Survey of the genus Diplotaenia (Umbelliferae), with description of two new species from Turkey

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Abstract

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The genus *Diplotaenia* currently includes two species: *D. cachrydifolia*, providing the type of the generic name, is disjunctly distributed in N Iran and S and E Turkey; *D. damavandica* is a narrow endemic of the Elburs Mts, being sympatric in this region with *D. cachrydifolia*. Studies of the *Diplotaenia* material from Turkey showed that the Bitlis and Taurus populations are morphologically divergent from each other as well as from the Iranian populations of *D. cachrydifolia* and they are described as two separate new species, *D. turcica* and *D. hayri-dumanii*. Analysis of nrITS sequences of all four species of *Diplotaenia* and presumably related *Umbelliferae* taxa of SW Asia provide support for the monophyly of *Diplotaenia* and its placement in the *Prangos-Ferulago* clade, but revealed almost no differences among the species of *Diplotaenia*. The fruit anatomical analysis of *Diplotaenia* also supports an *Apioae* rather than a *Peucedanaceae* relationship. A key to the four species of *Diplotaenia* is provided and the name *D. cachrydifolia* lectotypified.

Additional key words: *Apiaceae*, taxonomy, systematics, molecular phylogenetics, nrITS, carpology, Iran

Introduction

*Diplotaenia* was described by Boissier (1844) as a monotypic genus based on *D. cachrydifolia* Boiss. from Iran and stated to be similar to *Peucedanum orientale* (L.) Boiss. and *P. schlechtendalii* Boiss. (i.e. *Ferula orientalis* L. in modern treatments), but differing from them in characters of the calyx, stylopodium and secretory system. It was compared also with *Johrenia* DC. Bentham (1867), Boissier (1872), Baillon (1879) and Hedge & Lamond (1987) regarded *Diplotaenia* as a relative of *Peucedanum* L. (s.l.) and *Ferula* L. but having Cachrys- or Prangos-like leaves. With some hesitation, Koso-Poljansky (1916) included *Diplotaenia* into *Peucedanum*. Chamberlain (1972) stated that *D. cachrydifolia* is a distinctive species with no close affinity.

Rather recently, a second species, *D. damavandica* Mozaff. ex Hedge & Lamond (1987), was described, which is distributed in a limited area in the Elburs Mts. Both species of *Diplotaenia* have been investigated in their anatomy (Ghareman & Amin 1996) and chemical composition (Harkiss & Salehy Surmaghy 1988a, b) but no conclusions regarding the relationship of the genus were drawn. Similarly, Downie & al. (2001) listed *Diplotaenia* among genera of uncertain tribal or clade placement based on their DNA sequence analysis. According to more recent molecular analyses (Valiejo-Roman & al. 2006; Ajani & al. 2008), *Diplotaenia* is related to the genera *Prangos* Lindl., *Alococarpum* H. Riedl & Kuber, *Cachrys* L., *Bilacunaria* Pimenov & V. N. Tikhom. and *Azilia* Hedge & Lamond, whereas its former attribution to the *Peucedanaceae* received no confirmation.

Chamberlain (1972), when studying the *Umbelliferae* for the Flora of Turkey, found that *Diplotaenia cachrydifolia* is not only distributed in N Iran but also in the

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Bitlis Province in SE Turkey. Both occurrences are several hundred kilometres distant from each other. In 1993, Turkish collectors found the species then also in Antalya Vilayet and 1998 in Konya Vilayet, in S Turkey, again several hundred kilometres distant from the occurrence in SE Turkey.

The aims of the present contribution are as follows: (1) Testing the hypothesis of a systematic position of the genus outside the Peucedaneae by phylogenetic analysis of nrITS and fruit anatomical studies. (2) Revising the disjunct Turkish gatherings and their affinity to Diplotaenia cachrydifolia by morphological and molecular analyses. (3) Testing the hypothesis of the monophyly of Diplotaenia by the molecular phylogenetic analysis.

Material and methods

The study is based on specimens from the herbaria (abbreviations following Thiers 2008+) ANK, BM, EP, ESKI, HUB, GAZI, G-BOIS, K, LE, MW and TARI.

For molecular phylogenetic analysis, nrITS sequences of two new Diplotaenia species were generated and added to the sequences of closely related taxa revealed by previous molecular phylogenetic studies (Valiejo-Roman & al. 2006; Ajani & al. 2008). The sources of the samples for the molecular analysis are given in Table 1. The procedures used for isolation, amplification and sequencing of DNA are the same as described in Valiejo-Roman & al. (2006). The data set was analysed using Maximum Parsimony and the Bayesian Inference.

The parsimony analysis involved a heuristic search conducted with PAUP* (version 4.0b8; Swofford 2000) using TBR branch swapping with character states specified as equally weighted. 100 replicates with random addition of sequences were performed and all shortest trees were saved. Bootstrap (Felsenstein 1985) analysis was performed to assess the degree of support for particular branches on the tree. Bootstrap values were calculated from 100 replicate analyses with TBR branch swapping and random addition sequence of taxa. One thousand most parsimonious trees from each replicate were saved. In the parsimony analyses all gaps were treated as missing data.

The Bayesian analysis was carried out using the MrBayes program (version 3.1.2; Huelsenbeck & Ronquist 2001; Ronquist & Huelsenbeck 2003) with the SYM+G model. The model was selected by the Akaike Information Criterion in the program Modeltest (Posada & Crandall 1998). The analysis was performed with 2 parallel runs, three Markov chains were used for each run, and one tree for every 1000 generations was saved. A total of 20 000 000 generations were performed.

For the fruit anatomical studies, the standard methods used by the present authors in previous contributions were applied (Kljuykov & al. 2004).

Table 1. Voucher information and GenBank accession no. for plants used in the molecular analyses of the present study.

<table>
<thead>
<tr>
<th>Species</th>
<th>GenBank accession no.</th>
<th>Source or voucher</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alococarpum erianthum (DC.) H. Riedl &amp; Kuber</td>
<td>AY941264, AY941292</td>
<td>Valiejo-Roman &amp; al. (2006)</td>
</tr>
<tr>
<td>Azilia eryngioides (Pau) Hedge &amp; Lamond</td>
<td>EU169247</td>
<td>Ajani &amp; al. (2008)</td>
</tr>
<tr>
<td>Bilacunaria microcarpa (M. Bieb.) Pimenov &amp; V. N Tikhom.</td>
<td>AY941265, AY941293</td>
<td>Valiejo-Roman &amp; al. (2006)</td>
</tr>
<tr>
<td>Cachrys libanotis L.</td>
<td>EU169248</td>
<td>Ajani &amp; al. (2008)</td>
</tr>
<tr>
<td>Cachrys sicula L.</td>
<td>EU169249</td>
<td>Ajani &amp; al. (2008)</td>
</tr>
<tr>
<td>Diploentaenia cachrydifolia Boiss.</td>
<td>(1) EU169258; (2) AY941267, AY941295</td>
<td>(1) Ajani &amp; al. (2008); (2) Valiejo-Roman &amp; al. (2006)</td>
</tr>
<tr>
<td>Diploentaenia damavandica Mozaff. &amp; al.</td>
<td>EU169259</td>
<td>Ajani &amp; al. (2008)</td>
</tr>
<tr>
<td>Diploentaenia hayri-damanii Pimenov &amp; Kljuykov</td>
<td>GU182368</td>
<td>this paper; Turkey, C4, Konya, 15.7.1998, Baser &amp; al. 12660 (ESKI)</td>
</tr>
<tr>
<td>Diploentaenia turcica Pimenov &amp; Kljuykov</td>
<td>GU182367</td>
<td>this paper; Turkey, B9, Bitlis, 20.8.1972, Pesmen 3277 (HUB)</td>
</tr>
<tr>
<td>Ferulago angulata (Schltdl.) Boiss.</td>
<td>AY941272, AY941300</td>
<td>Valiejo-Roman &amp; al. (2006)</td>
</tr>
<tr>
<td>Ferulago galbanifera (Mill.) W. D. J. Koch (1)</td>
<td>AP077889</td>
<td>Valiejo-Roman &amp; al. (1998)</td>
</tr>
<tr>
<td>Ferulago galbanifera (Mill.) W. D. J. Koch (2)</td>
<td>AJ972385, AJ972386</td>
<td>Maras &amp; al. (2006)</td>
</tr>
<tr>
<td>Prangos acaulis (DC.) Bornm.</td>
<td>AY941281, AY941309</td>
<td>Valiejo-Roman &amp; al. (2006)</td>
</tr>
<tr>
<td>Prangos ferulacea (L.) Lindl.</td>
<td>EU169310</td>
<td>Ajani &amp; al. (2008)</td>
</tr>
<tr>
<td>Prangos goniocarpa (Boiss.) Zohary</td>
<td>EU169311</td>
<td>Ajani &amp; al. (2008)</td>
</tr>
<tr>
<td>Prangos pabularia Lindl.</td>
<td>AP077906</td>
<td>Valiejo-Roman &amp; al. (1998)</td>
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<tr>
<td>Prangos uloptera DC.</td>
<td>EU169312</td>
<td>Ajani &amp; al. (2008)</td>
</tr>
<tr>
<td>Seseli olivieri Boiss.</td>
<td>AY941289, AY941317</td>
<td>Valiejo-Roman &amp; al. (2006)</td>
</tr>
</tbody>
</table>
Results

Maximum Parsimony analyses resulted in 15 shortest trees with 227 steps (CI = 0.749, RI = 0.708). Bayesian Inference yielded a congruent tree that was better resolved. Therefore, only the Bayesian 50% majority rule tree is shown with posterior probability values above and parsimony bootstrap support values below the branches (Fig. 1).

Molecular analysis of all four species of *Diplotaenia* in comparison with presumably related SW Asian *Umbelliferae* taxa revealed that the *Diplotaenia* species form a statistically well-supported (posterior probability = 1; bootstrap support = 99) subclade of the *Prangos-Ferulago* clade, confirming the monophyly of the genus *Diplotaenia*.

Within *Diplotaenia*, *D. damavandica* appears as sister to the other three species, the relationships of which are not resolved, forming a polytomy (Fig. 1). Variation of the nrITS marker among the four *Diplotaenia* species is very low (Table 2). The sympatric *D. damavandica* and *D. cachrydifolia* differ in five nucleotide positions, whereas the vicariant *D. cachrydifolia*, *D. turcica* and *D. hayridumanii* with considerable geographical disjunctions differ only in a single nucleotide position. However, since except for *D. cachrydifolia* with two accessions (without any differences), only one accession each was available (Table 1), nothing can be said about infraspecific variation and the minor interspecific differences are therefore not conclusive.

The analysis of the fruit structure of *Diplotaenia* (Fig. 2) showed that attribution of the genus to the *Peucedanaceae* is not confirmed by carpological characters, too. In particular, the fruits are not strongly compressed dorsally and, in general, correspond to the carpological pattern of the *Apiaceae* rather than of traditional *Peucedanaceae*. The specific epithet “cachrydifolia” of the type species thus does not only indicate superficial resemblance with *Prangos* but a stronger affinity of *Diplotaenia* with the latter genus.

The distribution of the *Diplotaenia* species, based on all the known (very limited) material, is shown in Fig. 3.

### Taxonomic conspectus of Diplotaenia


Including two species newly described here, the genus comprises four species, two in Iran and two in Turkey.

#### Key to the species of Diplotaenia

1. Terminal leaf lobes arched; vallecular vittae 3–4, commissural vittae 1–2 .......................... 2
   - Terminal leaf lobes straight; vallecular vittae solitary (sometimes paired), commissural vittae 2 (sometimes 3) .................... 3
2. Terminal leaf lobes 10–20 mm long; umbel rays strongly unequal  ................. *D. hayridumanii*
   - Terminal leaf lobes 20–40 mm long; umbel rays ± equal  ..................... *D. turcica*
3. .................................................................. *D. turcica*
   - Terminal leaf lobes linear or filiform, 0.5–1 mm broad; mericarps

### Table 2. Summary of differences between the nrITS sequences of the examined *Diplotaenia* species.

<table>
<thead>
<tr>
<th>Species</th>
<th>Site position in the alignment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>22   89  165  471  478  542</td>
</tr>
<tr>
<td><em>D. damavandica</em></td>
<td>C    T    A    G    T    G</td>
</tr>
<tr>
<td><em>D. cachrydifolia</em></td>
<td>T    C    C    G    C    T</td>
</tr>
<tr>
<td><em>D. turcica</em></td>
<td>T    C    C    G    C    T</td>
</tr>
<tr>
<td><em>D. hayridumanii</em></td>
<td>T    C    C    A    C    T</td>
</tr>
</tbody>
</table>

Fig. 1. Bayesian tree of *Diplotaenia* and close allies based on the nrITS dataset. Only relationships with posterior probabilities greater than 50% are shown, branches of lower resolution are collapsed. Numbers above branches are posterior probabilities (>0.50), parsimony bootstrap support values are given below the branches.
slightly compressed dorsally; all mericarp ribs shortly keeled, marginal ribs slightly broader (Fig. 2A–B); styles 1.2–1.5 mm long.

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**Distribution.** — Iran (W: E Azarbayjan, Zanjan; N: Mazandaran; C: Tehran) – Fig. 3.

The distribution area of *Diplotaenia cachrydifolia* includes the Elburs Mts in northwestern Iran and adjacent regions (provinces Tehran, Mazandaran, Zanjan). The collections from Bitlis, SE Turkey, referred to this species by Chamberlain (1972) actually represent a different species, see *D. turcica*.

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Fig. 2. Fruit structure of *Diplotaenia* – A–B: *D. cachrydifolia*, dorsal view (A) and transect of mericarp (B), from Pimenov & al. 539, MW (Iran, prov. Tehran, Alborz Mts, valley of Karaj River, near Hassankadar, 36°01’N, 51°18’E, 2060 m, 29.6.2001); C: *D. damavandica*, transect of mericarp, from Mozaffarian 53805, TARI (Iran, prov. Tehran, Damavand, Garrubar valley, 2450 m, 13.8.1985); D–E: *D. turcica*, dorsal view (D) and transect of mericarp (E), from the holotype; F–H: *D. hayri-dumanii*, lateral view of young mericarp (F) and transect of mericarps (G–H), from Ilarslan & Dural 4070, ANK (Turkey, C4 Antalya; near Gündoğmuş, 1900–2200 m, 10.8.1993).

Distribution. — Iran (C: Tehran), Fig. 3.

3. Diplotaenia turcica Pimenov & Kljuykov, sp. nov.

Ho lotype: “Turkey: B9 Bitlis: Tatvan, Karz Dağ, 1800–2600 m”, 20.8.1972, Pe men 3277 (HUB!) – Fig. 4A.

A D. cachrydifolia, cui proxima est, lobis terminalibus foliorum vix brevioribus, arcuatis, radiis umbellis subaequilongis (non valde inaequilongis), vittis vallecularibus 3–4 (non solitariis), commissuralibus 4–7 (non binis) differt.

Plantae perennes polycarpicae. Radices ignotae. Stems 1.8–2 m high, c. 1 cm in diam. at the base, rounded at cross-section, branched from the middle, with alternate lower and verticillate upper branches overtopping the central umbels. Leaves long-petiolate, leaf blades up to 35 cm long, ovate at outline, 4-pinnatisect, leaflets 20–40 mm long, filiform, curved; stem leaves with lanceolate sheaths. Umbel rays 12–15, slightly unequal in length, 2.5–5 cm long, bracts 5–7, linear-lanceolate, entire, herbaceous, reflexed. Umbellets with c. 20 flowers; pedicels at fruiting 3–9 mm long, bracteoles lanceolate, entire, reflexed. Calyx teeth lanceolate, hooked. Petals white. Stylopods conic, styles 2.3–2.5 mm long, reflexed. Mericarps (Fig. 2D–E) 11 mm long, 5 mm broad, ovate, slightly narrowed towards stylopods, compressed dorsally; ribs obtusely triangular to narrowly winged, marginal broader than dorsal ones; commissure broad (exocarp interrupted at commissural side near the bases of marginal ribs, composed by small cells with thickened outer walls), mesocarp parenchymatous. Vitae in furrows per 3–4, at commissural side 4–7, long or short; rib secretory ducts solitary. Endosperm ± deeply emarginate.

Notes. — The first known collection of this species (Davis & Polunin 24750) was referred to Diplotaenia cachrydifolia by Chamberlain (1972). A later collection...
from Bitlis, from the same place, made by the Turkish botanist Hasan Peşmen, is preserved in the Hacettepe herbarium in Ankara (HUB) and chosen here as the holotype of *D. turcica*. The study of this gathering has been possible due to courtesy of Dr Ali Dönmez who sent us also a photo of the plant.

Additional collection studied. – Turkey, B9 Bitlis, Karz Da, above Kemer, 2200 m, rocky slope of limestone ravine, 24.8.1954, *Davis & Polunin 24570* (E!).

**Distribution.** — Turkey (E Anatolia: Bitlis), Fig. 3.

### 4. *Diplotaenia hayri-dumanii* Pimenov & Kljuykov, sp. nov.

Holotype: “Turkey: C4 Konya, Hadim, Beyreli, N 36°51’93.8”, E 82°21’56.4”, 1545 m”, 15.7.1998, *Duman & Aytaç 6715* (GAZI!; Fig. 4B; isotype [“Turkey: C4, Konya: Hadim, Beyreli, 1545 m”, 15.7.1998, *Baser & al. 12660*] ESKI!).

A *Diplotaenia turcicae*, cui proxima est, lobis terminalis foliorum brevioribus (10–20, non 20–40 mm longis), radiis umbellis valde inaequilongis (non plus minusve aequalibus) differt. Species haec nonnullis characteribus magis vergit ad *D. cachrydifoliae* quam ad *D. turcicae*, quae in regionem prope jacentem viget.

**Plantae** perennes, polycarpicae, radicibus palaribus. *Caules* 1.5–1.7 m alti, solidi, basi ad 5 mm in diam., fere omnino glabri, tenuiter striati, in parte inferiore sectione rotundi, sub inflorescentia costati, ramis inferioribus alternis, ramis superioribus oppositis vel verticillaribus, umbellis centralibus multo superantibus. *Folia radicata* rosulata, folia exterius cito marcescentia, vaginis longis angustis, laminis parvis, folia centrales petiolis sectione fere rotundis, ad 30 cm longis, laminis ambitu ovatis, ad 40 cm longis, 4–5-pinnatisectis, segmentis longepetiolulatis, lobis terminalibus filiformibus, plus minusve acruatis, divaricatis, 1–2 cm longis, ad 0.5 mm in diam. *Folia caulina* superius valde simplificata, vaginis triangulatis, margine anguste albo-membranaceis, fere sine laminis. *Umbellae* centrales pedunculis obsoletis vel valde brevibus; *umbellae radis* 8–12, valde inaequilongis, 2–5 cm longis, sulculatis, scabridulis, *bracteis* 7–8, integris, lanceolatis, herbaceis, margine anguste albo-membranaceis, reflexis. *Umbellulae* 20–25-floraes, pedicellis sub anthesis 1–5 mm longis, *bracteis* 5–7, integris, anguste lanceolatis, margine brevissime scabridis, reflexis. *Dentes calycini* bene evoluti, lanceolati, uncinati. *Petala* alba, ad 2 mm longa, obovata, basi cuneata, apice emarginata, lobis inflexis brevibus, laminae adnatis, canaliculis secretoriis subinconspicuis. *Fructus* (non omnino maturi) elongati, ad 4–5 mm longi;
Polycarpic perennials with taproot. Stems 1.5–1.7 m high, c. 5 mm in diam. at the base, almost completely glabrous, finely striate, rounded at cross-section in lower part, ribbed under inflorescence, with alternate lower branches and verticillate or opposite upper branches, much overtopping the central umbels. Basal leaves rosulate, outer soon withering but not falling off, with long sheaths and small blades; central leaves with petioles almost rounded in cross-section, to 30 cm long, their blades ovate at outline, to 40 cm long, 4–5-pinnatisect; their segments with long petiolules, terminal lobes filiform, ± curved, divericate, 1–2 cm long, up to 0.5 mm in diam. Upper stem leaves very simplified, their sheaths triangular, narrowly white-membranous at the margin, almost without blades. Central umbels without peduncles or with very short peduncles, 8–12-rayed; rays very unequal, 2–5 cm long, finely furrowed, somewhat scabrous; bracts 7–8, lanceolate, entire, herbarceous, white-membranous at the margin, reflexed. Umbelliferae.

Fruit well developed, lanceolate, hooked. Petals white, up to 2 mm long, obovate, cuneate at the base, emarginate at the tip, with short lobe bent inwards, attached to petal blade, with secretory ducts almost inconspicuous. Fruit (not completely mature) elongate, up to 4–5 mm long; stylolods conic, styles up to 2.5 mm long, reflexed at mericarp dorsal side. Mericarps (Fig. 2F–H) 4–5 mm long, 2 mm broad, elongate, slightly compressed dorsally; ribs approximately equal, obtusely triangular; commissure broad (exocarp interrupted at commissural side near the bases of marginal ribs, composed by small cells with slightly thickened outer walls), mesocarp parenchymatous. Vittae in furrows per 2–3, at commissural side 4, under vascular bundles not constant, rib secretory ducts solitary, ± broad. Endosperm flat at the commissural side.

Notes. — After publication of the Diplotaenia treatment in “Flora of Turkey” (Chamberlain 1972), the genus was found in a region, again remote from the previously known distribution area, in Toros Dağları in S Anatolia: R. Ilarslan & H. Dural found the plant in 1993 in Antalya Vilayet near Gündoğmuş, and later, in 1998, the team of Turkish botanists K. H. C. Başer, Z. Aytaç, H. Duman, T. Ekim, A. Güner & T. Özbek collected it in Konya Vilayet (Beyreli area). The latter collection was determined by Prof. H. Duman as D. cachrydifolia, the former was misidentified by the collectors as “Ferula lycia Boiss.” Comparison of these two gatherings from S Anatolia showed that the collections from the Taurus Mts are referable to the same species, which differs from both true D. cachrydifolia and D. turcica. This new Diplotaenia species is named in honour of Prof. Hayri Duman, a leading Turkish expert in the Umbelliferae.

The specimens in GAZI and ESKI from C4 Konya, Beyreli, made on 15.7.1998, are parts of the same collection in spite of different collecting numbers and the incomplete correspondence of the collector team designations on the two sheets. Therefore, the sheet kept in ESKI is regarded as an isotype.

Additional collection studied. — Turkey: C4 Antalya, near Gündoğmuş, 1900–2200 m, 10.8.1993, Ilarslan & Dural 4070 (ANK!).

Distribution. — Turkey (Central Anatolia: Konya; S Anatolia: Antalya), Fig. 3.

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