

1 **Analysis of wild *Taraxacum bicorne* Dahlst.** 2 **(Compositae-Crepidinae) as a potential alternative** 3 **natural rubber crop**

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26

27 **Abstract** *Taraxacum koksaghyz* Rodin, a dandelion from the steppes of south-eastern Kazakhstan, has been
28 known for long time as potential rubber producer, as a temperate region alternative to the tropical rubber tree
29 *Hevea brasiliensis*. In this work, we evaluate *Taraxacum bicorne* Dahlst. (wild populations), a closely related
30 congener of *T. koksaghyz*. The taxonomy of *T. bicorne* is reviewed, population genetic characteristics are
31 analysed by Simple Sequence Repeat markers (SSRs), and rubber biosynthesis genes are identified by
32 Kompetitive Allele Specific PCR (KASP). Rubber content of *T. bicorne* plants collected in wild is measured
33 using a modified extraction method to minimize amount of material. *Taraxacum bicorne* is shown to be an
34 outcrossing sexual diploid. Its rubber content is about half of that of *T. koksaghyz* (~3.2% vs. ~7.2% of root
35 dry weight), but *T. bicorne* could be used as a potential rubber source or as a source of germplasm for
36 production of hybrids to improve rubber yield in various environments, because *T. bicorne* has considerable
37 genetic variation, wider geographical range than *T. koksaghyz* and grows in different environments.

1

2 **Keywords** Natural rubber · population genetics · rubber content · rubber extraction · *Taraxacum* · taxonomy

3 **Introduction**

4 The vulnerability of the current production and economy of *Hevea brasiliensis* Müll. Arg. natural rubber and
5 the need for both emergency and economically viable alternatives were summarized by Mooibroek and
6 Cornish (2000), and more recently by van Beilen and Poirier (2007a, b) and Kirschner et al. (2013). In the
7 past, only two alternative sources of natural rubber were grown at a significant scale: *Parthenium*
8 *argentatum* A. Gray, also known as guayule (Ray 1993), and *Taraxacum koksaghyz* Rodin, also known as the
9 rubber dandelion, Kazakh or Russian dandelion (if we disregard shorter periods of industrial exploitation of
10 *Chondrilla ambigua* Kar. et Kir. or similar attempts with *Scorzonera tausaghyz* Lipsch. & Bosse and
11 *Landolphia owariensis* P. Beauv.; Ulmann 1951, Neuwinger 1996). However, at least 2,100 plant species
12 produce a certain amount of rubber (Ulmann 1951). There are several, either regional or global lists or
13 accounts of rubber producing plants, particularly that of Ulmann (1951), who also provided a digest of the
14 relevant Soviet literature, and two more recent but relatively unknown catalogues (Vakhrusheva 1988, 1990).

15 Although current breeding programmes are concentrated on the most promising alternative rubber
16 crops (Arias et al. 2016c), *Parthenium argentatum* and *T. koksaghyz* (and on hybrids of the latter), there are
17 good reasons not to neglect other taxa, particularly the closest relatives of the above two species (de
18 Rodriguez et al. 2005; Kirschner and Štěpánek 2008; Ray et al. 2010; Ilut et al. 2015). Inclusion of such taxa
19 into breeding and hybridization schemes may broaden the genetic basis of the future rubber crops, including
20 broader ecological and variation amplitudes.

21 Relatives of *Taraxacum koksaghyz* on the basis of morphology

22 The earlier studies dealing with *T. koksaghyz* included it in section (or subsection) *Macrocornuta* Soest
23 (Schischkin and Tzvelev 1964; Tzvelev 1987) or sect. *Scariosa* Hand.-Mazz. (Lipschitz 1934; Orazova
24 1975). In the recent study, Kirschner and Štěpánek (2008) analysed the morphology and ecology of what was
25 originally called *T. sect. Macrocornuta*, and recognized a group of taxa morphologically very similar to one
26 another, and deviating in a number of features from the rest of the section *Macrocornuta* (Kirschner and
27 Štěpánek 2008). The group was described as *T. sect. Ceratoidea* Kirschner & Štěpánek. The new section,
28 when contrasted with sect. *Macrocornuta*, was primarily diagnosed by light green, appressed horned outer
29 phyllaries, yellow stigmas and beak shorter than 7–8 mm; the two groups also differ in their habitats, sect.
30 *Ceratoidea* preferring (temporarily) wet subsaline sites, while sect. *Macrocornuta* growing on drier,
31 subsaline, often disturbed places.

32 The core of sect. *Ceratoidea*, i.e. *T. koksaghyz* and its closest relatives, is represented by four
33 predominantly sexual species quite similar to one another:

- 34 • *T. neolobulatum* Soest ex Schischk. et Tzvelev (type of the section, growing mainly in Iran and
35 adjacent regions),
- 36 • *T. koksaghyz* Rodin (growing in Kazakhstan and a narrow border area with Xinjiang, China),
- 37 • *T. monochlamydeum* Hand.-Mazz. (growing mainly in Uzbekistan and adjacent areas), and
- 38 • *T. bicornis* Dahlst. (for details, see below).

39 In accordance with the geographical parthenogenesis model (Štěpánek et al. 2011), marginal parts of
40 the section's range are occupied by agamosperous species: *T. glaucanthos* (C. A. Mey. ex Ledeb.) DC. and
41 *T. halophilum* Trautvetter ex Regel in the north, and *T. badachschanicum* Schischk. and *T. varsobicum*
42 Schischk. in the southeast.

43 We hypothesize that the core of sect. *Ceratoidea* is the source germplasm to search for further rubber
44 producing dandelions. We selected *T. bicornis* Dahlst. from among the Kazakh dandelion relatives to be
45 tested as a potential rubber plant in the present paper.

1 The reconstruction of phylogenetic relationships in *Taraxacum* is complicated by several factors,
2 primarily by widespread and complex hybridity (the parental taxa often not being extant), frequent allopolyploidy
3 (usually triploidy but occasionally up to hexa- or even dodecaploidy) and a high number of taxa to be
4 analysed (Kirschner and Štěpánek 1993, 2004; Kirschner et al. 2016). Attempts at the reconstruction of
5 evolutionary relationships thus mostly failed when cpDNA was included as the major information source
6 (Wittzell 1999; Kirschner et al. 2003), and only a few features of *Taraxacum* evolution could have been
7 inferred from the analysis of nrDNA of sexual species of *Taraxacum* (Kirschner et al. 2015). The latter work,
8 however, set up a framework to evaluate either the major coherent groups of sections, or on the contrary, to
9 define sections or their groups clearly unrelated to one another. We therefore used the published sequences
10 involving sexual representatives of 25 sections (including *T. koksaghyz* of sect. *Ceratoidea*) and compared it
11 with the new nrDNA sequences of *T. bicorne* Dahlst.

12 Rubber biosynthesis genes

13 Since *T. bicorne* may be used in the development of alternative rubber crops in the future, it is important to
14 know how similar the rubber biosynthesis pathway of *T. bicorne* is to that of *T. koksaghyz*. Several genes
15 directly involved in rubber biosynthesis have been identified in *T. koksaghyz*. Initially it was thought that *T.*
16 *koksaghyz* had three highly similar cis-prenyl transferases: CPT1, CPT2 and CPT 3 (Schmidt et al. 2010). It
17 later turned out that the material investigated belonged to a species closely related to *T. koksaghyz* (*T.*
18 *brevicorniculatum*, Post et al. 2012). True *T. koksaghyz* only has two cis-prenyl transferases, CPT2 and
19 CPT3. Since CPT1 is present in the poor rubber producer *T. brevicorniculatum* and the non-rubber producer
20 *T. officinale*, it may be the cause of low rubber production. It is therefore of interest to know whether CPT1
21 is present in *T. bicorne*. Rubber Transferase Activator (RTA; Epping 2015) or CPT-Like 2 (CPTL2) protein
22 (Qu et al. 2015) form together with CPT the rubber-transferase complex which catalyses the rubber
23 polymerization. Other important rubber biosynthesis proteins are the Small Rubber Particle Proteins (SRPPs)
24 and the Rubber Elongation Factor (REF), which are components of the rubber particles in the latex. Markers
25 were developed for these five genes encoding for these proteins in *T. koksaghyz*. In addition, a *T. koksaghyz*-
26 specific marker set for six genes not related to rubber biosynthesis was developed.

27 **Material and Methods**

28 Taxonomy

29 Our general strategy is to make our taxonomic treatments of *Taraxacum* uniform and comparable to the
30 modern standards, and we therefore follow principles briefly outlined by Richards (1973), Kirschner and
31 Štěpánek (1996), Kirschner et al. (2003) and Ge et al. (2011), and are derived from the peculiar features and
32 processes known in *Taraxacum*, particularly the regional coexistence of apomixis (agamospermy) and
33 sexuality, complicated hybridity and polyploidy, relatively low structural morphological differentiation and
34 numerous similar and mostly hybridogenous species. The principles inferred from the *Taraxacum* general
35 attributes include (i) different kinds of species to be recognized on the basis of the extent of variation and
36 modes of reproduction, (ii) distribution of sexuality is to be explored, (iii) variation within a family of
37 siblings should be studied for each taxon (to detect autonomous aberrant, facultative sexuality etc.), (iv) the
38 study should be started at the lowest variation level (within and among populations).

39 The complexity of the genus, primarily the incommensurable variation patterns of species with
40 different modes of reproduction, also requires a taxonomic rank placed between species and genus in the
41 traditional hierarchy, to make the population and taxonomic structure more easily understandable for non-
42 specialists, and the rank of section is used in the *Taraxacum* literature.

1 Plant material

2 *Taraxacum bicornе* Dahlst. was studied in the wild in two natural populations in the vicinity of Kokpek
3 (District of Enbekshikazakhstan Rayon, basin of Dolina Sogety, between hilly regions of Gory Sogety
4 (Sogeti) and Gory Toraigyr, near the SE foothills of the Kungei Alatau Range, Fig. 1): the first site (Site I)
5 was visited in 2008 (J. Kirschner and J. Štěpánek), at saline, temporarily wet banks of dry brook beds,
6 population centre at 43°26'38.58" N, 78°40'16.53" E (1115 m a.s.l.), the second site (Site II) was sampled in
7 2014 (J. Kirschner and J. Štěpánek), at sub-saline to saline, partly humid pastures and spring areas just below
8 the village, population centre at 43°26'49.68" N, 78°40'26.04" E. The material from Site I was used for flow
9 cytometry analyses and hybridization experiments, while that from Site II was analysed to get data about
10 rubber production and genetic make-up of the population and of the species.

11 Another important source of plant material comes from herbarium collections, the most important
12 ones having been those of TASH, LE and AA. The herbarium material was used to compile the description,
13 to assess the variation limits of *T. bicornе*, and to describe the geographic range of this species.

14 Cultivation and reproduction system

15 Details of the cultivation methods are given in Kirschner and Štěpánek (1993). The cultivation simulated
16 natural season conditions, i.e. achenes were sown in late June (in pots), transplanted in low wooden boxes
17 with a standard garden soil in late summer; the herbarium gatherings were performed during the natural
18 spring flower season. The half-sib families were collected and prepared for herbarium (a rapid drying at 35–
19 40 °C); all the major stages of development were covered, with emphasis on plants in full blossom. Several
20 plants per half-sib family yielded ripe achenes. The cultivation was performed on the Experimental Garden
21 of the Institute of Botany, Czech Academy of Sciences, Průhonice, Czech Republic (49°59'41.296" N
22 14°34'0.584" E, 306 m a.s.l.) in open beds using common local garden soil (see also Fig. 2). The cultivation,
23 especially repeated mass cultivation, reveals limits of morphological plasticity of individual taxa.

24 Moreover, it provided material for the study of reproduction systems of plants under study.
25 Determination of the reproduction system, an important background for taxonomic decisions, was performed
26 according to Kirschner et al. (2006).

27 Hybridization

28 For both the breeding purposes and to have another information source on the relationships between *T.*
29 *koksaghyz* and *T. bicornе*, we performed a series of reciprocal crosses between these two species. All the
30 experiments were carried out in the Experimental Garden (see previous chapter). When isolated, plants do
31 not set seed, suggesting that *T. bicornе* is self-incompatible, like other sexual *Taraxacum* species (Kirschner
32 et al. 2006). The half-sib families of experimental plants were cultivated in open beds, each in a separate
33 wooden box embedded in garden soil. Mother plant families were isolated by caging (sides of cages with a
34 mesh, each cage covered with plexiglass, see Fig. 2). Crosses were done by rubbing the flowering capitula
35 twice during the full blossom (see Fig. 3), within one or two days of flowering. Each individual cross, i.e. the
36 mother capitulum and the pollen donor plant, was identified by a unique number. We crossed 56 *T.*
37 *koksaghyz* mother plants with *T. bicornе* pollen donors and 81 *T. bicornе* mother plants with *T. koksaghyz*
38 pollen donors. The aim was to verify possible hybridization and viability of hybrids of the two species for
39 possible future breeding programme.

40 Molecular analysis

41 For taxonomic analysis we used 108 sequences of ITS1-5.8S rDNA-ITS2 from Kirschner et al. (2015, 2017).

42 All 32 plants from Site II were genotyped by 13 microsatellite loci (SSRs, Jarne and Lagoda 1996),
43 which were distributed over two multiplex PCR reactions (multiplex 1: MSTA 44B, 73, 78, 93, 103, 105 and
44 131, and multiplex 2: MSTA 53, 61, 85, 102, 133 and 143). Six microsatellite loci and relevant primers

1 (MSTA 44B, 53, 61, 73, 78 and 85) were taken from Falque et al. (1998) and seven microsatellite loci
2 (MSTA 93, 102, 103, 105, 131, 133 and 143) from Vašut et al. (2004). Primers were originally developed for
3 *T. officinale* agg. (sect. *Taraxacum*) and *T. laevigatum*, respectively. The PCR reaction was performed using
4 the QIAGEN Multiplex PCR kit (Qiagen, Venlo, Netherlands) according to manufacturer's protocol in a final
5 volume of 20 µl containing 10 µM of each primer and 30–50 ng of DNA. PCR protocol was as follows: 95°C
6 hot start for 15 min, 30× (30s 94°C, 90s 57°C, 60s 72°C) and 30 min 60°C. Final PCR products were
7 analysed using a 3130 ABI Genetic Analyser (Life Technologies, Carlsbad, CA, USA) and allele numbers
8 and sizes were subsequently scored using GeneMarker 2.4 (SoftGenetics LLC, State College, PA, USA) and
9 rewritten into the data matrix.

10 Species-specific Single Nucleotide Polymorphisms (SNPs) were mined in the sequences published in
11 Schmidt et al. (2010), and Epping et al. (2015), and the *T. koksaghyz* and *T. officinale* Expressed Sequence
12 Tag (EST) libraries at The Compositae Genome Project website (<http://compgenomics.ucdavis.edu/>). SNPs
13 were found for the five rubber synthesis genes *CPT1*, *CPT2*, *CPT3*, *RTA*, *SRPP5* and *REF*. SNPs were also
14 found in six genes, not related to rubber biosynthesis: *CTO_M1_Contig179*, *CTO_M1_Contig2608*,
15 *CTO_M1_Contig3593*, *CTO_M1_Contig5704*, *CTO_M1_Contig6303*, *CTO_M1_Contig6322*. In order to
16 screen effectively a high number of plants, KASP (Kompetitive Allele Specific PCR) assays were developed.
17 Based on 50 bp sequences immediately upstream and downstream of the SNP (Table 1), KASP assays were
18 designed by LGC (<https://www.lgcgroup.com/>).

19 Because of the high nucleotide similarity between the *CPT* genes, it was difficult to design
20 discriminating KASP markers. Two KASP markers were developed that could be used in combination.
21 Marker CPT-A does not amplify *CPT3* but distinguishes *CPT1* from *CPT2*. Marker CPT-B distinguishes
22 *CPT2* from *CPT1* and *CPT3*. If marker CPT-A indicates the presence of *CPT2* and the absence of *CPT1*, then
23 marker CPT-B can be used to interpret the presence of *CPT2* and *CPT3*. When *CPT1* is absent and both
24 *CPT2* and *CPT3* are present, the KASP assay will show a 'heterozygote' between two loci. This is always the
25 case in *T. koksaghyz* (Peter J. van Dijk, unpublished).

26 KASP assays were run and analysed in a Roche LightCycler® 480. To test for species specificity the
27 KASP assays were run on a panel of 10 diploid sexual *T. koksaghyz* and 10 diploid sexual *T. officinale* plants
28 originating from pure populations in Kazakhstan and Switzerland, respectively. All KASP markers showed a
29 species-specific polymorphism in this panel. All 32 *T. bicornis* plants from site II were analysed with the 11
30 KASP markers, including three controls: a *T. koksaghyz* plant and a *T. officinale* plant (2x sexual) and a 3x
31 apomictic *T. brevicorniculatum* plant.

32 Statistical evaluation

33 Neighbour Network from ITS sequences was constructed in SplitsTree 4.14 (Huson and Bryant 2006). The
34 figure was modified from Kirschner et al. (2017). Calculations based on SSRs as well as KASP loci were
35 performed in R 3.1 (R Core Team 2014). We used packages ade4 (Dray and Dufour 2007), adegenet
36 (Jombart 2008), APE (Paradis et al. 2004), pegas (Paradis 2010) and Poppr (Kamvar et al. 2014). We
37 computed the basic population statistics, i.e. observed and expected heterozygosity, departure from Hardy-
38 Weinberg equilibrium (HWE; Jombart 2008), allelic richness (Paquette 2012), distribution and diversity of
39 multilocus genotypes (MLGs), Shannon-Wiener Index of MLG diversity (H), E.5 (Evenness measuring
40 distribution of genotype abundances ranging from 0 where population is dominated by single genotype to 1
41 where all genotypes are equally distributed), inbreeding coefficient (as implemented in adegenet), and Index
42 of Association (Ia) and its standardized version (rbarD). Ia and rbarD detect clonal reproduction within
43 populations. Calculation based on the ratio of the variance of the raw number of differences between
44 individuals and the sum of those variances over each locus. It is the observed variance over the expected
45 variance — if they are the same, then the index is zero (=prevailing clonal reproduction) after subtracting
46 one — it rises with increasing differences. For details see Kamvar et al. (2014). Genotype diversity was
47 quantified according to Hughes and Richards (1988) $G = 1 - \sum x_i^2$, where x_i is the frequency of i-th MLG. This

1 parameter is useful for population sets with expected variation in reproduction systems (i.e. a substantial
2 departure from the Hardy-Weinberg expectations) and for situations where recombination is partially
3 suppressed as a consequence of allopolyploidy; it reasonably reflects both richness and evenness and closely
4 approaches the modified Simpson's index λ (Kirschner et al. 2016). Details about R work-flow, software
5 settings etc. are available from VZ upon request.

6 Flow cytometry (FCM)

7 The FCM analyses followed Závěský et al. (2005) and Trávníček et al. (2013); the PI measurements and
8 *Lycopersicon esculentum* cv. 'Stupické polní tyčkové rané' as a standard having been used. The FCM
9 screening was carried out on the material cultivated from wild roots (collected under no. 74/1 to 74/33) in
10 2008–2009, under no. JK 5276/1 through JK 5276/33, i.e. on 33 plants from the Kokpek locality.

11 Rubber content quantification

12 We modified gravimetric method of Post et al. (2012) based on extraction of rubber into toluene and its
13 subsequent precipitation by addition of methanol, as the method is feasible also in small volumes. Roots
14 were dried and ground into a fine powder. Powdered root samples (~0.5 g) were weighted (with accuracy to
15 10^{-4} g) into 10 ml glass vials, and 5.0 ml of toluene and a small magnetic stirring bar were added to each vial.
16 The vials were tightly closed and the samples were stirred in a thermoblock at 85 °C for 24 h. After cooling,
17 undissolved material was filtered off using a S3 glass frit and the solution was collected in 25 ml round-
18 bottom flask. The insoluble material remaining on the frit was washed by 2 ml of toluene, which was
19 previously used for vial rinse. The solid material was transferred into a weighted vial, dried in a drying oven
20 at 60 °C overnight and weighted; its weight was used for check of a mass balance. The toluene extract was
21 evaporated to dryness using a rotary evaporator (bath temperature 60 °C). A rubber appearing on the walls of
22 the flask was dissolved by portion-wise addition of toluene (4×0.75 ml) and the solution portions were
23 transferred into a weighted 15 ml centrifugation tube (accuracy to 10^{-4} g). The flask was 2× rinsed by 3.0 ml
24 of methanol and the washings were transferred into the tube with toluene extract. On addition of methanol,
25 the rubber precipitated. The tubes with individual samples were closed, shaken and left to stand overnight.
26 After standing, the suspensions were centrifuged at 3000 G ($r = 7.5$ cm, $f = 6000$ min⁻¹) for 1 h. Liquid
27 phases were discarded and the solid rubber in each sample was washed by ca. 5 ml of acetone and 5 ml of
28 water. Tubes were then dried in a drying oven at 60 °C overnight and weighted; amount of rubber in each
29 sample was determined differentially.

30 In total, 4 samples of *T. bicornis* and 12 of *T. koksaghyz* were analysed. Samples of *T. bicornis* were
31 sampled in the field (Site II, Fig. 1 and Plant material chapter), samples of *T. koksaghyz* were cultivated in
32 the Experimental Garden. Plants were sown at spring and harvested second autumn. This timing has usually
33 the best rubber yield (e.g. Kreuzberger et al. 2016).

34 Nuclear magnetic resonance (NMR) analysis

35 Solid material which was previously dried as aforementioned was dissolved in CDCl₃ (0.7 ml) under
36 ultrasound irradiation. The fine suspension was filtered through PVDF microfilter (0.2 µm) directly into
37 NMR tube. The samples were measured on VNMRS 300 (7.0 T) and Bruker Avance III 400 (9.7 T) using
38 basic 1H and 13C{1H} pulse sequences. The NMR spectra were referenced on non-deuterated residual
39 solvent (CHCl₃, δ H = 7.26 ppm and δ C = 77.0 ppm) and evaluated using MestReNova program.

40 Selected specimens studied

41 Studied specimens from Afghanistan, China, Kazakhstan, Kyrgyzstan, Mongolia, Pakistan, Turkmenistan,
42 Tajikistan and Uzbekistan are list in Appendix 1.

1 Reliable literature records

2 According to the determination labels in the herbarium collections of LE, AA and TASH, we identified S.
3 Kovalevskaya and T. Vainberg as reliable authors of *T. bicornе* records. We therefore add literature records of
4 *T. bicornе* from the following sources: Kovalevskaya (1962) and Vainberg (1991, 1993). When only regions
5 or districts are given in these sources, we place a map dot in the centre of the region (Fig. 12).

6 Results

7 Evolutionary relationships of *Taraxacum koksaghyz* and *T. bicornе*

8 The analysis of ITS region in SplitsTree (Fig. 4) unanimously shows *T. bicornе* as a very close relative of *T.*
9 *koksaghyz*. The hypothesis set up on the basis of shared morphological characters was therefore supported
10 from an independent data source. Furthermore, these results also support the taxonomic conclusions about
11 the separate status of sect. *Ceratoidea* (Kirschner and Štěpánek 2008).

12 KASP markers

13 Table 1 shows the KASP genotyping results for the 32 *T. bicornе* plants from site II. All primer pairs
14 amplified a PCR product, although the dropout rate was higher than in TKS and TO plants. From marker
15 CPT-A it can be concluded that all *T. bicornе* plants lack the CPT1 gene and have, like *T. koksaghyz* the
16 CPT2 gene. However, in contrast to *T. koksaghyz*, marker CPT-B indicates that CPT3 is absent in *T. bicornе*.
17 This may explain the intermediate rubber production in *T. bicornе*: it lacks the CPT1, which is associated
18 with the poorer rubber producers, but only has one of the two CPTs of the high rubber producer *T. koksaghyz*.
19 Concerning the other rubber biosynthesis genes, the markers suggest that *T. bicornе* has the RTA allele of *T.*
20 *officinale* and is almost fixed for the *T. officinale* form of REF. For SRPP5 *T. bicornе* in this population is
21 almost fixed for the *T. koksaghyz* variant. For five of the six non-rubber genes, *T. bicornе* is fixed for the *T.*
22 *koksaghyz* variant. There is a trend that the rubber biosynthesis genes of *T. bicornе* are more different from *T.*
23 *koksaghyz* than the non-rubber genes.

24 Population genetic statistics

25 Our aim was to characterize the heterozygosity (and the H-W equilibrium relationships) to infer reproductive
26 attributes of *T. bicornе* populations on one hand, and on the other hand, genotype diversity and the overall
27 level of genetic diversity in the population.

28 Results obtained by 13 microsatellite (SSRs) showed high differences of expected and observed
29 heterozygosity (Fig. 5) and departure from Hardy-Weinberg equilibrium (p-value of significant departure <
30 0.05 for 9 loci, > 0.05 for 3 loci and not available for 1 locus). SSRs also exhibit low inbreeding (Fig. 6).

31 Table 2 shows population-genetic indices summed over all SSRs loci. Shannon-Wiener (H) shows
32 much high allelic diversity. Evenness (E.5) of proportional occurrence of genotypes is low. Index of
33 association (Ia and its standardized version rbarD) shows significantly prevailing outcrossing. Genotype
34 diversity (G) is extremely high for SSRs data.

35 According to the SSRs data, *T. bicornе* is an outcrossing sexual species, with a high SSR genotype
36 diversity. The conclusion on the mode of reproduction is supported by the absence of achenes in isolated
37 inflorescences indicating the absence of autonomous autogamy.

38 FCM

39 According to flow cytometry measurements using PI, all our plants of *T. bicornе* are diploids ($2n = 16$). The
40 sample size (33 plants) did not show substantial variation, the sample/standard ratio ranged from 1.29 to
41 1.399, with coefficient of variation (standard deviation/mean) being 2.31–5.07. These figures correspond to

1 those ascertained in *T. koksaghyz* (but are much higher than those found in *T. stevenii*, a species with
2 remarkably small genome, T. Černý, unpubl.).

3 Rubber content of *T. bicornne*

4 Mean content of rubber in dry root biomass was 3.2% for *T. bicornne* (4 measurements) and 7.1% for *T.*
5 *koksaghyz* (12 measurements, Fig. 7). Although there is an obvious difference of the rubber content between
6 these two species, because values of *T. bicornne* do not fit normal distribution (tested by Shapiro test), we had
7 to use non-parametric Kruskal-Wallis test, which is weaker than T-test, but still it was able to verify the
8 significant difference ($p = 0.029$).

9 NMR analysis

10 NMR spectra of measured samples contained natural rubber as a main component (approx. 75–90 % based
11 on ¹H NMR spectra integration). Representative ¹H and ¹³C{¹H} spectra with signal assignment are shown
12 in Fig. 8. The observed resonance frequencies (Fig. 8) are in agreement with published data for natural
13 rubber (Thuong et al. 2016). All randomly chosen measured samples exhibited the same composition (i.e.
14 majority of natural rubber) with only minor changes of additional impurities observable in NMR spectra
15 (Fig. 9). The results suggest all prepared samples contains the natural rubber as the main component.

16 Hybridization between *T. koksaghyz* and *T. bicornne*

17 Table 3 summarizes hybridization between *T. koksaghyz* and *T. bicornne*. We can conclude that both reciprocal
18 crossing experiments showed a high yield of hybrid achenes and were generally very successful, which is
19 primarily due to the sexual diploidy of both parents and the evolutionary proximity of these two species.

20 Taxonomy of *T. bicornne*

21 The protologue of the name *T. bicornne* appeared in an early study of sect. *Borealia* Hand.-Mazz. (as the
22 group of *T. ceratophorum*, Dahlstedt 1905). Since then, this species name was seldom mentioned, mostly as
23 a member of *T. sect. Ceratophora* auct. (a synonym of the name *T. sect. Borealia*), sometimes (Russian
24 authors, e.g. Schischkin and Tzvelev 1964) as belonging to sect. *Macrocornuta* Soest, but usually without
25 indication of its relationships. Most importantly, it was Tzvelev (1987) who listed *T. bicornne* among
26 members of sect. *Ceratophora*.

27 As a result of the above confusion, *T. bicornne* was only seldom considered as a potential rubber crop.
28 According to our records, only Il'in and Yakimov (1950) and Il'in (1953) mentioned *T. bicornne* (see also
29 Vakhrusheva 1990).

30 Together with *T. koksaghyz*, *T. bicornne* was listed among members of the sect. *Ceratoidea* (Kirschner
31 and Štěpánek 2008, with nomenclatural details). In order to put our study on a solid taxonomic basis, we give
32 a full description of *T. bicornne*, with notes on its ecology and distribution, and with a selection of herbarium
33 specimens studied.

34 ***Taraxacum bicornne*** Dahlst., Ark. Bot., 5/9: 29, Tab. 17 (1906)

35 Type: Plantae Turkestanicae, Alpes Alexandri, in valle fl. Kaschkara [Kyrgyzstan] locis graminosis
36 humidis, 5. vi. 1896, V. F. Brotherus 103 (syn: H, B [the latter probably destroyed]; isosyn: LE, no. det.
37 8053; [fragment] S, no. det. 18357).

38 Illustrations: Fig. 10 in the present paper; Dahlstedt, Ark. Bot. 5/9: Plate 17, 1906 (Fig. 11).

39 Description: Plants small, usually 7–10 cm tall at grazed sites, or up to 12 (–20) cm tall at sheltered
40 places (e.g. among shrubs). Root not conspicuously thickened, usually branched above, when broken, the
41 parts of root connected with thin threads of rubber. Petiole narrowly winged, usually ± green above with pale
42 green to purple mid-vein, or suffused lividoid. Leaves vivid greyish green, usually with lividoid hue, linear
43 oblanceolate in outline, usually 4.5–8 × 0.5–1.3 cm, occasionally to 12 cm long, rarely undivided, usually
44 pinnatilobed, with 2–4 pairs of ± patent triangular-deltoid, broadly triangular to linear-triangular lateral

1 lobes, entire or with a few minute teeth on distal margin; terminal lobe usually larger, often elongated,
2 sometimes acute with concave sides. Scapes pale green or suffused purple, aranose, usually overtopping
3 leaves. Capitulum usually 2–2.5 cm wide, lighter yellow. Involucre light green, ca 6–8 mm in wide and \pm
4 rounded at base. Outer phyllaries appressed, loosely appressed or erect, usually 10–13, light yellowish green,
5 with distinct, anastomosing venation, narrowly lanceolate to ovate, relatively short, usually $5\text{--}6.5 \times 1.5\text{--}2.3$
6 mm, with 0.2–0.4 mm wide whitish border, upper part of phyllaries usually suffused pink or dirty pink to
7 purplish, with a thick obtuse grey-pink horn below apex, the apex itself bent perpendicularly and having an
8 appearance of another horn (thus ‘bicorne’), margin entire, not ciliate, occasionally denticulate at apex; inner
9 phyllaries ca 9–11, pale green, pinkish above, initially 11–12 mm long, later conspicuously elongating to
10 reach at least 16–17 mm. Outer ligules flat, lighter yellow inside, striped dirty pinkish below and grey-pink
11 above, outer ligule teeth greyish-pinkish, inner ones \pm yellow or pinkish yellow. Stigmas yellow. Anthers
12 polliniferous, pollen grains of regular, \pm equal size. Achenes light stramineous-brown to light greyish so,
13 (3.2–) $3.5\text{--}3.8 \times 0.8\text{--}0.9$ mm, body with numerous conspicuous ridges, upper half of achene body,
14 particularly on ridges, covered with erect-patent to upwards sickle-shaped coarse spinules, body subgradually
15 narrowing into \pm subconical cone 0.7–1.0 mm long, with a few basal spinules; beak thin, usually 8–9.5 mm
16 long, pappus \pm pure white, 8–9 mm long. Sexual.

17 Distribution and ecology

18 Fig. 12 shows distribution range of *T. bicorne* based on herbarium specimens and literature records. It is
19 distributed in Kazakhstan, Kyrgyzstan and Uzbekistan. Limited number of records come from Turkmenistan,
20 Mongolia and China. It was also recorded in Pakistan, Afghanistan and northern Iran (the latter occurrence is
21 based on literature record only).

22 As regards the ecology, *T. bicorne* grows under relatively harsh, continental conditions. The
23 humidity and available ground water are only seasonal (not relatively permanent as *T. koksaghyz*), the span
24 of diurnal and annual temperature fluctuations is broader, and the salinity is high. *T. bicorne* grows in
25 temporarily wet depressions in saline steppes or a degraded steppe, often in the vicinity of *Sophora*
26 *alopecuroides* L. (as in the vicinity of Kokpek, Kazakhstan).

27 Discussion

28 Relatedness of *T. bicorne* and *T. koksaghyz*

29 Our phylogenetic analysis of ITS sequences confirmed the morphological placement of *T. bicorne* into
30 section *Ceratoidea* (Fig. 4) as close relative of well-known rubber producer, *T. koksaghyz*. Successful
31 amplification of SSR and KASP markers developed for *T. officinale* and *T. koksaghyz* further supports the
32 close relationships between these *Taraxacum* species. The high number of successful hybridizations is
33 consistent with this conclusion. In nature hybridization may be restricted by ecological differentiation.

34 The population genetics of *T. bicorne* compared to other sexual dandelions

35 Genus *Taraxacum* has been known for high incidence of clonality, hybridization, polyploidy and
36 combination of sexual and asexual reproduction (e.g. Kirschner and Štěpánek 1994, 1996; Kirschner et al.
37 2003; Závěská Drábková et al. 2009; Kirschner et al. 2015).

38 Comparison of our results with other species of the genus *Taraxacum* is not straightforward as most
39 of the *Taraxacum* species are agamosperous polyploids, and there are only a few population-genetic studies
40 on diploid sexual species of the genus done with variable genetic markers. Older studies are mainly based on
41 allozyme polymorphisms, more recent studies on SSR. We confine our comparisons to diploid sexual
42 *Taraxacum* taxa.

43 Older studies used to use allozymes, e.g. Hughes and Richards (1988) found percentage of
44 polymorphic loci 40–50 (mean 45.7) for sexual and 27–47 (mean 38) for triploid agamosperous

1 populations. Genotype diversity (G in our study) was 0.14–0.17 (mean 0.16) for sexual and 0.27–0.40 (mean
2 0.38) for agamosperous populations. Kirschner and Štěpánek (1994) found in 20 sexual populations of *T.*
3 *bessarabicum* and related species of *T.* sect. *Piesis* proportion of polymorphic loci 0–0.89 (mean 0.31) and
4 heterozygote frequency per population over all loci 0–0.36 (mean 0.13). Kashin et al. (2005) detected by
5 allozymes mean observed heterozygosity of 0.52 for sexual *T. serotinum*. Zeisek et al. (2015) characterized
6 sexual diploids of *Taraxacum* sect. *Dioszegia* (when excluding probably autogamous *T. serotinum* subsp.
7 *tomentosum*) observed heterozygosity 0.52–0.63 (mean 0.56) and expected heterozygosity 0.43–0.71 (mean
8 0.57). Departure from Hardy-Weinberg equilibrium was not significant. Study of Zeisek et al. (2015) used
9 SSRs primers from same set as the study presented here (Falque et al. 1998; Vašut et al. 2004). Matsuyama et
10 al. (2018), using SSRs, studied the hybridization between the native *T. japonicum* (sexual diploid) and an
11 introduced *T. officinale*. They reported clonal diversity of *T. japonicum* 0.98, *T. officinale* 1 and 3× and 4×
12 hybrids 0.7–0.9 (mean 0.85); and evenness of *T. japonicum* 0.98, *T. officinale* 1 and 3× and 4× hybrids 0.51–
13 0.92 (mean 0.79). Similar problem was addressed by Iaffaldano et al. (2018), who studied possible
14 hybridization between *T. koksaghyz* and *T. officinale* and they found only very little introgression of *T.*
15 *officinale* pollen into *T. koksaghyz*. Some apomictic hybrids were able to produce viable seeds, nonapomictic
16 hybrids were sterile (Zhang et al. 2017; Iaffaldano et al. 2018).

17 *Taraxacum koksaghyz* occupies a medium-sized geographical range in south-eastern Kazakhstan
18 (Kirschner et al. 2013), comparable in size to that of *T. haussknechtii* (Zeisek et al. 2015). Both are obligate
19 out-crossers with the absolute predominance of within-population genetic variation (Kirschner et al. 2013)
20 and their levels of genetic variabilities are comparable. Regarding *T. koksaghyz*, large population sampling
21 (175 individuals) was genotyped by McAssey et al. (2016) by 17 EST-SSRs primers. They found F_{ST}
22 (depending on the loci) 0.10–0.19 (mean 0.11) and G_{ST} 0.03–0.11 (mean 0.06). Population observed
23 heterozygosity 0.28–0.47 (mean 0.37) and unbiased expected heterozygosity 0.28–0.50 (mean 0.43). PCoA
24 did not reveal any structure among their 17 populations (they were sampled from relatively small region in
25 Kazakhstan). In our study, we detected in *T. bicornne* slightly higher observed (mean 0.64) as well as expected
26 (mean 0.63) heterozygosity. Overall we conclude that levels of variation in *Taraxacum bicornne* are
27 comparable to that of other outcrossing sexual *Taraxacum* species.

28 KASP markers

29 The KASP markers indicate that *T. bicornne* lacks the *CPT1* gene, which is associated with the poorer rubber
30 producers *T. officinale* and *T. brevicorniculatum*. However, *T. bicornne* has one of the two *CPT* genes of the
31 high rubber producer *T. koksaghyz*. *CPT3* seems to be absent in *T. bicornne*. However, full length gene
32 sequencing is necessary to confirm this. For two of the three other rubber biosynthesis genes analysed, *T.*
33 *bicornne* was (nearly) fixed for the allele of the poor rubber producers (for *RTA* and *REF*). Only the *SRPP5*
34 allele was shared with *T. koksaghyz*. A lower *CPT* gene dose and non-*T. koksaghyz* alleles at the *RTA* and
35 *REF* loci may explain the lower rubber production of *T. bicornne* in comparison to *T. koksaghyz* (see below).
36 Whereas the rubber biosynthesis genes seems to differ considerably between *T. bicornne* and *T. koksaghyz*, *T.*
37 *bicornne* was fixed for five of the six non-rubber *T. koksaghyz* genes not related to rubber biosynthesis. This is
38 consistent with the good crossability of the two species. The difference between the two group of genes may
39 be due to a difference in gene function, for example if high rubber content is stronger selected for in habitats
40 where *T. koksaghyz* grows and in habitats where *T. bicornne* grows. Rubber production is seen as an
41 adaptation to (root) herbivory, hence herbivore pressure may be higher in *T. koksaghyz* habitats.

42 Rubber content in *T. bicornne* and *T. koksaghyz*

43 Rubber is stored mainly in outermost layer in roots, protecting inulin (storage polysaccharide) from
44 herbivores, thus physiological state and sampling season must inevitable play an important role regarding
45 yield of rubber from the roots. A surprisingly low proportion of *Taraxacum* rubber percentage reports gives a
46 satisfactory description of methods and circumstances of sampling to allow comparability or even

1 repeatability of the data. The following factors should be taken into account in order to have interpretable
2 data:

- 3 • The age of cultivated plants, particularly when cultivated as a winter crop (the rubber yield in the
4 second year is much higher, e.g. Suomela 1950, pp. 64–66), plants cultivated in Scandinavia (see the
5 list of herbarium specimens), using a nutrient-rich compost and a regular irrigation, developed in
6 very robust individuals;
- 7 • storage of harvested roots (much increased amount of rubber after storage, Neiman and
8 Dobrovol'skaya 1940);
- 9 • ecological conditions, or agronomy in the case of planted material (e.g. Arias et al. 2016b;
10 Kreuzberger et al. 2016; Hodgson-Kratky et al. 2017a; Eggert et al. 2018);
- 11 • levels of natural variation in individual rubber production (e.g. Arias et al. 2016c);
- 12 • breeding success and cultivars available (e.g. Hodgson-Kratky et al. 2017b);
- 13 • health and physiological condition of plants;
- 14 • allelic make up regarding the rubber-relevant loci, including (poly)ploidy (e.g. Warmke 1945; Luo et
15 al. 2018);
- 16 • accuracy of the analytical methods of rubber quantification and the reliability of results to vary (e.g.
17 Sikandar et al. 2017); and
- 18 • homogeneity of the dry root sample.

19 In most works that involve rubber quantification, the above factors are not properly considered, which has a
20 negative impact on the reliability of rubber quantity figures. Some works referenced in the following
21 paragraphs show substantial variability of rubber content according to season of sampling, planting
22 conditions, etc. Another issue, not addressed here or elsewhere, is exactness of the used analytical methods,
23 their comparisons and limitations.

24 So far, *T. bicornis* was overlooked as potential rubber source (if we disregard possible
25 misidentification or taxonomical confusion). Il'in and Yakimov (1950) briefly mentioned *T. bicornis* among
26 rubber producing plant and reported it to contain 1.3–5% of rubber in roots (probably DW); later (Il'in
27 1953), he gave slightly higher figures repeated by Vakhrusheva (1990): 1.3–8.1%. Our laboratory tests
28 showed a similar DW content of rubber as that reported in the literature: 1.4–6.2%.

29 As regards the comparison of our measurements of rubber content in roots of *T. koksaghyz*, Eggert et
30 al. (2018) reported around 4–5%, but the number was strongly varying among years and higher
31 concentrations were obtained when plants were planted in ridges than on the flat bed. Plant density also
32 played role (Eggert et al. 2018). Luo et al. (2018) reported average concentration of rubber in roots around
33 3% in natural diploids, 5.8% in colchicine-induced tetraploids and 3.5% in colchicine-treated diploids.
34 Similarly, Warmke (1945) found 2.95% of rubber content in roots of colchicine-treated tetraploids and 2.14%
35 in diploids. Kreuzberger et al. (2016) found significant variability (ca. 4–9%) depending on planting and
36 harvest season and trials. Season also influenced degree of polymerization of inulin. Arias et al. (2016a)
37 measured 2.14–6.5% (in one case even 11.5%). They found slightly higher content of rubber in plants
38 planted under lower irrigation dose. No significant variability was found among populations or in planting
39 date. Bobkov (1939) reported 1.65% of rubber in fresh roots, Ignatiev (1939) 4–7% in dry root weight,
40 Brandes (1941) 2–2.5% in fresh roots and up to 7% in dry roots on the end of the season. According to
41 Kolachov (1941), rubber content may reach 26% of dry weight. Reichert (1942) reported around 12% of dry
42 root weight and Drobkov (1945) 4–5% after one season and up to 12% after second season. These records
43 show high variability, obviously depending on a number of factors (see above). Moreover, majority of
44 available ex situ germplasm of *T. koksaghyz* belongs to closely related, but asexual poor rubber produce *T.*
45 *brevicorniculatum* (Kirschner et al. 2013), making many recent reports problematic. The results are
46 comparable to our own (mean rubber content in root dry weight 3.25% for *T. bicornis* and 7.12% for *T.*
47 *koksaghyz*) obtained from wild plants sampled in the field (Fig. 7).

1 The percentage of rubber in root dry weight of *T. bicornis* is on average about half of that of *T.*
2 *koksaghyz*, but plants of the latter are rather slender. Our results are to be taken as preliminary, because we
3 used roots of *T. bicornis* collected in the wild (with variable age and health condition), characterized by a
4 quite low weight. Both species can be easily cultivated and it is more a question for field experiments which
5 of these two species would be the better rubber producer in practice. *T. bicornis* is a widespread species
6 without any conservation concern, and it has much wider ecological amplitude than *T. koksaghyz* promising a
7 wide range of regions suitable for cultivation. *T. bicornis* has a considerable amount of genetic variation and
8 therefore this species could be exploited either as a new source of natural rubber, or, since it is easily
9 crossable with *T. koksaghyz*, as a source for introgression to enrich the *T. koksaghyz* germplasm.

10
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15 the field.

16 **Compliance with ethical standards**

17 **Conflict of interest** The authors declare that they have no conflict of interest.

18 Appendix 1 – Selected specimens studied

19 Kazakhstan: Yuzhnyy Kazakhstan, Chu District, Chu River valley, Kush-tyube, 28 May 1926, V. Drobov &
20 P. Gololitskiy 226 (TASH, no. det. 15772). — Syr-Darya Region, Aulizatskiy Uyezd., left bank of Chu
21 River, between villages of Gulyaevka and Alekseevka, 3 May 1916, M. Sovetkina 456 (TASH, no. det.
22 15775). — Syr-Darya Region, Chu River left bank, 4.2 km SW of Alekseevka, 4 May 1916, M. Sovetkina &
23 S. Chausova 481 (TASH!). — Syr-Darya Region, Aulizatskiy District, Alekseevka, 4 May 1916, M.
24 Sovetkina 526 (LE, no. det. 20490). — Syr-Darya Region, Aulizatskiy District, Talas, sands of Karagundy
25 basin, 28 May 1916, M. Sovetkina (AA, no. det. 20097). — Syr-Darya Region, Aulizatskiy District, Talas,
26 sands of Karagundy basin, 28 May 1916, M. Sovetkina 607 (TASH, no. det. 15774). — Chu District, Chu R.
27 valley, Kum-tyube, 28 May 1926, V. Drobov & P. Gomolickiy (LE, no. det. 20487). — Chu R. district,
28 Talasa R. valley, Kara-bakyr, 19 May 1926, V. Drobov & P. Gomolickiy (LE, no. det. 20486). — Turgai
29 Region, Irgiz District, B. Barsuki, W margin of sands of Chin'-Davlip, 25 May 1914, N. Dessiatoff 715 (LE,
30 no. det. 20484). — E shore of Biylilikul' (Dzhambul Region), 22 May 1963, V. Goloskokov (AA!). — Syr-
31 Darya Region, Perovski District, 26 May 1910, O. Knorring & Z. Minkwitz (LE, no. det. 20488).

32 Kyrgyzstan: [Bishkek district] right bank of Kochkur River, upper Chu River, Bektenov winter
33 camp, 1 Jun 1915, M. Sovetkina 607 (S, no. det. 18356). — Kaschkara [Kochkor River], Kyrgyz Alatau
34 [Alpes Alexandri], 5 Jun 1896, V. F. Brotherus 103 (LE, no. det. 8053). — [Bishkek Region] W shore of
35 Lake Issyk-kul', ca. 2 km of Rybach'e, 29 May 1915, M. Sovetkina 535 (LE, no. det. 20496).

36 Uzbekistan: Margelan [Margilon], Karl Marx Street, 19 Apr 1959, Kovalevskaya 90 (TASH, no. det.
37 15781). — Yad'yavanskiy r-n [district], kolkhoz Stalinabad, 19 May 1957, U. Saidaliev (TASH, no. det.
38 15780). — Amu-Dar'ya (Urgen'—Chardzhuy), Dzhagiribentskiy Tugay, 25–26 Mar 1913, M. Popov 602
39 (TASH, no. det. 15779). — Samarkand—Bukhara, km 122, 24 Apr 1957, Vvedenskiy & Kovalevskaya 159
40 (TASH, no. det. 15778). — Middle Zeravshan, Khatyrchinskiy District, 5 May 1928, S. Lepeshkin (TASH,
41 no. det. 15773). — Andizhan Region, Nazar-Makhram, road from Shary-Yulduz kolkhoz to Sharykhan,
42 along the bank of Fergana channel, 23 Apr 1959, Kovalevskaya 190 (TASH, no. det. 15767). — Kokand—
43 Buvaydy road, settlement Palakhan, 18 Apr 1959, Kovalevskaya 60 (TASH, no. det. 15765). — Kokand, old
44 town, 18 Apr 1959, Kovalevskaya 52 (TASH, no. det. 15766). — Bukhara Region, valley of Zarovshan
45 River, in the vicinity of Kermine [Karmana], 12 May 1956, V. Sedov 786 (TASH, no. det. 15762). — Kara-
46 Kalpakiya, Kegeili, 16 May 1967, [collector illegible] (TASH, no. det. 15763). — Golodnaya step' [Steppe

1 of Starving, Mirzachol], along the road to Krasnoarmeisk, 11 Jun 1957, Mel'nikova & Ergashev 68 (TASH,
2 no. det. 15761). — Termez, 15 Apr 1958, Vvedenskiy et al. 219 (TASH, no. det. 15759). — Uzbekistan:
3 prope opp. Urgenč, Čalyš., 22 Apr 1979, E. Hadač & H. Rambousková (PRC, no. det. 26804). — Zeravshan
4 Expedition, Bukhara Region, Kermishskiy raion [district], Kemish, 14 May 1956, V. Sedov (TASH, no. det.
5 15950). — Katta-Kurgan Reservoir, 21 Apr 1957, Vvedenskiy & Kovalevskaya 115 (TASH, no. det. 15758).
6 — Kosh-Tagirman, near the road from Denau to Shurchi, along Surkhan R., 20 Apr 1958, Vvedenskiy et al.
7 303 (TASH, no. det. 15760). — Saryoshei [Sariosiyo] to Uzunom [Uzun] road, 18 Apr 1958, Vvedenskiy et
8 al. 288 (TASH, no. det. 15757). — Tashkent highway, km 9 along the road, 15 Apr 1958, Vvedenskiy et al.
9 220 (TASH, no. det. 15755, 15756). — Plants of Kashka-Darya Region of UzSSR, district of Bek-budi,
10 Dzhuta-bazar, a road to Bek-budi, 1 Jun 1927, M. Kultiasov & A. Granitov 188 (TASH, no. det. 15753). —
11 Between Dusar-Kurgan and Surkhan, 16 Apr 1958, Vvedenskiy et al. 236 (TASH, no. det. 15754). — Plants
12 of Bukhara, sands of Sundukli (right bank of Amu-Darya), Assiya, right bank of Taikyr R., 5 Mar 1911, A. K.
13 Golbek 26 (LE, no. det. 20493). — Delta of Amu-Darya, bank of Ak-Darya below Dzhalsan, 17 Aug 1928,
14 N. I. Kuznetsov 858 (LE, no. det. 20495). — Syr-Darya Region, Chimkent District, Sary-kamysh, near Lake
15 Tugul' (bank of Syr-Darya), 7 Jun 1908, Z. Minkwitz 616 (LE, no. det. 20494). — Desertum Aralense, regio
16 fluv. Ssyrdarja, in humidis pr. Var-kaga, Aug 1856, E. Borsczow 660 (LE, no. det. 20489).

17 Turkmenistan: Herb. Horti botanici Turcomanici, near Charshanga, 193(?), Anonymus 1689 (LE, no.
18 det. 20491).

19 Tajikistan: Leninabad [Khujand, Khudzhand], botanical garden, 16 Apr 1959, Kovalevskaya 33
20 (TASH, no. det. 15768).

21 China: prov. Kansu, ca 3 km ad septentr. vers. a Camp LXV, 9 Jun 1930, B. Bohlin, Exped. S. Hedin
22 (S, no. det. 23060). — Kansu, in montibus Richthofen (Nan-Shan) [Qilian Mountains], Tung Wei Ch'ü kou,
23 17 Jun 1931, B. Bohlin, Exped. S. Hedin 2134 (S, no. det. 23057, 23061). — [Xinjiang] Turkestanica sinensis,
24 in deserto Taklamakan, Domoku, ad fossam, 1500 m, 'Kök kuk', 28 Apr 1933, N. Ambolt, Exped. S. Hedin
25 6030 (S, no. det. 23059). — [Xinjiang] Turkestanica sinensis, Jarkend, in agro, 1300 m s.m., 13 May 1930, C.
26 Persson 170 (S, no. det. 9252). — [Xinjiang] Pamir: distr. Kashgar, in oppido., 12 May 1935, C. Persson
27 629a (S, no. det. 9255). — [Xinjiang] Turkestanica sinensis, Kashgar, 12 May 1935, C. Persson no. 629a, cult.
28 in Lund, no. 231, leg. G. Haglund 12 Jun 1937 (S, no. det. 9253). — [Xinjiang, Kumul] Hami, 28 May 1877,
29 G. N. Potanin s.n. (S, no. det. 9254). — [Ningxia] Mongolia, Gobi, inter Sha-hu [Shahu Lake] et Fui-ting, 5
30 Jun 1886, G. N. Potanin (LE!). — Mongolia austral.: prov. Ming-hsia, ca. 8 li ad orient. versus a Camp. LIX,
31 in prato humido., 25 May 1930, B. Bohlin 1865 (S, no. det. 9251). — Qinghai, Golmud, 2740 m, 14 Jun
32 1955, S.-Y. Li, C.-J. Wang & D.-L. Huang [Qaidam Basin Expedition] 40 (PE 318457, no. det. 24838).

33 Mongolia: Bajanchongor-Aimak, Oase Echin-gol, Rand der Solontschakwiese., 14 Jun 1962, C.
34 Davažamc & P. Hanelt 1022 (PE, no. det. 24836, GAT, no. det. 26468).

35 Pakistan: Quetta, Pishin Forest, 7 May 1965, S.M.A. Kazmi 1442 (RAW, no. det. 33765).

36 Afghanistan: prov. Kabul, Paghman, 7 May 1964, Neubauer 4708 (M, no. det. 33766). — Kandahar,
37 Pirzada, 900 m s.m., 25 Feb 1949, M. Köie 3439 (C, no. det. 31007, cf.).

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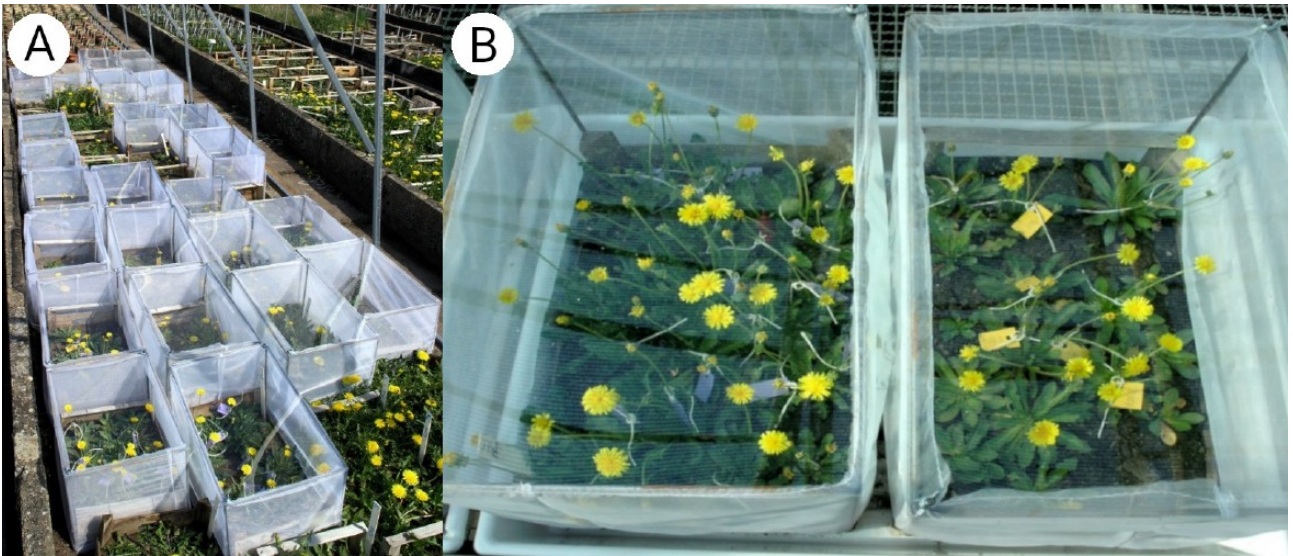
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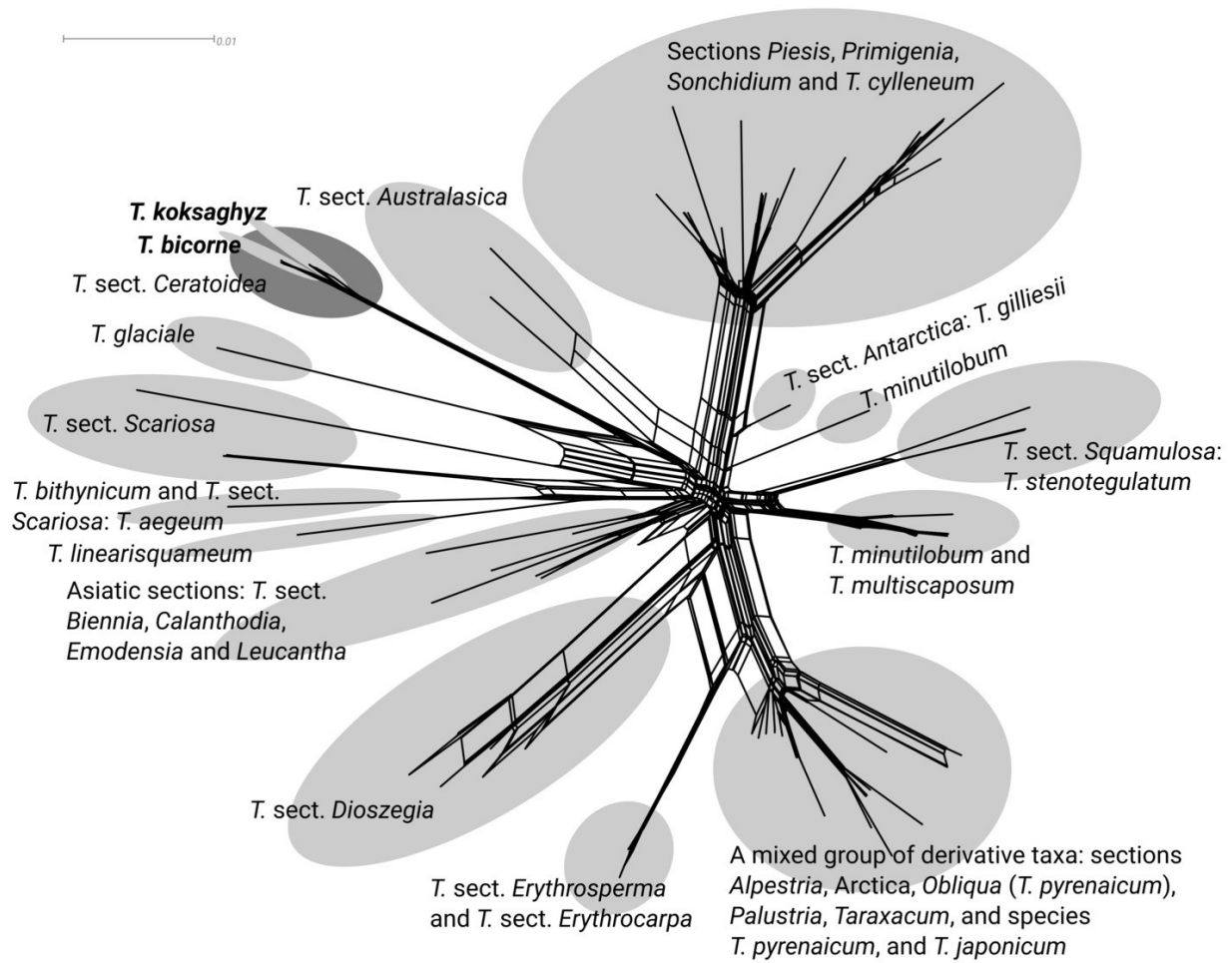
- 1 **Fig. 1** The Kokpek region in the SE Kazakhstan, with Site I (2008, yellow) and Site II (2014, red) indicated.
- 2 Scale bar marks 300 m. Data are from OpenStreetMap (accessed through portal Mapy.cz).
- 3



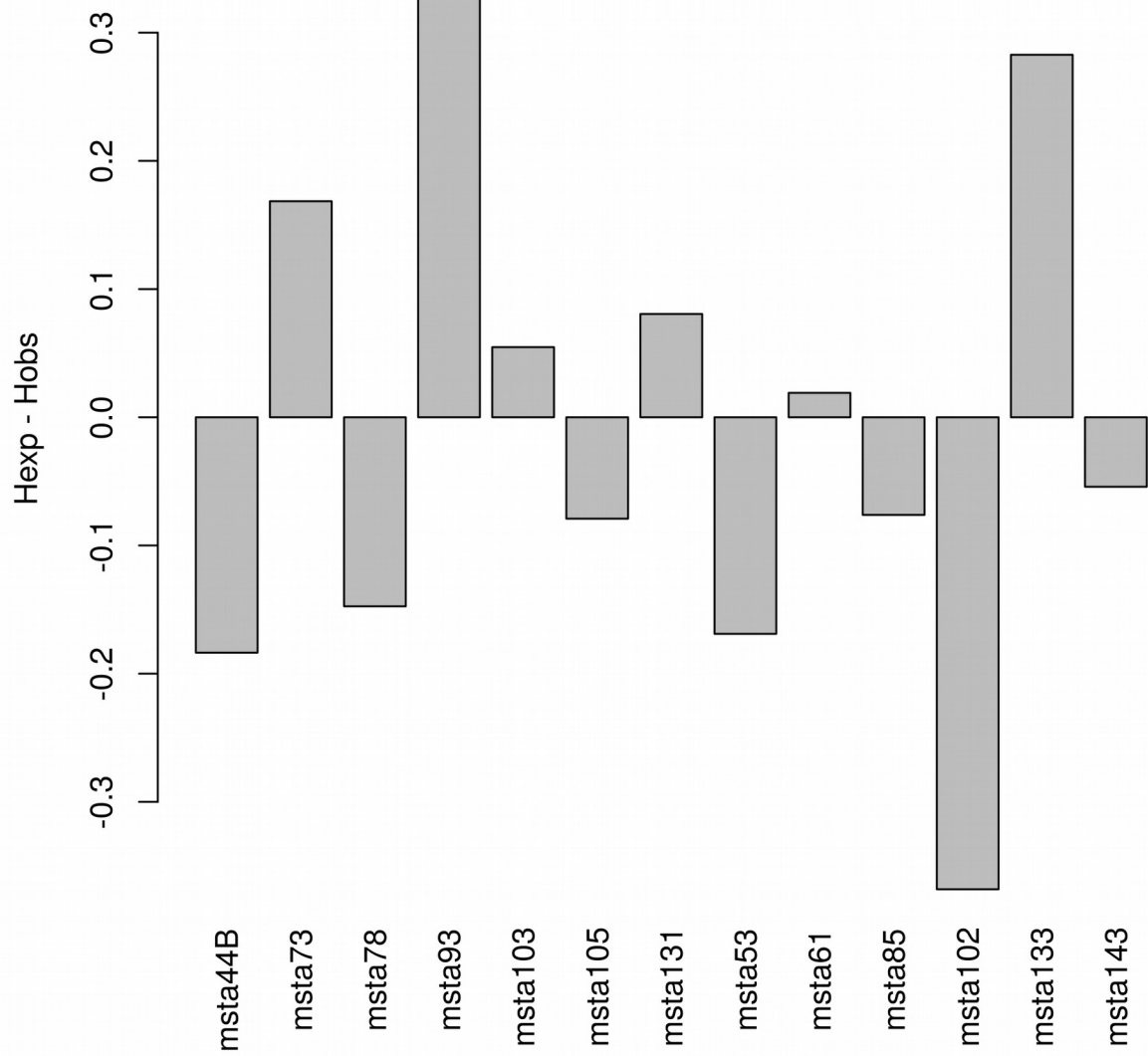
1 **Fig. 2** A, flower beds with dandelion samples at the experimental garden. B, cages covered with a plexiglass,
2 each with a half-sib family of cultivated plants.
3



1 **Fig. 3** The method of hand crossing.
2

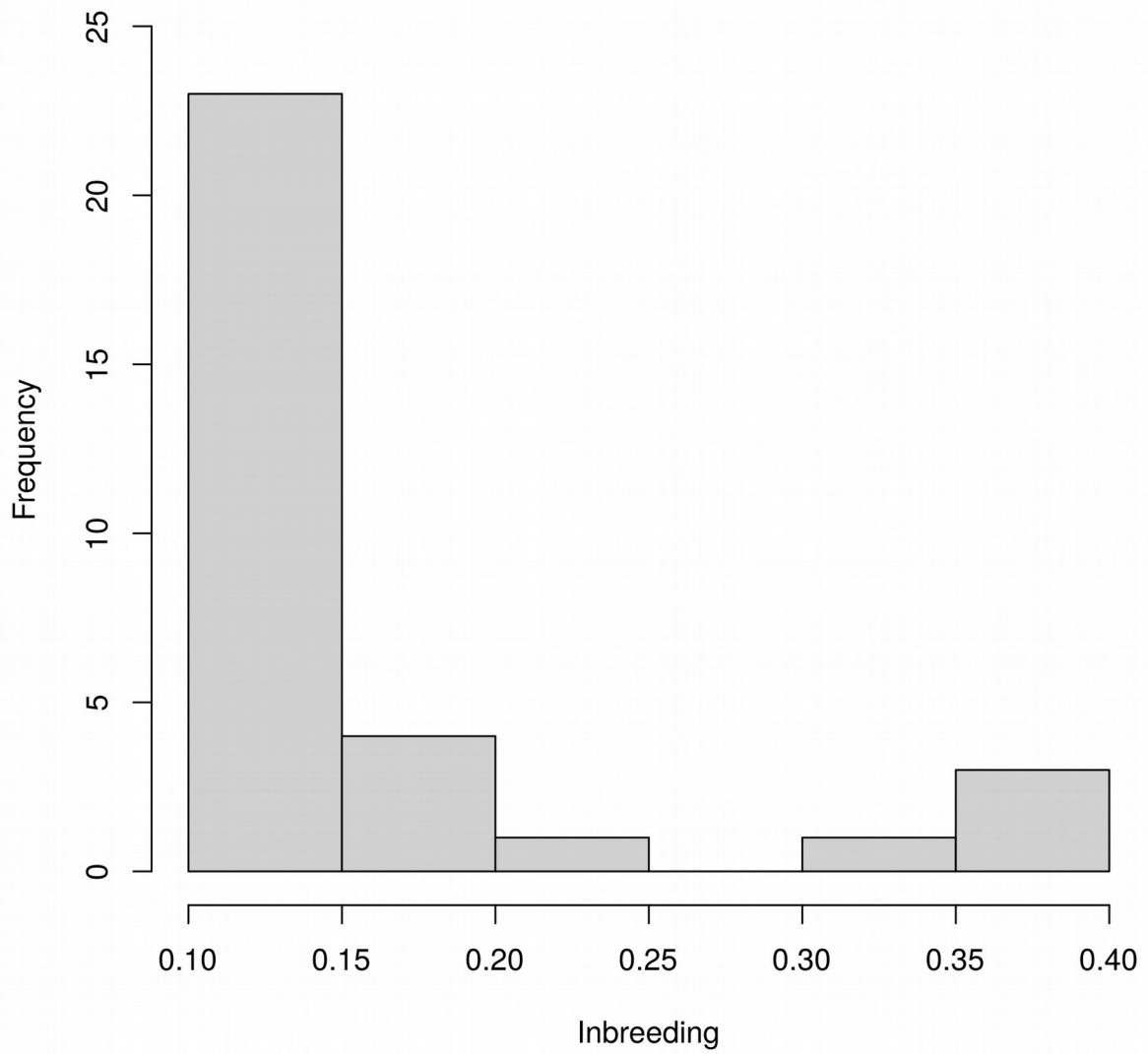


1 **Fig. 4** Neighbour Net constructed from uncorrected P-distances in SplitsTree based on nrDNA ITS sequences
 2 of sexual members of 24 *Taraxacum* sections. The position of the section *Ceratoidea* is marked by dark grey;
 3 two species under study are in bold. Modified from Kirschner et al. (2017) where also lists of samples are
 4 given.
 5

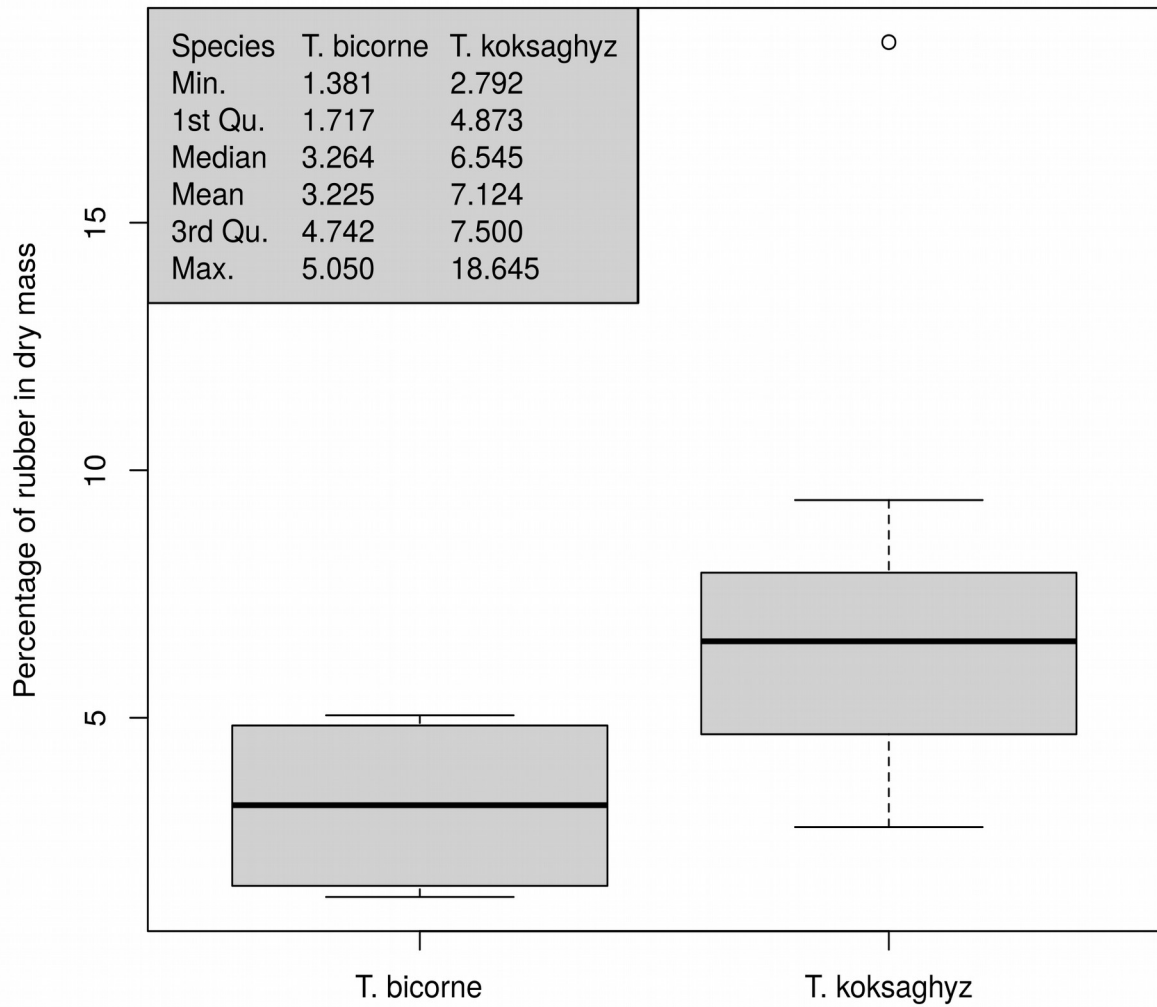


1 **Fig. 5** Difference of expected and observed heterozygosity for individual loci of SSRs markers. Negative
 2 bars show higher observed than expected heterozygosity.

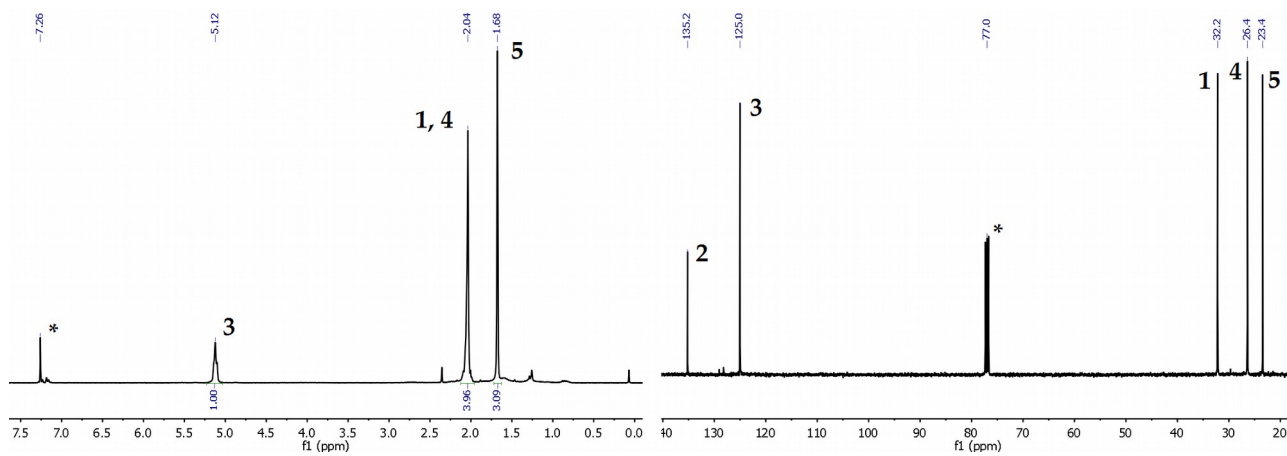
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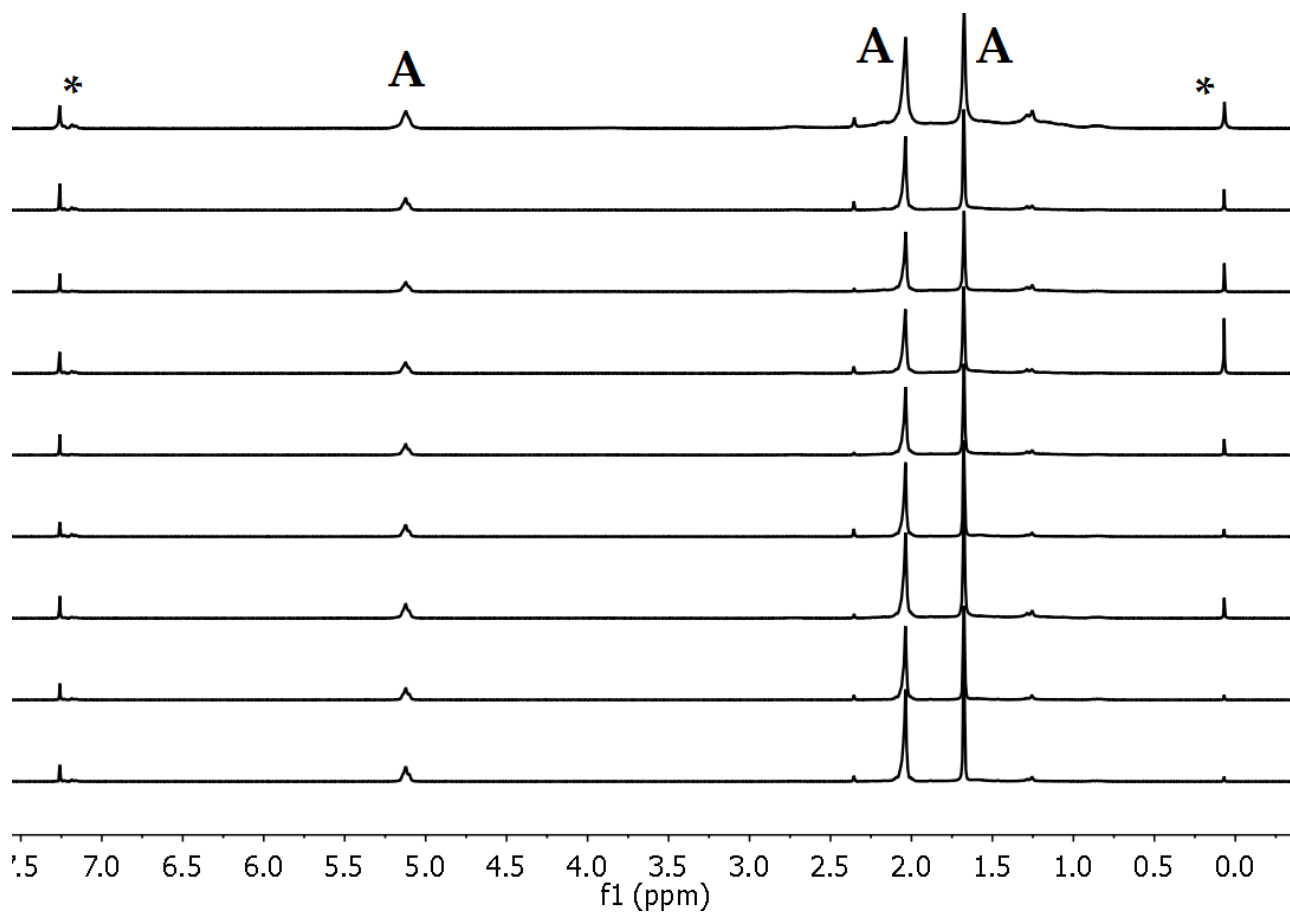
1 **Fig. 6** Frequency histograms of level of inbreeding across all SSRs markers. Horizontal axes show level
2 (prevalence) of inbreeding and vertical respective frequency.
3



1 **Fig. 7** Percentage of content of rubber in dry root mass of *T. bicornne* (left) and *T. koksaghyz* (right). Inset
 2 legend shows basic statistics of the measurements. Content of rubber is significantly different in both species
 3 (tested by Kruskal-Wallis test, $p = 0.029$).
 4



1 **Fig. 8** ^1H (left) and $^{13}\text{C}\{^1\text{H}\}$ (right) NMR spectra of a sample with signal assignment of the major
 2 component (i.e. natural rubber). The star corresponds to the solvent signals.
 3



1 **Fig. 9** The stacked ^1H NMR spectra of the randomly chosen (9 out of 25) prepared rubber samples. All
 2 exhibit the same composition with varying amount of impurities. The star corresponds to the solvent and
 3 internal standard signals and „A“ to natural rubber signals.
 4



1 **Fig. 10** Flowering capitula of *T. bicornis* from the Kokpek locality (2014).
2

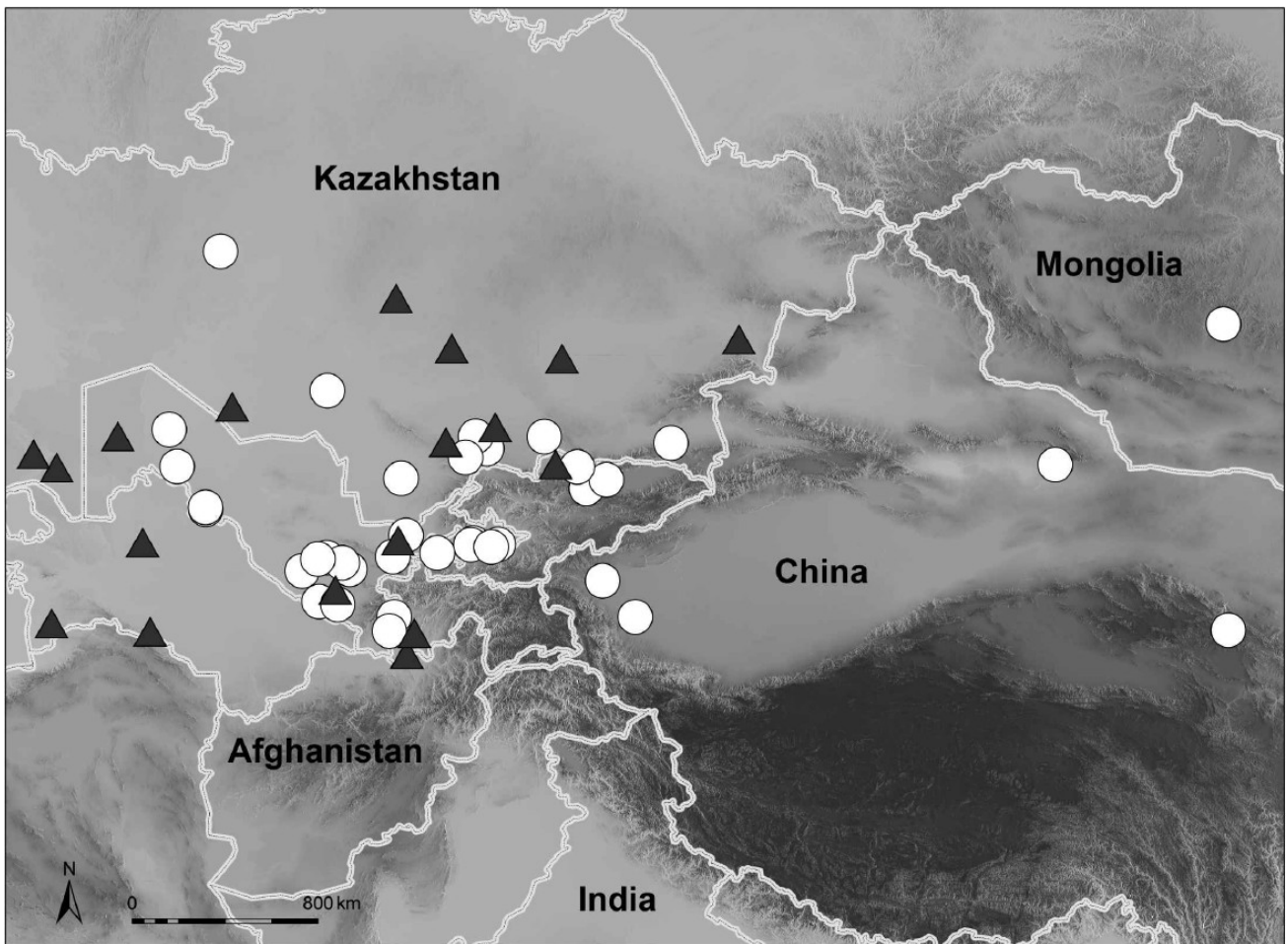


Fl. Ekblom del.

Taraxacum bicorne Dahlst

J. Cederquist foto. o. tr.

1 Fig. 11 A reproduction of the figure in the protologue of the name *T. bicorne* (Dahlstedt, Ark. Bot. 5/9: Plate
2 17, 1906).
3



1 **Fig. 12** Distribution of *T. bicorne* in Central Asia. Grey triangles mark literature records and white circles
2 herbarium vouchers.
3

1 **Table 1** KASP genotypes in the *T. bicornis* population. The three controls at the top are *T. koksaghyz* (diploid,
2 AA), *T. officinale* (diploid, BB) and an artificial hybrid between these two species (diploid AB). ‘- -’ stands
3 for unknown, due to a drop out.

Marker	CPT1 vs CPT2/3	CPT2 and CPT3	RTA	SRPP5	REF	CTO_M1_Contig179_397	CTO_M1_Contig2608_372	CTO_M1_Contig3593_566	CTO_M1_Contig5704_271	CTO_M1_Contig6303_344
Plant										
TKS	AA	AB	AA	AA	AA	AA	AA	AA	AA	AA
TO	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB
TKS x TO	AB	AB	AB	AB	AB	AB	AB	AB	AB	AB
Kz_1-TBI	AA	--	BB	AA	BB	AA	AA	BB	AA	AA
Kz_2-TBI	--	--	--	--	AB	--	--	--	--	--
Kz_3-TBI	AA	BB	BB	AA	BB	AA	AA	BB	AA	AA
Kz_4-TBI	AA	BB	BB	AA	BB	AA	AA	BB	AA	AA
Kz_5-TBI	AA	BB	BB	AA	BB	AA	AA	BB	AA	AA
Kz_6-TBI	AA	BB	BB	AA	BB	AA	AA	BB	AA	AA
Kz_7-TBI	AA	BB	BB	AA	BB	AA	AA	--	AA	AA
Kz_8-TBI	AA	--	BB	--	--	AA	AA	BB	AA	AA
Kz_9-TBI	AA	--	BB	AA	BB	AA	AA	BB	AA	AA
Kz_10-TBI	AA	--	BB	AA	BB	AA	--	--	--	--
Kz_11-TBI	AA	BB	BB	AA	BB	AA	AA	BB	AA	AA
Kz_12-TBI	AA	BB	BB	AA	BB	AA	AA	BB	AA	AA

Kz_13-TBI	BB	--	--	--	--	AA	AA	BB	AA	AA
Kz_14-TBI	--	--	--	--	--	AA	AA	BB	AA	AA
Kz_15-TBI	--	--	--	--	--	AA	AA	BB	AA	AA
Kz_16-TBI	AA	--	--	--	BB	AA	AA	BB	AA	AA
Kz_17-TBI	AA	--	BB	AB	BB	AA	AA	BB	AA	AA
Kz_18-TBI	AA	BB	BB	AA	BB	AA	AA	BB	AA	AA
Kz_19-TBI	AA	--	--	--	--	AA	AA	BB	AA	AA
Kz_20-TBI	AA	BB	BB	AA	BB	AA	AA	BB	AA	AA
Kz_21-TBI	AA	--	BB	AA	BB	AA	AA	BB	AA	AA
Kz_22-TBI	AA	BB	BB	AA	BB	AA	AA	BB	AA	AA
Kz_23-TBI	AA	BB	BB	AA	BB	AA	AA	BB	AA	AA
Kz_24-TBI	AA	BB	BB	AA	BB	AA	AA	BB	AA	AA
Kz_25-TBI	AA	--	BB	AA	BB	AA	AA	BB	AA	AA
Kz_26-TBI	AA	--	BB	AA	BB	AA	AA	BB	AA	AA
Kz_27-TBI	AA	BB	BB	AA	BB	AA	AA	BB	AA	AA
Kz_28-TBI	AA	BB	BB	AA	BB	AA	AA	BB	AA	AA
Kz_29-TBI	AA	--	BB	AA	BB	AA	AA	BB	AA	AA
Kz_30-TBI	AA	--	--	AB	BB	AA	AA	BB	AA	AA
Kz_31-TBI	--	--	--	--	AB	--	--	--	--	--
Kz_32-TBI	AA	--	--	--	AB	AA	--	--	--	AA
TKS	AA	AB	AA	AA	AA	AA	AA	AA	AA	AA

TO	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB
TKS x TO	AB	AB	AB	AB	AB	AB	AB	AB	AB	AB
Kz_1-TBI	AA	--	BB	AA	BB	AA	AA	BB	AA	AA

1 **Table 2** Population-genetic indices for the population of *T. bicorne* summed over all SSRs loci. See text for
2 detailed explanations.

Number of multilocus genotypes (MLG) among 32 genotyped individuals	24
H (Shannon-Wiener Index of MLG diversity)	3.02
E.5 (Evenness)	0.493
Ia (Index of Association)	3.03
P-value for Ia	1e-04
rbarD (standardized Ia)	0.26
P-value for rbarD	1e-04
G (Genotype diversity)	0.892

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1 **Table 3** Yielded results of the experimental crosses (number of crosses is represented by the number of
2 capitula used).

Mother plant	<i>T. koksaghyz</i>	<i>T. bicorne</i>
Pollen donor	<i>T. bicorne</i>	<i>T. koksaghyz</i>
Overall number of crosses	56	81
Successful crosses	49	54
Total number of hybrid achenes	2425	1499
Mean number of achenes per capitulum	51.6	27.8

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1 This is Author's Accepted Manuscript. See <https://link.springer.com/article/10.1007/s10722-019-00788-4> for
2 published version.
3 This is a post-peer-review, pre-copyedit version of an article published in Genetic Resources and Crop
4 Evolution. The final authenticated version is available online at: <https://doi.org/10.1007/s10722-019-00788-4>
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