Karyomorphological Study of Taraxacum in Northeast China

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Abstract

Background: Dandelions (Taraxacum) comprise more than 2,000 species distributed worldwide, including 70 species in China and over 20 species in Northeast China. It is an edible plant used also in traditional Chinese medicine. Methods: Chromosome number was determined by conventional tableting, and karyotypes were analyzed. We determined the number of mitotic chromosomes, karyotype characteristics, and morphology of Taraxacum germplasm resources collected in Northeast China. The data were compared with those in the Chromosome Counts Database (CCDB) using principal component analysis (PCA) and cluster analysis. Results: The collected Taraxacum species included diploids, triploids, and tetraploids, with chromosome numbers of 2n = 16, 24, and 32, respectively. The karyotypes showed metacentric and submetacentric chromosomes and satellites. The absolute length of a single chromosome was 3.56–31.68 μm, and the karyotype asymmetry coefficient was 59.68%–67.45%. PCA of karyotype data showed that chromosome size, long arm length, and short arm length formed PC1. Cluster analysis based on karyotype data of the 18 species supported the taxonomic classification of this genus in the Flora of China . Conclusions: Our results indicate that karyotype analysis can be used as cytological evidence for Taraxacum taxonomy. We also determined that among the 18 Taraxacum species, triploid species were more evolved than diploid and tetraploid species. We believe that our results provide a basis for further research in Taraxacum germplasm resources and lay the experimental foundation for further development of Taraxacum in traditional Chinese medicine.

Background

Dandelions, *Taraxacum* F.H. Wigg. (family *Compositae*), are one of the most evolved taxa in the subfamily *Liguliflorae* DC. *Taraxacum* plants have a unique mode of reproduction—they can reproduce both asexually (apomixis) and sexually. The *Flora of China* reports that many *Taraxacum* species often form new populations by apomixis or natural hybridization [1,2]. However, there is no gene exchange among these apomictic individuals, and therefore, it is difficult to determine the biological species and establish the species boundaries. Consequently, classification and identification of *Taraxacum* resources based on morphological characteristics have always been challenging and their taxonomic identification controversial. The Herbaceous Flora of Northeast China recognizes 19 species, 1 variety, and 3 forms in the genus *Taraxacum* present in Northeast China, whereas the *Flora of China* records 11 species of *Taraxacum* in the same region [3]. The substantial differences in the types of germplasm resources recorded in the two authoritative botanical texts highlight the need for standardization of botanical texts. The northeast region of Chine where *Taraxacum* is commonly found is also one of the main supply bases for the Chinese medicine market. Therefore, in this study, we sampled plant populations from this region for the taxonomic identification of *Taraxacum* plants.

The chromosome is the principal component and genetic material of the cell nucleus. Chromosome number is typical for each species, and it remains relatively stable between generations and between individual cells (except the endosperm). Therefore, the chromosome number can be used as a taxonomic character for species delimitation [4]. Plant karyotype analysis is used to study the variation patterns of plants and further explore the genetic relationships and evolutionary trends among various populations. Aquaro et al. [5,6] compared the morphology of four systematic units of *Taraxacum* from Italy and, in combination with genomic studies, identified four species, two of which were new to science. Their chromosome numbers and karyotype formulae, which were provided for the *Taraxacum* germplasm resources, laid a foundation for the further development and utilization of these four *Taraxacum* species. Verhoeven et al. reported the interference effects of geographical parthenogenesis in *T. officinale* W.H. Wigg. on the spread of invasive plants and determined the mode of parthenogenesis in *Taraxacum* [7]. Mitsuyuki et al. reported that hybridization of the native diploid Japanese *T. platycarpum* Dahlst. with European triploid species *T. officinale* and *T. laevigatum* DC. produced triploid plants capable of sexual reproduction [8]. Musiał analyzed the ovule development process in *T. udum* Jord. (sect. Palustria), a species from Poland, and identified three modes of reproduction, namely, apomixis,

parthenogenesis, and autonomous endosperm formation [9]. These complex reproduction patterns further complicate the classification and identification of *Taraxacum* germplasm resources. In this study, we conducted nearly 10-year-long field investigations in Northeast China and identified 18 *Taraxacum* germplasms. Karyotype analysis of seven of those *Taraxacum* species was previously published [10], whereas the karyotype of the remaining 11 species is analyzed here for the first time. Furthermore, we performed karyotype cluster analysis of the 18 species distributed in Northeast China and elucidated their phylogenetic relationships and evolutionary trends, thereby laying an experimental foundation for further identification of *Taraxacum* germplasm resources in Northeast China. This study will also provide a basis for further development of *Taraxacum* resources and the rational use of this genus in traditional Chinese medicine.

Methods

Plant material

The test material included 18 *Taraxacum* species distributed in Northeast China (including seven species previously reported by our laboratory). Taxonomic criteria were based on the *Flora of China* and *Herbaceous Flora of Northeast China*. All collected specimens were identified by Jiyun Li of the Shenyang Institute of Applied Ecology, Chinese Academy of Sciences, and deposited in the Experimental Station of Jinzhou Medical University. *Taraxacum* resources were collected from Heilongjiang, Jilin, Liaoning, and other provinces from 2008 to 2019 and planted in the Experimental Base of Jinzhou Medical University following routine management protocols. Voucher information of sampled materials is provided in Table 1 and Figure1.

Table.1. Collection locations of 18 Taraxacum species in Northeast China

No.	Species nam	Voucher		
A	*T. antungense* Kitag	Dandong City, Liaoning Province	X.Zhao	011(SYAB)
B	*T. asiaticum* Dahlst	Panshi City, Jilin Province	X.Zhao	043(SYAB)
C	*T. urbanum* Kitag.	Chaoyang City, Liaoning Province	B.Zhang	047(SYAB)
D	*T. ohwianum* Kitag.	Dandong City, Liaoning Province	B.Zhang	023(SYAB)
E	*T. variegatum* Kitag.	Dandong City, Liaoning Province	X.Zhao	034(SYAB)
F	*T. asiaticum var.lonchophyllum* Kitag.	Chaoyang City, Liaoning Province	X.Zhao	029(SYAB)
G	*T. junpeianum* Kitam. In Act.	Dandong City, Liaoning Province	X.Zhao	014(SYAB)
H	*T. mongolicum* Hand. -Mazz.	Dandong City, Liaoning Province	X.Zhao	014(SYAB)
I	*T. liaotungense* Kitag.	Songyuan City, Jilin province	J.Wu	010(JZKY)
J	*T.coreanum* Nakai	Shenyang City, Liaoning province	J.Wu	005(JZKY)
K	*T.formosanum* Kitam.	Tongliao City, Neimengu province	J.Wu	009(JZKY)
L	*T. sinomongolicum* Kitag.	Dandong City, Liaoning Province	J.Wu	012(JZKY)
M	*T.heterolepis* Nakai et Koidz.ex Kitag.	Panshi City, Jilin Province	J.Wu	013(JZKY)
N	*T.brassicae folium* Kitag.	Chaoyang City, Liaoning Province	J.Wu	014(JZKY)
O	*T.platypecidum* Diels	Dandong City, Liaoning Province	J.Wu	015(JZKY)
P	*T. falcilobum* Kitag.	Dandong City, Liaoning Province	J.Wu	016(JZKY)
Q	*T.borealisinense* Kitam.	Chaoyang City, Liaoning Province	J.Wu	017(JZKY)
R	*T.erythopodium* Kitag.	Dandong City, Liaoning Province	J.Wu	018(JZKY)

Resource: investigated on location by authors.

Methods

The achenes were germinated and cultivated using hydroponics to generate root tips. Ten representative achenes of each species were evenly placed in a glass culture dish with a diameter of 90 mm; 5–10 culture dishes were used for each species. Appropriate volumes of water were added to partially soak the achenes, and the culture dishes with filter paper were incubated at 23 °C to germinate the seeds. Root tips were collected when the primary roots reached 0.5–1.5 cm length. Samples were prepared by the crushing method for observation of chromosome characteristics. Specifically, the samples were treated with 0.0038 mol·L^{-1} 8-hydroxyquinoline for 4–5 h and then fixed in Carnoy's solution I (anhydrous ethanol and glacial acetic acid

mixed in a volume ratio of 3:1). After fixing at 4 °C for 12 h, the samples were washed with 70% ethanol solution and stored in 70% ethanol solution at 4 °C. The fixed root tips were dissociated in 1 mol·L^{-1} hydrochloric acid for 15 min in a thermostatic water bath at 60 °C, stained with basic fuchsin, crushed, and observed, as well as photographed using a Moficam 2206 (Olympus, Japan) microscope. Permanently mounted slides were prepared. At least 30 cells with clearly visible chromosomes were selected to determine the chromosome number. The chromosomes at metaphase were analyzed according to the protocol proposed by Li[10]. Measurements were performed by using Motic Images Advanced 3.2 (Motic Asia, Hong Kong, China). Karyotype classification was based on Stebbins' criteria. [11-13] The relative length of chromosomes (RL) and arm ratio (AR; ratio of long arm length to short arm length) were calculated, and the type of centromere position (CP) was determined. The karyotype asymmetry coefficient (As.K%) was calculated using the method proposed by Arano [14-16]. The relative chromosome length was calculated according to the method of Kuo [17-19], and the kinetochore index and arm index were calculated according to the method of Li [20].

Data analysis

The karyotypes of seven previously reported *Taraxacum* species were included to ensure the scientific accuracy of the results. We also queried the plant chromosome number indexes and Chromosome Counts Database (CCDB, http://ccdb.tau.ac.il/search/) and updated and verified each taxon (including species and subspecies) of *Taraxacum* in this study. Principal component analysis (PCA) was performed on the data from the karyotype analysis using Origin 2018 (OriginLab, Northampton, MA, USA) to identify the main factors contributing to karyotype differences. The same data were subjected to cluster analysis using SPSS 21.0 (IBM Corp., Armonk, NY, USA) to determine phylogenetic relationships of the sampled taxa [21]. The Minimum Standards of Reporting Checklist includes details of the experimental design, statistics, and resources used in this study.

Results

Chromosome numbers of *Taraxacum* plants

The chromosome numbers of the 18 dandelion species distributed in Northeast China (including the seven previously published species) were 16, 24, and 32 for diploid, triploid, and tetraploid germplasm resources, respectively. Chromosome maps and karyotype analysis revealed two composition types of chromosomes, the metacentric (m) and submetacentric (sm).

Karyotype analysis of 18 *Taraxacum* species

We described the karyotype characteristics of 11 *Taraxacum* species and combined these with the karyotype characteristics of the seven *Taraxacum* species previously reported by Ning (2012). In addition, by comparing with the plant chromosome number indexes and the CCDB, we found that karyotype information was recorded for the first time for the following 11 species: *T. antungense*, *T. urbanum*, *T. variegatum*, *T. asiaticum* var. *lonchophyllum*, *T. liaotungense*, *T. pingue*, *T. heterolepis*, *T. brassicifolium*, *T. falcilobum*, *T. junpeianum*, and *T. erythropodium*. The previously reported chromosome numbers of *T. asiaticum* Dahlst. (16 chromosomes), *T. ohwianum* Kitam. (24 chromosomes), and *T. platypecidum* Diels (12 chromosomes) differed from those observed in this study (24, 16, and 24 chromosomes, respectively). Hence, we recorded these new counts in the CCDB.

Table.2. The chromosome parameters of 11 *Taraxacum*

No	*T. mongolicum* Hand. -Mazz.						*T. liaotungense* Kitag. in Bot.					
	Relative length (%)			CI	AR	CP	Relative length (%)			CI	AR	CP
1	LL	SL	TL	(%)			LL	SL	TL	(%)		
	15.80	14.76	15.16	40.67	1.45	m	16.07	14.52	15.58	35.86	1.78	sm
2	13.88	14.73	14.40	37.62	1.65	m	14.45	15.50	14.95	39.90	1.50	m
3	13.15	14.43	13.93	36.84	1.71	sm	14.59	14.12	14.51	37.47	1.66	m
4	11.55	12.04	11.85	38.07	1.62	m	12.20	13.62	12.83	40.86	1.24	m
5	12.73	11.07	11.72	42.41	1.35	m	11.89	12.14	11.41	40.97	1.44	m
6	12.80	10.33	11.29	44.25	1.25	m	10.63	11.18	10.91	39.44	1.53	m
7	9.70	11.95	11.07	34.20	1.92	sm	10.52	9.55	10.21	35.96	1.98	sm
8	10.34	10.65	10.53	38.34	1.60	m	9.60	9.32	9.56	37.53	1.66	m

No	*T. coreanum* Nakai.						*T. formosanum* Kitam.					
	Relative length (%)			CI	AR	CP	Relative length (%)			CI	AR	CP
1	LL	SL	TL	(%)			LL	SL	TL	(%)		
	17.43	13.55	15.87	34.32	1.91	sm	15.17	13.63	14.59	35.73	1.79	sm
2	12.95	14.45	13.55	42.84	1.33	m	12.52	14.19	13.91	38.98	1.56	m
3	12.38	12.73	12.52	40.86	1.44	m	13.85	13.54	13.73	37.68	1.65	m
4	12.08	11.83	11.98	39.69	1.52	m	12.05	13.90	12.76	41.64	1.40	m
5	11.16	13.18	11.97	44.24	1.26	m	11.86	11.44	11.70	37.38	1.67	m
6	11.99	12.90	12.36	41.94	1.38	m	11.47	12.08	11.70	39.44	1.53	m
7	10.95	12.43	11.55	43.28	1.31	m	11.99	9.89	11.19	33.78	1.95	sm
8	11.02	8.89	10.17	35.15	1.84	sm	9.84	11.30	10.40	41.53	1.40	m

No	*T. sinomongolicum* Kitag.						*T. heterolepis* Nakai et Koidz.ex Kitag.					
	Relative length (%)			CI	AR	CP	Relative length (%)			CI	AR	CP
	LL	SL	TL	(%)			LL	SL	TL	(%)		
1	10.34	10.11	10.22	35.23	1.24	m	10.21	10.02	10.11	34.65	1.55	m
2	11.12	10.97	11.04	37.61	1.26	m	11.69	10.39	11.04	39.23	1.94	sm
3	11.87	11.46	11.66	38.48	1.32	m	12.37	12.12	12.24	37.18	2.11	sm
4	13.18	13.12	13.15	37.74	1.78	sm	13.26	12.56	12.91	40.24	2.31	sm
5	12.43	12.17	12.32	40.14	1.26	m	13.78	14.38	14.08	40.97	1.52	m
6	12.64	12.93	12.78	41.37	1.49	m	14.43	15.21	14.82	39.44	1.87	sm
7	13.78*	14.36*	14.07	38.18	1.79	sm*	15.08*	16.31*	15.69	35.96	1.68	m*
8	14.64	14.88	14.76	35.64	1.82	sm	9.18	9.01	9.09	37.53	1.11	m

No	*T.brassicae folium* Kitag.						*T. platypecidum* Diels.					
	Relative length (%)			CI	AR	CP	Relative length (%)			CI	AR	CP
	LL	SL	TL	(%)			LL	SL	TL	(%)		
1	10.43	10.14	10.28	32.38	1.41	m	9.11	8.69	8.9	34.16	1.24	m
2	11.86	12.13	11.99	37.81	1.66	m	10.01	10.97	10.49	38.39	1.74	sm
3	12.45	13.39	12.92	38.45	2.28	sm	11.57*	11.94*	11.75	35.38	2.15	sm*
4	10.98	10.67	10.82	36.54	1.24	m	12.21	12.73	12.47	36.24	1.78	sm
5	11.27	11.68	11.47	40.26	1.29	m	13.06	13.15	13.11	38.72	1.97	sm
6	13.67	13.67	13.67	44.21	2.89	sm	13.94	13.68	13.81	36.92	2.53	sm
7	14.45	13.95	14.20	41.32	2.18	sm	14.69	14.26	14.48	40.19	1.72	sm
8	14.87*	14.37*	14.62	35.31	1.82	sm*	15.41	14.58	14.99	39.33	2.24	sm

No	*T. falcilobum* Kitag.						*T. borealisinense* Kitam.					
	Relative length (%)			CI	AR	CP	Relative length (%)			CI	AR	CP
	LL	SL	TL	(%)			LL	SL	TL	(%)		
1	10.06	9.87	9.97	32.67	1.21	m	9.16	9.01	9.08	33.86	1.63	m
2	10.98*	10.91*	10.95	35.12	1.86	sm*	13.94	13.61	13.77	42.91	1.54	m
3	11.42	11.47	11.45	36.48	1.63	m	10.98*	11.42*	11.2	37.47	2.16	m*
4	12.37	12.59	12.48	39.08	1.91	sm	12.02	12.43	12.23	40.86	1.48	m
5	12.68	13.28	12.98	40.43	1.86	sm	13.29	12.85	13.07	40.25	2.44	sm
6	13.53	13.52	13.52	42.26	1.83	sm	10.13	10.25	10.19	32.24	1.53	m
7	14.32	14.09	14.20	43.27	1.79	sm	14.56	14.92	14.74	42.36	1.78	sm
8	14.64	14.27	14.45	45.14	1.93	sm	15.92	15.51	15.71	45.13	1.92	sm

No	*T. erythopodium* Kitag.					
	Relative length (%)			CI	AR	CP
	LL	SL	TL	(%)		
1	10.13	10.25	10.19	38.13	2.27	sm
2	11.27	11.55	11.41	40.25	2.05	sm
3	12.16	11.43	11.79	38.22	1.96	sm
4	13.74	12.42	13.08	37.68	1.87	sm
5	13.89	14.26	14.08	42.22	1.61	m
6	14.54	15.74	15.14	41.79	1.73	sm
7	14.15*	15.64*	14.89	38.42	1.31	m*
8	10.12	8.71	9.42	34.31	1.98	sm

Resource: LL: long arm length; SL: short arm length; TL: total length; CI: centromeric index; AR: arm ratio; CP: position of centromere; *Chromosome with satellite, whose length was not included in the chromosome length.

Table.3. Karyotype comparison of 18 *Taraxacum*

Species	A.A.R	Lt/St	P.C.A	As.k (%)	Karyotype	Satellite number	Karyotype formula
T. antungense Kitag.	1.61	1.54	0	61.32%	1A	3	2n = 3x = 12 m + 3 sm(3SAT) + 9sm
T. asiaticum Dahlst.	1.50	2.03	12.5	59.98%	1A	3	2n = 3x = 3m (3SAT) + 18 m + 3 sm
T. urbanum Kitag.	1.61	1.65	0	61.43%	1A	3	2n = 3x = 12 m + 3 sm(3SAT) + 9sm
T. ohwianum Kitag.	1.78	1.82	0.25	63.81%	2A	3	2n = 2x= 2m(SAT) + 6m + 8sm
T. variegatum Kitag.	1.78	1.62	0.37	64.02%	2A	0	2n =4x = 12m + 20sm
T. asiaticum var. lonchophyllum Kitag.	1.49	1.62	0	59.68%	1A	3	2n = 3x = 3m(3SAT) + 18 m + 3 sm
T. junpeianum Kitam. In Act.	1.70	1.49	0.25	61.63%	2A	3	2n = 3x = 3m(SAT) + 9m + 12sm
T. mongolicum Hand.-Mazz.	1.56	1.43	0	66.96%	1A	0	2n = 4x = 24m + 8sm
T. liaotungense Kitag.	1.59	1.59	0	64.17%	1A	0	2n = 4x = 24m + 8sm
T. coreanum Nakai	1.49	1.56	0	59.80%	1A	0	2n = 4x = 24m + 8sm
T. formosanum Kitam.	1.61	1.40	0	62.77%	1A	0	2n = 4x = 24m + 8sm
T. sinomongolicum Kitag.	1.49	1.83	0	60.18%	1A	3	2n = 3x = 15m + 6sm+3sm□3SAT□
T. heterolepis Nakai et Koidz. ex Kitag.	1.76	2.41	0.25	67.34%	2B	3	2n = 3x = 9m +3m (3SAT)+ 9sm
T. brassicae folium Kitag.	1.85	1.97	0.37	65.32%	2A	3	2n = 3x = 9m +3m (3SAT)+ 9sm
T. platypecidum Diels	1.92	2.14	0.37	66.41%	2B	3	2n = 3x = 3m +3sm(3SAT) +18sm
T. falcilobum Kitag.	1.75	2.27	0	61.18%	1B	3	2n = 3x = 6m +3sm(3SAT) +15sm
T. borealisinense Kitam.	1.81	2.49	0.25	61.67%	2A	3	2n = 3x = 9m +3sm(3SAT) +12sm
T. erythopodium Kitag.	1.84	2.18	0.25	67.45%	2B	3	2n = 3x = 3m(SAT) + 3m + 18sm

Resource: A.A.R: average arm ratio; Lt/St: ratio of the longest chromosome to the shortest; P.C.A: ratio of long arms to short arms is greater than 2; As.k(%): karyotype asymmetry coefficient.

T. mongolicum Hand.-Mazz.

The chromosome number for this species was $2n = 4x = 32$. The karyotype formula was K($2n$) = $4x$ = 24 m + 8 sm. The absolute length varied between 16.32 to 25.83 µm. The arm ratio varied between 1.25 to 1.92, and the average arm ratio was 1.56. The longest: shortest chromosome ratio was 1.43. None of the chromosomes had an arm ratio greater than 2. The karyotype asymmetry coefficient was 66.96%, which corresponds to 1A karyotype. Satellite chromosomes were absent. The 3 and 7 pairs of chromosomes were of sm type, and the remaining six pairs were of m type. The morphological characteristics of the chromosomes are shown in Table 2 and Figs. 2 and 3.

T. liaotungense Kitag.

The chromosome number for this species was $2n = 4x = 32$. The karyotype formula was K($2n$) = $4x$ = 24 m + 8 sm. The absolute length varied between 16.78 to 25.21 µm. The arm ratio varied between 1.24 to 1.98, and the average arm ratio was 1.59. The longest: shortest chromosome ratio was 1.59. None of the chromosomes had an arm ratio greater than 2. The karyotype asymmetry coefficient was 64.17%, which corresponds to 1A karyotype. Satellite chromosomes were absent. The 1 and 8 pair of chromosomes was of sm type, and the remaining six pairs were of m type. The morphological characteristics of the chromosomes are shown in Table 2 and Figs. 2 and 3.

T. coreanum Nakai.

The chromosome number for this species was $2n = 4x = 32$. The karyotype formula was K($2n$) = $4x$ = 24 m + 8 sm. The absolute length varied between 18.56 to 28.32 µm. The arm ratio varied between 1.31 to 1.91, and the average arm ratio was 1.49. The longest: shortest chromosome ratio was 1.56. None of the chromosomes had an arm ratio greater than 2. The karyotype asymmetry coefficient was 59.80%, which corresponds to 1A karyotype. Satellite chromosomes were absent. The 1 and 7 pair of chromosomes was of sm type, and the remaining six pairs were of m type. The morphological characteristics of the chromosomes are shown in Table 2 and Figs. 2 and 3.

T. formosanum Kitam.

The chromosome number for this species was $2n = 4x = 32$. The karyotype formula was K($2n$) = $4x$ = 24 m + 8 sm. The absolute length varied between 17.21 to 27.64 μm. The arm ratio varied between 1.40 to 1.95, and the average arm ratio was 1.61. The longest: shortest chromosome ratio was 1.40. None of the chromosomes had an arm ratio greater than 2. The karyotype asymmetry coefficient was 62.77%, which corresponds to 1A karyotype. Satellite chromosomes were absent. The 1 and 7 pair of chromosomes was of sm type, and the remaining six pairs were of m type. The morphological characteristics of the chromosomes are shown in Table 2 and Figs. 2 and 3.

T. sinomongolicum Kitag.

The chromosome number for this species was $2n = 3x = 24$. The karyotype formula was K($2n$) = $3x$ = 15m + 6sm + 3sm (3SAT). The absolute length varied between 6.32 to 9.24 μm. The arm ratio varied between 1.24 to 1.82, and the average arm ratio was 1.49. The longest: shortest chromosome ratio was 1.83. None of the chromosomes had an arm ratio greater than 2. The karyotype asymmetry coefficient was 60.18%, which corresponds to 1A karyotype. Satellite chromosomes were absent. Among them, chromosome 7 has a satellite chromosome (SAT)，the 4, 7, 8 pair of chromosomes was of sm type, and the remaining five pairs were of m type. The morphological characteristics of the chromosomes are shown in Table 2 and Figs. 2 and 3.

T. heterolepis Nakai et Koidz.ex Kitag.

The chromosome number for this species was $2n = 3x = 24$. The karyotype formula was K($2n$) = $3x$ = 9m +3m (3SAT) + 9sm. The absolute length varied between 6.25 to 9.56 μm. The arm ratio varied between 1.11 to 2.31, and the average arm ratio was 1.76. The longest: shortest chromosome ratio was 2.41. The chromosomes had an arm ratio greater than 2 ratio 25%. The karyotype asymmetry coefficient was 67.34%, which corresponds to 2B karyotype. Satellite chromosomes were absent. Among them, chromosome 7 has a satellite chromosome (SAT)，the 2, 3, 4, 6 pair of chromosomes was of sm type, and the remaining four pairs were of m type. The morphological characteristics of the chromosomes are shown in Table 2 and Figs. 2 and 3.

T. brassicae folium Kitag.

The chromosome number for this species was $2n = 3x = 24$. The karyotype formula was K($2n$) = $3x$ = 9m +3m (3SAT) + 9sm. The absolute length varied between 4.97 to 9.32 μm. The arm ratio varied between 1.24 to 2.98, and the average arm ratio was 1.85. The longest: shortest chromosome ratio was 1.97. The chromosomes had an arm ratio greater than 2 ratio 37%. The karyotype asymmetry coefficient was 65.32%, which corresponds to 2A karyotype. Satellite chromosomes were absent. Among them, chromosome 8 has a satellite chromosome (SAT)，the 1, 2, 4, 5 pair of chromosomes was of m type, and the remaining four pairs were of sm type. The morphological characteristics of the chromosomes are shown in Table 2 and Figs. 2 and 3.

T. platypecidum Diels.

The chromosome number for this species was $2n = 3x = 24$. The karyotype formula was K($2n$) = $3x$ = 3m +3sm (3SAT) +18sm. The absolute length varied between 5.27 to 10.67 μm. The arm ratio varied between 1.24 to 2.53, and the average arm ratio was 1.92. The longest: shortest chromosome ratio was 2.14. The chromosomes had an arm ratio greater than 2 ratio 37%. The karyotype asymmetry coefficient was 66.41%, which corresponds to 2B karyotype. Satellite chromosomes were absent. Among them, chromosome 3 has a satellite chromosome (SAT)，the 1 pair of chromosomes was of m type, and the remaining seven pairs were of sm type. The morphological characteristics of the chromosomes are shown in Table 2 and Figs. 2 and 3.

T. falcilobum Kitag.

The chromosome number for this species was $2n = 3x = 24$. The karyotype formula was K($2n$) = $3x$ = 6m +3sm (3SAT) +15sm. The absolute length varied between 6.25 to 12.47 μm. The arm ratio varied between 1.21 to 1.93, and the average arm ratio was 1.75. The longest: shortest chromosome ratio was 2.27. None of the chromosomes had an arm ratio greater than 2. The

karyotype asymmetry coefficient was 61.18%, which corresponds to 1B karyotype. Satellite chromosomes were absent. Among them, chromosome 2 has a satellite chromosome (SAT)，the 1, 3 pair of chromosomes was of m type, and the remaining six pairs were of sm type. The morphological characteristics of the chromosomes are shown in Table 2 and Figs. 2 and 3.

T. borealisinense **Kitam.**

The chromosome number for this species was $2n = 3x = 24$. The karyotype formula was K($2n$) = $3x$ = 9m +3sm (3SAT) +12sm. The absolute length varied between 6.00 to 10.68 μm. The arm ratio varied between 1.53 to 2.44, and the average arm ratio was 1.81. The longest: shortest chromosome ratio was 2.49. The chromosomes had an arm ratio greater than 2 ratio 25%. The karyotype asymmetry coefficient was 61.63%, which corresponds to 2A karyotype. Satellite chromosomes were absent. Among them, chromosome 3 has a satellite chromosome (SAT)，the 5, 7, 8 pair of chromosomes was of sm type, and the remaining five pairs were of m type. The morphological characteristics of the chromosomes are shown in Table 2 and Figs. 2 and 3.

T. erythopodium **Kitag.**

The chromosome number for this species was $2n = 3x = 24$. The karyotype formula was K($2n$) = $3x$ = 3m (SAT) + 3m + 18sm. The absolute length varied between 5.27 to 8.69 μm. The arm ratio varied between 1.31 to 2.27, and the average arm ratio was 1.84. The longest: shortest chromosome ratio was 2.18. The chromosomes had an arm ratio greater than 2 ratio 25%. The karyotype asymmetry coefficient was 67.45%, which corresponds to 2B karyotype. Satellite chromosomes were absent. Among them, chromosome 7 has a satellite chromosome (SAT)，the 5, 7 pair of chromosomes was of m type, and the remaining six pairs were of sm type. The morphological characteristics of the chromosomes are shown in Table 2 and Figs. 2 and 3.

PCA of karyotype-related data

The karyotype indexes obtained by karyotype analysis of the 18 species were subjected to PCA. We found that the relative total length, relative long arm length, and relative short arm length of the chromosomes formed principal component (PC) 1. In other words, the size of chromosomes was the main factor for interspecific classification. The kinetochore index, percentage with arm ratio greater than 2, and average arm ratio contributed to PC2, indicating that the position of horizontal kinetochore splitting determined the interspecific differences among *Taraxacum* chromosomes. The remaining traits dominated PC3. There were substantial differences in total chromosome length, total long arm length, and total short arm length among diploid, triploid, and tetraploid species, which explains the contribution of the indexes related to chromosome length to the PCs.

Cluster analysis of 18 *Taraxacum* species

The cluster analysis using karyotype data of the 18 species grouped the taxa based on their chromosome numbers and ploidy levels into three clusters, a diploid, a triploid, and a tetraploid clade. The diploid *T. ohwianum* was located between triploid and tetraploid species. Based on genetic distance, it was closer to the triploid species, and its rescaled distance threshold was between 10 and 15. Among the tetraploid species, *T. coreanum* formed a separate clade in the cluster topology, distant from other tetraploid species, confirming its independent species status. This may be related to the white color of *T. coreanum*, which differentiates this species from other *Taraxacum* species. The genetic distance among many triploid species was very small, supporting the interspecific merging of *T. urbanum* into *T. antungense*, *T. asiaticum* var*. lonchophyllum* into *T. asiaticum*, and the inclusion of *T. formosanum* and *T. liaotungense* into *T. mongolicum* in the *Flora of China*. In addition, the cluster analysis results supported the formation of a single group comprising *T. sinicum*, *T. asiaticum*, *T. lamprolepis*, *T. antungense*, *T. heterolepis*, and *T. stenolobum*, which are listed as valid species in the *Flora of China*, i.e., the division of Sect. 3 Sinensia V. Soest.

Discussion

Cytological evidence for taxonomic identification of *Taraxacum*

Levan suggested that chromosome morphology, which he termed karyotypes, corresponds to taxonomic units [22]. Accordingly, biological populations can be roughly delimited using karyotypes as a unit for systematics and taxonomy. During PCA of *Taraxacum* karyotype data, we found that the total length of the chromosome is PC1. This is related to plant evolution, and there is a consensus that changes in chromosome length, which have been clearly indicated, are the main evolutionary trend. We found that the length of dandelion chromosomes changes and that the tetraploid *Taraxacum* shows slow evolution [23]. From cytological analysis of the 18 *Taraxacum* species distributed in Northeast China, we found that karyotype analysis of *Taraxacum* could be used as the basis for taxonomy of *Taraxacum*. The results of cluster analysis of *Taraxacum* karyotype were consistent with the classification results of *Flora of China*, supporting interspecific merges of some *Taraxacum* distributions in northeast China. Thus, this study provides cytological evidence for the classification of *Taraxacum.*

The cytological analysis of the 18 *Taraxacum* species distributed in Northeast China identified diploid, triploid, and tetraploid taxa, with chromosome numbers of 2*n* = 16, 24, and 32, respectively. PCA of the karyotype indexes of the 18 species showed that PC1 was associated with the total long arm length, total short arm length, and total chromosome length. This clearly indicates the evolutionary trend leading to changes in chromosome length in this genus [24,25].

The comparison of karyotype data of the 18 dandelion species obtained in this study with those available in the CCDB verified that karyotype information of 11 dandelion species has been reported for the first time herein. In addition, the chromosome numbers in this study for previously identified species were incongruous with those in previous studies. Plant chromosomes, while stable, can produce different types of heritable variations under certain conditions, which is conducive to increasing species diversity and adaptability. Therefore, the differences in chromosome number among *Taraxacum* species may be caused by genetic variations, unique reproductive modes (facultative parthenogenesis) [26], or result from misidentification of *Taraxacum* germplasm resources, the specific reasons for which are yet to be determined. Only on the basis of correct interspecific identification of *Taraxacum* plants can the corresponding species be developed and maximally utilized, thus, providing basic germplasm characteristics for optimal application of *Taraxacum* resources.

Karyotype evolutionary trends of dandelion chromosomes and their application

Studies have shown that chromosome shortening is the main cause of karyotype evolution in taxonomic systems. Older species and genera in a taxonomic system have longer chromosomes, while newer species and genera have shorter chromosomes. Karyotypes gradually change among species and genera. They are not static and can be replaced by genomic units, which corresponds to the genome theory in genetics [27]. The basic trend in karyotype evolution in higher plants is from a symmetrical to asymmetrical karyotype. Plants that are relatively old or evolutionarily primitive often harbor relatively symmetrical karyotypes, whereas asymmetrical karyotypes usually appear in more evolved or specialized plants [28]. The karyotypes of the 18 dandelion species were asymmetric, with the asymmetry coefficients approximately in the range of 60%–70%, i.e., they belonged to relatively evolved species. This may be related to the reproductive modes of *Taraxacum* species. Among the 18 species studied, *T. erythropodium* and *T. heterolepis*, both having the highest karyotype asymmetry coefficients, are triploid. Therefore, we conclude that triploid *Taraxacum* species are more evolved than diploid and tetraploid species and are richer in species. This is also consistent with the *Flora of China*, according to which “many *Taraxacum* species often form new plants without syngamy or natural hybridization. However, there is no gene exchange among these apomictic individuals, which complicates the determination of their biological species and identification of species boundaries.”

The length and centromere position of homologous chromosomes are relatively constant among different chromosome pairs and different species, which can reflect the genetic stability of the species. However, the number, morphology, and structure of chromosomes, the main carriers of genetic material, may vary owing to interference and destruction by

specific genetic and environmental factors. Therefore, the results of karyotype analysis of *Taraxacum* chromosomes may represent the cytological characteristics of a certain germplasm resource in a certain period. This may explain the differences in chromosome numbers for *Taraxacum* species in the CCDB. However, this characteristic is of importance for protecting the genetic diversity and promoting the evolution of *Taraxacum* species.

Conclusion

Karyotype analysis was carried out on 18 *Taraxacum* species distributed in Northeast China. We found that cytological karyotype analysis can be used as cytological evidence for plant taxonomy. The main factor affecting the karyotype analysis of this genus was chromosome size. We analyzed the karyotype data of 18 species by cluster analysis, and the results supported the classification and clustering of *Taraxacum* germplasms as reported in the *Flora of China*. We believe that the cytological results of karyotype characteristics presented in this study provide the basis for future research of apomixis in *Taraxacum*. Furthermore, using fixed heterosis, we can complete the screening for high-quality dandelion germplasm resources and provide cytological evidence for *Taraxacum* breeding.

Abbreviations

RL: relative length; LL: long arm length; SL: short arm length; TL: total length; CI: centromeric index; AR: arm ratio; CP: position of centromere; A.A.R: average arm ratio; Lt/St: ratio of the longest chromosome to the shortest; P.C.A: ratio of long arms to short arms is greater than 2; As.k(%): karyotype asymmetry coefficient; SAT: satellite chromosome; CCDB: Chromosome Counts Database; PCA: Principal component analysis; LMH: Liaoning Medicinal Herbarium; SYAU: Shenyang agricultural University; JZMU, JZKY: Jinzhou Medical University; EWVG: Exsitu Conservation Garden Evaluation; FRPS: Flora Reipublicae Popularis Sinicae

Declarations

Author Contributions

Conceptualization Writing – review & editing, Jie Wu; Formal analysis Yanping Xing; Methodology, Ran Li, Wei Ning, Wei Cao; Software, Qun Liu, Jing Li and Yuefei Li; Writing – original draft, Jie Wu.

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Not applicable.

Competing interests

The authors declare that they have no competing interests.

Availability of data and materials

All data generated or analyzed during the course of this study are included in this document or obtained from the appropriate author(s) at reasonable request.

Consent for publication

Not applicable.

Ethics approval and consent to participate

Not applicable.

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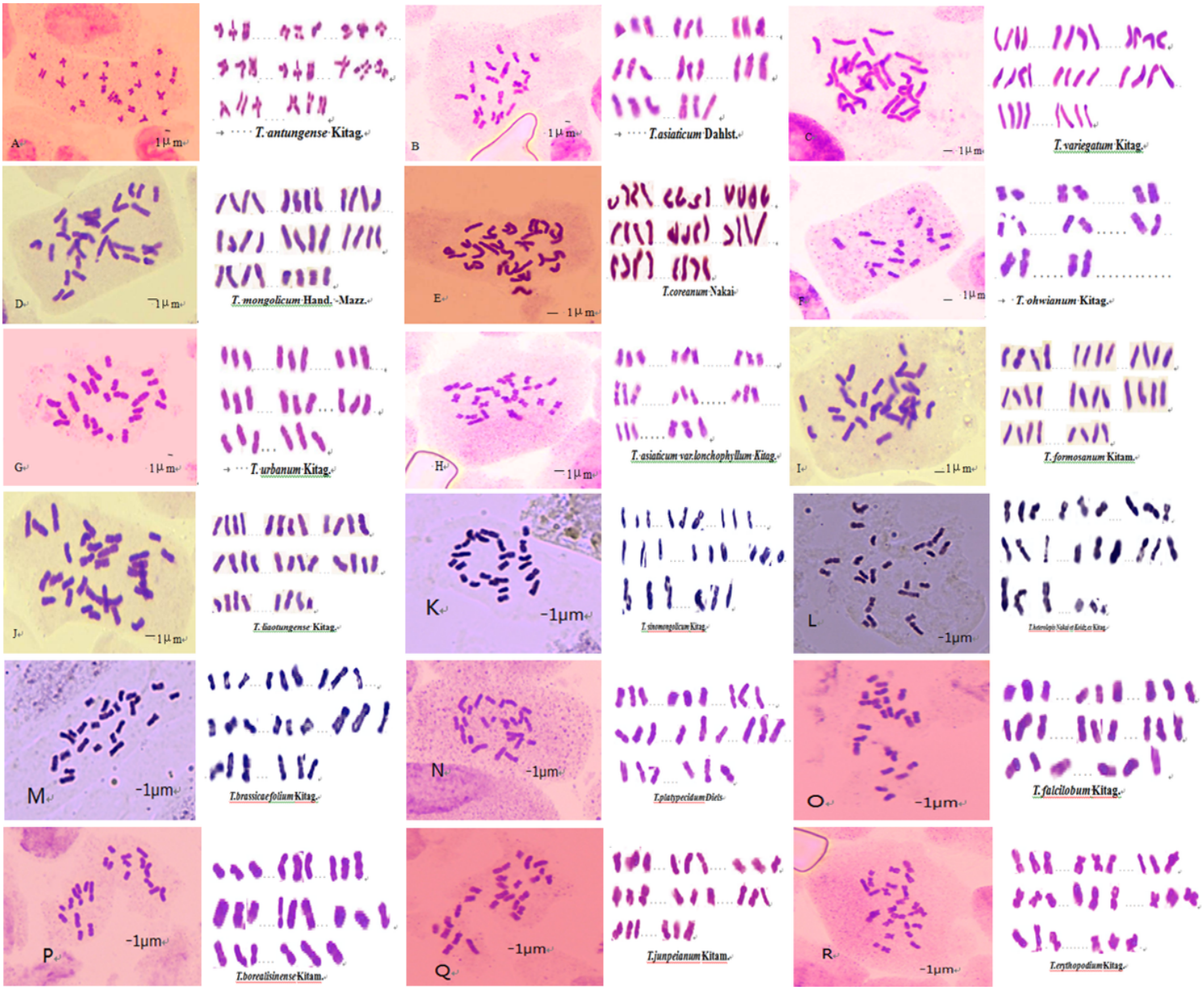
Figures


A
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T. antungense Kitag.
B
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T. asiaticum Dahlst.
C
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T. variegatum Kitag.
D
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T. mongolicum Hand.-Mazz.
E
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T. ohwianum Kitag.
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P
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Q
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T. junpeianum Kitam.
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T. erythropodium Kitag.

Figure 1


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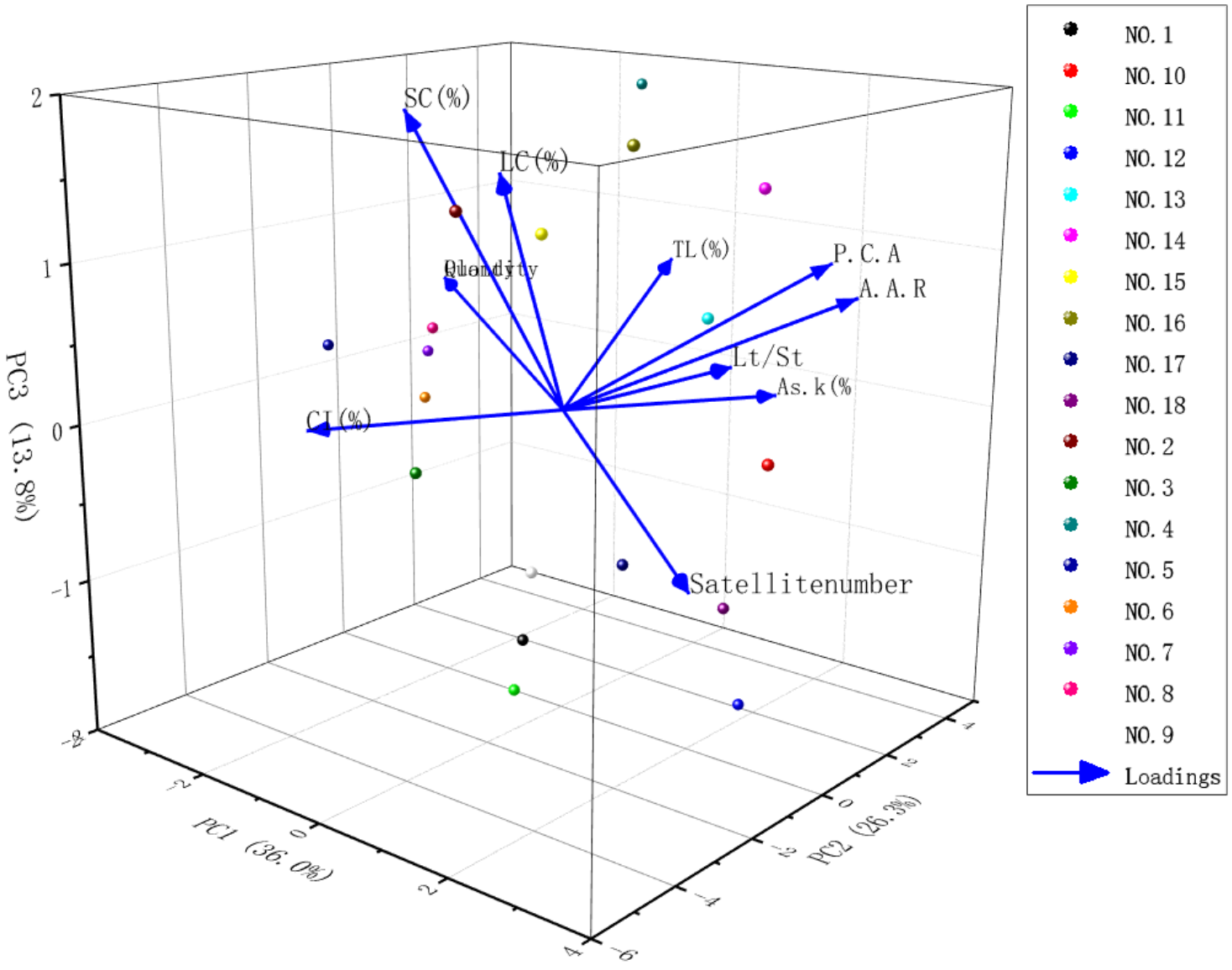
Figure 2


SC (%)
LC (%)
TL (%)
P. C. A
A. A. R
Lt/St
As. k (%
CI (%)
Satellitenumber
PC3 (13.8%)
PC1 (36.0%)
PC2 (26.3%)
NO. 1
NO. 10
NO. 11
NO. 12
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NO. 14
NO. 15
NO. 16
NO. 17
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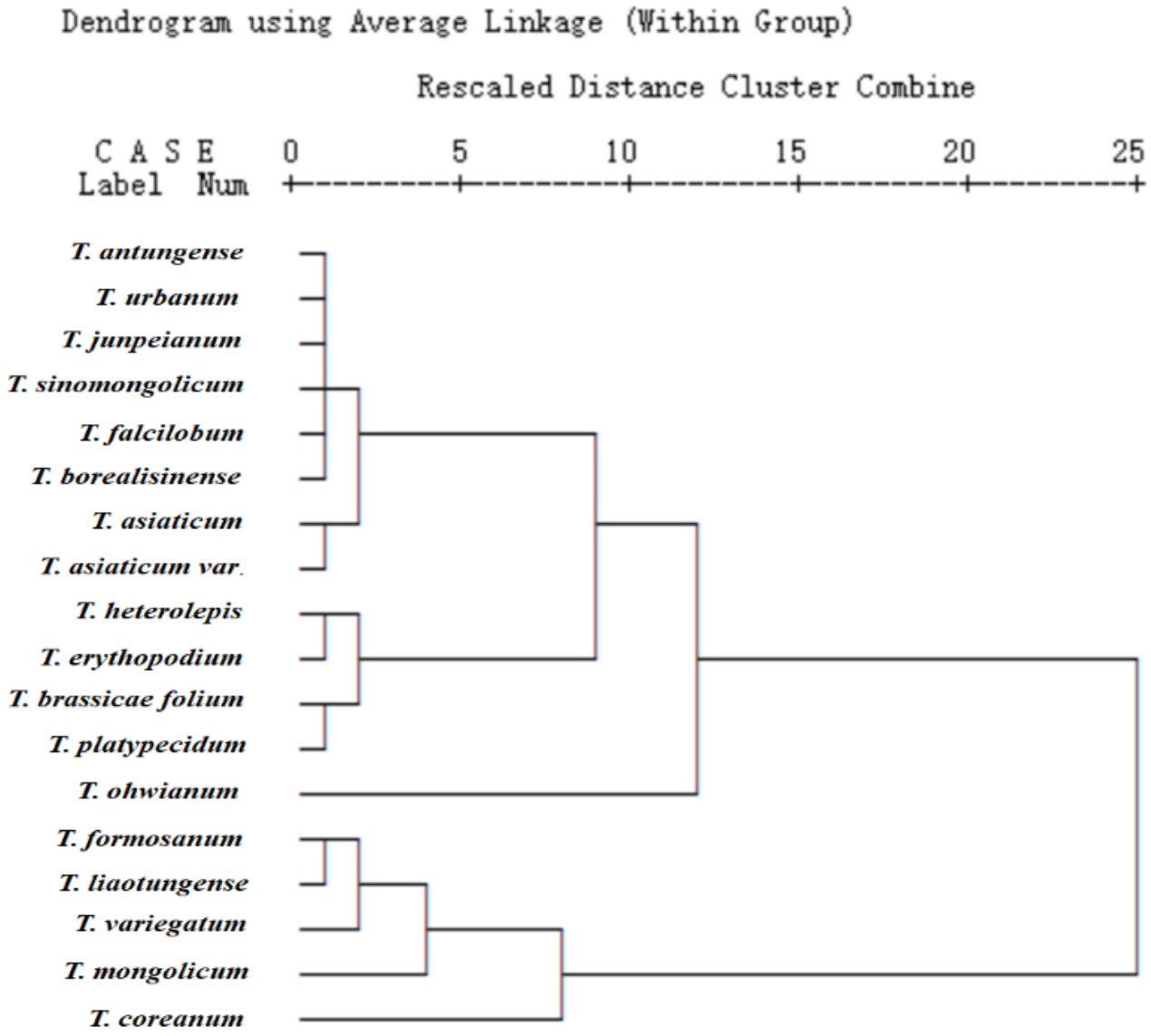
Loadings

Figure 3


Dendrogram using Average Linkage (Within Group)
Rescaled Distance Cluster Combine
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25
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T. urbanum
T. junpeianum
T. sinomongolicum
T. falcilobum
T. borealisinense
T. asiaticum
T. asiaticum var.
T. heterolepis
T. erythopodium
T. brassicae folium
T. platypecidum
T. ohwianum
T. formosanum
T. liaotungense
T. variegatum
T. mongolicum
T. coreanum

Figure 4

Figure 5

Pictures of the 18 taxa of northeast China native Taraxacumspecies.

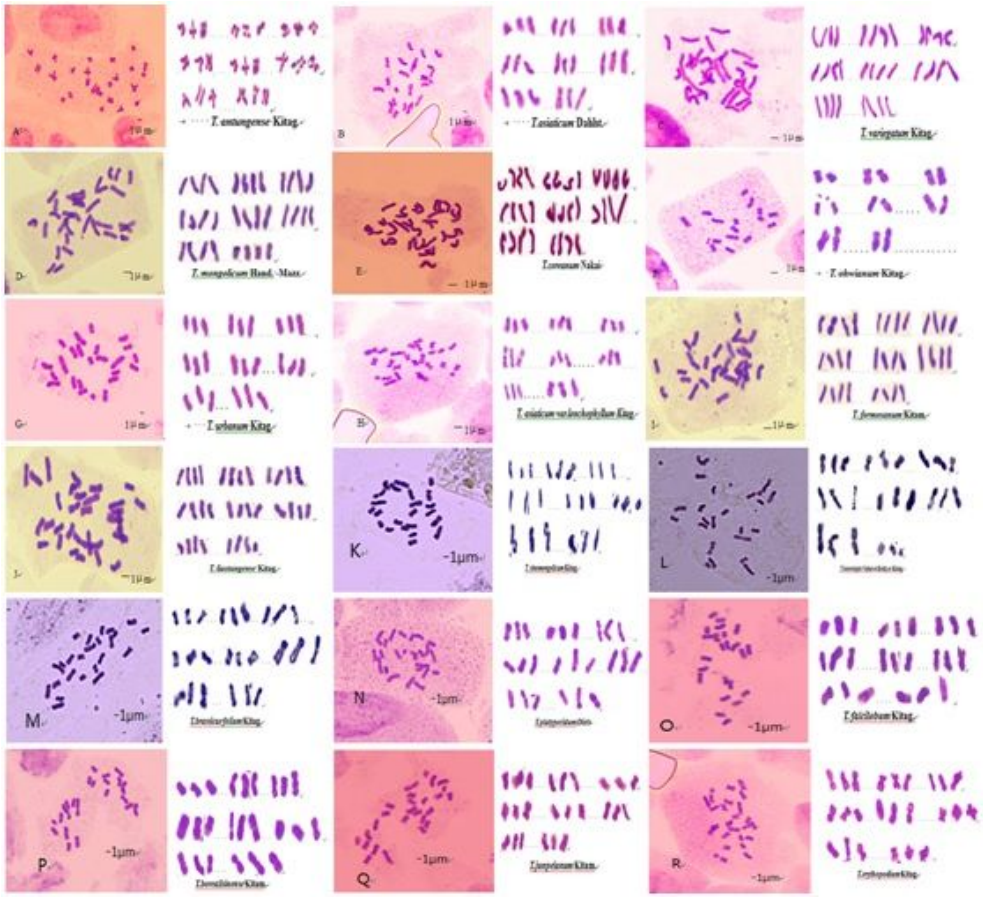

Figure 6

Chromosomes of cells of 18 Taraxacum

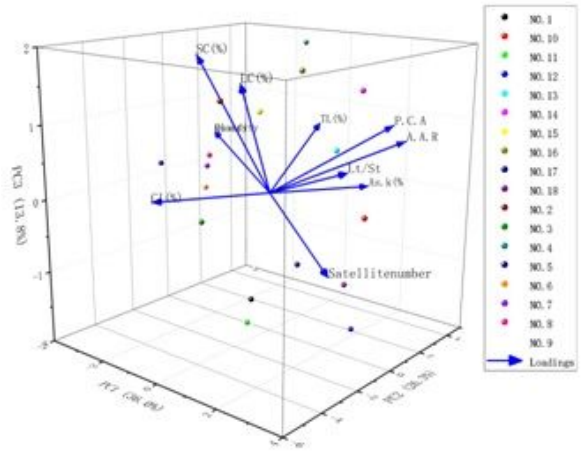

Figure 7

Principal component analysis (PCA) of 18 Taraxacumkaryotype data

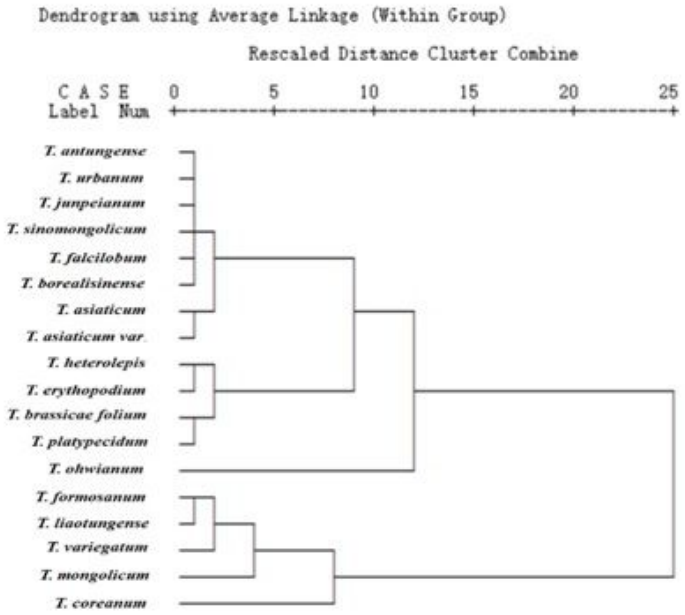

Figure 8

Cluster analysis chart of 18 Taraxacumkaryotype data

Supplementary Files

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