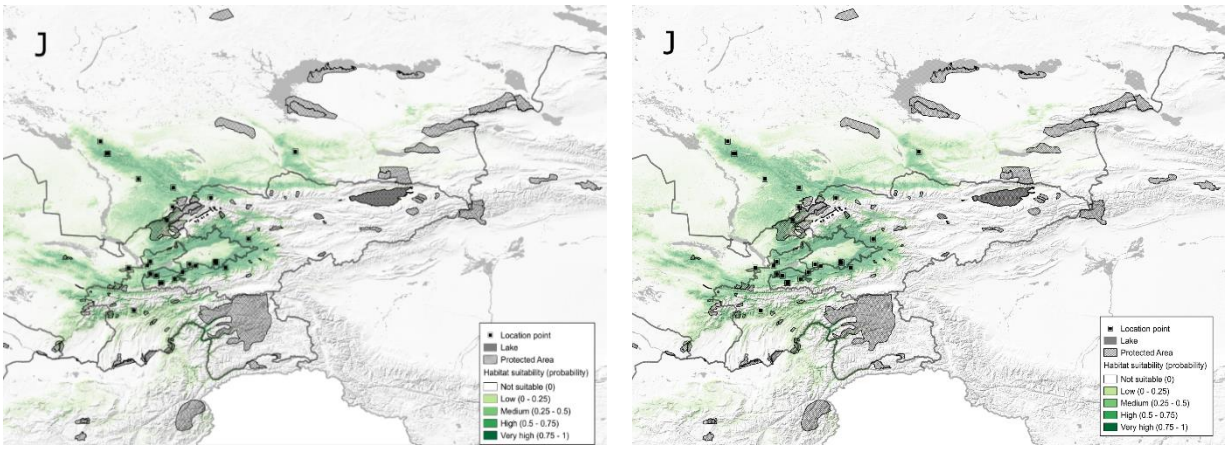
Regularization parameters of 0.5, 1.0, 1.5, and 2.0 were tested for *T. kaufmanniana* and *T. dasystemon*. Although several regularisation parameters produced similar TestAUC values we selected 1.0 for modelling as it matched the current distribution knowledge of the species most closely. Parameters above and below 1.0 narrowed or broadened the range too much and not infrequently had lower TestAUC values. Presented here in wgs84 ESPG4326.

Appendix 9. Assessment of effectiveness of bias file




D
D
E
E
F
F
Location point
Lake
Protected Area
Habitat suitability (probability)
Not suitable (0)
Low (0 - 0.25)
Medium (0.25 - 0.5)
High (0.5 - 0.75)
Very high (0.75 - 1)


G
G
H
H
I
I
Location point
Lake
Protected Area
Habitat suitability (probability)
Not suitable (0)
Low (0 - 0.25)
Medium (0.25 - 0.5)
High (0.5 - 0.75)
Very high (0.75 - 1)
Location point
Lake
Protected Area
Habitat suitability (probability)
Not suitable (0)
Low (0 - 0.25)
Medium (0.25 - 0.5)
High (0.5 - 0.75)
Very high (0.75 - 1)



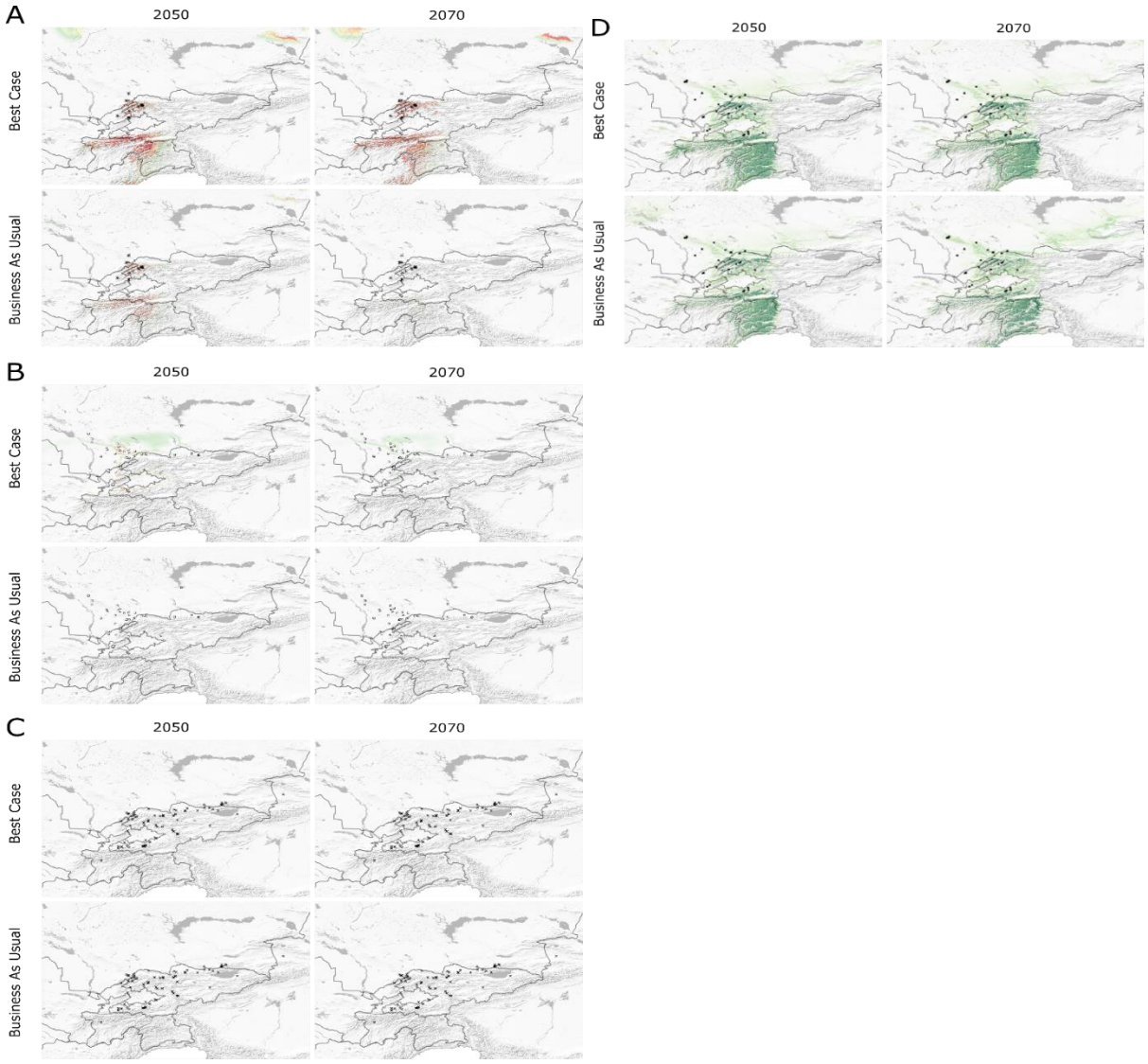
MaxEnt Species distribution models produced with a bias file on the left and without a bias file on the right. Panels correspond to different species: A – *T. bifloriformis*, B - *T. dasystemon*, C – *T. ferganica*, D - *T. greigii*, E – *T. heterophylla*, F - *T. jacquesii*, G – *T. kaufmanniana*, H – *T. kolpakowskiana*, I – *T. korolkowii,* and J – *T. turkestanica*. Presented here in wgs84 ESPG4326.

The test data AUC values for each species present day distribution both with and without a bias file.

	T. bifloriformis	***T. dasystemon***	***T. ferganica***	***T. greigii***	***T. heterophylla***
Bias file	0.983 (0.008)	0.976 (0.007)	0.991 (0.007)	0.985 (0.005)	0.976 (0.018)
No Bias file	0.990 (0.003)	0.980 (0.007)	0.991 (0.008)	0.989 (0.005)	0.979 (0.026)
	T. jacquesii	***T. kaufmanniana***	***T. kolpakowskiana***	***T. korolkowii***	***T. turkestanica***
Bias file	0.992 (0.006)	0.996 (0.005)	0.983 (0.013)	0.993 (0.004)	0.978 (0.008)
No Bias file	0.995 (0.002)	0.996 (0.005)	0.993 (0.005)	0.994 (0.003)	0.985 (0.006)

We tested modelling using a bias file generated for the Liliaceae clade using the procedure found here: https://scottrinnan.wordpress.com/2015/08/31/how-to-construct-a-bias-file-with-r-for-use-in-maxent-modeling/. TestAUC values were generally lower and the bias file led to slightly larger areas of suitable habitat for all models. Importantly when using a bias file, areas where the species is not known to occur were highlighted. We believe that the modelling undertaken without the bias file represents more accurate distributions given current knowledge. The general similarity of both modelling efforts could be suggestive that collected *Tulipa* data is relatively unbiased, or that due to the small number of location points sampling bias plays a lesser role in the limitations of these models. We favour the latter argument.

Appendix 10. Climate models built using Miroc ESM climate model



Climate modelling of suitable habitat for A – *T. kaufmanniana*, B – *T. greigii*, C – *T. dasystemon*, and D – *T. bifloriformis*.using the Miroc ESM climate models. Presented here in wgs84 ESPG4326.

Miroc ESM models were similar to CCSM4 GCM models. However, there were some minor discrepancies between them with some Miroc ESM models showing smaller suitable habitat areas, especially for *T. dasystemon* where the low suitability habitat highlighted in CCSM4 GCM models was completely lost. We have used both models to make overall conclusions but focus on presenting the CCSM4-GCM models and we have sued these for all calculations

Appendix 11. Cell numbers and area of distributions for models

Number of cells of suitable habitat in the present data and best-case climate change scenario of 2050 and 2070 under the CCSM4 GCM model

			T. bifloriformis	T. dasystemon	T. ferganica	T. greigii	T. heterophylla	T. kaufmanniana	T. kolpakowskiana	T. korolkowii	T. turkestanica
Present Day	Low suitability	Total	6133959	11041350	3256182	5779958	11723712	3032240	8317675	2930187	5684183
		Native	569271	437737	68682	334786	311769	121140	171526	123771	317692
		Non-native	5564688	10603613	3187500	5445172	11411943	2911100	8146149	2806416	5366491
	Medium suitability	Total	166407	248926	51142	197570	159095	41563	132114	88881	193107
		Native	129962	134231	20849	101445	90828	24920	73356	33170	114337
		Non-native	36445	114695	30293	96125	68267	16643	58758	55711	78770
	High suitability	Total	97289	146166	27596	110266	75198	14594	78501	42090	124090
		Native	82498	108590	14714	60587	55398	12693	49496	20656	108795
		Non-native	14791	37576	12882	49679	19800	1901	29005	21434	15295
	Very high suitability	Total	90215	124676	22093	89612	61362	10124	42692	27802	77566
		Native	82619	117183	16315	63097	53774	10074	28978	15909	69726
		Non-native	7596	7493	5778	26515	7588	50	13714	11893	7840
2050	Low suitability	Total	5004737	3351133	0	470172	61964	643406	4793	533	253160
		Native	693152	637366	0	71969	45620	14267	3994	2	65655
		Non-native	4311585	2713767	0	398203	16344	629139	799	531	187505
	Medium suitability	Total	174334	8590	0	23941	0	119974	0	0	155
		Native	35413	6733	0	15939	0	2614	0	0	104
		Non-native	138921	1857	0	8002	0	117360	0	0	51
	High suitability	Total	89866	713	0	14407	0	85078	0	0	43
		Native	22273	17	0	9046	0	2441	0	0	33
		Non-native	67593	696	0	5361	0	82637	0	0	10
	Very high suitability	Total	113240	399	0	14157	0	43638	0	0	2
		Native	44057	0	0	9654	0	5546	0	0	1
		Non-native	69183	399	0	4503	0	38092	0	0	1
2070	Low suitability	Total	4712239	3059804	0	399407	62748	577721	3474	0	259467
		Native	689161	643830	0	71893	46511	35607	3254	0	56238
		Non-native	4023078	2415974	0	327514	16237	542114	220	0	118426
	Medium suitability	Total	125485	5280	0	9240	0	108104	0	0	147
		Native	34441	4456	0	5973	0	3470	0	0	92
		Non-native	91044	824	0	3267	0	104634	0	0	55
	High suitability	Total	76820	158	0	4002	0	28703	0	0	44
		Native	23141	0	0	2802	0	2231	0	0	31
		Non-native	53679	158	0	1200	0	26472	0	0	13

	Very high suitability	Total	100341	89	0	150	0	35323	0	0	4
		Native	41824	0	0	54	0	3906	0	0	2
		Non-native	58517	89	0	96	0	31417	0	0	2

Area of suitable habitat (in km^2) for present data and best-case climate change scenario of 2050 and 2070 under the CCSM4 GCM model based on an average cell size of 0.648km^2 calculated using the area function of the raster library in R.

			T. biflorifor mis	T. dasystem on	T. fergani ca	T. greigii	T. heterophy lla	T. kaufmannia na	T. kolpakowski ana	T. korolko wii	T. turkestani ca
Prese nt Day	Low suitabili ty	Total	3974805	7154795	211000 6	37454 13	7596965	1964892	5389853	189876 1	3683351
		Nativ e	368888	283654	44506	21694 1	202026	78499	111149	80204	205864
		Non-nativ e	3605918	6871141	206550 0	35284 71	7394939	1886393	5278705	181855 8	3477486
	Mediu m suitabili ty	Total	107832	161304	33140	12802 5	103094	26933	85610	57595	125133
		Nativ e	84215	86982	13510	65736	58857	16148	47535	21494	74090
		Non-nativ e	23616	74322	19630	62289	44237	10785	38075	36101	51043
	High suitabili ty	Total	63043	94716	17882	71452	48728	9457	50869	27274	80410
		Nativ e	53459	70366	9535	39260	35898	8225	32073	13385	70499
		Non-nativ e	9585	24349	8348	32192	12830	1232	18795	13889	9911
	Very high suitabili ty	Total	58459	80790	14316	58069	39763	6560	27664	18016	50263
		Nativ e	53537	75935	10572	40887	34846	6528	18778	10309	45182
		Non-nativ e	4922	4855	3744	17182	4917	32	8887	7707	5080
2050	Low suitabili ty	Total	3243070	2171534	0	30467 1	40153	416927	3106	345	164048
		Nativ e	449162	413013	0	46636	29562	9245	2588	1	42544
		Non-nativ e	2793907	1758521	0	25803 6	10591	407682	518	344	121503
	Mediu m suitabili ty	Total	112968	5566	0	15514	0	77743	0	0	100
		Nativ e	22948	4363	0	10328	0	1694	0	0	67
		Non-nativ e	90021	1203	0	5185	0	76049	0	0	33
	High suitabili ty	Total	58233	462	0	9336	0	55131	0	0	28
		Nativ e	14433	11	0	5862	0	1582	0	0	21
		Non-nativ e	43800	451	0	3474	0	53549	0	0	6
	Very high suitabili ty	Total	73380	259	0	9174	0	28277	0	0	1
		Nativ e	28549	0	0	6256	0	3594	0	0	1
		Non-nativ e	44831	259	0	2918	0	24684	0	0	1
2070	Low suitabili ty	Total	3053531	1982753	0	25881 6	40661	374363	2251	0	168135
		Nativ e	446576	417202	0	46587	30139	23073	2109	0	36442
		Non-nativ e	2606955	1565551	0	21222 9	10522	351290	143	0	76740
		Total	81314	3421	0	5988	0	70051	0	0	95

Medium suitability	Native	22318	2887	0	3871	0	2249	0	0	60
	Non-native	58997	534	0	2117	0	67803	0	0	36
High suitability	Total	49779	102	0	2593	0	18600	0	0	29
	Native	14995	0	0	1816	0	1446	0	0	20
	Non-native	34784	102	0	778	0	17154	0	0	8
Very high suitability	Total	65021	58	0	97	0	22889	0	0	3
	Native	27102	0	0	35	0	2531	0	0	1
	Non-native	37919	58	0	62	0	20358	0	0	1

Appendix 12. Cell numbers and area of distributions inside protected areas for models

The number of cells of high and very high suitable habitat inside of protected areas across the native area of each species for present data and best-case climate change scenario of 2050 and 2070 under the CCSM4 GCM model.

	T. bifloriformis	T. dasystemon	T. ferganica	T. greigii	T. heterophylla	T. kaufmanniana	T. kolpakowskiana	T. korolkowii	T. turkestanica
Present day	7936	20889	445	8087	11334	7176	3348	722	5846
2050	12020	0	0	721	0	2157	0	0	0
2070	12724	0	0	226	0	2074	0	0	0

The estimated area (in km^2) of high and very high suitable habitat inside of protected areas across the native area for each species for present data and best-case climate change scenario of 2050 and 2070 under the CCSM4 GCM model based on an average cell size of 0.648km^2 calculated using the area function of the raster library in R

		T. bifloriformis	T. dasystemon	T. ferganica	T. greigii	T. heterophylla	T. kaufmanniana	T. kolpakowskiana	T. korolkowii	T. turkestanica
Present day	Area (km^2)	5143	13536	288	5240	7344	4650	2170	468	3555
	% of present native range	4.8	9.3	1.4	6.5	10.4	31.5	4.3	2.0	3.1
2050	Area (km^2)	7789	0	0	467	0	1398	0	0	0
	% of present native range	7.3	0	0	0.6	0	9.5	0	0	0
2070	Area (km^2)	8245	0	0	147	0	1344	0	0	0
	% of present native range	7.7	0	0	0.2	0	9.1	0	0	0

Appendix 13. Mean altitude of distributions at different times for species with future predicted habitat

Mean altitude and standard deviation for the four species with predicted suitable habitat in CCSM4 GCM best case model across 2020, 2050, and 2070.

	Year	*T. bifloriformis*	*T. dasystemon*	*T. greigii*	*T. kaufmanniana*
Mean (m) [Standard Deviation]	2020	1225.894 [607.5094]	2251.479 [762.6774]	1039.499 [499.2809]	2052.31 [479.3261]
	2050	3228.455 [1014.581]	2972.738 [115.9974]	788.7337 [303.8581]	3071.84 [149.4073]
	2070	3258.808 [1022.229]	No data	965.7797 [229.2731]	3051.716 [152.5075]

Appendix 14. National species lists

* Indicates an endemic species in all tables

Species of Kazakhstan

Tulipa* subgenus *Eduardoregelia (Popov) B.D.Wilson & Christenh., ined
Section Eduardoregelia B.D.Wilson & Christenh., ined
Tulipa heterophylla (Regel) Baker

Tulipa* subgenus *Orithyia (D.Don) Baker J. Linn. Soc. Bot. 14: 277. (1874)
Section *Orithyia* (D.Don)Vved.
Tulipa heteropetala Ledeb.
Tulipa uniflora (L.) Besser

Tulipa* subgenus *Eriostemones (Boiss.) Hall, Book of the Tulip: 60 (1929), as *Eriostemon*.
Section *Sylvestres* (Baker) Baker
Tulipa patens C.Agardh ex Schult&Schult. f
Tulipa regelii Krassn.*
Tulipa sylvestris L. subsp. *australis* (Link) Pamp.
Tulipa sylvestris L. subsp. *sylvestris* (type)
Tulipa turgaica Perezhogin*
Section *Biflores* A.D.Hall ex Zonn. & Veldk.
Tulipa auliekolica Perezhogin.*
Tulipa biflora Pall.
Tulipa bifloriformis Vved.
Tulipa dasystemon (Regel) Regel
Tulipa dianaeverettiae J.de Groot & Zonn. *
Tulipa kolbintsevii Zonn.*
Tulipa orthopoda Vved.*
Tulipa turkestanica Regel
Tulipa urumiensis Stapf

Tulipa* subgenus *Tulipa
Section *Kolpakowskianae* Van Raamsd. Ex Zonn.& Veldk.
Tulipa brachystemon Regel
Tulipa iliensis Regel
Tulipa kolpakowskiana Regel
Tulipa korolkowii Regel
Tulipa lemmersii Zonn., Peterse & J.de Groot*
Tulipa ostrowskiana Regel
Tulipa tetraphylla Regel
Tulipa zenaidae Vved.
Section *Tulipa* (Van Raamsd.) Zonn.
Tulipa alberti Regel*
Tulipa altaica Pall.
Tulipa borszczowii Regel
Tulipa dubia Vved.
Tulipa greigii Regel
Tulipa ivasczenkoae Epiktetov & Belyalov*
Tulipa kaufmanniana Regel
Tulipa lehmanniana Merckl.
Tulipa suaveolens Roth

Species of Kyrgyzstan

Tulipa* subgenus *Eduardoregelia (Popov) B.D.Wilson & Christenh., ined
Section Eduardoregelia B.D.Wilson & Christenh., ined
Tulipa heterophylla (Regel) Baker

Tulipa* subgenus *Eriostemones (Boiss.) Hall, Book of the Tulip: 60 (1929), as *Eriostemon*.
Section *Biflores* A.D.Hall ex Zonn. & Veldk.
Tulipa biflora Pall.
Tulipa bifloriformis Vved.
Tulipa dasystemon (Regel) Regel
Tulipa jacquesii Zonn.*
Tulipa neustruevae Pobed.*
Tulipa orithyioides Vved.
Tulipa turkestanica Regel
Tulipa urumiensis Stapf

Tulipa* subgenus *Tulipa

Section *Kolpakowskianae* Van Raamsd. Ex Zonn.& Veldk.

Tulipa brachystemon Regel
Tulipa kolpakowskiana Regel
Tulipa korolkowii Regel
Tulipa ostrowskiana Regel
Tulipa platystemon Vved.*
Tulipa talassica Lazkov*
Tulipa tetraphylla Regel
Tulipa toktogulica B.D.Wilson & Lazkov*
Tulipa zenaidae Vved.

Section *Multiflorae* (Van Raamsd.) Zonn.

Tulipa ferganica Vved.

Section *Tulipa* (Van Raamsd.) Zonn.

Tulipa affinis Botschantz.
Tulipa anadroma Botschantz.*
Tulipa dubia Vved.
Tulipa greigii Regel
Tulipa kaufmanniana Regel
Tulipa zonneveldii J.de Groot & Tojibaev*

Species of Tajikistan

Tulipa* subgenus *Clusianae (Baker) Zonn. & Veldkamp, Pl. Syst. Evol. 298: 89. (2012)

Section *Clusianae* Baker

Tulipa linifolia Regel

Tulipa* subgenus *Eriostemones (Boiss.) Hall, Book of the Tulip: 60 (1929), as *Eriostemon*.

Section *Biflores* A.D.Hall ex Zonn. & Veldk.

Tulipa biflora Pall.
Tulipa bifloriformis Vved.
Tulipa dasystemon (Regel) Regel
Tulipa orithyioides Vved.
Tulipa turkestanica Regel

Tulipa* subgenus *Tulipa

Section *Kolpakowskianae* Van Raamsd. Ex Zonn.& Veldk.

Tulipa korolkowii Regel

Section *Multiflorae* (Van Raamsd.) Zonn.

Tulipa anisophylla Vved.
Tulipa boettgeri Regel*
Tulipa praestans H.B.May*

Section *Tulipa* (Van Raamsd.) Zonn.

Tulipa affinis Botschantz.
Tulipa carinata Vved.
Tulipa dubia Vved.
Tulipa fosteriana W.Irving
Tulipa greigii Regel
Tulipa hissarica Popov & Vved.
Tulipa ingens Hoog
Tulipa kaufmanniana Regel
Tulipa lanata Regel
Tulipa lehmanniana Merckl.
Tulipa micheliana Hoog
Tulipa subquinquefolia Vved.*
Tulipa tubergeniana Hoog
Tulipa undulatifolia Boiss.
Tulipa vvedenskyi Botschantz.

Species of Uzbekistan

Tulipa* subgenus *Eriostemones (Boiss.) Hall, Book of the Tulip: 60 (1929), as *Eriostemon*.

Section *Biflores* A.D.Hall ex Zonn. & Veldk.

Tulipa biflora Pall.
Tulipa bifloriformis Vved.
Tulipa dasystemon (Regel) Regel
Tulipa orithyioides Vved.
Tulipa turkestanica Regel

Tulipa* subgenus *Tulipa

Section *Kolpakowskianae* Van Raamsd. Ex Zonn.& Veldk.

Tulipa korolkowii Regel

Section *Multiflorae* (Van Raamsd.) Zonn.

Tulipa anisophylla Vved.
Tulipa ferganica Vved.
Tulipa scharipovii Tojibaev*

Section *Tulipa* (Van Raamsd.) Zonn.

Tulipa affinis Botschantz.
Tulipa bactriana J.de Groot & Tojibaev*
Tulipa borszczowii Regel
Tulipa butkovii Botschantz.*
Tulipa carinata Vved.
Tulipa dubia Vved.
Tulipa fosteriana W.Irving
Tulipa greigii Regel
Tulipa hissarica Popov & Vved.
Tulipa ingens Hoog
Tulipa kaufmanniana Regel
Tulipa lanata Regel
Tulipa lehmanniana Merckl.
Tulipa micheliana Hoog
Tulipa × tschimganica Botschantz.*
Tulipa tubergeniana Hoog
Tulipa undulatifolia Boiss.
Tulipa uzbekistanica Botschantz. & Scharipov*
Tulipa vvedenskyi Botschantz.

Appendix 15. Workshop agenda

Day 1 – 10th of May, 2022 (Training)

Time	Workshop Session & Content
08:45	Workshop room open for participants' arrival and registration
09:00	*Session 1:* Welcome and Introduction to the Workshop Welcoming remarks from workshop host (Professor Kaiyrkul Shalpykov) Welcome & introduction to IUCN (Katharine Davies, BGCI) Workshop objectives, schedule & structure Participant introductions
	Session 2: From Raw Data to Red List: Introduction to the IUCN Red List Assessment Process and the Role of the Assessor Presentation: An introduction to the IUCN Red List of Threatened Species Presentation: From Raw Data to Red List: the Red List assessment process and role of the Red List Assessor
	Session 3: Key Terms and Concepts used in the Red List Criteria Presentation: Terms used in the IUCN Red List Criteria
10:50	Coffee Break (10 minutes)
11:00	*Session 3 (continued)* Recap: Terms used in the IUCN Red List Criteria
	Session 4: Red List Categories Presentation: IUCN Red List Categories Presentation: Data Quality & Uncertainty
	Session 5: Criterion A Presentation: Criterion A
12:00	Lunch (45 min)
12:45	*Session 6:* Criterion B Presentation: Criterion B
	Session 7: Criteria C, D & E Presentation: Criteria C, D & E
	Session 8: Red List Assessment: Case Study Exercise: Applying the IUCN Red List Criteria – a case study
15:00	Coffee Break (15 minutes)
15:15	*Session 8:* Practicing the Red List assessments Presentation: Example of a tulip Red List assessment Practice: Go through tulip Red List assessment together
17:30	End of Workshop

Day 2 and 3 – 11th/12th of May 2022 (Red Listing)

Time	Sessions
08:45	Workshop room opens for participants

09:00	*Session 1* Red Listing
10:50	Coffee Break (10 minutes)
11:00	*Session 2* Red Listing
12:00	Lunch (45 min)
12:45	*Session 3* Red Listing
15:00	Coffee Break (15 minutes)
15:15	*Session 4* Red Listing
17:30	End of Day

Day 4 – 13th of May 2022 (Strategy Day)

Time	Sessions
08:45	Workshop room opens for participants
09:00-09:15	Presentation – Brett Wilson Current sites in consideration and criteria
09:15-09:45	Group work by country, identifying 3 priority sites based on criteria
09:45-10:25	Presentation of each group as to their priority sites and why (10 mins per country)
10:25-11:15	Regional scale priority sites (facilitated by Ormon) (Label priority sites)
11:15-11.25	Coffee Break (10 minutes)
11:25-12:00	Presentation of objectives and discussion of missed objectives. Q&A
12:00	Lunch (45 min)
12:45-12:55	Wake up game
12:55-13:30	Rank objectives (top 5)
13:30-15:00	Break into mixed groups to discuss actions across sites and objectives (prepare flipcharts with Objective, Site, Actions columns)
15:00-15:15	Coffee Break (15 minutes)
15:15-16:15	Present actions for each objective (someone type in One Drive)
16:15-17:30	Summarising actions, next steps (Assessing actions to teams, working group (contact details), Regional MoU)
17:30	End of Day

Day 5-14th of May 2022 Field Trip

Appendix 16. List of workshop participants

№	Name	Country	Place of work	Participation
1	Buzurmanov B.M.	Kyrgyzstan	Ministry of natural resources, ecology and technical supervision, Kyrgyzstan	Offline
2	Shalpykov K.T.	Kyrgyzstan	National Academy of Sciences, Kyrgyzstan	Offline
3	Lazkov G.A	Kyrgyzstan	Institute of Botany, NAS Kyrgyzstan	Offline
4	Ganybaeva M.	Kyrgyzstan	Institute of Botany, NAS Kyrgyzstan	Offline
5	Usupbaev A.K.	Kyrgyzstan	Institute of Botany, NAS Kyrgyzstan	Offline
6	Ivashenko A.A.	Kazakhstan	Institute of Zoology, Kazakhstan	Offline
7	Epiktetov V.G.	Kazakhstan	Institute of Botany, Kazakhstan	Offline
8	Beshko N.Yu.	Uzbekistan	Institute of Botany, Academy of Sciences of Uzbekistan	Offline
9	Boboev M.	Tajikistan	Kulob Botanical Garden, Tajikistan	Online
10	Turakulov I.	Tajikistan	Khudjant State university, Tajikistan	Online
11	Doolotbakov A.K.	Kyrgyzstan	National Academy of Sciences, Kyrgyzstan	Offline
12	Christenhusz M.	UK	Royal Botanical Garden Kew	Offline
13	Gill D.	UK	Acting deputy director, Eurasian program, FFI	Offline
14	Brockington S.	UK	Cambridge Botanical Garden	Online
15	Wilson B.	UK	PhD student, Cambridge University	Offline
16	Davies K.	UK	IUCN red-listing specialist	Offline
17	Samanchina J.B.	Kyrgyzstan	FFI Kyrgyzstan, branch director	Offline
18	Sultangaziev O.E.	Kyrgyzstan	FFI, Central Asia ecologist	Offline
19	Kabaeva A.M.	Kyrgyzstan	FFI Kyrgyzstan, IWT regional coordinator	Offline
20	Tagaev B.A.	Kyrgyzstan	FFI Kyrgyzstan, sustainable livelihood coordinator	Offline
21	Cherniavskaia M.V.	Kyrgyzstan	FFI Kyrgyzstan, project officer	Offline
22	Bekenova Zh.	Kyrgyzstan	FFI Kyrgyzstan, project assistant	Offline
23	Dekhonov Davron	Uzbekistan	Institute of Botany, Academy of Sciences of Uzbekistan	Offline
24	2 Interpreters+ technical support	Kyrgyzstan		Offline

Appendix 17. IUCN summary criteria sheet

SUMMARY OF THE FIVE CRITERIA (A-E) USED TO EVALUATE IF A TAXON BELONGS IN AN IUCN RED LIST THREATENED CATEGORY (CRITICALLY ENDANGERED, ENDANGERED OR VULNERABLE).[1]

A. Population size reduction. Population reduction (measured over the longer of 10 years or 3 generations) based on any of A1 to A4

	Critically Endangered	Endangered	Vulnerable
A1	≥ 90%	≥ 70%	≥ 50%
A2, A3 & A4	≥ 80%	≥ 50%	≥ 30%

A1 Population reduction observed, estimated, inferred, or suspected in the past where the causes of the reduction are clearly reversible AND understood AND have ceased.

A2 Population reduction observed, estimated, inferred, or suspected in the past where the causes of reduction may not have ceased OR may not be understood OR may not be reversible.

A3 Population reduction projected, inferred or suspected to be met in the future (up to a maximum of 100 years) *[(a) cannot be used for A3]*.

A4 An observed, estimated, inferred, projected or suspected population reduction where the time period must include both the past and the future (up to a max. of 100 years in future), and where the causes of reduction may not have ceased OR may not be understood OR may not be reversible.

based on any of the following:

(a) direct observation *[except A3]*
(b) an index of abundance appropriate to the taxon
(c) a decline in area of occupancy (AOO), extent of occurrence (EOO) and/or habitat quality
(d) actual or potential levels of exploitation
(e) effects of introduced taxa, hybridization, pathogens, pollutants, competitors or parasites.

B. Geographic range in the form of either B1 (extent of occurrence) AND/OR B2 (area of occupancy)

	Critically Endangered	Endangered	Vulnerable
B1. Extent of occurrence (EOO)	< 100 km²	< 5,000 km²	< 20,000 km²
B2. Area of occupancy (AOO)	< 10 km²	< 500 km²	< 2,000 km²
AND at least 2 of the following 3 conditions:			
(a) Severely fragmented **OR** Number of locations	= 1	≤ 5	≤ 10
(b) Continuing decline observed, estimated, inferred or projected in any of: **(i)** extent of occurrence; **(ii)** area of occupancy; **(iii)** area, extent and/or quality of habitat; **(iv)** number of locations or subpopulations; **(v)** number of mature individuals			
(c) Extreme fluctuations in any of: **(i)** extent of occurrence; **(ii)** area of occupancy; **(iii)** number of locations or subpopulations; **(iv)** number of mature individuals			

C. Small population size and decline

	Critically Endangered	Endangered	Vulnerable
Number of mature individuals	< 250	< 2,500	< 10,000
AND at least one of C1 or C2			
C1. An observed, estimated or projected continuing decline of at least (up to a max. of 100 years in future):	25% in 3 years or 1 generation (whichever is longer)	20% in 5 years or 2 generations (whichever is longer)	10% in 10 years or 3 generations (whichever is longer)
C2. An observed, estimated, projected or inferred continuing decline AND at least 1 of the following 3 conditions:			
(a) (i) Number of mature individuals in each subpopulation	≤ 50	≤ 250	≤ 1,000
(ii) % of mature individuals in one subpopulation =	90–100%	95–100%	100%
(b) Extreme fluctuations in the number of mature individuals			

D. Very small or restricted population

	Critically Endangered	Endangered	Vulnerable
D. Number of mature individuals	< 50	< 250	**D1.** < 1,000
D2. *Only applies to the VU category* Restricted area of occupancy or number of locations with a plausible future threat that could drive the taxon to CR or EX in a very short time.	-	-	**D2.** typically: AOO < 20 km² or number of locations ≤ 5

E. Quantitative Analysis

	Critically Endangered	Endangered	Vulnerable
Indicating the probability of extinction in the wild to be:	≥ 50% in 10 years or 3 generations, whichever is longer (100 years max.)	≥ 20% in 20 years or 5 generations, whichever is longer (100 years max.)	≥ 10% in 100 years

1 Use of this summary sheet requires full understanding of the *IUCN Red List Categories and Criteria* and *Guidelines for Using the IUCN Red List Categories and Criteria*. Please refer to both documents for explanations of terms and concepts used here.

Appendix 18. Activities discussed for priority objectives

Objective 1 - Climate change will mean that tulip species will likely decrease in abundance and may be forced to migrate to track shifting habitats, increasing extinction risk

Activities	**Site/Sites**
KG • Conduct climate monitoring at different altitudes (long-term), annual surveys (high altitude and low altitude) • Study the plant community where wild tulips grow	
UZ • Link monitoring plots to weather stations • Establish a seed collection • Cooperate with entomologists (zoologists) and observe insect (mammal) damage to wild tulips • Climate modelling on climate, soil, rainfall changes for wild tulips survival • Include a sub-component "tulip conservation" in climate change projects	
TJ • Annual monitoring and population size. • Phenological observations • Introduction and reintroduction of rare and endangered species • At regional level, establishment of a seed gene bank for storage	
KZ • Observe endangered species (see different altitude amplitudes) • Create ex situ collections to be duplicated in CA countries • Build seed collections • Study on soils • Create seed gene bank at regional level	At priority sites

Objective 2 - Unsustainable grazing in low altitude pastures is causing large-scale degradation of tulip habitat and significant reduction of tulip abundance in these areas

Activities	**Site/Sites**
KG • Develop a Pasture Management Plan (indicate where not to graze animals during tulip blooming)	
UZ • Create micro-seeded areas where mother tulips can persist (fencing, shrub planting) and produce seeds • Conduct research on different degrees of grazing pressure on wild tulip populations • Control grazing time on pastures, rotational grazing • Integrate "tulips" into grazing projects carried out in UZ	

TJ • Regulated grazing, grazing rotation • Fencing of areas where tulips are abundant • Separate areas collection of forage seeds to establish a micro reserve and sow forage plants where wild tulips are not found	
KZ • Special programme for the rehabilitation of degraded pastures to relieve the pressure on tulip pastures • Regulating the grazing time on the pastures • Study on the impact of grazing on tulips and shift the grazing period (after tulip flowering)	

Objective 3 - Mining of natural resources is leading to damage of areas where important tulip populations grow

Activities	**Site/Sites**
KG • Transplanting of red-listed tulips to other locations. If tulips are endemic, legislation needs to be improved	Chatkal, Terek Sai
UZ • Inform mining companies about wild tulip species, compensation for destruction of wild tulips • Inclusion of wild species in IUCN red-list • Transplant red-listed, endemic tulips to other areas • Negotiate, persuade mining companies to remove mining sites if wild tulips are found. • Integrate tulips into other projects, as part of oil and gas development	All three priority sites chosen for Uzbekistan
TJ • Inform mining companies (together with the Committee) and fence off certain areas for endemic tulip species	
KZ • For IUCN-listed wild tulips, our recommendations will have weight	

Objective 4: Urban development and agriculture does not account for importance of rare or threatened tulip species

Activities	**Site/Sites**
KG	
UZ A) Introduction of 'wild plots' 'islands' within settlements. Use of wild tulips in landscaping. Eco-education in schools, establishing demonstration plots in schools B) Seed collection from populations where there is habitat loss and relocation to more suitable sites	

TJ A) Organise meetings, workshops on preserving rare tulip species in the communities and encourage them to grow wild tulips in their plots (good results on growing wild onions, disseminate this experience) To educate schools, to organise school (mountain schools) plots with wild tulips and other species to inform them about the status of tulip species Organise demo plots in forestry units	
KZ A) Informing, educating the local population Ecological expertise in areas of wild tulips growing near settlements Introduction of wild tulips to the urban area, "wild tulip plots" in Almaty and Nur-Sultan Fencing "wild" areas of tulips in the city and close to settlements where there is a threat of destruction. Setting up demonstration plots for schoolchildren in schools and protected areas Developing a series of small grants for preserving specific tulip species, setting up tulip nurseries, tulip festival, competitions, crafts, embroidery. In rural areas. Development of tulip tourism B) Design adjustments for infrastructure construction plans	Aksy-Zhabagly(Tulipa greigii, Tulipa kaufmanniana) Almaty

Objective 5 - Wild collection of tulips in areas near human settlements

Activities	Site/Sites
KG • Produce videos, educate schoolchildren, set up patrol groups, school grounds, demo plots • Install signs in tourist areas with information about tulips	
UZ • Tulip tourism development • Show people that there is money to be made from preserving and showing tulips to tourists. • Increase knowledge of law enforcement agencies so they know the species in KK so they can better control collection and trade • Improve the legal framework and introduce economic mechanisms • Introduce a month of tulip conservation in CA countries • There is no data on illegal trade of tulips on the international market. If there is then these species should be included in the CITES Appendix.	
TJ • Who collects tulips? Conduct training sessions for school children on how to grow tulips • Grow in-vitro tulips in botanical gardens. Then distribute them to schools and villages so they can plant them in nature • Create a green patrol	
KZ	Zaili mountains (Almaty

• Identify key points of collection and sale (regions, villages) and hold festivals, events, competitions, small grants and inform about the illegality of collection and sale • Involve school children to protect wild tulips, educate school children • Install signs at collection points indicating CC and rare species of tulips • Take control over sale of tulip Regel tulip bulbs • Buy back a film about tulips filmed in KZ and show it in the media (Tulip Motherland) • Monitor populations at collection sites near human settlements	and Dzhambul regions)

Objective 6 - Limited information about the extent of all threats to tulip populations

Activities	**Site/Sites**
KG	
UZ • Develop a unified methodology for studying wild tulips • Publications, joint review publications on species (involving foreign specialists)	
TJ • Organise regional expeditions to study rare and endangered tulip species • Regional research project on border priority sites (many sites in the border areas have not been explored so far) • Joint publications	
KZ • Surveillance, long-term monitoring, develop a long-term monitoring programme • Cross-border project on long-term monitoring and population studies • Establish a regional working group, commission, expert group to exchange information, knowledge etc. • Create a unified database on wild tulips of Central Asia	