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RESEARCH

Reassessment of Practical Subspecies Identifications of the USDA *Daucus carota* L. Germplasm Collection: Morphological Data

David M. Spooner,* Mark P. Widrechner, Kathleen R. Reitsma, Debra E. Palmquist, Slim Rouz, Zeineb Ghrabi-Gammar, Mohamed Neffati, Béchir Bouzbida, Hassan Ouabbou, Mohammed El Koudrim, and Philipp W. Simon

ABSTRACT

The genus *Daucus* includes about 20 recognized species. The most widespread and economically important species, *Daucus carota* L., occurs on almost every continent. The cultivated carrot, subsp. *sativus* (Hoffm.) Schübl. and G. Martens, has been selected from wild populations that are extremely diverse, especially in the western Mediterranean. The predominant outcrossing and the lack of sexual isolating mechanisms among recognized infraspecific taxa complicate the taxonomy and identification of the wild populations, resulting in widely different interpretations of the number of infraspecific taxa. We measured 36 morphological characters from multiple individuals within each of 155 accessions of *D. carota* and from the morphologically similar species *D. capillifolius* (both species $2n = 18$) alongside other species for comparison (*D. aureus* Desf., $2n = 22$; *D. broteri* Ten., $2n = 20$; *D. involuocratus* Sm., $2n = 20$; and *D. littoralis* Sm., $2n = 20$) in an experimental field plot. Within *D. carota*, multivariate analyses were able to identify only two subspecies, but even these showed great overlap of individual characters. Because of the ease of crossability of wild *D. carota* to the domestic landraces and cultivars and because of the taxonomic challenges, the purpose of our study is to explore morphological support for subspecies within *D. carota*, including the phenetically similar *D. capillifolius*, which is part of the same clade as *D. carota*, with the long-term goal of resolving taxonomic disagreements and developing a practical system to classify variation within this economically important species.

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Abbreviations: GRIN, Germplasm Resources Information Network; NCRPIS, North Central Regional Plant Introduction Station.

THE Umbelliferae comprise some 300–455 genera and 3000–3750 species (Constance, 1971; Pimenov and Leonov, 1993). The family is cosmopolitan but most diverse in the Northern Hemisphere, particularly in the Mediterranean region. The cultivated carrot (*Daucus carota* L. subsp. *sativus*) is by far its economically most important member, but the family also contains many other vegetables, flavorings, and herbs, including angelica, anise, caraway, celeriac, celery, chervil, coriander (cilantro), cumin, dill, fennel, lovage, parsley, and parsnip (Rubatzky et al., 1999).

While it is easy to place species within the Umbelliferae, existing generic boundaries within this large family are unnatural. Recent molecular investigations based on DNA sequences from nuclear ribosomal internal transcribed spacers, plastid *rpoC1* intron and *rpl16* intron sequences, plastid *matK* coding sequences,

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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) do not support many genera within the Umbelliferae as monophyletic. This is clearly the case with *Daucus*, as molecular data from these papers above place species from the genera *Agrocharis*, *Athamanta*, *Cryptotaenia*, *Margotia*, *Melanoselinum*, *Monizia*, *Pachyctenium*, *Pseudorlaya*, and *Tornabenea* within a monophyletic *Daucus* clade.

The latest taxonomic monograph of *Daucus* was by Sáenz Laín (1981), who recognized 20 species. The haploid chromosome number for *Daucus* ranges from $n = 9$ to $n = 11$. Most species are diploids with $2n = 20$ or $2n = 22$, but two tetraploid species have been reported (Grzebelus et al., 2011). There are only four $2n = 18$ chromosome species of *Daucus*: the widespread *D. carota* (all subspecies) and three North African species—*D. capillifolius* Gilli, *D. syrticus* Murb., and *D. sahariensis* Murb. (Grzebelus et al., 2011). DNA sequencing data of multiple nuclear orthologs (Arbizu et al., 2013; Spooner et al., 2013) place all subspecies of *D. carota* and *D. capillifolius* as a monophyletic clade, with *D. sahariensis* and *D. syrticus* as sisters to this clade. *Daucus carota* and *D. capillifolius*, but excluding *D. sahariensis* and *D. syrticus* (here referred to as the *D. carota* clade, or the $2n = 18$ accessions), are clearly interrelated based on the molecular studies mentioned above, shared karyotypes (Iovene et al., 2008), and crossability data (McCollum, 1975, 1977). *Daucus carota* is strongly outcrossing, and its populations are genetically heterogeneous (Simon, 1984). All known crosses among the subspecies of *D. carota* and *D. capillifolius* are interfertile, as evidenced by the results of manual crosses (Krickl, 1961; McCollum, 1975, 1977; Umiel et al., 1975; Ellis et al., 1993; Steinborn et al., 1995; Nothnagel et al., 2000). In addition, morphological intermediates among sympatric subspecies of *D. carota* are common and have been ascribed to natural interspecific hybridization (Nehou, 1961; Heywood, 1968; Wijnheijmer et al., 1989; Magnussen and Hauser, 2007).

This ease of crossing and great morphological variation within *D. carota* have resulted in confusing patterns of natural variation and widely different classifications. Within *D. carota*, two groups are phenotypically coherent: (i) plants with a relatively short stature, thick, broad leaf segments, and usually flat or convex fruiting umbels, distributed in the coastal regions of the central and western Mediterranean and Atlantic coasts of northern Africa, Portugal, Spain, France, and the UK; and (ii) taller plants with thinner narrower leaf segments and fruiting umbels that are frequently curved upward and that close into a characteristic “bird’s nest” form, occurring in coastal regions as above but also in inland regions and over a greater distributional range that includes Asia, Australia, and the Americas. Onno (1937) classified populations of the first group as *D. gingidium* L., containing eight subspecies, and the latter as *D. carota*, including four subspecies. Small (1978) and

Reduron (2007) recognized two “species aggregates,” or “subgroups,” within the single species *D. carota* corresponding to the above two groups. Reduron (2007) recognized five species within subgroup *carota* and four subspecies within subgroup *gummifer*, our first group above. Heywood (1968), Sáenz Laín (1981), and Pujadas Salvà (2003) recognized only a single species, but without the division into subgroups. They divided *D. carota* into subspecies but differed in the number of their recognized subspecies.

Daucus identifications made at the USDA-ARS North Central Regional Plant Introduction Station (NCRPIS) in Ames, Iowa, have typically been based on the sole comprehensive taxonomic treatment by Sáenz Laín (1981) supplemented by 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), Tunisia (Le Floc’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 and character states 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 *D. carota*, and identification of the accessions conserved by the NCRPIS is often problematic.

The NCRPIS conserves 1381 accessions of *Daucus*. Of these, 566 are classified as landraces, cultivars, cultivated populations, or breeding lines. Improvement status for the remaining accessions include 570 wild, 17 uncertain, and 227 accessions have 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.

Because of the ease of crossability of wild *D. carota* to the domestic landraces and cultivars, and because of the taxonomic challenges noted earlier, the purpose of our study was to explore morphological support for various subspecies within *D. carota*, with the long-term goal of developing a practical system to classify variation within this economically important species. To this end, we also included *D. capillifolius* because it shares the same chromosome number ($2n = 18$) and crossability pattern as *D. carota* and because it is part of the *D. carota* clade (Arbizu et al., 2013; Spooner et al., 2013). We also included four morphologically distinct species of *Daucus* with different base chromosome numbers as comparator species.

MATERIALS AND METHODS

Accessions Examined

We examined a total of 155 accessions of *D. carota*, all of wild origin except for one accession of *D. carota* subsp. *sativus* var. *atrorubens* Alef., including those with no subspecies designation and those previously identified 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., subsp. *maximus* (Desf.) Ball, *D. capillifolius*, and putative interspecific hybrids between *D. capillifolius* and *D. carota* subsp. *carota* (all above presumed to be $2n = 18$, based on identifications of these accessions). For comparison, we also examined accessions morphologically distinctive from *D. carota* and *D. capillifolius* and outside of the *D. carota* clade, including *D. aureus* Desf. ($2n = 22$), *D. broteri* Ten. ($2n = 20$), *D. guttatus* Sibth. and Sm. ($2n = 20$), *D. involucratus* Sm. ($2n = 20$), and *D. littoralis* Sm. ($2n = 20$). We measured different accessions in 2010 and 2011; 15 replicates were measured in both years (Table 1), raising the total from 155 to 170 examinations.

Daucus Observation Plots

Different accessions were measured in 2010 and 2011 except that 15 accessions were measured in both years to ensure that character development was consistent in different years (Table 1). For the 2010 observations, one 6-m row of each accession was direct seeded in field plots using a V-belt push planter with 3-m alleys between rows. Accessions were thinned to 20 plants per row, and traits (descriptors) were measured on at least three plants per accession. Plant and umbel descriptor data were collected during the growing season. Field plots were maintained with small plot tillers and hand weeding.

To better ensure sufficient plant populations in the 2011 observation plot, biennial and mixed life-cycle accessions were planted in the greenhouse in early November 2010. 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 2011. A fungicide spray (Rubigan, DuPont, Wilmington, DE) 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. Twenty plants per accession were transplanted into 6-m rows, one row per accession in each of two field plots in early May. Annual accessions were direct seeded into two field plots as described for the 2010 observation plot. Field plots were maintained with small plot tillers and hand weeding.

Characters Measured

Thirty-six characters were measured from at least three individuals per accession (Table 2). These characters were chosen to represent all those used in prior keys and morphological analyses (Small, 1978) to distinguish subspecies within *D. carota* and between *D. carota* and morphologically similar species. Size characters were measured in the field with a ruler or calipers, and floral and fruit characters were measured in the laboratory with the aid of a dissecting microscope. For both year's

observations, 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 all images are available on the Germplasm Resources Information Network (GRIN; www.ars-grin.gov/). Herbarium vouchers and alcohol-preserved inflorescences in flower and fruit of all of the accessions are deposited at the herbarium of the Potato Introduction Station, Sturgeon Bay, WI.

Analytical Methods

Thirty-two of the 36 characters were scored and analyzed as continuous variables; the remaining four were treated as nominal variables (Table 2). Means were taken of the former and modes for the latter. All analyses were conducted in JMP 9.0.3 software (SAS Institute, 2010). We ran two types of analyses to explore the best ways to distinguish the groups. We first performed hierarchical cluster analyses, all with standardized data, exploring five distance methods: average, centroid, ward, single, and complete. Second, we performed stepwise discriminant analyses (linear, common covariance) using all 32 continuous variables to obtain a model whose variables were significant in correctly identifying accession composition, with characters removed one at a time until the model F-test p value was ≤ 0.05 . This process was conducted in three iterations until a combination of reidentifications resulted in all taxa being confidently identified by this method, but with reidentifications verified only after examination of herbarium vouchers and photographs of the accessions. Once the taxa were reidentified (Table 1), we conducted stepwise discriminant analysis of all taxa (Fig. 1), and subsequently with all $2n = 18$ taxa only (Fig. 2). Histograms were then constructed to show character-state distributions of the 10 characters exhibiting the highest F-values (all with $p \leq 0.05$) within each of the above two methods (Fig. 3 and 4).

Field Observations in Morocco, Tunisia, and the United States

In addition to our common-garden studies, field trips to collect *Daucus* germplasm from wild and weedy populations were made by coauthors Simon and Spooner to Tunisia in 2009, the western United States in 2010 and 2011, and Morocco in 2012 and 2013. *Daucus carota* was collected extensively on all trips, and numerous collections of *D. capillifolius* made in Tunisia. Trips were taken in August or September in 2009–2012, when we could observe late-flowering and mature-fruited plants, and in June 2013 for flowering plants in Morocco. *Daucus carota* and *D. capillifolius* generally occur in disturbed areas along roadsides, in cultivated fields, and in peri-urban locales. Plant populations were frequently very large near the Mediterranean Sea (Tunisia and Morocco) and the Pacific Ocean (Washington, Oregon, and California of the United States) and also inland where natural rainfall was relatively plentiful or near irrigated agriculture. Populations reidentified as *D. carota* subsp. *gummifer* were all confined to areas within a few kilometers of the Mediterranean Sea or the Pacific Ocean.

Table 1. The 170 accessions (including one landrace, *D. carota* var. *atrorubens*) of wild carrot examined in this study, the year examined, the generalized locality, and original and proposed new identifications.

Original identification	Accession	Year	Location of collection [†]	Proposed new identifications [‡]
<i>Daucus aureus</i>	PI 295854	2010	Israel, Wadi Rubin (HaMerkaz)	
<i>D. aureus</i>	PI 319403	2010	Israel, Mediterranean region	
<i>D. aureus</i>	PI 478858	2010	France, Dijon	
<i>D. broteri</i>	Ames 25721	2010	Syria, Younesiya	
<i>D. broteri</i>	Ames 25729	2010	Syria, Qastal	
<i>D. broteri</i>	PI 652329	2010	Greece, Peloponnese	
<i>D. broteri</i>	PI 652385	2010	Turkey, Antalya	
<i>D. capillifolius</i>	Ames 30198	2010	Tunisia, Medenine	
<i>D. capillifolius</i>	Ames 30225	2010	Sfax, Tunisia	
<i>D. capillifolius</i>	Ames 30233	2010	Tunisia, Mahdia	
<i>D. capillifolius</i>	PI 279764	2010	Libya, near Jefren	
<i>D. carota</i>	Ames 25612	2010	Greece, Macedonia	<i>D. carota</i> subsp. <i>carota</i>
<i>D. carota</i>	PI 242384	2011	USA, Maryland	<i>D. carota</i> subsp. <i>carota</i>
<i>D. carota</i>	PI 242385	2011	USA, Maryland	<i>D. carota</i> subsp. <i>carota</i>
<i>D. carota</i>	PI 274298	2011	Pakistan, Parachinar	<i>D. carota</i> subsp. <i>carota</i>
<i>D. carota</i>	PI 287518	2011	Iran, Khoiy	<i>D. carota</i> subsp. <i>carota</i>
<i>D. carota</i>	PI 344446	2011	Iran, Khoiy	<i>D. carota</i> subsp. <i>carota</i>
<i>D. carota</i>	PI 344447	2011	Iran, Hamadan	<i>D. carota</i> subsp. <i>gummifer</i>
<i>D. carota</i>	PI 652213	2011	Kazakhstan, Chimkent	<i>D. carota</i> subsp. <i>carota</i>
<i>D. carota</i>	PI 652214	2011	Portugal, Peso da Regua	<i>D. carota</i> subsp. <i>carota</i>
<i>D. carota</i>	PI 652215	2011	USA, Colorado	<i>D. carota</i> subsp. <i>carota</i>
<i>D. carota</i>	PI 652219	2011	Hungry, Lake Balaton	<i>D. carota</i> subsp. <i>carota</i>
<i>D. carota</i>	PI 652220	2011	Poland, Chelm	<i>D. carota</i> subsp. <i>carota</i>
<i>D. carota</i>	PI 652221	2011	Poland, Lublin	<i>D. carota</i> subsp. <i>carota</i>
<i>D. carota</i>	PI 652222	2011	Portugal, Vila Real	<i>D. carota</i> subsp. <i>carota</i>
<i>D. carota</i>	PI 652223	2011	Poland, Nowy Sacz	<i>D. carota</i> subsp. <i>carota</i>
<i>D. carota</i>	PI 652224	2011	Poland, Lomza	<i>D. carota</i> subsp. <i>carota</i>
<i>D. carota</i>	PI 652292	2011	Greece, Macedonia	<i>D. carota</i> subsp. <i>carota</i>
<i>D. carota</i>	PI 652295	2011	Greece, Epirus	<i>D. carota</i> subsp. <i>carota</i>
<i>D. carota</i>	PI 652297	2011	Greece, Epirus	<i>D. carota</i> subsp. <i>carota</i>
<i>D. carota</i>	PI 652299	2011	Greece, Ionian Islands	<i>D. carota</i> subsp. <i>carota</i>
<i>D. carota</i>	PI 652301	2011	Greece, Ionian Islands	<i>D. carota</i> subsp. <i>carota</i>
<i>D. carota</i>	PI 652304	2011	Greece, Peloponnese	<i>D. carota</i> subsp. <i>carota</i>
<i>D. carota</i>	PI 652306	2011	Greece, Peloponnese	<i>D. carota</i> subsp. <i>carota</i>
<i>D. carota</i>	PI 652309	2011	Greece, Peloponnese	<i>D. carota</i> subsp. <i>carota</i>
<i>D. carota</i>	PI 652311	2011	Greece, Central Greece	<i>D. carota</i> subsp. <i>carota</i>
<i>D. carota</i>	PI 652313	2011	Greece, Central Greece	<i>D. carota</i> subsp. <i>carota</i>
<i>D. carota</i>	PI 652335	2011	Syria, Damascus	<i>D. carota</i> subsp. <i>carota</i>
<i>D. carota</i>	PI 652344	2011	Syria, Alratbeh	<i>D. carota</i> subsp. <i>carota</i>
<i>D. carota</i>	PI 652346	2011	Syria, Crac des Chevaliers	<i>D. carota</i> subsp. <i>carota</i>
<i>D. carota</i>	PI 652347	2011	Syria, Sweida	<i>D. carota</i> subsp. <i>carota</i>
<i>D. carota</i>	PI 652348	2011	Turkey, Izmir	<i>D. carota</i> subsp. <i>carota</i>
<i>D. carota</i>	PI 652353	2011	Turkey, Izmir	<i>D. carota</i> subsp. <i>carota</i>
<i>D. carota</i>	PI 652358	2011	Turkey, Izmir	<i>D. carota</i> subsp. <i>carota</i>
<i>D. carota</i>	PI 652361	2011	Turkey, Mugla	<i>D. guttatus</i>
<i>D. carota</i>	PI 652364	2011	Turkey, Mugla	<i>D. carota</i> subsp. <i>carota</i>
<i>D. carota</i>	PI 652369	2011	Turkey, Mugla	<i>D. carota</i> subsp. <i>carota</i>
<i>D. carota</i>	PI 652373	2011	Turkey, Mugla	<i>D. carota</i> subsp. <i>carota</i>
<i>D. carota</i>	PI 652375	2011	Turkey, Mugla	<i>D. guttatus</i>
<i>D. carota</i>	PI 652378	2011	Turkey, Mugla	<i>D. carota</i> subsp. <i>carota</i>
<i>D. carota</i>	PI 652384	2011	Turkey, Antalya	<i>D. carota</i> subsp. <i>carota</i>
<i>D. carota</i>	PI 652395	2011	Turkey, Konya	<i>D. guttatus</i>
<i>D. carota</i>	PI 652398	2011	Turkey, Isparta	<i>D. carota</i> subsp. <i>carota</i>
<i>D. carota</i>	PI 652406	2011	Turkey, Denizli	<i>D. carota</i> subsp. <i>carota</i>

Table 1. Continued.

Original identification	Accession	Year	Location of collection [†]	Proposed new identifications [‡]
<i>D. carota</i>	PI 652407	2011	Turkey, Denizli	<i>D. carota</i> subsp. <i>carota</i>
<i>D. carota</i>	PI 652408	2011	Turkey, Denizli	<i>D. guttatus</i>
<i>D. carota</i> subsp. <i>carota</i>	Ames 25740	2010	Syria, As Samra	
<i>D. carota</i> subsp. <i>carota</i>	Ames 26371	2011	Portugal, Braga	
<i>D. carota</i> subsp. <i>carota</i>	Ames 26372	2011	Portugal, Braga	
<i>D. carota</i> subsp. <i>carota</i>	Ames 26376	2010	Portugal, Castelo Branco	
<i>D. carota</i> subsp. <i>carota</i>	Ames 27397	2010	Uzbekistan, between Yalangoch and Sobir Raximova	
<i>D. carota</i> subsp. <i>carota</i>	Ames 27401	2010	Uzbekistan, Tashkent to Bolta	
<i>D. carota</i> subsp. <i>carota</i>	Ames 27403	2010	Uzbekistan, Angren to Tashkent	
<i>D. carota</i> subsp. <i>carota</i>	Ames 27410	2010	Uzbekistan, Between Kitab and Samarkand	
<i>D. carota</i> subsp. <i>carota</i>	Ames 27416	2010	Uzbekistan, between Angren and Nurobad	
<i>D. carota</i> subsp. <i>sativus</i>	Ames 30234	2010	Tunisia landrace	
<i>D. carota</i> subsp. <i>carota</i>	Ames 30242	2010	Tunisia, Ben Arous	
<i>D. carota</i> subsp. <i>carota</i>	Ames 30244	2010	Tunisia, Zaghouan	
<i>D. carota</i> subsp. <i>carota</i>	Ames 30245	2010	Tunisia, Zaghouan	
<i>D. carota</i> subsp. <i>carota</i>	Ames 30248	2010	Tunisia, Zaghouan	<i>Daucus</i> hybrid (<i>D. carota</i> , <i>D. capillifolius</i>)
<i>D. carota</i> subsp. <i>carota</i>	Ames 30249	2010	Tunisia, Nabeul	
<i>D. carota</i> subsp. <i>carota</i>	Ames 30256	2010	Tunisia, L'Ariana	
<i>D. carota</i> subsp. <i>carota</i>	Ames 30258	2010	Tunisia, Bizerte	
<i>D. carota</i> subsp. <i>carota</i>	Ames 30262	2010	Tunisia, Beja	
<i>D. carota</i> subsp. <i>carota</i>	Ames 30265	2010	Tunisia, Jendouba	
<i>D. carota</i> subsp. <i>carota</i>	Ames 30272	2010	Tunisia, Jendouba	
<i>D. carota</i> subsp. <i>carota</i>	PI 274297	2010	Pakistan, Northern Areas	
<i>D. carota</i> subsp. <i>carota</i>	PI 279788	2010	Austria, Vienna	
<i>D. carota</i> subsp. <i>carota</i>	PI 295861	2010	Spain, El Viso	
<i>D. carota</i> subsp. <i>carota</i>	PI 390887	2010	Israel, central Israel	
<i>D. carota</i> subsp. <i>carota</i>	PI 421301	2010	USA, Kansas	
<i>D. carota</i> subsp. <i>carota</i>	PI 430525	2010	Afghanistan, Zardek	
<i>D. carota</i> subsp. <i>carota</i>	PI 478369	2010	China, Xinjiang	
<i>D. carota</i> subsp. <i>carota</i>	PI 478859	2010	Italy, Rimini	
<i>D. carota</i> subsp. <i>carota</i>	PI 478860	2010	France, Seine et Oise	
<i>D. carota</i> subsp. <i>carota</i>	PI 478861	2010	France, Seine et Oise	
<i>D. carota</i> subsp. <i>carota</i>	PI 478863	2010	Collection site unknown	
<i>D. carota</i> subsp. <i>carota</i>	PI 478869	2010	Germany, Juterbog	
<i>D. carota</i> subsp. <i>carota</i>	PI 478873	2010	Italy, Sardinia	
<i>D. carota</i> subsp. <i>carota</i>	PI 478875	2010	Italy, Molise	
<i>D. carota</i> subsp. <i>carota</i>	PI 478876	2010	Italy, Latium	
<i>D. carota</i> subsp. <i>carota</i>	PI 478878	2010	Switzerland, Geneva	
<i>D. carota</i> subsp. <i>carota</i>	PI 478881	2010	USA, Oregon	
<i>D. carota</i> subsp. <i>carota</i>	PI 478884	2010	The Netherlands, South Holland	
<i>D. carota</i> subsp. <i>carota</i>	PI 652191	2010	Poland, Okolice	
<i>D. carota</i> subsp. <i>carota</i>	PI 652218	2010	Hungary, Békés	
<i>D. carota</i> subsp. <i>carota</i>	PI 652236	2010	Bulgaria, Lovech	
<i>D. carota</i> subsp. <i>carota</i>	PI 652296	2010	Greece, Epirus	
<i>D. carota</i> subsp. <i>carota</i>	PI 652303	2010	Greece, Central Greece	
<i>D. carota</i> subsp. <i>carota</i>	PI 652320	2010	Greece, Macedonia	
<i>D. carota</i> subsp. <i>carota</i>	PI 652341	2010	Syria, Ash Sheik Hasan	
<i>D. carota</i> subsp. <i>carota</i>	PI 652351	2010	Turkey, Izmir	
<i>D. carota</i> subsp. <i>carota</i>	PI 652379	2010	Turkey, Mulga	
<i>D. carota</i> subsp. <i>carota</i>	PI 652393	2010	Turkey, Konya	
<i>D. carota</i> subsp. <i>carota</i>	PI 652409	2010	Turkey, Aydin	
<i>D. carota</i> subsp. <i>commutatus</i>	Ames 7674	2011	Italy, Tuscany	<i>D. carota</i> subsp. <i>gummifer</i>
<i>D. carota</i> subsp. <i>commutatus</i>	PI 478883	2010	France, Finistere	<i>D. carota</i> subsp. <i>gummifer</i>
<i>D. carota</i> subsp. <i>commutatus</i>	PI 652291	2010	Portugal, Faro	<i>D. carota</i> subsp. <i>gummifer</i>

Table 1. Continued.

Original identification	Accession	Year	Location of collection [†]	Proposed new identifications [‡]
<i>D. carota</i> subsp. <i>drepanensis</i>	PI 279794	2010	Spain, Madrid	<i>D. carota</i> subsp. <i>gummifer</i>
<i>D. carota</i> subsp. <i>fontanesii</i>	PI 652387	2010	Turkey, Antalya	<i>D. guttatus</i>
<i>D. carota</i> subsp. <i>gummifer</i>	Ames 26382	2010 and 2011	Portugal, Faro	
<i>D. carota</i> subsp. <i>gummifer</i>	Ames 26383	2010 and 2011	Portugal, Faro	
<i>D. carota</i> subsp. <i>gummifer</i>	Ames 26384	2010 and 2011	Portugal, Beja	
<i>D. carota</i> subsp. <i>gummifer</i>	PI 652411	2010	France, Finistere	
<i>D. carota</i> subsp. <i>hispanicus</i>	PI 652139	2010	Italy, Apulia	<i>D. carota</i> subsp. <i>gummifer</i>
<i>D. carota</i> subsp. <i>hispanicus</i>	PI 652150	2010	Collection site unknown	<i>D. carota</i> subsp. <i>gummifer</i>
<i>D. carota</i> subsp. <i>major</i>	Ames 24682	2010 and 2011	Portugal, Coimbra	<i>D. carota</i> subsp. <i>carota</i>
<i>D. carota</i> subsp. <i>major</i>	Ames 25017	2010 and 2011	Germany, Saxony-Anhalt	<i>D. carota</i> subsp. <i>carota</i>
<i>D. carota</i> subsp. <i>major</i>	Ames 25898	2011	Turkey, Konya	<i>D. carota</i> subsp. <i>carota</i>
<i>D. carota</i> subsp. <i>major</i>	PI 652226	2011	Greece, 10 km N of Kassandra	<i>D. carota</i> subsp. <i>carota</i>
<i>D. carota</i> subsp. <i>major</i>	PI 652229	2011	Tunisia	<i>D. carota</i> subsp. <i>carota</i>
<i>D. carota</i> subsp. <i>maritimus</i>	Ames 26386	2011	Portugal, Braganca	<i>D. carota</i> subsp. <i>carota</i>
<i>D. carota</i> subsp. <i>maritimus</i>	Ames 26387	2011	Portugal, Braganca	<i>D. carota</i> subsp. <i>carota</i>
<i>D. carota</i> subsp. <i>maritimus</i>	Ames 26388	2011	Portugal, Braganca	<i>D. carota</i> subsp. <i>carota</i>
<i>D. carota</i> subsp. <i>maritimus</i>	Ames 26389	2011	Portugal, Guarda	<i>D. carota</i> subsp. <i>carota</i>
<i>D. carota</i> subsp. <i>maritimus</i>	Ames 26393	2011	Portugal, Branco	<i>D. carota</i> subsp. <i>carota</i>
<i>D. carota</i> subsp. <i>maritimus</i>	Ames 26398	2011	Portugal, Faro	<i>D. carota</i> subsp. <i>carota</i>
<i>D. carota</i> subsp. <i>maritimus</i>	Ames 26399	2011	Portugal, Faro	<i>D. carota</i> subsp. <i>carota</i>
<i>D. carota</i> subsp. <i>maritimus</i>	Ames 26400	2011	Portugal, Beja	<i>D. carota</i> subsp. <i>carota</i>
<i>D. carota</i> subsp. <i>maritimus</i>	PI 502244	2010 and 2011	Portugal, Coimbra	<i>D. carota</i> subsp. <i>carota</i>
<i>D. carota</i> subsp. <i>maritimus</i>	PI 652225	2010 and 2011	Collection site unknown	<i>D. carota</i> subsp. <i>carota</i>
<i>D. carota</i> subsp. <i>maximus</i>	Ames 26401	2011	Portugal, Portalegre	<i>D. carota</i> subsp. <i>carota</i>
<i>D. carota</i> subsp. <i>maximus</i>	Ames 26403	2011	Portugal, Evora	<i>D. carota</i> subsp. <i>carota</i>
<i>D. carota</i> subsp. <i>maximus</i>	Ames 26404	2011	Portugal, Evora	<i>D. carota</i> subsp. <i>carota</i>
<i>D. carota</i> subsp. <i>maximus</i>	Ames 26405	2011	Portugal, Beja	<i>D. carota</i> subsp. <i>carota</i>
<i>D. carota</i> subsp. <i>maximus</i>	Ames 26406	2011	Portugal, Beja	<i>D. carota</i> subsp. <i>carota</i>
<i>D. carota</i> subsp. <i>maximus</i>	Ames 26407	2011	Portugal, Faro	<i>D. carota</i> subsp. <i>carota</i>
<i>D. carota</i> subsp. <i>maximus</i>	Ames 26409	2011	Portugal, Faro	<i>D. carota</i> subsp. <i>carota</i>
<i>D. carota</i> subsp. <i>maximus</i>	PI 478866	2010 and 2011	Collection site unknown	<i>D. carota</i> subsp. <i>carota</i>
<i>D. carota</i> subsp. <i>maximus</i>	PI 478872	2010 and 2011	Germany, Wolferode	<i>D. carota</i> subsp. <i>carota</i>
<i>D. carota</i> subsp. <i>maximus</i>	PI 478874	2010 and 2011	Italy, Sicily	<i>D. carota</i> subsp. <i>carota</i>
<i>D. carota</i> subsp. <i>maximus</i>	PI 652227	2011	Croatia, between Hvar and Milna	<i>D. carota</i> subsp. <i>carota</i>
<i>D. carota</i> subsp. <i>maximus</i>	PI 652228	2011	Italy, Calabria	<i>D. carota</i> subsp. <i>carota</i>
<i>D. carota</i> subsp. <i>maximus</i>	PI 652230	2010 and 2011	Albania, Lushnje	<i>D. carota</i> subsp. <i>carota</i>
<i>D. carota</i> var. <i>atorrubens</i>	PI 279777	2010 and 2011	Egypt, Giza	
<i>D. guttatus</i>	Ames 25608	2010 and 2011	Greece, Central Greece	
<i>D. guttatus</i>	Ames 25724	2010 and 2011	Syria, Younesiya	
<i>D. guttatus</i>	Ames 25807	2011	Turkey, Izmir	
<i>D. guttatus</i>	PI 279763	2011	Israel, Jerusalem	
<i>D. guttatus</i>	PI 652343	2011	Syria, Halwah	
<i>Daucus</i> hybrid (<i>D. carota</i> , <i>D. capillifolius</i>)	Ames 30211	2010	Tunisia, Gabes	<i>D. capillifolius</i>
<i>Daucus</i> hybrid (<i>D. carota</i> , <i>D. capillifolius</i>)	Ames 30215	2010	Tunisia, Gafsa	
<i>Daucus</i> hybrid (<i>D. carota</i> , <i>D. capillifolius</i>)	Ames 30253	2010	Tunisia, Nabuel	
<i>D. involucratus</i>	PI 652350	2011	Turkey, Izmir	
<i>D. littoralis</i>	PI 295857	2010 and 2011	Israel, Beit Alpha	

[†]Complete locality data can be obtained at www.ars-grin.gov/.

[‡]We agree with the identifications for the accessions for which this column is left blank.

Table 2. The 36 morphological characters measured in this study, modeling type, and F-test *p* values of characters retained in a stepwise discriminant analysis for 1: all accessions identified as in Table 1; 2: a subset of the accessions containing and reidentified as *D. capillifolius*, *D. carota* subsp. *gummifer*, and *D. carota* subsp. *carota*; and 3: all accessions of *D. capillifolius* and *D. carota* (all subspecies) designated as one group and all other *Daucus* designated as another group.

Character†	Modeling type‡	F-test <i>p</i> values			Character†	Modeling type‡	F-test <i>p</i> values		
		1	2	3			1	2	3
Plant					Bract width (mm)	C	0.0015	0.0001	
Plant height (cm)	C	0.0000		0.0009	Involucral bract position (deflexed, not deflexed [outward or upward])	N			
Stem diameter (mm)	C	0.0166			Number of bract lobe points	C	0.0001		0.0100
Leaf					Number of bract lobe pairs	C	0.0008	0.0004	
Leaf length (cm)	C	0.0269			Number of umbel rays	C	0.0183		0.0080
Leaf width (cm)	C				Pigmented central umbel (concolorous to outer [uniform color], 1; differently pigmented, 2)	C	0.0001	0.0001	0.0001
Stipule width (mm)	C	0.0019			Pigmented central umbel color (yellow, green, pink, purple, dark purple, red)	N			
Petiole length (cm)	C	0.0000		0.0000	Petal color (white, cream, yellow [only], pink)	N			
Petiole diameter (mm)	C	0.0000		0.0000	Anther color (white, cream, yellow, pink, purple, brown)	N			
Petiole shape (round, 1; semi-round, 2; flat, 3)	C	0.0170			Peripheral petal length (mm)	C			
Leaf type (celery, 1; normal, 2; parsley, 3; other, 4)	C				Central petal length (mm)	C			0.0287
Leaf and petiole pubescence (smooth, 1; intermediate, 2; very hairy, 3)	C	0.0000	0.0000		Stamen length (mm)	C			
Leaf Color (light green, 1; medium green, 2; grey green, 3; dark green, 4)	C	0.0000	0.0052	0.0129	Seed				
Flower					Seed length (mm)	C	0.0001	0.0001	0.0018
Peduncle pubescence (glabrous, 1; soft hairs, 2; scabrous, 3; very scabrous, 4)	C				Seed width (mm)	C	0.0001		0.0001
Primary umbel shape, full bloom (convex, 1; flat, 2; concave, 3)	C	0.0110	0.0051		Confluency of seed spines (separate, 1; little confluency, 2; much confluency, 3)	C			
Primary umbel shape, mature seed (convex, 1; flat, 2; concave, 3)	C	0.0000	0.0000		Width of secondary seed rib confluency (mm)	C	0.0001		
Primary umbel height (cm)	C	0.0000	0.0000	0.0000	Number spines on the secondary seed ribs	C	0.0070		
Primary umbel diameter (cm)	C	0.0000		0.0000	Length of secondary seed spines (mm)	C	0.0001	0.0067	0.0014
Secondary umbel diameter (cm)	C								
Bract length (mm)	C								

RESULTS

Phenetic Analyses

Hierarchical cluster analyses using all data with the five types of distance methods (analyses not shown) failed to group the subspecies as initially identified. Stepwise discriminant analyses, however, aided the reidentifications of some specimens. Some were changed from one subspecies to another within *D. carota*, and some *D. carota* were changed to *D. guttatus* (Table 1). Reiterative analyses produced stable results only after *D. carota* was divided into *D. carota* subsp. *carota* sensu lato (in a broad or taxonomically expanded sense), and *D. carota* subsp. *gummifer* (also sensu lato). These two taxa correspond to Onno's (1937) *D. gigidium* and *D. carota* and to Small's (1978) and Reduron's (2007) two "species aggregates," or "subgroups," respectively, within the single species *D. carota*. Stepwise discriminant analysis was conducted for three subsets of the accessions, each using a different number of characters: Analysis 1 included all taxa examined for 23 continuously

†Additional details on these descriptors can be found in IPGRI (1998).

‡N, nominal; C, continuous.

variable morphological characters (F-test $p \leq 0.05$); Analysis 2 included just *D. carota*, all subspecies, and *D. capillifolius* in the *D. carota* clade analyzed for only 10 characters; Analysis 3 included all members of the *D. carota* clade as one group and all other accessions as a second group and analyzed 13 characters (Table 2).

Analysis 1 placed most specimens in three groups, as is evident from a canonical variates plot that shows the points and multivariate means in two dimensions that best separate the groups (Fig. 1). The first group corresponds to *D. carota* subsp. *gummifer*, the second group corresponds to *D. carota* subsp. *carota* sensu lato, and the third group includes *D. broteri* and *D. guttatus*. *Daucus capillifolius* and putative *D. capillifolius* × *carota* hybrids formed a group that partly overlaps with second group above, and the remaining taxa were scattered around the edge of the diagram.

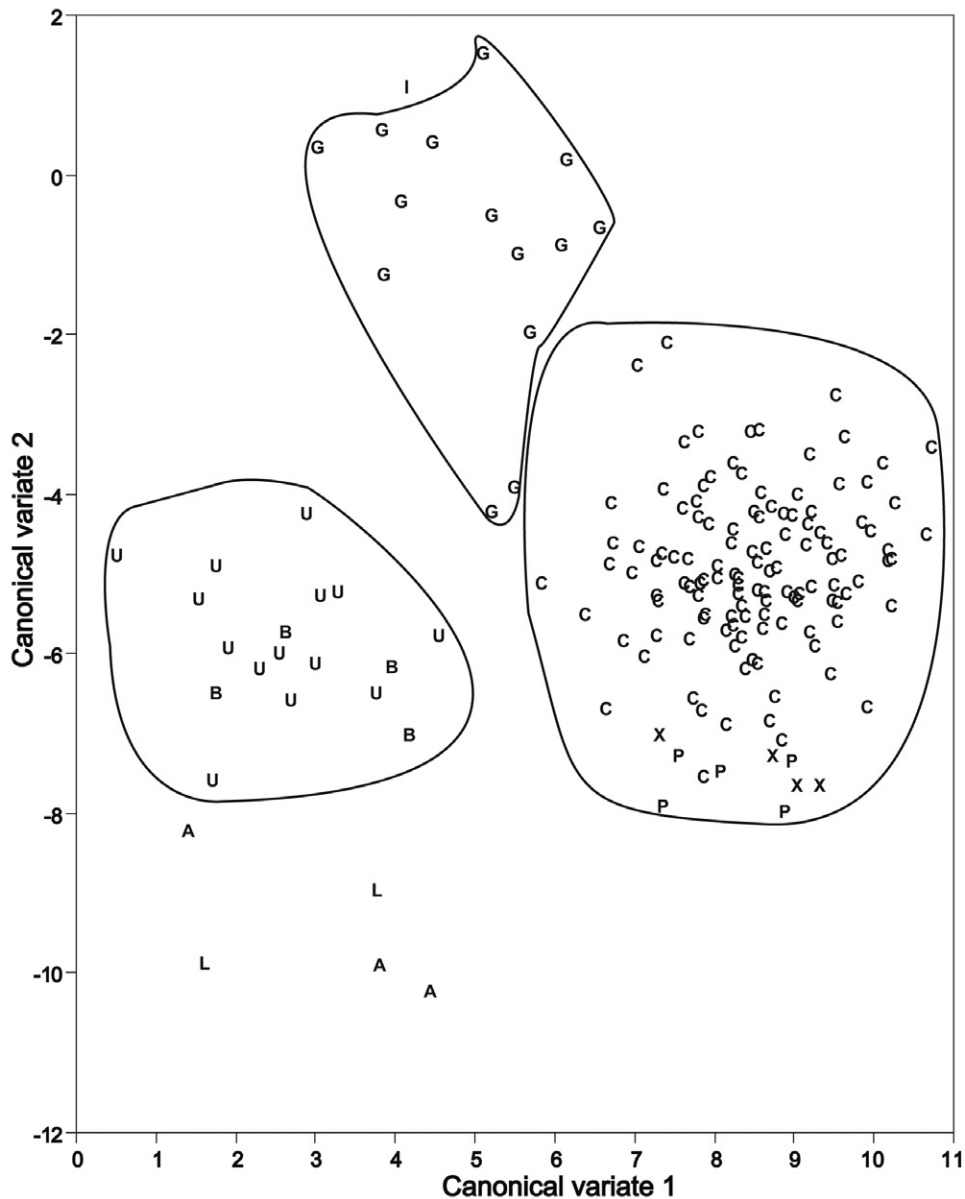


Figure 1. Plots of the first two canonical variates from discriminant analysis of all taxa based on the proposed new identifications of taxa (Table 1). A, *Daucus aureus*; B, *D. broteri*; C, *D. carota* subsp. *carota*; G, *D. carota* subsp. *gummifer*; I, *D. involucratus*; L, *D. littoralis*; P, *D. capillifolius*; U, *D. guttatus*; X, hybrids between *D. capillifolius* and *D. carota*, no subspecies.

Analysis 2 produced better separation of the $2n = 18$ species than did the analysis of all taxa (Fig. 2 and 3). The 15 replicate accessions examined in both 2010 and 2011 were all consistently assigned to their respective taxonomic groups. Analysis 3 clearly separated these two groups (Fig. 4; canonical variates analyses not shown). See Table 2 for F values of all three analyses.

Character-State Distributions

An examination of the 10 strongest variable characters (all $p \leq 0.05$) separating *D. carota* subsp. *carota*, *D. carota* subsp. *gummifer*, and *D. capillifolius* accessions (Fig. 3), and these $2n = 18$ accessions compared with all other species (Fig. 4), shows considerable overlap within the character-state distributions supporting these groups. The best characters

separated the $2n = 18$ species from the others on size characters (plant height, petiole length, primary umbel height and diameter) and number of plant parts (number of umbel rays), but with considerable overlap. An analysis within the $2n = 18$ accessions shows a similar pattern of character-state overlap, with the most obvious characters being the color of the central umbel (concolorous yellow in *D. capillifolius*; concolorous white, to pink to purple in all subspecies of *D. carota*), bract width (always <15 mm in *D. capillifolius*, but sometimes also <15 mm in *D. carota*), and seed length and secondary seed spine length (longest in *D. capillifolius*). Of these characters, only seed length separates the two species with little overlap.

The four nominal morphological characters (of 36 total; Table 2) are inappropriate for discriminant analyses,

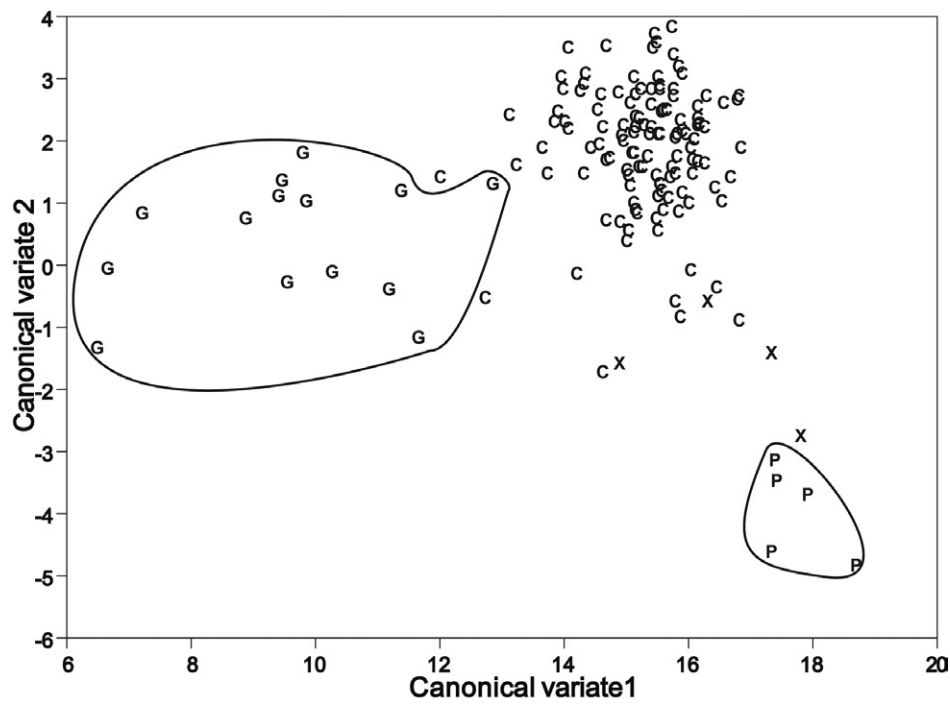


Figure 2. Plots of first two canonical variates from discriminant analysis of just the $2n = 18$ taxa based on the proposed new identifications of taxa (Table 1). C, *Daucus carota* subsp. *carota*; G, *D. carota* subsp. *gummifer*; P, *D. capillifolius*; X, hybrids between *D. capillifolius* and *D. carota* subsp. *carota*.

but three of them show clear separations of some taxa. *Daucus capillifolius* is the only taxon with yellow-pigmented central umbels and petal colors. *Daucus capillifolius* and *D. carota* subsp. *gummifer* have more involucre bracts pointed upward or outward than do the other taxa.

DISCUSSION

Our analysis, which is focused on representative accessions of *D. carota* subspecies and the related species *D. capillifolius*, with a few unrelated *Daucus* species for comparison, shows great overlap of character-state distributions among taxa. It highlights the great morphological similarity among these taxa for most characters and suggests that for wild *D. carota* only two subgroups can be separated morphologically. Morphological definition of even a limited number of subspecific taxa within *D. carota*, therefore, relies entirely on polythetic support, that is, grouping taxa that have the greatest number of shared features, no single feature of which is essential to group membership or is sufficient to make an organism a member of a group (Sokal and Sneath, 1963). Such concepts have been used in many complex groups exhibiting poorly defined isolating mechanisms but great within-group morphological variation, such as wild potatoes (Van den Berg et al., 1998) and indeed in many other difficult taxonomic groups.

The lack of agreement on circumscription of subspecies within *D. carota*, combined with the lack of a comprehensive taxonomic treatment of the subspecies throughout their ranges, has precluded stable and reliable identifications at the NCRPIS and other carrot gene banks. The

best we can do at present with representative samples at the NCRPIS is to define two taxa, *D. carota* subsp. *carota* sensu lato and subsp. *gummifer*, corresponding to the two species (*D. carota* and *D. gingidium*) recognized by Onno (1937) or to the two “species aggregates,” or “subgroups,” recognized by Small (1978) and Reduron (2007) although without recognizing subspecies within these groups.

This study was designed to discover clear and practical methods to identify germplasm collections of *D. carota* and related species, but we await additional studies for a monographic level treatment of final taxonomic names. We attribute this to four factors requiring additional information. First, one of the characters used in the literature for *D. carota* subsp. *gummifer*, leaves that are stiff and shiny, was impossible to assess in an efficient manner because of so much pertinent phenotypic variation within and among accessions. Second, we found much variation in the subspecies or undescribed distinctive forms of *D. carota*. For example, in Tunisia, we identified two forms of *D. carota*, a typical form found in the United States and worldwide, identified as subsp. *carota*, and also another, which was encountered only in northwestern Tunisia and had inflated, leathery stipule bases and relatively large spherical umbels with tightly appressed and sclerified bracts in fruit. Further to the west, in Morocco, this was also a common morphotype (along with subsp. *gummifer* along the coast). However, these stipule-base and umbel characters vary greatly across Tunisia and Morocco, and it is difficult to assign collections to this morphotype. Third, the accessions that we evaluated in our study are not as comprehensive as we would have preferred,

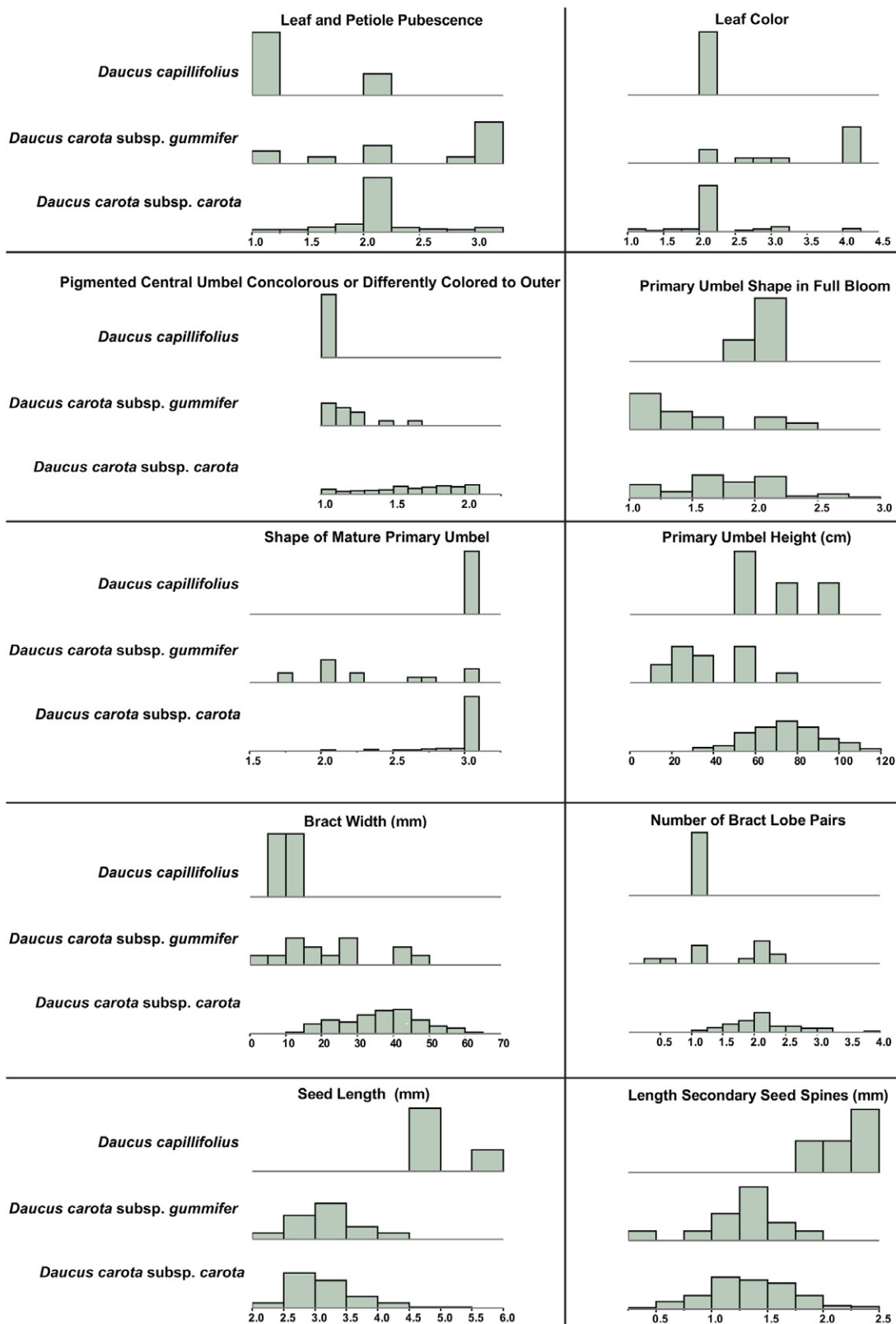


Figure 3. Histograms of character-state distributions of the 10 strongest characters (Table 2) separating the $2n = 18$ taxa: *D. capillifolius* ($n = 5$), *D. carota* subsp. *gummifer* ($n = 14$), *D. carota* subsp. *carota* and subsp. *sativus* ($n = 126$); excludes the three interspecific hybrids of *D. carota* \times *D. capillifolius* ($n = 3$) and other species ($n = 22$).

and we lack much of the subspecific variation described for the Iberian Peninsula by Pujadas Salvà (2003). Fourth, single nucleotide polymorphism examination of 81 accessions

of cultivated and wild *D. carota* and closely related species (Iorizzo et al., 2013) was able to distinguish *D. carota* subspecies and even geographic subsets in subsp. *carota* better

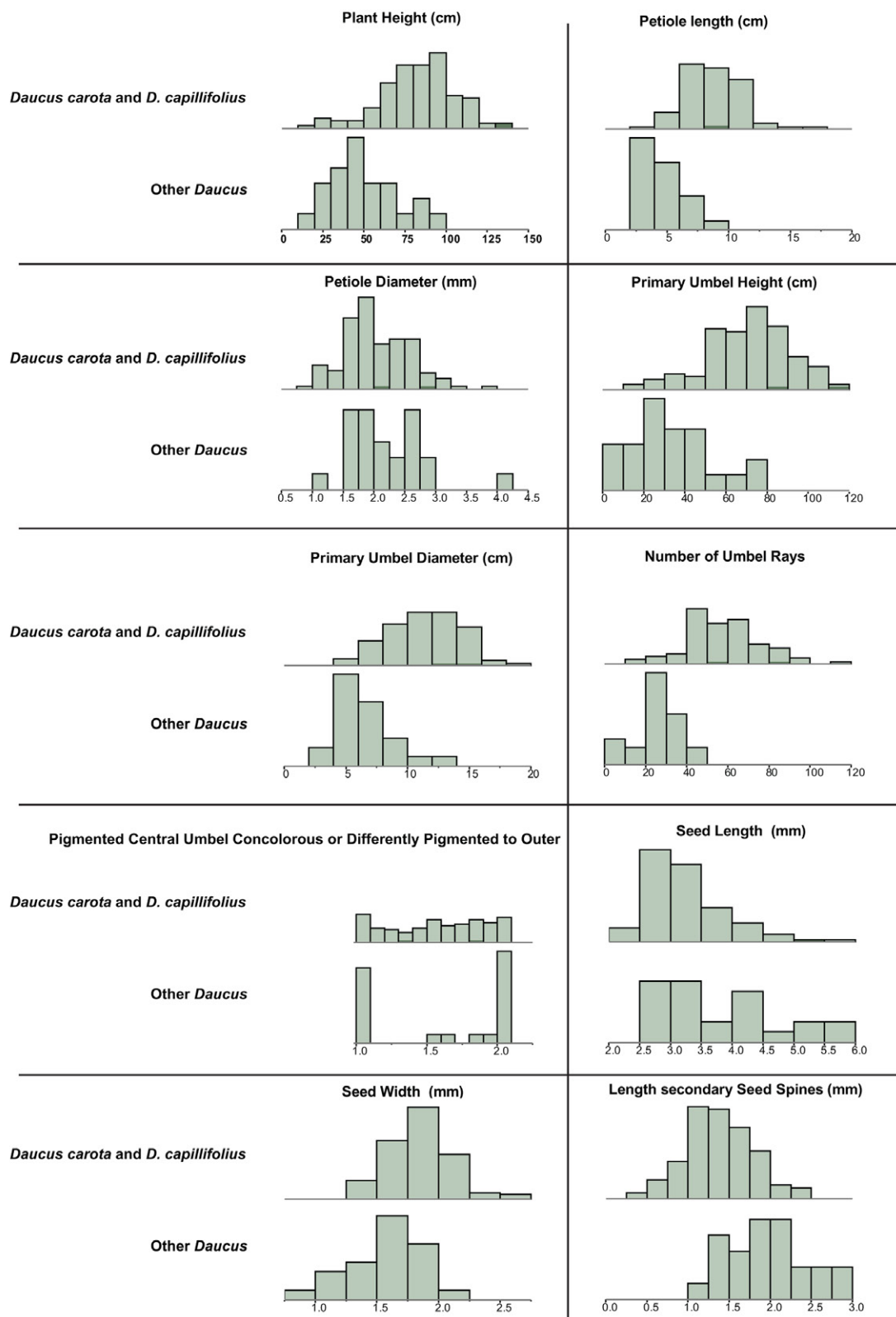


Figure 4. Histograms of character-state distributions of the 10 strongest (of 13 total) characters (Table 2) separating *Daucus capillifolius* and *D. carota* ($n = 148$) from all other *Daucus* ($n = 22$).

than our present study. Interestingly, Iorizzo et al. grouped *D. capillifolius* with *D. carota* subsp. *carota*, a result concordant with the DNA sequence data of multiple nuclear orthologs (Spooner et al., 2013) and with an amplified study using

more accessions and nuclear orthologs (Arbizu et al., 2013), suggesting the need for a reclassification of *D. capillifolius* to *D. carota*, as suggested by the crossing studies of McCollum (1975). The phylogenetic studies of Spooner et al. (2013) and

Arbizu et al. (2013), which used nuclear ortholog sequencing, also failed to distinguish even the two subgroupings of *D. carota* that we distinguish here. However, the same accessions were not always used in those molecular studies and the present morphological study. Our definitive conclusions await additional field experience and access to additional materials in different geographic regions for further morphological and molecular studies.

We analyzed accessions from many areas worldwide, with a concentration in the Mediterranean region, where *D. carota* is most diverse. Our proposed new identifications (Table 1) are of two main types: (i) those resulting from reduced numbers of taxa we support here, that is, *D. carota* subsp. *carota* sensu lato to include the names subsp. *carota*, subsp. *major*, subsp. *maritimus*, and subsp. *maximus*; and *D. carota* subsp. *gummifer* to include the names *D. carota* subsp. *commutatus*, subsp. *drepanensis*, subsp. *gummifer*, and subsp. *hispanicus*; (ii) unexpected identifications outside of these groups, including *D. carota* without subspecies to *D. carota* subsp. *gummifer*, or *D. carota* without subspecies designation and subsp. *fontanesii* to *D. guttatus*. Most proposed new identifications to subsp. *gummifer* are concentrated in the Mediterranean regions because these morphotypes are endemic there. Proposed new identifications of *D. carota* to *D. guttatus* are concentrated in Turkey, are unexpected, and require further morphological analyses of all available *Daucus* species (currently in progress).

At present, we will apply our morphological results as a basis for verification and possible reidentification of *Daucus* accessions in the GRIN database, noting that GRIN does retain former identifications to alert users of prior status. Our long-term plan is to use an integrated approach of morphological and molecular studies to clarify substructure in *D. carota*, as has been done in other groups such as cultivated potatoes (Spooner et al., 2007) and sorghum (Brown et al., 2011). However, we suspect that these additional studies will also conclude there are only two subspecies of *D. carota*.

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