

MORPHOMETRICS OF *DAUCUS* (APIACEAE): A COUNTERPART TO A PHYLOGENOMIC STUDY¹

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- *Premise of study:* Molecular phylogenetics of genome-scale data sets (phylogenomics) often produces phylogenetic trees with unprecedented resolution. A companion phylogenomics analysis of *Daucus* using 94 conserved nuclear orthologs supported many of the traditional species but showed unexpected results that require morphological analyses to help interpret them in a practical taxonomic context.
- *Methods:* We evaluated character state distributions, stepwise discriminant analyses, canonical variate analyses, and hierarchical cluster analyses from 40 morphological characters from 81 accessions of 14 taxa of *Daucus* and eight species in related genera in an experimental plot.
- *Key results:* Most characters showed tremendous variation with character state overlap across many taxa. Multivariate analyses separated the outgroup taxa easily from the *Daucus* ingroup. Concordant with molecular analyses, most species form phenetic groups, except the same taxa that are problematical in the molecular results: (1) the subspecies of *D. carota*, (2) *D. sahariensis* and *D. syrticus*, and (3) *D. broteri* and *D. guttatus*.
- *Conclusions:* Phenetic analyses, in combination with molecular data, support many *Daucus* species, but mostly by overlapping ranges of size and meristic variation. The subspecies of *D. carota* are poorly separated morphologically, are paraphyletic, and all could be recognized at the subspecies rank under *D. carota*. *Daucus sahariensis* and *D. syrticus* are so similar morphologically that they could be placed in synonymy. Combined molecular and morphological data support three species in accessions previously identified as *D. broteri* and *D. guttatus*. Molecular and morphological results support the new combination *Daucus carota* subsp. *capillifolius*.

Key words: Apiaceae; *Daucus*; germplasm; morphological phenetics; species boundaries; Umbelliferae.

Recent phylogenomic analyses are producing unprecedented phylogenetic resolution (e.g., Rokas et al., 2003; Burki et al., 2008; Christelová et al., 2011; Dos Reis et al., 2012; Egan et al., 2012). An analysis of 92 accessions of 13 species and two subspecies of *Daucus* and an additional 15 accessions of related genera [*Ammi* L., *Astrodaucus* Drude, *Caucalis* L., *Rouya* Coincy (incorrectly identified in our prior papers as *Margotia* Boiss.), *Oenanthe* L., *Orlaya* Hoffm., *Pseudorlaya* (Murb.) Murb., *Torilis* Adans., *Turgenia* Hoffm.] was examined with DNA sequences of 94 nuclear orthologs of average length of 1180 bp, with an aligned length of 111 166 bp (Arbizu et al., 2014). It provided 100% bootstrap support for most of the external and many of the internal clades, grouped different accessions of most of the species with strong support, but failed to support others such as (1) the subspecies of *D. carota* and *D. capillifolius*, (2) *D. sahariensis* and *D. syrticus*, and (3) *D. broteri* and

D. guttatus (Table 1 lists authors of taxa investigated here). Distinguishing characters of *D. broteri* and *D. guttatus* are unclear from the taxonomic literature, and they fell into three clades that were labeled as *D. guttatus* (the earliest name) 1, 2, and 3 (Arbizu et al., 2014) (Fig. 1). *Rouya polygama* Coincy ($2n = 20$) and *Pseudorlaya pumila* ($2n = 16$) were resolved as ingroups to *Daucus*, sister to a $2n = 18$ clade composed of *D. carota*, *D. capillifolius*, *D. sahariensis*, and *D. syrticus* (clade A' of Fig. 1), with the remaining species of *Daucus* being $2n = 20$, 22, and 44. Discordant or confusing phylogenetic results, no matter how strongly supported by molecular methods, are of little value without corroborative studies, here examined using morphological data.

The necessity of corroborative phenotyping studies in phylogenetics is analogous to its need in high-resolution linkage mapping and genome-wide association studies, where “phenomics” is emerging as a time-consuming and expensive constraint needed with next-generation DNA sequencing data (Cobb et al., 2013; Dhondt et al., 2013; Fiorani and Schurr, 2013). The purpose of the present study was to phenotype the accessions examined by Arbizu et al. (2014), to examine the support for species in *Daucus*, to provide a morphological counterpart to the phylogenomic results of Arbizu et al. (2014), and to place both sets of data in a practical taxonomic context. It is an extension of a similar study examining the taxonomic boundaries of the subspecies of *Daucus carota* and *D. capillifolius* (Spooner et al., 2014). That morphological study suggested the failure to provide molecular support for the subspecies of *D. carota* was partly a result of too many recognized subspecies and supported

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TABLE 1. Accessions examined in this study.

Taxon ^a	Tentative new identifications	Accession ^b	Location or source ^c
Ingroup			
<i>Daucus aureus</i> Desf.		PI 295854	Israel. Wadi Rubin (HaMerkaz).
<i>D. aureus</i>		PI 319403	Israel. Mediterranean Region.
<i>D. aureus</i>		PI 478858	France. Dijon.
<i>D. broteri</i> Ten.	<i>D. guttatus</i> 1	PI 652233	Iran. Mazandaran: Dhalus Road, Dasht-e Nazir, Kandalus.
<i>D. broteri</i>	<i>D. guttatus</i> 2	PI 652329	Greece. Peloponnese: 4 km from Skoura, toward Leonidion, Laconia Prefecture.
<i>D. broteri</i>	<i>D. guttatus</i> 1	PI 652340	Syria. Kassab.
<i>D. broteri</i>	<i>D. guttatus</i> 3	PI 652367	Turkey. Mugla.
<i>D. capillifolius</i> Gilli		PI 279764	Libya. Near Jefren.
<i>D. capillifolius</i>		Ames 30198	Tunisia. Medenine.
<i>D. capillifolius</i>		Ames 30202	Tunisia. Medenine.
<i>D. capillifolius</i>		Ames 30207	Tunisia. Medenine.
<i>D. carota</i> L. subsp. <i>carota</i>		Ames 25017	Germany. Saxony-Anhalt.
<i>D. carota</i> subsp. <i>carota</i>		Ames 26393	Portugal. Castelo Branco.
<i>D. carota</i> subsp. <i>carota</i>		Ames 26394	Portugal. Portalegre near Monforte.
<i>D. carota</i> subsp. <i>carota</i>		Ames 26401	Portugal. Portalegre near Monforte.
<i>D. carota</i> subsp. <i>carota</i>		Ames 27397	Uzbekistan. Between Yalangoch and Sobir Raximova.
<i>D. carota</i> subsp. <i>carota</i>		Ames 30250	Tunisia. Nabuel: along Route 28 at junction of road to Takelsa.
<i>D. carota</i> subsp. <i>carota</i>		Ames 30251	Tunisia. Nabuel: Route 26, between Takelsa and El Haouaria, 26 km from El Haouaria.
<i>D. carota</i> subsp. <i>carota</i>		Ames 30252	Tunisia. Nabuel: Sidi Daoud, 1 km from Route 27.
<i>D. carota</i> subsp. <i>carota</i>		Ames 30253	Tunisia. Nabuel: between El Haouarcae and Dor Allouche.
<i>D. carota</i> subsp. <i>carota</i>		Ames 30254	Tunisia. Nabuel: between El Haouarcae and Dor Allouche.
<i>D. carota</i> subsp. <i>carota</i>		Ames 30255	Tunisia. Nabuel: along road between Korba and Beni Khalled.
<i>D. carota</i> subsp. <i>carota</i>		Ames 30259	Tunisia. Bizerte: south side of Ischkeul.
<i>D. carota</i> subsp. <i>carota</i>		Ames 30260	Tunisia. Bizerte: along Route 51, west of Ghzab.
<i>D. carota</i> subsp. <i>carota</i>		Ames 30261	Tunisia. Bizerte: grounds of Direction Regionale Mogods, Khroumerie Sejnane.
<i>D. carota</i> subsp. <i>carota</i>		Ames 30262	Tunisia. Beja: road from Route 7, just west of Sejnane to Cap Negro.
<i>D. carota</i> subsp. <i>carota</i>		PI 274297	Pakistan. Northern Areas.
<i>D. carota</i> subsp. <i>carota</i>		PI 279762	Source: Denmark. Copenhagen.
<i>D. carota</i> subsp. <i>carota</i>		PI 279775	Source: Hungary. Pest. Botanical Garden.
<i>D. carota</i> subsp. <i>carota</i>		PI 279777	Source: Egypt. Giza: Orman Botanic Garden.
<i>D. carota</i> subsp. <i>carota</i>		PI 279788	Austria. Vienna.
<i>D. carota</i> subsp. <i>carota</i>		PI 279798	Spain. Madrid.
<i>D. carota</i> subsp. <i>carota</i>		PI 295862	Spain.
<i>D. carota</i> subsp. <i>carota</i>		PI 390887	Israel. Central Israel: from Bet Elazari.
<i>D. carota</i> subsp. <i>carota</i>		PI 421301	USA. Kansas: Elk County.
<i>D. carota</i> subsp. <i>carota</i>		PI 430525	Afghanistan. Zardek.
<i>D. carota</i> subsp. <i>carota</i>		PI 478369	China. Xinjiang: near Chou En Lai Monument Stone River, Sinkiang.
<i>D. carota</i> subsp. <i>carota</i>		PI 478873	Italy. Sardinia: St. Elia Beach, 50 m from sea, Cagliari.
<i>D. carota</i> subsp. <i>carota</i>		PI 478881	USA. Oregon: roadside between Echo and Pendleton.
<i>D. carota</i> subsp. <i>carota</i>		PI 478884	Source: The Netherlands, South Holland: Botanical Garden, Leiden.
<i>D. carota</i> subsp. <i>carota</i>		PI 502244	Portugal. Coimbra: Louisa.
<i>D. carota</i> subsp. <i>carota</i>		PI 652225	Source: France. Collection site unknown.
<i>D. carota</i> subsp. <i>carota</i>		PI 652226	Greece. N. Khalkidiki: 10 km N of Kassandra on coast road.
<i>D. carota</i> subsp. <i>carota</i>		PI 652229	Source: Tunisia.
<i>D. carota</i> subsp. <i>carota</i>		PI 652230	Albania. Lushnje.
<i>D. carota</i> subsp. <i>carota</i>		PI 652393	Turkey. Konya: 10–15 km to Seydisehir, between Yarpuz and Konya.
<i>D. carota</i> subsp. <i>gummifer</i> (Syme) Hook.f.		Ames 7674	Source: Italy. Tuscany: Botanic Garden.
<i>D. carota</i> subsp. <i>gummifer</i>		Ames 26381	Portugal. Faro: Near Portunao.
<i>D. carota</i> subsp. <i>gummifer</i>		Ames 26382	Portugal. Faro: Near Sagres.
<i>D. carota</i> subsp. <i>gummifer</i>		Ames 26383	Portugal. Faro: Near Aljezur.
<i>D. carota</i> subsp. <i>gummifer</i>		Ames 26384	Portugal. Beja.
<i>D. carota</i> subsp. <i>gummifer</i>		Ames 31193	France.
<i>D. carota</i> subsp. <i>gummifer</i>		PI 478883	France. Finistere: maritime turf, Le Conquet.
<i>D. carota</i> subsp. <i>gummifer</i>		PI 652411	France. Finistere: Pointe de Rospico, Navez.
<i>D. carota</i> subsp. <i>major</i> (Vis.) Arcang.	<i>D. guttatus</i> 1	Ames 25898	Turkey. Konya: Konya, toward Beysehir.
<i>D. carota</i>	<i>D. guttatus</i> 1	PI 286611	Source: Lebanon. Faculty of Agricultural Sciences.
<i>D. crinitus</i> Desf.		PI 652412	Portugal. Braganca: near Zava.
<i>D. crinitus</i>		PI 652413	Portugal. Guarda: near Barca de Alva.
<i>D. crinitus</i>		PI 652414	Portugal. Faro: near Bengado.

TABLE 1. Continued.

Taxon ^a	Tentative new identifications	Accession ^b	Location or source ^c
<i>D. glochidiatus</i> (Labill.) Fisch., C.A.Mey. & Avé-Lall.		PI 285038	Source: CSIRO, Australia. Capital Territory.
<i>D. guttatus</i> Sibth. and Sm.	<i>D. guttatus</i> 1	PI 279763	Source: Israel. Jerusalem Department of Botany.
<i>D. guttatus</i>	<i>D. guttatus</i> 2	PI 652331	Greece. Peloponnese: village of Loutra Agias Elenis, 17 km S of Korinthos, Korinthia Prefecture.
<i>D. guttatus</i>	<i>D. guttatus</i> 1	PI 652343	Syria. Halwah.
<i>D. guttatus</i>	<i>D. guttatus</i> 2	PI 652360	Turkey. Mugla: between Soke and Milas.
<i>D. involucratus</i> Sm.		PI 652332	Greece. Peloponnese: village of Loutra Agias Elenis, 17 km S of Korinthos, Korinthia Prefecture.
<i>D. involucratus</i>		PI 652350	Turkey. Izmir.
<i>D. involucratus</i>		PI 652355	Turkey. Izmir: 5 km N of Kusadasi.
<i>D. littoralis</i> Sm.		PI 295857	Israel. Beit Alpha.
<i>D. littoralis</i>		PI 341902	Israel.
<i>D. littoralis</i>	<i>D. guttatus</i> 3	PI 652375	Turkey. Mugla: between Dalaman-Gocik and Fethiye.
<i>D. muricatus</i> L.		Ames 25419	Portugal. Coimbra: Pitanca de Baixo-Condeixa.
<i>D. muricatus</i>		Ames 29090	Tunisia. South of Tunis along Hwy. 3 toward Zaghuan.
<i>D. muricatus</i>		PI 295863	Spain. Cordoba. From Villa del Rio (Cordoba).
<i>D. pusillus</i> Michx.		PI 349267	Uruguay. Montevideo. Near La Colorado Beach.
<i>D. sahariensis</i> Murb.		Ames 29096	Tunisia. between Tataouine and Bir Lahmer.
<i>D. sahariensis</i>		Ames 29097	Tunisia. between Tataouine and Remada.
<i>D. sahariensis</i>		Ames 29098	Tunisia. between Remada and Chenini.
<i>D. syrticus</i> Murb.	<i>D. sahariensis</i>	Ames 29107	Tunisia. near Beni Kdache to the south.
<i>D. syrticus</i>		Ames 29108	Tunisia. between Medenine and Matmatas.
<i>D. syrticus</i>		Ames 29109	Tunisia. between Medenine and Matmatas.
<i>D. syrticus</i>		Ames 29110	Tunisia. between Matmatas and El Hamma, near the Gabes airport.
<i>Rouya polygama</i> Coincy		Ames 30292	Tunisia. Jendouba: road to Tabarka, near Tabarka airport.
Outgroups			
<i>Ammi visnaga</i> (L.) Lam.		Ames 30185	Tunisia. Bizerte: National Park Ischkeul on road to Eco Museum.
<i>Astrodaucus littoralis</i> Drude		PI 277064	Source: Azerbaijan. Baku Botanical Garden.
<i>Caucalis platycarpus</i> L.		PI 649446	Germany. Saxony-Anhalt: Mannsdorf.
<i>Oenanthe virgata</i> Poir.		Ames 30293	Tunisia. Beja: Route 11, 41 km from Eudiana, 254 km from Beja.
<i>Orlaya daucooides</i> (L.) Greuter		PI 649477	Turkey. Aydin: Dilek Peninsula Reserve.
<i>Orlaya daucorlaya</i> Murb.		PI 649478	Greece. Epirus: 8 km from Aristi, toward Ioannina.
<i>Torilis leptophylla</i> Rchb.f.		Ames 25750	Syria. Salma.

^a These names correspond to those in the Germplasm Resources Information Network (GRIN) website (see methods section), except for the proposed new identifications of the subspecies of *D. carota* listed by Spooner et al. (2014).

^b Plant Introduction (PI) numbers are permanent numbers assigned to germplasm accessions in the National Plant Germplasm System (NPGS). Germplasm centers in the NPGS assign temporary site-specific numbers to newly acquired germplasm (Ames numbers for carrots and other Apiaceae maintained at the North Central Regional Plant Introduction Station in Ames, Iowa, USA) until an accession's passport data and taxonomy is verified, it is determined not to be a duplicate accession, and it has been determined the accession can be successfully maintained. These accessions may or may not be assigned a PI number after the assessment period.

^c Location refers to where the germplasm was collected in the wild, while source refers to germplasm acquired through another entity such as a market vendor or genebank.

only subsp. *carota* and subsp. *gummifer*, not the 9–12 subspecies of *D. carota* recognized by other authors.

Daucus is an economically important genus, but is in need of modern taxonomic and monographic studies. The genus includes about 20 recognized species mostly centered in the Mediterranean area in contrast to the widespread *Daucus carota* that occurs on almost every continent. The haploid chromosome number for *Daucus* ranges from $n = 9$ to $n = 11$. Most species are diploids with $2n = 18, 20,$ and 22 , but two polyploid species have been reported (Grzebelus et al., 2011). The latest taxonomic monograph of *Daucus* by Sáenz Laín (1981) lacks complete synonymies, distribution maps and phylogenetic data and cites few specimens. Practical identifications have relied more on floristic treatments such as those from Algeria (Quezel and Santa, 1963), Europe (Heywood, 1968), the Iberian Peninsula and Balearic Islands (Pujadas Salvà, 2003), Libya (Jafri and El-Gadi, 1985), Morocco (Jury, 2002; Faris and Ibn Tattou, 2007),

Tunisia (Le Flocc'h et al., 2010), Palestine (Zohary, 1972), Syria (Mouterde, 1986), and Turkey and the East Aegean Islands (Cullen, 1972). However, identifications in these taxonomic treatments frequently use different characters in their taxonomic keys and descriptions, have incomplete synonymies which preclude comparison of their taxonomic concepts, often have little information about geographic ranges, and lack distribution maps. In addition, there has been no single compilation of type specimens and many of the types lack the full range of plant parts necessary for unambiguous identification. In summary, there has been no accepted standard to quantify and describe the huge range of variation in *Daucus*, and identifications are often problematic.

The present study expands the morphological analysis of Spooner et al. (2014) to include all *Daucus* species available as germplasm and used the same accessions examined by Arbizu et al. (2014). In addition to phylogenetic insights needed for

crop improvement, these combined molecular and morphological analyses are needed to organize the world's germplasm collections of *Daucus*. The US collection of *Daucus* is maintained at the North Central Regional Plant Introduction Station (NCRPIS) in Ames, Iowa. This genebank conserves 1381 accessions of *Daucus*. Of these, 569 are classified as landraces, cultivars, cultivated populations, or breeding lines. Improvement status for the remaining accessions includes 571 wild, 17 uncertain, and 224 accessions with no status designated (though many of these most likely are cultivated). Taxonomically, there are 917 accessions identified as *D. carota*, with 247 of these identified as *D. carota* with a variety or subspecies designation (1164 *D. carota* total), leaving 217 accessions identified as other *Daucus* species.

MATERIALS AND METHODS

Study species—We examined 81 accessions of 14 taxa of *Daucus* (*D. aureus*, *D. broteri*, *D. capillifolius*, *D. carota* subsp. *carota*, *D. carota* subsp. *gummifer*, *D. crinitus*, *D. glochidiatus*, *D. guttatus*, *D. involucratus*, *D. littoralis*, *D. muricatus*, *D. pusillus*, *D. sahariensis*, *D. syrticus*), seven species in other genera in the Apiaceae supported as outgroups (*Ammi visnaga*, *Astrodaucus littoralis*, *Caucalis platycarpus*, *Oenanthe virgata*, *Orlaya daucoides*, *Orlaya daucorlaya*, *Torilis leptophylla*), and one species in another genus supported as an ingroup (*Rouya polygama*; Spalik and Downie, 2007; Spooner et al., 2013) (Table 1). We did not examine *Pseudorlaya pumila*, another ingroup species, because it died in the field plot, and *D. tenuisectus* because it was acquired too late for planting. On the basis of the morphological analysis of Spooner et al. (2014), we labeled all accessions of *D. carota* as either subsp. *carota* or subsp. *gummifer*, not as their current listing in the Germplasm Resources Information Network (GRIN; <http://www.ars-grin.gov/>) as subsp. *carota*, subsp. *commutatus* (Paol.) Thell., subsp. *drepanensis* (Arcang.) Heywood, subsp. *fontanesii* Thell., subsp. *gummifer* (Syme) Hook.f., subsp. *hispanicus* (Gouan) Thell., subsp. *major* (Vis.) Arcang., subsp. *maritimus* (Lam.) Batt., and subsp. *maximus* (Desf.) Ball. This classification of *D. carota* into two subspecies is similar to that of Onno (1937), who classified the “subsp. *gummifer*” taxon as *D. gingidium* L., and the “subsp. *carota*” taxon as *D. carota*. It is also similar to the classifications of Small (1978) and Reduron (2007), who recognized two “species aggregates”, or “subgroups,” within the single species *D. carota*. However, these authors recognized more subspecies than our two. On the basis of Arbizu et al. (2014), we labeled accessions formerly identified as *D. broteri* or *D. guttatus* as *D. guttatus* 1, 2, or 3.

***Daucus* observation plots**—To ensure sufficient plant populations in the observation plot, biennial and mixed life-cycle accessions were planted in the greenhouse in early November 2012. Seedlings were thinned to one per pot, and plants were fertilized weekly with a commercial liquid fertilizer (NPK 20–10–20). Roots were vernalized in the dark (4–5°C, 50–70% relative humidity) for approximately 60 d beginning in February 2013. A fungicide spray (Rubigan, DuPont, Wilmington, Delaware, USA) was applied at the beginning of vernalization and reapplied as necessary to prevent Botrytis blight. Roots were moved outside to a protected area in mid-April to allow them to develop new foliage. Annual accessions were planted in the greenhouse in late February 2013 and maintained using the same protocols as with the biennials without vernalization. Twenty plants per accession were transplanted into 6-m rows, one row per accession in each of two field plots in late April. Harsh weather conditions (excessive rain, snow, and cold temperatures) following transplanting damaged or killed many of the annual accessions. As a result, additional seeds of the affected accessions were direct seeded by hand into a parallel furrow 30 cm from the transplanted row in late May. Field plots were maintained with small plot tillers and hand weeding.

Characters recorded—Forty characters were recorded from at least three individuals per accession (Table 2), and character sets were always recorded by the same individual. These characters were chosen to represent all those used in prior morphological analyses (Small, 1978; Spooner et al., 2014), the latest comprehensive monograph of *Daucus* (Sáenz Laín, 1981), and regional floras outlined in the introduction. Size characters were recorded in the field with a ruler or calipers, and floral and fruit characters were recorded in the laboratory

with the aid of a dissecting microscope. As part of normal genebank operations at the NCRPIS, electronic images of leaves were generated on a flatbed scanner; images of various plant parts were made from plants in the field with a digital camera; and images will be available on the GRIN website (<http://www.ars-grin.gov/>). These serve as useful resources for others to conveniently check the morphology of our accessions and as supplements to the voucher specimens. Herbarium vouchers collected for this morphological study are a subset of the same accessions from Arbizu et al. (2014) but different specimens (Table 1) and are deposited at the herbarium of the Potato Introduction Station, Sturgeon Bay, Wisconsin, USA.

Analytical methods—Thirty-eight of the 40 characters were scored and analyzed as continuous variables; the remaining two were treated as nominal variables (Table 2). All analyses were conducted in JMP software version 10.0.0 (SAS Institute, Cary, North Carolina, USA). To examine character state distributions, we analyzed the accessions with the box plot or histogram functions of Graph Builder in JMP (Appendix S1; see Supplemental Data with the online version of this article).

For multivariate analyses, means were assessed for the continuous variables. We first performed stepwise discriminant analyses (linear, common covariance) using all 38 continuous variables to obtain a model whose variables were significant in identifying accession composition with characters removed one at a time until the model *F* test *P* value was ≤ 0.05 . We then performed canonical variate analysis (CVA) and hierarchical cluster analyses (HCA) of (1) all taxa; (2) *Daucus* ingroup (all *Daucus* and *Rouya polygama*); (3) *D. capillifolius* and *D. carota*; (4) clade B species (see Fig. 1 that shows the species in different clades) *D. glochidiatus*, *D. guttatus* (subsets 1, 2, 3), *D. involucratus*, *D. littoralis*, *D. pusillus*; (5) *D. guttatus* (subsets 1, 2, 3); and (6) *D. sahariensis* and *D. syrticus*. These analyses use only the characters identified by stepwise discriminant analyses as significant in the *F* test, $P \leq 0.05$. The HCA uses standardized data and average similarity.

RESULTS

Character state distributions—Graphical analyses of all 40 character state distributions are shown in Appendix S1. Stepwise discriminant analyses of the 38 characters coded as continuously variable showed all but five of them (stipule width, foliage color, bract length, length of longest peripheral ray, barbs at tips of spines) to be significant in the *F* test, $P \leq 0.05$, in at least one of the six analyses of different groups of species (Table 2). Of the two nominal characters (petal color, anther color), yellow petals are unique to *D. capillifolius*, and red anthers are unique to *Oenanthe virgata*. Some characters showed little variation and little to no overlap or ranges of character states with many other characters, but most characters showed tremendous variation within some taxa and overlap of ranges across taxa. Examination of additional accessions, especially for those species with only few available accessions (e.g., *D. glochidiatus*, *D. pusillus*, *Rouya polygama*) will be needed to make more definitive conclusions of character state variation. Figure 2 illustrates one of these 40 characters, plant height, showing cases of both narrow and wide ranges of overlap among characters. Despite character overlap, many characters were useful to distinguish taxa, but often only in combination with others.

Multivariate analyses—We employed both CVA and HCA to analyze our data because both distinguish taxa using different methods and assumptions and both are useful to visualize results and infer group membership, here inferred to be potentially valid taxa. The CVA is an ordination method that uses assigned groups to derive a linear combination of the variables (morphological characters) that produces the greatest separation of the groups. The HCA, in contrast, makes no assumptions about group membership; it produces trees based on average

TABLE 2. The 40 morphological characters recorded in this study, modeling type, and *F*-test *P* values of characters retained in a stepwise discriminant analysis for (1) all taxa; (2) *Daucus* ingroup (all *Daucus* and *Rouya polygama*); (3) *D. capillifolius* and *D. carota*; (4) *D. glochidiatus*, *D. guttatus* (subsets 1, 2, 3), *D. involucratus*, *D. littoralis*, *D. pusillus*; (5) *D. guttatus* subsets 1, 2, 3; (6) *D. sahariensis* and *D. syrticus*.

Character ^a	Model type ^b	<i>P</i> ¹	<i>P</i> ²	<i>P</i> ³	<i>P</i> ⁴	<i>P</i> ⁵	<i>P</i> ⁶
Plant							
Plant height (cm)	C	0.0001	0.0001	0.0180			
Stem diameter (mm)	C		0.0022				0.0007
Leaf							
Leaf length (cm)	C	0.0001	0.0008				
Leaf width (cm)	C	0.0053	0.0086				
Stipule width (mm)	C						
Petiole length (cm)	C	0.0008	0.0033				
Petiole diameter (mm)	C	0.0003					
Petiole shape (round, 1; semiround, 2; flat, 3)	C	0.0037	0.0028				
Leaf type (celery, 1; normal, 2; parsley, 3; other, 4)	C	0.0240	0.0324				
Leaf and petiole pubescence (smooth, 1; intermediate, 2; very hairy, 3)	C	0.0001	0.0001				
Foliage color (light green, 1; medium green, 2; gray green, 3; dark green, 4)	C						
Flower							
Peduncle pubescence (glabrous, 1; soft hairs, 2; scabrous, 3; very scabrous, 4)	C	0.0353		0.0001		0.0067	
Primary umbel shape, full bloom (convex, 1; flat, 2; concave, 3)	C	0.0009	0.0005				
Primary umbel shape, mature seed (convex, 1; flat, 2; concave, 3)	C	0.0001		0.0010			
Primary umbel height (cm)	C			0.0142			
Primary umbel diameter (cm)	C	0.0001	0.0028				0.0004
Secondary umbel diameter (cm)	C	0.0001	0.0083				
Bract length (mm)	C						
Bract width (mm)	C		0.0204				
Involucral bract posture (deflexed, not deflexed [outward or upward])	C	0.0001	0.0001				
Number of bract lobe points	C	0.0001	0.0001	0.0112	0.0001		0.0001
Number of bract lobe pairs	C	0.0001	0.0001	0.0020	0.0005	0.0001	
Number of umbel rays	C	0.0005			0.0370		
Pigmented central umbel (concolorous to outer [uniform color], 1; differently pigmented, 2)	C	0.0003	0.0100	0.0002			
Length of longest peripheral ray (cm)							
Length of shortest peripheral ray (cm)		0.0001	0.0392				
Petal color (white, cream, yellow [only <i>D. capillifolius</i>], pink)	N						
Anther color (white, cream, yellow, pink, purple, brown)	N						
Peripheral petal length (mm)	C	0.0001			0.0021	0.0005	
Central petal length (mm)	C						0.0001
Stamen length (mm)	C	0.0001	0.0001				0.0062
Style length (mm)		0.0001	0.0001				
Stylopodium length (mm)		0.0015	0.0213				
Stylopodium width (mm)		0.0001	0.0001				
Seed							
Seed length (mm)	C	0.0001	0.0023	0.0001			
Seed width (mm)	C	0.0001		0.0070			
Confluency of seed spines (separate, 1; little confluency, 2; much confluency, 3)	C				0.0030		
Barbs at tips of spines of secondary seed ribs (3, 2 barbs; 7, 4–8)	C						
Number spines on the secondary seed ribs	C	0.0001	0.0001				
Length of secondary seed spines (mm)	C	0.0001	0.0001				

^a Additional details on these descriptors can be found at the USDA, ARS, National Genetic Resources Program, Germplasm Resources Information Network (GRIN) website [online database], National Germplasm Resources Laboratory, Beltsville, Maryland, <http://www.ars-grin.gov/cgi-bin/npgs/html/crop.pl?70> [accessed 10 October 2014].

^b N, nominal; C, continuous.

similarity of all data. Because the clustering results differ with different sets of accessions, we performed both analyses (and stepwise discriminant analyses) with six different groups of putatively related taxa. We present the *F*-test *P* values of characters retained in a stepwise discriminant analysis of all six analyses in Table 2, and all 12 CVA and HCA in Figs. 3–6 or Appendix S2 (see online Supplemental Data). For space considerations, we here present only the CVA and HCA results of analysis 2 (*Daucus* ingroup) and analyses 4 (clade B species:

D. glochidiatus, *D. guttatus* [subsets 1, 2, 3], *D. involucratus*, *D. littoralis*, *D. pusillus*).

All taxa—The sole purpose of this analysis was to see how well the *Daucus* outgroups (i.e., all non-*Daucus* species except *Rouya polygama*) were separated from the ingroup. Stepwise discriminant analyses identified 28 of the 38 continuous characters as significant discriminators within all taxa at the *F*-test *P* value ≤ 0.05 (Table 2). The HCA separated all outgroups

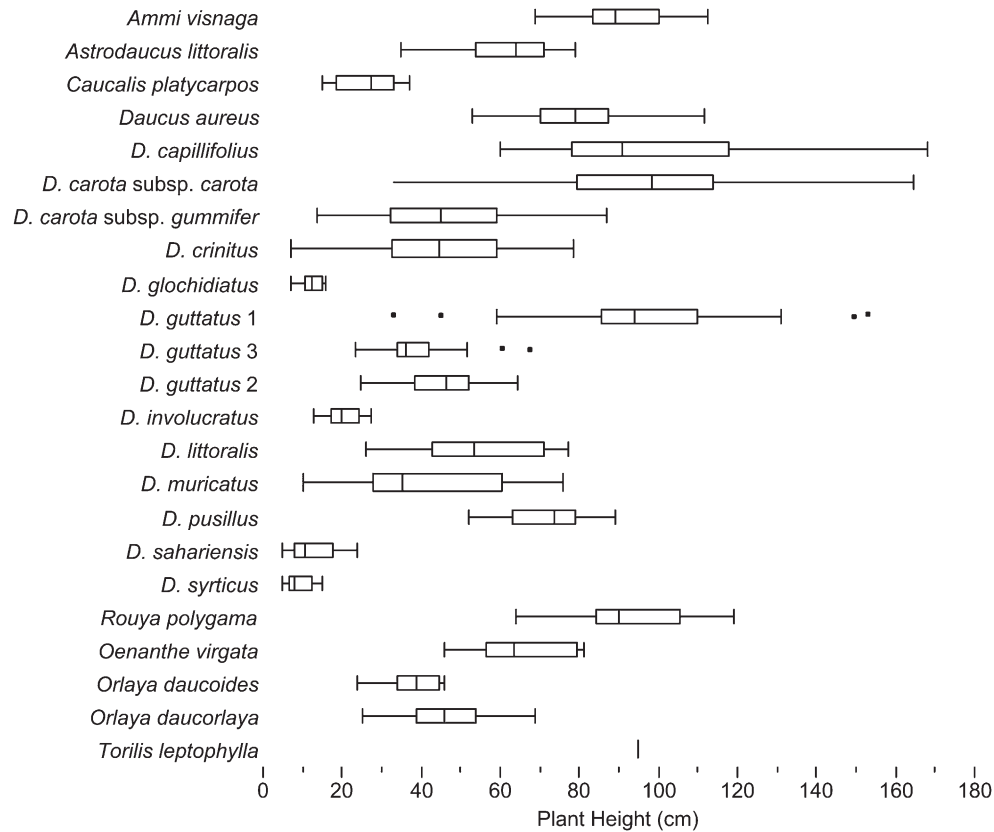


Fig. 2. Box plot of plant height for all taxa examined in this study showing individual plant values for median, 25% and 75% quantiles, range, and outliers.

except *Orlaya daucooides*, which grouped with *D. muricatus*. The CVA analysis, however, separated *Orlaya daucooides* from all *Daucus* species well, close to *Oenanthe virgata* and *Rouya polygama* (Appendix S2). Within the ingroup, *Rouya polygama* is distinct from other *Daucus* ingroups by both analyses.

Daucus ingroup (all *Daucus* and *Rouya polygama*)—Deletion of the outgroups significantly changed the phenetic structure of the ingroup in both the HCA (Fig. 3) and CVA (Fig. 4). *Rouya polygama* and *D. pusillus* appear far from others in the HCA, while *Rouya polygama* and *D. muricatus* are phenetically most separate in the CVA (Fig. 4). Many ingroup taxa cluster in these analyses, concordant to their grouping in the multiple nuclear orthology phylogeny (*D. aureus*, *D. crinitus*, *D. involucratus*, *D. littoralis*, *D. muricatus*). As in the phenetic analysis (Spooner et al., 2014), *D. carota* subsp. *carota* and *D. carota* subsp. *gummifer*, are difficult to separate in the HCA and CVA, *D. capillifolius* is similar in the CVA, and all the accessions of *D. capillifolius* cluster together in the HVA. *Daucus guttatus* 2 and 3 cluster separately in the CVA, but none of the three forms do so consistently in HCA. *Daucus sahariensis* and *D. syrticus* form their own clusters near each other in CVA, but fail to form species-specific clusters in the HCA.

Daucus capillifolius, *D. carota* subsp. *carota*, and *D. carota* subsp. *gummifer*—The HCA and CVA analyses of *D. capillifolius*, *D. carota* subsp. *carota*, and *D. carota* subsp. *gummifer*

(Appendix S2) cluster well with the elimination of other taxa except for one accession of subsp. *gummifer* (Ames 26381) that clusters near *D. carota* subsp. *carota*. Although not used in these analyses, the yellow petal trait easily separates *D. capillifolius* from *D. carota*, and when used in combination with the longer seeds of *D. capillifolius* (Table 2, Appendix S2), these two species are easily distinguished.

Clade B species—The HCA clusters *D. guttatus* 2, *D. guttatus* 3, *D. littoralis*, and *D. pusillus*. *Daucus glochidiatus* and *D. involucratus* cluster together, and *D. guttatus* 1 clusters with *D. guttatus* 2 and *D. guttatus* 3 (Fig. 5). The CVA clusters all species separately, with *D. muricatus* being the phenetically most distinctive species (Fig. 4).

Daucus guttatus subsets 1, 2, and 3—All three groups of *D. guttatus* are distinguished with the elimination of all other species (Appendix S2). They are best distinguished by number of bract lobe pairs (highest number in *D. guttatus* 3), peduncle pubescence (harshes in *D. guttatus* 1) and peripheral petal length (longest in *D. guttatus* 1) (Table 2, Appendix S1).

Daucus sahariensis and *D. syrticus*—*Daucus sahariensis* and *D. syrticus* do not cluster in the HCA when analyzed separately, but do so in the CVA. The best characters separating these two species are stem diameter, primary umbel diameter, number of bract lobe points, central petal length, and stamen length (Table 2). However, all of these characters overlap considerably in range (Appendix S1).

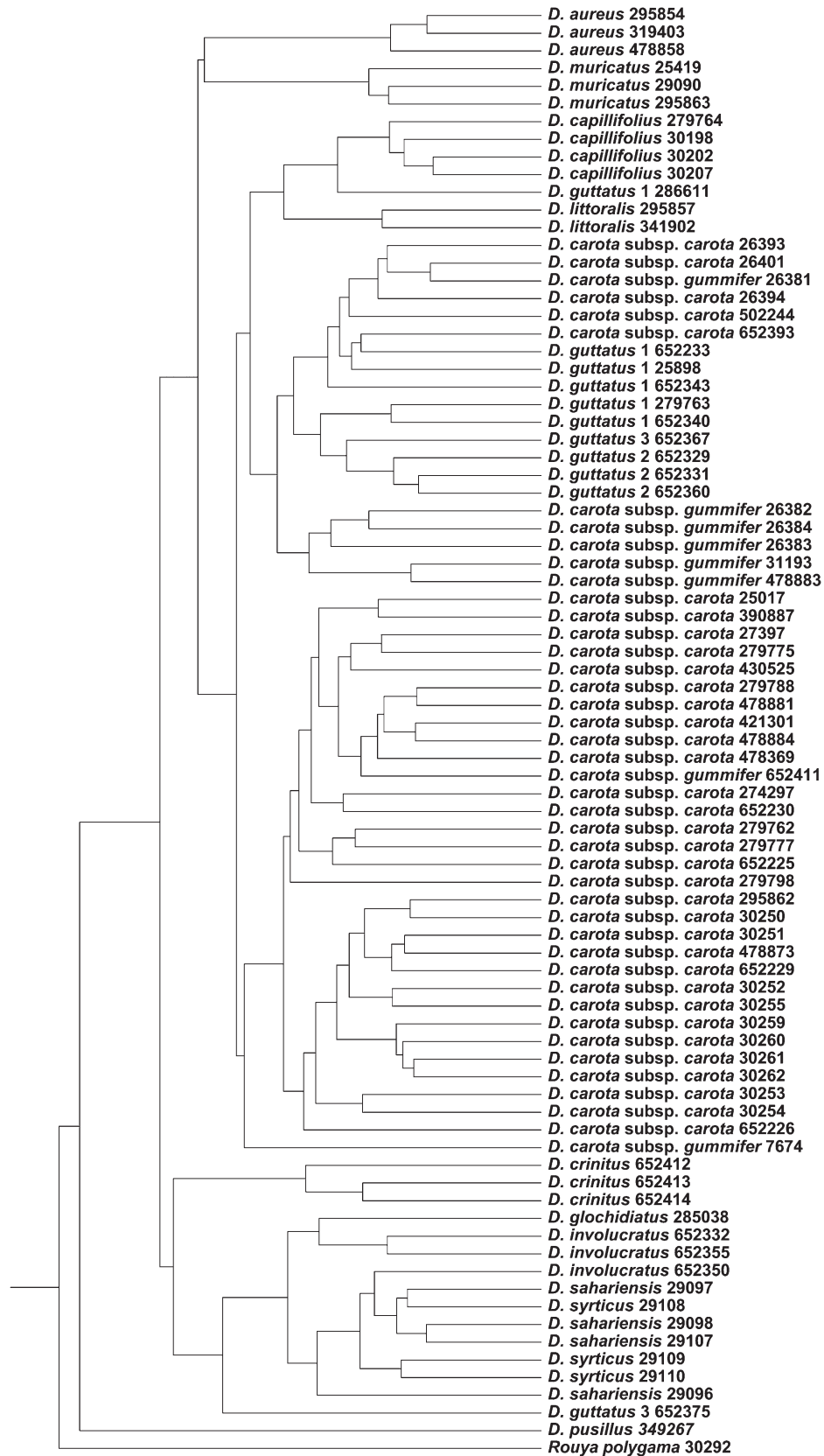


Fig. 3. Hierarchical cluster analyses of the *Daucus* ingroup including *Rouya polygama*.

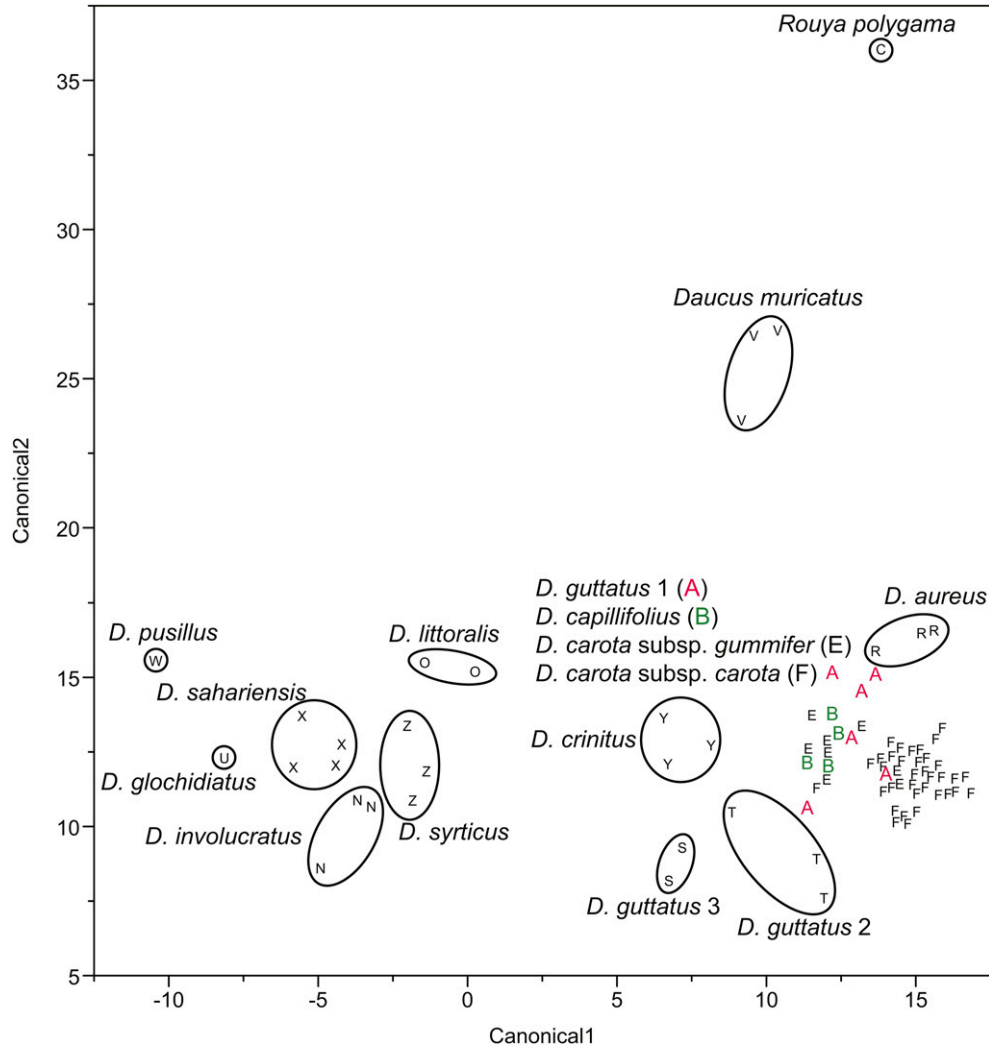


Fig. 4. Canonical variate analysis of the *Daucus* ingroup including *Rouya polygama*; (A) *D. guttatus* 1 and (B) *D. capillifolius* are highlighted in larger colored type solely to distinguish them from their intermingled *D. carota* subspecies.

DISCUSSION

Daucus taxonomy traditionally has been difficult, as we infer from our continuing challenges with identifications at the NCRPIS and from different and often overlapping sets of character states provided in regional floras for the same species. Our raw data confirm such a pattern of overlapping character states, traditionally used as species identifiers (Fig. 2, Appendix S1). Similarly, our multivariate analyses (Figs. 3–6; Appendix S2) show difficulty in distinguishing some taxa. Comparison of the multiple nuclear ortholog study of Arbizu et al. (2014; Fig. 1 of this paper) and the analyses presented here shows both data sets are concordant regarding problems with distinguishing the subspecies of *D. carota*, *D. sahariensis* and *D. syrticus*, and *D. broteri* and *D. guttatus*, here considered as three putatively different taxa and labeled as *D. guttatus* groups 1, 2, and 3. While some characters showed little variation and little to no overlap with many other characters, most characters showed tremendous variation within some taxa and overlap of ranges across taxa, demonstrating that most *Daucus* species are distinguished by size and meristic variation, not the possession of unique traits (Appendix S1).

Arbizu et al. (2014) confirmed that *Rouya polygama* and *Pseudorlaya pumila* are part of the *Daucus* clade, and our results show that *Rouya* (*Pseudorlaya* not examined here) was indeed distinct and reasonably excluded from *Daucus* on morphological criteria. Weitzel et al. (2014) recently showed, with ITS data, that *Rouya polygama* (misidentified as *Margotia gummifera* in the studies of Spooner et al. [2013] and Arbizu et al. [2014]) was a *Daucus* ingroup. They made the transfer of this species to *Thapsia gummifera* (Desf.) Spring. Problems in such paraphyletic genera in the Apiaceae are common. The Apiaceae comprise some 300–455 genera and 3000–3750 species (Constance, 1971; Pimenov and Leonov, 1993). Many generic boundaries within the Apiaceae are unnatural, as documented by molecular investigations based on DNA sequences from nuclear ribosomal internal transcribed spacers, plastid *rpoC1* intron and *rpl16* intron sequences, plastid *matK* coding sequences, plastid DNA restriction-site data, and DNA sequences from nuclear orthologs (Plunkett et al., 1996; Downie et al., 2000; Lee and Downie, 2000; Spalik and Downie, 2007; Spooner et al., 2013). Generic boundaries are particularly difficult in *Daucus*, as molecular data from the above studies place species from the genera *Agrocharis*,

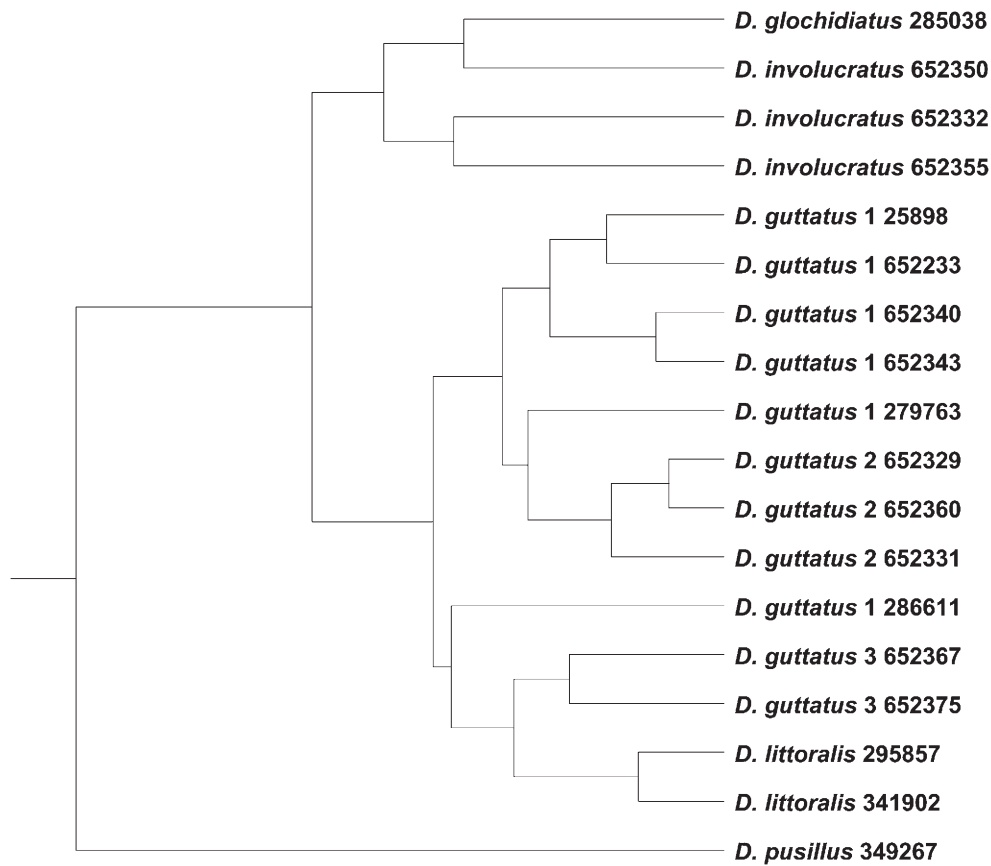


Fig. 5. Hierarchical cluster analyses of clade B species *Daucus glochidiatus*, *D. guttatus* (subsets 1, 2, 3), *D. involucratus*, *D. littoralis*, *D. pusillus*.

Athamanta, *Cryptotaenia*, *Melanoselinum*, *Monizia*, *Pachyctenium*, *Pseudorlaya*, *Rouya*, and *Tornabenea* within a monophyletic *Daucus* clade. However, we do not yet have access to living collections of many of these non-*Daucus* genera, which are necessary for our nuclear ortholog studies.

Taxonomic concepts—While the identification of most *Daucus* species relies on a variety of traits with overlapping ranges of values (polythetic support), in contrast to possessing unique traits (Sokal and Sneath, 1963), many species are supported by molecular data and can be distinguished by morphology (e.g., *D. aureus*, *D. capillifolius*, *D. crinitus*, *D. glochidiatus*, *D. involucratus*, *D. littoralis*, *D. muricatus*, *D. pusillus*, *Rouya polygama*). However, for some species, the taxonomy of *Daucus* remains complicated by the lack of sufficient germplasm for definitive morphological and molecular analyses, lack of comprehensive herbarium studies to associate names to type specimens, unsettled generic affiliations, and undefined species boundaries. Our combined molecular and morphological studies indicate particular problems in the subspecies of *D. carota*, species distinctions of *D. sahariensis* and *D. syrticus* and the subgroups of *D. broteri*/*D. guttatus*.

We are pursuing these remaining problems with additional fieldwork; collaborations with other *Daucus* investigators to share germplasm, DNA, and herbarium data; and additional focused morphological and molecular studies. Arbizu et al. (2014) identified a subset of nuclear orthologs that give a topology nearly identical to the use of 94 nuclear orthologs, and we are

examining additional collections of *D. guttatus* 1, 2, and 3 with these markers, in concert with an expanded morphological study. Iorizzo et al. (2013) demonstrated the utility of single nucleotide polymorphism (SNP) data to distinguish *D. carota* subsp. *carota* (wild) from *D. carota* subsp. *sativus* (cultivated), and even major cultivar groups of the latter (eastern vs. western carrot). We are extending these analyses by generating single nucleotide polymorphisms (SNPs) via genotyping by sequencing to examine the subspecies of *D. carota* that cannot be distinguished by multiple nuclear orthologs (Arbizu et al., 2014). Longer term, we plan morphological analyses of these subspecies in a field trial in a maritime environment, where *D. carota* subsp. *gummifer* (sensu lato) grows, as an extension and comparison to the study by Spooner et al. (2014), using additional collections of these subspecies. Until we have access to these data, and because of our desire to make taxonomic decisions that are stable, we are reluctant to make comprehensive taxonomic changes now, following our desire to follow a phylogenetic species concept.

One taxonomic relationship is well supported, however. *Daucus capillifolius* is morphologically distinct and diagnosable (Spooner et al., 2014) (Appendix S2), yet nested in a clade of *D. carota* (Arbizu et al., 2014; Iorizzo et al., 2013) (Fig. 1). It shares the same number of chromosomes as all subspecies of *D. carota* ($2n = 18$) and is fully intercussable with the other subspecies (McCollum, 1975, 1977), supporting its inclusion within *D. carota*. It has its own range, confined to western Libya and adjacent northeastern Tunisia. Coauthors P. W. Simon and D. M. Spooner collected *D. capillifolius* throughout most of its range in

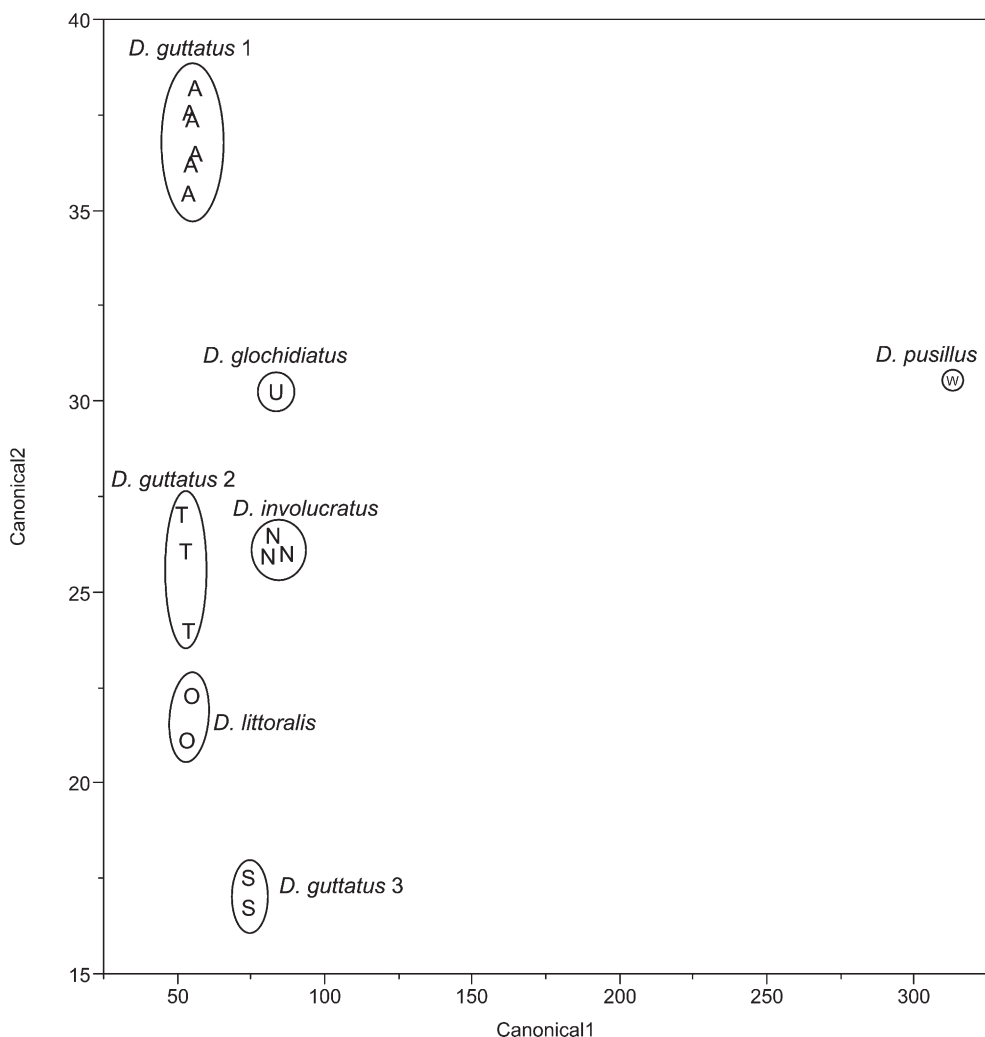


Fig. 6. Canonical variates analysis of clade B species *Daucus glochidiatus*, *D. guttatus* (subsets 1, 2, 3), *D. involucratus*, *D. littoralis*, *D. pusillus*.

northern Tunisia and have good knowledge of its variation. We here recognize it as a subspecies of *D. carota*.

Daucus carota* subsp. *capillifolius (A. Gilli) C. Arbizu, comb. et stat. nov. *Daucus capillifolius* A. Gilli, Oesterreichische Botanische Zeitschrift 104: 574. 1958.

TYPE: Libya, Tripolitania, hills northwest of Jefren (Yaf-ran), 24 Jul 1956, in sandy soil with *Foeniculum vulgare*, *Echinops spinosus*, and *Aristida pungens*, Elfrid Gerhart, s.n. (holotype: W).

LITERATURE CITED

- ARBIZU, C., H. RUESS, D. SENALIK, P. W. SIMON, AND D. M. SPOONER. 2014. Phylogenomics of the carrot genus (*Daucus*, Apiaceae). *American Journal of Botany* 101: 1666–1685.
- BURKI, F., K. SHALCHIAN-TABRIZI, AND J. PAWLOWSKI. 2008. Phylogenomics reveals a new ‘megagroup’ including most photosynthetic eukaryotes. *Biology Letters* 4: 366–369.
- CHRISTELOVÁ, P., M. VALÁRIK, E. HRIBOVÁ, E. DE LANGHE, AND J. DOLEŽEL. 2011. A multi gene sequence-based phylogeny of the Musaceae (banana) family. *BMC Evolutionary Biology* 11: 103.
- COBB, J. N., G. DECLERCK, A. GREENBERG, R. CLARK, AND S. MCCOUCH. 2013. Next-generation phenotyping: Requirements and strategies for enhancing our understanding of genotype–phenotype relationships and its relevance to crop improvement. *Theoretical and Applied Genetics* 126: 867–887.
- CONSTANCE, L. 1971. History of the classification of Umbelliferae (Apiaceae). In V. H. Heywood [ed.], *The biology and chemistry of the Umbelliferae*, 1–8, Academic Press, New York, New York, USA.
- CULLEN, J. 1972. *Daucus*. In P. H. Davis [ed.], *Flora of Turkey and the East Aegean Islands*, vol. 4, Flora of Turkey, 531–536. Edinburgh University Press, Edinburgh, UK.
- DHONDT, S., N. WUYTS, AND D. INZÉ. 2013. Cell to whole-plant phenotyping: The best is yet to come. *Trends in Plant Science* 18: 428–439.
- DOS REIS, M., J. INOUE, M. HASEGAWA, R. J. ASHER, P. C. DONOGHUE, AND Z. YANG. 2012. Phylogenomic datasets provide both precision and accuracy in estimating the timescale of placental mammal phylogeny. *Proceedings of the Royal Society, B, Biological Sciences* 279: 3491–3500.
- DOWNIE, S. R., D. S. KATZ-DOWNIE, AND M. F. WATSON. 2000. A phylogeny of the flowering plant family Apiaceae based on chloroplast DNA *rpl16* and *rpoc1* intron sequences: Towards a suprageneric classification of subfamily Apioideae. *American Journal of Botany* 87: 273–292.
- EGAN, A., J. SCHLUETER, AND D. M. SPOONER. 2012. Applications of next-generation sequencing in plant biology. *American Journal of Botany* 99: 175–185.

- FARIS, F. E., AND M. IBN TATTOU. 2007. Umbelliferae. In M. Fennane, M. Ibn Tattou, A. Ouyahya, and J. El Oualidi [eds.], *Flore Pratique du Maroc*, vol. 2, Angiospermae (Leguminosae–Lentibuliaceae), Série Botanique no. 38, 281–336. L'Institut Scientifique, Rabat, Morocco.
- FIORANI, F., AND U. SCHURR. 2013. Future scenarios for plant phenotyping. *Annual Review of Plant Biology* 64: 267–291.
- GRZEBELUS, D., R. BARANSKI, K. SPALIK, C. ALLENDER, AND P. W. SIMON. 2011. *Daucus*. In C. Kole [ed.], *Wild crop relatives: Genomic and breeding resources*, vol. 5, Vegetables, 129–216. Springer-Verlag, Berlin, Germany.
- HEYWOOD, V. H. 1968. *Daucus*. *Feddes Repertorium* 79: 1–68.
- IORIZZO, M., D. SENALIK, S. ELLISON, D. GRZEBELUS, P. CAVAGNARO, C. ALLENDER, J. BRUNET, D. M. SPOONER, A. VAN DEYNZE, AND P. W. SIMON. 2013. Genetic structure and domestication of carrot (*Daucus carota* subsp. *sativus* L.) (Apiaceae). *American Journal of Botany* 100: 930–938.
- JAFRI, S. M. H., AND A. EL-GADI. 1985. *Daucus*. In S. M. H. Jafri and A. El-Gadi [eds.], *Flora of Libya*, vol. 117, Apiaceae, 130–144, Al Faatth University, Faculty of Science, Department of Botany, Tripoli, Libya.
- JURY, J. L. 2002. *Daucus*. In B. Valdés, M. Rejdali, A. Achhal El Kadmiri, J. L. Jury, and J. M. Montserrat [eds.], *Catalogue des plantes vasculaires du Nord de Maroc, incluant des clés d'identification*, vol. 1, 467–469. Consejo Superior de Investigaciones Científicas, Madrid, Spain.
- LEE, B. Y., AND S. R. DOWNIE. 2000. Phylogenetic analysis of cpDNA restriction sites and *rps16* intron sequences reveals relationships among Apiaceae tribes Caucalideae, Scandiceae and related taxa. *Plant Systematics and Evolution* 221: 35–60.
- LE FLOC'H, É., L. BOULOS, AND E. VÉLA. 2010. *Catalogue synonymique commenté de la flore de Tunisie*. Ministère de l'Environnement et Développement Durable Banque Nationale de Gènes, Tunis, Tunisia.
- MCCOLLUM, G. D. 1975. Interspecific hybrid *Daucus carota* × *D. capillifolius*. *Botanical Gazette* 136: 201–206.
- MCCOLLUM, G. D. 1977. Hybrids of *Daucus gingidium* with cultivated carrots (*D. carota* subsp. *sativus*) and *D. capillifolius*. *Botanical Gazette* 138: 56–63.
- MOUSTERDE, P. 1986. *Nouvelle flore du Liban et de la Syrie*, part 2. Dar El-Machreq Editeurs, Beirut, Lebanon.
- ONNO, M. 1937. Die wildformen von *Daucus* sect. *Carota*. *Beihefte zum Botanischen Centralblatt* 56(B): 83–136.
- PIMENOV, M. G., AND M. V. LEONOV. 1993. The genera of Umbelliferae: A nomenclator. Royal Botanic Gardens, Kew, UK.
- PLUNKETT, G. M., D. E. SOLTIS, AND P. S. SOLTIS. 1996. Evolutionary patterns in Apiaceae: Inferences based on *matK* sequence data. *Systematic Botany* 21: 477–495.
- PUJADAS SALVÀ, A. J. 2003. *Daucus*. In G. Nieto Feliner, S. L. Jury, and A. Herrero [eds.], *Flora iberica: Plantas vasculares de la península Ibérica e islas Baleares*, vol. 10, Alaliaceae-Umbelliferae, 97–125. Real Jardín Botánico, CSIC, Madrid, Spain.
- QUEZEL, P., AND S. SANTA. 1963. *Daucus*. In P. Quezel and S. Santa [eds.], *Nouvelle flore de L'Algérie et régions désertiques méridionales*, 659–663. Éditions du Centre National de la Recherche Scientifique, Montpellier, France.
- REDURON, J. P. 2007. *Ombellifères de France*, vol. 2. Société Botanique du Centre-Ouest, Nercillac, France.
- ROKAS, A., B. L. WILLIAMS, N. KING, AND S. B. CARROLL. 2003. Genome-scale approaches to resolving incongruence in molecular phylogenies. *Nature* 425: 798–804.
- SÁENZ LAÍN, C. 1981. Research on *Daucus* L. (Umbelliferae). *Anales del Jardín Botánico de Madrid* 37: 481–534.
- SMALL, E. 1978. A numerical taxonomic analysis of the *Daucus carota* complex. *Canadian Journal of Botany* 56: 248–276.
- SOKAL, R. R., AND R. R. SNEATH. 1963. *Principles of numerical taxonomy*. W. H. Freeman, San Francisco, California, USA.
- SPALIK, K., AND S. R. DOWNIE. 2007. Intercontinental disjunctions in *Cryptotaenia* (Apiaceae, Oenantheae): An appraisal using molecular data. *Journal of Biogeography* 34: 2039–2054.
- SPOONER, D. M., P. ROJAS, M. BONIERBALE, L. A. MUELLER, M. SRIVASTAV, D. SENALIK, AND P. SIMON. 2013. Molecular phylogeny of *Daucus*. *Systematic Botany* 38: 850–857.
- SPOONER, D. M., M. P. WIDRLECHNER, K. R. REITSMA, D. E. PALMQUIST, S. ROUZ, Z. GHRABI-GAMMAR, M. NEFFATI, ET AL. 2014. Reassessment of practical subspecies identifications of the USDA *Daucus carota* germplasm collection: Morphological data. *Crop Science* 54: 706–718.
- WEITZEL, C., N. RØNSTED, K. SPALIK, AND H. T. SIMONSEN. 2014. Resurrecting deadly carrots: Towards a revision of *Thapsia* (Apiaceae) based on phylogenetic analysis on nrITS sequences and chemical profiles. *Botanical Journal of the Linnean Society* 174: 620–636.
- ZOHARY, M. 1972. *Flora Palaestina*, part 2. Israel Academy of Sciences and Humanities, Jerusalem, Israel.