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ORIGINAL ARTICLE

Updated-extended molecular time and molecular phylogeny of *Gundelia* species native to Turkey

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Abstract

Generally, *Gundelia tournefortii* is considered as the sole representative of the genus and the other species of *Gundelia* as synonyms. Recent studies suggest that the genus is rich with 22 species. Off these, thirteen are endemic to Turkey. To date, no comprehensive molecular study dealing with speciation exists in the genus. To address the speciation at the molecular level, fresh leaves from 57 samples representing 15 species from their natural ranges in Turkey were obtained by sampling over several years and studied with respect to *nr*DNA (ITS) and the *cp*DNA (*ndh*F) gene regions. Molecular data from *cp*DNA and *nr*DNA revealed that there were two major clades. One of these clades consists of *G. anatolica, G. glabra* and *G. asperrima* when the *ndh*F data were used (or just *G. anatolica* based on ITS data*)* while the other major clade included the remaining species with subclades. Divergence time and geographical phylogenetic reconstruction analysis indicated that there may be 3 major ancestral associations existing in the studied *Gundelia* species. Based on *ndh*F data, the oldest ancestral group included *G. anatolica, G. glabra and G. asperrima* with the divergence time about 21.27 MYA, while *G. tournefortii* var. *tenuisecta and G. dersim* were the most recently diverged group (about 4.66 MYA). Ancestral history of diversity analysis suggested that the dispersal and vicariance events involved in the speciation in the genus *Gundelia* with complex events of natural hybridization, introgression, geographical isolations or some other weak isolation mechanisms.

Keywords *Gundelia* · ITS · Molecular clock · Molecular systematics · *Ndh*F · RASP · Speciation

Introduction

In the historical treatment of the genus, *Gundelia tournefortii* was referred as the only species of the genus, while the other species of the genus were considered as synonyms. However, numerous taxonomic studies in recent years reported that there have been 22 species of *Gundelia* described over the years from the East Mediterranean region, Asia Minor, Transcaucasia, Iran and Afghanistan (Nersesyan 2014; Fırat 2016, 2017a, b, c; Tarikkahya Hacıoglu and Fırat 2017; Vitek et al. 2017a,b; Vitek and Nooroozi 2017a, b; Vitek 2018; Fırat 2018a, b, 2021b).

There have been attempts to classify *Gundelia* sp. using morphological data on flower types and colors (Fırat 2017a; 2018a, b; 2019a, b; 2021a). The classification of the species is mainly based on flower aggregate and corolla colors in lesser extent (Supplementary Figure 1) (Vitek et al. 2010). According to this approach, *G. anatolica* with 6 cephaloid flower heads was found to be distinct, while *G. rosea, G. mesopotamica, G. tournefortii, G. cilicica, G. purpurascens* and *G. dersim* with presence of cephaloid flowers from 5 to 8 were considered to be similar. *Gundelia colemerikensis, G. glabra*, *G. munzuriensis* having flowers with 3 to 6 flower heads were treated together. The remaining species such as *G. vitekii, G. komagenensis* and *G. asperrima* with 3 cephaloid flower heads were put in the same group. However, these classifications need to be explored further with the data from numerical taxonomy and molecular systematics since variations in these traits seem to be influenced heavily by environmental conditions and segregation or accumulations of genes. Even if

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there are studies dealing with morphological differences between *G. tournefortii* and *G. rosea* (Vitek and Jarvis 2007) and taxonomical revision of *G. tournefortii* (Vitek et al. 2017b), clear morphological delineation of *Gundeli*a species has not been reported enough to date. Although, Vitek (2019) attempted to identify the species of *Gundelia* genus in diversity center, there were still some species need to be studied further for a clear description since information on these problematic species lacks (lack of photos and information which were found in the internet). Where divergence or speciation among taxonomic units becomes unclear and variability of morphological features affected by geographical and climatic variables, molecular systematic studies could be valuable resources for taxonomists to address taxonomic problems in plant species (Zhang and Jiang 2019).

Vitek et al. (2009) studying ITS region of selected *Gundelia* species with limited sampling reported that phylogenetic tree indicated numerous clades. In the study, clear identifications of species in the genus could not be done due to lack of informative parts in the herbarium material. Nevertheless, the ITS region is widely used in many plant species, even closely related taxa, due to presence of high degree of variation and its highly conserved nature (Hamby and Zimmer 1992). The *ndh*F gene (NADH dehydrogenase) of chloroplast DNA is another widely used gene region to understand infraspecific phylogenetic relationships of different taxa. The *ndhF* is considerably longer and evolve faster compared to the other chloroplast genes (i.e., *rbcL* (ribulose-bisphosphate carboxylase), *rpoC*l (RNA polymerase C1). Kim and Jansen (1995) reported that *ndh*F region provides more information (informative sites) than other *cp*DNA gene regions for phylogenetics involving species of Angiosperms.

Including *Gundelia tournefortii*, there are 22 species in the world. Nineteen of these species are naturally growing in Turkey (Fırat 2021b). Also, thirteen of these are endemics to the country. These new species were basically characterized with respect to selected morphological traits which may be heavily influenced by wide range of variations occurring in their natural ranges. Although few studies have addressed molecular phylogenetic of *Gundelia* species (Vitek et al. 2009; Asadi-Samani et al. 2013; Tarikahya Hacıoğlu and Fırat 2017), information provided on the taxonomy of the genus has been limited due restricted sampling and choice of studied gene regions. With the current study, we aimed to investigate the phylogenetic relationships within the genus, the evolutionary divergence time and historical biogeographic distributions in *Gundelia* species using *ndh*F region of *cp*DNA and ITS region of *nr*DNA, with an extensive and representative sampling of species from their natural ranges in Turkey.

Materials and methods

Plant material

Fresh leaves from 57 samples representing 15 species from their natural ranges in Turkey were collected through field trips made over several years, in April–June from 2015 to 2017 (Fig. 1). The detail information about samples, their locations and collection numbers were provided in the Supplementary Table 1, Supplementary Fig. 1.

DNA extraction, amplification and sequencing

Optimized 2XCTAB method was used for total DNA isolation (Doyle 1990). To amplify the ITS (ITS1 + 5.8S + ITS2) (nuclear ribosomal internal transcribed spacers) and the *ndh*F (NADH dehydrogenase) gene regions, the primers designed by Hsiao et al. (1995) and by Karis et al. (2001) were used, respectively. For polymerase chain reaction (PCR) amplifications, 5X HOT FIREPol Blend PCR Mix (with 15 Mm $MgCl_2$) (Solis Byodyne, Estonia) was used. For all primers, the PCR reactions were performed in a total volume of 20 µl containing 3 µl PCR Mix, 0.5 *µl* each primer pairs (0.5 µl + 0.5 µl), 4 µl template DNA and 12 µl water in 0.2 ml sterile tubes.

PCRs were performed with a thermo cycler (Eppendorf Mastercycler, Canada) by optimized cycling parameters as: initial denaturation at 95 °C for 5 min followed by of 1 min at 94 °C, 30 cycles at 95 °C for 30 s of denaturation, at 50.7 °C for 40 s of annealing, at 72 °C for 90 s of extension, and at 72 °C for 10 min of final extension. Agarose gels with 1%, and 1.5% concentrations were used to run the PCR samples. The PCR products were stored at −20 °C until they were sent for sequencing. The purification and sequencing procedure were carried out by the BM Company (Çankaya, Ankara). An ABI3730XL (Applied Biosystems, Hitachi, U.S.) 96 capillary automatic sequencer was used for sequencing of amplified DNA products.

Data analysis

All sequences were checked for base calls before data analysis. They were examined and edited by Finch TV software (Version 1.4.0-manufactured by Geopiza Research Team; Patterson et al. 2004, 2006) to correct ambiguous reflections. The sequences of *Gundelia* species were aligned by MUSCLE option (Edgar 2004) of MEGA X (Molecular Evolutionary Genetics Analysis) software (Kumar et al. 2018).

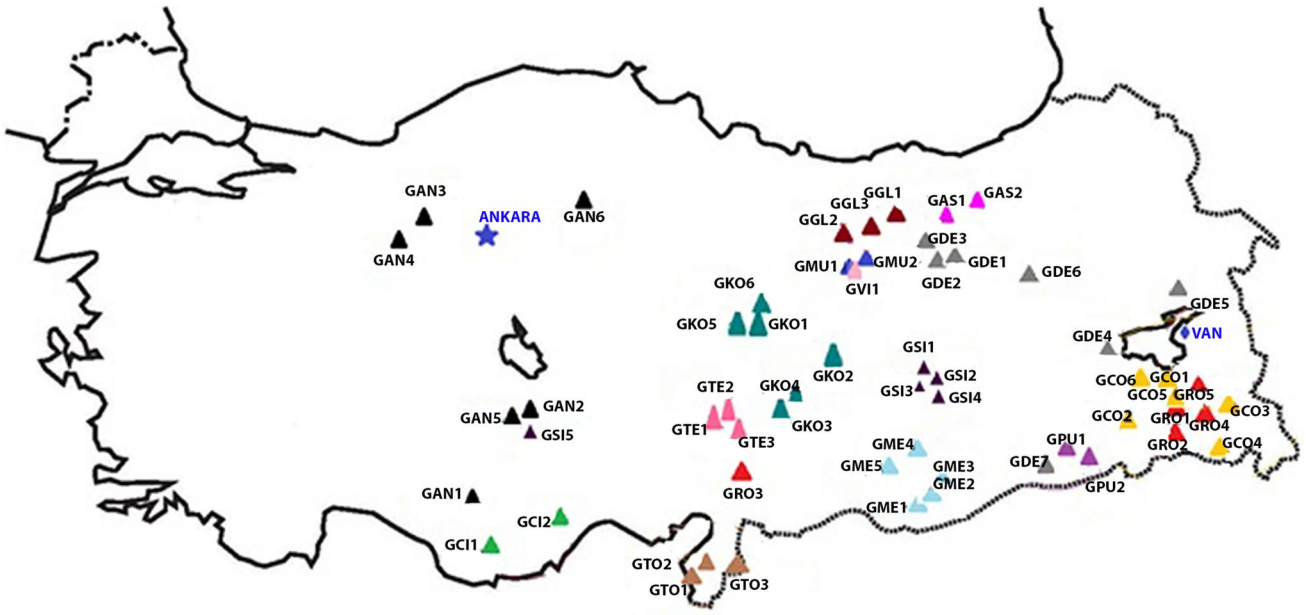

Fig. 1 The map of Turkey showing the location of samples of *Gundelia* species in the study. The species were coded as *GANGundelia anatolica*, *GCIG. cilicica*, *GGLG. glabra, GASG. asperrima, GCOG. colemerikensis, GDEG. dersim, GKOG. komagenensis, GMEG. mesopotamica, GMUG. munzuriensis, GPUG. purpurascens, GTEG. tournefortii* var. *tenuisecta, GROG. rosea, GTOG. tournefortii, GVIG. vitekii* and *GSIG. siirtica,* that numbers following codes indicate the sample number for a given species. Please see supplementary table for details of sampling

Molecular diversity and evolutionary statistics such as total nucleotide length (base pairs), GC contents (%), nucleotide deletions and insertions, conserved and variable sites, parsimony informative sites, transition/transversion (tr/tv) ratio and overall nucleotide diversity were computed using the sequences of *ndh*F and ITS regions via MEGA X software (Kumar et al. 2018). To evaluate evolutionary relationship among the species of the genus, the phylogenetic trees were constructed with the use of the *ITS* (Internal transcribed spacer of *nr*DNA), the *ndh*F (NADH dehydrogenase gene) sequences of *Gundelia* species. The sequences of the same gene regions for *Hoplophyllum spinosum* (JN837106.1(Funk et al. 2012) &AF303925.1 (Karis et al. 2001)) and *Wariona saharae* (AY190608.1 & EU385216.1(Panero and Funk 2008)) were selected as outgroups since they are the members of the same family, but different tribe (Karis et al. 2001; Tarikahya Hacıoğlu and Fırat 2017). The ITS and the *ndh*F sequences of *W. saharae* and *H. spinosum* were retrieved from the NCBI database (https://www.ncbi.nlm.nih.gov/).

Both studied *cp*DNA and *nr*DNA regions were analyzed separately to understand phylogenetic relationships of *Gundelia* species at both *cp*DNA and *nr*DNA level in detail. The MEGA X software was used for testing the best suitable substitution model. The program suggested GTR (Generalized Time Reversible) substitution model to evaluate the *nr*DNA sequences and JC (Jukes Cantor) model for *cp*DNA sequences based on the AICc (Akaike Information Criterion) values. The BEAST v 2.5.1 (Bayesian Evolutionary Analysis by Sampling Trees) package program was used with GTR and JC substitution model of base substitution for both data partitions, with a Yule tree prior and a randomly generated starting tree (Suchard et al. 2018). After these analysis, phylogenetic complex trees were summarized and combined with Tree Annotator v1.8.0 program with a posterior probability limit of 1 (Drummond et al. 2012). For estimating molecular divergence time, strict clock model of the BEUTI program with the mutational substitution value of 2×10^{-9} for *cp*DNA (Pevsner 2009) and 10.7×10^{-9} for *nr*DNA (Tremetsberger et al. 2013) per year was used. The Markov Chain Monte Carlo (MCMC) chains were run with parameters sampled every 10000th generations. Tracer 1.6 (Rambaut et al. 2014) was applied to examine effective sample sizes (ESS) for estimated parameters. For both data, ESS values were calculated far beyond 200 for both calibrations resulting in reasonable-looking bell-shape posterior probability density curves. All estimated trees were combined with TreeAnnotator 1.8 and Maximum clade credibility (MCC) trees with posterior probability (pp) values presented with FigTree 1.4.0. Moreover, all phylogenetic trees were also constructed with same substitution model in MEGA program to estimate bootstrap values with 1000 replicates in Maximum Likelihood (ML) analysis. The Maximum Parsimony method was automatically applied to obtain

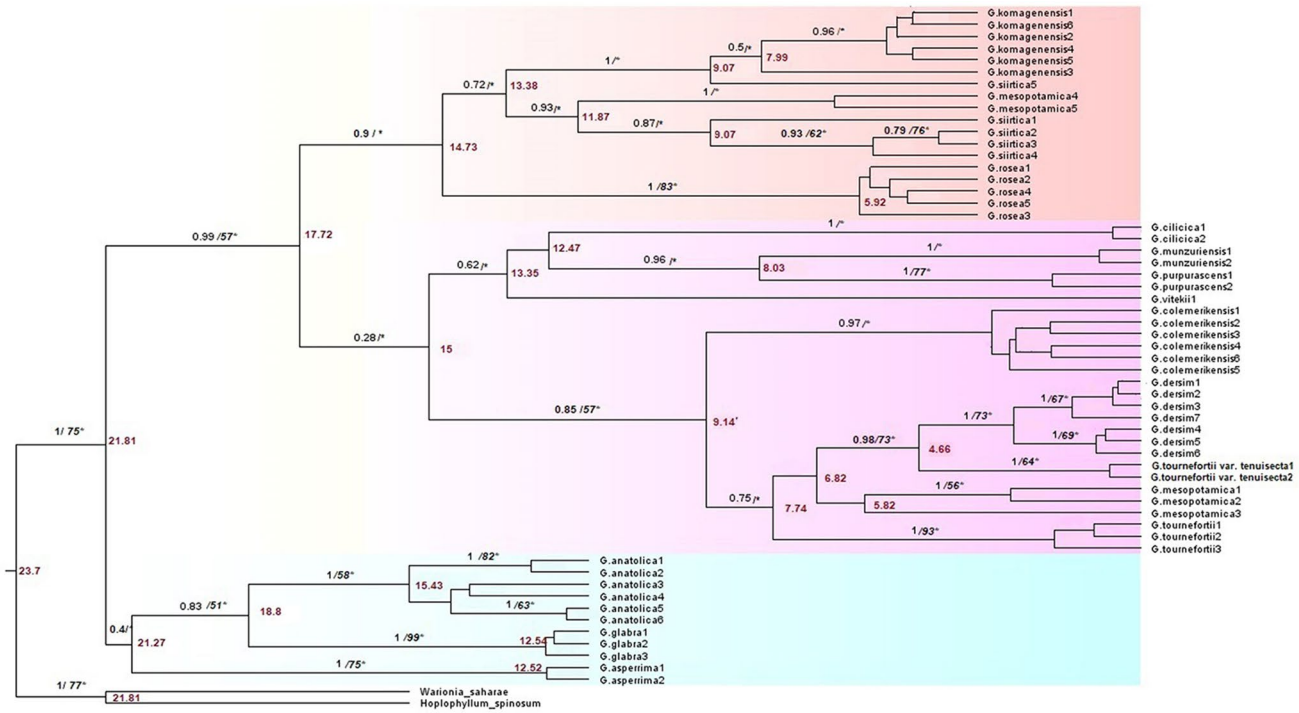

Fig. 2 Bayesian consensus tree from *ndh*F gene region. Posterior probabilities (PP), which are pointed out the important branches, were given above each branch with black colored numbers. The results of ML analysis with subsequent optimization were given with italic black colored numbers with star (*) next to pp values (bootstrap values with 1000 replicates and lower 50 was given as only star (*)). Molecular clock estimations were provided below each node with red colored numbers (the root was optimized at 23.7MYA according to the calculations of the study done by Tremetsberger et al (2013))

initial trees for the heuristic search in the program. The bootstrap values were added to the phylogenetic trees of BEAST analysis (Fig. 2 and Fig. 3).

Additionally, with the use of both ITS and *ndh*F sequences data (samples from different regions), phylogenetically based historical biogeographical reconstructions of *Gundelia* species were performed with the RASP (Reconstruct Ancestral State in Phylogenies) software (Yu et al. 2015, 2020). The BioGeoBEARS package was applied to the sequences to test the best model test for analysis. According to the AICc values, the S-DEC with Bayesian Binary MCMC and maximum parsimony (MP) analysis was chosen and applied to the sequences with 50,000 burning stages in the RASP program to get ancestral probability ranges (%) at each node from each sampled region. Also, the states were determined as related with the regions where specimens were collected.

Results

Due to the large size of *ndh*F gene region, amplifications were accomplished on two fragments. One of them was 800 bp in length and the other fragment was 1200 bp which comprise more variable region (Karis et al. 2001). Therefore, due to save time and money consume, only second part of the region were amplified and sequenced by using primers of Karis et al. (2001). Moreover, after trimming the ends of the sequences, the length of the region was about 1167 bp in *Gundelia* genus with 74 variable sites. Fifty-six of these were parsimony informative sites. Singletons were found to be 18. Overall nucleotide diversity of the genus was estimated as 0.01. The ITS region in *Gundelia* genus was found to be 627 bp in length with 46 variable sites. Great majority of these sites (43) were parsimony informative. There were a few singleton sites. The ITS1 region appeared to be more diverse and informative than the ITS2. The ITS1 region provided 30 of total parsimony informative sites which contributed greatly to overall nucleotide diversity estimation (0.013) (Table 1).

Molecular phylogenetic tree constructed using *ndh*F gene sequences from 15 *Gundelia* species revealed two main clades (Fig. 2). One of these clades (main clade 1) included samples of *G. asperrima,* G. *anatolica* and *G. glabra*. Within the clade, each species formed distinct subclades with high posterior probability values, providing clear identity patterns of the species. The other main clade (main clade 2) had two noticeable subclades in which there are further

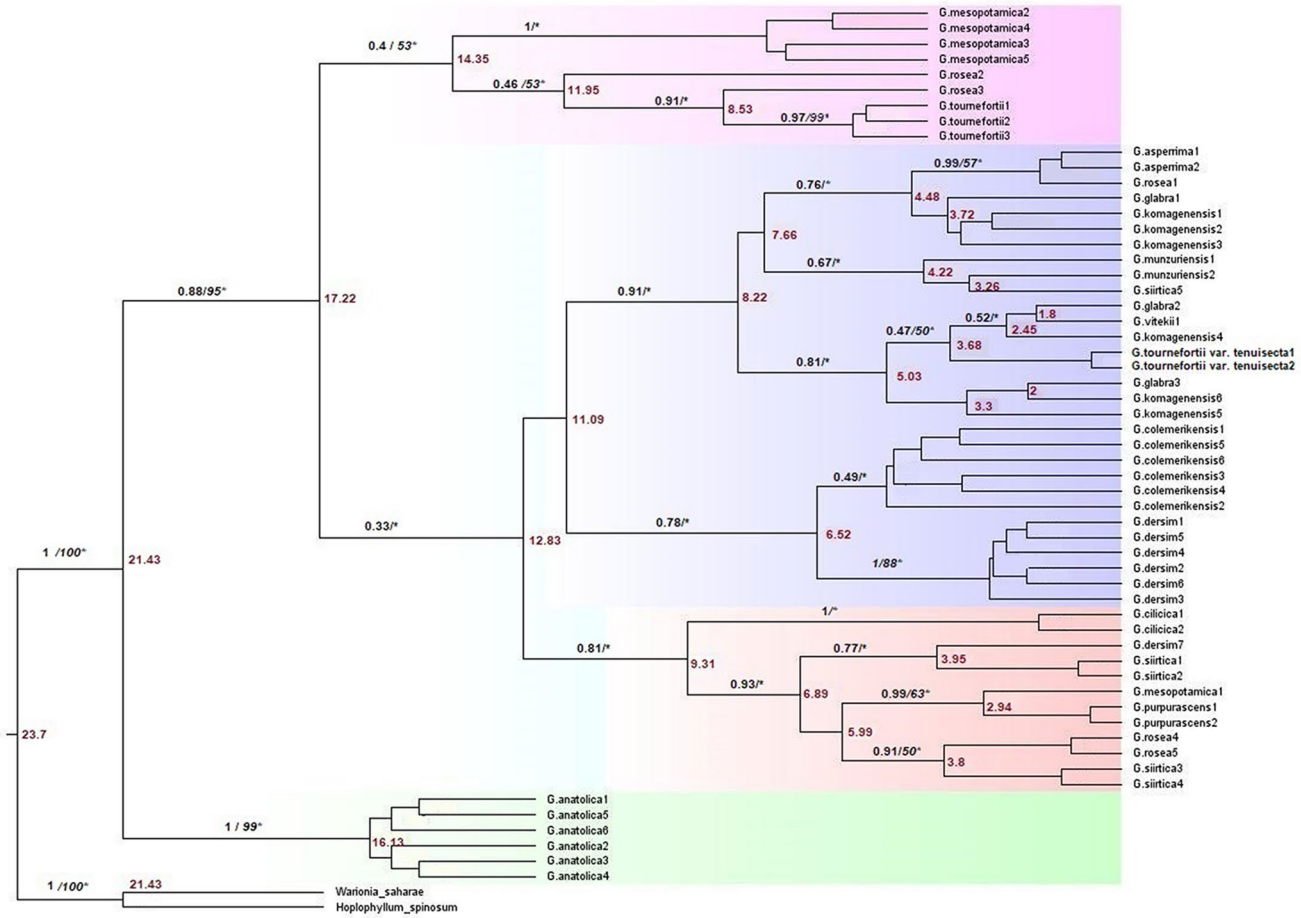

Fig. 3 Bayesian consensus tree from ITS region. Posterior probabilities (PP), which are pointed out the important branches, were given above each branch with black colored numbers. The result of ML analysis with subsequent optimization were presented with italic black colored numbers with star (*) next to pp values (bootstrap values with 1000 replicates and lower 50 was given as only star (*)). Molecular clock estimations were provided below each node with red colored numbers (the root was optimized at 23.7MYA according to the calculations of the study done by Tremetsberger et al (2013))

substructures (Fig. 2). In this clade, *G. komagenesis*, *G. rosea, G. siirtica and G. mesopotamica* samples ended up in a subclade with high posterior probabilities though one *G. siirtica* sample appeared to be closer to the *G. komagenesis* group. The remaining of *Gundelia* species in the main clade 2 were structured three subclades which were *G.cilicica-G. munzuriensis-G.vitekii-G.purpurascens, G. colemerikensis, G.dersim- G. tournefortii* var. *tenuisecta* (Fig. 2). Interestingly, the samples identified morphologically as *G. mesopotamica* appeared to be originated from two distinct entities.

Table 1 Molecular statistics for *ndhF* and ITS regions

Diversity parameters	*ndhF*	ITS (ITS1 + *5.8S* + ITS2)
Number of *Gundelia* taxa	15	15
Number of sequences	57	57
Total length	1167	627
Conserved sites	1093	581
Variable sites	74	46
Parsimony informative sites	56	43
Singletons	18	3
GC ratio (%)	34.7	58
Transition/Transversion bias (R)	0.53	1.37
Overall nucleotide diversity	0.01	0.013

Molecular phylogenetic analysis with the ITS sequences of 15 *Gundelia* species also yielded two main clades (Fig. 3). One of these main clades (main clade 1) included only *G. anatolica* samples with very high posterior probabilities and bootstrap values. The other main clade (main clade 2) included the remaining species of *Gundelia* and formed weakly associated subclades though some of the species grouping had strong associations within their respective subclades. One of the subclades of main clade 2 was formed with the samples of *G. tournefortii, G. rosea* and

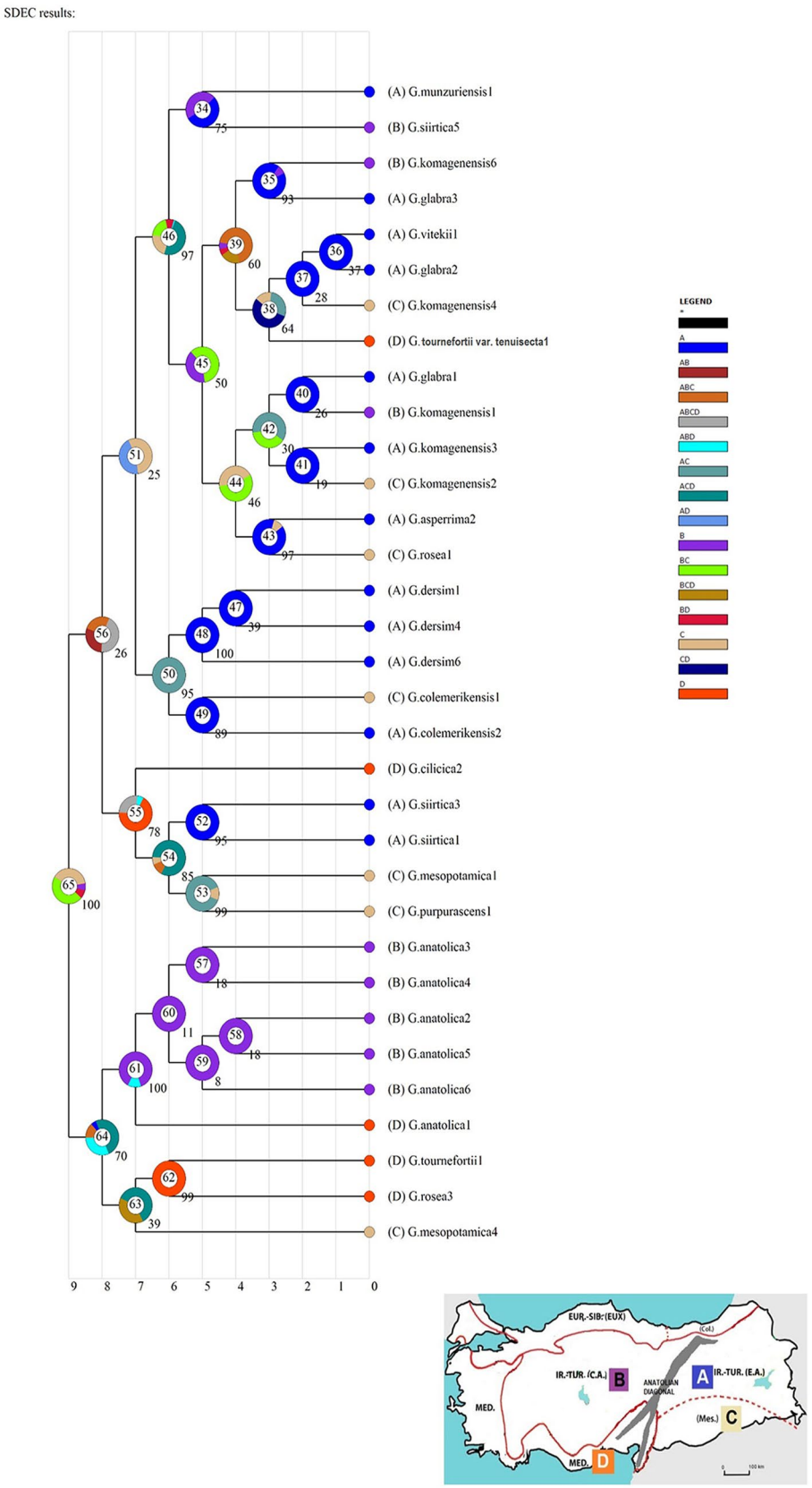

Fig. 4 Tree output of RASP analysis with S-DEC & Bayesian Traits with the ITS data. Bayesian Credibility values are shown above the pies and the most probable region was shown with color of the pies at each node. The color legends of the map indicate the possible ancestral ranges at nodes is above the tree and described phytogeographical regions from where the samples were originated

G. mesopotamica. The other subclade of the main clade had three distinct groups, namely they are *G. asperrima -G. tournefortii- G. glabra- G. munzuriensis-G. vitekii-G. komagenensis, G. colemerikensis—G. dersim*, and *G. cilicia—G. purpurascens – G. siirtica*. Some samples initially characterized as the *G. rosea* and *G. mesopotamica* were not grouped as they were expected (Fig. 3).

The RASP analysis using ITS sequences, with selection of the S-DEC and Bayesian traits models were carried out to estimate a biogeographical history in which divergence and vicariance have been important in speciation of *Gundelia* genus (Fig. 4). The results of the S-DEC analysis suggest that biogeographical history was determined with dispersal and vicariance events and these were seemed to be important in the shaping of the current distribution pattern in *Gundelia* genus. From the S-DEC results, mainly 13 dispersals and 13 vicariance events have been postulated with ITS data. At the node 65, the event route starts with ACD and continued with ABCD region, meaning that there are 3 possible ancestral ranges. Moreover, with *ndh*F data mainly 16 dispersals and 12 vicariance events have been postulated and at the node 65 the event route starts with ACD like ITS data and continued with the region A, yielding the results similar to the results from the *ndh*F data, that is, the existence of 3 possible ancestral ranges (Supp. Fig. 2). The RASP analysis revealed that the ancestors of all members of *Gundelia* species in Turkey are likely to be evolved in generally continental climates of eastern part of Anatolian Diagonal (Iranion Turanian Region (A)) and Southern Turkey (Mesopotamian part of Irano-Turanian Region(C)) or transition zones between southeastern and eastern Turkey (AC) or southeastern and southern Turkey (CD) (Fig. 4). The current distribution of *G. anatolica* is limited to only in Central Turkey, while *G. purpurascens*, *G. mesopotomica* and *G. siirtica* samples are adapted to southeastern Turkey. On the other hand, *G. dersim, G. colemerikensis, G. vitekii,* and *G. glabra* are mainly found in eastern Turkey. Only *G. cilicica* and *G. tournefortii* appear to be originated from the Mediterranean region (D) (Fig. 4).

The results of molecular clock estimations with the *ndh*F gene region of cpDNA revealed that the earliest diverging species appear to be *G. anatolica, G. glabra* and *G. asperrima,* dated back in late Miocene (about 21.27 MYA) (Fig. 2). The other species were diverged from this group about 17.72 MYA. The most recent diverging species of the genus is likely to be *G. dersim* and *G. tournefortii* var. *tenuisecta* (Pliocene period ~4.66 MYA). Similarly, molecular clock estimations with the ITS region of *nr*DNA showed that earliest diverging species was *G. anatolica* (21.43 MYA). The divergence time of the remaining species was about 17.22 MYA. With respect to *ITS* data, the most recent diverging species of the genus appears to be *G. vitekii* (Pleistocene period ~1.8 MYA) (Fig. 3). However, due natural hybridization, in turn, gene flow among many species of the genus, the species-specific samples did not end up in the same clades in the ITS based phylogenetic tree, that further complicated the molecular clock estimation for these species. Nevertheless, it seems that speciation in the genus still continues.

Discussion

The current study with comprehensive sampling of *Gundelia* species, which has biodiversity center in Turkey, provided invaluable molecular diversity information that can shed a light to taxonomy of the genus. Both studied chloroplast (*ndh*F) and nuclear DNA (ITS) regions varied in number of variable and parsimony informative sites which were useful in phylogenetic analysis. It appeared that the phylogenetic relationships among studied *Gundelia* species were better explained by the *ndh*F data than the data from the ITS gene region. The ITS data based phylogenetic tree indicated that evolutionary relations among closely related species may be further complicated by possible natural hybridization and introgression events. Especially, gene flow among *G. rosea, G. komagenensis* and *G. mesopotomica* species have been reflected in phylogenetic tree that morphologically identified samples of these species were allocated in different subclades. Although, samples of *G. mesopotamica* species were collected at closely related region, the species position in the phylogenetic trees were sometimes polyphyletic. Inconsistency in clustering pattern with the samples of these species could be due the characterization of samples from the species which harbor vast amount of variation created in morphology by blending of traits among closely related taxa due to gene flow among species through natural hybridizations.

As suggested by Vitek et al. (2010), *Gundelia* genus is not a monospecific genus and the interspecific relationships were presented with low posterior probability values. These findings have been also supported by the results of the current study. From both *ndh*F and ITS based phylogenetic trees, two major clades exist. In the main clades of both ITS and *ndh*F phylogenetic trees, *G. anatolica* appeared to be significantly different from the other species of the genus that this species deserves to be investigated further with respect to its reproductive and pollination biology to understand the speciation mechanism of it.

Turkey is located the intersection of three major phytogeographic regions as Mediterranean, Euro-Siberian, and Irano-Turanian (Avci 1993). Moreover, the Anatolian Diagonal which is a major mountain ranges that run from the north (Gümüşhane- Bayburt) to southwest (the Taurus Mountains) across Turkey (Davis 1971; Ekim and Güner 1986) have been proposed as a significant geographic barrier shaping many species compositions naturally distributed in the country (Davis 1971; Ekim and Güner 1986; Çıplak et al.

1993; Mutun 2010, 2016). The Anatolian Diagonal divides the Irano-Turanian phytogeographic region of Turkey into the east and west (Mutun 2016) which played important role in species divergence due to geographical isolation mechanism. In the current study, the results from the RASP analysis supported that the Anatolian Diagonal affects the species divergence of the genus. The diagonal forms a barrier to the genus and effects the distribution route of them (ACD and continued with A|ABCD region explained in result part). Especially, the east part of the diagonal was strictly isolated from the other part of the Irano-Turanian Region.

The genus *Gundelia* is certainly a diverse genus. Recently several new species have been identified and described in the literature (Fırat 2016, 2017a;2019a; Vitek 2018; Vitek and Noroozi 2017a, b; Vitek et al 2014). For example, Fırat (2019a) studied morphologically different species which were consistently grouped in the same clade of the *ndh*F based tree in the current study and introduced as a new species of *G. siirtica*. Also, there have been attempts to group the species of *Gundelia* genus based on mainly number of cephaloid flowers and corolla colors in lesser extent (Fırat 2017b, 2018a, b, 2019a, b). The results of the current study did not support this type of groupings. Tarikahya-Hacıoğlu and Fırat (2017) studied the ITS region on some *Gundelia* species in Turkey and reported a similar result with the current study that *G. anatolica* formed a separate major clade, while other species formed weakly associated groups. Molecular dating from both *ndh*F and ITS data confirmed that *G. anatolica* is earliest diverging species while the divergence time of other species varied from Miocene (15-17MYA) to Pleistocene period (1.27 MYA).

In a recent study Fırat (2021a), a new taxonomic arrangement of the genus *Gundelia* into subgenera and sections has been attempted using morphological traits of samples obtained from the Turkish herbaria and field studies. In the study, the genus was divided into 2 sub genera as *Anatolia* and *Gundelia*. Molecular data in the current study were indicated this separation that *G.anatolia* always positioned alone and gave clues for the section separations as Anatolia section consisted of *G.anatolica* taxa as a sub genus. Therefore, phylogenetic information from the current study will be very useful in future taxonomic revisions or new arrangements of the genus as subgenera or sections.

In times, it was clearly seen that only taxonomic classifications are not adequate a firm taxonomic separation in many species. This is not a surprise since morphological quantitative traits are not only controlled by many genes with small effects, but also environmental variables. The natural ranges of the species of *Gundelia* genus studied in the current study are so diverse with respect to elevation, annual temperature, precipitation, mountain ranges and growing season lengths. These topographic and climatic variables certainly created diverse habitats for diverse *Gundelia* taxa in Turkey. Moreover, variation and mutations are common especially in flower features like bracts or flowers number in *Gundelia* genus (Fırat 2021a). Therefore, the occurrence of natural hybridizations among *Gundelia* species and molecular evidence should be considered while one describes new species.

The results from phylogenetic trees (especially *ndh*F based tree), molecular clock estimation and RASP analysis suggest that there may be three ancestral groups among the studies species. These groups are *G.anatolica-G.glabra-G. asperrima*, *G.komagenesis-G.siirtica-G.rosea* and the remaining species with weak association. These associations may be maintained by either geographical isolations or some other weak isolation mechanisms which currently operate on speciation of the genus. Like the species of *Gundelia,* low levels of genetic differentiation among species in nature especially in sympatric habitats suggest that difficulties with morphological identification are likely due to shared ancestral gene pool along interspecific gene flow shared through pollinators (Mesquita-Neto et al. 2018; Albarrán-Lara et al. 2019). To explore this issue further, future studies dealing with the reproductive biology and population genetics of three ancestral groups of *Gundelia* will be needed.

Information on Electronic Supplementary Material

Online Resource 1. Pictures of studied species of *Gundelia* taxa from their natural habitats.
Online Resource 2. Tree output of RASP analysis with S-DEC & Bayesian Traits with the *ndhF* data.
Online Resource 3. Geographic and topographic information on studied *Gundelia* species and samples.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s00606-021-01771-2.

Declarations

Conflict of interest No known or potential conflicts of interest exist for any author.

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