**Extended studies of interspecific relationships in *Daucus* (Apiaceae) using DNA sequences from ten nuclear orthologues**

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*Daucus* has traditionally been estimated to comprise 21–25 species, but a recent study expanded the genus to c. 40 species. The present study uses ten nuclear orthologues to examine 125 accessions, including 40 collections of 11 species (*D. annuus, D. arcanus, D. decipiens, D. durieua, D. edulis, D. gracilis, D. minusculus, D. montanus, D. pumilus, D. setifolius* and *D. tenuissimus*) newly examined with nuclear orthologues. As in previous nuclear orthologue studies, *Daucus* resolves into two well-defined clades, and groups different accessions of species together. Maximum likelihood and maximum parsimony analyses provide concordant results, but SVD quartets reveals many areas of disagreement of species within these two major clades. With maximum likelihood and maximum parsimony analyses *Daucus montanus* (hexaploid) is resolved as an allopolyploid between *D. pusillus* (diploid) and *D. glochidiatus* (tetraploid), whereas with SVD quartets it is resolved as an allopolyploid between *D. glochidiatus* and an unknown *Daucus* sp. We propose the new combination *Daucus junceus* (*Durieua juncea*) for a neglected species endemic to the south-western Iberian Peninsula often referred to as *D. setifolius*, and we place *D. arcanus* in synonymy with *D. pusillus*. Three lectotypes are also designated.


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**INTRODUCTION**

The latest comprehensive taxonomic monograph of *Daucus* L. by Sáenz (1981) recognized 21 species; Rubatzky, Quirós & Simon (1999) later estimated 25 species. Recent morphological and molecular studies using plastid and nuclear DNA sequences have been drastically changing our understanding of the species boundaries (Arbizu et al., 2014b, 2016; Spooner et al., 2014) and ingroup and outgroup relationships of *Daucus* (e.g. Špalik & Downie, 2007; Arbizu et al., 2014a; Banasiak et al., 2016; Spooner et al., 2017). Banasiak et al. (2016) used DNA sequences from nuclear ribosomal ITS and the three plastid markers (the *rps16 intron, rpoC1 intron* and *rpoB-trnC* intergenic spacer) to redefine and expand *Daucus* to include the following genera and species into its synonymy: *Agrocharis* Hochst. (four species); *Melanoselinum* Hoffm. (one species); *Monizia* Lowe (one species); *Pachycytenium*...
Maire & Pamp. (one species); Pseudorlaya (Murb.) Murb. (two species); Rouya Coincy (one species); Tornabenea Parl. (six species); Athamanta della-cellae Asch. & Barbey ex E.A. Durand & Barratte and Cryptotaenia elegans Webb ex Bolle. They made the relevant nomenclatural transfers into Daucus, expanding the genus to c. 40 species. In addition, they divided Daucus into four sections, three of which [section Daucus, section Melanoselinum (Hoffm.) Spalik, Wojew., Banasiak & Reduron, section Anisactis DC.] we examine here; we do not examine section Agrocharis (Hochst) Spalik, Banasiak & Reduron that is sister to the sections above and comprised of the former genus Agrocharis. This expansion of Daucus and new sectional classification renders Daucus difficult to characterize morphologically, and it was based solely on molecular data.

Arbizu et al. (2014a) identified 94 nuclear orthologues in Daucus, constructed a phylogenetic tree with these and determined ten of them to provide essentially the same result as all 94, paving the way for additional cost-effective nuclear orthologue studies in Daucus. These ten nuclear orthologues were then successfully used in a focused study of the species boundaries of the D. guttatus Sibth. & Sm. complex (Arbizu et al., 2016), which, in concert with data from type specimens (Martínez-Flores et al., 2016), redefined the species boundaries and nomenclature of this group. The focus of the present study is to expand further these studies by adding 40 accessions of taxa from 11 species and from areas not examined with these ten nuclear orthologues. We provide suggestions on further taxonomic research on Daucus and new taxonomic decisions and synonymies.

MATERIAL AND METHODS

ACCESSIONS EXAMINED

Previously sequenced amplicons were obtained from Arbizu et al. (2014a, 2016) and are listed in those publications. The 40 new accessions (Table 1) were obtained from (1) the Agrocampus Ouest – IRHS, France, (2) the Warwick Crop Centre, UK and (3) an expedition in Spain in 2016. Newly examined taxa are D. annuus (Bég.) Wojew., Reduron, Banasiak & Spalik (=Tornabenea annua Bég.), D. arcanus García Martín & Silvestre, D. decipiens (Schrad. & J.C. Wendl.) Spalik, Wojew., Banasiak & Reduron (=Melanoselinum decipiens) (Schrad. & J.C. Wendl.) Hoffm.), D. durieu Lange, D. edulis (Lowe) Wojew., Reduron, Banasiak & Spalik (=Monizia edulis Lowe), D. gracilis Steinh., D. minusculus Pau ex Font Quer (=Pseudorlaya minuscula) (Pau ex Font Quer) M. Lainz], D. montanus Humb. & Bonpl. ex Schult., D. pumilus (L.) Hoffmanns. & Link (=Pseudorlaya pumila (L.) Grandel], D. setifolius Desf. and D. tenuissimus (A.Chev.) Spalik, Wojew., Banasiak & Reduron (=Tornabenea tenuissima (A.Chev.) A. Hansen & Sunding). We lack vouchers for three accessions that grew as young plants sufficient to obtain DNA, but failed to grow to flowering stage as is typical for some Daucus sp. pl. that are biennial or have other problems relating to flowering in cultivation.

CHROMOSOME COUNTS

We obtained chromosome counts of all the species examined in this study from the literature using the Missouri Botanical Garden Index to Plant Chromosome Numbers (IPCN; Goldblatt & Johnson, 1979; (https://www.tropicos.org/Project/IPCN) (Table 2) and other sources from the literature on Daucus. We found chromosome number references for all examined species except for D. bicolor Sm., D. conchitae Greuter and D. gracilis.

DNA EXTRACTION AND SEQUENCE GENERATION

New accessions were grown in a greenhouse at the University of Wisconsin-Madison, tissue harvested and freeze-dried leaves, and DNA extracted with CTAB (Doyle & Doyle, 1990). For markers dc10366, dc10966, dc15347, dc16308, dc16577, dc3374, dc3902 and dc35097, 200 ng of DNA was amplified in 20 μL volume containing 1x Buffer, 1.5 mM MgCl₂, 0.2 mM dNTP (Promega Corporation, USA), 0.2 μM each forward and reverse primer and 1 U taq (GoTaq, Promega Corporation, USA) with the following program steps: 94 °C for 5 min, 35 cycles of 94 °C for 30 s, 55 °C for 1 min and 72 °C for 2 min, followed by 72 °C for 10 min. For markers dc16645 and dc32914, and any unamplified product from the previous eight markers, the magnesium concentration was doubled to 3 mM and the annealing step lowered from 55 °C to 50 °C. The forward and reverse strands were Sanger sequenced for each amplicon at the Biotechnology Center of the University of Wisconsin-Madison. Amplicons with SNP differences or an insertion or deletion, discovered during Sanger sequencing, were further processed with SSCP following Cai et al. (2012), using the MDE gel solution and 72 h run times.

PHYLOGENETIC ANALYSES

We deposited all sequences in GenBank (Supplementary File 1); sequences were assembled with PreGap4 and Gap4 (Staden, 1996) and aligned with MUSCLE v.3.8.31 (Edgar, 2004). Alignments were refined in Mesquite v.3.31 (Maddison & Maddison, 2017). A major analytical problem, most critical for analyses with...
Table 1. The 40 additional accessions of *Daucus* examined in this study, vouchers and locality information

<table>
<thead>
<tr>
<th>Species and GenBank number</th>
<th>Voucher</th>
<th>GenBank or new collections in 2016 (Spain)</th>
<th>Accession</th>
<th>Location</th>
</tr>
</thead>
<tbody>
<tr>
<td>Outgroup</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Athananta sicula</em> 1301</td>
<td>PTIS</td>
<td>France</td>
<td>France 1301</td>
<td>Morocco</td>
</tr>
<tr>
<td>Traditional <em>Daucus</em> ingroups</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Daucus arcanus</em> 33768 E-29</td>
<td></td>
<td></td>
<td>F. Martínez-Flores E-029</td>
<td>Spain, Ciudad Real, c. 25 km NW of Piedrabuena, top of hill with antenna, mountain Navalagruela, in fenced private area of Finca de Nuestra Señora del Rosario, S of CR-721, c. 500 m NNE of towers on dirt logging road</td>
</tr>
<tr>
<td><em>Daucus aureus</em> 33769</td>
<td>PTIS</td>
<td>Spain</td>
<td>Ames 33769</td>
<td>Spain: Sevilla: leaving Sanlúcar la Mayor, c. 10 km W of Sevilla, just W of town on S side of road, Rt A472</td>
</tr>
<tr>
<td><em>Daucus aureus</em> 33770</td>
<td>PTIS</td>
<td>Spain</td>
<td>Ames 33770</td>
<td>Spain: Córdoba: junction of gravel road and road from Santa Cruz and Espejo (N432), near salt evaporation ponds, 5 m SE of Santa Cruz</td>
</tr>
<tr>
<td><em>Daucus aureus</em> 33771</td>
<td>PTIS</td>
<td>Spain</td>
<td>Ames 33771</td>
<td>Spain: Córdoba: N of Baena on Rt 325 near junction of CO 284, adjacent to Guadajoz River</td>
</tr>
<tr>
<td><em>Daucus aureus</em> 33772</td>
<td>PTIS</td>
<td>Spain</td>
<td>Ames 33772</td>
<td>Spain: Jaén: near Laguna Honda, side of Vía Verde del Aceite</td>
</tr>
<tr>
<td><em>Daucus aureus</em> 33773</td>
<td>PTIS</td>
<td>Spain</td>
<td>Ames 33773</td>
<td>Spain: Jaén: on Rt. JV-3054, c. 600 m W of junction with Rt. A-316 at Estación de Begijar</td>
</tr>
<tr>
<td><em>Daucus guttatus</em> 1303</td>
<td>PTIS</td>
<td>France</td>
<td>France 1303</td>
<td>France. Sillans la Cascade</td>
</tr>
<tr>
<td><em>Daucus crinitus</em> 33833</td>
<td>PTIS</td>
<td>Spain</td>
<td>Ames 33833</td>
<td>Spain: Sevilla: leaving Sanlúcar la Mayor, c. 10 km W of Sevilla, just W of town on S side of road, Rt A472</td>
</tr>
<tr>
<td><em>Daucus crinitus</em> 33834</td>
<td>PTIS</td>
<td>Spain</td>
<td>Ames 33834</td>
<td>Spain: Córdoba: along road from Baena to Alcaudete (Rt. N-432), at junction of Rt. A-333, by bridge</td>
</tr>
<tr>
<td><em>Daucus crinitus</em> 33835</td>
<td>PTIS</td>
<td>Spain</td>
<td>Ames 33838</td>
<td>Spain: Ciudad Real: c. 10 km SW of Manzanares on CR-5212, at the spring of Siles</td>
</tr>
<tr>
<td><em>Daucus crinitus</em> 33836</td>
<td>PTIS</td>
<td>Spain</td>
<td>Ames 33836</td>
<td>Spain: Ciudad Real: along the roadside and up steep slope by road</td>
</tr>
<tr>
<td><em>Daucus crinitus</em> 33838</td>
<td>PTIS</td>
<td>Spain</td>
<td>Ames 33838</td>
<td>Spain: Badajoz: below Cornalvo dam, c. 15 km NE of Mérida</td>
</tr>
<tr>
<td><em>Daucus durieua</em> 33839</td>
<td>PTIS</td>
<td>Spain</td>
<td>Ames 33839</td>
<td>Spain: Ciudad Real: on dirt road 100 m N and also 1 km N of CM-413, c. 3.5 km W of Aldea del Rey</td>
</tr>
<tr>
<td><em>Daucus durieua</em> 33840</td>
<td>PTIS</td>
<td>Spain</td>
<td>Ames 33840</td>
<td>Spain: Ciudad Real: 3.5 km NE of Villamayor de Calatrava, then 1 km NW of road on the way to Volcán del Morrón de Villamayor</td>
</tr>
</tbody>
</table>
### Table 1. Continued

<table>
<thead>
<tr>
<th>Species and GenBank number</th>
<th>Voucher</th>
<th>GenBank or new collections in 2016 (Spain)</th>
<th>Accession</th>
<th>Location</th>
</tr>
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<tbody>
<tr>
<td><em>Daucus durieua</em> 33841</td>
<td>PTIS</td>
<td>Spain</td>
<td>Ames 33841</td>
<td>Spain. Ciudad Real: c. 2.3 km NW of outskirts of Piedrabuena, on Camino de la Reguerilla</td>
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<tr>
<td><em>Daucus durieua</em> 33842</td>
<td>PTIS</td>
<td>Spain</td>
<td>Ames 33842</td>
<td>Spain. Ciudad Real: c. 25 km NW of Piedrabuena, La Charca de las Colmenas, S of CR-721</td>
</tr>
<tr>
<td><em>Daucus durieua</em> 33845</td>
<td>PTIS</td>
<td>Spain</td>
<td>Ames 33845</td>
<td>Spain. Toledo: Rt. CM-5001, N of Talavera de la Reina, c. 1 km N of N end of San Román de los Montes</td>
</tr>
<tr>
<td><em>Daucus durieua</em> 33846</td>
<td>PTIS</td>
<td>Spain</td>
<td>Ames 33846</td>
<td>Spain. Toledo: hills above El Real de San Vicente, on Rt. TO-9045V</td>
</tr>
<tr>
<td><em>Daucus glochidiatus</em></td>
<td>HRIGRU 8251</td>
<td>England</td>
<td>HRIGRU 8251</td>
<td>Australia</td>
</tr>
<tr>
<td><em>Daucus gracilis</em></td>
<td>HRIGRU 6677</td>
<td>England</td>
<td>HRIGRU 6677</td>
<td>Morocco. Region Goumina: 18 km NE of Goumina on road to Ksar es Souk</td>
</tr>
<tr>
<td><em>Daucus montanus</em> 816</td>
<td>PTIS</td>
<td>France</td>
<td>France 816f</td>
<td>Chile. Juan Fernandez Islands, Camote</td>
</tr>
<tr>
<td><em>Daucus muricatus</em> 33850</td>
<td>PTIS</td>
<td>Spain</td>
<td>Ames 33850</td>
<td>Spain. Málaga: road from Álora to Carratraca (A7007), c. 5 km W of N end of Álora, c. 1 km W of km 4 road sign</td>
</tr>
<tr>
<td><em>Daucus muricatus</em> 33851</td>
<td>PTIS</td>
<td>Spain</td>
<td>Ames 33851</td>
<td>Spain. Málaga: c. 1 km up hill on unnamed farm road ascending mount El Hacho, off of road from Álora to Carratraca (A7007), beginning just E of Restaurant Los Conejitos</td>
</tr>
<tr>
<td><em>Daucus muricatus</em> 33852</td>
<td>Field photograph E5</td>
<td>Spain</td>
<td>Ames 33852</td>
<td>Spain. Cádiz: c. 1 km N of San Roque in fenced field just after going through gate, between Fuente Maria España and Arroyo (stream) de la Mujer</td>
</tr>
<tr>
<td><em>Daucus muricatus</em> 33853</td>
<td>PTIS</td>
<td>Spain</td>
<td>Ames 33853</td>
<td>Spain. Badajoz: below Cornalvo dam, c. 15 km NE of Mérida</td>
</tr>
<tr>
<td><em>Daucus setifolius</em> 33891 E-107</td>
<td>ABH78370</td>
<td>Spain</td>
<td>F. Martinez-Flores E-107</td>
<td>Spain. Málaga: along road from Estepona to Jubrique, between km 6–7 (at water collection station by road</td>
</tr>
<tr>
<td><em>Daucus junceus</em> 33890, E-101</td>
<td>ABH78369</td>
<td>Spain</td>
<td>Ames 33890</td>
<td>Spain. Huelva: Rd. N-435 c. 8 km N of La Nava</td>
</tr>
<tr>
<td><em>Daucus junceus</em> 33893 E-122</td>
<td>ABH56276</td>
<td>Spain</td>
<td>Ames 33893</td>
<td>Spain. Jaén: Rumblar river, next to Rd. JV-3151, near bridge (Puente del Rumblar), c. 700 m S of Zocueca</td>
</tr>
<tr>
<td><strong>Daucus ingroups recently transferred to Daucus</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Daucus decipiens = Melanoselinum decipiens</em> 1306</td>
<td>PTIS (photograph)</td>
<td>France</td>
<td>France 1306</td>
<td>Portugal. Quinta do Jardim da Serra, Madère</td>
</tr>
<tr>
<td><em>Daucus edulis = Monizia edulis</em> 1307</td>
<td>PTIS (photograph)</td>
<td>France</td>
<td>France 1307</td>
<td>Portugal. Madère</td>
</tr>
</tbody>
</table>

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multiple and highly unlinked nuclear orthologues, is how to align allelic data across marker data in concatenated analyses. There is no logical solution to concatenate such widely dispersed orthologue data unambiguously. We addressed this challenge by separating the alleles into two randomly chosen sets in concatenated matrices and examining each of these two datasets separately. As detailed below, maximum likelihood (ML) and maximum parsimony (MP) results were unambiguous in showing that the two analyses positioned all 124 accessions in nearly equal positions on the tree, except for the allopolyploid *D. montanus*, that in one set resolved with *D. durieua* and in the other set with *D. glochidiatus* (Labill.) Fisch., C.A.Mey. & Avé-Lall. Based on this result we analysed each of the 20 orthologue trees separately (ten for set A and ten for set B) and these individual analyses clearly showed the orthologues supporting an allopolyploid origin from *D. durieua* and *D. glochidiatus*. Only when *D. montanus* is included do we find internal conflict of A and B allele sets.

The aligned file A (see below) is deposited in TreeBASE (submission ID 32618). We rooted our trees on *Athananta sicula* L., based on Downie, Katz-Downie & Spalik (2000). MP analysis was conducted in PAUP* v.4.0a145 (Swofford, 2002). Missing data and gaps were all scored as missing data. All characters were treated as unordered and weighted equally (Fitch, 1971). The most parsimonious trees were found using a heuristic search (Farris, 1970) by generating 100 000 random taxon addition sequence replicates using tree-bisection reconnection (TBR) and holding one tree for each replicate. Then, we ran a final heuristic search of the most parsimonious trees from this analysis using TBR and MULPARS. Bootstrap values (Felsenstein, 1985) for the clades were estimated using 1000 replicates with a heuristic search, TBR and MULPARS, setting MAXTREES to 1000.

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**Table 1.** Continued

<table>
<thead>
<tr>
<th>Species and GenBank number</th>
<th>Voucher</th>
<th>GenBank or new collections in 2016 (Spain)</th>
<th>Accession&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Location</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Daucus minusculus</em> = <em>Pseudorlaya miniscula</em> 33854</td>
<td>PTIS</td>
<td>Spain</td>
<td>Ames 33854</td>
<td>Spain. Cádiz: Punta Paloma</td>
</tr>
<tr>
<td><em>Daucus minusculus</em> 33855</td>
<td>Field photograph E9, PTIS</td>
<td>Spain</td>
<td>Ames 33855</td>
<td>Spain. Cádiz: Cabo Roche, sand dunes about lighthouse</td>
</tr>
<tr>
<td><em>Daucus pumilus</em> = <em>Pseudorlaya pumila</em> 33856</td>
<td>PTIS</td>
<td>Spain</td>
<td>Ames 33856</td>
<td>Spain. Málaga: Beach Cabopino c. 10 km W of Fuengirola</td>
</tr>
<tr>
<td><em>Daucus pumilus</em> 33858</td>
<td>PTIS</td>
<td>Spain</td>
<td>Ames 33858</td>
<td>Spain. Cádiz: Beach Tarifa, c. 500 M N of harbor</td>
</tr>
<tr>
<td><em>Daucus pumilus</em> 33859</td>
<td>Field photograph E8, PTIS</td>
<td>Spain</td>
<td>Ames 33859</td>
<td>Spain. Cádiz: Punta Paloma</td>
</tr>
<tr>
<td><em>Daucus pumilus</em> 33861</td>
<td>Field photograph E13, PTIS</td>
<td>Spain</td>
<td>Ames 33861</td>
<td>Huelva. at the SE end of Matalascañas, on sand dunes</td>
</tr>
<tr>
<td><em>Daucus pumilus</em> 817</td>
<td>PTIS</td>
<td>France</td>
<td>France 817</td>
<td>France. Corcica, Corse Bonifacio</td>
</tr>
<tr>
<td><em>Daucus annua</em> = <em>Tornabenea annua</em> 819</td>
<td>PTIS</td>
<td>France</td>
<td>France 819</td>
<td>Cape Verde. Sao Tiago Malagueta</td>
</tr>
<tr>
<td><em>Daucus tenuissimus</em> = <em>Tornabenea tenuissima</em> 818</td>
<td>PTIS</td>
<td>France</td>
<td>France 818</td>
<td>Cape Verde. Fogo Chãdas Caldeiras</td>
</tr>
</tbody>
</table>

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<sup>a</sup>DNA sequences and locality data of the 85 accessions not listed here were obtained from Arbizu et al. (2014, 2016).

<sup>b</sup>Ames numbers are assigned for carrots and other Apiaceae in the US National Plant Germplasm System temporarily to newly acquired germplasm until passport data of an accession and taxonomy is verified. In addition, for accessions with Ames numbers it has to be determined that they are not duplicate accessions and that they can be successfully maintained. These accessions may or may not be assigned a PI number after the assessment period.
ML analysis was conducted via the CIPRES (Miller, Pfeiffer & Schwartz, 2010) portal at the San Diego Supercomputer Center (http://www.phylo.org) with the GTR + G nucleotide substitution model using RAxML v.8.2.4 (Stamatakis, 2014). The most common model of evolution for DNA analysis is general time-reversible (GTR) (Sumner et al., 2012) being the main reason only GTR-based models are implemented in RAxML (Stamatakis, 2014). We obtained the best-scoring ML tree from 100 independent ML trees.
searches, and then 1000 non-parametric bootstrap inferences were performed with the same program. As for MP, we rooted our tree on A. sicula. We also conducted a singular-value decomposition (SVD) quartets analysis using the multispecies coalescent model (Chifman & Kubatko, 2014, 2015) (to produce a lineage tree) with 100 bootstrap replicates, also using PAUP* v.4.0a131.

RESULTS

MAXIMUM Parsimony

There were one or two alleles for each of the ten nuclear orthologues in our new data. Some failed to amplify, with an average of 9.2% missing data across all markers (Supplementary File 2). Tree statistics for the four main analyses under MP (A allele and B allele, each with and without D. montanus) are presented in Supplementary File 3. The MP A (Supplementary File 4) and B set (Fig. 1) trees were extremely similar, except for D. montanus that in one set resolved in a clade with D. pusillus Michx. and in another with D. glochidiatus. Hence, we examined each of the ten genes separately for each set, with and without D. montanus (20 analyses in total), and found that the alleles of D. montanus resolved into different species (Supplementary File 5; Table 3), supporting an allopolyploid origin between D. pusillus and D. glochidiatus.

Figure 1 is of the B set run without D. montanus, which was then added manually relative to D. pusillus and D. glochidiatus. Relative to previous studies using these markers, Daucus resolves into two well-defined clades A and B, and the species with 2n = 18 chromosomes into subclade A’ (except for D. tenuissimus, 2n = 16). The newly examined taxa resolve in clade B as D. arcanus sister to D. pusillus and D. durieua sister to D. glochidiatus. In clade A, D. annuus, D. gracilis and D. tenuissimus resolve into clade A; D. decipiens, D. edulis, D. minusculus and D. pumilus (along with previously examined D. rouyi Spalik & Reduron) sister to clade A’ and D. setifolius sister to D. crinitus Desf. Daucus montanus (a hexaploid) is an apparent allopolyploid between D. pusillus (diploid) and D. glochidiatus (tetraploid). These results partly match the new sectional classification of Banasiak et al. (2016) except that the Macronesian endemics D. decipiens and D. edulis are embedded in clade A (their section Daucus), not as a separate early-diverging clade to clade A that they recognized as section Melanoselinum. The remaining species of clade B match the placement of their section Anisactis, but not with the same cladistic structure in these clades.

Figure 1 shows the chromosome numbers (Table 2) supporting clades. In some cases, there is chromosome number support for clades, for example D. arcanus and D. pusillus or D. aureus Desf. and D. muricatus L. In many other cases, there is poor association of chromosome numbers and clades.

MAXIMUM Likelihood

The ML tree of allele set B is presented in Supplementary File 6. Except for minor differences in the topology of some duplicate accessions in species exhibiting low bootstrap values in both ML and MP analyses (e.g. D. conchitae, D. guttatus), and in the topology of D. aureus and D. muricatus, there are no substantive differences between the ML and MP analyses. In the MP analysis D. aureus and D. muricatus are sister taxa with moderate (77%) bootstrap support, but in the ML analysis D. muricatus is sister to a clade containing D. aureus, other members of the A’ clade, D. rouyi, D. minusculus and D. pumilus.

SVD QUARTETS MULTISPECIES COALESCENT ANALYSIS

As molecular systematics progressed, multiple datasets using different genes or gene regions for the same accessions became common. It was soon discovered that the results typically showed some incongruence (Wendel & Doyle, 1998; Rokas et al., 2003). A number of solutions were advanced for this problem, one a total evidence analysis of a single concatenated dataset, which essentially joins all of the independent data together as a single locus to resemble one large supergene (Springer & Gatesy, 2016) as we perform here with ML and MP. Others, however, argued that incongruence was expected with a history of incomplete lineage sorting (e.g. Linz, Radtke & von Haeseler, 2007) and that the data should be analysed by multispecies coalescent procedures that took this into account (e.g. Edwards et al., 2016). Which procedure is more appropriate, or whether different procedures are appropriate for different groups is still a matter of debate, and we performed both here.

Figure 2 shows the SVD quartets multispecies coalescent results of allele set B run without D. montanus. Supplementary File 7 shows the allele set A and B trees with and without D. montanus and allele set B with D. montanus. In Figure 2, there are many points of concordance and discordance with the ML and MP analysis (these two treated here as a single largely concordant result). Although SVD maintains the same major clades (A, A’, B), there are many areas of disagreement of species within these clades. For example, ML and MP resolve D. montanus as an allopolyploid between D. pusillus and D. glochidiatus, whereas SVD supports D. glochidiatus as one parent, but the other allele is sister
to *D. pusillus*, *D. bicolor*, *D. guttatus* and *D. glochidiatus*. In clade A, ML and MP resolve *D. tenuisectus* as sister to *D. crinitus*, *D. junceus* comb. nov. and *D. setifolius*, but SVD as sister to all of clade A. Similar discordances occur between analyses of *D. decipiens* + *D. edulis* and *D. aureus* and *D. muricatus*. In clade B, between analyses discordances also occur with *D. pusillus* + *D. arcanus* and *D. durieua* + *glochidiatus* and indeed other groups of species. In addition, bootstrap support values throughout *Daucus* are in general reduced relative to ML and MP.

### DISCUSSION

*Daucus* has been the focus of numerous phylogenetic studies. Most of these have been based on one or few DNA regions, mainly nuclear internal transcribed spacer regions (nrITS) (Spalik & Downie, 2007; Spalik et al., 2010; Lee & Park, 2014; Banasiak et al., 2016) that produced trees greatly helping to redefine relationships in *Daucus* and outgroups, but sometimes with moderate to weak support for some terminals. Recently, phylogenetic trees constructed with single-to low-copy nuclear orthologous gene sequences (Arbizu et al., 2014a) have yielded phylogenetic trees with stronger support that are more consistent with morphological relationships among clades. In previous studies, incorrect identifications existed for members of the *D. guttatus* complex (including *D. bicolor*, *D. broteri* Ten., *D. guttatus* and *D. setulosus* Guss. ex DC.) which hindered the interpretation of trees based on morphology. Those misidentifications were corrected in Arbizu et al. (2016) and Martínez-Flores et al. (2016), and we here use this corrected nomenclature.

Previous studies found that members of *Daucus* s.l. are arranged into two well-supported (96–100% bootstrap) clades (hereafter referred to as clades A and B, with the species with 2n = 18 resolving into a subclade A'). Species in clade A were sister to the ‘Macaronesian endemics group’, section Melanoselinum (Spalik & Downie, 2007; Banasiak et al., 2016) including taxa usually ascribed to the genera *Cryptotaenia* DC., *Melanoselinum* and *Monizia*. These three genera are native to the Macaronesian region and have been classified in other genera due to morphological traits strikingly deviating from traditionally circumscribed *Daucus* sp. pl. (detailed below). Species in clade B are sister to members of *Agrocharis*, a genus native to central tropical Africa. According to Lee (2002), characters of fruit morphology of *Agrocharis* (i.e. primary ridges with numerous papillate hairs, well-developed secondary ridges and single-rowed stout spines with a glochidiate apex) support a close relationship to *Daucus*, although with some striking differences (i.e. petals yellow; yellowish-greenish or dark vs. white or pinkish; most spines of each ridge straight and retrorse) that allow easy separation from *Daucus* (Martínez-Flores, 2016). *Agrocharis* species are tetraploids with 2n = 44, a chromosome number infrequent in *Daucus* and related taxa that is shared only by *D. glochidiatus* (Constance, Chuang & Bell, 1976; Constance & Chuang, 1982; Iovene et al., 2008).

Banasiak et al. (2016) obtained the most complete phylogenetic trees to date for the expanded *Daucus* s.l. clade, with nuclear (nrITS) and three plastid markers. Comparative studies of concordance and discordance in phylogenetic trees built from various molecular markers (e.g. Wendel & Doyle, 1998) have shown that plastid phylogenetic trees are the most discordant relative to other molecular markers, for various reasons. Spooner et al. (2017) demonstrated that these nuclear and plastid markers sometimes produce incongruent results. Our present results show additional significant incongruences that we interpret with morphological, chromosome and biogeographic data as discussed below.

### CLADE B, *Daucus setulosus* AND *D. arcanus*

In clade B, most major subclades showed weak bootstrap support values in the combined nrDNA and plastid DNA tree of Banasiak et al. (2016). In our ML and MP phylogenetic trees, clade B is arranged into three subclades with strong support (BS ≥ 90%), but, as mentioned above, SVD trees often reveal different cladistic relationships in these clades, with lower bootstrap supports. According to Banasiak et al. (2016), *D. setulosus* (from Greece) is sister to the ‘guttatus-littoralis’ clade (from Israel and Egypt), but that relationship is weakly supported (BS = 46%). Conversely, our ML and MP results show *D. setulosus* as sister to the ‘arcanus-pusillus’ clade (from the Americas and the Iberian Peninsula) with a strong support (BS ≥ 90%). Although results by Banasiak et al. (2016) show low resolution for *D. setulosus*, some similarities are found with our tree, since their nrITS and combined plastid trees show the ‘arcanus-pusillus’ clade sister to a clade including the ‘setulosus’ plus the ‘guttatus-littoralis’ group (BS = 49%), and *D. setulosus* is again nested with the ‘arcanus-pusillus’ clade (BS = 31%) in their nrITS tree. Our SVD results, in contrast, resolve *D. setulosus* with *D. conchitae* and *D. littoralis*, but with low (52%) bootstrap support.

Separately, all three of our results highlight a close relationship between the Iberian *D. arcanus* and *D. pusillus*, previously noted by Lee & Park (2014) based on nrITS data. After studying the morphology of these taxa, and despite of the often smaller size of *D. arcanus* (Fig. 3), we assume that the Iberian species fits well within the wide morphological plasticity observed for *D. pusillus* throughout South
Gene sequences of allele set B, without, but with this species drawn in manually based on individual allele

Figure 1. Strict consensus maximum parsimony tree of 48 trees (tree length 8341) constructed with ten nuclear orthologous gene sequences of allele set B, without *Daucus montanus*, but with this species drawn in manually based on individual allele
Table 3. Summary of the cladistic placement of both alleles of *Daucus montanus* in the A and B random allele sets examined here.

<table>
<thead>
<tr>
<th>Marker and allele set</th>
<th>With all three <em>D. pusillus</em></th>
<th>With both <em>D. glochidiatus</em></th>
<th>With at least one <em>D. glochidiatus</em></th>
<th>Insufficient structure to determine</th>
<th>Not amplified in <em>D. montanus</em></th>
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<tbody>
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and North America. Thus, both taxa are treated here as conspecific, with *D. pusillus* being the name with priority. *Daucus arcanus* is only known from a few distant localities in southern Spain and Portugal (García-Martín & Silvestre, 1990; Martín-Blanco & Carrasco, 1997; Martínez-Flores, 2016; Porto & Pereira, 2018; E. Sánchez-Gullón, pers. comm.) (Fig. 4). The connection between the New World to the Iberian Peninsula remains unclear. The mericarps of *D. pusillus* (including *D. arcanus*) are the smallest in *Daucus* (Martínez-Flores, 2016) (Fig. 3B), and the fruits of *Daucus* are easily dispersed by wind and animals (Lacey, 1981; Okeke, 2015). Thus, the introduction of *D. arcanus* to the Iberian Peninsula could be the result of a long-distance dispersal similar to recent introductions of *D. glochidiatus* into Europe (Okeke, 2015), and the dispersal of *D. carota* to South Africa and Australia (Burtt, 1991; Groves, 2003); this dispersal mechanism is common in Apiaceae (Banasiak et al., 2013). The type locality of *D. arcanus* (Matalascañas, Doñana National Park) is c. 40 km south-west of the ancient harbour of Palos de Moguer, in Huelva province and c. 30 km north-west of Sanlúcar de Barrameda (the entry to the harbour of Seville through the Guadalquivir river), in the province of Seville (Spain). After 1492, the whole of this area received a large volume of maritime traffic from America, a fact that could explain the introduction of *D. pusillus* to southern Spain and Portugal.

CLADE B, *DAUCUS CONCHITAE AND D. INVOLUCRATUS* Sm.

The relationship between *D. conchitae* and *D. involucratus* is strongly supported in all our analyses (BS ≥ 90%) and that of Banasiak et al. (2016). Banasiak et al. (2016) found that the ‘conchitae-involucratus’ clade was closely related to *D. bicolor* (BS = 99%). These three species grow in Turkey. Our ML and MP results resolve the ‘conchitae-involucratus’ clade as sister to all other species in clade B, whereas *D. bicolor* is included in a strongly supported (BS ≥ 90%) wider clade also including *D. guttatus*, *D. littoralis* Sm., *D. durieua* and *D. glochidiatus*. Species in this wider clade mainly inhabit the eastern Mediterranean region (with *D. durieua* also present in the Iberian Peninsula and *D. glochidiatus* being endemic to Oceania). SVD, in contrast, resolved *D. conchitae + D. involucratus* as the weakly supported (52% bootstrap) sister group of *D. setulosus* (these three species growing along the lands surrounding the Aegean Sea).
Figure 2. Singular-value decomposition (SVD) quartets analysis using the multispecies coalescent model with 100 bootstrap replicates constructed with ten nuclear orthologous gene sequences of allele set B, without Daucus montanus.
**Clade B, Daucus Durieua and the Allopolyploid D. Montanus**

In Banasiak et al. (2016) and in all three analyses of our present study, as in other recent phylogenetic analyses, the position of D. durieua remains unclear. According to Banasiak et al. (2016), it is sister to the 'glochidiatus-montanus' clade in a weakly supported relationship (BS = 64%). Our results (Fig. 1) are similar, but our ML and MP evidence supports an allopolyploid origin of D. montanus from D. glochidiatus and D. pusillus. Daucus montanus and D. glochidiatus are morphologically similar (i.e. pollen shape and ornamentation, leaf indumentum, bract number, petal shape and size, mericarp length, style length, relative size of glochidia). Daucus montanus and D. pusillus also share several morphological characteristics such as petal shape and size, style length, anther size and pollen features (Sáenz, 1981; Okeke, 2015; Martínez-Flores, 2016). Daucus glochidiatus is found in Oceania and its chromosome number is 2n = 22. Finally, D. montanus occurs in Central and South America and its chromosome number is 2n = 22. The three species therefore share the basic number x = 11 (Constance et al., 1976; Iovene et al., 2008; Okeke, 2015). Spalink et al. (2010) suggested several dispersal events in Daucus from the Old World to the New World and, successively, a dispersal from South America to Australia (during the late Pliocene). Our ML and MP results highlight a relationship between the Australian D. glochidiatus and the American D. pusillus. Nevertheless, there are no records of D. glochidiatus today in South America where D. pusillus and D. montanus grow, so the likely mechanism of the hybrid origin of D. montanus involving these species is unclear and would remain entirely speculative. This hypothesis is better supported by our results than autopolyploidy in D. pusillus as suggested by Okeke (2015).

**Clade A – the Macaronesian taxa**

Clade A groups several Daucus sp. pl., encompassing the type of the genus, D. carota. As well, taxa traditionally included in other genera, such as Cryptotaenia, Melanoselinum, Monizia, Pseudorlaya and Tornabenea, also occur in clade A. According to the phylogenetic tree of Banasiak et al. (2016), the ‘Melanoselinum-Monizia’ clade is sister to the remaining species in clade A. The rest of that clade forms a group with weak support (BS = 67%), including Cryptotaenia elegans in a early-diverging position. These relationships remain unresolved in their plastid DNA trees.

Relative to Banasiak et al. (2016), our ML and MP results show a strongly supported ‘crinitus-setifolius-tenuisectus’ clade sister to the ‘Melanoselinum-Monizia’ clade and the remaining groups of clade A (Cryptotaenia was not analysed). The SVD results are quite different with D. tenuisectus as sister to all remaining species in clade A, and quite different interspecific relationships among the remaining species, but the Macaronesian species maintained in its own well-supported clades. These Macaronesian taxa show characters different from the classic concept of Daucus. All three produce mericarps without spines. Cryptotaenia elegans is distinguished by a reduced or absent involucre and mericarps lacking secondary ridges and trichomes on the primary ridges (Martínez-Flores, 2016). Its chromosome number 2n = 16 (Suda, Kyncl & Jarolimová, 2005) is only shared by both Pseudorlaya spp. Melanoselinum decipiens and Monizia edulis differ from the rest of species in several characteristics as long-life span, small tree-sized habit, long (7.6–16.8 mm) dorsally compressed mericarps with dorsal secondary ridges clearly smaller than the lateral ones and hypertrophied commissural vitae (Martínez-Flores, 2016).

**Clade A, Daucus Muricatus L. and D. Tenuisectus.**

In Banasiak et al. (2016), a ‘muricatus-tenuisectus’ clade (BS = 70% in their study) is sister to the remaining Daucus spp. in clade A, except for the ‘Macaronesian group’ being successive sister groups of the whole aggregate.

Conversely, our ML and MP results place D. tenuisectus in a strongly supported clade (92% BS) containing D. crinitus and D. setifolius (s.l.), whereas D. muricatus and D. aureus are sister in a separate moderately supported clade (Fig. 1). Our SVD analysis resolves D. tenuisectus as sister to the other species in clade A (Fig. 2).

Daucus aureus and D. muricatus have many morphological features suggesting they are sister, such as the extreme reduction of vitae, the ray indumentum, the reflexed bracts (Martínez-Flores, Juan & Crespo, 2012), the pollen ornamentation and the primary commissural ridges crowded together (Martínez-Flores, 2016). The position of D. tenuisectus, although strongly supported in our ML and MP analysis, is ambiguous in previous studies. The nrITS phylogenetic tree in Banasiak et al.
Figure 3. Specimen of *Daucus pusillus* (including *D. arcanus*) collected in Ciudad Real, Spain (ABH78367): A, habit; B, detail of fruiting umbels.
(2016) placed *D. tenuisectus* with *D. muricatus* in a weakly supported clade (BS = 60%). However, their plastid DNA data indicated that *D. tenuisectus* could be closer to *Daucus rouyi* Špalik & Reduron, *D. syrticus* and *D. carota*, although resolution is weak (BS = 54%). The most evident morphological features seem insufficient to clarify *D. tenuisectus* affinities, and further detailed studies are therefore needed.

**CLADE A – DAUCUS CRINITUS AND D. SETIFOLIUS**

Both Banasiak et al. (2016) and our phylogenetic analysis demonstrate that *D. crinitus* and *D. setifolius* are strongly supported sister taxa (BS ≥ 90%). These species show distinctive morphological characters from other *Daucus*. They are the only species to be polycarpic hemicryptophytes, producing leaves with filiform to narrow apparently verticillate (pseudoverticillate) segments, and producing mericarps elongate (up to 7.2–8.0 mm long; mean ratio length/width ± 4.3–4.9), with soft spines, glochidia reduced to a short straight apex and long styles (up to 3.5–3.9 mm) (Martínez-Flores, 2016). Both species are found in the western Mediterranean (Tunisia, Algeria, Morocco, Spain and Portugal; Fig. 5), and they share the diploid chromosome number 2n = 22 [Aparicio & Silvestre, 1985; Silvestre, 1993 (as ‘*D. brachylobus* Boiss.’)]. We here distinguish two distinct species formerly referred to as *D. setifolius* (Fig. 6). They have similar mericarps, but they can be easily differentiated by the indumentum of stems and leaf sheaths, the branching pattern and their allopatric distribution. The typical *D. setifolius* shows an indumentum of minute retrorse trichomes on the basal third of stem and leaf sheaths; it produces numerous long primary and secondary branches in a common Apiaceae branching pattern with long-pedunculate umbels (Fig. 6A, B), and grows in northern Morocco, Algeria and Tunisia, along the Atlas mountains, with only a single Spanish population in the western Penibetic mountains (Málaga province) (Fig. 5, squares). In contrast, *D. junceus* exhibits an indumentum of antrorse trichomes on the basal third
of stem and leaf sheaths (an exclusive character not found in any other representative of clade A), and it has short-pedunculate to sessile umbels, with few or no primary branches, forming a distinctive branching pattern in Daucus (Fig. 6C, D), resembling a species of Juncus L. That rush-like morphology led Willkomm (1851) to describe it as Durieua juncea.

According to our research, D. junceus is endemic to the south-western Iberian Peninsula along the river basins and surrounding mountains (Fig. 5, circles and asterisks). Our phylogenetic results are congruent with the recognition of two morphologically distinct species. The plastid DNA tree of Banasiak et al. (2016) also analysed D. junceus (Spanish D. setifolius 237 collected in Seville province) and is likewise separated from D. setifolius (Agrell 467 and Spanish G56 collected in Málaga province). These species were not clearly separated in their combined analysis, possibly due to plastid/nrITS incongruence, or different taxa analysed for plastid DNA and nrITS.

Clade A, Daucus main group plus clade A’

Regarding the remaining groups in clade A, our phylogenetic analysis coincides with results in Banasiak et al. (2016). The ‘Pseudolaya clade’ (D. minusculus and D. pumilus) is strongly supported (BS ≥ 90%) and sister to the remaining clades. Both of these species have a distinctive morphological syndrome: annual habit, zygomorphic umbels (both involucres and rays arrangement), short styles (0.4–0.9 mm), mericarps with two rows of spines per ridge and sparse indumentum in primary commissural ridges (0–5 trichomes/mm) (Martínez-Flores, 2016) and they share the same chromosome number 2n = 16 (García-Martín & Silvestre, 1985; Vogt & Oberprieler, 1994; Mohamed, 1997).

Daucus rouyi is placed between the ‘Pseudolaya clade’ and the ‘Daucus main group’ plus clade A’. Daucus rouyi formerly was the sole member of a monotypic genus, Rouya, found in a small area in northern Corsica, Sardinia, north-eastern Algeria and northern Tunisia. It is characterized by its polycarpic chamaephytic habit, the small erect petals (±1.3 mm), the widely winged mericarps (1.9–3.1 mm width), lack of spines on secondary ridges and lack of trichomes on primary ridges (Martínez-Flores, 2016) and a chromosome number 2n = 20 (Constance et al., 1976). The remaining Daucus sp. pl. (clade A’) share a chromosome number 2n = 18 (Owens, 1974; Grosso et al., 2008; Iovene et al., 2008), patent petals usually longer than 1.3 mm, mostly spiny and not clearly winged secondary ridges, primary ridges with indumentum and according to our molecular phylogenetic studies are nested in a strongly supported clade (BS ≥ 90%). Daucus gracilis (unknown chromosome number) is sister to these species, whereas D. syriicus Murb. is sister to the ‘carota-Tornabenea’ (D. annuus and D. tenuissimus) clade. The former genus Tornabenea includes several species endemic of Cape Verde, which are characterized mainly by fruits with narrowly ‘winged’ secondary ridges. However, Martínez-Flores (2016) found that morphological characters in Tornabenea such as the mericarp size, vitae shape, style length and pollen size and ornamentation, are similar to those in D. carota, and even extremely reduced spines can be observed on the mericarp narrow ‘wings’, as in some taxa in the D. carota complex (Pujadas-Salvá, 2003). Different chromosome numbers were reported within these species. Brochman et al. (1997) and Grosso et al. (2008) indicated 2n = 18 for D. annuus and D. insularis [=T. insularis (Parl.) Parl.], and Bramwell & Murray (1972) reported 2n = 18 for T. hirta J.A.Schmidt (no name in Daucus to date). However, Borgen (1980) reported 2n = 16 for T. hirta and Borgen (1974) indicated the same number for D. tenuissimus, whereas Bramwell & Murray (1972) found 2n = 22 in T. bischoffii J.A.Schmidt (no name in Daucus to date). According to our results, 2n = 18 is congruent with the former members of Tornabenea closely related to Daucus carota, and, based on those facts, some of those species may be included in Daucus, as formally transferred by Banasiak et al. (2016). In summary, notable discrepancies exist in the taxonomy of these former members of Tornabenea, both in morphology (Grosso et al., 2008) and in chromosome numbers (Bramwell & Murray, 1972; Borgen, 1974, 1980; Brochman et al., 1997; Grosso et al., 2008). No molecular and morphological revision of these species is available, and they are in need of further detailed analyses.

Nomenclatural proposals

Daucus setifolius Desf., Fl. Atlant. 1: 244, t. 65. 1798


Lectotype (designated here): [ALGERIA]. Prope Mascar in collibus incultis, Desfontaines (P-00320312!). Isolectotypes: G-00023270 (digital image!), MPU-021024 (digital image!). Note: previous indication of ‘holotype’ in the electronic publication
of Okeke's (2015) PhD thesis should be regarded as 'lectotype', but such typification is not effective according to Art. 29.1 of the ICN (Turland et al., 2018) since that publication lacks ISBN, ISSN or doi number. The lectotype designated here is a specimen from Desfontaines's 'Herbier de la Flore Atlantique' among the historical collections at P.

**Ind. loc.:** ‘Habitat prope Mascar in collibus incultis' [Algeria]

**Brief description:** polycarpic hemicryptophyte; stems minutely pubescent in the basal third, with retrorse trichomes; primary and secondary stem branches numerous and elongated, with long-pedunculate umbels; leaves with basal sheath covered with retrorse trichomes, and with filiform to narrow segments apparently verticillate (pseudoverticillate); mericarps elongate cylindric-ovoid, mostly covered with a dense indumentum, secondary ridges with soft spines and glochidia reduced to a short straight apex, styles long, vittae subtriangular in section.

**Chromosome number:** $2n = 22$ [$n = 11$; Silvestre, 1993 (as *D. brachylobus* Boiss.).]

**Ecology:** degraded brushwood areas, often in rocky places. Usually on ultrabasic substrates (i.e. peridotites, serpentines), but sometimes on acid substrates (i.e. basalts, sandstones).

**Distribution:** Morocco, northern Algeria and northern Tunisia along the Atlas Mountains, with only a relict Spanish population in the western Penibetic Mountains (Málaga province).

**Selected specimens:** see Appendix.

**DAUCUS JUNCEUS (WILLK.) MART.FLORES & M.B.CRESPO, COMB. NOV.**

*Durieua juncea* Willk. in Flora 34: 711. 1851 [basionym]

*Lectotype (designated here):* [SPAIN]. “76. d. (coll. sel.) / Durieua ?” [cross outs literal transcription of the label]
Figure 6. Specimens of *Daucus junceus* and *D. setifolius*. *Daucus setifolius* A, herbarium specimen ABH78370 collected in Málaga, Spain; B, natural population of *D. setifolius* from Málaga, Spain, accession Martínez-Flores et al. E-107; *D. junceus* C, herbarium specimen ABH53906 collected in Seville, Spain; D, natural population of *D. junceus* from Jaén, Spain, accession Ames 33893.
Diagnosis: similar to D. setifolius from which it differs mainly by the stems covered for the basal third with antrorse trichomes, identical to those coating the leaf sheaths (an exclusive character not found in any other representative of clade A) and by the primary branches absent or scarce, bearing subsessile to shortly pedunculate umbels (often several small umbels grouped together).

Chromosome number: 2n = 22 [n = 11; Aparicio & Silvestre, 1985 (as ‘D. setifolius’)].

Ecology: roadsides, borders of fields and degraded brushwood areas, often near rivers. Usually on acid substrates (i.e. granites, schists etc.).

Distribution: endemic to the south-western Iberian Peninsula along the river basins (Guadalquivir, Guadiana, Tajo) and surrounding mountains. Only one population had been reported to the north of that indicated area (Almeida et al., 2009), but that population was destroyed during the construction of a dam (C. Aguiar, pers. comm.).

Selected specimens: see Appendix.

DAUCUS PUSILLUS Michx., Fl. Bor.-Amer. 1: 164. 1803

Babiron pusillus (Michx.) Raf., New Fl. 4: 23. 1836.
Daucus brevifolius Raf., New Fl. 4: 26. 1836.
Daucus hispidifolius Clos, Fl. Chil. 3(2): 135. 1848.
Daucus scaber Larrañaga, Escritos Damaso Antonio Larrañaga 2: 113. 1923, nom. illeg.
Daucus scadiophylus Raf., New Fl. 4: 24. 1838.

Lectotype (designated here): [USA]. ‘Daucus pusillus’ / in sabulosis / Caroliniae (P-00320342!). Note: previous indication of ‘holotype’ in the electronic publication of Okeke’s (2015) PhD thesis should be regarded as ‘lectotype’, but such typification is not effective according to Art. 29.1 of the ICN (Turland et al., 2018) since that publication lacks ISBN, ISSN or doi number. Furthermore, the Canadian voucher selected by that author, ‘In America bor. occid. ad Nootka-Soud’, ‘Haenke (G-DC)’, is not acceptable since it cannot be regarded as original material of D. pusillus (Michaux, 1803). The lectotype designated here is a specimen from ‘Herbier de l’Amérique septentrionale d’André Michaux’ at P, collected in Carolina and matching with the protologue of that name.

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APPENDIX

Localities of selected specimens of Daucus pusillus (Fig. 4) and of D. junceus and D. setifolius (Fig. 5).

DAUCUS JUNCEUS (WILLK.) MART. FLORES & M.B. CRESPO

PORTUGAL. Beja: Moura, matorral exposición N, junto a ribera del Arilda, 14.x.1994, M. Lousã et al. (LISI596); Moura, ribera de Ardila, 107 m, 12.x.2009, J. Calvo & S. Hantson #JC4246 (MA794634); Estimated coordinates: 37°27′14.1″N 8°42′53.5″W, 37°42′33.5″N 8°59′46.6″W, 38°11′17.1″N 8°11′39.8″W and 38°11′40.4″N 7°07′25.5″W, 38°04′04.8″N 8°36′11.6″W, M. Porto, F. Clamote, A.J. Pereira, U. Schwarzer. Daucus setifolius Desf. – mapa de distribuição. Flora-On: Flora de Portugal Intercativa, Sociedade Portuguesa de Botânica. http://www.flora-on.pt/#wDaucus+setifolius [accessed 22/08/2018];

DAUCUS PUSILLUS Michx. (incl. D. ARCANUS
GARCÍA-MARTÍN & SILVESTRE)

SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

**Supplementary File 1.** GenBank deposition numbers.
**Supplementary File 2.** Number of alleles in each of the ten nuclear orthologous gene sequences examined here.
**Supplementary File 3.** Maximum parsimony tree statistics for the four main analyses (A and B set allele trees, with and without *Daucus montanus*) conducted here.
**Supplementary File 4.** The eight A and B set allele trees (equally parsimonious and majority rule strict consensus) constructed with ten nuclear orthologous gene sequences.
**Supplementary File 5.** The individual nuclear orthologue bootstrap consensus trees of the A allele set (ten trees) and B allele set (ten trees; 20 trees in total).
**Supplementary File 6.** The maximum likelihood tree constructed with ten nuclear orthologous gene sequences of allele set B, without *Daucus montanus*. Bootstrap support values are given above the branches.
**Supplementary File 7.** The allele set A and B SVD trees with and without *D. montanus* and allele set B with *D. montanus*.