Evaluation and Identification of Morphological Characters Suitable for Delimitation of *Taraxacum* Species Distributed in Northeastern China

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Abstract

Background The genus *Taraxacum* F.H. Wigg. (fam. *Compositae*, subfam. *Liguliflorae*) includes about 300 species globally. Seventy-nine are widely distributed throughout China, mostly in southwestern and northwestern regions. The genus has adopted different reproductive strategies, and there is weak reproductive isolation and differentiation between species. Unresolved species boundaries make classification and identification of *Taraxacum* species is difficult. *Taraxacum* germplasm resources in northeastern China are not current and therefore, do not accurately reflect actual distribution. The objective of this study was to investigate the morphological traits of *Taraxacum* species distributed in northeastern China and identify those that will facilitate classification of *Taraxacum* species in this region.

Methods Leaf, flower, and achene characteristics of 18 species were used for morphological classification. Scanning electron microscopy was used to examine pollen morphology. Leaf extracts were analyzed by high performance liquid chromatography to measure the caffeic acid, chlorogenic acid, and luteolin content. Internal transcribed spacer (ITS) sequences were used to determine the sequence pairwise differences among the species and their utility in delimitation.

Results The taxa were classified into groups based on morphology. Beak length to achene length ratio was a new taxonomically informative morphological trait for delimitation. The ITS sequence analysis supported the classification of the taxa, but genetic distances among the taxa did not reflect morphological differences. Phylogenetic analysis resolved the eighteen species into three groups. Group I: *T. coreanum*, (which has white flowers). Group II: *T. heterolepis*, *T. sinomongolicum*, *T. variegatum*, *T. asiaticum*, *T. lonchophyllum*, *T. falcilobum*, *T. brassicaefolium*, and *T. erythropodium*. Group III: *T. formosanum*, *T. liaotungense*, *T. mongolicum*, *T. borealisinense*, *T. ohwianum*, *T. platyepidum*, *T. urbanum*, *T. antungense*, *T. asiaticum*, and *T. junpeianum*. The chemical composition of the species is not suitable for their delimitation; *T. antungense* is a potential medicinal plant with therapeutic properties since it had the highest concentration of phytochemicals.

Conclusions This study completes the classification and delimitation of the *Taraxacum* germplasm resources present in northeastern China and supplements the "*Flora of China*". The study thus provides information that will help to further develop *Taraxacum* medicinal resources and regulate *Taraxacum* medicinal use in traditional Chinese medicine.

1. Background

The genus *Taraxacum* F.H. Wigg in the aster family (*Compositae*) includes More than 2,500 species: mainly in the Arctic and temperate zones of the N Hemisphere with main diversity in mountains of Eurasia, a few species in temperate regions of the S Hemisphere; 116 species (81 endemic, 3 introduced) in China[1]. "*Flora of China*" states that of those, approximately 11 species are recorded in northeastern China. "*Flora Plantarum Herbacearum Chinae Boreali-Oientalis*" [2, 3] divided the genus dandelion distributed in northeast China into 19 species, 1 variety, and 3 variants. The interaction between genetic variation and the environment produces morphological variation in *Taraxacum* and formation of agamospermous complexes [4]. Many species of *Taraxacum* are apomictic, and some asexual genotypes may be more predisposed to undergo genomic changes than other asexual genotypes [5]. Their various ploidy levels [6] and intraspecific morphological variation [7] complicates the classification of *Taraxacum* species.

Previous studies have employed the morphological characters related to achene shape and color, overall achene length, achene beak length [8–10], leaf shape, leaf length, and leaf color [11] to distinguish between *Taraxacum* species. Several taxonomic studies have characterized the morphological variations in leaves, flowers, and roots, examined genetic characteristics based on random amplified polymorphic, analyzed somatic chromosomes and karyotypes, and reported chemical composition [12–15] in species from China. Principal component analysis (PCA) and cluster analysis have been used to assess morphological variation within and between species in many other plant genera and to delimit species [16].

The internal transcribed spacer (ITS) region of the nuclear ribosomal DNA consists of ITS-1, the 5.8S gene, and ITS-2. The region has been widely used in molecular phylogenetic analyses of many plant taxa such as *Asarum sieboldii* Miq. *Withania somnifera* (L.) Dunal., *Convolvulus prostrates* Linn, and *Evolvulus alsinoides* (L.) L. var. [17–20] because of its small size (± 700 base pairs), highly conserved flanking regions, high copy number, high mutation rate of ITS-1 and ITS-2, and rapid concerted evolution. In addition, a conserved 14 bp motif (5'-GAA TTG CAG AAT CC-3') was found in the 5.8S gene. This motif is useful to differentiate between flowering plants and other plant groups such [21–27]. Jan Kirschner revised the classification of dandelion in central Asia by using color digital pictures to compare with the known characteristics of dandelions, which is an important advancement in the classification of dandelions in central Asia. The article describes in detail each dandelion's morphological characteristics, origin, habits, and has a digital image to define each species of dandelion. It is convenient see the comparison research merged with existing text classification information for central Asia dandelion. It also points out the advantages and disadvantages of current classifications, in order to further important dandelion plant taxonomic study [28].

Taraxacum species native to northeastern China have garnered a lot of attention as important medicinal and ornamental plants. Chlorogenic acid, caffeic acid, and luteolin are the most important secondary metabolites in *Taraxacum*, and they have diuretic, antioxidant, cholagogic, antirheumatic, anti-allergic, anti-inflammatory, analgesic, anticoagulant, antibiotic, choleric, angiogenic, and anticarcinogenic properties [29–31]. The study aimed to categorize 18 *Taraxacum* taxa from northeastern China based on DNA data and cluster analysis of morphological characteristics and test whether the latter support the molecular-based phylogeny.

2. Materials And Methods

2.1 Plant materials and growing conditions

The flora of northeastern China harbors 18 native *Taraxacum* taxa, which are defined based on the leaf margin, flower color, shape and texture of outer bracts, shape of inner bracts, achene length, and other characters. Their distribution in this region is shown in Fig. 1. The 18 taxa were collected from their natural habitats from April to October each year from 2011 to 2018; more than 30 individuals were collected for each taxon in total. (Table 1 and FigS1.). The classification and nomenclature of *Taraxacum* species were mainly in accordance with the Flora Plantarum Herbacearum Chinae Boreali-Oientalis [3]. Voucher specimens were deposited in the Liaoning Medicinal Herbarium at Shenyang Agricultural University and Jinzhou Medical University, in the Exsitu Conservation Garden Evaluation Centre of Wild Vegetable Germplasm in Northeast China, Ministry of Agriculture. Individual plants were grown in the greenhouse (41°49'N, 123°33'E, altitude 74 m above sea level). The plants were collected at anthesis, processed into herbarium specimens, and deposited in JZKY. Five individuals from each taxon were randomly selected and assessed for morphological characteristics.

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

No.	Latin	Population name	Origin	Voucher
A	<i>T. antungense</i> Kitag.	Dandong dandelion	Dandong City, Liaoning province	001(JZKY)
B	<i>T. asiaticum</i> Dahlst.	Yazhou dandelion	Jilin City, Jilin Province	002(JZKY)
C	<i>T. variegatum</i> Kitag.	Banye dandelion	Dandong City, Liaoning Province	003(JZKY)
D	<i>T. mongolicum</i> Hand.-Mazz.	Menggu dandelion	Chifeng City, Neimengu province	004(JZKY)
E	<i>T. coreanum</i> Nakai.	Chaoxian dandelion	Shenyang City, Liaoning province	005(JZKY)
F	<i>T. ohwianum</i> Kitag.	Dongbei dandelion	Heihe City, Heilongjiang province	006(JZKY)
G	<i>T. urbanum</i> Kitag.	Juanbao dandelion	Changchun City, Jilin province	007(JZKY)
H	<i>T. asiaticum</i> var. <i>lonchophyllum</i> Kitag.	Xiajipian dandelion	Chaoyang City, Liaoning province	008(JZKY)
I	<i>T. formosanum</i> Kitam.	Taiwan dandelion	Tongliao City, Neimengu province	009(JZKY)
J	<i>T. liaotungense</i> Kitag.	Liaodong dandelion	Songyuan City, Jilin province	010(JZKY)
K	<i>T. sinomongolicum</i> Kitag.	Tujian dandelion	Yichun City, Heilongjiang province	011(JZKY)
L	<i>T. heterolepis</i> Nakai et Koidz.ex Kitag.	Yibao dandelion	Sipin City, Jilin province	012(JZKY)
M	<i>T. brassicae folium</i> Kitag.	Jieye dandelion	Anshan City, Liaoning province	013(JZKY)
N	<i>T. platyepidum</i> Diels.	Baiyuan dandelion	Tongliao City, Neimengu province	014(JZKY)
O	<i>T. falcilobum</i> Kitag.	Xingan dandelion	Yichun City, Heilongjiang province	015(JZKY)
P	<i>T. borealisinense</i> Kitam.	Hua dandelion	Songyuan City, Jilin province	016(JZKY)
Q	<i>T. junpeianum</i> Kitam.	Changchun dandelion	Shenyang City, Liaoning province	017(JZKY)
R	<i>T. erythopodium</i> Kitag.	Honggeng dandelion	Xinganmeng City, Neimengu province	018(JZKY)

Resource: investigated on location by authors.

2.2 Measurement of morphological characteristics

Morphological traits that distinguish species of *Taraxacum*, both vegetative and reproductive, were selected based on the treatment of the genus by Ge et al. [32–35]. Plant height was measured from the highest vertical point (stem or leaf apex) to the root tip. The largest leaves with normal morphological appearance were selected for measurements. Leaf length was measured from the lamina tip to its base, excluding the petiole. Leaf width was measured across the widest point. Leaf thickness was measured across the thickest part. The shape, size, color, and ornamentation characteristics of achenes, (e.g., the shape and amount of ornamentation) were observed on the fruit wall. The shape and size of the coracoid base and the transition of the fruit body to the coracoid base were constricted suddenly or contracted gradually; beak thickness and length; the color of the crested hair, beak length ratio (BL /AL: beak base length achene length)[36–39].

Pollen morphology was examined with a scanning electron microscopy (SEM; KYKY 10000B; Science Instrument Company, Beijing, China) in natural mode then all discs were observed with SEM (KYKY SBC-12I ion sputter coater). Pollen size (polar length [P], equatorial diameter [E], and P/E ratio) was calculated with Motic Images Advanced 3.2 software (Motic, Hong Kong, China). Size measurements were based on 20 pollen grains. Pollen grains underwent acetolysis [40] and were mounted in glycerin jelly. Mature achenes were collected from each species two months after flowering and photographed with a camera (Moficam 2206, Olympus, Japan) attached to a light microscope.

2.3 High Performance Liquid Chromatography (HPLC) analysis for chlorogenic acid, caffeic acid, and luteolin

Chlorogenic acid and caffeic acid were acquired from the National Institute for the Control of Pharmaceutical and Biological Products; luteolin was obtained from Shanghai Tauto Biotech Co., Ltd. (Shanghai, China). A single standard was created by dissolving 2.20 mg caffeic acid, 0.90 mg chlorogenic acid, and 1.75 mg luteolin in 25 mL of methanol. The solution was filtered through a 0.45 µm microsieve before HPLC analysis.

Chlorogenic acid, caffeic acid, and luteolin were extracted from the whole dandelion grass using a method described elsewhere [41]. The standards of chlorogenic acid, caffeic acid, and luteolin were identified by HPLC using a reverse-phase C18 column (Pinnacle, 250 mm × 4.6 mm; inner diameter, 5 µm) (Restek, Bellefonte, PA, USA). We chose a mixture of methanol (solvent A) and water containing 0.5% glacial acetic acid (99.5:0.5, v/v) (solvent B) as the mobile phase. The sample injection volume was 5 µL, and the column temperature was maintained at 30 °C. The gradient elution of caffeic acid and chlorogenic acid was increased from 10% to 30% within 44 min; the gradient elution of luteolin was increased from 5% to 50% within 77 min. The flow rate was maintained at 0.5 mL/min, and the UV spectra were recorded at 323 nm, for identification of caffeic acid, chlorogenic acid, and luteolin, respectively. Compare calibration curves and correlation coefficients of different chemical compositions in *Taraxacum* species are shown.

2.4 ITS sequence analysis

Genomic DNA from *Taraxacum* leaf tissue was extracted and purified using a modified CTAB method [42]. The quality and quantity of isolated DNA were verified by agarose gel electrophoresis. The universal forward and reverse primers ITS-F (5'-AGG TGA ACC TGC GGA AGG ATC ATTG-3') and ITS-R (5'-CTT CTC CTC CGC TTA TTG ATA TGCT-3') were used to amplify the ITS region. A total of 25 ng genomic DNA and 5 pmol of each primer were used in the reaction, 1 µ mol·L⁻¹ primer, 2.0 m mol·L⁻¹ MgCl₂, 1.6 m mol·L⁻¹ dNTP, 0.4 m mol·L⁻¹ Taq polymerase, 50 ng template DNA. Which was carried out in a thermocycler (Bio-rad PTC-100 PCR, USA) programmed as follows: 95 °C for 5 min; 35 cycles of 95 °C for 30 s, 60 °C for 30 s, and 72 °C for 1 min, with a final extension at 72 °C for 10 min. The amplicons were purified with a PCR Cleanup Kit (Axygen Scientific, Inc., Union City, CA, USA) and sequenced by

Sangong Biotech (Shanghai, China). In order to determine the ITS sequence of dandelion more completely and effectively, sequences were checked against other *Taraxacum* ITS sequences in GenBank with the Basic Local Alignment Search Tool, to construct the database model of molecular biomolecule (BLAST; National Center for Biotechnology Information, Bethesda, MD, USA).

2.5 Data analysis

Several statistical procedures were conducted to evaluate morphological diversity and establish the relationships between species. Data for 32 qualitative and quantitative morphological characteristics of the 18 taxa were subjected to analysis of variance (ANOVA) using SPSS v.19.0 (Chicago, IL, USA) at significance levels of $P \leq 0.01$ and $P \leq 0.05$. Two multivariate analyses, principal component analysis (PCA) and cluster analysis, in SPSS v.21.0 were used for species identification based on quantitative and qualitative morphological characteristics data.

The obtained sequences of the ITS region were aligned with Clustal X [43] and adjusted manually. Phylogenetic tree reconstruction based on parsimony method was performed using PAUP* version 4.0b10. Insertions and deletions were treated as missing data. All characters were equally weighted and unordered. Clades support was evaluated by bootstrap analysis using 1,000 replicates. The number of steps, consistency indices, and retention indices were calculated using the TREE SCORE command in PAUP* [44–45]. The Minimum Standards of Reporting Checklist includes details of the experimental design, statistics, and resources used in this study.

3. Results

3.1 Growth characteristics

Thirty-two variable morphological characteristics were evaluated (Table 1). Plant height was divided into three categories: short (5–10 cm), medium (10–15 cm), and tall (15–20 cm). Leaf length ranged from 6 to 20 cm, and leaf width from 1.2 to 2.9 cm (Table 2). Leaf thickness ranged from 0.61 to 1.26 mm. The leaf anatomy in cross-section and floral and achene traits differed among the 18 taxa. *T. antungense* Kitag., *T. urbanum* Kitag., *T. mongolicum* Hand.-Mazz., *T. coreanum* Nakai., and *T. asiaticum* Dahlst. have thinner, pubescent leaves compared with thicker, glabrous leaves in other species (Figs. 1–3). Three types of leaf margin were observed: deeply pinnatifid (pinnatisect), moderately pinnatifid (pinnatifid to pinnatisect), and shallowly pinnatifid. The apical lobe shape of the leaves is primarily triangular, but shapes range from triangular, triangular-oblong, diamond-shaped triangle, to triangular-hastate (Table 2). In *T. asiaticum*, *T. coreanum*, *T. ohwianum*, *T. erythropodium*, and *T. formosanum*, the leaf margins of middle leaves have lateral lobes.

Taraxacum coreanum has white flowers, while the other species have yellow flowers (Fig. 2). The abaxial corolla surface is concolorous or has green, purple, or black stripes, depending on the species (Table 2). The involucres were described in terms of the pubescence, arrangement, and structure of outer phyllaries and inner bracts. The outer phyllaries are ovate-lanceolate, lanceolate, or ovate (Table 2 and Fig. 2). A Minority of the outer bracts are lanceolate, for example in *T. antungense*, *T. urbanum*, *T. asiaticum* var. *lonchophyllum* Kitag., and *T. brassicaefolium* Kitag. Most outer bracts are ovoid or linear-lanceolate most of the inner bracts are lanceolate. Inner bracts are corniculate. The scape length to leaf length ratio (S/L) is lowest in *T. antungense*. The scape pubescence was categorized as dense, sparse, or lacking (scape glabrous). The scape base is purple in *T. mongolicum*, *T. formosanum*, *T. heterolepis* Nakai & Koidz. ex Kitag., and *T. erythropodium* and green in other taxa.

Table 2

Mean values of 32 horticultural qualitative and quantitative traits in the 18 taxa of northeast China native *Taraxacum* species used for multivariate analysis.

Species ^a	Plant	Leaf						
	plant height(cm)	leaf length(cm)	leaf width(cm)	leaf thickness(mm)	hair on leaf ^b	Leaf margin	apical lobe shape	lateral lobes
A	12.71	10.21	1.71	0.61	-	1	2	-
B	6.11	5.42	1.32	0.65	+	2	1	+
C	8.21	7.31	2.11	0.69	-	1	1	+
D	8.12	11.23	1.32	1.04	+	1	2	-
E	8.51	7.61	0.81	1.26	+	2	3	+
F	19.41	14.41	2.91	1.13	+	2	4	-
G	12.61	11.42	1.62	0.61	-	1	3	-
H	10.92	7.21	1.53	0.75	+	1	1	+
I	11.81	10.41	1.71	1.03	+	2	1	-
J	12.73	11.71	1.22	1.05	+	3	2	-
K	10.21	15.32	0.94	0.75	-	3	1	+
L	8.22	7.33	3.41	1.02	-	1	1	-
M	11.33	17.21	1.13	0.84	-	1	2	+
N	8.61	9.51	2.81	1.12	+	3	3	-
O	7.62	8.42	1.82	0.62	+	1	2	-
P	12.91	18.51	3.23	1.78	-	1	1	+
Q	11.81	18.42	0.71	0.42	-	1	1	+
R	6.71	8.71	1.12	1.08	+	2	2	-

Explanatory Comment: Leaf margin of Deep "1"; Medium "2"; Shallow "3"; Triangle of Triangular hastate "1"; Triangle "2"; Triangular oblong "3"; Diamond shaped triangle "4".

species	Flower		Outer phyllaries					
	flower color	abaxial corolla background color	abaxial corolla stripe color	pubescence of outer phyllaries	membranous	corniculate	outer bracts shape	imbricate of outer phyllaries
A	1	1	1	-	+	-	1	+
B	1	2	1	+	+	+	2	+
C	1	2	1	+	-	+	2	-
D	1	3	2	+	-	+	2	-
E	0	2	1	-	-	+	2	-
F	1	2	1	+	+	-	2	-
G	1	1	-	+	+	-	1	+
H	1	1	1	-	-	-	1	-
I	1	1	1	-	-	+	2	-
J	1	2	2	-	+	+	3	+
K	1	1	1	+	+	+	2	+
L	1	1	-	+	+	+	2	+
M	1	1	1	-	+	+	1	+
N	1	1	1	+	-	-	3	-
O	1	2	2	-	-	+	2	-
P	1	1	1	+	+	+	3	+
Q	1	1	1	+	+	-	2	+
R	1	3	2	+	-	+	2	-

Explanatory Comment: flower color of white “0”; yellow “1”; abaxial corolla background color of green“1”; black“2”; purple “3”; abaxial corolla stripe color of purple “1”; black “2”; outer bracts shape of lanceolate “1”; ovate-lanceolate “2”; oval “3”.

species	Inner bracts		Scape			Pollen			
	inner bracts shape	corniculate	S/L radio ^c	pubescence on scape	color of scape base	length of the polar axis (μm)	equatorial axis(μm)	P/E radio	pollen shaped
A	1	-	0.7	1	1	19.81 – 22.32	16.74 – 18.41	1.19	1
B	1	+	1.2	2	1	21.93 – 24.84	20.63 – 22.76	1.06	2
C	1	+	1.1	3	1	20.51 – 22.75	18.72 – 20.33	1.09	2
D	1	+	1.2	2	2	19.97 – 22.16	17.25 – 17.91	1.16	1
E	1	+	1	3	1	30.53 – 31.21	22.56 – 23.44	1.35	3
F	2	-	1.2	3	1	18.95 – 20.54	16.57 – 17.95	1.14	1
G	1	-	1	1	1	20.86 – 23.53	17.93 – 19.66	1.16	1
H	3	+	1	1	1	26.32 – 27.15	22.51 – 24.18	1.17	1
I	1	+	1.1	1	2	23.41 – 24.68	19.96 – 21.62	1.07	1
J	3	+	1	3	1	24.23 – 25.27	21.76 – 22.31	1.11	2
K	1	+	1	2	1	21.43 – 23.25	17.02 – 18.53	1.26	2
L	1	-	0.8	1	2	20.07 – 22.08	17.28 – 18.02	1.16	1
M	1	+	1	2	1	20.43 – 21.21	21.21 – 22.34	0.96	1
N	2	-	0.8	1	1	19.05 – 20.14	18.54 – 19.35	1.03	1
O	3	+	1	3	1	20.46 – 21.53	18.73 – 19.26	1.09	1
P	2	-	1	3	1	26.12 – 27.05	22.31 – 23.78	1.17	2
Q	2	-	1.2	2	1	20.42 – 21.68	19.66 – 20.82	1.04	2
R	2	+	1	2	2	20.23 – 21.27	19.56 – 20.21	1.03	1

Explanatory Comment: inner bracts shape of Lanceolate “1”; Ovate-lanceolate “2”; Linear “3”; pubescence on scape of glabrous “1”; sparsely “2”; densely “3”; color of scape base of green “1”; purple “2”; pollen shaped of subprolate “1”; spheroidal “2”; prolate “3”.

species	Achene						pappus
	color of achene	achene length [cm]	achene width [cm]	beak length [cm]	BL/AL ratio ^e	Achene shape	
A	1	2.91-3.22	0.55-0.57	0.60-0.62	0.21	1	-
B	1	3.01-3.22	0.71-0.93	1.02-1.04	0.34	1	-
C	1	3.83-4.21	1.33-1.62	2.41-2.43	0.63	2	+
D	3	4.41-4.71	1.32-1.51	1.07-1.09	0.24	2	-
E	2	3.82-4.23	1.55-1.60	1.16-1.19	0.30	1	-
F	2	3.01-3.52	0.85-0.92	1.08-1.11	0.36	1	-
G	1	2.85-2.92	0.55-0.60	0.52-0.54	0.18	1	-
H	2	3.13-3.42	0.81-1.09	1.16-1.19	0.37	1	-
I	3	4.42-4.82	1.24-1.53	1.11-1.14	0.25	3	-
J	3	4.32-4.71	1.12-1.25	1.04-1.06	0.24	4	-
K	2	4.03-4.21	1.33-1.62	2.78-2.93	0.69	1	+
L	2	3.41-3.71	1.31-1.41	1.27-1.29	0.37	1	+
M	2	3.74-3.81	1.38-1.42	1.16-1.19	0.31	1	-
N	1	3.31-3.42	0.86-0.91	2.08-2.11	0.63	1	+
O	1	2.88-2.95	1.85-1.87	1.16-1.19	0.41	1	-
P	1	3.23-3.35	1.81-1.89	0.82-0.84	0.26	1	-
Q	3	2.94-3.05	0.84-0.88	1.11-1.14	0.38	1	-
R	3	3.05-3.11	1.12-1.15	1.14-1.16	0.37	1	-

Explanatory Comment: color of achene of Light brown “1”; Brown “2”; Dark brown “3”; Achene shape of Lanceolate-oblong “1”; lanceolate-obovate “2”; Obovate oblong “3”; Narrowly obovate “4”.

^a A, *T. antungense* Kitag.; B, *T. asiaticum* Dahlst.; C, *T. variegatum* Kitag.; D, *T. mongolicum* Hand.-Mazz.; E, *T. coreanum* Nakai.; F, *T. ohwianum* Kitag.; G, *T. urbanum* Kitag.; H, *T. asiaticum* var. *lonchophyllum* Kitag.; I, *T. formosanum* Kitam.; J, *T. liaotungense* Kitag. K, *T. sinomongolicum* Kitag. L, *T. heterolepis* Nakai et Koidz. ex Kitag. M, *T. brassicae folium* Kitag. N, *T. platyepidum* Diels O, *T. falcilobum* Kitag. P, *T. borealisinense* Kitam. Q, *T. junpeianum* Kitam. R, *T. erythropodium* Kitag.

^b “-” means for not exist and “+” means for exist, the same in blow.

^c S/L ratio: scape length/leaf length ratio

^d Pollen shape: spheroidal = 0.88–1.14, subprolate = 1.14–1.33, prolate = 1.33–2.00.

^e BL/AL ratio means Achene length/Beak length.

Common achene micromorphological features include the achene body with spines and spines with white spots; spines arranged in regular rows form a higher consistency (Table 2 and Fig. 3). The color of achenes varies from light brown, brown to dark brown, and is crimson in *T. liaotungense* Kitag. The longest achenes are found in *T. formosanum* (4.41–4.82 mm) and the shortest in *T. urbanum* (2.85–2.92 mm). Achene shape is lanceolate-obovate in *T. variegatum* and *T. mongolicum*, obovate oblong in *T. formosanum*, narrowly obovate in *T. liaotungense* Kitag., and lanceolate-oblong in other species. The smallest achenes are present in *T. falcilobum* Kitag. (2.88 cm × 1.85 cm) and the largest in *T. formosanum* (4.82 cm × 1.24 cm) (Table 3 and Fig. 4). The longest achene beak was measured in *T. sinomongolicum* Kitag. (2.78–2.93 cm). The achene length to beak length ratio (BL/AL) was the highest in *T. sinomongolicum* (0.69) and the lowest in *T. urbanum* (0.18). White pappus was found only in *T. variegata* Kitag. The size and micromorphological characteristics of the achenes were relatively stable within a species, but significantly different among the species. Shallow or deep depressions observed on the achene surface is an unstable feature and therefore of little significance in the identification of *Taraxacum* species. The achenes of dandelion generally consist of three parts: the achenes body, the achenes beak base and the three beak parts. The achene features in detail and distinguishing criteria are as follows, 1. Achene shape and size: *Taraxacum* achene fusiform, linear, or inverted cone, slightly compressed, with longitudinal groove, the upper part of the fruit or almost all with spiny or verrucous projections, thin smooth, ribbed or keeled, achenes generally come in two sizes, that is, achene length > 4 mm (*T. mongolicum*, *T. formosanum*, *T. liaotungense* and *T. sinomongolicum*) and achene length < 4 mm (the other 14 species). 2. Achenes long beak: the beak length of dandelion was divided into two domains with a limit of 8 mm, crown hair multilayer, the white tail easily falls off. Among them > 8 mm includes, *T. antungense*, *T. urbanum*, *T. ohwianum*, while the other 15 species all had beak lengths < 8 mm. 3. Beak length ratio (BL/AL), can be used as a reference for the characteristic of *Taraxacum*. It can be divided into three types, BL/AL < 0.3 (*T. antungense*, *T. urbanum*, *T. mongolicum*, *T. formosanum*, *T. liaotungense*, *T. borealisinense*); BL/AL 0.3–0.5 (*T. asiaticum*, *T. coreanum*, *T. ohwianum*, *T. asiaticum* var. *lonchophyllum*, *T. heterolepis*, *T. brassicaefolium*, *T. falcilobum*, *T. junpeianum*, *T. erythropodium*); BL/AL > 0.5 the other 3 species. *Taraxacum urbanum* had the lowest ratio (0.18) and *T. sinomongolicum* had the highest (0.69). 4. Achenes ribbed or not: the presence or absence of achene ribs divided the index into non – ribbed (*T. heterolepis*, *T. sinomongolicum*, *T. variegatum*, *T. asiaticum*, *T. lonchophyllum*, *T. falcilobum*, *T. brassicaefolium*, and *T. erythropodium*.) and ribbed (*T. formosanum*, *T. liaotungense*, *T. mongolicum*, *T. borealisinense*, *T. ohwianum*, *T. platyepidum*, *T. urbanum*, *T. antungense*, *T. asiaticum*, and *T. junpeianum*). 5. Achene color: achenes come in three colors: reddish brown (*T. antungense*, *T. asiaticum*, *T. variegatum*, *T. urbanum*, *T. platyepidum*, *T. falcilobum*, *T. borealisinense*), brown (*T. coreanum*, *T. ohwianum*, *T. asiaticum* var. *lonchophyllum*, *T. sinomongolicum*, *T. heterolepis*, *T. brassicaefolium*), and tawny (the other 5 species). 6. Achenes shape. Achene shape of lanceolate-oblong (the other 14 species); lanceolate-obovate (*T. variegatum*, *T. mongolicum*); Obovate–oblong (*T. formosanum*); Narrowly obovate (*T. liaotungense*). The survey revealed some common features in epidermis micromorphology: the achenes were covered entirely with spines, which were classified into: small or big spines, and further into: big spines, small dense spines, and small sparse spines.

Table 1

List of 34 horticultural qualitative and quantitative traits and their range and parameter code in the 18 taxa of northeast China native *Taraxacum* species.

Plant part	Trait	Unit	Mean $\pm$ SD	F value ^a	Range and parameter code ^b
Plant	Plant height	cm	11.3 $\pm$ 4.47	5.92**	6.1-19.4
Leaf	Leaf length	(cm)	9.88 $\pm$ 2.72	26.85*	5.4-14.4
	Leaf width	(cm)	1.67 $\pm$ 0.69	6.43*	0.8-2.9
	leaf thickness	(mm)	0.87 $\pm$ 0.25	4.2NS	0.60-1.26
	hair on leaf	degree	1.7 $\pm$ 0.48	2.36*	Non (1), Yes (2)
	Leaf margin	degree	3.8 $\pm$ 1.4	0.82NS	Shallow(1),mieium(3),Deep(5)
	Apical lobe shape	degree	3.2 $\pm$ 2.2	10.44*	Triangle(1),Triangular oblong (3), Triangular hastate(5), Diamond shaped triangle(7)
	lateral lobes	degree	1.4 $\pm$ 0.51	2.13	Non (1), Yes (2)
Flower	Flower color	degree	1.2 $\pm$ 0.63	0.41NS	yellow(1),white(3)
	abaxial corolla background color	degree	3.2 $\pm$ 1.99	3.55NS	green(1),purple(3),black(5)
Outer bracts	abaxial corolla stripe color	degree	3.8 $\pm$ 2.35	4.17 ^{NS}	no(1),red(3)purple(5)black(7)
	pubescence of outer phyllaries	degree	1.7 $\pm$ 0.48	2.23*	Non (1), Yes (2)
	membranous	degree	1.5 $\pm$ 0.53	1.43*	Non (1), Yes (2)
	corniculate	degree	1.4 $\pm$ 0.52	1.65*	Non (1), Yes (2)
	outer bracts shape	degree	2.8 $\pm$ 1.48	0.67NS	lanceolate(1),ovate-lanceolate(3),Oval(5)
	imbricate of outer phyllaries	degree	1.3 $\pm$ 0.48	0.87NS	Non (1), Yes (2)
Inner bracts	inner bracts shape	degree	2.4 $\pm$ 1.9	0.42NS	lanceolate(1),ovate-lanceolate(3),Linear(5)
	corniculate	degree	1.4 $\pm$ 0.52	0.55*	Non (1), Yes (2)
Scape	S/L radio	radio	1.06 $\pm$ 0.126	6.25*	0.8-1.2
	pubescence on scape	degree	2.60 $\pm$ 1.84	7.42*	no(1),Non bushy (3), bushy (5)
	color of scape base	degree	1.60 $\pm$ 0.966	0.52*	green(1),purple(3)
Pollen	length of the polar axis	(um)	23.34 $\pm$ 3.57	3.12NS	18.95-31.21
	equatorial axis	(um)	20.10 $\pm$ 2.57	4.55NS	16.57-24.18
	P/E radio	radio	1.17 $\pm$ 0.075	2.78*	1.1-1.36

	pollen shape	degree	4.00±1.7	0.44*	spheroidal (3), subprolate (5), prolate (9)
Achene	color of achene	degree	2.90±2.60	14.22*	Light brown(1),brown(3),Dark brown(5),Crimson(9)
	achene length	(mm)	3.75±0.71	25.46*	2.91-4.71
	achene width	(mm)	1.09±0.38	37.41*	0.55-1.56
	beak length	(mm)	8.54±0.68	8.65*	8.2-10.45
	BL/AL radio	radio	0.34±0.092	218.55**	0.2-0.5
	achene shape	degree	2.40±2.12	18.24*	Lanceolate-oblong (1), lanceolate-obovate (3), Obovate oblong (5),Narrowly obovate(7)
Pappus	Crested color	degree	1.60±0.966	0.36NS	No (1), yes (3)

^aThe variables in 10 China native *Taraxacum* species expressed NS non-significant, * significant at $P<0.05$, or** significant at $P<0.01$.

^bThe Chrysanthemum Test Guidelines Criteria by the Seed and Variety Service.

Pollen from the 18 taxa was observed with light microscopy and SEM. Pollen morphological structures of the 18 *Taraxacum* species have conspicuous characteristics of *Compositae* pollen, namely the pollen is nearly spherical, with three indehiscent ditches and a spiny surface. *T. coreanum* pollen has the longest polar axis (P) at 30.53–31.21 μm and *T. ohwianum* the shortest at 18.95–20.54 μm (Table 2 and Fig. 4). The pollen varies from spheroidal to subprolate or prolate. The pollen of the 18 species is prolate to spheroidal type ($P/E = 0.95\text{--}1.36$). The P/E max ratio of *T. ohwianum* is 1.36 (prolate); the P/E min ratio of *T. brassicae folium* is 0.96 (subprolate); subprolate pollen shape (the other 11 species); spheroidal (*T. asiaticum*, *T. variegatum*, *T. liaotungense*, *T. sinomongolicum*, *T. borealisinense*, *T. junpeianum*); and prolate (*T. coreanum*).

Table 4
Principal component analysis of 32 morphological characteristics in 18 taxa *Taraxacum* species of northeast China.

Total Variance Explained						
Component	Initial Eigenvalues			Extraction Sums of Squared Loadings		
	Total	% of Variance	Cumulative %	Total	% of Variance	Cumulative %
1	9.483	29.635	29.635	9.483	29.635	29.635
2	5.566	17.393	47.029	5.566	17.393	47.029
3	4.594	14.356	61.385	4.594	14.356	61.385
4	3.485	10.892	72.277	3.485	10.892	72.277
5	2.802	8.755	81.032	2.802	8.755	81.032
6	2.167	6.771	87.803	2.167	6.771	87.803
7	1.535	4.798	92.602	1.535	4.798	92.602
8	1.316	4.113	96.715	1.316	4.113	96.715
9	1.051	3.285	100	1.051	3.285	100

Extraction Method: Principal Component Analysis.

3.2 Taxonomic relationships inferred from morphological characteristics based on PCA and cluster analysis

Thirty-two qualitative and quantitative morphological traits were subjected to multivariate analyses (Table 3). Of those, 21 variables were significantly different among the taxa. The PCA returned nine principal components that explained 96.7% of the variation (Table 4). The eigenvalues of each principal component indicated that the first, second, and third principal component were associated with 21, 6, and 4 variables of the 32 morphological characteristics, respectively. The three major principal components covered 31 characteristics and explained 61.4% of the results (Table 4). The first principal component (PC 1) was associated with 21 morphological characteristics including: plant height, leaf length, leaf width, BL/AL, leaf thickness, leaf pubescence, abaxial corolla background color, abaxial corolla stripe color, membranous, corniculate of flower, outer bracts shape, inner bracts shape, corniculate (Table 5). Cluster analysis using average linkage (between groups) of the standardized first three principal components produced three groups (Fig. 5). Group I included one species, *T. coreanum* with white flowers, indicating the contribution of flower color to the classification. Group II comprised seven species: *T. heterolepis*, *T. sinomongolicum*, *T. variegatum*, *T. asiaticum* var. *lonchophyllum*, *T. falcilobum*, *T. brassicaefolium*, and *T. erythropodium*; Group III included 10 species: *T. formosanum*, *T. liaotungense* Kitag., *T. mongolicum*, *T. borealisinense*, *T. ohwianum*, *T. platyepidum* Diels., *T. urbanum*, *T. antungense*, *T. asiaticum*, and *T. junpeianum* Kitam.

Table 5

Component Matrix of 9 components of Principal Component Analysis of 32 morphological characteristics in the 18 taxa of northeast China native *Taraxacum* species

Component Matrix ^a									
morphological characteristics	Component								
	1	2	3	4	5	6	7	8	9
Plant height	-0.419	0.801	-0.336	0.092	-0.022	0.049	-0.165	-0.094	-0.147
Leaf length	-0.165	0.883	-0.088	0.014	-0.263	0.258	0.164	-0.116	-0.099
Leaf width	-0.427	0.46	0.621	0.257	0.031	0.092	0.01	-0.216	-0.313
leaf thickness	0.676	0.398	-0.288	0.483	-0.167	0.081	0.064	-0.141	-0.099
hair on leaf	0.556	0.295	-0.376	0.38	0.322	-0.185	-0.416	0.026	-0.079
Leaf margin	-0.346	-0.372	0.369	-0.333	-0.248	0.395	-0.365	-0.327	0.192
Apical lobe shape	-0.135	-0.017	0.512	0.647	0.317	0.093	-0.261	0.15	-0.318
lateral lobes	0.221	-0.792	0.187	0.295	0.422	-0.062	-0.051	-0.074	0.108
Flower color	0.395	-0.452	-0.412	0.538	-0.28	0.143	0.238	-0.116	0.112
abaxial corolla background color	0.362	0.101	0.19	0.509	0.265	-0.409	0.441	-0.128	0.34
abaxial corolla stripe color	0.235	0.464	-0.462	0.261	0.402	-0.068	0.088	-0.52	0.062
pubescence of outer phyllaries	0.645	-0.119	0.008	-0.323	0.005	0.617	0.095	0.265	0.069
membranous	-0.722	0.365	-0.249	0.047	0.152	-0.333	0.333	0.132	0.138
corniculate	0.81	0.237	-0.391	-0.072	-0.319	-0.038	0.037	0.158	-0.012
outer bracts shape	-0.143	0.451	0.193	0.494	0.193	0.513	0.001	0.337	0.284
imbricate of outer phyllaries	-0.799	-0.255	-0.167	-0.16	-0.153	-0.281	-0.016	0.257	0.272
inner bracts shape	-0.236	0.288	-0.297	-0.168	0.617	0.552	0.188	0.152	-0.008
corniculate	0.507	0.072	0.237	-0.55	0.482	0.162	0.053	-0.308	0.149
S/L ratio	0.319	0.383	0.456	0.41	0.176	-0.166	-0.407	0.185	0.343
pubescence on scape	0.601	0.147	0.587	0.085	-0.475	0.064	-0.173	-0.052	-0.057
color of scape base	0.432	0.276	-0.298	-0.51	0.486	0.157	-0.192	-0.265	0.144
length of the polar axis	0.493	-0.646	-0.407	0.303	0.035	0.242	-0.069	0.084	-0.095
equatorial axis	0.423	-0.547	-0.371	0.061	0.531	0.059	-0.054	0.259	-0.159
P/E ratio	0.137	-0.376	-0.554	0.411	-0.42	0.35	0.155	-0.193	0.064
pollen shape	-0.512	0.357	0.413	0.35	-0.021	0.466	0.238	0.095	0.182

color of achene	0.521	0.58	-0.169	-0.168	-0.274	-0.088	-0.298	0.139	0.379
achene length	0.915	0.262	0.041	-0.178	-0.207	-0.017	0.049	0.121	-0.021
achene width	0.955	-0.061	0.213	0.091	-0.159	0.028	0.027	-0.051	0.041
beak length	0.946	0.07	0.266	-0.023	-0.057	-0.056	0.152	0.008	-0.007
BL/AL radio	0.779	0.09	0.558	-0.021	0.209	-0.15	0.065	0.008	-0.063
achene shape	0.578	0.433	0.003	-0.426	0.072	-0.168	0.312	0.327	-0.244
Crested color	0.169	-0.304	0.841	-0.119	0.045	0.025	0.379	-0.097	0.023

Extraction Method: Principal Component Analysis. ^a. 9 components extracted.

3.3 Cluster analysis of chlorogenic acid, caffeic acid, and luteolin concentrations

The chromatographic peaks of the 18 taxa were detected within 90 min and their chromatographic charts were recorded (Fig.S2). The number of common peaks was 22; common peak ratio was 100%; relative standard deviation of the 18 dandelion samples was less than 0.39%. The chlorogenic acid peak corresponds to the peak at 29.388 min (No. 5, caffeic acid to the peak at 33.299 min (No. 11), and luteolin to the peak at 78.059 min (No. 22) in the chromatogram; all these peaks coincided with on-line UV spectra. The above contents were classified based on retention time and on-line UV qualitative attribution. Chlorogenic acid peak (No. 5), with moderate intensity, was well separated from adjacent peaks and therefore was selected as the reference peak.

The 18 taxa were significantly different in chlorogenic acid, caffeic acid, and luteolin concentrations (Table 6 and Table 7). The sum of chlorogenic acid, caffeic acid, and luteolin concentrations was highest in *T. antungense* and lowest in *T. mongolicum*, *T. liaotungense*, and *T. formosanum*; the latter three were not significantly different from each other. The results of the cluster analysis using phytochemical ingredients were incongruent with the results based on morphological data, except for *T. mongolicum*, *T. formosanum*, and *T. liaotungense*, which were clustered in the same group (Fig. 6).

Table 6
Concentration of 3 phytochemical ingredients in northeast China native 18 *Taraxacum* species.

<i>Taraxacum</i> species	chlorogenic acid	caffeic acid	luteolin
<i>T. antungense</i> Kitag.	16.04±0.223a	9.08±0.193a	4.10±0.183a
<i>T. asiaticum</i> Dahlst.	13.88±0.105c	5.12±0.112e	1.26±0.108e
<i>T. variegatum</i> Kitag.	15.82±0.101a	7.88±0.168b	1.96±0.115c
<i>T. mongolicum</i> Hand.-Mazz.	7.88±0.152e	4.10±0.117h	1.82±0.128c
<i>T. coreanum</i> Nakai	15.58±0.193a	7.34±0.126c	1.30±0.105e
<i>T. ohwianum</i> Kitag.	15.1±0.264b	5.72±0.165d	1.58±0.109d
<i>T. urbanum</i> Kitag.	8.76±0.140d	4.88±0.109f	3.98±0.120a
<i>T. asiaticum</i> var <i>lonchophyllum</i> Kitag.	8.12±0.127e	4.50±0.141g	2.86±0.146b
<i>T. formosanum</i> Kitam	6.76±0.116f	5.68 ±0.114d	0.78±0.122f
<i>T. liaotungense</i> Kitag.	7.88±0.175e	4.10±0.137h	1.82±0.121c
<i>T. sinomongolicum</i> Kitag.	14.82±0.101a	6.94±0.167c	1.98±0.115c
<i>T. heterolepis</i> Nakai et Koidz.ex Kitag.	10.68±0.152c	4.15±0.117h	1.85±0.128c
<i>T. brassicae folium</i> Kitag.	12.32±0.193c	7.38±0.126c	1.37±0.105e
<i>T. platyepidum</i> Diels	15.11±0.264b	5.81±0.165d	1.51±0.109d
<i>T. falcilobum</i> Kitag.	10.76±0.140d	4.74±0.109f	3.92±0.120a
<i>T. borealisinense</i> Kitam.	9.32±0.127e	4.61±0.141g	2.81±0.146b
<i>T. junpeianum</i> Kitam.	6.87±0.116f	5.86±0.114d	0.72±0.122f
<i>T. erythropodium</i> Kitag.	8.02±0.175e	4.12±0.137h	1.88±0.121c

Data are means ± standard error (n=6). Numbers followed by the same letter within a column indicate that the values do not differ significantly between treatments at P<0.05 (Duncan's test). Concentration of 3 phytochemical ingredients (mg (g DW)⁻¹). DW: dry weight.

Table 7
Calibration curves and correlation coefficients of different chemical composition in *Taraxacum* species

Chemical composition	Calibration curve ^a	r ²
chlorogenic acid	Y = 17993X - 4.6433	0.9996
caffeic acid	Y = 11892X + 0.0291	0.9991
luteolin	Y = 11796 X + 9.6504	0.9997

^aY and X are the peak area and concentration of the chemical composition, respectively.

Chemical composition differed between the 18 taxa, but it primarily contributed to the content and peak time of caffeic acid, chlorogenic acid, and luteolin. If the similarity of fingerprint was greater than 0.9, the similarity was considered to be good, and the similarity of HPLC fingerprint was high (> 0.96), which may have contributed to the large area of peak No. 1 and No. 12 in the sample and little effect of other small chromatographic peaks, resulting in overestimated overall similarity. In other words, some characteristic peaks were shadowed by larger peaks, thus affecting the accuracy and reliability of chemical classification of *Taraxacum*. Reverse-phase HPLC fingerprints of 70% ethanol extracts from the 18 taxa reflected the specificity and integrity of dandelion quality control, and thus it can provide reference for quality control of this genus (acid, caffeic acid, and luteolin). Chlorogenic acid, caffeic acid, and luteolin content vary with plant growth and environmental factors and can be used only as an auxiliary taxonomy character in species delimitation of the genus *Taraxacum*.

3.4 ITS sequence analysis

The general ITS sequence primers were used to amplify *Taraxacum*. Most of the materials obtained bright and clear bands around 730 bp, and the amplification products were between 750 bp and 500 bp, all kinds of *Taraxacum* ITS amplification bands were recovered, cloned, and sequenced using T vector. The length of the ITS region ranged from 728 to 733 bp (FigS3.). The aligned ITS matrix was 733 bp long; ITS-1 region was 276 bp, ITS-2 region was 295 bp, and the 5.8S rDNA sequence was 162 bp long. BLAST search of all the sequences showed 99% similarity to a partial sequence of the ITS region in *Taraxacum coreanum* (accession number JF837599.1). Of these, 162 (22%) were constant and 569 (78%) were potentially informative. Consistency index of the maximum parsimonious tree was 0.051, and the retention index was 0.007. The ITS-based topology of 18 species was consistent with the morphological characteristics of the plants. The total G + C content varied among the taxa from 51.23% in *T. ohwianum* to 52.53% in *T. coreanum*. Few base variation sites were identified in *Taraxacum*, and the information sites were about 3–4. The pairwise difference of ITS base composition between the 18 taxa was within 1% and ranged from 0.057 between *T. coreanum* and *T. formosanum* to 0.007 between *T. liaotungense* and *T. variegatum*.

4. Discussion

4.1 Trend of evolution in *Taraxacum* species

Morphological classification is the basis of plant taxonomy, based on morphological classification and assisted by palynology, chemistry, and ITS sequence analysis, we classified, and germplasm identified 18 *Taraxacum* species in northeast China. In “*Flora of China*” the floral organs of dandelion were recorded as the main basis for morphological classification. The present study found that the characteristics of achenes of dandelion can also be used as the basis of classification to identify species useful in traditional Chinese medicine. Achenes, as reproductive organs, are in a relatively enclosed environment, less affected by external environmental factors compared with floral organ, and harbor stable genetic traits. This trait in achene micromorphology may provide new evidence for phylogenetic studies of *Taraxacum*. The micromorphological characteristics of *Taraxacum* species examined here, which are characterized by whole achene body spiny, are complex, which belongs to a relatively evolutionary group. The main evolutionary features of *Taraxacum* are achenes with short and thick beak that is not obvious, achene wall without strumae or small spines, or partially or completely covered with tumor or spines. Therefore, achene morphology indicate that *Taraxacum* is a well-evolved genus. It's worth mentioning that beak length ratio (BL/AL), the ratio is a fixed value in the same species of *Taraxacum*, indicating that beak base size and achene length are quality traits which are not easily affected by the environment and stable genetic characters, so they can be used as the basis for species classification. In the study of palynology, pollen size, pollen shape, type of germ pore, and exine patterns were specific to the species and therefore suitable for species delimitation of

Taraxacum species growing in northeastern China. Morphological characteristics and growth habits are not sufficient criteria for classification of *Taraxacum* species. The data characteristics of pollen size, spine-width, spine-density, germination pore size, and pore size can be used as the auxiliary basis for classification of *Taraxacum*. The results of the ITS sequence analysis were supported by the morphological traits of the sampled taxa. The 18 taxa from the northeastern China were divided into three groups. The taxa within Group II are characterized by closely arranged outer bracts with hornlike protuberances, large achenes shaped like a spindle or inverted cone and covered with spines. Group III is characterized by outer involucre basally unrolled or rolled, outer bracts without hornlike projections, achenes that are smaller, achene lower half with tuberos projections. The results of this study support the taxonomic treatment of the genus in the “*Flora of China*”.

In addition, the concentrations of the three phytochemicals were significantly higher in *T. antungense* than in the other studied species, suggesting that this species has medicinal properties. The concentrations of chlorogenic acid, caffeic acid, and luteolin in the studied taxa were incongruent with the results of morphology-based cluster analysis. The synthesis of different phytochemicals in plants, including the three compounds evaluated in this study, is strongly related to the environment, growing conditions, and regulation of gene expression, and therefore, it is not a reliable trait for *Taraxacum* categorization.

Plants of the 18 *Taraxacum* taxa were identified and classified using traditional methods based on morphological traits. In addition to morphological observations, this study evaluated the reliability of palynological and chemical data for taxonomic identification of *Taraxacum* and conducted phylogenetic analysis of the taxa using ITS sequence data. The results of morphological and palynological clustering analysis were consistent with the results of ITS sequence analysis. The pairwise genetic distances of *Taraxacum* species were congruent with the results of the PCA based on morphological characters and the ITS analysis.

The results of integrated whole analysis and clustering show that, the phylogenetic analysis resolved the 18 species into three groups. Group I includes *T. coreanum*, the only species with white flowers among the sampled taxa. Group II includes seven species: *T. heterolepis*, *T. sinomongolicum*, *T. variegatum*, *T. asiaticum*, *T. lonchophyllum*, *T. falcilobum*, *T. brassicaefolium*, and *T. erythropodium*. Group III includes 10 species: *T. formosanum*, *T. liaotungense*, *T. mongolicum*, *T. borealisinense*, *T. ohwianum*, *T. platyepidum*, *T. urbanum*, *T. antungense*, *T. asiaticum*, and *T. junpeianum*. The chemical composition of the species is not suitable for their delimitation; the highest concentration of phytochemicals was detected in *T. antungense*, and the species therefore is a potential medicinal plant with therapeutic properties.

4.2 The causes for complicated nature of *Taraxacum* classification

Under different environmental condition during the seven years of study, these taxa differed in the morphology of the leaves, flowers, inflorescences, pollen, and achenes. Of the 32 analyzed morphological characters, 31 exhibited significant differences among the taxa. The PCA and cluster analyses of those characters arranged the taxa into three groups. In particular, the BL/AL ratio of the achene morphological characteristics was not used for species delimitation in the “*Flora of China*”; our study suggests that this character is potentially useful in identification of *Taraxacum* species.

The classifications of *Taraxacum* species in “*Herbaceous Flora of Northeast China*” and “*Flora of China*” are in disagreement as to the approximate number of species in northeastern China (19 species, 1 variety, and 3 variants in the former and 11 species in the latter), although the classification in both flora guides is based on plant

morphology; the results of palynological analysis in this study are consistent with the taxonomic treatment of the genus presented in “Flora of China.” The possible reasons for the discrepancy between the two flora guides are as follows: 1. There are significant intraspecific variations in *Taraxacum* species. Due to high plasticity of the genus, it is found throughout China, resulting in high species and intraspecific variations that hinder their classification. 2. Apomixis, as one of the relatively primitive reproductive modes, contributes to geographic isolation of *Taraxacum* species, whereas the lack of gene exchange between populations of the same species impedes the delimitation of biological species. 3. Traditional taxonomic evidence other than morphology, such as palynology, cytology, embryology, chemistry, and molecular biology alone, are insufficient for species identification. The palynological evidence presented in this study shows that pollen morphological characteristics and clustering analyses support the treatment of *Taraxacum* species from the northeastern China as presented in the “Flora of China.” 4. Human factors as different authors adopt different interpretations of the species concept and species range of distribution, causing controversy.

5. Conclusions

Chinese medicine uses dandelion plants, which belong to the Compositae family, they are one of the most evolutionary diverse subfamilies and are widely distributed in China. The vast majority of areas in China were surveyed but dandelion resource classification identification is more difficult because species boundaries are confused. For example, dandelion germplasm resources in northeast China do not accurately reflect actual species distribution [1, 2]. First, species were identified through the morphological classification method, using dandelion classification identification of germplasm resources. Secondly, identified utilizing chemical taxonomy through a high performance liquid chromatography (HPLC) method adopted to establish the dandelion chemical fingerprint. Finally, we verified molecular systematics, complete the dandelion classification evaluation. This study completed the northeast dandelion germplasm resource classification and evaluation, and can supplement and perfect "Flora of China" dandelion classification key points and the distribution of the species resources, in order to further develop the dandelion medicinal resources, regulate the dandelion medicinal value, and validate experimental basis of potential medicinal species for use in traditional Chinese medicine (TCM).

Declarations

Ethics approval and consent to participate

The authors declare that they have no ethics approval conflict of interest.

Consent for publication

The authors declare that they have consent for publication.

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.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

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

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Specimen vouchers

The specimen certificate of this paper has been placed in Institute of Applied Ecology, Chines Academy of Sciences, Shenyang, Liaoning, China.

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Figures

Figure 1

Pictures of the 18 taxa of northeast China native *Taraxacum* species. A, *T. antungense* Kitag.; B, *T. asiaticum* Dahlst.; C, *T. variegatum* Kitag.; D, *T. mongolicum* Hand.-Mazz.; E, *T. coreanum* Nakai.; F, *T. ohwianum* Kitag.; G, *T. urbanum* Kitag.; H, *T. asiaticum* var. *lonchophyllum* Kitag.; I, *T. formosanum* Kitam.; J, *T. liaotungense* Kitag. K, *T. sinomongolicum* Kitag. L, *T. heterolepis* Nakai et Koidz. ex Kitag. M, *T. brassicae folium* Kitag. N, *T. platycedum* Diels O, *T. falcilobum* Kitag. P, *T. borealisinense* Kitam. Q, *T. junpeianum* Kitam. R, *T. erythopodium* Kitag. Bar = 1 cm.

Figure 2

Pictures of flower organ of the 18 taxa of northeast China native *Taraxacum* species. A, *T. antungense* Kitag.; B, *T. asiaticum* Dahlst.; C, *T. variegatum* Kitag.; D, *T. mongolicum* Hand.-Mazz.; E, *T. coreanum* Nakai.; F, *T. ohwianum* Kitag.; G, *T. urbanum* Kitag.; H, *T. asiaticum* var. *lonchophyllum* Kitag.; I, *T. formosanum* Kitam.; J, *T. liaotungense* Kitag. K, *T. sinomongolicum* Kitag. L, *T. heterolepis* Nakai et Koidz. ex Kitag. M, *T. brassicae folium* Kitag. N, *T. platyepidum* Diels O, *T. falcilobum* Kitag. P, *T. borealisinense* Kitam. Q, *T. junpeianum* Kitam. R, *T. erythopodium* Kitag. Bar = 1 cm.

Figure 3

Achene of 18 taxa of northeast China native *Taraxacum* species. A, *T. antungense* Kitag.; B, *T. asiaticum* Dahlst.; C, *T. variegatum* Kitag.; D, *T. mongolicum* Hand.-Mazz.; E, *T. coreanum* Nakai.; F, *T. ohwianum* Kitag.; G, *T. urbanum* Kitag.; H, *T. asiaticum* var. *lonchophyllum* Kitag.; I, *T. formosanum* Kitam.; J, *T. liaotungense* Kitag. K, *T. sinomongolicum* Kitag. L, *T. heterolepis* Nakai et Koidz. ex Kitag. M, *T. brassicae folium* Kitag. N, *T. platyepidum* Diels O, *T. falcilobum* Kitag. P, *T. borealisinense* Kitam. Q, *T. junpeianum* Kitam. R, *T. erythropodium* Kitag. Bar = 1 cm

Figure 4

Micrographs of pollen grains in 18 *Taraxacum* species, examined by SEM A, *T. antungense* Kitag.; B, *T. asiaticum* Dahlst.; C, *T. variegatum* Kitag.; D, *T. mongolicum* Hand.-Mazz.; E, *T. coreanum* Nakai.; F, *T. ohwianum* Kitag.; G, *T. urbanum* Kitag.; H, *T. asiaticum* var. *lonchophyllum* Kitag.; I, *T. formosanum* Kitam.; J, *T. liaotungense* Kitag. K, *T. sinomongolicum* Kitag. L, *T. heterolepis* Nakai et Koidz. ex Kitag. M, *T. brassicae folium* Kitag. N, *T. platyepidum* Diels O, *T. falcilobum* Kitag. P, *T. borealisinense* Kitam. Q, *T. junpeianum* Kitam. R, *T. erythopodium* Kitag.

Figure 5

Dendrogram of grouping based on morphological characteristics of the 18 taxa of northeast China native *Taraxacum* species by cluster analysis using Average linkage (Between Groups).

Figure 6

Dendrogram of chemical composition of the 18 taxa of northeast China native *Taraxacum* species by cluster analysis using Average linkage (Between Groups).

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