

Morphometric and molecular evidence for taxonomic recognition of a new subspecies of *Armeria filicaulis* (Plumbaginaceae)

por

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Abstract

A new subspecies of the Betic-Riffian species *Armeria filicaulis* is formally described –subsp. *alfacarensis*– from crystalline dolomitic sands NE of Granada (SE Spain). Its representatives exhibit a consistent morphological combination of characters on the inflorescence and flowers that support its taxonomic recognition. This is shown by means of a multivariate morphometric analysis. In one of the sites of the new taxon, where it is sympatric with *A. villosa* subsp. *bernisii*, nuclear ribosomal ITS sequences reveal gene flow between the two species, which is congruent with RAPD data and chloroplast *trnL-F* haplotypes. It is discussed that gene flow detected is not responsible for the origin of the new subspecies but a recent event. According to this, a differentiated biological entity related to other forms of *A. filicaulis* inhabiting dolomitic sands in eastern Andalusia, existed previously to gene flow events. The patterns detected (and inferred events) in *A. filicaulis* throughout its area suggest that the compilospecies model fits the evolution of this species as does that in *A. villosa* (as reported elsewhere) although not as closely.

Key words: *Armeria*, compilospecies, ITS, morphometrics, Plumbaginaceae, RAPDs, taxonomy, *trnL-F*.

Introduction

Armeria filicaulis (Boiss.) Boiss. is endemic to the SE quadrant of the Iberian Peninsula with a small disjunct area in the Moroccan Rif (Nieto Feliner, 2002). Although no intraspecific taxa were recognized in the last treatment of the genus for the Iberian Peninsula (Nieto Feliner, 1990), two subspecies were described in subsequent works to account for part of the morphological variability displayed by *A. filicaulis* in different areas.

Resumen

Se describe una nueva subespecie de la bético-rifeña *Armeria filicaulis*, que habita en arenas dolomíticas cristalinas del nordeste de Granada (sudeste de España): subsp. *alfacarensis*. Su reconocimiento taxonómico se apoya en una combinación constante de caracteres relativos a la inflorescencia y flores, como se comprueba en el análisis morfométrico multivariante que presentamos. En una de las localidades donde vive la nueva subespecie de forma simpátrica con *A. villosa* subsp. *bernisii*, las secuencias de ITS del ADN ribosómico nuclear revelan flujo génico entre ambos táxones, lo cual es congruente con los resultados que se presentan de RAPDs y con los haplotipos detectados de secuencias cloroplásticas (*trnL-F*). Se argumenta que el flujo génico detectado no es responsable del origen de la nueva subespecie sino consecuencia de un suceso más reciente. De acuerdo con esta interpretación, con anterioridad al flujo génico aludido, existiría una entidad biológica diferenciada en la zona, afín a otras formas de *A. filicaulis* que habitan en arenas dolomíticas del este de Andalucía. Los patrones detectados en *A. filicaulis* a lo largo de su área de distribución, así como los procesos inferidos, sugieren que el modelo de evolución de esta especie se ajusta al de compilospecie, tal como propusimos para *A. villosa* en otro trabajo, aunque no de forma tan patente.

Palabras clave: *Armeria*, compilospecie, ITS, morfometría, Plumbaginaceae, RAPDs, taxonomía, *trnL-F*.

The first one, subsp. *trevenqueana* Nieto Fel., includes populations from the dolomitic enclaves of the Cerro Trevenque and Alayos del Dilar, in the western end of the Sierra Nevada massif (Nieto Feliner, 1990; Blanca, 2002). The second taxon, subsp. *nevadensis* Nieto Fel. & al., was proposed to include populations from high altitudes (2000-2500 m) in Sierra Nevada that occur mostly on schist soils (Nieto Feliner & al., 1998; Blanca, 2002). There is evidence that the latter originated from hybridization between the alpine *A. splendens* (Lag. &

Rodr.) Webb and *A. villosa* subsp. *bernisii* (Gutiérrez Larena & al., 2002).

Our study focused on the Sierra Nevada massif detected populations NE of Granada (roughly between Alfacar and La Peza, Fig. 1) that exhibit distinct characters as compared to other populations of *A. filicaulis*. Alike with subsp. *trevenqueana*, these populations have leaves that are indistinguishable from those in the core of the species and occur on crystalline dolomitic sands. As subsp. *trevenqueana*, they differ from the rest of the populations by characters in inflorescence, bracts and flowers. This paper aims at presenting the morphological evidence that justifies taxonomic recognition at the subspecific level for the populations of Alfacar-La Peza as well as the molecular information provided by three markers (nuclear ribosomal, chloroplastic and total DNA). In addition, the possible origin of these populations is discussed taking into account the background knowledge of evolutionary patterns and processes in *Armeria*, and specifically the compilospecies model, which fits another species, *A. villosa* Girard (Fuertes Aguilar & al., 1999b).

Material and methods

Sampling. In accordance with the strategy followed in other studies in *Armeria* (e.g., Gutiérrez Larena & al.,

2002; Nieto Feliner & al., 2002), the neighbour territories have been sampled in order to minimize confounding molecular uniqueness with hybridization between related species. This is justified by the weak internal reproductive barriers in the genus (Nieto Feliner & al., 1996). Besides *A. filicaulis*, different populations of *A. villosa* subsp. *longiaristata* and *A. villosa* subsp. *bernisii* were sampled. The latter is sympatric to the populations of *A. filicaulis* in Alfacar-La Peza. Twenty individuals of the new subspecies from Sierra de Alfacar, representing 6 populations, have been used in the multivariate morphometric study. The geographic origin of those as well as the rest of the material used for the morphological and the molecular studies are shown in Table 1.

Molecular markers (ITS, *trnL-F*, RAPDs). Detailed results on the three molecular markers can be seen elsewhere. Protocols for DNA extraction, amplification, sequencing, data analysis, and specifically for ribotype (ITS repeat type) definition have been described in Fuertes Aguilar & Nieto Feliner (2003) and Nieto Feliner & al. (2004). Those for *trnL-F* in Gutiérrez Larena & al. (2002), The RAPD study is in Fuertes Aguilar & al. (unpubl.; Gutiérrez Larena, unpubl. PhD). Phenetic similarity from RAPD bands (Williams & al., 1990) was analyzed by Principal Coordinates Analysis and Cluster analysis applied to a similarity matrix constructed using the Dice coefficient. A minimum spanning tree based on the Euclidean distance was superimposed on the scatter plot of the samples against the first three PCO axes. Phenetic analyses of RAPD data were conducted using NTSYSpc (Rohlf, 2000).

Morphology. Thirty morphological characters have been used in the multivariate morphometric study (Table 2). Fifteen of them are quantitative continuous, seven are ratios, one is quantitative discrete (number of bracts) and seven are qualitative. For the continuous characters, values in the data matrix are averages of two or three measurements per specimen. Most of the metric characters were measured with the aid of a Brown & Sharpe Plus digital calliper (model 599-571-3). The morphometric study was conducted using NTSYSpc (Rohlf, 2000). Two different analyses have been carried out. A principal components analysis (PCA), based on the correlation matrix, was used as an ordination procedure to reduce the number of original variables to a few representative uncorrelated ones (the PCs). A minimum spanning tree based on the Euclidean distance was superimposed on the scatter plot of the samples against the first three PCA axes. An UPGMA cluster analysis was also conducted as a classificatory procedure, based on a similarity matrix constructed using Euclidean distance. All measurements have been made on dried specimens, which are kept in MA.

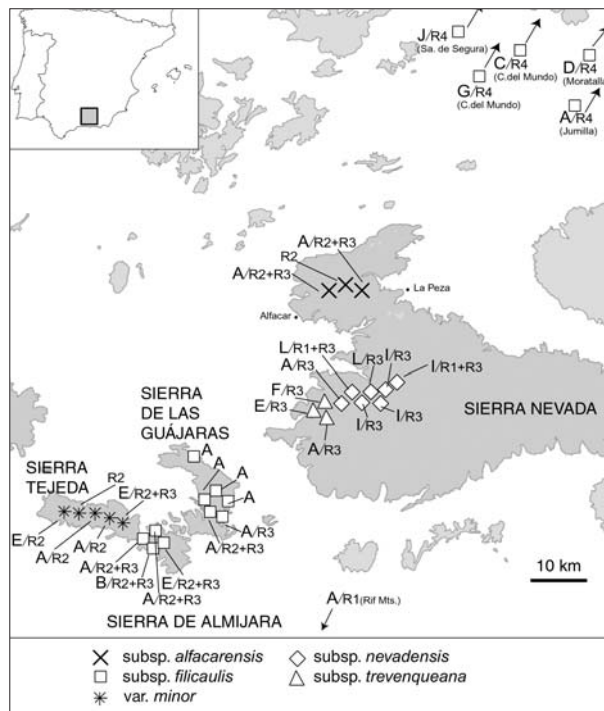


Fig. 1. Distribution of ITS ribotypes (R1 to R4; according to Nieto Feliner & al., 2004) and *trnL-F* chlorotypes (A to L; according to Gutiérrez Larena & al., 2002) in *Armeria filicaulis*. Detailed results in those two papers.



Fig. 2. Representative herbarium specimen of *Armeria filicaulis* subsp. *alfacarensis*, with enlarged glomerule (183 BG, see Table 1).

Results

Morphological characters. The main differences between populations of *A. filicaulis* from Sierra de Alfacar and the remaining are the following. The scape is 20-40 cm long, distinctly larger than the rest of the species (Fig. 2). The tubular involucre sheath, 14-22 mm, is also longer than in other populations. The involucre diameter, 21-28 mm, is also the largest within *A. filicaulis*. Involucre bracts are smooth straw-colored (not yellowish-cream and minutely rugose), the inner ones are long (> 10 mm), narrower than in subsp. *filicaulis*, with a narrower scarious margin. Unlike the usual spikelet bracts in *A. filicaulis* subsp., those in Alfacar-La Peza are longer than (or at least equalling) the inner involucre bracts. Spikelets also differ in being more or less stipitate, non sessile. The pedicels of every individual flower within each spike are longer than the standards and almost lack bracteoles, resem-

bling species in the section *Macrocentron* such as *A. birta* Willd. etc. Calyces are also longer (14-22 mm) that in other subspecies, mainly due to the larger lobule and awn.

Morphometric multivariate analyses. In the PCA, the first three axes account for 62.2% of the total variance (35.9%, 18.2% and 8.0%, respectively). The first axis is contributed significantly by almost half of the variables but mostly by calyx length, calyx-lobe length, involucre diameter, length of outer involucre bracts and spikelet bract length while the second is by ratio of the outer to the longest inner involucre bracts, width of inner involucre bracts and ratio of the involucre diameter to the length of the involucre sheath. In the scatter plot of the samples against the first three axes, specimens of *A. filicaulis* from Alfacar-La Peza are distinctly separated from the rest although with respect to the first axis they are intermingled with *A. villosa* (Fig. 3). The superimposed mini-

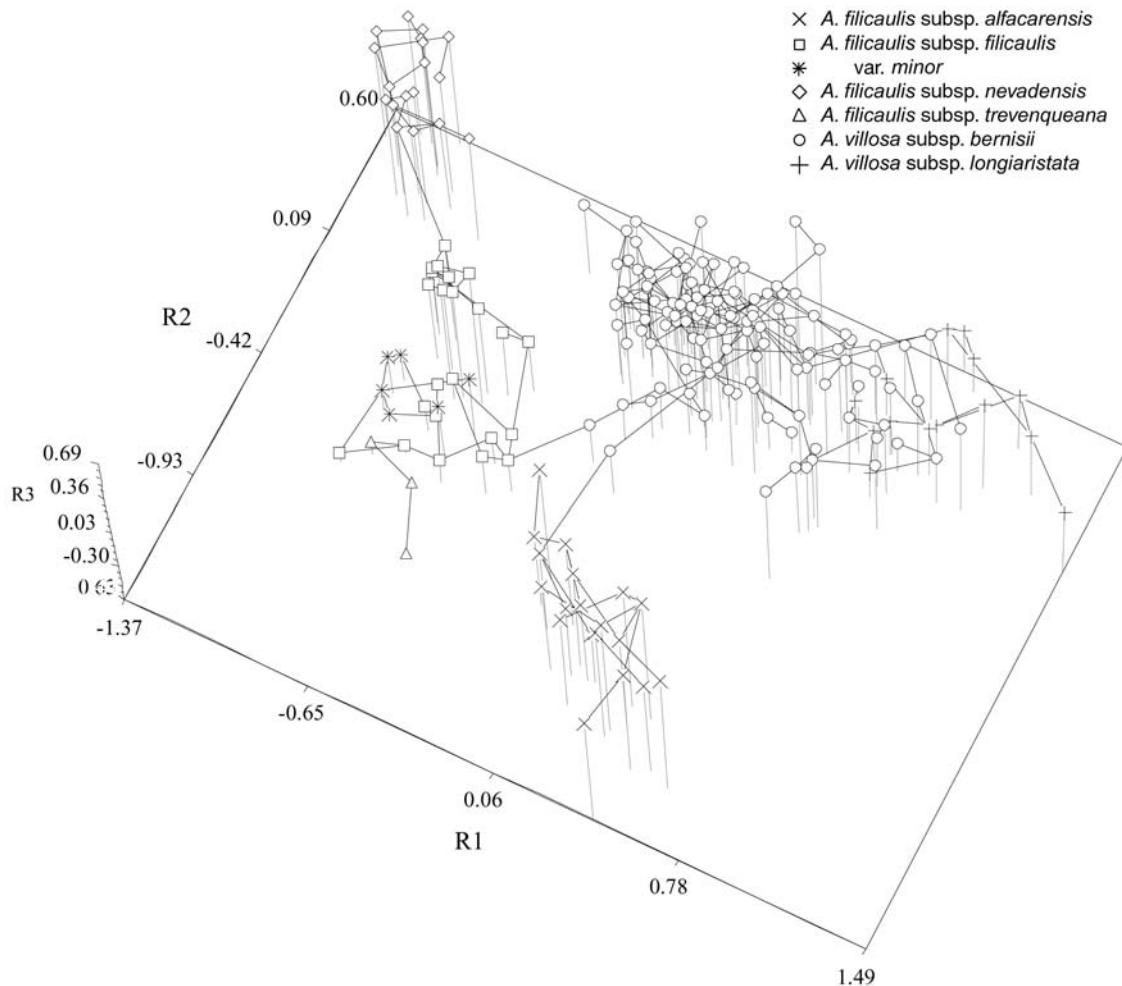


Fig. 3. Principal components analysis of *Armeria* spp. from SE Spain based on 30 morphometric characters. Plot of 205 specimens in the space defined by the first three axes, with a superimposed minimum spanning tree (based on Euclidean distance).

mum spanning tree connects one of the samples of Alfacar-La Peza with one of *A. villosa* subsp. *bernisii* from Sierra de Gádor. Samples of *A. filicaulis* subsp. *nevadensis* also cluster separately in the scatter plot,

while those of subsp. *filicaulis* (including var. *minor*) and subsp. *trevenqueana* form a lax cluster. The UPGMA phenogram provides a representation of the taxonomic groupings (Fig. 4). Two large clusters arise,

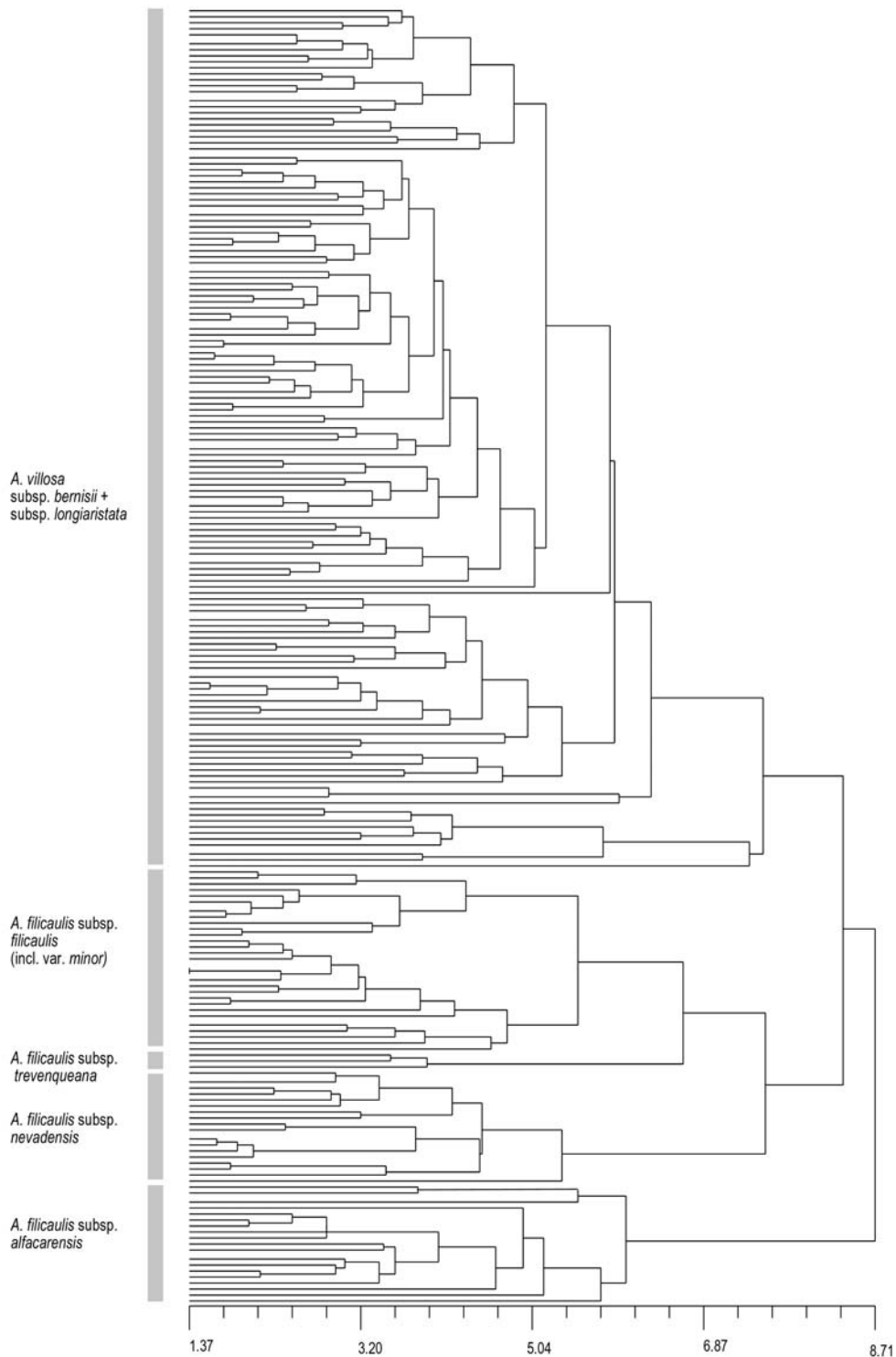


Fig. 4. UPGMA phenogram of 205 specimens of *Armeria* spp. from SE Spain based on Euclidean distance matrix computed from 30 morphometric characters from SE Spain

one including all samples of *A. villosa* (subsp. *bernisii* and subsp. *longiaristata*) and the other including all samples of *A. filicaulis*. Within the *A. filicaulis* cluster, the samples from Alfacar-La Peza are the first ones to split off, being all included in their own sub-cluster.

Molecular markers, ITS. The geographic structure shown by this marker (Fuertes & al., 1999b; Fuertes Aguilar & Nieto Feliner, 2002) has been confirmed to occur also at a fine-scale in the Sierra Nevada massif (Nieto Feliner & al., 2004; Gutiérrez Larena, unpubl. PhD). Of the three samples of *A. filicaulis* from Alfacar-La Peza, one has the ribotype 2 (R2) from central Andalusia, and the other two have R2 and R3 (from Sierra Nevada) co-occurring intraindividually (Fig. 1). Of the samples of *A. villosa* subsp. *bernisii*,

three from Alfacar also exhibit R2+R3 while two from La Peza present R3 (Nieto Feliner & al., 2004).

trnL-F. The two samples of *A. filicaulis* from Alfacar have a chloroplast trnL-F haplotype A (Fig. 1). This is the most frequent in the species, occurring in more than 50% of its samples (Gutiérrez Larena & al., 2002). Of the sequences of *A. villosa* subsp. *bernisii*, the two sampled from Alfacar correspond to haplotype L while those two from La Peza do to haplotype I. These are the most frequent haplotypes in *A. villosa* subsp. *bernisii*, occurring in 35% and 41% of its samples, respectively (Gutiérrez Larena & al., 2002).

RAPDs. PCO based on RAPD phenotypes provides a picture that is not readily interpretable (Fig. 5). In the scatter plot of the samples against the first three

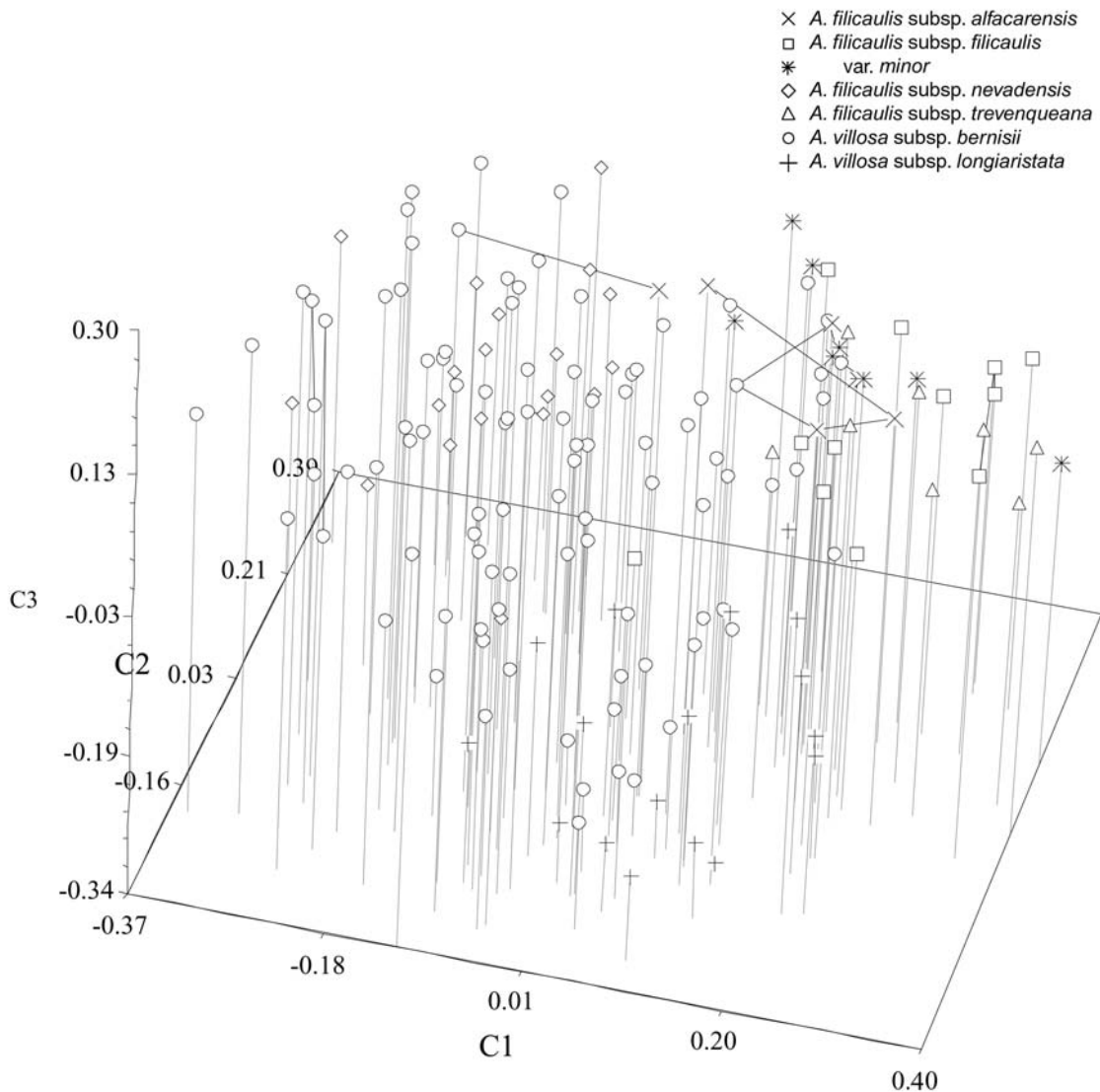


Fig. 5. Principal Coordinate analysis of RAPD bands of *Armeria* from SE Spain based on a Dice similarity matrix. Plot of 167 samples in the space defined by the first three principal coordinate axes with a superimposed minimum spanning tree (based on Euclidean distance).

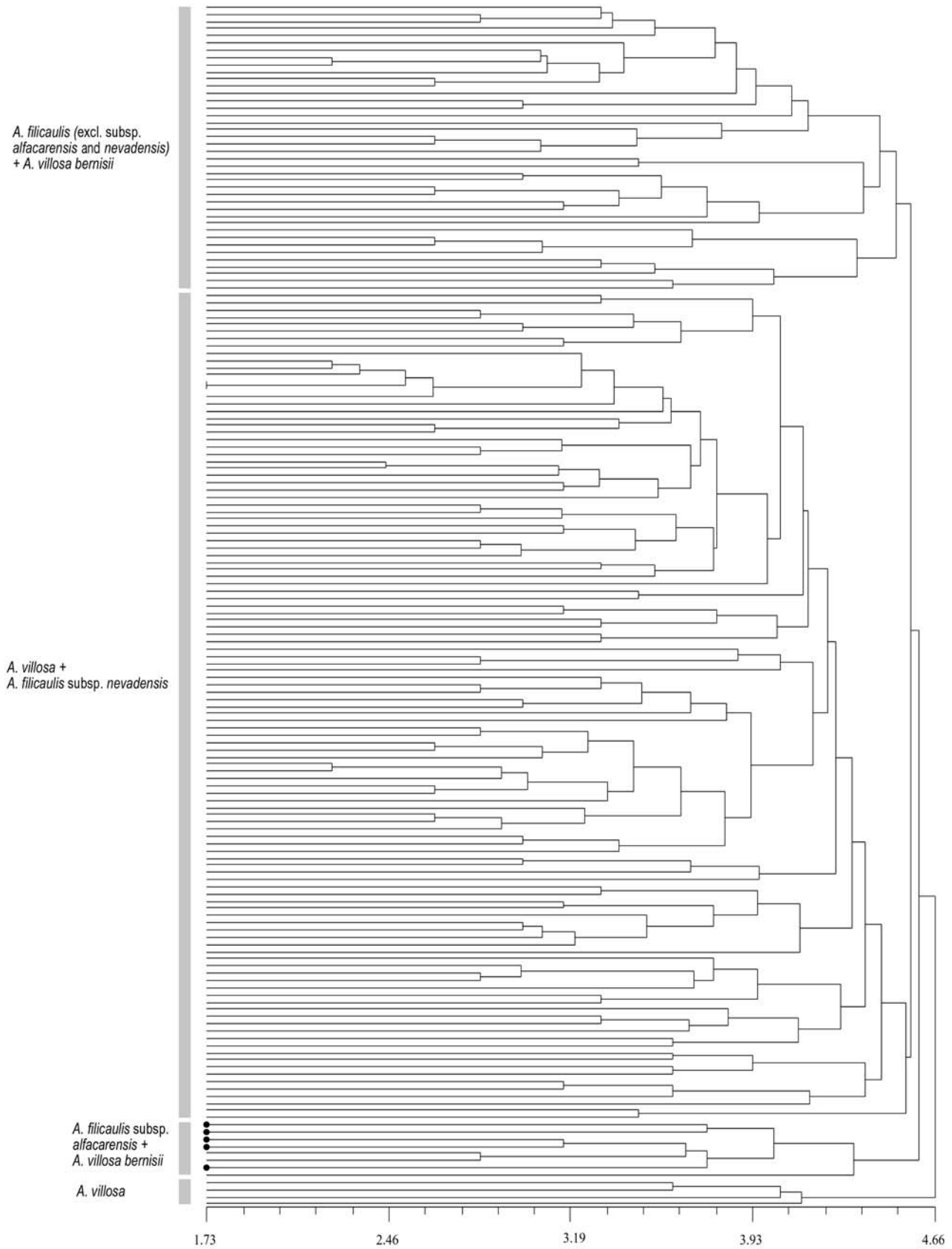


Fig. 6. UPGMA phenogram of 167 samples of *Armeria* from SE Spain based on a Dice similarity matrix constructed from RAPD bands. Samples of *Armeria filicaulis* subsp. *alfacarensis* marked with a solid circle.

Table 1. Geographic origin of the samples of *Armeria* used for morphometrics and RAPDs. Of the sequence data (ITS and *trnL-F*) only those referred to *A. filicaulis* (shown in Fig. 1) are specified. The rest can be found in Nieto Feliner & al. (2004) and Gutiérrez Larena & al. (2002), respectively.

Taxon	Origin	Voucher specimens	RAPD	ITS	<i>trnL-F</i>	Morphometrics
<i>A. filicaulis</i> subsp. <i>alfacarensis</i>	Granada: Beas de Granada, puerto de la Mora, carretera al Pozuelo a 3 km del puerto, 30SWG6124, 1400 m, laderas pedregosas de dolomías cristalinas.	GN 4070-4074 GN 2722	+	+	+	+
	Granada: Beas de Granada, puerto de la Mora, carretera al Pozuelo a 2,7 km de la A-92, 400 m antes de la explotación minera, 30SVG6025, 1400 m, dolomías cristalinas en zonas muy expuestas.	BG 183-185 BG 186-187				+
	Granada: Beas de Granada, puerto de la Mora, carretera al Pozuelo, pasado el Cortijo El Pozuelo, a 5,6 km de la A-92, 30SVG6126, 1420 m, en rambal con suelos un poco desarrollados sobre dolomías.	BG 194-197				+
	Granada: Alfacar, Sierra de Alfacar, cerro de las Higuierillas, 30SVG5124, 1400 m, en calizas bajo pinar.	BG 198-199 BG 202				+
	Granada: Beas de Granada, carretera al Pozuelo, pista forestal derecha posterior a la explotación minera, 30SVG6224, 1400 m, dolomías cristalinas muy expuestas.	BG 203-205				+
	Granada: Beas de Granada, carretera al Pozuelo, altos del Calar Blanco, pista forestal derecha posterior a la explotación minera cerca de la cantera El Pozuelo, 30SVG6024, 1380 m, dolomías cristalinas muy expuestas.	BG 208-209				+
	Granada: Beas de Granada, carretera al Pozuelo en la explotación minera, 30SVG6025, 1400 m, dolomías cristalinas muy expuestas.	BG 218-219				+
	Granada: La Peza, Cerro del Salto del Caballo, 30SVG62, 1300 m, arenas dolomíticas.	GDA17703				+
	Granada: Sierra de Alfacar, El Puerto, 30SVF52.	GDA11514				+
	<i>A. filicaulis</i> subsp. <i>filicaulis</i>	Málaga: Camillas de Albaída, Sierra Tejada, pista al puerto Blanquillo, 30SVF1479, 900 m, arenas dolomíticas, en tojal, bajo pinar.	GN 4026-4030 GN 4031	+	+	+
Granada: Canillas de Albaída, Sierra Tejada, pista a puerto Blanquillo, 30SVF1680, 1200 m, arenas dolomíticas.		GN 4032-4035	+	+	+	+
Granada: Albuñuelas, Sierra de las Guájaras, 30SVF3884, 1180 m, derrubios arenosos dolomíticos.		GN 4041-4045	+	+	+	+
Albacete: Calar del Mundo, 30SWH4753, 1300 m, grietas de rocas.		10SPV99				+
Granada: carretera Suspiro del Moro-Almunécar, km 17, 30SVF3691, junto a cantera, roquedo.		11VW00				+
Morocco: Chefchauen, Bab de Lars, 37°11' N, 5°12' W, 1800 m, pinsapar entre rocas.		157PV00				+
Morocco: Yebel Tsuka, 1900 m.		MA145713				+
Murcia: Jumilla, El Carché, 30SXH6055, 1350 m, calizas.		MA208105				+
Albacete: Los Chorros del Mundo, 30SWH4956, calizas; en pastizales de sitios altos.		MA319954				+
Jaén: Sierra de Cazorla, Cortijos Nuevos, el Yelmo, 30SWH3034, 1200 m, calizas.		MA490911				+
<i>A. filicaulis</i> subsp. <i>filicaulis</i> var. <i>minor</i>	Málaga: Alcaucín, Sierra Tejada, senda al pico Maroma, 30SVF0834, 1550 m, grietas de dolomías.	GN 4006-4010		+		+
	Málaga: Alcaucín, Sierra Tejada, senda al pico Maroma, 30SVF0584, 1700 m, litosuelos y grietas de dolomías.	GN 4015	+	+		+
	Málaga: Alcaucín, Sierra Tejada, senda al pico Maroma, 30SVF0684, 1800 m.	GN 4016-4018 GN 4019	+	+	+	+
	Málaga: Alcaucín, Sierra Tejada, senda al pico Maroma, 30SVF0285, 1200 m, litosuelos y grietas de dolomías.	GN 4020-4023 GN 4024	+	+	+	+
	Málaga: Alcaucín, Sierra Tejada, senda al pico Maroma, barranco de los Polvilleros, 30SVF0584, 1700 m, litosuelos y grietas de dolomías.	AP-1		+	+	+
	Málaga: Alcaucín, Sierra Tejada, senda al pico Maroma, 30SVF0834, 1550 m, grietas de dolomías.	BG 6	+	+	+	+
<i>A. filicaulis</i> subsp. <i>nevadensis</i>	Granada: Alcaucín, Sierra Tejada, senda al pico Maroma, 30SVF0285, 1200 m, litosuelos y grietas de dolomías.	BG 58-61	+	+		+
	Málaga: Alcaucín, Sierra Tejada, pico Maroma, 30SVF0684, 1800 m.	BG 10-13	+	+		+
	Granada: Güéjar-Sierra, Sierra Nevada, Barranco de las Ánimas, 30SVG6407, 2280 m, matorral bajo esquistoso.	BG 14	+	+		+
	Granada: Güéjar-Sierra, Sierra Nevada, cerca del Albergue de San Francisco, 30SVG6506, 2200 m, matorral bajo, esquistos.	BG 62-63	+	+		+
	Granada: Güéjar-Sierra, Sierra Nevada, Barranco de las Ánimas, 30SVG6407, 2280 m, matorral bajo esquistoso.	BG 15	+	+		+
	Granada: Monachil, Sierra Nevada, Peñones de San Francisco, 30SVG6407, 2340 m, matorral bajo, esquistos.	BG 64-66	+	+		+
	Granada: Monachil, Sierra Nevada, Peñones de San Francisco, 30SVG6407, 2340 m, matorral bajo, esquistos.	BG 67				+
	Granada: Dilar, Sierra Nevada, Hoya de la Mora, 30SVG6605, 2550 m, pastos secos.	BG 33				+
	Granada: Dilar, Sierra Nevada, Hoya de la Mora, 30SVG6605, 2550 m, pastos secos.	BG 68				+

Table 1. (continuation)

Taxon	Origin	Voucher specimens	RAPD	ITS	trnL-F	Morphometrics
<i>A. filicaulis</i> subsp. <i>trevenqueana</i>	Granada: Guéjar-Sierra, Sierra Nevada, pista a la Loma de los Cuartos, Barranco de las Castillas, 30SVG7010, 2200 m.	BG 145				+
	Granada: Dílar, Sierra Nevada, Peñón del Dílar, 30SVG6103, 2460 m, pastos secos esquistosos.	BG 28 BG 29-32 BG 119 BG 121	+	+	+	+
	Granada: Guéjar-Sierra, Sierra Nevada, pista a la Loma de los Cuartos, pasada la cadena forestal, Loma de las Cunas de los Cuartos, 30SVG6910, 2100 m.			+	+	
	Granada: Monachil, base del cerro Trevenque, 30SVG5803, 1850 m, laderas arenosas de dolomías cristalinas, vertiente NE.	GN 4084 GN 4085-4088 GN 4090-4092 CN 2248 MA 460125	+	+	+	+
<i>A. villosa</i> subsp. <i>bernisii</i>	Granada: Monachil, Cerro del Trevenque, 30SVG5703, 1900 m, laderas arenosas de dolomías cristalinas, vertiente NE.		+	+	+	+
	Granada: Dílar, Macizo del Trevenque, Los Alayos, 30SVG5501, dolomías, graveras.		+	+	+	+
	Granada: Sierra Nevada, pista al Canal del Espartal, Trevenque, 30SVG50, 1700 m, crestas descarnadas y venteadas con suelo arenoso-pedregoso dolomítico.					
	Granada: La Peza, carretera a Quénitar, 1 km antes del puerto Blancares, 30SWG6723, 1250 m, suelos algo desarrollados, sobre dolomías, en sotobosque.	GN 4048-4050 GN 4051-4055 GN 4056 GN 4061 GN 4063-4065 GN 4066-4068	+			
	Granada: Beas de Granada, puerto de la Mora, carretera al Pozuelo a 1 km del puerto, 30SWG5925, 1300 m, herbazales, suelos pedregosos calizos.		+			
	Granada: Beas de Granada, carretera al Pozuelo a 1 km del puerto, 30SWG6025, 1350 m, suelos pedregosos calizos, en sotobosque de pinar.					
	Granada: Beas de Granada, puerto de la Mora, carretera al Pozuelo a 1,3 km de la A-92, 30SVG6025, 1360 m, en zonas más expuestas del pinar, dolomías	BG 181-182				
	Granada: La Zubia, pista al Cerro Trevenque, 30SVG5404, 1350 m, matorrales calizos junto a torrentera.	GN 4076-4080 GN 4094-4098	+	+		
	Granada: Monachil, E del Cerro Trevenque, cerca del collado Ruquino, 30SVG5803, 1800 m, sotobosque de pinar, sobre dolomías.					
	Granada: Cañar, Sierra Nevada, barranco del río Chico, cerca de zona de acampada Fuente Palo, 30SVF6391, 1800 m, talud esquistoso junto a un arroyo.	GN 4104-4105 GN 4107-4108 GN 4109 GN 4112-4113, GN 4115 GN 4114, 4116 GN 4120				
	Granada: Sopotújar, Sierra Nevada, Barranco Frio, 30SVF6489, 1590 m, laderas esquistosas, junto a un juncal y un arroyo.					
	Granada: Bubión, Sierra Nevada, pista de Capileira al Veleta, 30SVF6991, 1950 m, matorrales ralos sobre esquistos.		+	+		
Granada: Portugos, Sierra Nevada, loma de Piedra Blanca, 30SVF7192, 2230 m, céspedes pedregosos, sobre esquistos en zonas aclaradas.	Granada: Portugos, Sierra Nevada, pista de Capileira al Veleta, Cascajar Negro, 30SVF7295, 2630 m, matorral xeroacántico bajo sobre esquistos.	GN 4126				+
	Granada: Portugos, Sierra Nevada, pista de Capileira al Veleta, Loma de Piedra Blanca, 30SVF7192, 2230 m, céspedes pedregosos sobre esquistos, en lomas aclaradas.	GN 4121-4124 GN 4125 GN 4128-4132	+	+		
	Granada: Bérchules, Sierra Nevada, pista forestal por encima del pueblo, 30SVF8092, 1830 m, claros de matorral sobre esquistos, en vegaada.		+			
	Granada: Bérchules, Sierra Nevada, pista forestal por encima del pueblo, 30SVF8194, 1920 m, matorral sobre esquistos.	GN 4133 GN 4134-4137 GN 4139-4143	+	+		
	Granada: Bérchules, Sierra Nevada, pista forestal, barranco de las Angosturas por encima del pueblo, 30SVF8199, 2060 m, praderas alpinas húmedas, nitrificadas, sobre esquistos.		+			
	Granada: Bérchules, Sierra Nevada, pista forestal, próximo a la Cruz de los Caminos, 30SVF8496, 1950 m, laderas esquistosas.	GN 4146-4147 GN 4151 GN 4152-4156 GN 4157	+			
	Granada: Bayárcal, Sierra Nevada, puerto de la Ragua, barranco del Hornillo, 30SVG9705, 1880 m, claros de matorral sobre esquistos.					
	Granada: Bayárcal, Sierra Nevada, próximo a Las Minillas, 30SWG0101, 1920 m, claros de matorral sobre esquistos, en vegaadas.		+			

Table 1. (continuation)

Taxon	Origin	Voucher specimens	RAPD	ITS	trnL-F	Morphometrics
<i>A. villosa</i> subsp. <i>longianistata</i>	Granada: Ferreira, Sierra Nevada, cr. del puerto de la Ragua, 30SVG9513, 1600 m, laderas esquistosas secas.	GN 4172-4175	+			+
	Almería: Berja, Sierra de Gádor, Balsa Bermeja, 30SWF1587, 1800 m, laderas pedregosas calizas.	GN 3986	+			+
		GN 4160-4161	+			+
	Granada: Aldeire, Sierra Nevada, pista forestal, 30SVG9510, 1790 m, cunetas esquistosas secas, bajo pinar.	GN 4162	+			+
		GN 4176-4180	+			+
		GN 4181-4185	+			+
	Granada: Aldeire, Sierra Nevada, pista forestal, junto al barranco de los Tejos, 30SVG9007, 2060 m, zonas aclaradas, sobre esquistos, cerca de arroyo.	GN 4186	+			+
	Granada: Aldeire, Sierra Nevada, pista forestal, junto al barranco de los Tejos, 30SVG9007, 2100 m, zonas removidas y cunetas, sobre esquistos.	GN 4187-4191	+			+
	Granada: Aldeire, Sierra Nevada, pista forestal, Loma del Collado del Pino, 30SVG8909, 1990 m, claros de matorral xeroacántico, sobre esquistos.	GN 4192	+			+
		GN 4193-4197	+			+
	Granada: Lanteira, Sierra Nevada, pista forestal, Loma del Collado del Pino, 30SVG8910, 1900 m, laderas pedregosas en lugares abiertos, sobre esquistos.	GN 4198	+			+
		GN 4200-4202	+			+
	Granada: Aldeire, Sierra Nevada, pista forestal, 30SVG9010, 1820 m, laderas pedregosas en lugares abiertos, sobre esquistos.	GN 4203-4207	+			+
		GN 4208, GN 4210-4212	+			+
	Granada: Monachil, Sierra Nevada, El Dornajo, 30SVG6008, 2020 m, calizas, NO.	GN 4209	+			+
		GN 4213	+			+
	Almería: Bacares, Sierra de los Filabres, Fuente de la Venta Luisa, 30SWG4320, 1950 m, pizarras, borde de la carretera, pastos secos de pinar de repoblación.	BG 1, BG 3-4	+			+
BG 2, BG 5		+			+	
BG 37, BG 39, BG 41		+			+	
Granada: Sedella-Alhama de Granada, "Las Llanadas", 1600 m, sotobosque de <i>Quercus pyrenaica</i> y <i>Sorbus aria</i> , suelo desarrollado.	BG 38, BG 40	+			+	
	AP-2	+			+	
Granada: Olivár, Sierra Almijara, subida al Navachica por cara W, 30SVF2976, dolomías.	Apberm 1-5	+			+	
	MGC 44193	+			+	
Granada: Alhama de Granada, Sierra Almijara, carril de la Resinera al Navachica, 30SVF2781, dolomías.	MGC 44202	+			+	
<i>A. villosa</i> subsp. <i>longianistata</i>	Albacete: Villaverde de Guadalimar, pista al Calar del Mundo, 30SWH4654, 1200 m, zona nitrificada al lado de la pista.	BG 77				+
	Albacete: Villaverde de Guadalimar, pista al Calar del Mundo, fuente de las Raigadas, 30SWH4653, 1240 m, zona nitrificada al lado de la pista.	BG 85				+
		BG 87, BG 91	+			+
	Jaén: Siles, carretera desde Segura de la Sierra al cruce con la carretera JF-7012, a 7 km del Campamento Juvenil Río Madera, 30SWH9540, 1300 m, claros de pinar.	BG 88-90	+			+
		BG 101-102	+			+
	Jaén: Segura de la Sierra, carretera JF-7012, cerca del Yelmo, 30SWH3134, 1300 m, claros de pinar en el borde de la carretera.	BG 108-111	+			+
		BG 112	+			+
	Jaén: Cazorla, Sierra de Cazorla, a 200 m de la Nava de San Pedro, 30SWG1093, 1320 m, claros de pinar.	BG 117-118	+			+
		GN 4002-4004	+			+
	Jaén: Cazorla, pista de Nava de San Pedro a El Cerezo entre km 27-28, 30SWG1193, 1400 m, claros de pinar.	GN 4232	+			+
	Córdoba: Cabra, la Nava, el Registro, 30SUG7851, 1000 m, pastos temporalmente inundados, sobre terreno arcilloso.	GN 4234	+			+
GN 4236		+			+	
Málaga: Alfarnate, Sierra de Alhama, por encima del puerto del Sol, 30SUF9292, 1250 m, paredes y canales de roquedo calizo, orientación W.	GN 4237	+			+	
	GN 4239,2	+			+	

Table 2. Morphometric characters used in the multivariate analyses

Number	Character description
1	leaf length
2	leaf width
3	ratio leaf length to leaf width
4	scape length
5	diameter of the scape at base
6	diameter of the involucre
7	ratio of the involucre diameter to the length of the involucre sheath
8	number of involucre bracts
9	length of outer involucre bracts
10	length of longest inner involucre bracts
11	ratio of the outer to the longest inner involucre bracts
12	width of inner involucre bracts
13	length of mucro of inner involucre bracts
14	length of mucro of intermediate involucre bracts
15	length of spikelet bracts
16	ratio of the length of spikelet bracts to the length of inner involucre bracts
17	calyx length
18	calyx lobe length (including awn)
19	ratio of calyx lobe length (including awn) to total calyx length
20	calyx tube length
21	calyx limb length
22	ratio of calyx tube length to calyx limb length
23	length of calyx pedicel scar
24	white salt crystals (absence, 0; presence, 1)
25	cilia on leaf margins (absence, 0; presence, 1)
26	glabrous (0) vs. pubescent (1) leaves
27	slender vs. firm (1) involucre bracts
28	calyx with 10 (0) vs. 5 (1) rows of longitudinal hairs
29	involucre bracts colour: yellowish-cream (0), straw-colored (1), tawny or copper (2)
30	petal color: white (0), pink (1), purplish (2)

principal coordinate axes, samples of *A. filicaulis* from Alfacar-La Peza do not cluster. The minimum spanning tree connects two of those samples with one of *A. villosa* subsp. *bernisii* (see discussion). An UPG-MA cluster based on the same Dice similarity matrix provides also a complex picture in which the samples of *A. filicaulis* from Alfacar-La Peza are among the first ones to split off from our data set that includes both *A. villosa* and *A. filicaulis* (Fig. 6).

Taxonomic proposal. Morphological distinctiveness confirmed also by the multivariate morphometric analyses, together with the restricted distribution and

the consistent results from the molecular marker distribution (see discussion), supports taxonomic recognition for the populations of *A. filicaulis* from Alfacar-La Peza.

Armeria filicaulis* subsp. *alfacarensis Nieto Fel., Gut. Larena & Fuertes, **subsp. nov.**

A subspécie típica scapis longiores –usque 40 cm–, vaginis involucralibus maioribus –14-22 mm–, involucris maioribus –20-28 cm diametro–, bracteis involucralibus laevibus, internae valde elongatis (> 10 mm,

obovato-oblongis nec late-obovatis), bracteis autem spicularibus longis aequalibusque aut maioribus quam bracteis involucralibus internis, pediculis spicularibus bracteolis deficientibus, lobulis calycinalibus maioribus (>2 mm), praecipue differt.

Holotypus: Granada, puerto de la Mora, carretera al Pozuelo, a 2.5 km de la carretera N-342, 3°26'10"W, 37°16'0"N (UTM 30SVG5921), 1300 m, dolomías, Nieto Feliner 2722 & Izuzquiza (MA 712146; right specimen).

Additional specimens studied are listed in Table 1.

Key to the subspecies of *Armeria filicaulis*

1. Involucral bracts straw-coloured or cream white, often with a somewhat waxy surface; spicular bracts almost entirely scarious; leaves triquetrous, acuminate, densely ciliate on the margins or less frequently pubescent 2
1. Involucral bracts tawny or copper-coloured; spicular bracts with a firm and tawny dorsal part; leaves somewhat flattish, subacute to subobtusate, glabrescent or sparsely ciliate subsp. **nevadensis**
2. Internal involucral bracts 4-8 mm, widely orbicular to transversely depressed, rounded or notched at apex, minutely rugose (under the lens), cream white, often with a somewhat waxy surface; plant usually with numerous scapes subsp. **filicaulis**
2. Internal involucral bracts 8-13 mm, oblanceolate to oblong, with a rounded or subacute apex, smooth and somewhat glossy (under the lens), straw-coloured; plant with a few scapes 3
3. Spikelet bracts distinctly shorter than the inner involucral bracts; petals pink; involucre 13-20 mm in diameter; tubular involucral sheath 6-7 mm long; scapes 4-6 cm, more or less curved; calyx 5-6 mm subsp. **trevenqueana**
3. Spikelet bracts equalling or longer than the inner involucral bracts; petals white; involucre 21-28 mm in diameter; tubular involucral sheath 14-22 mm long; scapes 20-40 cm, straight; calyx 6-9 mm subsp. **alfacarensis**

Discussion

Taxonomic integrity and distinctiveness of subsp. alfacarensis. The integrity and distinctiveness of *A. filicaulis* subsp. *alfacarensis* is mainly based on the morphological data. It is supported by the following facts. The PCA of morphometric characters cluster the specimens sampled as a distinct entity. This implies a cohesive combination of characters in all the representatives of this taxon. This pattern is paralleled in the UPGMA phenogram, where all the samples of subsp. *alfacarensis* form a single group that is not included within the *A. filicaulis* cluster. These facts, together with the peculiarity of the substrate, support taxonomic recognition of subsp. *alfacarensis* per se. However, inferring the possible origin of the taxon is more complex and molecular data should be used.

Recent Gene flow and co-occurring ITS repeat types. The most likely explanation for co-occurrence of ITS ribotypes within the same genome in *Armeria* is gene flow, either intra- or interspecific (Nieto Feliner & al., 2004). This interpretation is particularly reliable for individuals fitting the geographical structure of ITS variation, that is, those lying in areas where two ribotypes meet. The region between Alfacar and La Peza, north of Sierra Nevada, is one of those contact areas, where ribotypes 2 and 3 meet (Nieto Feliner & al., 2004). There are 5 differing nucleotides between R2 and R3. Of these positions, one has been found to be homogenized in four of the five specimens sampled from Sierra de Alfacar that have co-occurring ribotypes. Since homogenization in ITS regions has proved to be fast in artificial hybrids of *Armeria* (Fuertes Aguilar & al., 1999a), to explain the maintenance of nucleotide polymorphisms (reflective of co-occurring ribotypes), either gene flow is frequent or bias in the homogenization has favoured persistence of copies of the two ribotypes within genomes. There is some evidence of bias for R1 but not for R2 or R3 (Fuertes Aguilar & al., 1999a, 1999b; Nieto Feliner & al., 2004). Therefore, it is more parsimonious to assume that gene flow affecting plants from near Alfacar, both *A. filicaulis* subsp. *alfacarensis* (3 samples, 2 co-occurring ribotypes) and *A. villosa* subsp. *bernisii* (3 samples, 3 co-occurring ribotypes), is recent. There is additional evidence for this besides the ITS. Interspecific gene flow is a frequent phenomenon in *Armeria* (Arrigoni, 1970; Philipp, 1974; Fuertes Aguilar & al., 1999b; Gutiérrez Larena & al., 2002) and plants of *A. villosa* subsp. *bernisii* are at most 3 km away (but probably much less) from those of *A. filicaulis* subsp. *alfacarensis* in Alfacar. Taking into account the unspecific pollinators (Woodell & Dale, 1993) and the low reproductive barriers in *Armeria* (Nieto Feliner & al., 1996) the possibility of recent or contemporary gene flow is high. RAPD data, specifically the minimum spanning tree, supports the suggestion that gene flow suffered by *A. filicaulis* subsp. *alfacarensis* in Alfacar and revealed by the ITS patterns, is from *A. villosa* subsp. *bernisii*, as suggested above. On this same line, based on the relative frequencies and distribution of R2 and R3 (Nieto Feliner & al., 2004), it is likely that in the inferred gene flow events between *A. filicaulis* subsp. *alfacarensis* and *A. villosa* subsp. *bernisii* near Alfacar, the former provided R2 and the latter did R3.

Origin of subsp. alfacarensis. But if recent gene flow provides a likely explanation for the co-occurring ribotypes, it appears to be insufficient to account for the morphological patterns detected and is unlikely to

have been involved in the origin of *A. filicaulis* subsp. *alfacarensis*. Specifically, if standard undifferentiated populations of *A. filicaulis* subsp. *filicaulis* pre-existed in Alfacar-La Peza and only in recent times suffered gene flow from *A. villosa* subsp. *bernisii*, it would be reasonable to find standard forms of *A. filicaulis* subsp. *filicaulis* in the range too, not only subsp. *alfacarensis*. Also, morphological cohesiveness would be difficult to explain unless all the individuals were recent, close descendants of the individuals that suffered gene flow from *A. villosa* subsp. *bernisii*. This seems unlikely. Only specimens consistently matching the taxon here described are found in the crystalline dolomitic sands of Alfacar-La Peza (specimens cited in table 1 and personal observations). It can be therefore concluded that the origin of subsp. *alfacarensis* is probably not related to the occurrence of recent gene flow. In other words, there was some cohesive biological entity already differentiated prior to recent gene flow events. Further, from the eastern end of subsp. *alfacarensis* range, we lack sequence data, but we do have for *A. villosa* subsp. *bernisii* from this area. They reveal no co-occurring ribotypes but only the one from Sierra Nevada (R3), thus suggesting that recent gene flow events may have affected only plants from the eastern end (Alfacar).

¿What is then the origin of *A. filicaulis* subsp. *alfacarensis*? Two possibilities can be envisaged. Either populations from this area diverged from standard morphs of *A. filicaulis* subsp. *filicaulis* or the taxon was originated by hybridization and one of the progenitors was *A. filicaulis* subsp. *filicaulis*. Both possibilities match the scenario of pre-existing populations of *A. filicaulis* (similar to subsp. *filicaulis* or even subsp. *trevenqueana*, cf. Fuertes Aguilar & al., unpubl.) inhabiting the massif before the formation of subsp. *alfacarensis*. In fact, these kinds of xeric substrata exist in nearby areas of Eastern Andalusia (Cerro Trevenque in Sierra Nevada, c. 20 km south of Sierra de Alfacar; Sierra de las Guájaras, c. 50 km SE; Sierra de Almirajara, c. 60 km SE). In all these spots, the predominant or exclusive species of *Armeria* found is *A. filicaulis*, the most xerophytic one in Andalusia and probably the whole Iberian Peninsula. There are other spots with crystalline dolomitic sands in the province of Málaga, where another species of *Armeria* occur, *A. malacitana* Nieto Fel. Interestingly, this taxon has a very similar involucre to *A. filicaulis* subsp. *alfacarensis* although its leaves are wider; those in the new taxon are indistinguishable from *A. filicaulis* subsp. *filicaulis*. Furthermore, the basionym for *A. malacitana* is *A. filicaulis* var. *longifolia*, and it was suggested that both *A. filicaulis* and *A. hirta* were involved in

its origin (Nieto Feliner, 1987). Also noteworthy is the spikelets structure in subsp. *alfacarensis*, with scarce bractlets, and the size and shape of the involucre, both characters resembling those in *A. hirta*. A detailed scenario for this link between *A. malacitana* and *A. filicaulis* subsp. *alfacarensis* cannot be provided, but one possibility is that in both dolomitic areas (Sierra de Alfacar in Granada, Sierra de Mijas, etc. in Málaga) there were forms affine to *A. hirta* that hybridized with *A. filicaulis*. The occurrence of endemics to some of these dolomitic areas (*Anthyllis tejedensis* Boiss., *Convolvulus boissieri* Steudel, *Arenaria armerina* Bory subsp. *caesia* (Boiss.) C. Díaz, C. Morales & F. Valle) indicate floristic links between those areas that are supported by the high floristic similarity values found between some of them (Mota & al., 1993, 2002).

Armeria filicaulis as a *compilospecies*. The possibility of a hybrid origin for *A. filicaulis* subsp. *alfacarensis*, the third of three subspecies described in the last years raises the possibility that *A. filicaulis* follows an evolutionary model –*compilospecies* (Harlan & De Wet, 1963)– such as the one proposed for *A. villosa* (Fuertes Aguilar & al., 1999b). As *A. villosa*, *A. filicaulis* has a relatively wide distribution from the Rif mountains to, at least, Sierra de Alcaraz in Albacete. In addition, samples of *A. filicaulis* also display different ITS repeat copies depending on the geographic origin, namely ribotype 1 (R1) in Morocco, R2 in Sierras de Tejada or Almirajara, R3 in Sierra Nevada, R4 in Cazorla-Segura, Calar del Mundo or Murcian ranges, and a mixture of two ribotypes in intermediate areas (Nieto Feliner & al., 2004). Therefore, the behaviour of the different samples from the species is representative of the geographic structure exhibited by the ITS variation at the genus level and thus reveals important amounts of gene flow (Nieto Feliner & al., 2001). The chloroplast *trnL-F* haplotypes exhibited by *A. filicaulis* seem to be influenced by the sympatric congeners (Gutiérrez Larena & al., 2002) and thus part of them are explained by gene flow, e.g., populations from Sierra de Tejada-Almirajara, or Sierra Nevada populations of *A. filicaulis* subsp. *nevadensis*. However, such an interpretation is not always straightforward. The narrow endemic *A. filicaulis* subsp. *trevenqueana* displays three different haplotypes in a very restricted area, which might be consistent with a gene flow scenario on itself. But one of those haplotypes is a singleton (Gutiérrez Larena & al. 2002) suggesting that subsp. *trevenqueana* has remained isolated for a long period. The morphological evidence is not as revealing of hybridization and introgression as in the case of *A. villosa*. If morphological features of subsp. *nevadensis* do reveal its likely hy-

brid origin from *A. splendens* and *A. villosa* subsp. *bernisii*, a hybrid explanation for diagnostic features of subsp. *trevenqueana* and *alfacarensis* is unclear, as is for pink-flowered populations from Sierra Tejada named var. *minor*. In summary, although not so much of the intraspecific diversification of *A. filicaulis* can be unequivocally attributed to introgressive hybridization as in *A. villosa*, traces of this phenomenon are solid and thus part of the morphological and ecological diversity shown by *A. filicaulis* does seem to have been acquired by this means. Therefore, the species fit to a greater or lesser extent the compilospecies model.

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References

- Arrigoni, P.V. 1970. Contributto alla conoscenza delle Armerie sardo-corse. *Webbia* 25:137-182.
- Blanca, G. 2002. *Flora amenazada y endémica de Sierra Nevada*. Granada.
- Fuertes Aguilar, J., & Nieto Feliner, G. 2003. Additive polymorphisms and reticulation in an ITS phylogeny of thrifts (*Armeria*, Plumbaginaceae). *Molecular Phylogenetics and Evolution* 28: 430-447.
- Fuertes Aguilar, J., Rosselló, J.A. & Nieto Feliner, G. 1999a. Nuclear ribosomal DNA (nrDNA) concerted evolution in natural and artificial hybrids of *Armeria* (Plumbaginaceae). *Molecular Ecology* 8: 1341-1346.
- Fuertes Aguilar, J., Rosselló, J.A. & Nieto Feliner, G. 1999b. Molecular evidence for the compilospecies model of reticulate evolution in *Armeria* (Plumbaginaceae). *Systematic Biology* 48: 735-754.
- Gutiérrez Larena, B., Fuertes Aguilar, J. & Nieto Feliner, G. 2002. Glacial-induced altitudinal migrations in *Armeria* (Plumbaginaceae) inferred from patterns of cpDNA haplotype sharing. *Molecular Ecology* 11: 1965-1974.
- Harlan, J.R., & De Wet, J.M.J. 1963. The compilospecies concept. *Evolution* 17: 497-501.
- Mota, J.F., Valle, F. & Cabello, J. 1993. Dolomitic vegetation of South Spain. *Vegetatio* 109: 29-45.
- Mota, J.F., Pérez García, F.J., Jiménez, M.L., Amate, J.J. & Peñas, J. 2002. Phytogeographical relationships among high mountain areas in the Baetic Ranges (South Spain). *Global Ecology and Biogeography* 11: 497-504.
- Nieto Feliner, G. 1987. El género *Armeria* (Plumbaginaceae) en la Península Ibérica: aclaraciones y novedades para una síntesis. *Anales del Jardín Botánico de Madrid* 44: 319-348.
- Nieto Feliner, G. 1990. *Armeria*. In: Castroviejo, S. & al. (eds.), *Flora iberica* 2: 642-721. Madrid.
- Nieto Feliner, G. 2002. *Armeria*. In: B. Valdés & al. (eds.), *Checklist of Vascular Plants of N Morocco with Identification keys*: 183-185. Madrid.
- Nieto Feliner, G., Izuzquiza, A. & Lansac, A.R. 1996. Natural and experimental hybridization in *Armeria* (Plumbaginaceae): *A. villosa* ssp. *carratracensis*. *Plant Systematics and Evolution* 201: 163-177.
- Nieto Feliner, G., Fuertes Aguilar, J. & Rosselló, J.A. 2001. Can extensive reticulation and concerted evolution result in a cladistically structured molecular data set? *Cladistics* 17: 301-312.
- Nieto Feliner, G., Fuertes Aguilar, J. & Rosselló, J.A. 2002. Reticulation or divergence: the origin of a rare serpentine endemic assessed with chloroplast, nuclear and RAPD markers. *Plant Systematics and Evolution* 231: 19-38.
- Nieto Feliner, G., Gutiérrez Larena, B. & Fuertes Aguilar, J. 2004. Fine-scale Geographical Structure, Intra-individual Polymorphism and Recombination in Nuclear Ribosomal Internal Transcribed Spacers in *Armeria* (Plumbaginaceae). *Annals of Botany* 93: 189-200.
- Nieto Feliner, G., Rosselló, J.A. & Fuertes Aguilar, J. 1998. A new subspecies of *Armeria filicaulis* (Plumbaginaceae) from Sierra Nevada (Southern Spain). *Anales del Jardín Botánico de Madrid* 56: 162-164.
- Philipp, M. 1974. Morphological and genetical studies in the *Armeria maritima* Aggregate. *Botanisk tidsskrift* 69: 40-51.
- Rohlf, F.J. 2000. *NTSYS-pc. Numerical taxonomy and multivariate analysis system*. New York.
- Williams, J.G.K, Kubelik, A.R., Livak, K.J, Rafalski, J.A. & Tingey, S.V. 1990. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. *Nucleic Acids Research* 18: 6531-6535.
- Woodell, S.R.J. & Dale, A. 1993. Biological Flora of the British Isles: *Armeria maritima* (Mill.) Willd. (*Statice armeria* L.; *S. maritima* Mill.). *Journal of Ecology* 81: 573-588.

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