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

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Abstract

Tulipa toktogulica (*T.* sect. *Kolpakowskianae*; Liliaceae) is a cryptic new species from the Toktogul area of the Jalal-Abad Region, Kyrgyzstan. It is similar to *T. talassica*, with which it shares the characteristically elongated tunic, but it is genetically and geographically distinct. It has a combination of morphological traits shared between the closely related species *T. talassica*, *T. tetraphylla* and *T. ferganica*, but the combination of its traits is unique. Unlike most other members of the genus, this new species has a scent. It is here also assessed as endangered due to its narrow range and continuing threats from overgrazing.

Keywords: Central Asian flora, *Tulipa* sect. *Kolpakowskianae, Tulipa* subgenus *Tulipa*, *Tulipa talassica*, tulips

Introduction

Tulips are one of the most recognisable flowering plants, and their horticultural varieties support a billion Euro industry (Christenhusz *et al.* 2013). The flower is of cultural importance across many regions worldwide (Orlikowska *et al.* 2018). *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). *Tulipa* has been historically regarded as a complex genus with many taxonomic alterations (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 distinguish 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, an integrated taxonomic approach would be preferred (Dayrat 2005), 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). In addition, twelve sections have been proposed based on morphology, biogeography and genome size (Zonneveld, 2009). However, there remains significant uncertainty about their monophyly, and recent genetic work indicates further analyses are required to assess sectional integrity (Christenhusz *et al.* 2013). In addition, some cases of over-splitting of species has occurred (Hajdari *et al.* 2021), and many previously described 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, 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,

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primarily from this region, based exclusively on morphology (De Groot & Tojibaev 2020, De Groot & Zonneveld 2020, Rukđāns & Zubov 2022) with many species endemic and occurring in small gorges, pastureland and remote areas (Zonneveld 2009, 2015, Zonneveld & de Groot 2012). The conservation status of species from Central Asia has not been assessed on a global level (IUCN 2022), although national level assessments suggest a large proportion may be threatened (Davletkeldiev 2006, Baitulin 2014, Tojibaev & Beshko 2015, Nowak *et al.* 2020), with new species highly prone to extinction (Liu *et al.* 2022).

Here, we present evidence of a cryptic new species that is morphologically similar to *Tulipa talassica* Lazkov (2011: 11), the latter a relatively recently described species from the Talas Region of Kyrgyzstan (Lazkov and Pashinina 2011), but this new taxon is geographically and genetically distinct and in urgent need of conservation intervention.

Material & Methods

Plant material:—Plant material for all Kyrgyz specimens was collected primarily by BW, KS and GL in springs of 2019–2022. This includes *Tulipa biflora* Pallas (1776: 727) *s.l.*, *T. greigii* Regel (1873: 290) and *T. heterophylla* (Regel 1868: 440) Baker (1874: 295), specimens used as references (Table 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 (Fig. 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 was collected in silica gel. A voucher specimen was also made 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, 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 (1879: 162) [= *T. thianschanica* Regel (1879: 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.

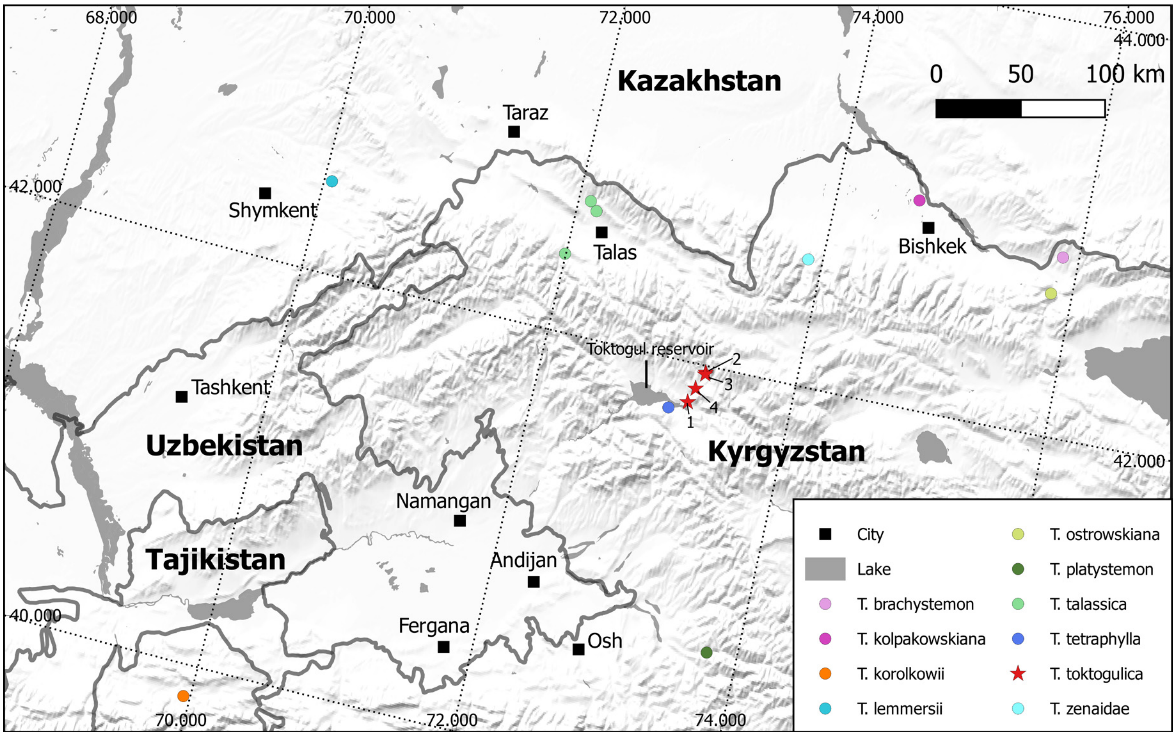

FIGURE 1. Map of specimens collected for the phylogenetic analysis in this study, excluding *Tulipa iliensis and T. altaica*, which both lacked GPS information. Populations of the new species *T. toktogulica* are labelled in order of discovery.

TABLE 1. *Tulipa* specimens used in the phylogenetic analyses (all from Kyrgyzstan, except where noted).

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

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.

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 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 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 2020.2.5 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 2. Plastomes downloaded from GenBank for use as references.

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

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 the *Lilium tsingtauense* Gilg (1904: 24) sequence (KM117263) downloaded from GenBank using the map to reference function on Geneious Prime 2020.2.5. 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 2020.2.5 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 & 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.

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 2020.2.5 and then aligned using MAFFT (Katoh and Standley 2013). These alignments were then cleaned using the pxclsq in the phyx package (Brown *et al.* 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 *et al.* 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 (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)

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 (Fig. 2), with the three plastid trees identical in topology (Appendix 1). 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 base the discussion on this result. Specifically, the new species is a member of a clade comprising *Tulipa 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) and diverges from the common ancestor of the *Kolpakowskianae* clade after *T. tetraphylla* Regel (1875: 296) (Fig. 2).

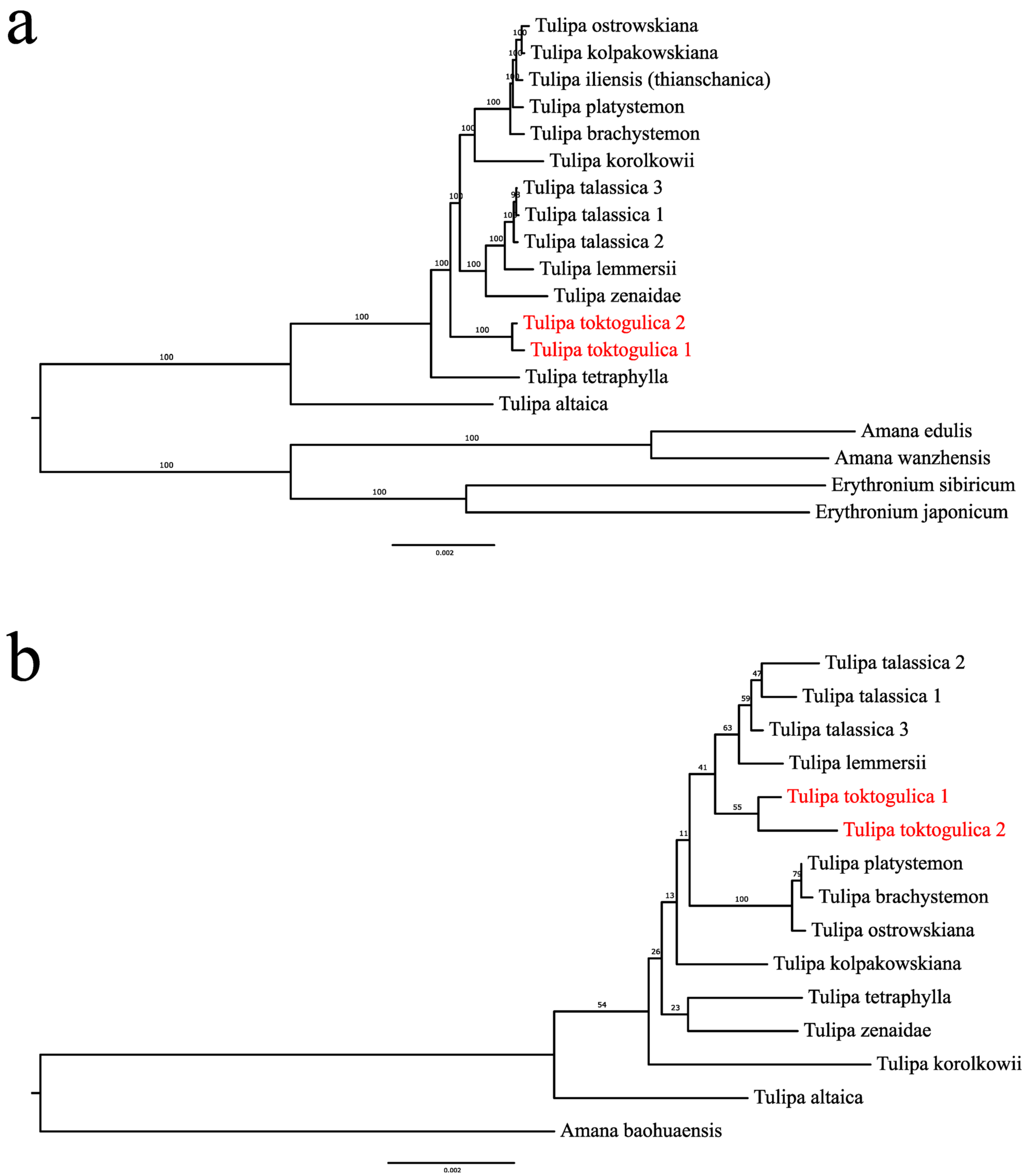

FIGURE 2. A. Multi-partition plastome tree. B. 35S rDNA tree. New species shown in red on both trees.

TABLE 3. Morphology of *Tulipa toktogulica* compared to the most similar species.

	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

Discussion

Tulipa toktogulica is most like *T. talassica* but has a unique combination of traits that supports its recognition as distinct from the other taxa (Table 3). Specifically, it has a prolonged tunic, broad stamens and a slightly fragrant flower. Importantly, this new species is clearly genetically and geographically distinct from other morphologically similar species. *Tulipa talassica* is genetically more closely related to *T. lemmersii* and *T. zenaidae*, whilst this new species is genetically distinct from closely related *T. tetraphylla* and *T. ferganica* Vvedensky (1935: 148) which occur in the same area (Wilson, unpublished). 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.

Taxonomy

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

FIGURE 3. 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.

This species is most similar to *Tulipa talassica* in its prolonged tunic but it has broad stamens like those of *T. tetraphylla* and 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 × 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 (Fig. 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.

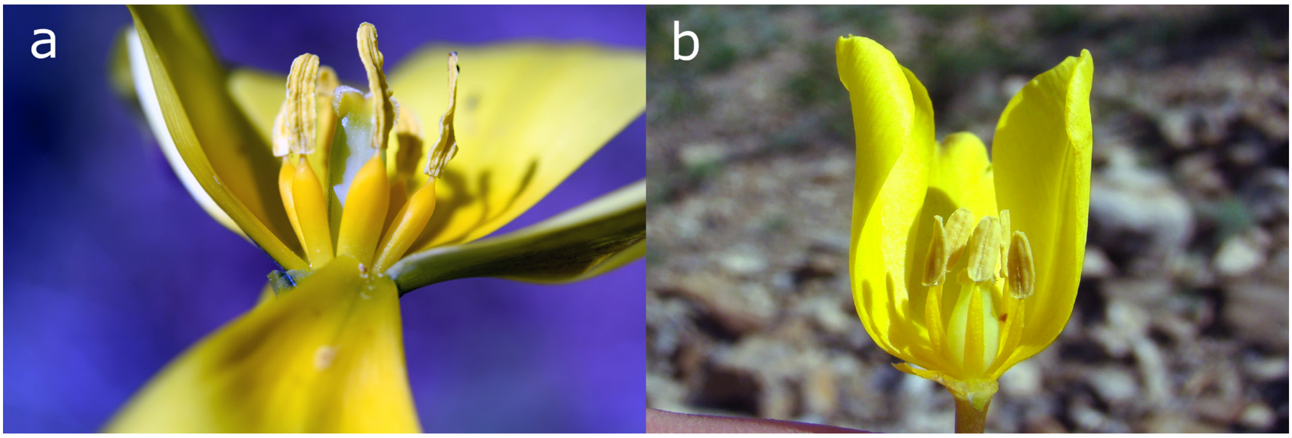

FIGURE 4. Photos showing the broader stamens in *Tulipa toktogulica* compared to *T. talassica*. A. *Tulipa toktogulica*. B. *Tulipa talassica*. Photos by Brett Wilson and Georgy Lazkov

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.

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 the villages of Sary-Seget and Bel-Aldy, 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.

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.

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*.

Conservation status:—Occurring at three locations consisting of four populations (Fig. 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 (Fig. 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 and likely only a minor threat for this species.

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Appendix 1: All trees produced in this paper.

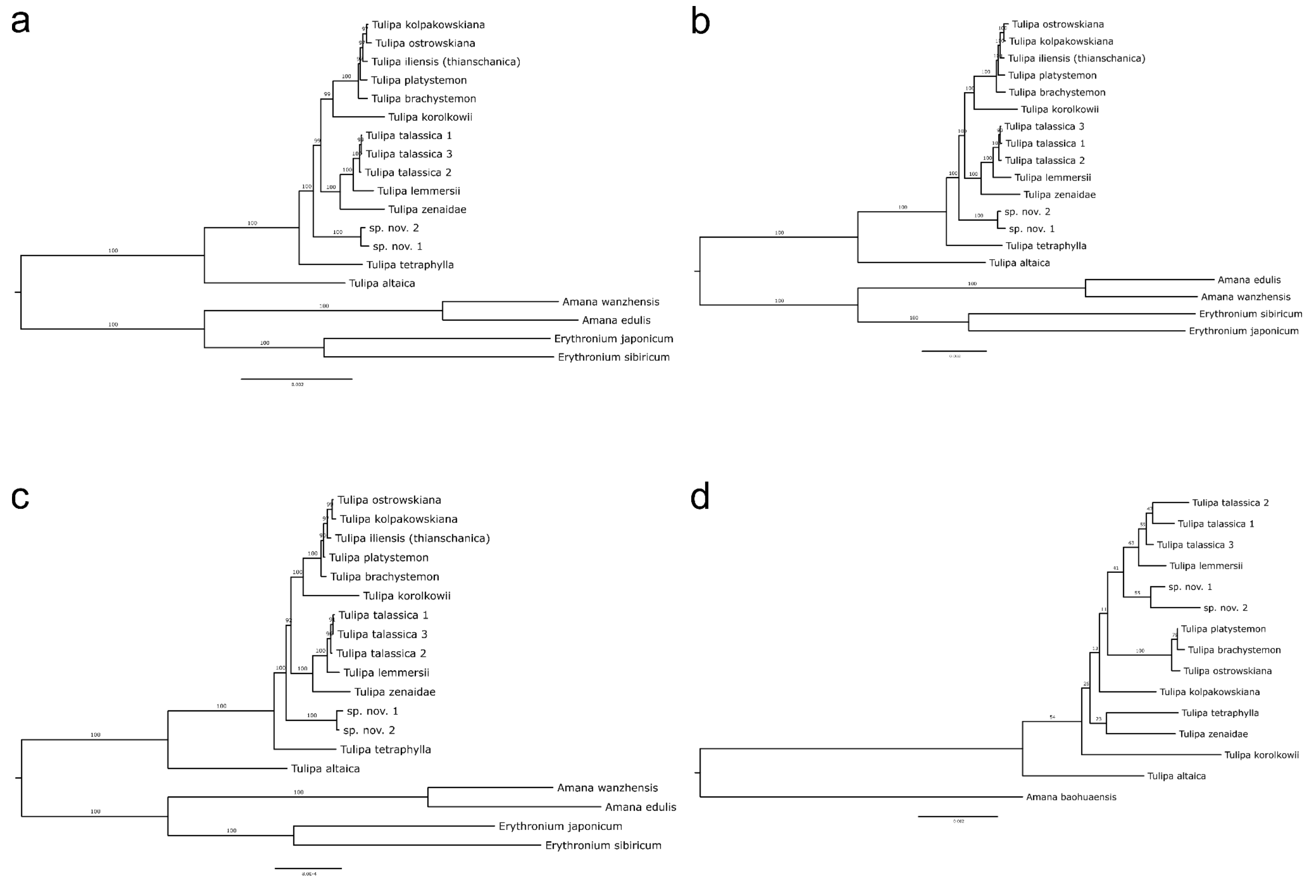

Figure 1a: (a) Single partition plastome tree, (b) multi-partition plastome tree, (c), multi-partition CDS tree, (d) 35S rDNA tree.