# یافته‌های نو در طایفه Cardueae از تیره کاسنیان

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**چکیده.** بر اساس این پژوهش، گونه *Jurinea cartilaginea* از سرده *Jurinea* جدا می شود و در زیرطایفه Centaureinae قرار می گیرد. مطالعات مولکولی بر پایه نواحی هسته ای قابل رونویسی بین ژنی ریبوزومی (nrITS)، اطلاعات کروموزومی و مورفولوژی دانه گرده از مطالعات مورفولوژی حمایت می کنند. بر پایه این نتایج، *J. cartilaginea* به سرده *Karvandarina* نزدیک است و به این سرده منتقل می شود. به علت تفاوت در ویژگی های ریخت شناسی بین گونه مذکور و گونه *Karvandarina aphylla*، یک زیرسرده نو به نام *Pseudojurinea* شرح داده می‌شود. همچنین مطالعات مولکولی از تک نیایی بودن سرده *Jurinea* حمایت می کنند.

**واژه‌های کلیدی.** تبارشناسی، دانه گرده، ژورینئا، سنتورینه، فاصله‌انداز رونویسی‌شونده داخلی، کاروان‌درینا

# Novelties in Cardueae (Asteraceae)

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**Abstract.** The current investigations suggest the segregation of *Jurinea cartilaginea* from *Jurinea*, and its placement among Centaureinae. Molecular studies, based on the nuclear ribosomal internal transcribed spacers (nrITS), chromosome number data and pollen morphology, support the morphological results. Based on these results, *J. cartilaginea* is related to and, therefore, transferred to the genus *Karvandarina*. Due to the morphological characters differing from *Karvanderina aphylla*, a new subgenus, *Pseudojurinea*, is described. Molecular study also supports the monophyly of *Jurinea*.

**Keywords.** Centaureinae, ITS, *Jurinea*, *Karvandarina*, Phylogeny, Pollen grain

## INTRODUCTION

Asteraceae, with about 1600 genera and 24000 species, is one of the largest and most widespread families of the plants with a worldwide distribution (Funk *et al*., 2009a).

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The tribe Cardueae Cass. is one of the largest tribes in Asteraceae. It contains 72 genera and about 2400 species (Funk *et al*., 2009b). This tribe contains large genera with various morphological characters. The members of the tribe are distinguished by mostly homogamous capitulum, variously chaffy or more often setose receptacle, tubular and somewhat curved corolla (Rechinger & Wagenitz, 1979; Funk *et al*., 2009b).

This tribe is divided into 5 subtribes according to the traits of the receptacle, achene, pappus and the form of the phyllaries. Carduinae (Cass.) Dumort. and Centaureinae (Cass.) Dumort. are the largest subtribes. In Carduinae, receptacle is mostly scaly or with bristles. Achenes are mostly hard and lignified and often have apical caruncle. Insertion areole of the achenes is straight and basal or basal-abaxial and without elaiosome. Pappus bristles lengths are more or less the same, except for *Jurinea-Saussurea* complex (Susanna & Garcia-Jacas, 2007). In the subtribe Centaureinae, insertion areole is concave and lateral-abaxial, often with elaiosome. Pappus bristles lengths are not the same (Rechinger & Wagenitz, 1979; Funk *et al.*, 2009b).

Genus *Jurinea* Cass., with about 200 species in the world, belongs to Carduinae. The species belonging to *Jurinea* are perennial herbs, sometimes woody at base. The leaves are herbaceous or somewhat leathery. Aerial parts are covered mostly with simple hairs or glands, rarely more or less glabrous. Involucre is globose, ovoid, cylindrical or ovoid-cylindrical. Phyllaries are lanceolate or linear-lanceolate, receptacles are scaly, achenes are mostly obpyramidal or obovoid-obpyramidal and pappi are scabrate or plumose (Rechinger & Wagenitz, 1979; Funk *et al*., 2009b; Mirtadzadini, 2011).

A taxonomic study of *Jurinea* specimens in several herbaria and a taxonomic study of species belonging to *Jurinea* from flora of Iran were carried out by the first author based on the morphological traits (Mirtadzadini, 2011). Karyotype analysis was carried out by Parishani *et al*. (2014). The karyotypic survey of *Jurinea* suggested the separation of *J. cartilaginea* Mozaff. from this genus, or the change of its sectional classification within *Jurinea*. Regarding the questionable differences of *J. cartilaginea*, a rigorous comparison with all other known species of Cardueae was conducted. For this, precise morphological studies as well as palynological, cytological and molecular studies were utilized. In addition, *Jurinea gedrosiaca* Bornm. and *Tricholepis edmondsonii* Rech.f. were included in the phylogenetic analysis to confirm the synonymy of these two names and the new combination *Tricholepis gedrosiaca* (Bornm.) Mirtadz., Parishani & Bordbar by Mirtadzadini *et al*. (In press).

## MATERIALS AND METHODS

**Morphological study. —** The morphological study was carried out using herbarium materials conserved in the herbaria G, MIR, W, WU and HUI (Thiers, 2018) as well as many individuals of several populations belonging to *Jurinea cartilaginea* and *Karvandarina aphylla* Rech.f., Allen et Esfand. in the field. This study focused on the reproductive parts, specially involucre and achene, because all previous studies were devoid of precise descriptions. Moreover, due to comprehensive collection of materials, morphology of vegetative parts was better studied. The information of the examined plant materials in each part is indicated in appendix 1.

**Molecular studies. —** New ITS sequences were provided in this study for 11 selected species belonging to *Jurinea* that were different in morphology and geographical distribution (appendix) as well as *J. cartilaginea*.

For a better comparison, additional ITS region sequences of selected species representing all major groups in Cardueae, mainly Centaureinae, were obtained from NCBI nucleotide database and included in the analyses. The ITS sequences belonging to *Carlina acaulis* L., *Amberboa turanica* Vail., *Cousinia argentea* Mehregan & Assadi, *Tricholepis gedrosiaca* (syn. *Jurinea gedrosiaca and Tricholepis edmondsonii*, Mirtadzadini *et al*., In press), and *Karvandarina aphylla* also sequenced in the present work. Phylogenetic reconstruction was performed on overall 55 species belonging to Cardueae in order to provide more reliable information of the taxonomic position of the questioned taxa. *Carlina acaulis*, *Atractylodes japonica* Koidz. ex Kitam. and *Atractylis carduus* (Forssk.) C. Chr. were defined as outgroups according to previous studies (Funk *et al*., 2009b). Voucher materials of sequenced specimens were deposited in the herbaria MIR and HUI. The detailed locality information of the examined materials and GenBank accession numbers are presented in the appendix. The taxonomic classification of Cardueae follows Susanna & Garcia-Jacas (2007).

Total genomic DNA was extracted from dried leaf tissue of herbarium specimens according to Gawel & Jarret (1991). DNA quality was estimated by electrophoresis in 1.2% (w/v) agarose.

For amplification of the ITS region (ITS1 and ITS2 spacers plus the 5.8S gene), primers ITS-A and ITS-B were used (Blattner, 1999). Polymerase chain reactions were performed according to the following cycling program: initial denaturation at 94°C for 3 min; 38 cycles of 94°C for 30 s, 53°C for 40 s, 68°C for 1 min; followed by a final elongation period at 70°C for 10 min. A final volume of 30 µl for each PCR reaction was prepared; containing 20 ml deionized water, 7 ml of the 2×Taq DNA polymerase master mix Red (Amplicon, Cat. No.180301, Tris-HCl pH 8.5, $(NH4)_2S0_4$, 3 mM $MgCl_2$, 0.2% Tween® 20, 0.4 mM of each dNTP, 0.2 units/µl Ampliqon Taq DNA polymerase, Inert red dye and stabilizer) 0.25 ml of each primer (50 pmol/ml), and 1.5 ml of template DNA.

Forward and reverse sequences obtained for examined samples were inspected and combined into unique consensus sequences. All the Nucleotide sequences were aligned using the program BioEdit sequence alignment editor v.7.2.5 (Hall, 1999), where necessary, manually edited. Sequences were stored in the NCBI nucleotide database (accession numbers MK226129, MK226407-21).

The best-fitted model of sequence evolution was estimated to be SYM+I+G with the Akaike information criterion (AIC) in jModelTest 2.0 (Darriba *et al*., 2012; Guindon & Gascuel, 2003). Bayesian analysis (BI) was performed with MrBayes 3.1 (Ronquist & Huelsenbeck, 2003) under the SYM+I+G model of sequence evolution, running two analyses for $2 \times 10^6$ generations, sampling a tree every 1000 generations. The initial 25% of the trees were discarded as burn-in, and posterior probabilities were calculated on the basis of the remaining trees. Tree visualization was carried out using FigTree v.1.3.1 (Rambaut, 2006-2009).

**Pollen morphology. —** Pollen morphology of two specimens belonging to *Jurinea cartilaginea* and *Karvandarina aphylla* conserved in MIR herbarium were analysed using scanning electron microscopy. For this purpose, pollen grains were taken from mature anthers, mounted on clean metallic stubs and coated with gold layer. The photographs were taken from whole pollen grain and the detailed surface ornamentation by means of a MIRA*II*LMU microscope. The terminology follows Erdtman (1952), Iversen & Troels-Smith (1950), Punt *et al*. (2007) and Wodehouse (1928).

**Chromosome counts. —** Metaphase cells of root tips obtained from germinating seeds were used to evaluate the chromosome number of *Karvandarina aphylla*. Methods for pre-treatment, fixation and staining were described by Bordbar *et al*. (2017). The roots were gently squashed in 45% acetic acid on a glass slide. The clearest mitotic metaphases cells were imaged using an Olympus BH-2 light microscope equipped with camera.

## RESULTS AND DISCUSSION

The karyotype of *Karvandarina aphylla* was studied for the first time (Fig. 1. A). It was revealed that 2n = 2x = 30 with x=15 should be assigned for this species. The mean chromosome lengths for *K. aphylla* and *Jurinea cartilaginea* were measured, which was found to be 1.39 ± 0.21 and 1.89 ± 0.33, respectively. For a better comparison, the karyotype of *J. cartilaginea* is presented as well (Fig. 1. B). This karyotype is provided with permission from Parishani *et al.* (2014).

The study of pollen morphology for *K. aphylla* and *J. cartilaginea* was done for the first time. The observations revealed that the shapes of the pollen in both species were oblate-spheroidal in equatorial view by P/E rate of 0.96-0.97 (Erdtman, 1952). The pollen grains were found to have echinate-perforate ornamentation and the length of echinus was found to be 2.95-3.3 µm. The pollen was found to be 3-zonocolporate with short colpus (11.52 ± 0.85 µm length) acute at the ends (Fig 1. C-F).

Molecular phylogenetic evidence, based on nuclear ribosomal internal transcribed spacer (ITS), was used to guarantee inferring taxonomic position of the taxa studied. The phylogenetic survey on nearly all genera of Centaureinae and other main groups of taxa belonging to Cardueae, based on the study of rDNA region, resulted in the exclusion of *J. cartilaginea* from the genus *Jurinea* and its placement within the tribe Centaureinae close to the so far monotypic genus *Karvandarina* Rech.f. (Fig. 2).

The phylogenetic results indicate that *J. cartilaginea* is closely related to *K. aphylla* with a higher support (BI=100), within the Centaureinae but far apart from other species of *Jurinea* (Fig. 2). The same result was gained for *Tricholepis gedrosiaca* which indicated a distinct clade together with *Tricholepis edmondsonii* among Centaureinae clade (Fig. 2).

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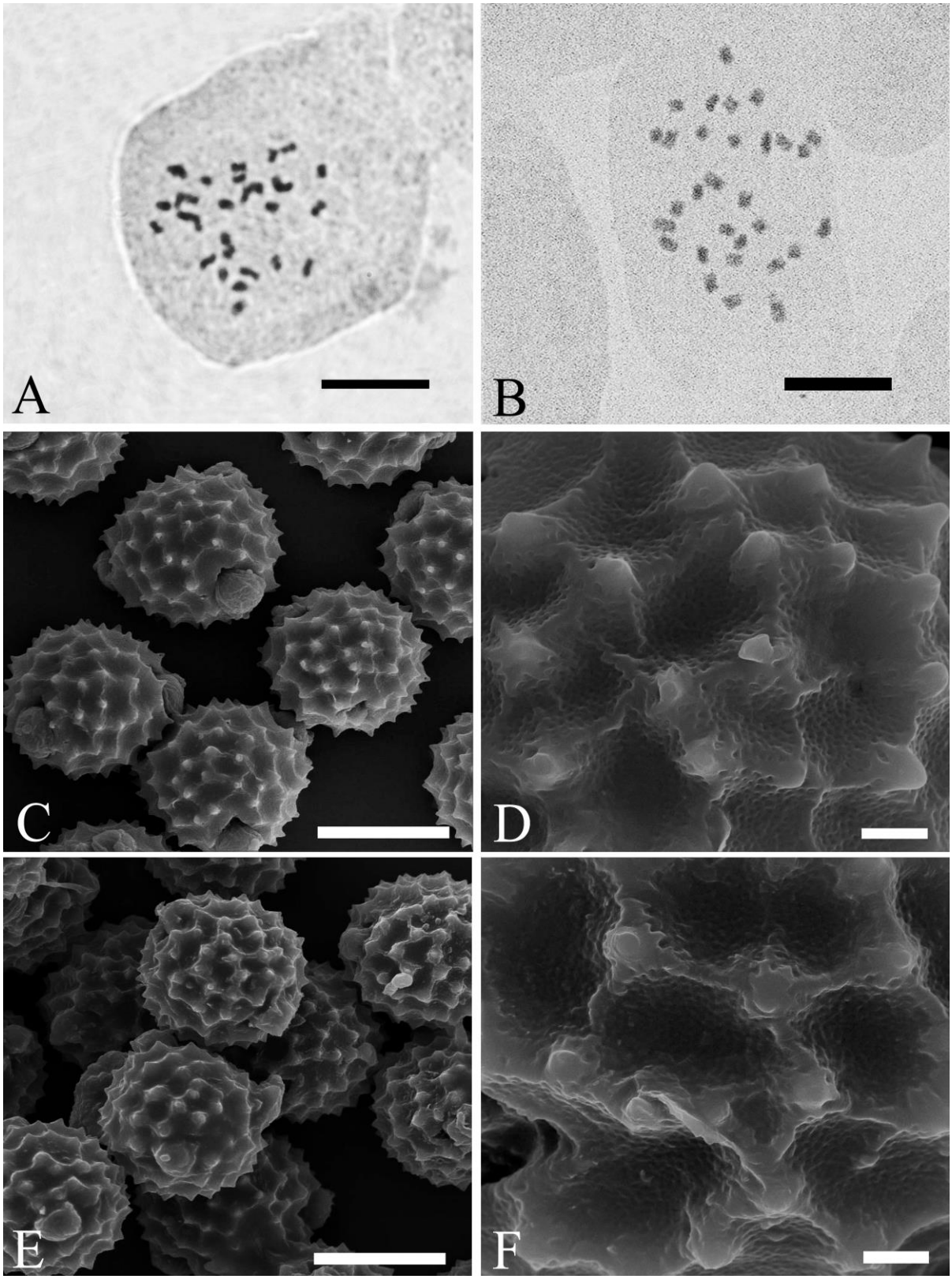



**Fig. 1. A, B:** Karyotype and **C-F:** SEM micrograph of taxa studied. **A:** Metaphase chromosomes of *Karvandarina aphylla*. **B:** Metaphase chromosomes of *Jurinea cartilaginea*. Obtained from Parishani & al. (2014) with permission. Scale bar =10 µm. **C,D:** *J. cartilaginea*. **E,F:** *Karvandarina aphylla*. **B:** Scale bars for the whole grain and enlarged view 20, 2 µm, respectively.

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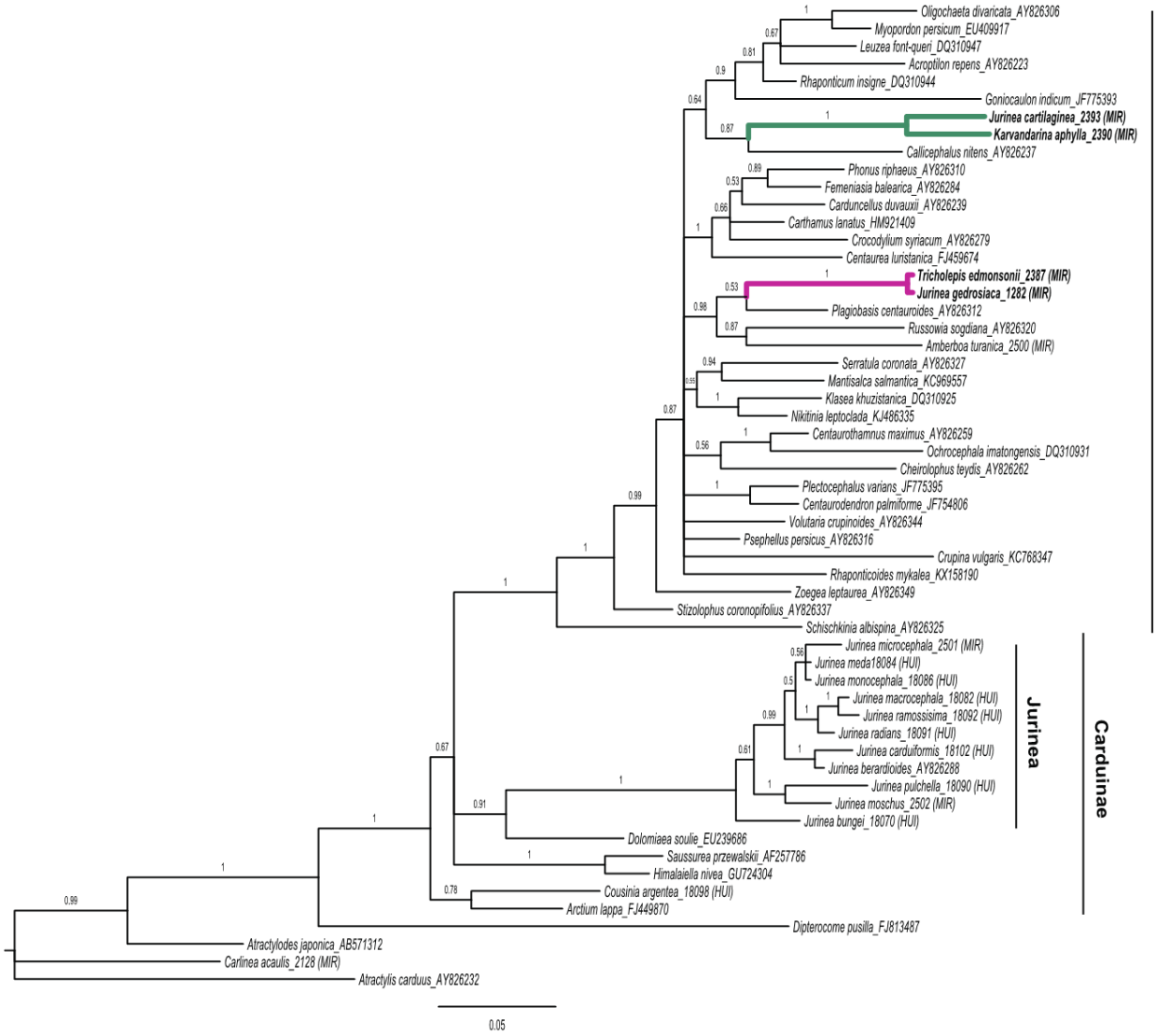



**Fig. 2.** Bayesian consensus tree from the nuclear ribosomal internal transcribed spacers (nrITS) of the taxa studied and related species from Cardueae. Data above branches are the value from Bayesian inference (BI).

The different studies brought together here and the complementary investigations carried out on species belonging to Cardueae, with special focus on *Jurinea cartilaginea*, resulted in a new picture of their relationship. The new information of morphology, ITS sequencing, pollen morphology and karyotypes are in accordance with one another.

**Relationships among *Jurinea cartilaginea* and other species of the genus. —** *Jurinea cartilaginea* was described on the basis of a specimen collected from a mountainous area in SW Iran, Khuzistan Province, near Behbahan (Mozaffarian, 1988). In spite of the traits of the inner parts of the involucre, it was included in the genus *Jurinea* probably because of its woody base and the many-branched subshrub form of the plants as well as the cylindrical capitulum with linear-lanceolate bracts in the general view. It looks similar to members of *Jurinea* sect. *Stechmannia* (DC.) Boiss. (Fig. 3. and 4).

Besides the mentioned characters, obpyramidal achene and scabrous and unequal pappus arms had confirmed this placement.

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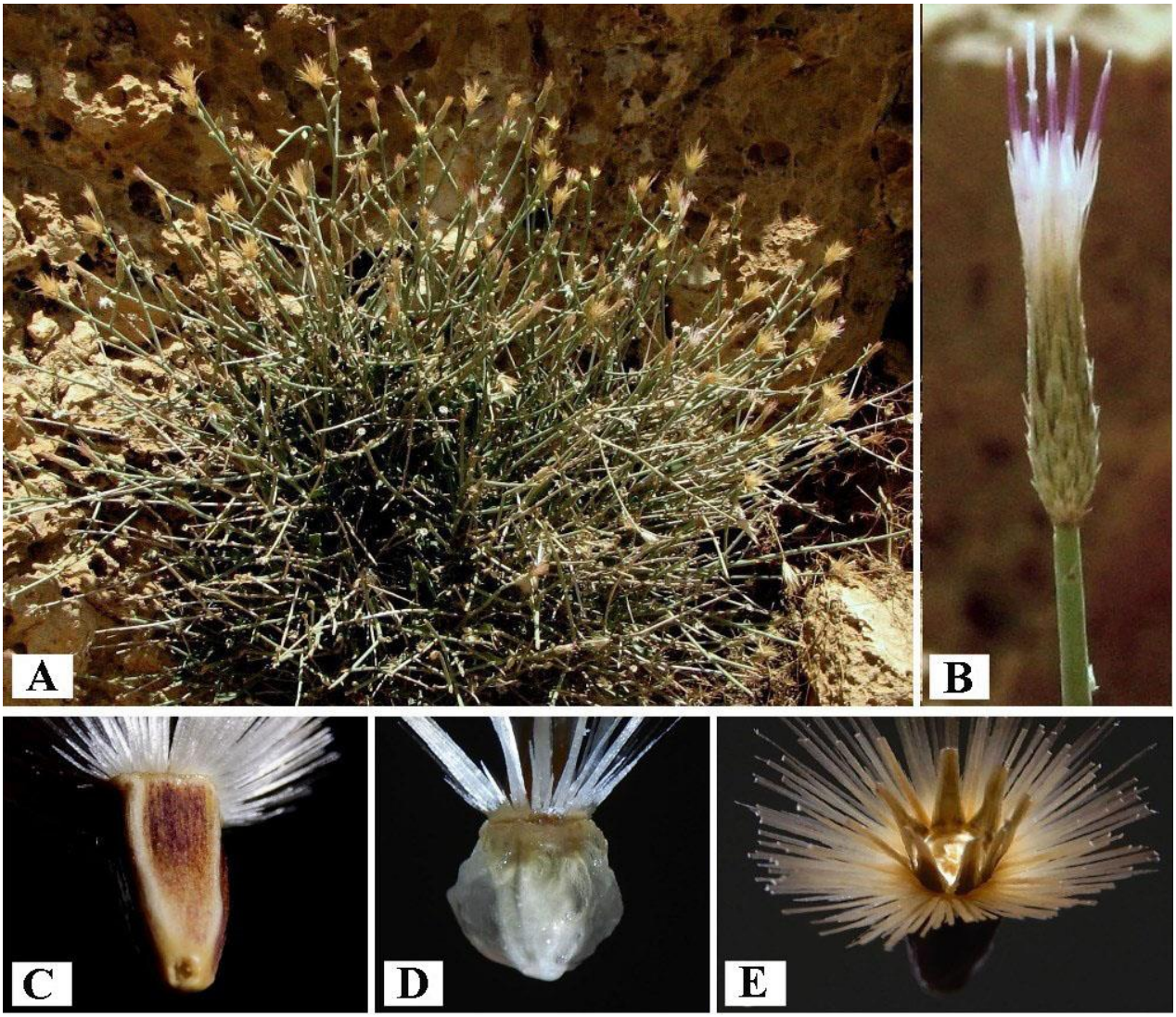



**Fig. 3.** *Jurinea cartilaginea*. **A:** Habit. **B:** Capitulum. **C:** Achene. **D:** Mucilaginous surface of achene after water absorption. **E:** Upside view of achene and pappus (the bristles were cut). Scale bar= 18 mm.

However, this species has some unique characters which cannot be seen in other species of the genus *Jurinea*, and this was not considered by its author. One of these characters is related to the traits of the leaves. The leaves have a blade with some lobes or large teeth. There are white and slightly hard sediments at their ends (Fig 4. C). Also, there are few simple scabrous hairs in this part that might be responsible for the secretion of the sediments. This kind of sediments as well as this type of glabrous leaves cannot be seen in *Jurinea* species. In comparison with the *Jurinea* species, leaf texture in this species is somewhat inflexible and partly cartilage texture. *Jurinea cartilaginea* has also evident differences in reproductive organs in comparison with other *Jurinea* species. The main difference is the existence of bristles on the receptacle (setose receptacles) in *J. cartilaginea*, while receptacles in other *Jurinea* species are covered with scales. The areole in the species is more or less lateral, a character fitting for members of Centaureinae (Fig. 3. C). Similar to most species belonging to *Jurinea*, the shape of achene is obpyramidal. But the surface of achene of *J. cartilaginea* is shining and it forms a mucilaginous layer in wet conditions probably due to existence of polysaccharide components on the surface (Fig. 3. D).

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**Fig. 4.** *Jurinea cartilaginea*, Mirtadzadini 2393. **A,B:** Habit. **C:** White and hard sediments at the margin of leaves. Scale bar = 5 cm

The pappus traits of *J. cartilaginea* are also different from those of other species. In *Jurinea* species with scabrous pappus, there are mostly three innermost bristles that are longer than others. In *J. cartilaginea*, the number of innermost bristles of pappus is at least five, which are wider than those in other *Jurinea* species (Fig. 3. E). The colour of the base of these pappus bristles in *J. cartilaginea* is brown, while the colour of the whole pappus in the majority of *Jurinea* species is white or milky. In the case of the indication of brown colour, it is dispersed uniform and pale in all of the arms of the pappus, e.g. *Jurinea* sect. *Stechmannia* (Mirtadzadini, 2011).

Based on the mentioned morphological characters, *J. cartilaginea* is different from other *Jurinea* species and has to be excluded from the genus. However, it remains in Centaureinae. The highest similarity in reproductive organs including traits of capitulum, involucre, flower and slightly in pappus and achene is found in the monotypic genus *Karvandarina*.

**Comparison of Jurinea cartilaginea with *Karvandarina aphylla*.** — The genus *Karvandarina* was described by Rechinger (1950) on the basis of materials collected from SE Iran, Baluchistan, Karvandar area with the only species *K. aphylla*. The shape and size of leaves and capitulum are similar to those of *J. cartilaginea*. Moreover, there are similarities in the inner parts of capitulum, such as the existence of bristles on the receptacle, the shape and size of corolla and achene (Fig. 5).

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**Fig. 5.** *Karvandarina aphylla*. **A:** Basal part of plant, Mirtadzadini 2390. **B:** Capitulum. **C:** Achene. Scale bar = 5 mm.

Despite the similarities between these two species, they have several morphological differences. The most important differences in the reproductive parts are differences in the traits of their achenes. One of the differences is the existence of hairs on the surface of achene in *K. aphylla* (Fig. 5. C.) while the surface of the achene in *J. cartilaginea* is glabrous, shiny and more or less smooth. These hairs are not lasting and if the achene is touched they will shed. The other difference in their achenes is mucilaginous state on the surface *of J. cartilaginea* due to water absorption (Fig. 3. D). In the vegetative parts, *J. cartilaginea* is a many-stemmed subshrub while *K. aphylla* has leafless, straight and long stems and is only woody at the lower part. Among the populations belonging to *J. cartilaginea*, some younger individuals could be observed that are fewer branched and show more similarity in appearance to *K. aphylla* (Fig. 4. B). In all of the populations belonging to *K. aphylla*, the leaves are apparent in the vegetative growth period but will be lost at the end of flowering phase before fruit ripening.

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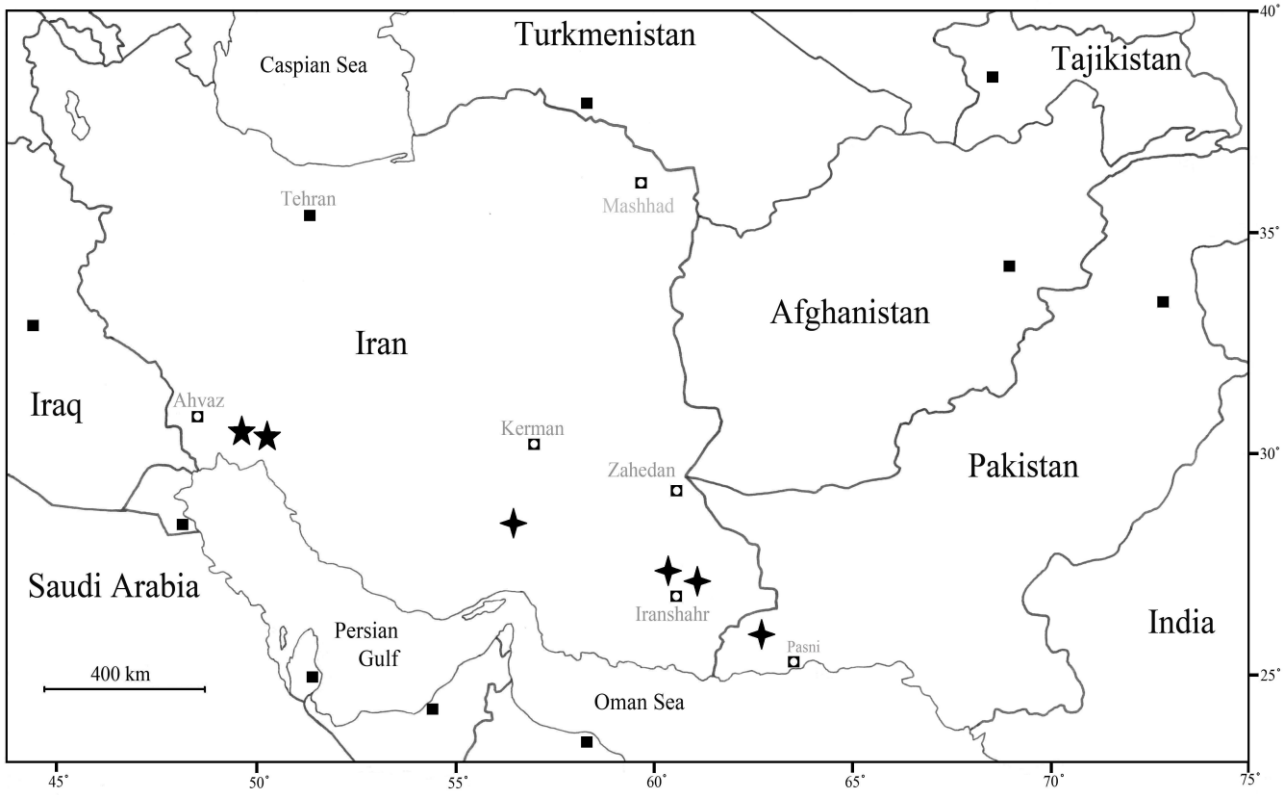



**Fig. 6.** Distribution map of *Jurinea cartilaginea* and *Karvandarina aphylla*. four-point star, *K. aphylla*, five-point star, *J. cartilaginea*.

**Palynology, karyotype and molecular studies.** — The Mean chromosomes length measured for *Karvandarina aphylla* and *Jurinea cartilaginea* species, provided by Parishani *et al*. (2014), are close to each other (Fig. 1. A and B). According to the literature, x = 17 is the most common basic chromosome number among the species belonging to *Jurinea* (Dogan *et al*., 2009, 2011; Parishani *et al*., 2014). Therefore, these two species with basic chromosome numbers of x=15 are close to each other and different from the majority of *Jurinea* species.

Also, a very high similarity between *K. aphylla* and *J. cartilaginea* was revealed in the pollen morphology (Fig. 1. C-F). Regarding to the pollen morphology, especially short colpus and the length of achinus, both species are more similar to other members of Centaureinae such as *Nikitinia* Iljin and *Serratula* L. than to *Jurinea* species (Bordbar & Mirtadzadini, 2015). Pollen evolution in Centaureinae proposed that the *Serratula* type with large spines was the most primitive (Wagenitz, 1955). According to Susanna *et al*. (1995), basic chromosome numbers in Centaureinae support the distinction between "primitive" and "advanced" pollen types means that the plants with high basic chromosome number (x = 15, 16 and 13) have primitive type of pollen.

In summary, a high relationship between *J. cartilaginea* and *Karvandarina aphylla* is supported by the results of morphological, molecular, palynological and karyotype studies. Consequently, these two taxa are phylogenetically closely related and have the same ancestor.

Based on the studied specimens, *K. aphylla* is distributed in SE Iran, Baluchistan and South Kerman. The species is reported from W Pakistan as well (Sales & Hedge, 2013). However, *J. cartilaginea* is distributed in SW Iran, Khuzistan with a 700 km interval from *K. aphylla* distribution area (Fig. 6). No specimen belonging to one of these species is reported from the area in-between. Therefore, no contact, nor any gene flow can be expected between these two species.

**Taxonomy**

Close relationship between *Jurinea cartilaginea* and *Karvandarina aphylla* is highly supported by the results of morphological, molecular, palynological and karyotype studies. The still-existing differences do not suffice to treat the two taxa as independent genera. This is also supported by the results of the sequencing of ITS. Consequently, we transfer *J. cartilaginea* to the genus *Karvandarina* - *K. cartilaginea*.

However, the following features are the morphological differences between these two

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species: plant height, type of stems branching, position of the leaves, leaf texture, the existence of white sediments on the leaf margin, the amount of lignification of the plant basal parts and indumentum of plant parts.

Accordingly, in order to show the phylogenetic distance of these two species, we place *Karvandarina cartilaginea* in a distinct subgenus.

***Karvandarina*** subgen. ***Pseudojurinea*** Mirtadz., ***subgen. nova.***

Type species: *Karvandarina cartilaginea*.

Diagnosis: Differs from *Karvandarina* subgen. *Karvandarina* by having more or less cartilage leaves with sediments on teeth not herbaceous leaves without sediments; surface of the achene glabrous and shiny not villous; areole basal-lateral not basal.

***Karvandarina cartilaginea*** (Mozaff.**)** Parishani, Mirtadz. & Bordbar, ***comb. nova.***

≡ *Jurinea cartilaginea* Mozaff. Iran. J. Bot. 4 (1): 62 (1988).

Holotype: Iran, Khuzistan, between Behbahan and Dehdasht, Tang-e Takab, 500 m. 14. 5. 1986, Mozaffarian 58838 [TARI].

Intricate and compact subshrub. Stems divided, glabrescent. Leaves at the lower parts, lanceolate or narrow elliptic or obovate, glabrescent, with large and remote teeth. Upper leaves decreased (diminished). Capitula homogamous and terminal. Involucre cylindrical or cylindrical-narrow ovoid, phyllaries ±lanceolate, veined, tomentose, slightly rigid, sparsely spiny. Bristles of receptacle scabridous. Corolla white or light pink. Achenes obpyramidal, glabrous, slightly constricted at above and terminated to a narrow and more or less entire or sub-dentate corona, areole basal-lateral. Pappus biseriate, scabrose, crème white, inner bristles with wider, brown and carinate base.

### Monophyly of *Jurinea* and monophyly of Centaureinae

With the ITS sequencing data we were able to estimate the phylogenetic relationships of the studied taxa within tribe Cardueae. In addition to the new position of *Karvandarina cartilaginea*, the cladogram confirms the segregation of *Tricholepis gedrosiaca* (syn. *Jurinea gedrosiaca)* from *Jurinea* species and the synonymy of this species with *Tricholepis edmondsonii* (Mirtadzadini *et al*., In press) (Fig 2.).

The results also support the monophyly of *Jurinea* after excluding the two mentioned species (BI=100) (Fig. 2). However, not all species belonging to *Jurinea* have been included in this study.

The phylogenetic studies of ITS region contained selected species of all genera belonging to Centaureinae. In accordance with the previous studies (Barres *et al.*, 2013), the monophyly of Centaureinae is also strongly confirmed (BI=100) (Fig. 2).

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**Appendix 1.** Specimens examined**.** GenBank accession numbers are indicated in brackets.
***Amberboa turanica*** Vail., Iran: S, Kerman, north of Golbaf, Fandoqa pass, 29.IV.1998, Mirtadzadini 2500 (MIR) [MK226415]; ***Carlina acaulis*** L., Austria, Rax, 23.VIII.2009, Mirtadzadini 2128 (MIR) [MK226413]; ***Cousinia argentea*** Mehregan & Assadi, Iran: NE, Khorasan, Torbat-e Heidarieh to Mashhad, near to Robat-e Sefid, 1100 m, N35º52'51", E59º23'31", Mirtadzadini and Parishani 18098 (HUI) [MK226129]; ***Jurinea bungei*** Boiss., Iran: C, Isfahan, Shahreza to Abadeh, NW of Aminabad, 2052 m, Mirtadzadini 18070 (HUI) [MK226409]; ***J. carduiformis*** Boiss., Iran: C, University of Isfahan campus, 1743 m, N32º35′45″, E51º39′32″, Mirtadzadini and Parishani 18102 (HUI) [MK226407]; ***J. macrocephala*** DC. subsp. ***elbursensis*** Wagenitz, Iran: N, Karaj to Qazvin, toward Kuhsar, Dobarzan village, 1699 m, N35º59'52", E50º50'04", Mirtadzadini 18082 (HUI) [MK226411]; ***J. meda*** Bornm., Iran: C, Isfahan, Khansar, 2650 m, N33º12'56", E50º19'53", Mirtadzadini and Parishani, 18084 (HUI) [MK226416]; *J. microcephala* Boiss., Iran: NE, Khorasan, Neyshabur, Darud, 2650 m, N36º20'34", E58º56'46", Mirtadzadini and Parishani 2501 (MIR) [MK226417]; ***J. monocephala*** Aitch. et Hemsl. subsp. ***sintenisii***, Iran: NE, Khorasan, 80 km of Daregaz from Qutshan, Darebadam, 2250 m, N37º26'13", E58º35'39", Mirtadzadini and Parishani 18086 (HUI) [MK226421]; ***J. moschus*** (Habl.) Bobrov, Iran: NW, Azarbaijan, 35 km to Khalkhal from Asalem, 2255 m, N37º35'40", E48º40'47", Mirtadzadini and Parishani 2502 (MIR) [MK226419]; ***J. pulchella*** DC., Iran: NW, Azarbaijan, Urumia, mt. Siv Daq, 1751 m, N37º29'01", E45º01'05", Mirtadzadini and Parishani 18090 (HUI) [MK226420]; ***J. radians*** Boiss., Iran: C, Semnan to Damghan, Ahowan pass, 1930 m, N35º44'39", E53º40'58", Mirtadzadini and Parishani 18091 (HUI) [MK226418]; ***J. ramosissima*** DC., Iran: C, University of Isfahan campus, 1743 m, N32º40′25″, E51º36′20″, Mirtadzadini and Parishani 18092 (HUI) [MK226412]; ***Karvandarina aphylla*** Rech.f., Allen & Esfand., Iran: S, Kerman, S Baft, 6 km of Giju village, N28°29'26.9", E56°29'31.3", 1259 m, 20.V.2017, Mirtadzadini 2391 (MIR); SW of Giju dam, 18.V.1997, Mirtadzadini 2389 (MIR); SW of Kerman, 75 km south of Baft, 12 km south of Dehsard village, 1574 m, 29.IV.2015, Mirtadzadini 2388 (MIR); SE, Balutshistan, Iranshahr to Nikshahr, 20 km after Espake crossroad, N26°45'55", E60°15'02", 969 m, 03.III.2014, Mirtadzadini 2390 (MIR) [MK226414]; ***Karvandarina cartilaginea*** (Mozaff.) Parishani, Mirtadz. & Bordbar (syn. ***Jurinea cartilaginea*** Mozaff**.**), Iran: SW, Khuzistan, NE of Behbahan, Tang-e Ban village, on rocky slopes, N30°50.810', E50°13.441', 602 m, 18.V.2013, Mirtadzadini 2393 (MIR) [MK226408]; Above Likak town, Tang-e Maghar, N31°00.203', E50°06.335', 956 m, 18.V.2013, Mirtadzadini 2392 (MIR); ***Tricholepis gedrosiaca*** (Bornm.) Mirtadz., Parishani & Bordbar (syn. ***Jurinea gedrosiaca*** Bornm.), Iran: S, Kerman, Orzu, Zanjiraviz, 03.V.2003, Mirtadzadini 2387 (MIR); Khabr, Chahkonar, 25.VII.2008, Mirtadzadini 1081 (MIR); Jiroft, Ramun, 17 km NW of Narab, Qale-Rigi, N28.95557°, E57.40255°, 1553 m, 24.V.2012, Mirtadzadini 2386 (MIR); 44 km from Jiroft to Esfandaqe, N of Tshelitan village, 1600 m, near the point N28°44'50.36", E57°23'28.47", 08.V.2014, Mirtadzadini 2385 (MIR); Bam, Seiedi hot spring road, 03.VI.1998, Mirtadzadini 1282 (MIR) [MK226410].

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