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Dichoropetalum alanyensis (Apiaceae), a new species from South Anatolia, Turkey

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Abstract: *Dichoropetalum alanyensis* Bilgili, Sağıroğlu & H.Duman, a new species from South Anatolia in Turkey (Antalya-Alanya), is described and illustrated. It is closely related to *D. chryseum* (Boiss. & Heldr. ex Boiss.) Pimenov & Kljuykov, from which it differs mainly by its habit, basal leaves and lobes, and mericarp features. Diagnostic morphological characters are discussed. Notes are also given on its ecology and conservation status, together with scanning electron micrographs of mericarp surface sculpturing pattern. ITS sequences were used for DNA fingerprinting. The data were analyzed with the SEAVIEW package. Standardized data were used to generate a dendrogram that revealed the phylogenetic relationships of taxa in tribes Selineae (*Dichoropetalum-Johrenia*), Tordylieae (*Cymbocarpum*), and Bupleureae (*Diplotaenia*) from different localities.

Key words: New species, *Dichoropetalum*, Apiaceae, ITS, Turkey

1. Introduction

The family Apiaceae includes approximately 450 genera and 3700 species (Pimenov, 2004). The Asian countries with the greatest Apiaceae diversity include China, Turkey, Iran, Russia, and Kazakhstan. Turkey shows the highest diversity in Asia and probably in the world, with about 160 endemic species in 44 genera (Davis, 1988; Güner, 2000; Pimenov, 2004, 2005, 2011; Duman, 2005; Duran, 2005, 2011; Özhatay, 2006; Kandemir, 2007; Sağıroğlu, 2007, 2010; Adıgüzel, 2011; Genç, 2012; Uzunhisarcıklı, 2013; Güner, 2013). The genus *Dichoropetalum* was recently resurrected by Pimenov, who assigned six sections within the genus: *Strida*, *Scoparia*, *Johreniopsis*, *Parajohrenia*, *Holandrea*, and *Dichoropetalum*. *Dichoropetalum* contains 30 species (including this new species), 14 of which are distributed in Turkey (Pimenov, 2007, 2011).

The Alanya region, from which the new species is described, is a very interesting area from the aspect of plant diversity as it is very rich in local endemics, including *Dichoropetalum longibracteolatum* (Parolly & Nordt) Pimenov & Kljuykov; *Ferula duranii* Sagiroglu & H.Duman; *Bilacunaria aksekiensis* A.Duran & B.Doğan; *Arenaria mcneillii* Aytaç & H.Duman; *Pentanema alanyense* H.Duman & Anderb; *Arabis alanyensis* H.Duman; *Aethionema alanyae* H.Duman; *Minuartia asiyeae* H.Duman; *Allium enginii* N.Özhatay & B.Mathew; *Origanum husnucan-baseri* H.Duman, Aytaç & A.Duran; and *Nonea dumanii* Bilgili & Selvi (Duman, 1994, 1999, 2001; Özhatay, 1995; Aytaç, 2004; Sağıroğlu, 2010; Duran, 2011; Pimenov, 2011; Bilgili, 2012).

For the first time, Dr Hayri Duman collected some interesting Apiaceae specimens in 1998 from the Alanya-Erik Stream region. Then, in 2006 and 2007, Dr Bilgili and Dr Sağıroğlu collected some similar specimens from several localities of the Alanya-Hisar Mountain. After critical literature reviews (Korovin, 1951; Zohary, 1966; Chamberlain, 1972; Rechinger, 1987) and herbarium surveys (ANK, HUB, GAZI, E), we decided that the specimens should be evaluated as a new species under the genus *Dichoropetalum* based on the taxonomic treatment of Pimenov et al. (2007).

The aim of this study is to describe a new *Dichoropetalum* species based on morphology, ecology, mericarp micromorphology, and molecular phylogenetic data.

2. Materials and methods

In the description below, each numerical value is the range from ten measurements from different specimens. The specimens of *Dichoropetalum alanyensis* were examined

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and compared taxonomically with specimens of *D. chryseum* (Boiss. & Heldr.) Pimenov & Kljuykov and *D. seseloides* (C.A.Mey.) Pimenov & Kljuykov.

Representative specimens in the tribe Selinae (Dichoropetalum *chryseum*, *D. depauperatum* (Boiss. & Balansa ex Boiss.) Pimenov & Kljuykov. comb. nov., *D. alpinum* Fenzl, and *Johrenia tortuosa* (Fisch. & Mey.) Chamb.), tribe Tordylieae (*Cymbocarpum wiedemannii* Boiss.), and tribe Bupleurae (*Diplotaenia turcica* Pimenov & Kljuykov) were used in molecular analyses and were collected from different localities. Please see the Appendix for details about specimens.

2.1. DNA isolation

Total DNA was obtained from 50–75 mg of dried leaf tissue from seven different individuals as listed in Table 1. An extraction followed the procedure of Doyle and Doyle (1987) (2X CTAB method), but some modifications were applied. DNA samples were isolated for every genotype and concentrations were determined by NanoDrop. DNA samples were diluted to 25 ng/µL. Stock DNAs were kept at –80 °C.

2.2. Sequencing reactions

The ITS1-5.8S-ITS2 rDNA region was amplified using the following primer pair (White et al., 1990): ITS-4 (50-TCCTCCGCTTATTGATATGC-30), ITS-5 (50-GGAAGTAAAAGTCGTAACAAGG-30). PCRs were performed in 25-µL reactions containing 12.9 µL of ddH_2O, 2.5 µL of 10X reaction buffer, 2.5 µL of 25 mM $MgCl_2$, 2.5 µL of 5 mM dNTPs, 1.25 µL of each 10 µM primer, 0.1 µL of Ampl. Taq (5 U μL^{-1}), and 1 µL of dimethyl sulfoxide (DMSO). PCR amplification began with initial denaturation (94 °C, 2 min), followed by 35 cycles of denaturation (94 °C, 1 min), annealing (60 °C, 1 min), and extension (72 °C, 2 min) with a final extension (72 °C, 5 min) in a thermocycler (Eppendorf, Applied Biosystems). The resulting PCR products were checked on a 1% agarose gel.

PCR amplification and sequencing procedures were performed for the nuclear regions used in this research. Purified PCR products (QIAquick Spin PCR Purification Kit, QIAGEN) were sequenced following the manufacturer's instructions. Sequencing was performed with either BigDye Terminator RR Mix (Applied Biosystems) and visualized on a CEQ 2000XL DNA Sequencer (Beckman Coulter), or with an ABI PRISM BigDye Terminator Premix Cycle Sequencing Kit and visualized on an ABI PRISM 377 Automated Sequencer (Applied Biosystems).

2.3. Alignment and phylogenetic analyses

Sequences for the above-mentioned taxa were edited using SEAVIEW (Galtier et al., 1996; Gouy et al., 2010) and were aligned with MUSCLE under default parameters (Edgar, 2004) followed by manual adjustment. The alignment of the datasets required the introduction of numerous single and multiple-base indels (insertions/deletions). Positions of indels were treated as missing data for all datasets. The ML analyses were performed for the datasets and parametric bootstrap values for ML were calculated in the program SEAVIEW based on 1000 replicates with one search replicate per bootstrap replicate.

3. Results

3.1. Morphology and taxonomy

Dichoropetalum alanyensis Bilgili, Sağıroğlu & H.Duman. sp. nov. (Figure 1)

Type: Turkey: C3 Antalya: Alanya-Gökbel plateau, rocky slopes, 1650 m, 06.07.2006, *B.Bilgili* 2416 & *M.Sağıroğlu* (holotype: GAZI, isotype: ANK, HUB).

Diagnosis: *Dichoropetalum alanyensis* is closely related to *D. chryseum* and *D. seseloides*. It differs from *D. seseloides* (Syn.: *Peucedanum meyeri*) by its dense inflorescence (not lax), number of central umbel rays 7–10 (not 10–20), bracteoles 1–3 (not 3–7), mericarps elliptic (not obovate). It differs from *D. chryseum* by its ultimate leaf segments 4–7(–10) mm (not 3–4 mm), dense inflorescence (not

Table 1. Taxa included in the nrDNA ITS analyses.

Taxon name	Voucher	DNA source (country, location)
Diplotaenia turcica	*A.Duran* 8650 & *M.Öztürk*	Turkey, B9 Bitlis
Cymbocarpum wiedemanni	*A.Duran* 6428 & *Hamzaoğlu*	Turkey, A7 Gümüşhane
Dichoropetalum alpinum	*A.Duran* 7711& *Bağcı*, *Dinc*	Turkey, C5 Mersin
Dichoropetalum depauperatum	*A.Duran* 7687& *Bağcı*, *Dinc*	Turkey, C6 Kahramanmaraş
Dichoropetalum chryseum	*A.Duran 3233*	Turkey, C3 Antalya
Dichoropetalum alanyensis	*B.Bilgili* 2416 & *M.Sağıroğlu*	Turkey, C3 Antalya
Johrenia tortuosa	*A.Duran* 7292	Turkey, B2 Bursa

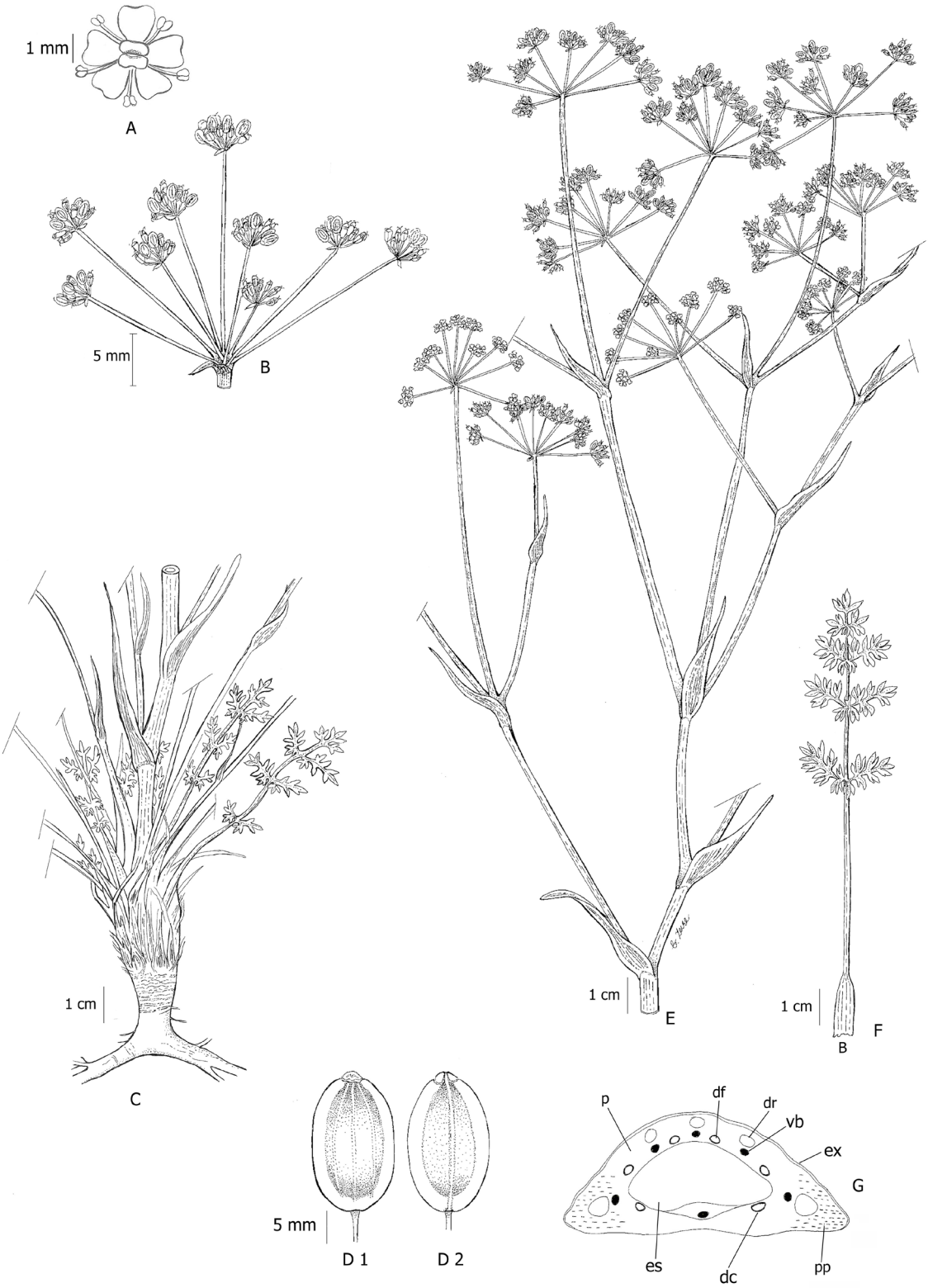

Figure 1. *Dichoropetalum alanyensis*. A: Flower, B: umbel, C: base of plant and basal leaves, D: fruits (1- dorsal surface, 2- commissural surface), E: stem and inflorescence, F: basal leaves, G: transverse section of mericarp. (dr = secretory duct in distal part of rib, dc = commissural secretory ducts (vittae), df = secretory ducts in furrows (vittae), es = endosperm, ex = exocarp, p = parenchyma cells without pits, pp = parenchyma cells with lignified pitted walls, vb = vascular bundles; *B.Bilgili* 3324 (GAZI) & *M.Sağıroğlu*).

lax), central umbel rays 7–10 (not 12–15), mericarps 3–5 × 1.5–3 mm (not 6–8 × 3–4 mm), and stylopodium conical (not depressed).

Description: Perennial, polycarpic, glaucescent, completely glabrous. Rootstock thin, with weakly fibrous collar, up to 1 cm in diameter. Stem 30–70 cm, intensively branching, terete, sulcate, glaucous, glabrous, 2–5 mm in diameter at base. Basal leaves numerous, linear in outline, 6–20 × 1–3 cm; petioles 2–6 cm; lamina bipinnatisect, glabrous; ultimate segments linear-lanceolate, 4–7(–10) × 1–2 mm, terminal segments deeply divided (2–3 pairs), acute. Lower cauline leaves reduced with few segments, ±amplexicaul. Middle and upper cauline leaves very reduced, semiamplexicaule, sheath-like, linear-lanceolate, 1–5 × 0.2–0.5 cm, membranous, glaucescent, lamina absent. Inflorescence dense paniculate-corymbose. Umbels up to 5 cm in diameter. Rays 7–10(–12), 1.5–3 cm long. Bract usually absent, rarely 1–2. Bracteoles 1–3, linear-lanceolate, 1–1.5 mm long. Umbellules with 7–10(–12) flowers. All flowers hermaphroditic; sepal inconspicuous; petal yellow, 1–1.5 mm deflexed. Fruiting pedicel 1–2 mm long, ±equal. Mericarps elliptic, 3–5 × 1.5–3 mm, glaucous when ripe; dorsal ridges conspicuously filiform, lateral wings 0.2–0.5 mm wide; stylopodium short conical, 1 mm long; styles c. 1 mm long reflexed; stigma capitate; dorsal vittae per vallecula 1, commissural 2. Fl. 6–7, Fr. 7–8.

Paratypes: Turkey: C3 Antalya: Alanya-Yaylacık mountain, 1600–1700 m, rocky slopes, 09.06.2006, *B.Bilgili* 2159 (GAZI) & *M.Sağıroğlu*; ibid., *B.Bilgili* 3324 (GAZI) & *M.Sağıroğlu*; Alanya-Hadim road, around Erikli Deresi, 1300–1350 m, rocky slopes, 24.07.2009, *H.Duman* 10085 (GAZI).

3.2. Distribution and ecology

Dichoropetalum alanyensis is endemic to South Anatolia (Antalya-Alanya region) and thus belongs to the East Mediterranean floristic element. The specimens were collected from Alanya (Antalya Province), where the species appears to be restricted and local (Figure 2). *D. alanyensis* grows on calcareous stony slopes in open areas of *Pinus nigra* Aiton forest in company with *Scrophularia myriopylla* Boiss. & Heldr., *Rosularia libanotica* (Lab.) Murhead., *Alyssum pateri* Nyar subsp. *pateri*, *Inula montbretiana* DC., *Campanula stellaris* Boiss., *Dianthus anatolicus* Boiss., *Silene anatolica* Melzheimer & A.Baytop, *Verbascum myrianthum* Boiss., *Sideritis erythrantha* Boiss. & Heldr. var. *cedretorum* Davis, and *Onosma frutescens* Lam.

3.3. Conservation status

Dichoropetalum alanyensis is an endemic species known only from two localities. According to our observations and field studies, we determined that the area of occupancy of the new species is less than 2 km^2, the population size of it was estimated to be fewer than 200 mature individuals, and the population size could be reduced in the near future based on local grazing pressure. Therefore, we concluded that the conservation status of the new species should be evaluated as 'Critically Endangered (CR)' in accordance with IUCN criteria (2011).

4. Discussion

According to recent taxonomic study and multivariate analysis of the genus *Peucedanum* s. amplo (Pimenov et al., 2007), the genus *Dichoropetalum* is resurrected and some species from the genus *Peucedanum* were transferred to the genus *Dichoropetalum* because of their inconspicuous sepals, smaller ultimate segments (shorter than <1 cm), sessile or petiolulate primary segments, and dentate or lobate leaf lamina. Our careful morphologic studies showed that our new species must be placed in the genus *Dichoropetalum* rather than the genus *Peucedanum*.

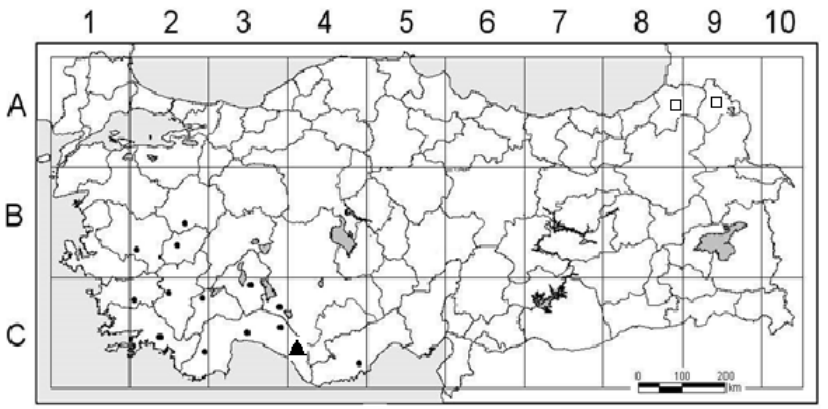

Figure 2. Distribution of *Dichoropetalum alanyensis* (▲), *D. seseloides* (□), and *D. chryseum* (●).

In accordance with literature and herbarium surveys and molecular data, we concluded that *Dichoropetalum alanyensis* is close to *D. chryseum* and *D. seseloides*. However, *D. alanyensis* clearly differs from them. In Table 2, diagnostic morphological differences among the species are given.

The distribution patterns of *Dichoropetalum alanyensis*, *D. seseloides*, and *D. chryseum* are given in Figure 2. As it is given, *D. alanyensis* is restricted in the Alanya region in South Anatolia. However, *D. seseloides* (Syn.: *Peucedanum meyeri*) is distributed in Northeast Anatolia, East Anatolia, Caucasia, North Iran, and Khorasan (Chamberlain, 1972). *D. chryseum* is an endemic species and distributed in West, South, and adjacent parts of Central Anatolia (Chamberlain, 1972) (Figure 2).

According to the SEM micrographs, the mericarp surface sculpturing pattern of *Dichoropetalum alanyensis* is regular reticulate with distinctly tetrangular or pentangular cells, whereas *D. chryseum* is irregularly reticulate-striate and colliculate to corrugate with inconspicuous and complicated cells (Figure 3).

The maximum likelihood tree derived from analyses of the combined nrDNA ITS sequences of *Dichoropetalum alanyensis* and related taxa with bootstrap value (Figure 4).

Table 2. Comparison of the diagnostic characters of *Dichoropetalum alanyensis*, *D. chryseum*, and *D. seseloides*.

Characters	*D. alanyensis*	*D. chryseum*	*D. seseloides*
Stem	Glabrous, sulcate	Puberulent at base, ribbed	Glabrous, round
Basal leaves	1–2 pinnate	2 pinnate	1–2 pinnate
Leaves lobes	Linear-lanceolate, glabrous	Linear, scabrid, scabrous	Linear, glabrous
Ultimate segment of leaves	4–7(–10) × 1–2 mm	3–4 × 1–2 mm	8–35 × 1–4 mm
Inflorescence	Dense	Lax	Lax
Rays	7–10(–12)	(12–)15–22	3–9
Fruiting pedicel	1–2 mm	5–8 mm	1–4(–6) mm
Bracteoles	1–3	6–7	3–7
Bract	Usually absent, rarely 1–3	Absent	Absent
Mericarp	Elliptic, 3–5 × 1.5–3 mm	Elliptic, 6–8 × 3–4 mm	Obovate-oblong, 5–7 × 9 mm

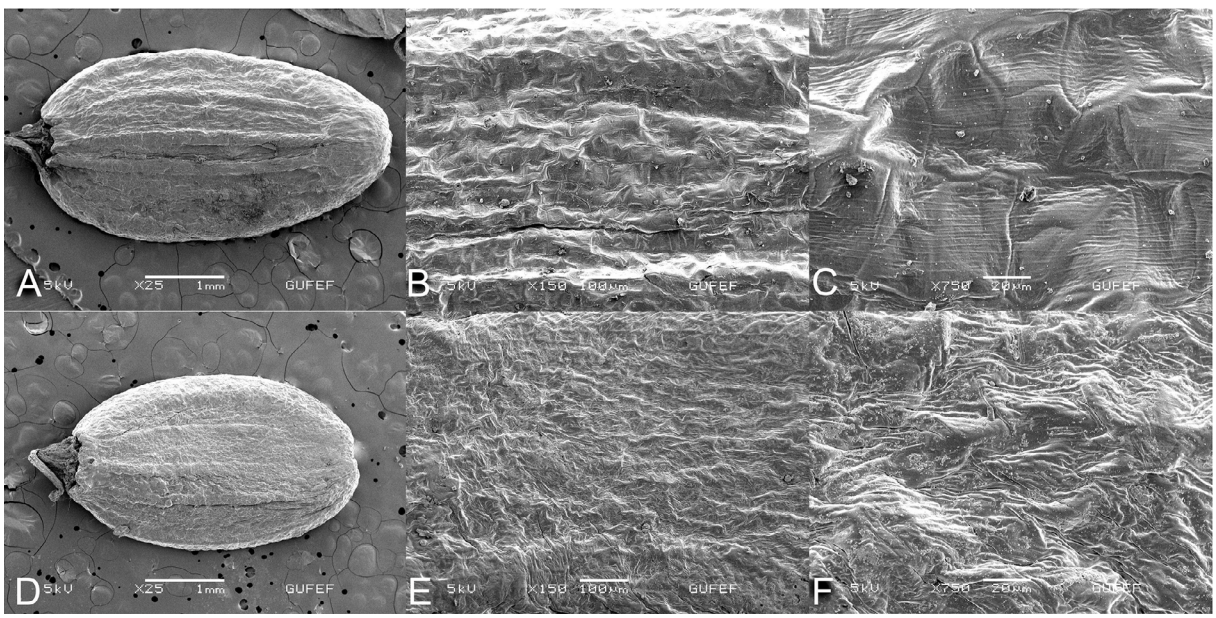

Figure 3. Fruit and fruit surface of *Dichoropetalum alanyensis* and *D. chryseum*. A: General view fruit of *D. alanyensis*, B–C: surface ornamentation of *D. alanyensis*. D: general view fruit of *D. chryseum*, E–F: surface ornamentation of *D. chryseum*.

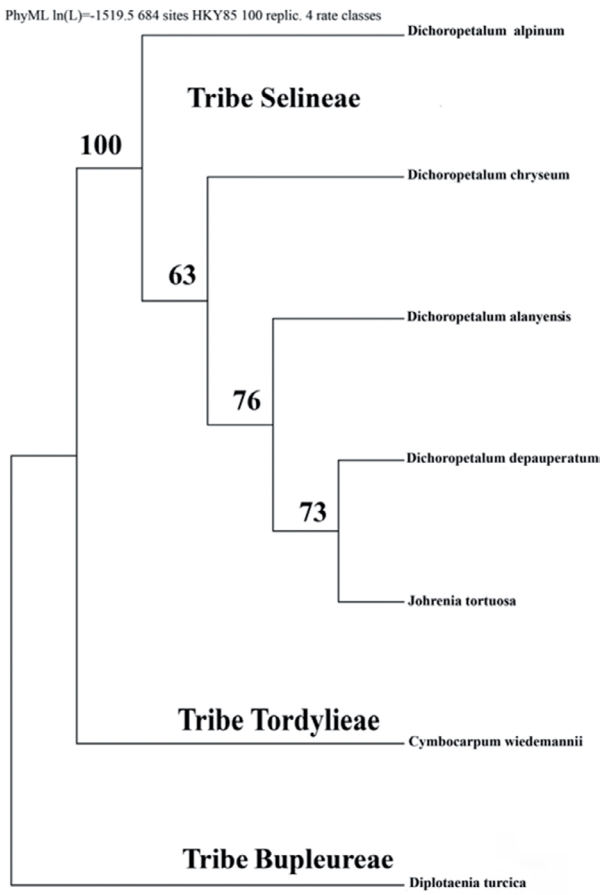

Figure 4. Phylogenic tree derived from maximum likelihood analyses of ITS data.

The length and composition of the ITS4/ITS5 gene region sequenced were used for analyses. Nucleotide sequences will be deposited in GenBank. The nuclear ITS4 and ITS5 sequences in *Dichoropetalum* varied with an average of 631 bp. After alignment, the final dataset consisted of 686 positions. The dataset was analyzed by ML method (PhyMl ln(L) = –1519.5 sites, HKY85 model in 1000 replicates) and the bootstrap value was calculated in SEAVIEW (Galtier et al., 1996; Gouy et al., 2010). According to the results, we infer 3 clades that contain the tribes Selineae, Tordylieae, and Bupleureae. Tribe *Selineae* includes *Johrenia tortuosa*, *Dichoropetalum alpinum*, *D. chryseum*, *D. alpinum*, *D. depauperatum*, and *D. alanyensis*; tribe *Tordylieae* includes *Cymbocarpum wiedemannii*; and tribe *Bupleureae* contains the Turkish local endemic *Diplotaenia turcica* (Figure 4).

The length and composition of the ITS4/ITS5 gene region sequenced were used for second analyses. Variation of the nrITS marker among *Dichoropetalum alpinum*, *D. chryseum*, and *D. depauperatum* had similar characters and differs from *D. alanyensis* in 214 characters. ITS sequence variation is very high in these species. These characters are given in Table 3.

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Table 3. Summary of differences between the nrITS sequences of *Dichoropetalum* species.

Site position in alignment	1, 2, 3, 6, 11	4, 7	5	8, 9, 10, 12, 13	14, 19	15, 20, 21, 22	16	17, 18	23, 40, 42	24, 30, 41	25, 27
1- *D. chryseum*	G	T	A	C	T	G	A	C	G	T	C
2- *D. depauperatum*	…	…	…	…	…	…	…	…	…	…	…
3- *D. alpinum*	…	…	…	…	…	…	…	…	G	T	C
4- *D. alanyensis*	…	…	…	…	T	G	A	C	G	T	C
Site position in alignment	29	31	32	33	34	35	36	37	38	39	45
1- *D. chryseum*	G	C	C	T	A	A	G	…	…	T	G
2- *D. depauperatum*	G	C	T	C	G	A	C	…	…	T	G
3- *D. alpinum*	…	A	C	C	G	A	A	…	…	C	G
4- *D. alanyensis*	G	C	C	C	G	G	A	G	A	T	…
Site position in alignment	48	49	50	51	52	92	207	208, 214	26, 28, 43	46	
1- *D. chryseum*	G	T	T	A	G	…	…	…	A	T	
2- *D. depauperatum*	A	A	G	A	A	C	…	…	…	C	
3- *D. alpinum*	G	T	G	G	A	T	G	A	A	T	
4- *D. alanyensis*	G	T	G	A	A	T	…	…	A	T	

Site position in alignment	53, 54, 59, 61, 65, 66, 68, 70, 71, 72, 84, 88, 89	55, 56, 63, 64, 76, 79, 80, 81, 82	57, 60, 67, 69, 73, 77, 83, 86, 87, 91, 93, 94, 101	62, 74, 75, 78, 85, 90	47, 191, 206, 209
1- *D. chryseum*	…	…	…	…	…
2- *D. depauperatum*	C	T	G	A	…
3- *D. alpinum*	C	T	G	A	C
4- *D. alanyensis*	C	T	G	A	…

Site position in alignment	159, 160, 171, 179, 184, 188, 192, 199, 145, 146, 147, 148, 149, 152, 154, 158, 116, 117, 125, 127, 131, 138, 139, 144, 95, 96, 98, 99, 100, 109, 110, 112, 113	201, 202, 174, 178, 180, 181, 182, 190, 198, 142, 143, 150, 153, 157, 166, 172, 121, 126, 129, 133, 134, 135, 136, 102, 103, 105, 107, 108, 119, 120	204, 205, 186, 187, 189, 193, 195, 196, 197, 165, 167, 168, 169, 175, 176, 183 106, 114, 123, 124, 155, 156, 164
1- *D. chryseum*	…	…	…
2- *D. depauperatum*	C	G	T
3- *D. alpinum*	…	…	…
4- *D. alanyensis*	…	…	…

Site position in alignment	151, 161, 162, 163, 170, 173, 177, 185, 194, 200, 203 44, 97, 104, 111, 115, 118, 122, 128, 130, 132, 137, 140, 141
1- *D. chryseum*	…
2- *D. depauperatum*	A
3- *D. alpinum*	…
4- *D. alanyensis*	…

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Appendix.

- ***Dichoropetalum chryseum*** (Syn.: *Peucedanum chryseum*): Turkey. C4 Konya: Ermenek, Kazancı Kasabası civarı, 650–850 m, 21.06.1984, *H.Sümbül* 3014 (HUB); Prov. Antalya: Distr. Marmaris, Poor Hills, Ak Dag, rocky knolls, 22.07.1960, Khan et al. 161 (ANK); Ankara: Kepekli Boğazı, 10.07.1972, *H.Peşmen* 1974 (ANK); Ankara: Çubuk Dere, Krause 4633 (ANK); Antalya: Gündoğmuş, Oğuz Bölgesi, *Pinus brutia* ormanı ve açıklığı, 1400–1500 m, 08.08.1991, *R.İlarslan* & *H.Dural* 3142 (ANK); Alanya: Hacıbelen, ca. 1100 m, 28.08.1947, *P.H.Davis* 14233 (ANK); C3 Antalya: Akseki, Güzelsu-Sadıklar Köyü arası, ekilmiş tarla, 1200 m, 20.08.1995, *A.Duran* 3233 (GAZI); C4 Antalya: Alanya, Derince çevresi, *Pinus nigra* açıklığı, taşlık alanlar, 800 m, 31.08.1993 *H.Duman* 5469 et al. (GAZI); C3 Antalya: Akseki, Geyran yaylası mevkii, tarla açıklıkları, 1250 m, 11.08.1994, *H.Duman* 2040-b (GAZI).
– ***Dichoropetalum seseloides*** (Syn.: *Peucedanum meyeri. Johreniopsis seseloides*): Iran. Kordeistan: c. 108 km from Zanjan on road to Bijar. Hot loam slopes, 1700 m, 30 June 1971, *J.Lamond* 4326 (E); East of Sanandağ, 6000 ft. alt. fallow, 22.07.1962, *P.Furse* 3356 (E); In umbrosis montains Georgiae caucascae. Jul Aug. *R.F.Hohenaker* 1832 (E), type of *Ferula seseloides* Meyer; Kurdistan: 16 km N. of Husayinabad, between Sanadağ and Sagezi dry exposed "hills of upland plateau in heavy stony clay among steppe vegetation", 2160 m, 16.071966, 3. *L.Archibald* 3138 (E). Turkmenistan. The Kopet Dagh Mts., canyon Pordere, 02.07.1978, *M.G.Pimenov*, *E.V.Klğnglov*, *L.P.Tomkorich*, *T.A.Oitroumova* 214 (E).