

# 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.

KEYWORDS: carrot – Daucinae – germplasm – nomenclature – phylogeny – taxonomy – typification.

## 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. Spalik & 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); *Pachytenium*

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Maire & Pamp. (one species); *Pseudorlaya* (Murb.) Murb. (two species); *Rouya Coincy* (one species); *Tornabenea* Parl. (six species); *Athamanta dellacellae* 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. durieua* 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.Laínz], *D. montanus* Humb. & Bonpl. ex Schult., *D. pumilus* (L.) Hoffmanns.

& Link [≡*Pseudorlaya pumila* (L.) Grande], *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 1× Buffer, 1.5 mM MgCl<sub>2</sub>, 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<sup>a</sup>

Species and GenBank number	Voucher	GenBank or new collections in 2016 (Spain)	Accession <sup>b</sup>	Location
<b>Outgroup</b>				
<i>Athamanta sicula</i> 1301	PTIS (photograph)	France	<i>France 1301</i>	Morocco
<b>Traditional <i>Daucus</i> ingroups</b>				
<i>Daucus arcanus</i> 33768 E-29	ABH 78367	Spain	<i>F. Martínez-Flores E-029</i>	Spain, Ciudad Real, c. 25 km NW of Piedrabuena, top of hill with antenna, mountain Navalagrulla, 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
<i>Daucus aureus</i> 33769	PTIS	Spain	<i>Ames 33769</i>	Spain. Sevilla: leaving Sanlúcar la Mayor, c. 10 km W of Sevilla, just W of town on S side of road, Rt A472
<i>Daucus aureus</i> 33770	PTIS	Spain	<i>Ames 33770</i>	Spain. Córdoba: junction of gravel road and road from Santa Cruz and Espejo (N432), near salt evaporation ponds, 5 m SE of Santa Cruz
<i>Daucus aureus</i> 33771	PTIS	Spain	<i>Ames 33771</i>	Spain. Córdoba: N of Baena on Rt 325 near junction of CO 284, adjacent to Guadajoz River
<i>Daucus aureus</i> 33772	PTIS	Spain	<i>Ames 33772</i>	Spain. Jaén: near Laguna Honda, side of Vía Verde del Aceite
<i>Daucus aureus</i> 33773	PTIS	Spain	<i>Ames 33773</i>	Spain. Jaén: on Rt. JV-3054, c. 600 m W of junction with Rt. A-316 at Estación de Begíjar
<i>Daucus guttatus</i> 1303		France	<i>France 1303</i>	France. Sillans la Cascade
<i>Daucus crinitus</i> 33833	PTIS (photograph)	Spain	<i>Ames 33833</i>	Spain. Sevilla: leaving Sanlúcar la Mayor, c. 10 km W of Sevilla, just W of town on S side of road, Rt A472
<i>Daucus crinitus</i> 33834	PTIS	Spain	<i>Ames 33834</i>	Spain. Córdoba: along road from Baena to Alcaudete (Rt. N-432), at junction of Rt. A-333, by bridge
<i>Daucus crinitus</i> 33835	PTIS (photograph)	Spain	<i>Ames 33835</i>	Spain. Ciudad Real: c. 10 km SW of Manzanares on CR-5212, at the spring of Siles
<i>Daucus crinitus</i> 33836	PTIS (photograph)	Spain	<i>Ames 33836</i>	Spain. Ciudad Real: along the roadside and up steep slope by road
<i>Daucus crinitus</i> 33838	PTIS	Spain	<i>Ames 33838</i>	Spain. Badajoz: below Cornalvo dam, c. 15 km NE of Mérida
<i>Daucus durieua</i> 33839	PTIS	Spain	<i>Ames 33839</i>	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
<i>Daucus durieua</i> 33840	PTIS	Spain	<i>Ames 33840</i>	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

**Table 1.** Continued

Species and GenBank number	Voucher	GenBank or new collections in 2016 (Spain)	Accession <sup>b</sup>	Location
<i>Daucus durieua</i> 33841	PTIS	Spain	<i>Ames 33841</i>	Spain. Ciudad Real: c. 2.3 km NW of outskirts of Piedrabuena, on Camino de la Reguerilla
<i>Daucus durieua</i> 33842	PTIS	Spain	<i>Ames 33842</i>	Spain. Ciudad Real: c. 25 km NW of Piedrabuena, La Charca de las Colmenas, S of CR-721
<i>Daucus durieua</i> 33845	PTIS	Spain	<i>Ames 33845</i>	Spain. Toledo: Rt. CM-5001, N of Talavera de la Reina, c. 1 km N of N end of San Román de los Montes
<i>Daucus durieua</i> 33846	PTIS	Spain	<i>Ames 33846</i>	Spain. Toledo: hills above El Real de San Vicente, on Rt. TO-9045V
<i>Daucus glochidiatus</i> HRIGRU8251		England	HRIGRU 8251	Australia
<i>Daucus gracilis</i> HRIGRU6677		England	HRIGRU 6677	Morocco. Region Goumina: 18 km NE of Goumina on road to Ksar es Souk
<i>Daucus montanus</i> 816	PTIS	France	<i>France 816f</i>	Chile. Juan Fernandez Islands, Camote
<i>Daucus muricatus</i> 33850	PTIS	Spain	<i>Ames 33850</i>	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
<i>Daucus muricatus</i> 33851	PTIS	Spain	<i>Ames 33851</i>	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
<i>Daucus muricatus</i> 33852	Field photograph E5	Spain	<i>Ames 33852</i>	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
<i>Daucus muricatus</i> 33853	PTIS	Spain	<i>Ames 33853</i>	Spain. Badajoz: below Cornalvo dam, c. 15 km NE of Mérida
<i>Daucus setifolius</i> 33891 E-107	ABH78370	Spain	<i>F. Martínez-Flores E-107</i>	Spain. Málaga: along road from Estepona to Jubrique, between km 6–7 (at water collection station by road)
<i>Daucus junceus</i> 33890, E-101	ABH78369	Spain	<i>Ames 33890</i>	Spain. Huelva: Rd. N-435 c. 8 km N of La Nava
<i>Daucus junceus</i> 33893 E-122	ABH56276	Spain	<i>Ames 33893</i>	Spain. Jaén: Rumblar river, next to Rd. JV-3151, near bridge (Puente del Rumblar), c. 700 m S. of Zocueca
<b><i>Daucus</i> ingroups recently transferred to <i>Daucus</i></b>				
<i>Daucus decipiens</i> = <i>Melanoselinum decipiens</i> 1306	PTIS (photograph)	France	<i>France 1306</i>	Portugal. Quinta do Jardim da Serra, Madère
<i>Daucus edulis</i> = <i>Monizia edulis</i> 1307	PTIS (photograph)	France	<i>France 1307</i>	Portugal. Madère

Table 1. Continued

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

<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.

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 *Athamanta 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.

**Table 2.** Chromosome numbers of the species examined here. We found no references for chromosome numbers for *Daucus bicolor*, *D. conchitae* and *D. gracilis*

Taxon	2n	References and available data for the specimens
<i>Athamanta sicula</i>	22	Colombo & Marcenó (1989), Italy; Shner, Alexeeva & Pimenov (2018), Italy.
<i>Daucus arcanus</i>	22	García-Martín & Silvestre (1990), Spain.
<i>Daucus aureus</i>	22	Constance <i>et al.</i> (1976); Silvestre (1986), Spain, (Seville, SEVF Cuesta de las Doblás).
<i>Daucus capillifolius</i>	18	Iovene <i>et al.</i> (2008), Libya (NPGS PI 279764).
<i>Daucus carota s.l.</i>	18	Gadella & Kliphuis (1966), Holland; Queirós (1974), Portugal; cf. Okeke (2015), Lago & Castroviejo (1993), Spain, (Santander); Iovene <i>et al.</i> (2008), several localities.
<i>Daucus crinitus</i>	22	Aparicio & Silvestre (1985), Spain (SEV 47250); Iovene <i>et al.</i> (2008), Portugal (NPGS PI 652413).
<i>Daucus decipiens</i> = <i>Melanoselinum decipiens</i>	22	Bell & Constance (1966), -.
<i>Daucus durieua</i>	20	Kapoor & Löve (1969), Spain (Palas del Rey); Queirós (1974), Portugal (Bragança).
	22	Owens (1974), <i>ut D. subsessilis</i> , Israel (Negev, Hinket Coll.); Silvestre (1986), Spain, (Barcelona, SEVF Tibidabo; Cádiz, SEVF 39806); Luque & Díaz-Lifante (1991), Spain (Granada, Jerez del Marquesado).
<i>Daucus edulis</i> = <i>Monizia edulis</i>	22	Dalgaard (1991), -.
<i>Daucus glochidiatus</i>	44	Constance <i>et al.</i> (1976), Australia; Iovene <i>et al.</i> (2008), Australia.
<i>Daucus guttatus</i>	20	Vogt & Aparicio (1999), Cyprus (Ayia Anna, UPA number Rec.It.: 0152); Iovene <i>et al.</i> (2008), <i>ut D. broteri</i> , Siria (NPGS PI 652342).
<i>Daucus involucratus</i>	22	Vogt & Aparicio (1999), Cyprus (Ayia Anna, MA495558).
<i>Daucus junceus</i>	22	Aparicio & Silvestre (1985), <i>ut D. setifolius</i> Spain, Seville (SEV 42820);
<i>Daucus littoralis</i>	20	Iovene <i>et al.</i> (2008), Israel (NPGS PI 341902).
<i>Daucus minusculus</i> = <i>Pseudorlaya minuscula</i>	16	Vogt & Oberprieler, (1994), -.
<i>Daucus montanus</i>	66	Constance <i>et al.</i> (1976), several localities; Iovene <i>et al.</i> (2008), <i>ut D. hispidifolius</i> Clos., Chile (HRI 7189).
<i>Daucus muricatus</i>	22	Queirós (1974), Portugal (several localities); Vogt & Oberprieler, (1994), -.
<i>Daucus pumilus</i> = <i>Pseudorlaya pumila</i>	16	García-Martín & Silvestre (1985), Spain (SEVF Chipiona); Ruíz de Clavijo (1994), Spain (Huelva COFC Isla Saltés); Mohamed (1997), Egypt.
<i>Daucus pusillus</i>	22	Constance <i>et al.</i> (1976), several localities; Iovene <i>et al.</i> (2008), Argentina and USA.
<i>Daucus rouyi</i> = <i>Rouya polygama</i>	20	Constance <i>et al.</i> (1976), -.
<i>Daucus setifolius</i>	22	Silvestre (1993), <i>ut D. brachylobus</i> Boiss., Spain (SEVF Jubrique- Estepona).
<i>Daucus setulosus</i>	20	Iovene <i>et al.</i> (2008), <i>ut D. guttatus</i> , Greece (NPGS PI 652326).
<i>Daucus syrticus</i>	18	Owens (1974), -.
<i>Daucus tenuisectus</i>	22	Constance <i>et al.</i> (1976), Morocco?
<i>Daucus</i> ( <i>Tornabenea</i> sp. pl.)	16	<i>T. tenuissima</i> : Borgen (1974), Cape Verde (Sao Nicolau); <i>T. hirta</i> : Borgen (1980), Cape Verde (Sao Nicolau).
	18	<i>T. hirta</i> : Bramwell & Murray (1972), Cape Verde (Santiago); <i>T. annua</i> and <i>T. insularis</i> : Brochman <i>et al.</i> (1997), Cape Verde; Ídem: Grosso <i>et al.</i> (2008), Cape Verde.
	22	<i>T. bischoffii</i> : Bramwell & Murray (1972), Cape Verde (Santo Antao).
<i>Orlaya daucoides</i>	16	Silvestre (1978), <i>ut O. kochii</i> Heywood, Spain (SEV27540, SEV27542, SEV27543); Engstrand (1970), Turkey.

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 tree

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).

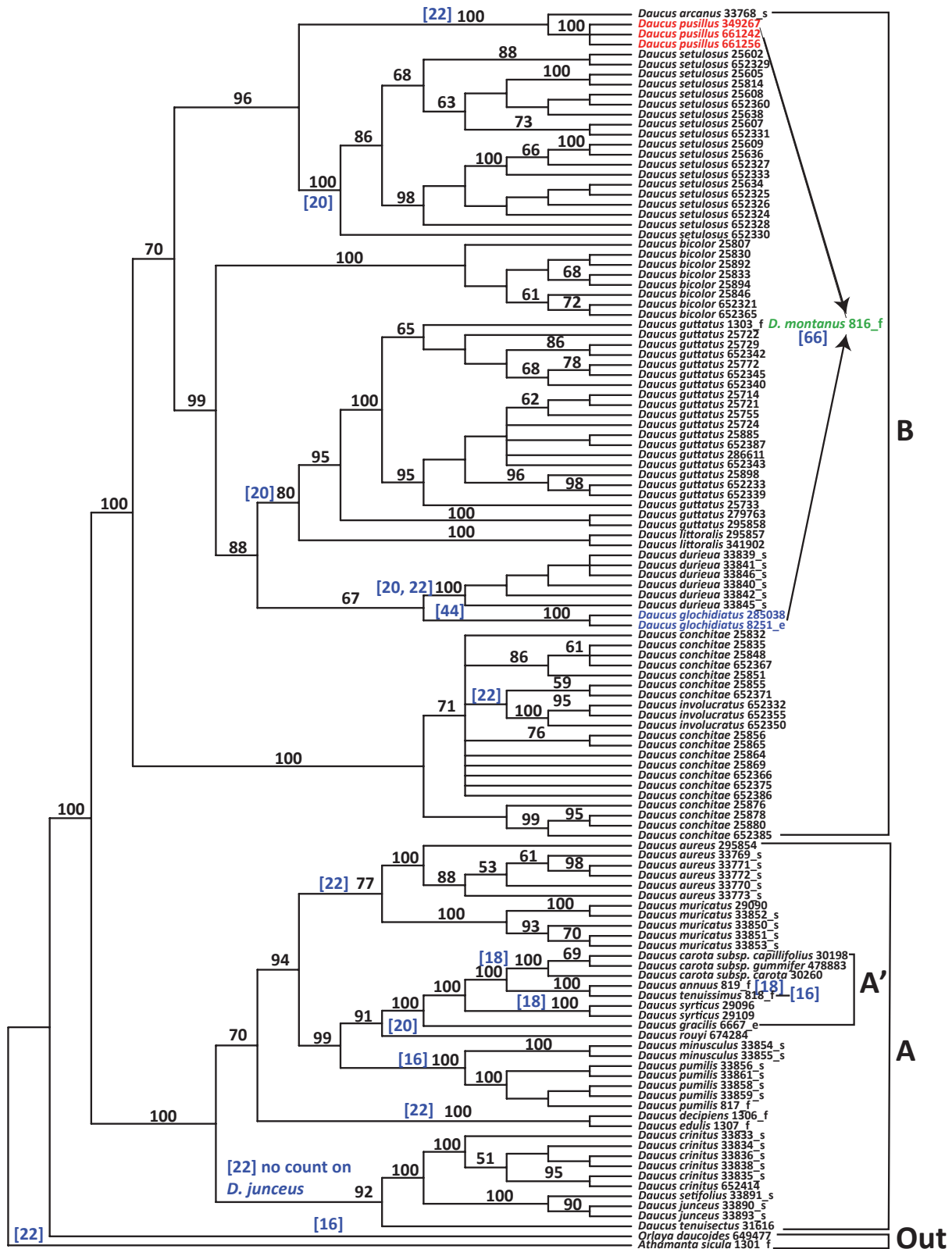
Banasiak *et al.* (2016) obtained the most complete phylogenetic trees to date for the expanded *Daucus s.l.* clade, with nuclear (nrITS) and three plastid markers. Comparative studies of concordance and discordance in phylogenetic trees built from various molecular markers (e.g. Wendel & Doyle, 1998) 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  $\geq 90\%$ ), 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  $\geq 90\%$ ). 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





**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

Marker and allele set	With all three <i>D. pusillus</i>	With both <i>D. glochidiatus</i>	With at least one <i>D. glochidiatus</i>	Insufficient structure to determine	Not amplified in <i>D. montanus</i>
3374 A set			X		
3374 B set		X			
3902 A set	X				
3902 B set	X				
10366 A set			X		
10366 B set		X			
10966 A set				X	
10966 B set				X	
15347 A set					X
15347 B set					X
16308 A set	X				
16308 B set		X			
16577 A set	X				
16577 B set		X			
16645 A set	X				
16645 B set		X			
32914 A set	X				
32914 B set	X				
35097 A set				X	
35097 B set	X				

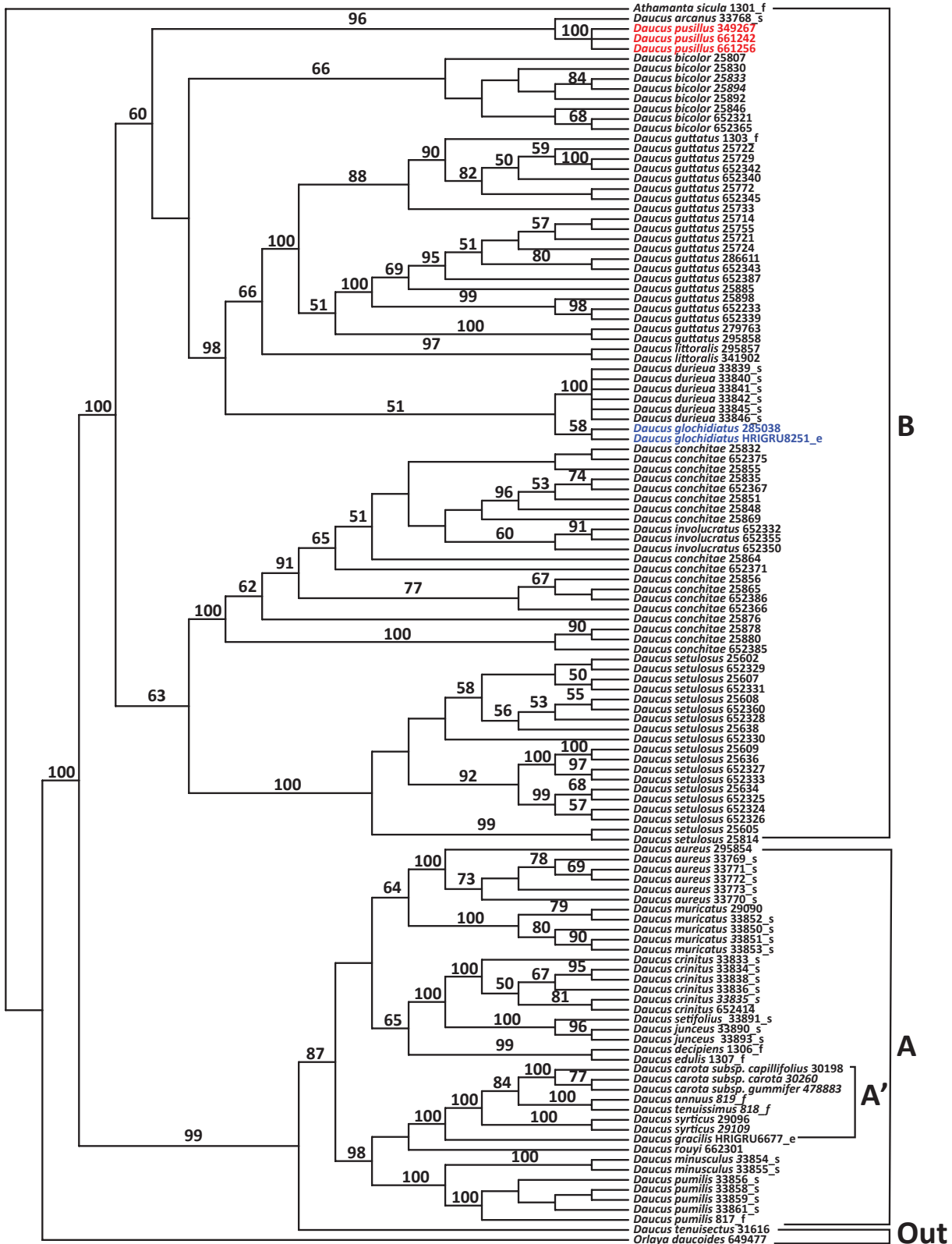
and North America. Thus, both taxa are treated here as conspecific, with *D. pusillus* being the name with priority. *Daucus arcanus* is only known from a few distant localities in southern Spain and Portugal (García-Martín & Silvestre, 1990; Martín-Blanco & Carrasco, 1997; Martínez-Flores, 2016; Porto & 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 (Burt, 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).

results supporting it as an allopolyploid species between *D. pusillus* and *D. glochidiatus*. Chromosome numbers supporting these clades (Table 2) are shown in blue bracketed type and bootstrap support values in black non-bracketed type.



**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  $2n = 44$ , whereas *D. pusillus* inhabits North and South America and its chromosome number is  $2n = 22$ . Finally, *D. montanus* occurs in Central and South America and its chromosome number is  $2n = 66$ . The three species therefore share the basic number  $x = 11$  (Constance *et al.*, 1976; Iovene *et al.*, 2008; Okeke, 2015). Spalik *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 & Jarolímová, 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).

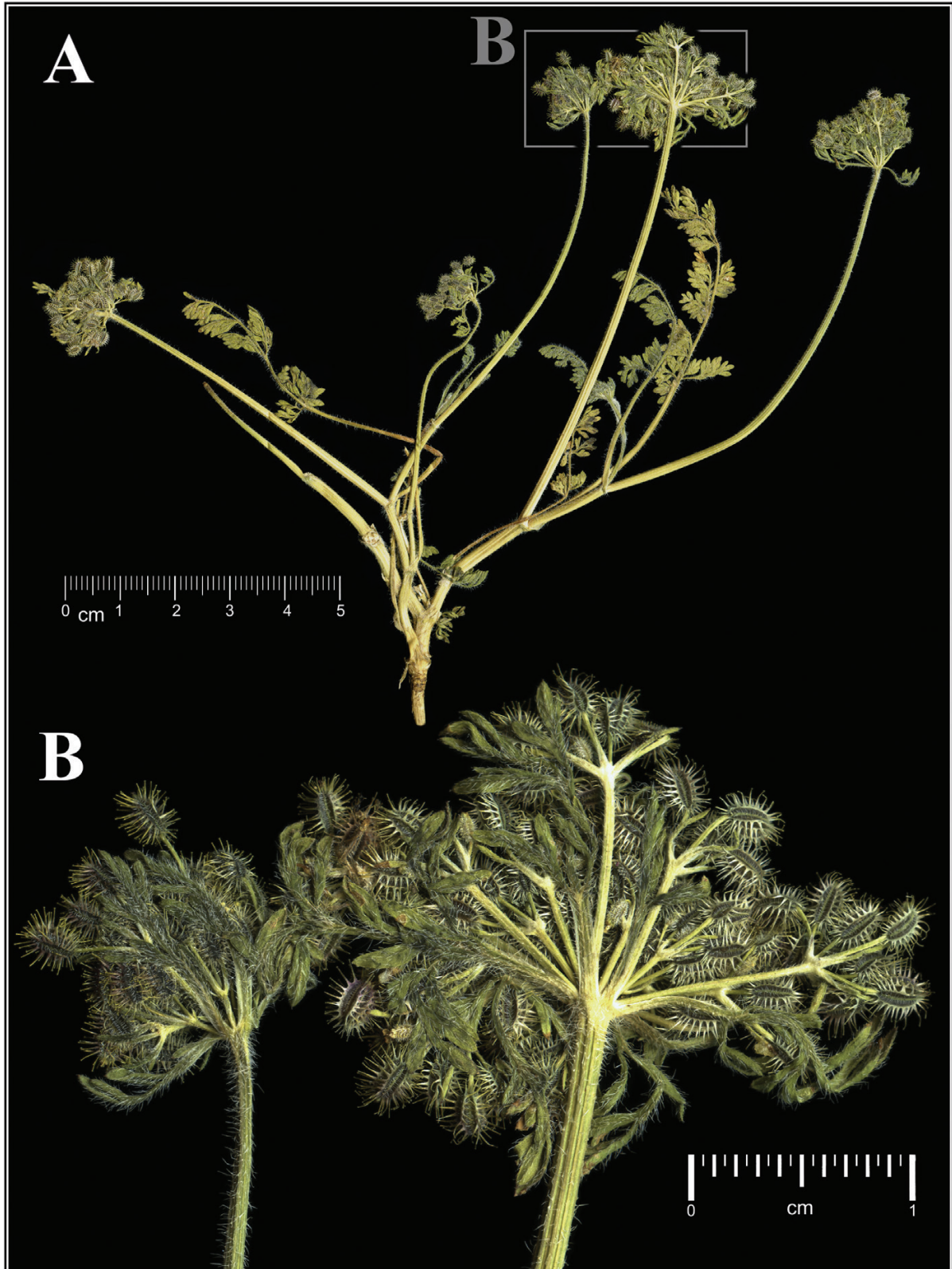
CLADE A, *DAUCUS MURICATUS* L. AND  
*D. TENUISECTUS* COSS. EX BATT.

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.

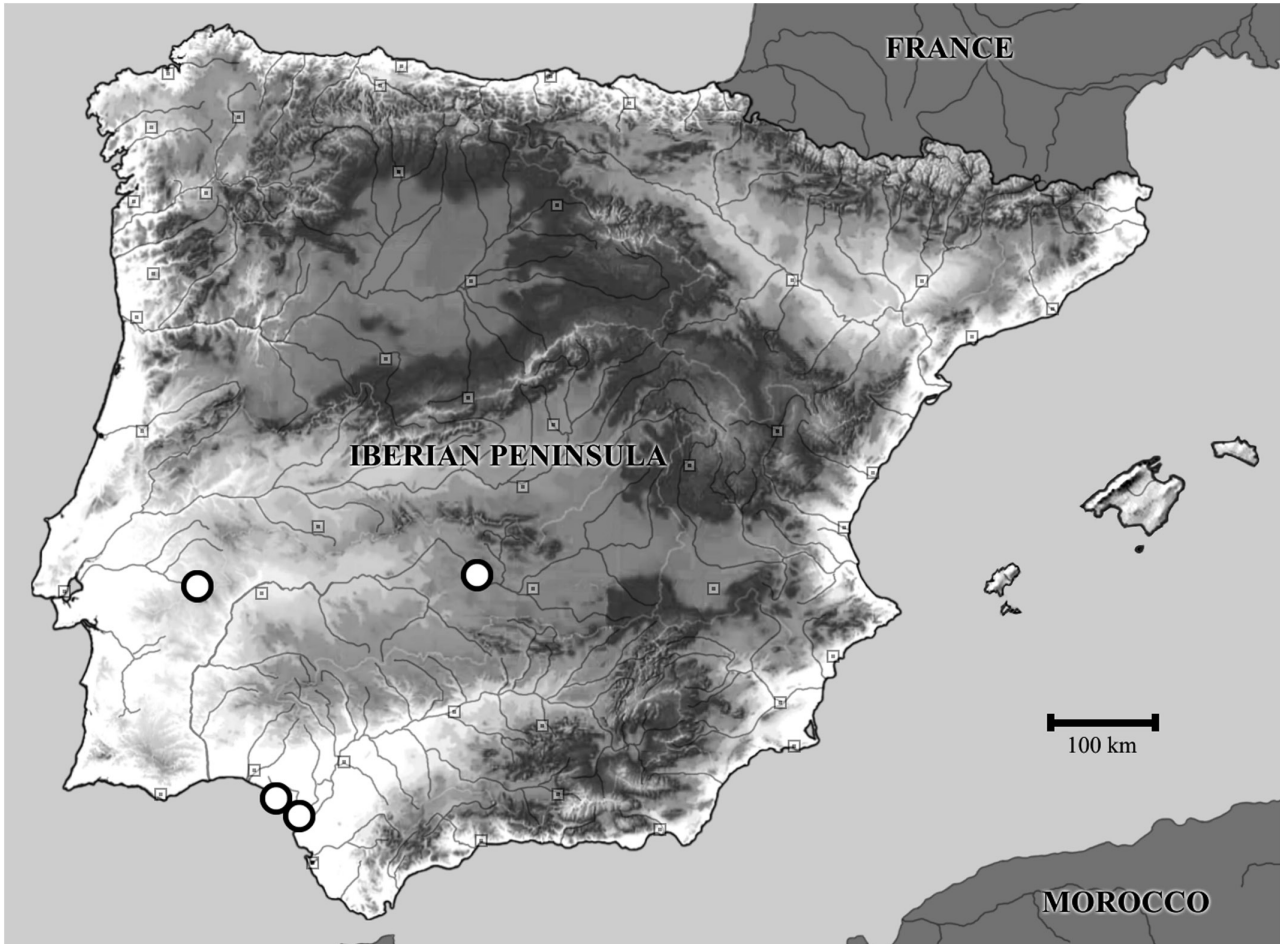
Conversely, our ML and MP results place *D. tenuisectus* in a strongly supported clade (92% BS) containing *D. crinitus* and *D. setifolius* (s.l.), whereas *D. muricatus* and *D. aureus* are sister in a separate moderately supported clade (Fig. 1). Our SVD analysis resolves *D. tenuisectus* as sister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

With *D. montanus* this species is supported as an allopolyploid between *D. pusillus* and an unidentified species. Chromosome numbers supporting these clades (Table 2) are shown in blue bracketed type and bootstrap support values in black non-bracketed type.



**Figure 3.** Specimen of *Daucus pusillus* (including *D. arcanus*) collected in Ciudad Real, Spain (ABH78367): A, habit; B, detail of fruiting umbels.



**Figure 4.** Distribution of *Daucus pusillus* (including *D. arcanus*) in the Iberian Peninsula. See [Appendix](#) for a detailed list of records.

(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* Spalik & 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 subsessile 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 *Durieuva juncea*. According to our research, *D. juncea* 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. juncea* (Spanish *D. setifolius* 237 collected in Seville province) and is likewise separated from *D. setifolius* (Algerian 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 'Pseudorlaya clade' (*D. minusculus* and *D. pumilus*) is strongly supported (BS  $\geq$  90%) and sister to the remaining clades. Both of these species have a distinctive morphological syndrome: annual habit, zygomorphic umbels (both involucre 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 'Pseudorlaya 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 ( $\pm 1.3$  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  $\geq$  90%). *Daucus gracilis* (unknown chromosome number) is sister to these species, whereas *D. syrticus* 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-Salvà, 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.

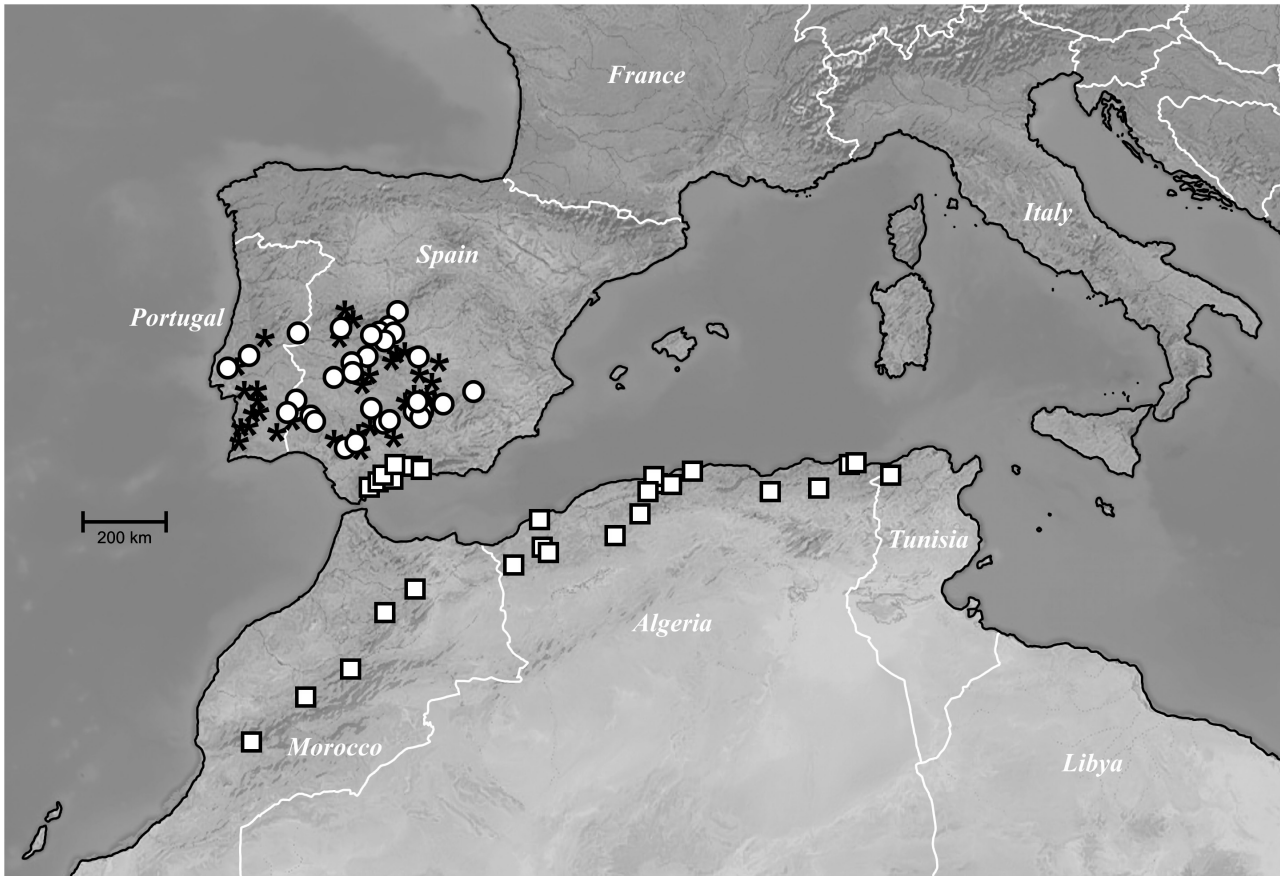
#### NOMENCLATURAL PROPOSALS

*DAUCUS SETIFOLIUS* DESF., FL. ATLANT. 1: 244, T. 65. 1798

*Staflinus setifolius* (Desf.) Raf., New Fl. 4: 28. 1838 = *Pomelia setifolia* (Desf.) Durando ex Pomel, Mat. Fl. Atl.: 7. 1860 = *Meopsis setifolia* (Desf.) Koso-Pol. in Trudy Tiflissk. Bot. Sada 16: 196. 1914.

*Daucus brachylobus* Boiss., Voy. Bot. Espagne 2: 258, t. 68. 1840.

*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



**Figure 5.** Distribution of *Daucus junceus* (circles) and *D. setifolius* Desf. (squares), based on studied herbarium specimens and reliable photographic records. Asterisks indicate trustworthy bibliographic references and databases for *D. junceus*. See Appendix for a detailed list of records.

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 cylindrical-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).

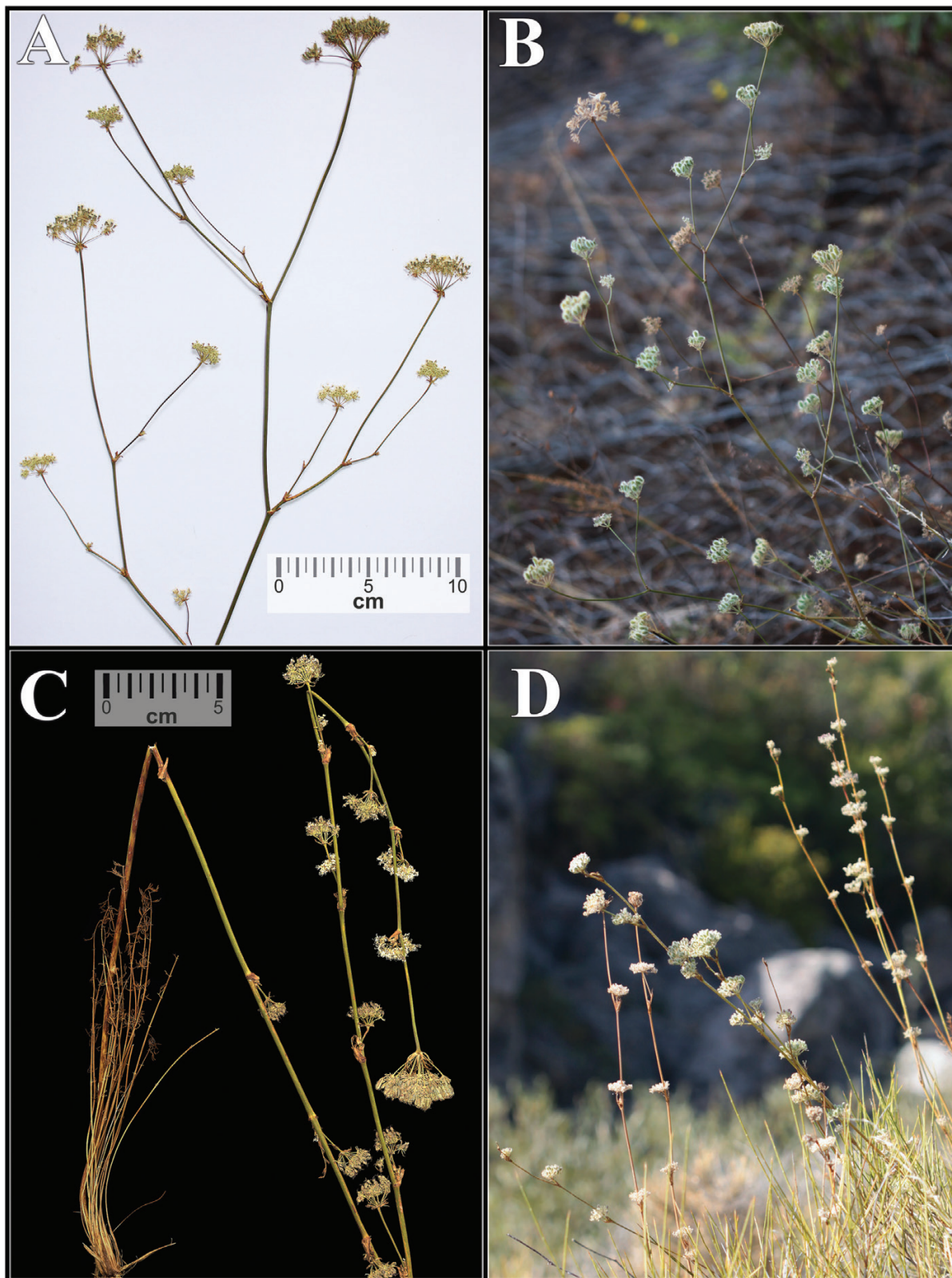
*Selected specimens*: see Appendix.

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

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

*Lectotype (designated here)*: [SPAIN]. “76. d. (coll. sel.) / *Durieuva* ? [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.

*/ juncea*, Willk! / in graminosis inter frutices / in solo granitico declivium vallis / fluvii Jerte pr. Plasencia in / Extremadura, 10/1850 / legit Willk.) (BM-000752080 [digital image!]). Isolectotype: P-02461299 [digital image!]).

*Ind. loc.*: ‘Hab. in graminosis inter saxa granitica in valle fluvii Jerte prope Plasencia in Extremadura hinc inde’ [Spain]. Pl. ex. coll. ven. select. n. 76 d.

*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, Gadiana, 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 pusillum* (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 microphyllus* Presl ex DC., Prodr. 4: 213. 1830 ≡ *Daucus pusillus* var. *microphyllus* (C.Presl ex DC.) Torr. & A.Gray, Fl. N. Amer. 1(4): 636. 1840.  
*Daucus montevidensis* Link ex Spreng., Syst. Veg., ed. 16, 4(2, Cur. Post.): 119. 1827.  
*Daucus pusillus* var. *scaber* Torr. & Gray, Fl. N. Amer. 1(1): 636. 1840. ≡ *Daucus scaber* Nutt. ex Torr. & A.Gray, Fl. N. Amer. 1(4): 636. 1840.  
*Daucus scaber* Larrañaga, Escritos Damaso Antonio Larrañaga 2: 113. 1923, nom. illeg.  
*Daucus scadiophyllus* Raf., New Fl. 4: 24. 1838.  
*Daucus arcanus* García-Martín & Silvestre in Lagasalia 15: 263. 1990, **syn. nov.**

*Lectotype (designated here)*: [USA]. ‘*Daucus pusillus* / in sabulosis / Carolinae’ (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 Ardila, 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 7°42'57.2"W, 37°50'11.9"N 8°28'48.2"W, 37°58'14.7"N 7°18'53.1"W, 38°04'40.4"N 7°07'25.5"W, 38°09'12.4"N 6°59'46.6"W, 38°11'17.1"N 8°11'39.8"W and 38°11'48.0"N 7°13'12.1"W, *M. Porto, F. Clamote, A.J. Pereira, U. Schwarzer. Daucus setifolius* Desf. – mapa de distribuição. Flora-On: Flora de Portugal Interactiva, Sociedade Portuguesa de Botânica. <http://www.flora-on.pt/#wDaucus+setifolius> [accessed 22/08/2018]; **Bragança:** Macedo de Cavaleiros, Lagoa, leito de cheias do rio Sabor, junto à foz do Rio Azibo, 225 m, 15.vi.2003, *C. Aguiar & J. Capelo* s/n (Herb. Esc. Sup. Agr. Bragança 5982), Extinct (C. Aguiar, pers. comm.); **Castelo Branco:** Idanha-a-velha, 11.ix.2015, *F. Clamote* (obotanicoaprendiznaterradosespantos.blogspot.com!); Sertã, Mosteiro de Sao Tiago, na serra, *J. Domingues de Almeida, Bot. Complut.* 30: 147–151 (2006) ([www.anthos.es](http://www.anthos.es) #2062096); **Évora:** Estimated coordinates: 38°25'57.8"N 8°14'02.0"W, *M. Porto, F. Clamote, A.J. Pereira, U. Schwarzer. Daucus setifolius* Desf. – mapa de distribuição. Flora-On: Flora de Portugal Interactiva, Sociedade Portuguesa de Botânica. <http://www.flora-on.pt/#wDaucus+setifolius> [accessed 22/08/2018]; **Lisboa:** Cabeço de Mil Regos, viii.1885, *J. Daveau* #721 (P02461302!, P04328984!); Serra da Arrábida, 1879, *J. Daveau* (P02461301!); Serra de Montejunto, 30.ix.2015, *F. Clamote* (obotanicoaprendiznaterradosespantos.blogspot.com!); **Portalegre:** Freixo do Meio, em solos compactos ricos em bases e biótopos medianamente nitrófilos, influenciados pelo homem e animais, 144–146 m, *M. Pereira, Guineana* 15: 5–316 (2009) ([www.anthos.es](http://www.anthos.es)

#2297084); Ribeira das Alcáçovas, nas fissuras terrosas de rochas graníticas e quartzíticas, 80–150 m, *M. Pereira, Guineana* 15: 5–316 (2009) ([www.anthos.es](http://www.anthos.es) #2295657); **Santarém:** Ribatejo, Entroncamento, ilegible (MA168162); Estimated coordinates: 39°10'39.1"N 8°49'06.9"W, *M. Porto, F. Clamote, A.J. Pereira, U. Schwarzer. Daucus setifolius* Desf. – mapa de distribuição. Flora-On: Flora de Portugal Interactiva, Sociedade Portuguesa de Botânica. <http://www.flora-on.pt/#wDaucus+setifolius> [accessed 22/08/2018]; **Setúbal:** Estimated coordinates: 38°07'20.7"N 8°21'54.8"W, 38°27'49.3"N 9°03'59.2"W and 38°40'04.8"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 Interactiva, Sociedade Portuguesa de Botânica. <http://www.flora-on.pt/#wDaucus+setifolius> [accessed 22/08/2018]; Cercal, *M. Ladero, Anales del Instituto Botánico Cavanilles* **31**(1): 119–137 (1974) ([www.anthos.es](http://www.anthos.es) #1180884); **SPAIN. Albacete:** Alcaraz, vii.1848, *F. Michael* (COI00058771!); **Ávila:** Cebreros, Laderas arenosas junto al Puente del Carpintero, en la carretera al Burguillo, 12.ix.1992, *V.J. Arán & M.J. Tohá* (FCO25111); Cebreros, Rd. AV-504, c. 1 km SW of town of Cebreros, environs of Puente del Carpintero and Arroyo de los Galayos, 2.ix.2016, *F. Martínez-Flores et al.*, #E-095 (ABH78368); **Badajoz:** Arroyo de Friegamuñoz entre Cheles y Villanueva del Fresno, no regolfo da albufeira de Alqueva, 13.x.1994, *M. Lousã et al.* (LISI570); Mérida, Trujillanos, proximidades del Embalse de Cornalvo, 310 m, 11.ix.2008, *A. Sánchez* (ABH53888); Zurbarán, 3 km al E., tomillar en raña pliocena, 15.ix.1979, *J.L. Pérez-Chicano* (MA309860); La Serena, *M. Ladero, Anales del Instituto Botánico Cavanilles* **31**(1): 119–137 (1974) ([www.anthos.es](http://www.anthos.es) #1171790); Puebla de Alcocer, *M. Ladero, Anales del Instituto Botánico Cavanilles* **31**(1): 119–137 (1974) ([www.anthos.es](http://www.anthos.es) #1171791); **Cáceres:** Guadalupe, taludes umbrosos de Guadalupe, 5.ix.1969, *S. Rivas-Goday et al.* (FCO02676); Plasencia, Extremadura, ad rupes graníticas in valle fluvii Jerte prope Plasencia, x.1850, *H.M. Willkomm* #76d (BM000752080!, COI00058767!); Plasencia, carretera de la Vera, 27.viii.1986, *E. Rico* (MA561136); Zorita, litosol sobre pizarras precámbricas 1 km al norte de Zorita, 14.ix.1997, *J.L. Pérez Chicano* (MA594719); Charco de la Torre, pastizales vivaces xerofíticos y termófilos, *M.D. Belmonte-López*, Ph. D. Thesis, Universidad Complutense de Madrid (1986) ([www.anthos.es](http://www.anthos.es) #1715410); Valle de Tornavacas, in collibus reg. calidae super. raro, *Graells, H.M. Willkomm & J.M.Ch. Lange. Prodrumus florum Hispanicae*, vol. **3** (1874–1880) ([www.anthos.es](http://www.anthos.es) #2372238); Villareal de

San Carlos, *M.D. Belmonte-López*, Ph. D. Thesis, Universidad Complutense de Madrid (1986) ([www.anthos.es](http://www.anthos.es) #1715420); **Ciudad Real**: Solanilla del Tamaral, Rd. CR-5002, between km 6–7, by bridge (Puente Mercedes) over Río Jándula, SE side of the bridge, 6.ix.2016, *F. Martínez-Flores et al.* #E-117 (ABH78371); Almuradiel-Venta de Cárdenas, *C.J. Martín-Blanco & M.A. Carrasco*. *Monografías de la AHIM* 1 (2005) ([www.anthos.es](http://www.anthos.es) #1842395); Calzada de Calatrava, cerro Calderón, 680 m., 15.ix.1991, *Fernández García-Rojo* (JAEN 914579) ([www.anthos.es](http://www.anthos.es) #1842393); Ciudad Real, La Atalaya, 670 m, *L. Pablos-Alcázar*, *Plantas silvestres de Ciudad Real-La Atalaya* (2004) ([www.anthos.es](http://www.anthos.es) #1869258); Comarca de Estena, 5.ix.1965, *S. Rivas-Goday et al.* (MAF 71752) ([www.anthos.es](http://www.anthos.es) #1842386); Herencia, Montes de Toledo, de Herencia a Villarta de San Juan, 800 m, 22.ix.1981, *C.J. Martín-Blanco & M.A. Carrasco*. *Monografías de la AHIM* 1 (2005) ([www.anthos.es](http://www.anthos.es) #1842385); Horcajo de los Montes, taludes de borde de caminos, 5.ix.1965, *Ladero* (MAF80390) ([www.anthos.es](http://www.anthos.es) #1842392); Mestanza, valle del río Jándula, 420 m, 14.ix.1996, *García-Río* ([www.anthos.es](http://www.anthos.es) #1842388); Moral de Calatrava, sierra de Moral de Calatrava, 25.v.1989, *Carrasco et al.* (MACB 37164) ([www.anthos.es](http://www.anthos.es) #1842387); **Córdoba**: Bélmez, 1869, *Ch. Rouques* (P02461300!); Castillo de la Albaida, 7.x.2007, *J. Trinado* (pers. comm.); Santa María de Trasierra en dirección al arroyo Bejarano, 20.ix.2009, *J.F. Moreno* (pers. comm.); subida a Cerro Muriano, 13.x.1978, *Pérez-Chiscano* (SEV55551!); Montilla, Fuente Rodas, 17.x.2001, *Garrido & Pérez-Porras*, *Acta Botanica Malacitana* 27: 295–308 (2002) ([www.anthos.es](http://www.anthos.es) #1654412); Sierra Morena, SW, desde Valle del Guadalquivir hasta N de Hornachuelos, embalses de La Breña y de Bembézar, Sierra de Córdoba, *A. Pujadas-Salvá*, *Flora arvense y ruderal de la Provincia de Córdoba*. Edit. Universidad de Córdoba (1986) ([www.anthos.es](http://www.anthos.es) #2310953); **Huelva**: La Nava, Rd. N-435 c. 8 km N of La Nava, *F. Martínez-Flores et al.*, #E-101 (ABH78369); idem, between km 124–125, near Barranco del Retamar and Casa del Retamar, *F. Martínez-Flores & A. Quílez-Méndez*, #E-123 (v.v.); La Nava a Encinasola, entre cerca Gavana y Puerto de las Adelfas, 315 m, 17.x.2017, *J. Fuentes*; carretera de La Nava a Encinasola, río Múrtigas, cerca Gavana, 326 m, 10.x.2016, *J. Fuentes* (GDA); **Jaén**: Andújar, hacia la Virgen de la Cabeza, en posíos de las viñas de Andújar, 11.ix.1951, *S. Rivas et al.* (FCO02675); Andújar, carretera JV-501, prox. Casa del Naranjal, 460 m, 11.ix.2015, *M. Cueto & G. Blanca* (GDA); Andújar, sierra Morena, entre casa Navandillos y cerro de Pedro López, 500 m, taludes y herbazales silicícolas, 5.v.2017, *J. Fuentes, G. Blanca & M. Cueto* (GDA); Bailén, paraje Piedras de Doña

Inés, margen del río Rumblar, 240 m, 23.xi.2009, *P. Rodríguez-Cobo* (ABH55115); Baños de la Encina, Barranco del Oso, orilla sureste del río, 16.x.2009, *P. Rodríguez-Cobo* (pers. comm.); Sierra Morena in dumetis apricis inter pagos Santisteban del Puerto et Aldeaquemada atque alibi frequens, 28.viii.1945, *H.M. Willkomm* (COI00058770!); Villanueva de la Reina, Zocueca, Puente del Rumblar, 240 m, 19.viii.2010, *F. Martínez-Flores* (ABH56276); idem, 6.ix.2016, *F. Martínez-Flores et al.*, #E-122 (v.v.); Viñas de Andújar, c. Santamaría de la Cabeza, 11.ix.1951, *S. Rivas-Goday & E.F. Galiano* (MA166100); Río Navalajeta, 11.vi.1986, *E. Cano-Carmona & F. Valle-Tendero*, *Monografías del Jardín Botánico de Córdoba* 4: 5–73 (1996) ([www.anthos.es](http://www.anthos.es) #1332792); Santa Elena, comarca, 700 m, *S. Rivas-Goday et al.*, *Anales del Instituto Botánico Cavanilles* 17(2): 285–403 (1959) ([www.anthos.es](http://www.anthos.es) #1764863); Vilches, Arroyo Galapagar, 440 m, *A. Payer-Martos, Blancoana* 14: 85–92 (1997) ([www.anthos.es](http://www.anthos.es) #1031813); **Salamanca**: Santibáñez de la Sierra, *F.J. Fernández-Díez*. *V Trabajos del Departamento de Botánica Salamanca* 4: 5–32 (1977) ([www.anthos.es](http://www.anthos.es) #60392); **Seville**: Alcalá de Guadaira, Hacienda de los Ángeles, 50 m, 10.ix.2008, *A. Aparicio* (ABH53906); Carmona, Cuatro Caminos, 24.ix.1977, *S. Silvestre* #8498 (MA359174, P04199154!, P04328983!, SEV42820!); Lora del Río, al N, 250 m, *S. Rivas-Goday et al.*, *Anales del Instituto Botánico Cavanilles* 17(2): 285–403 (1959) ([www.anthos.es](http://www.anthos.es) #1764866); Sevilla, Gerena, Corredor de la Plata, río Guadiamar, 80 m, *A.V. Pérez la Torre et al.*, *Acta Botanica Malacitana* 27: 189–228 (2002) ([www.anthos.es](http://www.anthos.es) #1653856); Paradas, 50 m, *S. Rivas-Goday et al.*, *Anales del Instituto Botánico Cavanilles* 17(2): 285–403 (1959) ([www.anthos.es](http://www.anthos.es) #1764865); **Toledo**: Alcaudete de la Jara, pizarroso silíceo seco, 1.ix.1982, *A. Segura-Zubizarreta* (MA359231); Cazalegas, 30.ix.2012, *R. Angulo* ([www.biodiversidadvirtual.org](http://www.biodiversidadvirtual.org) #197328); Hinojosa de San Vicente, encinar arenoso-granítico, 5.x.1979, *A. Segura-Zubizarreta* (MA359180); Los Yébenes, Montes de Toledo, Puerto del Comendador, 26.vii.1992, *V.J. Arán & M.J. Tohá* (FCO25110); Los Yébenes, 19.ix.1986, *Gómez-Manzaneque* (MAF349663); Oropesa, taludes sobre pizarras cámblicas, 2.ix.1976, *M. Ladero* (FCO08194); Velada, arenoso-silíceo, 16.viii.1980, *A. Segura-Zubizarreta* (MA359143); Velada-Gamonal, arenoso-granítico, 13.xi.1977, *A. Segura-Zubizarreta* (MA359209); Castell. San Pablo de Montes, *H.M. Willkomm & J.M.Ch. Lange*, *Prodromus Florae Hispanicae*, 3, Stuttgart. (1874–1880) ([www.anthos.es](http://www.anthos.es) #1564085); San Román de los Montes, arroyo de Guadamora, 430 m, *P. Cantó, Lázaroa* 25: 187–249 (2004) ([www.anthos.es](http://www.anthos.es) #1675177).

*DAUCUS PUSILLUS* MICHX. (INCL. *D. ARCANUS*  
GARCÍA-MARTÍN & SILVESTRE)

**PORTUGAL. Évora:** 38°54'01.8"N 7°40'38.3"W, *M. Porto & A.J. Pereira*. *Daucus arcanus* García Martín & Silvestre – mapa de distribuição. Flora-On: Flora de Portugal Interactiva, Sociedade Portuguesa de Botânica. <http://www.flora-on.pt/#wDaucus+arcanus> [accessed 22/08/2018]; **SPAIN. Huelva:** Almonte, Matalascañas, sabinas, 24.iv.1986, *F. García-Martín & Silvestre* (SEV126880!; SEV126881!); ídem, 11.iv.1989, *Aparicio et al.* (K000681200!, MA490772!, SEV126880!); Almonte, dunas del Asperillo, 15.v.1995, *E. Sánchez-Gullón & P. García-Murillo* (COA32418!); Matalascañas, carretera A-494 de Matalascañas a Mazagón, 30 m, 27.iv.2009, *F. García-Martín & J. Pastor* (ABH53887!); grown in Seville, 10.iii.1990, from mericarps collected by *Aparicio et al.*, vii.1975 (MA490773, SEV227030!); Las Marismillas, Parque Nacional de Doñana, 2016, *E. Sánchez-Gullón* (pers. comm.); **Ciudad Real:** Piedrabuena, Cerro Navalagrulla, canchal cuarcítico, 885 m, 17.vi.1993, *Burgaz et al.* (MACB56965!); c. 25 km NW of Piedrabuena, top of hill with antenna, mountain Navalagrulla, in fenced private area of Finca de Nuestra Señora del Rosario, S of CR-721, 15.vi.2016, *F. Martínez-Flores & D.M. Spooner* #E-029 (ABH78367!).

*DAUCUS SETIFOLIUS* DESF.

**ALGERIA. Ain Defla:** Zaccar–monte Zaccar, 1100 m, v.1919, *Ch. Alleizette* (P02517373!); **Annaba:** Djebel Edough près Bone, 20.viii.1860, *A.H. Letourneux* (COI00058768!, P02517382!, P02517390!, P02517397!, P02517398!, P02517399!, P02517400!, P02517401!, P02517403!, P02517404!); **Argel:** Reghaïa, rares seches en lisiere de la foret, 3.x.1937, *L. Faurel* (P04328042!); **Blida:** Blidah, 13.vii.1854, *E. Cosson* (P02517374!); Sidi Madani, Chiffa, 30.vi.1930, *L. Faurel* (P04328040!); **Constantine:** coteaux près du lac du Djebel-el-Ouach, 7.ix.1857, *S. Choulette* #236 (P02517375!, P02517376!, P02517379!, P02517388!, P04101340!); Djebel el Ouach près Constantine, 26.v.1880, *E. Cosson* (P02517377!); **Oran:** Oran, *F. Garrigues* (MO1606527!); Oran province, 1874, *A. Warious* (MO1789631!); Oran, in montibus, x.1850, *Munby* (P02517396!); Oran Hamarnah, près Sidi bel Abbis, ix.1863, *Lefrane* (P02517385!, P02517386! P02517389!); Maquis près Sidi bel Abbis, 28.ix.1874, *A. Warious* (P02517420!, P02517421!, P02517425!); **Setif:** Djebel Magris, lieux arides, sur le calcaire, 1600 m, 1898, *Juillet* #234 (P02517412!); Massif des Babors, 1500 m, 19.vii.1937, *L. Faurel* (P04328041!); **Sidi Bel Abbès:** Tenira, clairiers du forets, ix.1871, *A. Warious*

(P02517423!, P02517424!); **Skikda:** Stora, ix.1839, *Bové* (P02517367!, P02517369!, P02517370!); **Tiaret:** Tiaret, 1845, *Delastre* (P02517392!, P02517393!, P02517394!); **Tissemsilt:** Teniet el Haad, foret de cedrus, 23.vii.1854, *E. Cosson* (P02517380!); **Tipasa:** Marengo (Hadjout), 17.ix.1861, *J. Lefebre* (P02517387!); Tipaza, au sud de Desaix, 18.x.1963, *L. Faurel* (P04328038!); **Tlemcen:** Terny, en Aïn-Ghoraba, 1300 m, 11.viii.1932, *A. Faure* (MA89070, MA89071, MO1098506!); **MOROCCO. Fès-Boulemane:** Anocour, x.1913, *Mouret* #1579 (P02517427!); **Drâa-Tafilalet:** Road from El-Ksiba to Imilchil, c. 9 km N from Tizi-n-Islay, 1440 m, 5.vii.1997, *S.L. Jury et al.* #17514 (RNG!); **Souss-Massa-Drâa:** Aït Mahalla, 25.vii.1879, *Ibrahim* (P02517433!, P02517434!); **Tadla-Azilal:** Djebel Bouachfal, 3.viii.1882, *Ibrahim* (P02517429!, P02517431!); **Taza-Al Hoceima-Taounate:** Bab Azhar, 1500 m, 4.viii.1938, *L. Faurel* (P04328043!); **TUNISIA. Ben Arous:** Bou-Kournein pr. Hammam-El-Lif, 9.v.1883, *E. Cosson et al.* (P02517438!); **Jendouba:** Fedj El Sa-ha (Kroumirie), north of Ferrara, 30.vi.1883, *E. Cosson et al.* (P02517435!, P02517436!, P02517437!). **SPAIN. Málaga:** Inter pagum Alozaina et oppidulum Yunquera, 1845, *P. Prolongo y García* (COI00058769!); Coín, Sierra Alpujata, 500 m, taludes peridotíticos, 20.x.2016, *J. Fuentes, G. Blanca & M. Cueto* (GDA); Estepona to Jubrique, km 6–7, 4.ix.2016, *F. Martínez-Flores et al.*, #E-107 (ABH78370!); Estepona, Sierra Bermeja, bco. del Infierno, peridotitas, 300 m, 20.x.2016, *J. Fuentes, G. Blanca & M. Cueto* (GDA); Estepona, Sierra Bermeja, bco. del Infierno, 300 m, matorrales orientados al O., sustrato peridotítico, 11.x.2017, *J. Fuentes* (GDA); entre Alozaina y Jorox, casas del Arroyo de las Viñas, 510 m, taludes peridotíticos orientación O., 7.iv.2017, *J. Fuentes, G. Blanca & M. Cueto* (GDA); Istán, entre Cuesta del Alcornocal y río Verde, 250 m, matorral termófilo orientación O., peridotítico, 11.x.2017, *J. Fuentes* (GDA); San Pedro de Alcántara, río Guadaiza, Benavolá Alto, 200 m, taludes peridotíticos orientación E., bajo pinar entre matorral, 11.x.2017, *J. Fuentes* (GDA); San Pedro de Alcántara, río Guadaiza, El Colorao, 150 m, matorral termófilo en peridotitas, orientación O., 11.x.2017, *J. Fuentes* (GDA); Antequera, Sierra del Torcal, *B. Cabezudo et al.*, *Acta Bot. Malacitana* **17**: 145–166 (1992) ([www.anthos.es](http://www.anthos.es) #1046749); Circá Alhaurin, in collibus regionis calidae superioris, E. Boissier, Voyage botanique dans le midi de L'Espagne pendant l'anne 1837, vol. 2. Paris, Gide et Cie., Libraires-editeurs (1839) ([www.anthos.es](http://www.anthos.es) #1863783); Istán, Boornoque, *B. Cabezudo et al.*, *Acta Botanica Malacitana* **17**: 145–166 (1992) ([www.anthos.es](http://www.anthos.es) #1046746); Sierra Almirajara, *B. Cabezudo et al.*, *Acta Botanica Malacitana* **17**: 145–166 (1992) ([www.anthos.es](http://www.anthos.es) #1046745); Sierra Tejada, *B. Cabezudo et al.*, *Acta Botanica Malacitana* **17**: 145–166 (1992) ([www.anthos.es](http://www.anthos.es) #1046748).

## 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*.