APPLICATION OF ACHENE MORPHOLOGY IN SYSTEMATICS OF SOME IRANIAN TARAXACUM (ASTERACEAE TRIBE CICHORIEAE) SPECIES

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Achene micro-morphology of 17 species of *Taraxacum* (*Asteraceae*) is investigated using Scanning Electron Microscopy (SEM). In this study 12 qualitative and quantitative characters such as achene length, width and colour, ornamentation type and size, length of beak, length and colour of pappus were examined. Cluster Analysis (CA) and Principal Component Analysis (PCA) of data showed that micromorphology of achene has significant role in separating species from each other and it does support the delimitation at sectional level. Our results showed that ornamentation on achene body cannot be used in separation of taxa at species level as well as sectional rank, whereas ornamentation size showed to be a better tool for separation of species. Since fruit micromorphology provides some evidences for delimitation of species and sections, the results then were compared with the phylogenetic trees obtained from Maximum Parsimony (MP) and the Bayesian analysis (BA) of the nrDNA ITS region. Our results showed that current taxonomic system of the genus *Taraxacum* is not compatible with its phylogeny.

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Key words. Achene micromorphology, *Asteraceae*, Iran, nrDNA ITS, *Taraxacum* phylogeny.

کاربرد مورفولوژی فندقه در رده بندی چند گونه از قاصدک (Asteraceae tribe Cichorieae) در ایران

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میکرومورفولوژی هفده گونه متعلق به جنس قاصدک (تیره آفتابگردان) با استفاده از میکروسکوپ الکترونی مورد بررسی قرار گرفت. در این مطالعه دوازده صفت کمی و کیفی از قبیل طول، عرض و رنگ فندقه، نوع و اندازه تزئینات بدنه فندقه، طول منقار، طول و رنگ پاپوس بررسی شد. آنالیز خوشه‌ای و آنالیز مولفه اصلی داده‌ها نشان داد که میکرومورفولوژی فندقه در تفکیک گونه‌ها از یکدیگر نقش مهمی داشته در حالیکه جداسازی در سطح بخشه را تایید نمی‌کند. نتایج نشان داد که شکل و تزئینات خارها در سطح فندقه نمی‌تواند برای جداسازی تاکسون‌ها در سطح گونه به اندازه سطح بخشه مفید باشد، در حالیکه اندازه تزئینات نشان داد که می‌تواند به عنوان ابزار مناسبی برای تفکیک گونه‌ها باشد. با اینحال به نظر می‌رسد که میکرومورفولوژی میوه شواهدی را برای تعیین حدود گونه‌ها و بخشه‌ها فراهم می‌کند، سپس نتایج با درخت های فیلوژنی بدست آمده از آنالیزهای ماکزیمم پارسیمونی و Bayesian ناحیه فاصله انداز درونی هسته‌ای مقایسه شد. نتایج نشان داد که تاکسونومی جنس قاصدک با فیلوژنی آن سازگار نیست.

INTRODUCTION

The genus *Taraxacum* Wigg. belongs to the family *Asteraceae* (*Cichorieae*; *Crepidinae*). This genus, consisting of over 2500 species, divided into more than 50 sections (Kirschner & Štepanek 1997; Glowacki 2004). A comprehensive taxonomic treatment of all *Taraxacum* species has not published yet (Kirschner & Štepanek 1993, 1996, 1997, 1998, 2005; Glowacki 2004). Current taxonomy of the genus is mainly based on the morphological characters rather than phylogenetic evidences. Due to lack of sufficient morphological characters, sectional boundaries are sometimes unclear, and therefore resulted in disagreements over the number of sections in different literatures (Schischkin 1964; van Soest 1977; Kirschner & Štepanek 1996). Taxonomic treatment accepted in this study is that of van Soest (1977). According to Flora Iranica (van Soest 1977) there are 11 sections with 55 species, 2 subspecies and 6 forms growing in the different parts of Iran, of which nine species are endemic. *Taraxacum* generally grows in localities such as mountains, steppes, at riverside's, margin of lakes, field edges, margin of gardens, and road sides. The genus is distributed mainly in N, NW, W, C and NE of Iran and also in Afghanistan, Asia Minor, Lebanon, Syria, Iraq, Turkmenistan, SW Europe, W Asia, C Asia, Siberia (west part), Caucasus, Anatolia, India, Greece, and Cyprus (Jafari & Assadi 2007).

Taxonomy of the genus *Taraxacum* is very complicated. The co-occurrence of sexual and apomictic reproduction and common hybridization processes associated with polyploidy have resulted in evolution of a large number of microspecies (Kirschner & Stepanek 1997; Kirschner et. al. 2003; Zavesky et. al. 2005). Other factors leading to the complexity of the genus are morphological similarity and simultaneous high morphological variation, and wide geographical distribution including many unexplored areas (Kirschner et. al. 2003).

Among the more important characters used in discrimination of *Taraxacum* at sectional and species ranks are those of fruit morphology (Schischkin 1964; van Soest 1977; Kirschner & Stepanek 2011). Micromorphological characters of fruits in *Asteraceae* have been studied by a number of researchers and showed to be a useful tool in taxonomy (Lindberg 1935; Dittrich 1968; Kynclova 1970; Haque & Godward 1984; Abid & Qaiser 2002; Ritter & Miotlo 2006; Zhu et. al. 2006; Abid & Qaiser 2007 a, b; Kreitschitz & Valles 2007; Chehregani & Mahanfar 2007; Marciniuk et. al. 2009; Bednorz & Maciejewska 2010). Achenes are generally regarded as affording the most critical taxonomic characters, such as size, form, colour, beak length, pappus colour, ribbing and tubercles (Sears 1922).

We here primarily aim to examine the achene micro-morphology of some species of *Taraxacum* from different sections growing in Iran in order to assess its value in classification and delimitation of taxa at species and sectional level using SEM microscopy. More evidence will also be obtained from study of internal transcribed spacer (ITS) as a molecular marker, in order to clarify the phylogeny of the genus.

MATERIALS AND METHODS

Morphological and micromorphological studies

Mature achenes were collected from the field or taken from the herbarium material deposited at TARI herbarium. The voucher details of 17 sampled species are given in Table 1. Micro-morphological data were studied using a scanning electron microscope (LEO 440i). The achenes were coated with a 550 Å -thick layer of gold in a polaron SC7610 vacuum coating apparatus for three minutes. Five specimens were selected from each species to study morphological characters. The characters were measured using Carnoy, a digital measurement tool (Schols et. al. 2002). Numerical taxonomy of 12 qualitative and quantitative morphological characters (Tables 2 & 3) was also studied. To reveal species similarity, Cluster Analysis (CA) and Principal Component Analysis (PCA) of fruit characters were performed using SPSS software ver. 18 (2009).

Molecular studies

In total, 73 accessions belonging to 44 species of *Taraxacum* were examined (Table 1). Sixteen of these accessions (14 species) were newly sequenced. These were analyzed along with eight other accessions from the genera *Chondrilla*, *Crepis*, *Cichorium*, *Hieracium* and *Prenanthes* as outgroups (Drabkova 2009; Uhlemann et al. 2009). Total DNA was extracted from the fresh or silica-gel dried plant material after the modified 2X CTAB procedure of Doyle & Doyle (1987) and DNeasy plant mini kit extraction (Qiagen). Internal transcribed spacer (ITS) region of the nr DNA was amplified using either primer pairs AB101 (5'-ACGAAT TCA TGG TCC GGT GAA GTG TTC G-3') and AB102 (5'-TAG AAT TCC CCG GTT CGC TCG CCG TTA C-3') (Douzery et al. 1999), or ITS-18S (5'-CCT TMTCATYTAGAGGAAGGA G-3') and ITS-28S (5'-CCG CTTATTKATATGCTTAAA-3') (Muir & Schlötterer 1999). PCR protocol for amplification of the entire ITS region using ITS101/102 primers was: a 5 min initial preheating at 95°C, 35 cycles of 30″ denaturation (95°C), 30″annealing (50°C), and 90″elongation (72°C), followed by a final elongation for 7 min. PCR protocol for primers ITS-18S/ITS-28S is identical to that of

Table. 1. List of *Taraxacum* material included in this study. Exact localities are given for the Iranian material extensively examined in this study. For the material obtained from the genebank, only country of origin and GeneBank accession numbers are given.

Taxon	Sectional assignment	Locality	Type of data studied		
			Achene data	ITS	Gene Bank accession no.
T. afghanicum Soest	*T.* sect. *Macrocornuta*	Khuzestan: Ramhormoz, 30 km Haftgel road, 200 m, Foroughi 3015 (TARI)	*	-	-
T. juzepczkii Schischk.	*T.* sect. *Macrocornuta*	Khorasan: ca. 29 km West of Kalatenaderi, near Soltanabad (GC2),850 m, Assadi & Maassoumi 55779 (TARI)	*	-	-
T. stanjukoviczii Schischk.	*T.* sect. *Macrocornuta*	Khorasan: Torogh, Research Center of Agriculture and Natural Resources, 980 m, Saghafi & Borhan 370 (TARI)	*	*	KC312158
T. nevskii Juz.	*T.* sect. *Macrocornuta*	Azerbaiejan: Arasbaran Protected area Kaleibar to Hejrandust. 1350-1700 m, Assadi & Masoumi 20065 TARI	*	*	KC312159
T. monochlamydeum Hand. –Mazz. & G. E. Haglund	*T.* sect. *Macrocornuta*	Tehran: Kavir Protected area, North of Siah Kuh, 1300m, Wendelbo & Assadi 16057 (TARI)	-	*	KC312160
T. pseudo-calocephalum Soest.	*T.* sect. *Macrocornuta*	Azerbaiejan: Mountains of south-west of Ahar, 15 km from the village Mizan on the road to Haris (QH2), 2500 m, Assadi & Shahsavari 65938 (TARI)	*	-	-
T. caliops G. E. Haglund	*T.* sect. *Erythrocarpa*	Hamadan: Alvand mountain, near Ganjnameh, 2300 m, Wendelbo & Assadi 16829 (TARI)	*	*	KC312161
T. spinulosum Soest	*T.* sect. *Erythrocarpa*	Mazandaran, Gaduk to Veresk road, 1600-1700 m, Savadkoohi 13530 (IAUH).	*	*	KC312162
T. phaleratum G. E. Haglund	*T.* sect. *Erythrocarpa*	Azerbaiejan: Arasbaran Protected area, Doghrun and Kalan mountains, 2300-2500 m, Assadi & Sardabi 24074 (TARI)	*	*	KC312163
T. aurantiacum Dahlst.	*T.* sect. *Orientalia*	Hamadan: ca. 70 km from Bijar to Hamadan before the village Chahrod, 1800 m, Assadi 61018 (TARI)	*	*	KC312164
T. crepidiforme DC.	*T.* sect. *Orientalia*	Azarbayejan: Arasbaran protected area, mountains of Kharil, 2000-2500, Assadi & Masoumi 20260 (TARI)	*	*	KC312165
T. montanum Nutt.	*T.* sect. *Spuria*	Mazandaran: 21 km AbegarmLarijan to Mallard, 2350 m, Mozaffarian & Aboohamzeh 42610 (TARI)	*	-	-
T. syriacum Boiss.	*T.* sect. *Spuria*	Khorasan: Ghadamgah, Dizbad Village, 1700- 2200m, Mozaffarian 45588 (TARI)	-	*	KC312166
T. roseum Bornm.	*T.* sect. *Spuria*	Tehran: Firuzkuh to Semnan, North of Torud village, 1900-2000 m, Safavi 80558 (TARI)	*	*	KC312167

Continued Table. 1.

T. microcephaloides Soest	*T.* sect. *Rodotricha*	Azerbaiejan: Herodabad, 14 km Asalem road, 2080 m, Shirdelpur 10485 (TARI)	*	-	-
T. serotinum Poir. "Haraz"	*T.* sect. *Serotina*	Mazandaran: Haraz road, between Haraz and Lasem, 2700 m, Mehregan &Yeganeh 13531 (IAUH).	-	*	KC312170
T. serotinum Poir.	*T.* sect. *Serotina*	Mazandaran: Chalous road, above the Kandovan Tunnel, 2800 m, Mehregan &Yeganeh 13531 (IAUH).	-	*	KC312169
T. serotinum Poir.	*T.* sect. *Serotina*	Semnan: north of Semnan, Cheshme Asuran, 2000 m, Assadi & Ranjbar 82058 (TARI)	-	*	KC312168
T. oliganthum Hand. -Mazz	*T.* sect. *Oligantha*	Semnan: ca. 20km North West of Shahrud, above Nekarman, Kuh-e Shahvar, Assadi & Mozaffarian 40876 (TARI)	-	*	KC312171
T. iranicum Soest	*T.* sect. *Oligantha*	Khorasan: ca. 50 km Kashmar, Bezgh mountain (FE4), 2500 m, Assadi & Mozaffarian 35761 (TARI)	*	-	-
T. azerbaijanicum Soest	*T.* sect. *Erythrosperma*	Azerbaiejan: West of Rezaiyeh, hills West of Silvana village, 1550-1800 m, Runemark & Foroughi 19625 (TARI)	*	-	-
T. macrolepium Schischk.	*T.* sect. Vulgaria	Gilan: Asalem to Khalkhal, 800-1000 m, Wendelbo & Assadi 27695 (TARI)	*	*	KC312172
T. bessarabicum Hand. –Mazz	*T.* sect. Leptocephala	Azerbaiejan: ca. 50km North West of Khoy lake above the village Ghezelja.2300 m, Assadi & Olfat 68742 (TARI)	-	*	KC312173
T. mongoliforme R. Doll	*T.* sect. *Stenoloba*	Russia	-	-	EU637208-EU637210
T. formosissimum Kirschner & Štěpánek	*T.* sect. *Suavia*	Russia	-	-	EU637152
T. stupendum Kirschner & Štěpánek	*T.* sect. *Suavia*	Kirghizia	-	-	EU637295-EU637296
T. suasorium Kirschner & Štěpánek	*T.* sect. *Suavia*	Kirghizia	-	-	EU637302-EU637304
T. suave Kirschner & Štěpánek	*T.* sect. *Suavia*	Kirghizia	-	-	EU637316-EU637314
T. venustius Kirschner & Štěpánek	*T.* sect. *Suavia*	Kirghizia	-	-	EU637332-EU637334
T. nobile Kirschner & Štěpánek	*T.* sect. *Suavia*	Mongolia	-	-	EU637230-EU637232
T. armeriifolium Soest	*T.* sect. *Leucantha*	NW India	-	-	EU637116-EU637118
T. candidatum Kirschner, Štěpánek & Klimeš	*T.* sect. *Leucantha*	NW India	-	-	EU637132-EU637134
T. inimitabile Kirschner & Štěpánek	*T.* sect. *Leucantha*	Mongolia	-	-	EU637172-EU637174
T. leucanthum Ledeb.	*T.* sect. *Leucantha*	Russia	-	-	EU637192-EU637194

Continued Table. 1.

T. luridum G.E.Haglund	*T.* sect. *Leucantha*	NW India	-	-	EU637198-EU637200
T. niveum Kirschner & Štěpánek	*T.* sect. *Leucantha*	Russia	-	-	EU637220-EU637222
T. album Kirschner & Štěpánek	*T.* sect. *Leucantha*	Kirghizia	-	-	EU637110-EU637112
T. occultum Kirschner & Štěpánek	*T.* sect. *Leucantha*	Mongolia	-	-	EU637238-EU637240
T. sinicum Kitag.	*T.* sect. *Leucantha*	Siberia	-	-	EU637274-EU637276
T. virgineum Kirschner, Štěpánek & Klimeš	*T.* sect. *Leucantha*	NW India	-	-	EU637338-EU637340
T. tenuifolium H.Koidz.	*T.* sect. *Palustria*	Italy	-	-	EU637326-EU637328
T. stenocephalum Boiss. & Kotschy ex Boiss.	*T.* sect. *Piesis*	Russia	-	-	EU637284-EU637284
T. bessarabicum Hand.-Mazz.	*T.* sect. *Leptocephala*	Ukraine	-	-	EU637124-EU637126
T. cylleneum Fürnkranz.	*T.* sect. *Piesis*	Greece	-	-	EU637238-EU637240
T. glaucophyllum Soest	*T.* sect. *Sikkimensia*	NW India	-	-	EU637162-EU637164
T. sherriffii Soest	*T.* sect. *Sikkimensia*	NW India	-	-	EU637264-EU637166
T. serotinum poir.	*T.* sect. *serotina*	Bulgaria	-	-	EU637354-EU637356
T. arcticum Dahlst.	*T.* sect. *Arctica*	Greenland	-	-	AM946526
T. rubicundum Dahlst.	*T.* sect. *Erythrosperma*	Germany	-	-	AM946539
T. erythrospermum Andrz. ex Besser	*T.* sect. *Erythrosperma*	North of Mexico	-	-	AJ633291
T. nordstedtii Dahlst.	*T.* sect. *Celtica*	Germany	-	-	AM946531
T. officinale F.H.Wigg.	*T.* sect. *Taraxacum*	London	-	-	AY862583-HQ161934
Chondrilla juncea L.	-	Italy	-	-	AJ633348
Crepis biennis L.	-	Czech Republic	-	-	AJ633355
Hieracium pilosella L.	-	Germany	-	-	AM946982
Hieracium pilosella L.	-	Germany	-	-	AY879161
Cichorium intybus L.	-	Turkey	-	-	AJ746409
Cichorium intybus L.	-	Romania	-	-	AJ746407
Prenanthes purpurea L.	-	Bulgaria	-	-	AJ633342-AJ633343

(López-Vinyallonga et al. 2008). Electropherograms were analyzed with Sequencher 3.0 software (Gene Codes, AnnArbor, MI) and then aligned using MacClade 4 (Maddison & Maddison 2000). We excluded the species with intraindividual variation from the study.

Maximum Parsimony analysis of the obtained dataset were performed using PAUP* 4.0b10 (Swofford 2002). Bayesian analysis (BA) was performed using the MrBayes 3.1.2 (Ronquist & Huelsenbeck 2003). The settings appropriate for the best-fit model of nucleotide substitution was selected

Table 2. Achene quantitative morphological data of *Taraxacum* species (scales in mm). Abbreviation used: BL: Body length; BW: Body width; CL: Cone length; BEL: Beak length; PL: Pappus length.

Section	species	BL	BW	CL	BEL	PL
T. sect. *Macrocornuta* v.S.	*T. afghanicum* Soest	3.10	0.96	0.70	9.0	7.0
	T. juzepczkii Schischk.	3.60	0.90	1.00	10.0	7.0
	*T. stanjukoviczi i*Schischk.	4.10	0.86	1.00	8.0	8.0
	T. nevskii Juz.	2.90	0.91	0.70	8.0	6.0
T. sect. *Erythrocarpa* HAND.-MZT.	*T. calliops* G. E. Haglund	3.60	0.78	1.70	10.0	6.0
	T. pseudocalocephalum Soest	3.40	1.10	1.00	10.0	5.0
	T. spinulosum Soest	4.70	1.00	1.50	10.0	5.0
	T. phaleratum G. E. Haglund	4.40	1.00	1.50	11.0	6.0
T. sect. *Orientalia* HAND.-MZT.	*T .aurantiacum* Dahlst.	4.50	0.97	0	1.5	5.0
	T. crepidiforme DC.	3.90	0.84	0.80	6.0	4.2
T. sect. *Spuria* DC.	*T. montanum* Nutt.	7.00	1.30	2.50	18.0	11.0
	T. roseum Bornm.	4.00	0.80	1.00	14.0	10.0
T. sect. *Rodotricha* HAND.-MZT.	*T. microcephaloides* Soest	4.30	1.40	0.60	1.8	5.0
T. sect. *Serotina* v.S.	*T. serotinum* Poir.	4.90	0.83	1.30	7.0	6.0
T. sect. *Oligantha* v.S.	*T. iranicum* Soest	4.80	0.97	1.10	4.0	6.0
Erythrosperma Dahlst	*T .azerbaijanicum* Soest	3.40	0.74	1.30	9.0	5.5
T. sect. *Vulgaria* Dahlst	*T. macrolepium* Schischk.	4.40	0.95	0.60	12.0	7.0

by MrModeltest 3.7 (Posada 2008) under the AIC estimator as follows: Model selected: SYM+G; Base frequencies: R [A-C] = 0.9244, R [A-G] = 1.4773, R [A-T] = 2.1434, R [C-G] = 0.4855, R [Є T] = 4.0789, R [G-T] = 1.0000; proportion of invariable sites = 0; Gamma distribution shape parameter = 0.6029.

RESULTS AND DISCUSSION

Morphological and micromorphological studies

SEM micrographs of achenes studied are presented in Figs. 1-3 and listed in Table 2. The main features of the investigated achene are summarized in Table 2. Taxonomic treatment presented in table 2 is based on the work of van Soest (1977). Terminology of ornamentation and surfaces used in this article are based on Stearn (2004). Achene in *Taraxacum* is consisted from four parts, 1) body, 2) cone, 3) beak and 4) pappus. Nearly all species studied here (except *T. auratiacum* with no cone) possessed all four parts. Achene body is usually narrowly obovate or oblanceolate, attenuated at base, striated longitudinally and spiny at upper part. It is ended to a cone attenuating into a beak. The plumose pappus is attached on the top of beak.

Size of achene body

The size of achene body ranges from 2.9 mm (*T. nevskii* Juz.) to 7 mm (*T. montanum* Nutt.) in length (column 3, Table. 2) and 0.78 mm (*T. calliops* G. E. Haglund) to 1.4 mm (*T. microcephaloides* Soest) in width (column 4, Table. 2). Length and width of achene body also showed variable ranges in any sections, i.e sect. *Macrocornata* showed ranges from 2.9 mm to 4.1 mm and 0.86 mm to 0.96 mm and sect. *Erythrocarpa* showed ranges from 3.4 mm to 4.4 mm and 0.78 mm to 1.1 mm in length and width of achene body respectively.

Size of cone, beak and pappus

The size of cone and beak also showed variation. Most of the species investigated had sizes between 0.6 to 1.7 mm (column 5, Table. 2), among species studied *T. aurantiacum* Dahlst. lacks any cone and *T. montanum* Nutt. have the largest cone (2.5 mm), and also both species have the smallest and largest beak, respectively (column 6, Table 2). Pappus size varies from 4.2 mm in *T. crepidiforme* DC. to 11 mm in *T. montanum* Nutt. (column 7, Table. 2). In other species the pappus length ranged from 5 to 10 mm. The variation in size of cone, beak and pappus were also observed between sections, i.e. size of cone showed ranges from 0.7 mm to 1 mm in sect. *Macrocornuta* and ranges from 1 mm to 1.7 mm in sect. *Erythrocarpa.*

Type ofo rnamentation on achene body

The ornamentation on middle part of achene body showed some variation so that six basic types could be identified. These types were identified after analysis of their quantitative measures (Fig. 6) and qualitative data. In the first type, type A, the surface is covered by spines and gradually narrowed into a rather curved and free tip. The free part is 24-31 µm long and covered with granules (Fig. 1 A-L.). In type B, base of spines are wider and the free parts are short (14-19 µm), cuspidate and covered with tiny granules (Fig. 2 A-L.). Type C showed dense, irregularly arranged spines with smooth surface, tapering into a blunt apex (Fig. 1 M-P)

Table 3. Achene qualitative morphological data of *Taraxacum* species. Abbreviation used: TO: Type of ornamentation; AC: Achene colour; PC: Pappus colour; CE: Cone existence; OS: Ornamentation Size.

Section	species	TO	AC	PC	CE	OS
T. sect. *Macrocornuta* v.S.	*T. afghanicum* Soest	C	straw	snow-white	present	long
	T. juzepczkii Schischk.	B	light bown	snow-white	present	middle
	T. stanjukoviczii Schischk.	A	light brown	dingy white	present	long
	T. nevskii Juz.	C	dark brown	Snow-white	present	long
T. sect. *Erythrocarpa* HAND.-MZT.	*T. calliops* G. E. Haglund	A	dark brown	white	present	long
	T. pseudocalocephalum Soest.	A	straw	white	present	short
	T. spinulosum Soest	B	light brown	white	present	middle
	T. phaleratum G. E. Haglund	A	light brown	dingy white	present	short
T. sect. *Orientalia* HAND.-MZT.	*T. aurantiacum* Dahlst.	B	light brown	white	absent	long
	T. crepidiforme DC.	B	straw	white	present	short
T. sect. *Spuria* DC.	*T. montanum* Nutt.	D	yellowish	white	present	short
	T. roseum Bornm.	E	light brown	white	present	short
T. sect. *Rodotricha* HAND.-MZT.	*T. microcephaloides* Soest	F	yellowish	reddish	present	short
T. sect. *Serotina* v.S.	*T. serotinum* Poir.	A	yellowish	brownish	present	middle
T. sect. *Oligantha* v.S.	*T. iranicum* Soest	A	olive brown	white	present	short
T. Erythrosperma Dahlst	*T. azerbaijanicum* Soest	B	red	white	present	long
T. sect. *Vulgaria* Dahlst	*T. macrolepium* Schischk.	B	straw	white	present	long

In type D spines are very short, with 11-13 mm long free part, obtuse to round at apex, partly covered with dense tiny granules (Fig. 3 A-B). In type E spines are jointed at base in groups up to 4, terminated into acuminate free parts and covered with tiny granules (Fig. 2 M-N) and in type F, spines are irregularly distributed, terminated into acute- apiculate free parts, covered with tiny granules (Fig. 2 O-P). Two most common types i.e, A and B were observed in (*T. stanjukoviczii* Schischk., *T. calliop* G. E. Haglund, *T. pseudocalocephalum* Soest, *T. phaleratum* G. E. Haglund, *T. serotinum* Poir. and *T. iranicum* Soest.) (column 3 Table 2b) (Fig. 1 A-L) and (*T. juzepeczkii* Schischk.,*T. spinulosum* Soest, *T. aurantiacum* Dahlst., *T. crepidiforme* DC., *T. azerbaijanicum* Soest and *T. macrolepium* Schischk.) (Fig. 2 A-L; column 3 Table 3). *T. afghanicum* Soest and *T. nevskii* Juz. showed type C (Fig. 1 M-P; column 3 Table 3) and type D, E and F were observed in *T. montanum* Nutt. (Fig. 3 A, B), *T. roseum* Bornm. (Fig. 2 M, N) and *T. microcephaloides* Soest (Fig. 2 O, P), respectively. The variation in size of ornamentation were also observed in each species (Fig. 6). The longest ornamentation was observed in , *T. aurantiacum* Dahlst. (22-41 µm) and *T. macrolepium* (14-17 µm) had the widest ornamentation also two species *T. aurantiacum* Dahlst. and *T. afghanicum* Soest showed minimum size of ornamentation's length and width respectively.

Achene and pappus colour

Achene colour in most cases was from straw to light brown and yellowish while species *T. nevskii* juz. and *T. calliops* G. E. Haglund (dark brown), *T. iranicum* Soest (olive-brown) and *T. azerbaijanicum* Soest (red) were different from the other species (Table 3, column 4).

Species show low variation in pappus colour, *T. microcephaloides* Soest with reddish and *T. serotinum* with brownish colour separated from others that are snow-white to dingy white (Table. 3, column 5). Also there was no significant difference between species of the same section in terms of achene colour and pappus colour. Cone is present in all species studied except for *T. aurantiacum* Dahlst. (Table. 3, column 6) and the size of spines showed differences from short to long (Table. 3, column 7).

Analysis (CA) of 12 qualitative and quantitative

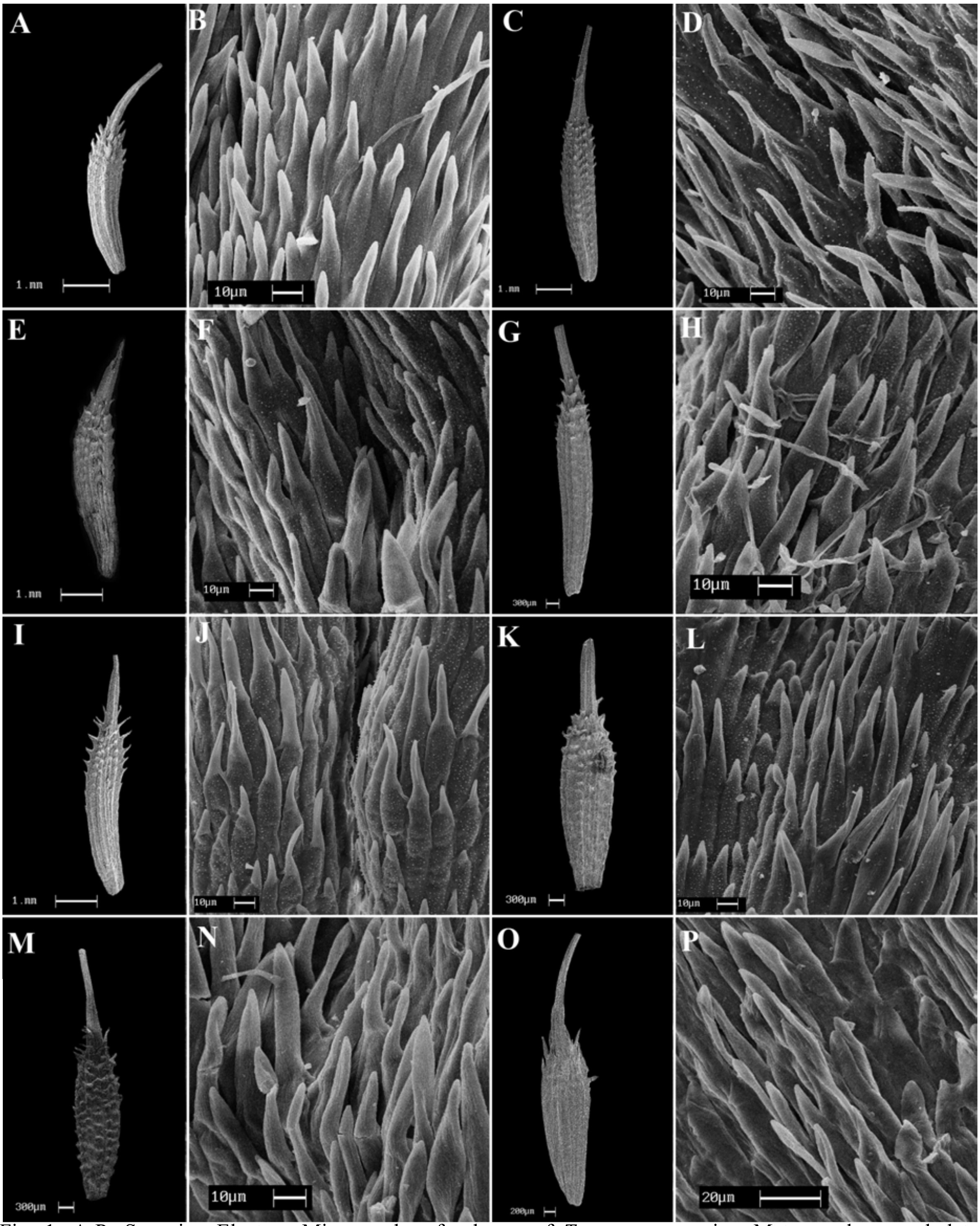

Fig. 1. A-P: Scanning Electron Micrographs of achenes of *Taraxacum* species: Mature achene and detail of ornamentation of achene body: A-B) *T. calliops*: A) Mature achene B) Ornamentation; C-D) *T. iranicum*; E-F) *T. phaleratum*; G-H) *T. serotinum*; I-J) *T. stanjukoviczii*; K-L) *T. pseudocalocephalum*; M-N) *T. nevskii*; O-P) *T. afghanicum*. Scale bar: Figs. A, C, E & I= 1 mm; Figs. B, D, F, H, J, L & N= 10µm; Figs. G, K & M= 300µm; Fig. O= 200µm; Fig. P=20µm.

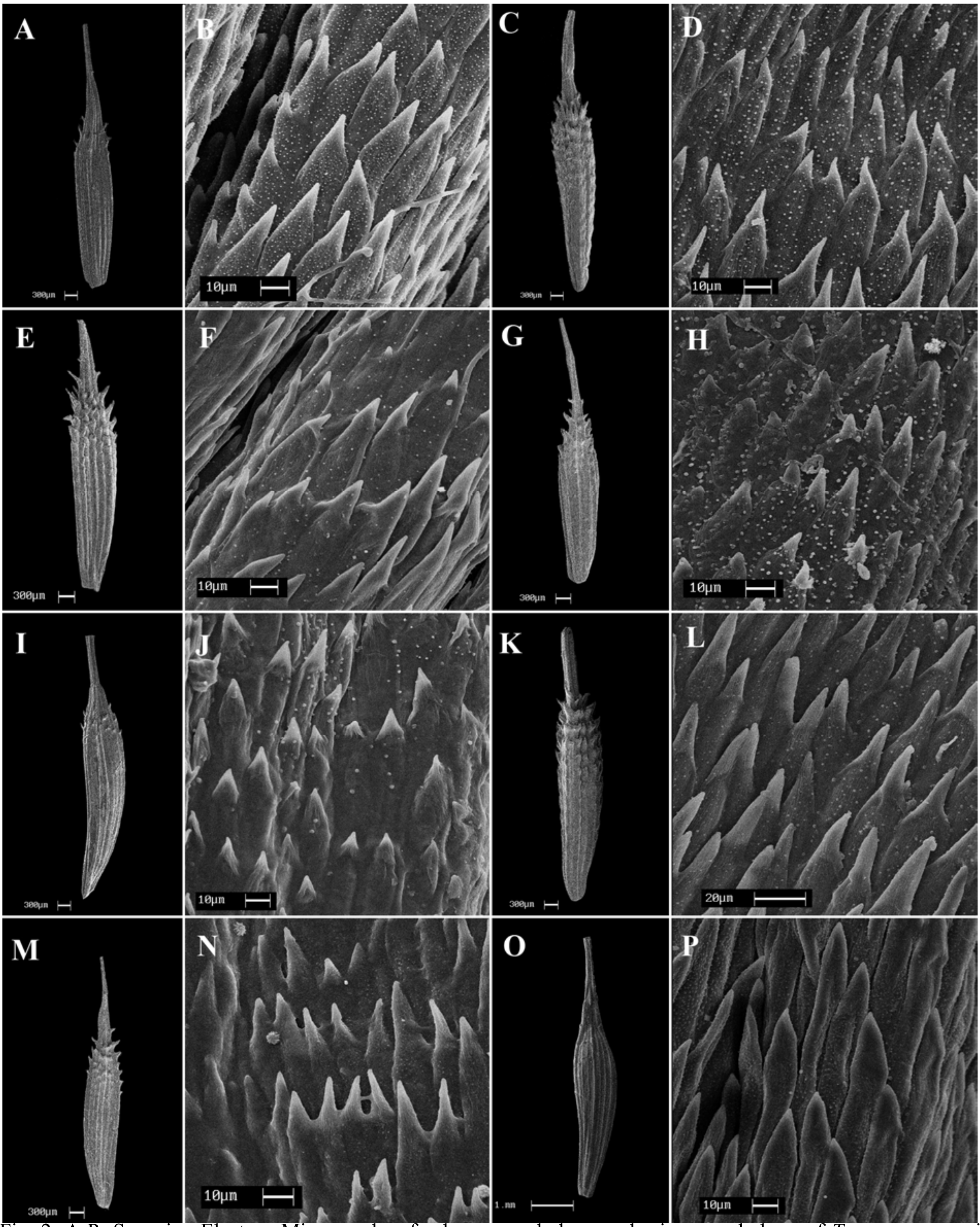

Fig. 2. A-P: Scanning Electron Micrographs of achene morphology and micromorphology of *Taraxacum* species: Mature achene and detail of ornamentation of achene body: A-B) *T. crepidiforme.*: A) Mature achene B) Ornamentation; C, D) *T.spinulosum*; E, F) *T. azerbaijanicum*; G, H) *T. juzepczkii*; I.J) *T. aurantiacum*; K, L) *T. macrolepium*; M, N) *T. roseum*; O, P) *T. microcephaloides*. Scale bar: Figs. B, D, F, H, J, N & P= 10µm; Figs. A, C, E, G, I, K & M= 300µm; Fig. O= 200µm; Fig. L=20µm; Fig. O= 1 mm.

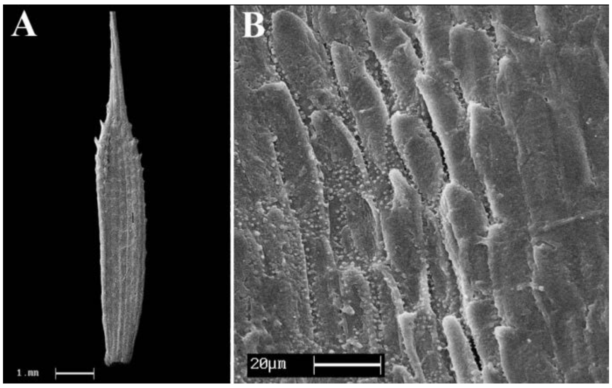

Fig. 3. A-B: Scanning Electron Micrographs of achene morphology and micromorphology of *Taraxacum* species: Mature achene and detail of ornamentation of achene body: A-B) *T. montanum*: A) Mature achene B) Ornamentation. Scale bar. Fig. A= 1 mm; Fig. B= 20μm.

morphological characters was performed using WARD method after standardization of quantitative data. The obtained dendrogram is represented in Fig. 4. Species are divided in two main groups ("A" and "B" in Fig. 4).

Both species of the branch "A", i. e. *T. montanum* Nutt. and *T. roseum* Bornm., belong to sect. *Spuria*.

Group B consists of 15 species representing eight sections. Two species of *Taraxacum* sect. *Macrocornuta*, i.e. *T. afghanicum* Soest and *T. nevskii* Juz., show high similarity and are correlated below the phenoline 5 (Fig. 4). *T. spinulosum* Soest, *T. phaleratum* G. E. Haglund and *T. pseudocalocephalum* Soest of sect. *Erythrocarpa* are clustered together. Two species of the sect. *Orientalia* (*T. aurantiacum* Dahlst. and *T. crepidiforme* DC.) were also clustered together.

Results from the ordination of taxa using Principal Component Analysis (PCA) of 13 qualitative and quantitative data (Fig. 5) is similar to that of cluster analysis. As seen in Fig. 5 species from same sections and also sections *Macrocornuta*, *Erythrocarpa*, *Vulgaria*, *Erythrosperma*, *Oligantha*, *Orientalia* and *Serotina* were placed near each other whereas sections *Spuria* and *Rodotricha* were placed far from the others.

Molecular studies

Characteristics of the nrDNA ITS sequences and the MP strict-consensus tree (Fig. 7) calculated from the 20000 shortest trees are summarized here: total characters=632; number of constant characters=310; number of parsimony-informative characters= 227(36 %); tree length= 774; CI= 0.570 and RI= 0.733. As illustrated in Fig. 8, all *Taraxacum* species form a monophyletic clade. The *Taraxacum* clade itself is mainly consisted of a polythomy of some subclades with different supports plus some individual species.

As phylogeny of the genus is not resolved clearly, we consider the species grouped in subclades. Of total eight main subclades forming the *Taraxacum* clade, only one (subclade I) is consisted of species belonging to same section (*T.* sect. *Leucantha*). All other subclades are formed by species belonging to different sections. For example, species from sect. *Leucantha* are scattered among all subclades except IV and VIII (Fig. 7). Some species of sect. *Leucantha* are grouped with species from other sections into subclades with high supports. In many cases, even accessions from same species but from different localities are not grouped together. We mapped achene types of some known species on the MP tree (Fig. 7). The results showed no clear correlation between the phylogeny and achene ornamentation. Different achene types (see earlier) can be found in subclades consisted of species belonging to different sections. In subclade IV, ornamentation types A (*T. stanjukovikzii* schischk.. *T. calliops* G. E. Haglund *and T. phaleratum* G. E. Haglund), B (*T. crepidiforme* DC*., T. spinulosum* Soest *and T. macrolepium* Schischk.), C (*T. nevskii* Juz.) and E (*T. roseum* Bornm) & in subclade V, types B (*T. aurantiacum*Dahlst) and F (*T. microcephaloides* Soest) were observed.

Incongruency between achene morphology and phylogeny

The micromorphology of only few *Taraxacum* species has been investigated yet. That is why we can not entirely compare our results with the data from literature. Wu et al. (2011) studied the morphology and micromorphology of achenes of 10 species of *Taraxacum* from northeastern China to provide evidence for classification. Their observation based on the size, shape, cone proportion, colour and surface sculpture of achenes revealed that the differences in achene morphological characteristics can be regarded as taxonomic evidence and they mentioned that cone proportion (B/A: cone length/achene length) is supplemented as a main point of taxonomy of *Taraxacum*.

Based on the results of this study the morphology and micro-morphology of achene showed some similarities and differences between species. Cluster Analysis (CA) and Principal Component Analysis (PCA) of data showed that micromorphological characteristics of the fruit has significant role in separating species from each other and it does support the delimitation at sectional level. Our results showed that shape and ornamentation of spines cannot be used in separation of taxa at species level as well as sectional level, whereas ornamentation size showed to be a better tool for separation of species.

The current taxonomy of the genus is mainly based on the morphological data. Boundaries between *Taraxacum* sections are mainly defined by some morphological characters. Comparing the phylogeny obtained from the analysis of nrDNA ITS dataset with current sectional classification of the genus indicates that no solid congruency between the phylogeny and current sectional concept of the genus can be observed (Fig. 7).

A comprehensive and accurate phylogeny of the genus based on the ITS dataset might never be possible due to complexities arisen from a large number of hybridization events leading to a reticulate evolution (Kirschner & Stepanek 1996, 2011). Kirschner et al. (2003) recommended that the nrITS region should be excluded from systematic studies in *Taraxacum*, due to ancestral polymorphisms which lead to an intraindividual sequence variation. The present study shows inter-individual sequence variation in some species, a phenomenon that was also observed by Drabkova et al. (2009).

It seems likely that the current classification of *Taraxacum* needs a comprehensive revision. Achene morphology assumed to be one of the constant characters of the genus, less affected by environment (Sears 1922). Mapping the achene morphology on the nrDNA ITS tree gives no better results. Phylogeny and the achene morphology are not congruent (Fig. 7). Achene types defined in this study are irregularly scattered in the subclades formed in the phylogenetic tree.

Since no clear correlation between the phylogeny, sectional classification and achene ornamentation was observed, fruit micromorphology alone could not provide a practical tool for the sectional delimitation in *Taraxacum*. However, it seems that fruit micromorphology provides some evidences for delimitation of species and sections. To achieve better results, a comprehensive study of achene micromorpholgy along with a better phylogeny of the group based on different molecular markers are required.

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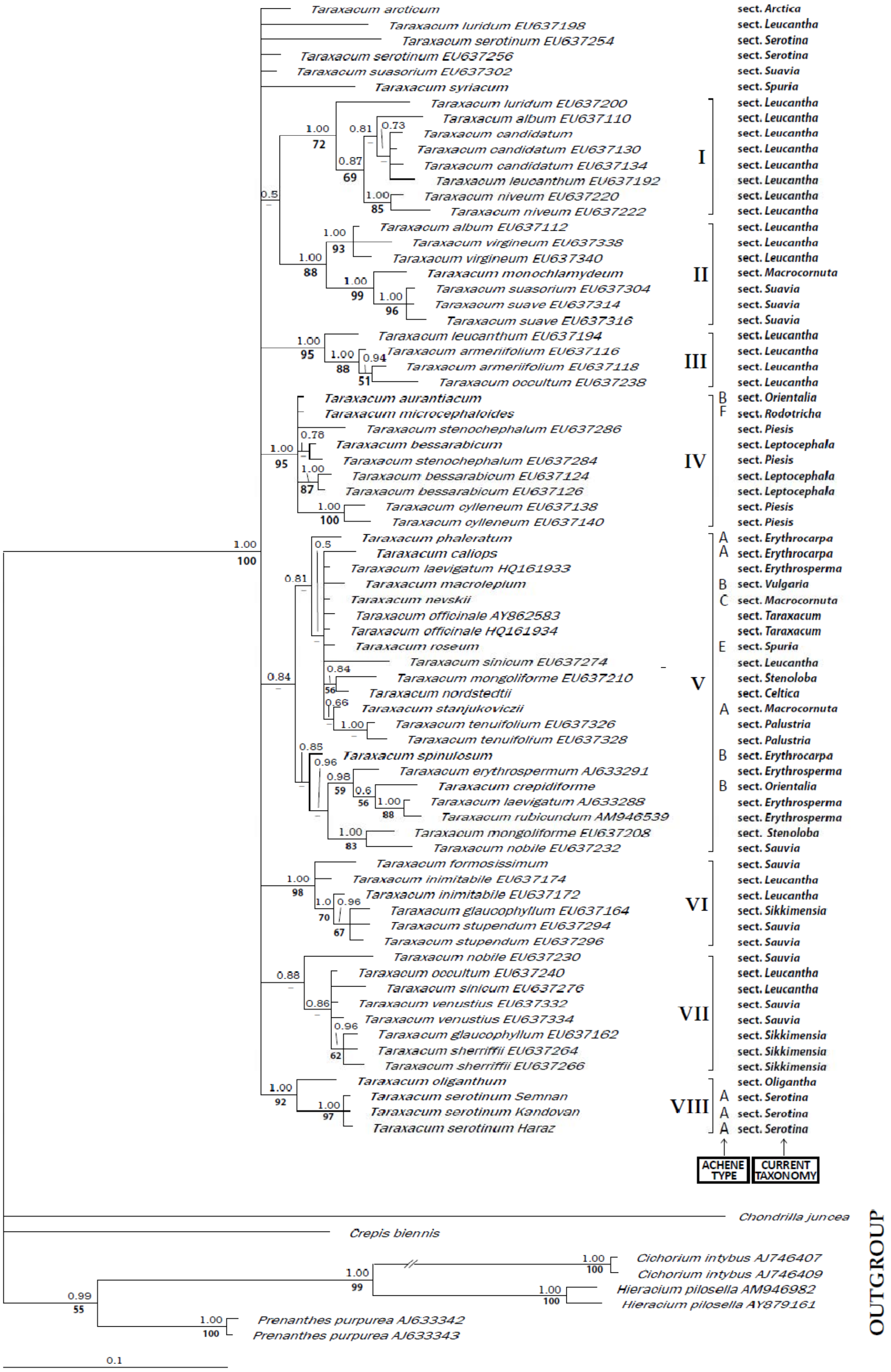
Fig. 7. Phylogenetic tree obtained from the Bayesian analysis (BA) of ITS data set. Numbers above clades indicate posterior probabilities. Number below clades are bootstrap supports (BS) obtained from the Maximum Parsimony (MP) analysis of dataset. Ornamentation types of achene (A-F) are in accordance with those indicated in the text (see results). Names in "***Bold-italics***" indicate species newly sequenced in this study. The bar below the tree shows amount of changes.


Taraxacum arcticum
sect. Arctica
Taraxacum luridum EU637198
sect. Leucantha
Taraxacum serotinum EU637254
sect. Serotina
Taraxacum serotinum EU637256
sect. Serotina
Taraxacum suasorium EU637302
sect. Suavia
Taraxacum syriacum
sect. Spuria
Taraxacum luridum EU637200
sect. Leucantha
Taraxacum album EU637110
sect. Leucantha
Taraxacum candidatum
sect. Leucantha
Taraxacum candidatum EU637130
sect. Leucantha
Taraxacum candidatum EU637134
sect. Leucantha
Taraxacum leucanthum EU637192
sect. Leucantha
Taraxacum niveum EU637220
sect. Leucantha
Taraxacum niveum EU637222
sect. Leucantha
I
Taraxacum album EU637112
sect. Leucantha
Taraxacum virgineum EU637338
sect. Leucantha
Taraxacum virgineum EU637340
sect. Leucantha
Taraxacum monochlamydeum
sect. Macrocornuta
Taraxacum suasorium EU637304
sect. Suavia
Taraxacum suave EU637314
sect. Suavia
Taraxacum suave EU637316
sect. Suavia
II
Taraxacum leucanthum EU637194
sect. Leucantha
Taraxacum armeriifolium EU637116
sect. Leucantha
Taraxacum armeriifolium EU637118
sect. Leucantha
Taraxacum occultum EU637238
sect. Leucantha
III
Taraxacum aurantiacum
B sect. Orientalia
Taraxacum microcephaloides
F sect. Rodotricha
Taraxacum stenochephalum EU637286
sect. Piesis
Taraxacum bessarabicum
sect. Leptocephala
Taraxacum stenochephalum EU637284
sect. Piesis
Taraxacum bessarabicum EU637124
sect. Leptocephala
Taraxacum bessarabicum EU637126
sect. Leptocephala
Taraxacum cylleneum EU637138
sect. Piesis
Taraxacum cylleneum EU637140
sect. Piesis
IV
Taraxacum phaleratum
A sect. Erythrocarpa
Taraxacum caliops
A sect. Erythrocarpa
Taraxacum laevigatum HQ161933
sect. Erythrosperma
Taraxacum macrolepium
B sect. Vulgaria
Taraxacum nevskii
C sect. Macrocornuta
Taraxacum officinale AY862583
sect. Taraxacum
Taraxacum officinale HQ161934
sect. Taraxacum
Taraxacum roseum
E sect. Spuria
Taraxacum sinicum EU637274
sect. Leucantha
Taraxacum mongoliforme EU637210
sect. Stenoloba
Taraxacum nordstedtii
sect. Celtica
Taraxacum stanjukoviczii
A sect. Macrocornuta
Taraxacum tenuifolium EU637326
sect. Palustria
Taraxacum tenuifolium EU637328
sect. Palustria
Taraxacum spinulosum
B sect. Erythrocarpa
Taraxacum erythrospermum AJ633291
sect. Erythrosperma
Taraxacum crepidiforme
B sect. Orientalia
Taraxacum laevigatum AJ633288
sect. Erythrosperma
Taraxacum rubicundum AM946539
sect. Erythrosperma
Taraxacum mongoliforme EU637208
sect. Stenoloba
Taraxacum nobile EU637232
sect. Sauvia
V
Taraxacum formosissimum
sect. Sauvia
Taraxacum inimitabile EU637174
sect. Leucantha
Taraxacum inimitabile EU637172
sect. Leucantha
Taraxacum glaucophyllum EU637164
sect. Sikkimensia
Taraxacum stupendum EU637294
sect. Sauvia
Taraxacum stupendum EU637296
sect. Sauvia
VI
Taraxacum nobile EU637230
sect. Sauvia
Taraxacum occultum EU637240
sect. Leucantha
Taraxacum sinicum EU637276
sect. Leucantha
Taraxacum venustius EU637332
sect. Sauvia
Taraxacum venustius EU637334
sect. Sauvia
Taraxacum glaucophyllum EU637162
sect. Sikkimensia
Taraxacum sherriffii EU637264
sect. Sikkimensia
Taraxacum sherriffii EU637266
sect. Sikkimensia
VII
Taraxacum oliganthum
sect. Oligantha
Taraxacum serotinum Semnan
A sect. Serotina
Taraxacum serotinum Kandovan
A sect. Serotina
Taraxacum serotinum Haraz
A sect. Serotina
VIII
ACHENE TYPE
CURRENT TAXONOMY
Chondrilla juncea
Crepis biennis
Cichorium intybus AJ746407
Cichorium intybus AJ746409
Hieracium pilosella AM946982
Hieracium pilosella AY879161
Prenanthes purpurea AJ633342
Prenanthes purpurea AJ633343
OUTGROUP
0.1