

## **ITS Phylogeny and Molecular Dating of some Gundelia (Asteraceae) of Anatolia**

**Burcu TARIKAHYA HACIOGLU<sup>1\*</sup> Mehmet FIRAT<sup>2</sup>**

<sup>1</sup>**MoFAL, Central Research Institute for Field Crops, Ankara / TURKEY**

<sup>2</sup>**Yüzüncü Yıl University, Faculty of Education, Department of Biology, TR-65080 Van / TURKEY**

\* Corresponding author (Sorumlu yazar): e-mail: btarikah@gmail.com

Received (Geliş tarihi): 28.07.2017 Accepted (Kabul tarihi): 21.11.2017

**ABSTRACT:** *Gundelia* L. is known to be a monotypic genus according to the flora books. *Gundelia tournefortii* L. was the only species of the genus *Gundelia* L. In our research we used the recently introduced species: *Gundelia aragatsi* Vitek, Fayvush, Tamanyan & Gemeinholzer, *Gundelia dersim* Vitek, Yüce & Ergin, *Gundelia munzuriensis* Vitek, Yüce & Ergin, *Gundelia vitekii* Armağan, *Gundelia komagenensis* Fırat, *Gundelia colemerikensis* Fırat, *Gundelia cilicica* Fırat and *Gundelia anatolica* Fırat, in order to reconstruct a phylogeny inference and molecular dating by using ITS region. The mentioned species were collected from Turkey and beside our collections, the ITS sequences belonging to *G. tournefortii* accessions of former studies were used in our research. According to the phylogeny inference, *G. anatolica* was different from all *Gundelia* accessions. Some *G. tournefortii* accessions belonging to former studies grouped with *G. cilicica*, *G. dersim* and *G. colemerikensis*, *G. munzuriensis* clustered with *G. vitekii*. *Gundelia komagenensis* and *G. aragatsi* accessions were grouped as close relatives. According to the molecular dating the distinction of the genus *Gundelia* from the relative genera was around 14.1 million years ago (mya).

**Keywords:** Asteraceae, *Gundelia*, ITS, molecular dating, phylogeny.

### **Anadolu'da Yetişen *Gundelia* (Asteraceae) Taksonlarının ITS Filogenisi ve Moleküler Tarihlendirilmesi**

**ÖZ:** Flora kitaplarına göre *Gundelia* L. monofiletik bir cins olarak bilinmektedir. *Gundelia tournefortii* L. cinsin tek türü olarak bilinmektedir. Çalışmamızda ITS bölgesine dayalı filogenetik analiz ve moleküler tarihlendirme yapmak için yeni tanımlanan türleri de kullandık. Bunlar: *Gundelia aragatsi* Vitek, Fayvush, Tamanyan & Gemeinholzer, *Gundelia dersim* Vitek, Yüce & Ergin, *Gundelia munzuriensis* Vitek, Yüce & Ergin, *Gundelia vitekii* Armağan, *Gundelia komagenensis* Fırat, *Gundelia colemerikensis* Fırat, *Gundelia cilicica* Fırat ve *Gundelia anatolica* Fırat. Adı geçen yeni türler Yürkiye'den arazi çalışmaları yaparak toplanmış ve bizim toplamalarımızın yanısıra *G. tournefortii* türüne ait daha önceki çalışmalarda ortaya çıkmış ITS dizileri de kullanılmıştır. Filogenetik ağaca göre, *G. anatolica* tüm *Gundelia* örneklerinden farklıdır. Daha önceki çalışmalarda toplanmış bazı *G. tournefortii* örnekleri *G. cilicica*, *G. dersim* ve *G. colemerikensis* ile birlikte kümelenebilir. *G. munzuriensis* *G. vitekii* ile grup oluşturmuştur. *Gundelia komagenensis* ve *G. aragatsi* örnekleri birbirine en yakın gruplanmışlardır. Moleküler tarihlendirmeye göre *Gundelia* cinsinin ayrılma zamanı 14.1 milyon yıl öncesine tarihlenmektedir.

**Anahtar Sözcükler:** Asteraceae, *Gundelia*, ITS, moleküler tarihlendirme, filogeni.

## INTRODUCTION

*Gundelia* L. belongs to the tribe Lactuceae according to the chloroplast *ndhF* gene (Karis *et al.*, 2001). In the flora books (Nikitin, 1960; Vasilchenko, 1961; Sofieva, 1961; Kupicha, 1975; Feinbrun-Dothan, 1978; Rechinger, 1989; Avetisian, 1995) *Gundelia tournefortii* L. is the only species of the genus *Gundelia*, and all other names were recorded as synonyms. Recently, several new species have been described, i.e. *G. aragatsi* Vitek, Fayvush, Tamanyan & Gemeinholzer, *G. armeniaca* Nersesian from Armenia, and *G. dersim* Vitek, Yüce & Ergin, *G. munzuriensis* Vitek, Yüce & Ergin, *G. vitekii* Armağan, *G. komagenensis* Firat, *G. colemerikensis* Firat, *G. cilicica* Firat, *G. anatolica* and *G. mesopotamica* Firat from Turkey and *G. tehranica* Vitek & Noroozi from Iran (Firat, 2016; Firat 2017a; Firat, 2017b).

For the taxonomic evaluation, molecular systematic approach was implemented to the genus *Gundelia* by Vitek *et al.* (2010). The researchers used ITS marker to investigate the infraspecific relations of the genus. In the phylogram they have constructed, *Gundelia* was clearly monomorphic and the infraspecific relations were supported with low posterior probability values. However, it was still probable to distinguish the new proposed taxa as different from others, with the help of the phylogeny tree. In our study we have used the ITS sequence data to reveal the phylogeny and molecular dating of the known *Gundelia* taxa.

## MATERIALS AND METHODS

### Plant material

A total of 10 herbarium specimens for each species were collected from the field and deposited in the herbaria ANK, E and VANF [acronyms according to Thiers (2016)], and in the personal herbarium of the author (Herb. Firat). The ITS sequences were gathered from NCBI GeneBank are as follows: *Scorzonera purpurea* AJ633477, *Tragopogon*

*albinervis* KF050360, *Warionia saharae* AY190608, *Scolymus maculatus* AJ633469, *Catananche caerulea* AJ633465 were used as outgroups and *Gundelia aragatsi* ssp. *steineri*\_1 FN582290, *G. aragatsi* ssp. *steineri*\_2 FN582289, *G. aragatsi* FN582288, *G. aragatsi* FN582283, *Gundelia* sp. FN582287, *G. tournefortii* FN582281, *G. tournefortii* FN582284, *G. tournefortii* FN582277, *G. tournefortii* FN582285, *G. tournefortii* FN582278, *G. tournefortii* AY504691, *G. tournefortii* FN582282, *G. tournefortii* FN582279, *G. tournefortii* FN582280, *G. tournefortii* FN582286 were used with our own collections to construct the phylogeny tree.

### DNA extraction, amplification, and sequencing

Total DNA was isolated with the DNAeasy Plant Mini Kit following the manufacturer's protocols (Qiagen, Germany). The amplification of ITS region, including 5.8S ribosomal RNA gene, was performed using primers ITS1 and ITS4 (White *et al.*, 1990). The PCR amplification of ITS followed Warwick *et al.* (2004). Purification and sequencing were performed by Bioeksen (Istanbul).

### Data Analysis

Sequences were aligned manually by using BioEdit (Hall, 1999). The aligned ITS sequences were analyzed using Bayesian approaches following BEAST1.8.0 (Drummond *et al.*, 2012). For the Bayesian analyses, GTR + Gamma model was chosen as best model according to Akaike Information Criterion (AIC). Two independent Markov Chain Monte Carlo (MCMC) runs were conducted with 10 million generations and sampled every 1,000 generations. Each run was checked in Tracer v1.5 (Rambaut and Drummond, 2007). Among the trees obtained 25% (5,000) were discarded as burn-in. Treeannotater v.1.7.5 programme was used to obtain the maximum clade credibility tree.

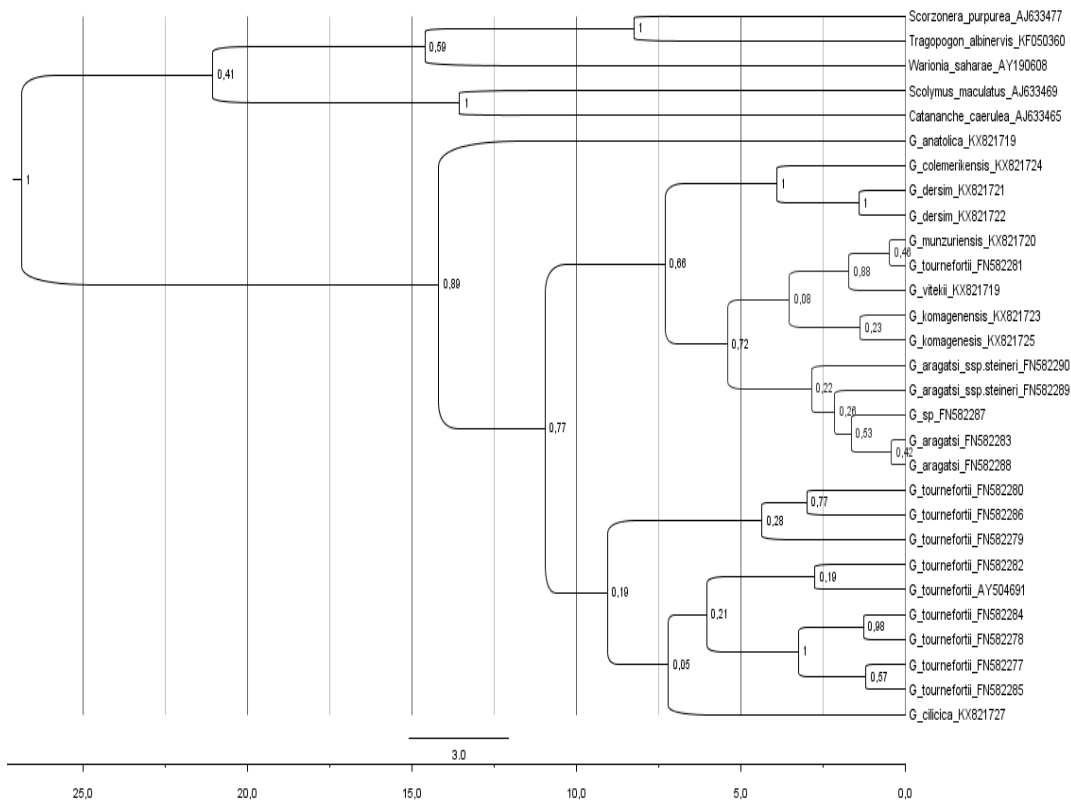
We used an external calibration point introduced by Tremetsberger *et al.* (2013). The age of

*Scolymus hispanicus* L. and *Catananche caerulea* L. was set to 13.6 Ma (million years ago) with  $\pm 0.42$  Ma standard deviation. Divergence time estimation was performed under the lognormal clock model. GTR with four gamma categories were used as a substitution model and Yule process of speciation was used as a tree prior. MCMC runs was conducted with 100 million generations and sampled every 10,000 generations. Each run was checked using Tracer v1.5 (Rambaut and Drummond, 2007) and then combined in Logcombiner v1.7.5. Treeannotater v.1.7.5 program was used to obtain a single posterior probability and maximum clade credibility tree was visualized using Figtree v1.3.1 (Anonymous, 2016).

## RESULTS AND DISCUSSION

According to the phylogeny reconstruction, *G. anatolica* derived from all other *Gundelias* (Fig.

1). The rest of the species grouped under two major clades. First group consisted *G. colemerikensis*, *G. dersim*, *G. munzuriensis*, *G. tournefortii*, *G. vitekii*, *G. komagenensis* and *G. aragatsi*. *Gundelia colemerikensis* clearly derived from *G. dersim* (posterior probability 1.0) in the first subclade. The species with ca.3 flowered cephaloid compound (*G. munzuriensis*, *G. vitekii*, *G. aragatsi*) grouped together in the second subclade. All other investigated taxa have ca. 6-flowered cephaloid compound. Second group comprised *G. tournefortii* accessions of Vitek *et al.* (2010) and *G. cilicica*. As described in the introduction part of this paper, formerly *Gundelia* was considered as a monotypic genus. The sequences gathered from the genebank were identified according to the flora books, therefore all *Gundelia* accessions were named as *G. tournefortii*. These *G. tournefortii* samples



**Figure 1.** The phylogeny tree of the *Gundelia* species; numbers on branches indicate clade credibility, x bar shows the time scale (mya).

**Şekil 1.** *Gundelia* türlerinin filogenetik ağacı; dalların üzerindeki sayılar clade credibility değerleridir, x eksenı zaman aralığını göstermektedir (milyon yıl önce).

could be identified with comparing the newly described species and our phylogeny tree could give an idea for naming the species. For example, FN582284, FN582277, FN582285, FN582278, AY504691, FN582282, FN582279, FN582280, FN582286 could be *G. cilicica* or could be some other species close to *G. cilicica*. FN582287 could be *G. aragatsi*. FN582281 could be either

*G. munzurensis* or *G. vitekii*. According to the molecular dating, the distinction of the genus *Gundelia* from the relative genera was around 14.1 mya mid-Miocene. The distinction of the ca.3 flowered cephaloid compound species group from the ca. 6-flowered cephaloid compound species group was around 7.3 mya late Miocene of the Cenozoic.

## REFERENCES

- Anonymous. 2016. Molecular evolution, phylogenetics and epidemiology. <http://tree.bio.ed.ac.uk/software/figtree>.
- Avetisyan, V. E. 1995. Gundelieae. In: A. L. Takhtajan (Ed.). Flora Armenii 9. Koeltz Scientific Books, Havlíčkův Brod. pp. 212-216.
- Drummond, A. J., M. A. Suchard, D. Xie, & A. Rambaut. 2012. Bayesian Phylogenetics with BEAUti and the BEAST 1.7. *Molecular Biology and Evolution* 29: 1969-1973.
- Feinbrun-Dothan, N. 1978. Flora Palaestina, 3. The Israel Academy of Sciences and Humanities, Jerusalem, 481 pp.
- Firat, M. 2016. Four new species of *Gundelia* L. (Asteraceae) from Anatolia: *G. komagenensis*, *G. colemerikensis*, *G. cilicica* and *G. anatolica*. Vamedia Ofset Press, Van, 32 pp.
- Firat, M. 2017a. *Gundelia rosea* (Asteraceae), a new record for the Flora of Turkey with contributions to its systematics. *Acta Biologica Turcica* 30 (2): 31-35.
- Firat, M. 2017b. *Gundelia mesopotamica* (Asteraceae), a new lactiferous species from Mardin (Turkey). *Acta Biologica Turcica* 30 (3): 64-69.
- Hall T. A. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symp.*, Ser. 41: 95-98.
- Karis P. O., P. Eldenäs, and M. Källersjö. 2001. New evidence for systematic position of *Gundelia* L. With notes on delimitation of Arctoteae (Asteraceae). *Taxon*, 50: 105-114.
- Kupicha, F. K. 1875. *Gundelia*. In: P. H. Davis (Ed.). Flora of Turkey 5. University Press, Edinburgh, pp. 325-326.
- Nikitin, V. V. 1960. *Gundelia* L. In: B. K. Schischkin (Ed.). Flora Turkmenii 7. Akademiia nauk Turkmenskoi SSR, Institut Botaniki, Ashkhabad, p. 141.
- Rambaut, A., and A. J. Drummond. 2007. Tracer, version 1.4. Available from <http://beast.bio.ed.ac.uk/Tracer>.
- Rechinger, K. H. 1989. *Gundelia*. In: K. H. Rechinger (Ed.). Flora Iranica 164. Akademische Druck- und Verlagsanstalt, Graz, pp. 107-109.
- Sofieva, R. M. 1961. *Gundelia* L. In: I. I. Karjagin (Ed.). Flora Azerbajdzana 8. Azerbaijan National Academy of Sciences Press, Baku, pp. 347-348.
- Thiers, B. 2016. Index Herbariorum: A Global Directory of Public Herbaria and Associated Staff. New York Botanical Garden's Virtual Herbarium. <http://sweetgum.nybg.org/science/ih/>
- Tremetsberger, K., Gemeinholzer, B., Zetzsche, H., Blackmore, S., Kilian, N., & Talavera, S. (2013). Divergence time estimation in Cichorieae (Asteraceae) using a fossil-calibrated relaxed molecular clock. *Organisms Diversity and Evolution*, 13 (1): 1-13.
- Vasilchenko, I. T. 1961. *Gundelia* L. In: V. L. Komarov (Ed.). Flora SSSR, 26. Nauka Publishers, Moscow & Leningrad, p. 862.
- Vitek, E., G. Fayvush, K. Tamanyan, and B. Gemeinholzer. 2010. New taxa of *Gundelia* (Compositae) in Armenia. *Annalen des Naturhistorischen Museums Wien, Serie B* 111: 85-99.
- Warwick, S. I., Al-Shehbaz, I. A., Sauder, C. A., Murray, D. F., Mummenhoff, K. 2004. Phylogeny of *Smelowskia* and related genera (Brassicaceae) based on nuclear ITS DNA and chloroplast trnL intron DNA sequences. *Annals of the Missouri Botanical Garden* 91: 99-123.
- White, T. J. T., Bruns, S. L., Taylor, J. W. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis, M. A., Gelfand, D. H., Sninsky, J. J. and White, T. J. (Ed.). PCR Protocols: A Guide to Methods and Applications, Academic Press, Inc., New York. pp. 315-322.