

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

F. Savadkoohi, T. Nejdassattari, M. Assadi, E. Jafari & I. Mehregan

Received 08.08.2012. Accepted for publication 05.09.2012.

Savadkoohi, F., Nejdassattari, T., Assadi, M., Jafari, E. & Mehregan, I. 2012 12 31: Application of achene morphology in systematics of some Iranian *Taraxacum* (Asteraceae tribe Cichorieae) species. -Iran. J. Bot. 18 (2): 249-261. Tehran.

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.

Faezeh Savadkoohi (correspondence <f.sa.biology87@gmail.com>), Taher Nejdassattari and Iraj Mehregan, Department of Biology, Science and Research Branch, Islamic Azad University, Tehran, Iran. -Mostafa Assadi, Research Institute of Forests and Rangelands, P.O. Box 13185-116, Tehran, Iran. -Efat Jafari, Research Center of Agricultural and Natural Resources of Fars province, P.O. Box 71555-617, Iran.

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

فائزه سوادکوهی، دانشجوی دکتری سیستماتیک گیاهی، دانشگاه آزاد اسلامی، واحد علوم و تحقیقات، گروه زیست‌شناسی، تهران، ایران.

طاهر نژاد ستاری، دانشیار گروه زیست‌شناسی، دانشگاه آزاد اسلامی، واحد علوم و تحقیقات، تهران، ایران.

مصطفی اسدی، استاد پژوهش، موسسه تحقیقات جنگلها و مراتع کشور، تهران، ایران.

عفت جعفری، عضو هیئت علمی مرکز تحقیقات کشاورزی و منابع طبیعی، فارس، ایران.

ایرج مهرگان، استادیار گروه زیست‌شناسی، دانشگاه آزاد اسلامی، واحد علوم و تحقیقات، تهران، ایران.

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

Continued Table. 1.

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

Continued Table. 1.

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

(López-Vinyallonga et al. 2008). Electropherograms were analyzed with Sequencher 3.0 software (Gene Codes, Ann Arbor, 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
<i>T. sect. Macrocornuta</i> v.S.	<i>T. afghanicum</i> Soest	3.10	0.96	0.70	9.0	7.0
	<i>T. juzepczkii</i> Schischk.	3.60	0.90	1.00	10.0	7.0
	<i>T. stanjukoviczi</i> iSchischk.	4.10	0.86	1.00	8.0	8.0
	<i>T. nevskii</i> Juz.	2.90	0.91	0.70	8.0	6.0
<i>T. sect. Erythrocarpa</i> HAND.-M.ZT.	<i>T. calliops</i> G. E. Haglund	3.60	0.78	1.70	10.0	6.0
	<i>T. pseudocalocephalum</i> Soest	3.40	1.10	1.00	10.0	5.0
	<i>T. spinulosum</i> Soest	4.70	1.00	1.50	10.0	5.0
	<i>T. phaleratum</i> G. E. Haglund	4.40	1.00	1.50	11.0	6.0
<i>T. sect. Orientalia</i> HAND.-M.ZT.	<i>T. aurantiacum</i> Dahlst.	4.50	0.97	0	1.5	5.0
	<i>T. crepidiforme</i> DC.	3.90	0.84	0.80	6.0	4.2
<i>T. sect. Spuria</i> DC.	<i>T. montanum</i> Nutt.	7.00	1.30	2.50	18.0	11.0
	<i>T. roseum</i> Bornm.	4.00	0.80	1.00	14.0	10.0
<i>T. sect. Rodotricha</i> HAND.-M.ZT.	<i>T. microcephaloides</i> Soest	4.30	1.40	0.60	1.8	5.0
<i>T. sect. Serotina</i> v.S.	<i>T. serotinum</i> Poir.	4.90	0.83	1.30	7.0	6.0
<i>T. sect. Oligantha</i> v.S.	<i>T. iranicum</i> Soest	4.80	0.97	1.10	4.0	6.0
<i>Erythrosperma</i> Dahlst	<i>T. azerbaijanicum</i> Soest	3.40	0.74	1.30	9.0	5.5
<i>T. sect. Vulgaria</i> Dahlst	<i>T. macrolepium</i> 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 [C 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. aurantiacum* 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. *Macrocornuta* 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 of ornamentation 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 μ m 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 μ m), 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
<i>T. sect. Macrocornuta</i> v.S.	<i>T. afghanicum</i> Soest	C	straw	snow-white	present	long
	<i>T. juzepczkii</i> Schischk.	B	light brown	snow-white	present	middle
	<i>T. stanjukoviczii</i> Schischk.	A	light brown	dingy white	present	long
	<i>T. nevskii</i> Juz.	C	dark brown	Snow-white	present	long
<i>T. sect. Erythrocarpa</i> HAND.-M.ZT.	<i>T. calliops</i> G. E. Haglund	A	dark brown	white	present	long
	<i>T. pseudocalocephalum</i> Soest.	A	straw	white	present	short
	<i>T. spinulosum</i> Soest	B	light brown	white	present	middle
	<i>T. phaleratum</i> G. E. Haglund	A	light brown	dingy white	present	short
<i>T. sect. Orientalia</i> HAND.-M.ZT.	<i>T. aurantiacum</i> Dahlst.	B	light brown	white	absent	long
	<i>T. crepidiforme</i> DC.	B	straw	white	present	short
<i>T. sect. Spuria</i> DC.	<i>T. montanum</i> Nutt.	D	yellowish	white	present	short
	<i>T. roseum</i> Bornm.	E	light brown	white	present	short
<i>T. sect. Rodotricha</i> HAND.-M.ZT.	<i>T. microcephaloides</i> Soest	F	yellowish	reddish	present	short
<i>T. sect. Serotina</i> v.S.	<i>T. serotinum</i> Poir.	A	yellowish	brownish	present	middle
<i>T. sect. Oligantha</i> v.S.	<i>T. iranikum</i> Soest	A	olive brown	white	present	short
<i>T. Erythrosperma</i> Dahlst	<i>T. azerbaijanicum</i> Soest	B	red	white	present	long
<i>T. sect. Vulgaria</i> Dahlst	<i>T. macrolepium</i> 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. calliops* G. E. Haglund, *T. pseudocalocephalum* Soest, *T. phaleratum* G. E. Haglund, *T. serotinum* Poir. and *T. iranikum* Soest.) (column 3 Table 2b) (Fig. 1 A-L) and (*T. juzepczkii* 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 μ m) and *T. macrolepium* (14-17 μ m) 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. iranikum* 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

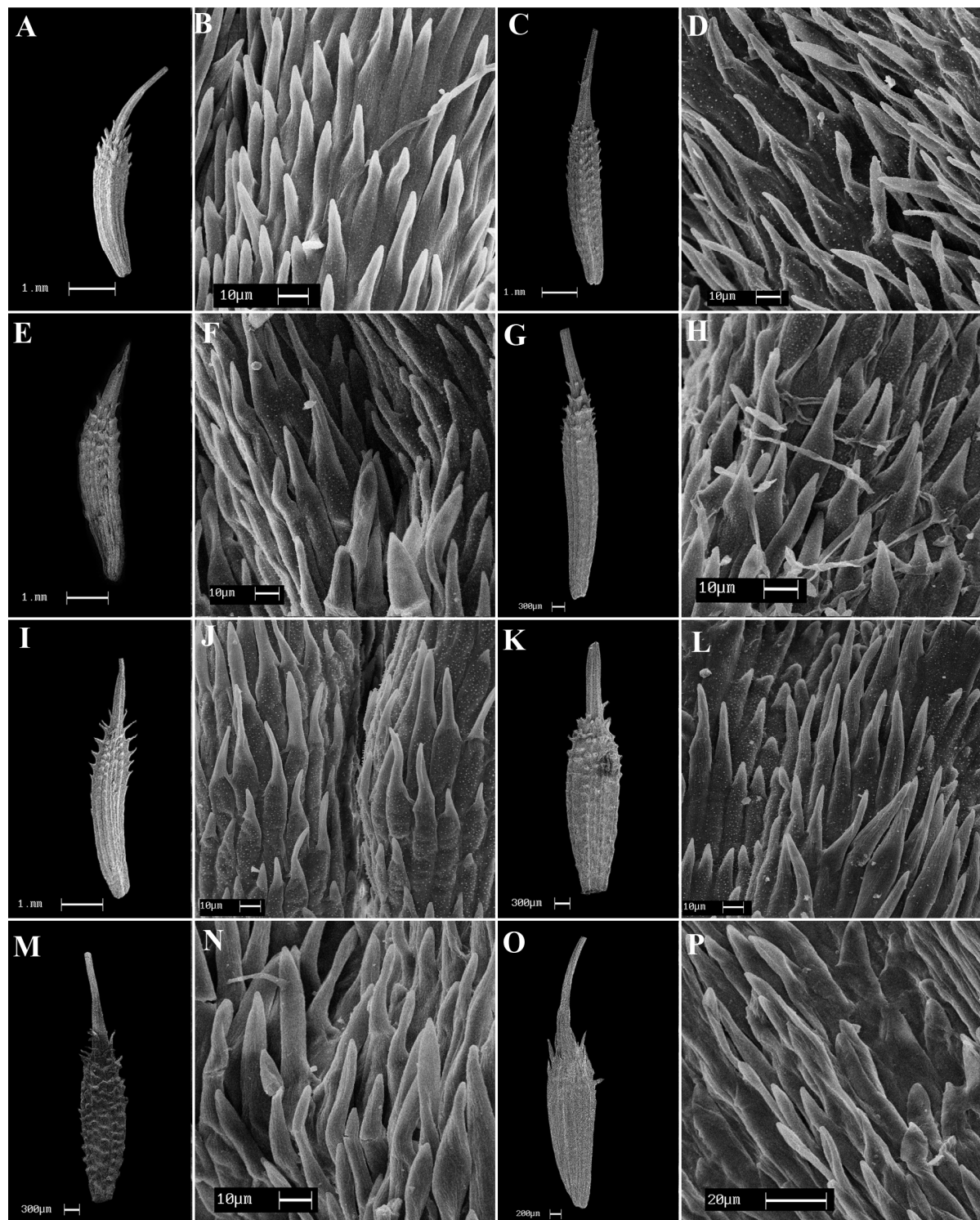


Fig. 1. A-P: Scanning Electron Micrographs of achenes of *Taraxacum* species: Mature achene and detail of ornamentation of achene body: A-B) *T. calliops*: A) Mature achene B) Ornamentation; C-D) *T. iranicum*; E-F) *T. phaleratum*; G-H) *T. serotinum*; I-J) *T. stanjukoviczii*; K-L) *T. pseudocalocephalum*; 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.

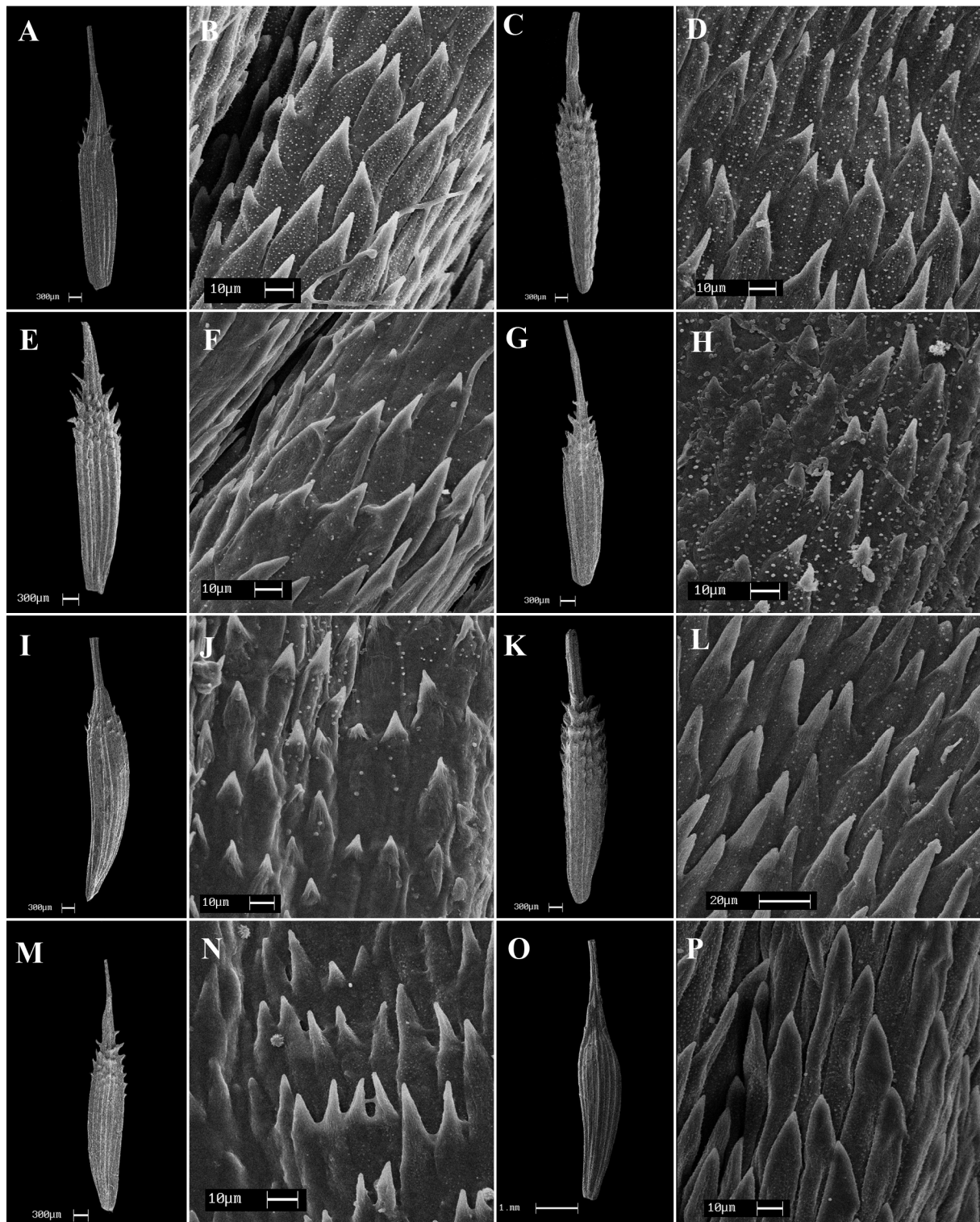


Fig. 2. A-P: Scanning Electron Micrographs of achene morphology and micromorphology of *Taraxacum* species: Mature achene and detail of ornamentation of achene body: A-B) *T. crepidiforme*: A) Mature achene B) Ornamentation; C, D) *T. spinulosum*; E, F) *T. azerbaijanicum*; G, H) *T. juzepczkii*; I, 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.

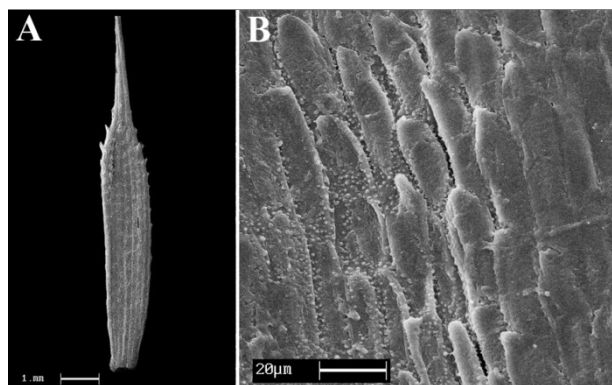


Fig. 3. A-B: Scanning Electron Micrographs of achene morphology and micromorphology of *Taraxacum* species: Mature achene and detail of ornamentation of achene body: A-B) *T. montanum*: A) Mature achene B) Ornamentation. Scale bar. Fig. A= 1 mm; Fig. B= 20μm.

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 micromorphology along with a better phylogeny of the group based on different molecular markers are required.

ACKNOWLEDGMENT

The authors wish to thank Mrs. S. Eshghi for preparing the SEM micrographs.

REFERENCES

- Abid R.D. & Qaiser, M. 2002: Cypsela morphology of *Inula* L. (s. str.) and its allied genera (Inuleae-Compositae) from Pakistan and Kashmir. -Pak. J. Bot. 34(3): 207-223.
- Abid R. & Qaiser, M. 2007 a: Micromorphology of cypsela in the tribe Pluceae from Pakistan. -Pak. J. Bot. 39(3): 671-677.
- Abid R. & Qaiser, M. 2007 b: Cypsela morphology of the genus *Pulicaria* Gaertn. (Inuleae-Asteraceae) from Pakistan. -Pak. J. Bot. 39 (4): 991-997.
- Bednorz, L. & Maciejewska-Rutkowska, I. 2010: SEM observation of pollen grains and fruits of the Pieniny Mountains Polish endemic species *Taraxacum pieninicum* (Asteraceae). -Biologia. 65 (2): 209-212.
- Chehregni, A. & Mahanfar, N. 2007: Achene micromorphology of *Anthemis* (Asteraceae) and its allies in Iran with emphasis on systematic. -IJAB. 3: 386-388.
- Dittrich, M. 1968: Morphologische Untersuchungen an den Früchten der Subtribus Cardueae- Centaureinae (Compositae). -Willdenowia. 5: 67-107.
- Doyle, J. J. & Doyle, J. L. 1987: A rapid DNA isolation procedure for small quantities of fresh leaf tissue. -Phytochem Bull. 19: 11-15.
- Douzery, E. J. P., Pridgeon, A. M., Kores, P., Linder, P., Kurzweil, H. & Chase, M. W. 1999: Molecular phylogenetics of *Dieseae* (Orchidaceae); a contribution from nuclear ribosomal ITS sequence. -Am. J. Bot. 86: 887-899.
- Drabkova, L. Z., Kirschner, J., Stepanek, J., Zavensky, L. & Vlcek, C. 2009: Analysis of nrDNA polymorphism in closely related diploid sexual, tetraploid sexual and polyploidy agamosperous species. -Plant Syst. Evol. 278: 67-85.
- Glowacki, Z. 2004: Morphology and taxonomy of the genus *Taraxacum* (Asteraceae). -Fragmenta Floristica et Geobotania. Polonica. 6: 59-64.
- Haque, M. Z. & Godward, M. B. E. 1984: New records of the *Carpopodium* in Compositae and its taxonomic use. -Bot J Linn Soc. 89: 321-340.
- Jafari, E. & Assadi, M. 2007: *Taraxacum aurantiacum* (Asteraceae), A new record for the flora of Iran. -Iran J Bot. 13 (1): 47-48.
- Kirschner, J. & Stepanek, J. 1993: The genus *Taraxacum* in the Caucasus. 1, Introduction. 2, The section *Porphyrantha*. -Folia Geobot Phytotax. 28: 295-320.
- Kirschner, J. & Stepanek, J. 1996: Modes of speciation and evolution of the sections in *Taraxacum*. -Folia Geobot Phytotax. 31: 415-426.
- Kirschner, J. & Štepanek, J. 1997: A nomenclatural checklist of supraspecific names in *Taraxacum*. -Taxon. 46: 87-98.
- Kirschner, J. & Štepanek, J. 1998: A Monograph of *Taraxacum* sect. *Palustria*. -Institute of Botany, Pruhonice. 281.
- Kirschner, J., Štepanek, J., Mes, T. H. M., Den Mijs, J. C. M., Oosterveld, P., Storchova, H. & Kuperus, P. 2003: Principal features of cpDNA evolution in

- Taraxacum (Asteraceae, Lactuceae): a conflict with taxonomy. -Pl Syst Evol. 239: 231-255.
- Kirschner, J. & Stepanek, J. 2004: New sections in Taraxacum. -Folia Geobot. 39: 259-274.
- Kirschner, J. & Stepanek, J. 2005: Dandelions in Central Asia: Taraxacum sect. Suavia. -Preslia. 77: 263-276.
- Kirschner, J. & Stepanek, J. 2011: Dandelions in Central Asia: a revision of Taraxacum section Stenoloba. -Preslia. 83: 491-512
- Kreitschitz, A. & Valle's, J. 2007: Achene morphology and slime structure in some taxa of Artemisia L. and Neopallasia L. (Asteraceae). -Flora. 202:570-580
- Kynclova, M. 1970: Comparative morphology of achenes of the tribe Anthemidae Cass. (Asteraceae) and its taxonomic significance. -Preslia (Praha). 42: 33-53.
- Lindberg, H. 1935: Die Früchte der Taraxacum-Arten Finnlands. -Acta Bot Fenn. 17: 1-60.
- Lopez-Vinyallonga, S., Mehregan, I., Garcia-Jacas, N., Tscherneva, O., Susanna, A. & Kadereit, J. W. 2009: Phylogeny and evolution of the Arctium-Cousinia complex (Compositae, Cardueae-Caruinae). Taxon. 58: 153-171.
- Maddison, W. P. & Maddison, D. R. 2010: Mesquite (version 2.74): A modular system for evolutionary analysis.
- Marciniuk, J., Vasut, R. J., Marciniuk, P. & Czarna, A. 2009: Taraxacum scanicum Dahlst. group (Section Erythrosperma) in Poland: Chorology and seed and pollen morphology of the Microspecies. -Acta Soc. Bot. Pol. 78 (2): 115-121.
- Muir, M., Schlötterer, C. 1999: Limitations to the phylogenetic use of ITS sequences in closely related species and populations, a case study in Quercus petraea (Matt.) Liebl. [Online at: <http://webdoc.gwdg.de/ebook/y/1999/whichmarker/ml1/Chap11.htm>]
- Posada, D. 2008: Model Test: Phylogenetic Model Averaging. Mol. Biol. Evol. 25: 1253-1256.
- Ritter, M. R & Miotlo, S. T. 2006: Micromorphology of fruit surfaces in species of Mikania Willd. (Asteraceae) occurring in Rio Grande dosul state, Brazil. Acta Bot. Bras. 20 (1): 241-247.
- Ronquist, F. & Huelsenbeck, J. P. 2003: MRBAYES 3: Bayesian phylogenetic inference under mixed models. -Bioinformatics. 19: 1572-1574.
- Schischkin, B. K. [& Tzvelev, N. N., ed.]. 1964: Rod 1667. Oduvančik – Taraxacum Wigg. – In: Komarov V. L. (ed.), Flora SSSR. 29: 405-560, 728-754, Moskva & Leningrad.
- Scholes, P., Dessein, S., D'hondt, C., Huysmans, S. & Smets, E. 2002: CARNOY: a new digital measurement tool for palynology. -Grana. 41: 124-126.
- Sears, P. 1922: Variations in cytology and gross morphology of Taraxacum II. Senescence, Rejuvenescence, and Leaf Variation in Taraxacum. -Botanical Gazette. 73 (6): 425-446.
- Stearn, W. T. 2004: Botanical Latin: History, Grammar, Syntax, Terminology & Vocabulary. eds. 4. -Timber Press.
- Swofford, D. L. 2002: Phylogenetic Analysis Using Parsimony (PAUP). -Sinauer Associates, Sunderland, Massachusetts.
- Uhlemann, I., Ritz C. M. & Penailillo, P. 2009: Relationships in Taraxacum sect. Arcticas.l. (Asteraceae, Cichorieae) and allies based on nrITS. -Feddes Repert. 120: 35-47.
- van Soest, J. L. 1977: Taraxacum Wiggers in Rechinger K. H. Flora Iranica. 122: 223-285. -Graz: Akademische Druck & verlagsanstalt Graz.
- Wu, J., Zhao, X. & Ning, W. 2011: Micro-morphological characteristics of Taraxacum F. H. Wigg. seeds from Northeastern China and taxonomic significance. -Chin. Bull. Bot. 46: 437-446.
- Zavesky, L., Jarolimova, V. & Stepanek, J. 2005: Nuclear DNA content variation within the genus Taraxacum (Asteraceae). -Folia Geobot. 40: 91-104.
- Zhu, S. X., Qin, H. N. & Shih, C. 2006: Achene wall anatomy and surface sculpturing of Lactuca L. and related genera (Compositae: Lactuceae) with notes on their systematic significance. -J. Integ. Pl. Bio. 48 (4): 390-399.

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.

