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Overlooked singularity and tiny plants: the *Filago desertorum* clade (Gnaphalieae, Asteraceae)

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Filago desertorum, as traditionally circumscribed, is a species that shows high levels of morphological variation. Previous authors have even suggested that this taxon should represent a heterogeneous assembly of true biological species. A taxonomic revision of the species included in the *Filago desertorum* clade was performed, and amplified fragment length polymorphism (AFLP) markers were used to explore the phylogenetic relationships among the members of the clade. Three species are recognized in the group, one of which is newly described. A full description of ***F. castroviejoi* sp. nov.**, a complete nomenclatural treatment and a key to the species included in the clade are provided. © 2015 The Linnean Society of London, *Botanical Journal of the Linnean Society*, 2015, **179**, 742–754

ADDITIONAL KEYWORDS: AFLPs – Compositae – identification key – Morocco – Spain – species delimitation – taxonomy – typification.

INTRODUCTION

The genus *Filago* L. (Asteraceae) is widely represented in the Northern Hemisphere. The Iberian Peninsula, northern Morocco and northern Algeria, and the Aegean Region, Middle East and Egypt are particularly species-rich areas and putative centres of diversity for this genus. *Filago* has been recircumscribed recently on the basis of sequence data from nuclear and plastid DNA (Galbany-Casals *et al*., 2010), morphological characters (Galbany-Casals *et al*., 2010; Andrés-Sánchez *et al*., 2011, 2015) and further evidence including genome size (Andrés-Sánchez *et al*., 2013c). The genus is monophyletic and is considered to be independent from *Logfia* Cass. (Galbany-Casals *et al*., 2010; Andrés-Sánchez, Martínez-Ortega & Rico, 2013a) and *Bombycilaena* (DC.) Smoljan. (Galbany-Casals *et al*., 2010; Andrés-Sánchez, Martínez-Ortega & Rico, 2014). According to this new classification, the genus comprises *c*. 40 taxa.

Filago is composed of small annual plants with a tomentose to villose eglandular indumentum, alternate, sessile leaves, mainly sessile, heterogamous capitula grouped in glomerula often surrounded by a ray of leaves, paleae arranged in a few rows subtending the external female florets (these filiform and often outnumbering the hermaphrodite ones, which are tubular and perfect or functionally male), female and hermaphrodite florets apically attached to an oblong achene, and a pappus, when present, composed of scabrid bristles. The delimitation of species in *Filago* is not an easy task because of the scarcity of morphological characters that have been considered to be relevant for the taxonomy of the genus, and also because many of these characters are probably affected by homoplasy (Galbany-Casals *et al*., 2010; Andrés-Sánchez *et al*., 2015). Furthermore, the general aspect of the individuals, even within one population, varies greatly, probably in connection with environmental factors (Authors, pers. observ.),

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which has further complicated the identification of morphological traits of potential taxonomic value.

Traditionally, particularly after the detailed studies conducted by Wagenitz (1968), the individuals of *Filago* from the semi-desert areas of south-eastern Spain, the Canary Islands, North Africa and south-western Asia, that have hairs at the margin of the internal paleae, have been included under the variability of the species *Filago desertorum* Pomel. Wagenitz (1968) had already commented on the high level of morphological variation among plants that, based on this character, could be determined as *F. desertorum* and had suggested that this taxon represented a heterogeneous assembly of true biological species. However, he was unable to find any correlation among morphological traits, geographical areas and/or ecological conditions, and therefore avoided the description of new species or subspecies in this taxonomic group.

A relatively recent DNA sequence-based phylogenetic analysis of the *Filago* group (Galbany-Casals *et al*., 2010) included, among others, two different samples identified as *F. desertorum*. Some level of sequence divergence was found between these two samples and, surprisingly, the species was not recovered as monophyletic. The specimen named '*F. desertorum* 1' from Israel was found to be closely related to the morphologically divergent species *F. mareotica* Delile, whereas '*F. desertorum* 2' from north-eastern Morocco showed a sister group relationship to the clade formed by the two preceding samples (i.e. *F. mareotica* + '*F. desertorum* 1'). These specimens comprised a clade, named Clade F by Galbany-Casals *et al*. (2010), and will hereafter be referred to as the '*Filago desertorum* clade'. Moreover, whilst preparing a taxonomic revision of *Filago* for *Flora iberica* [Castroviejo (Coord. Gen.), 1986–2014], we found several morphological differences between individuals previously determined as *F. desertorum* from the Iberian Peninsula and north-western Morocco and those from the remaining areas in which the plant is present.

In this work, an accurate revision of herbarium specimens has been carried out in order to search for characters with taxonomic and/or phylogenetic value in the study group. The use of genomic data to provide insight into the systematics and evolution of *Filago* has proved to be a requirement in a genus in which the widespread presence of homoplasy in morphology has obscured the taxonomic boundaries between species and has repeatedly led to unnatural classifications (Galbany-Casals *et al*., 2010). Considering this fact, we have selected an anonymous whole-genome fingerprinting method, i.e. amplified fragment length polymorphism (AFLP), to try to examine the phylogenetic relationships among the closely related species included in the *Filago desertorum* clade and to establish taxonomic limits between them accurately. This method has been repeatedly shown to be a useful tool in similar studies (e.g. Kardolus, Van Eck & Van den Berg, 1998; Werres *et al*., 2001; Bottini *et al*., 2002; Martínez-Ortega *et al*., 2004; Meudt & Clarke, 2007; Van den Berg & Groendijk-Wilders, 2007; Duminil *et al*., 2012; Prebble, Meudt & Garnock-Jones, 2012; Paul, Nandi & Palni, 2013; Magauer *et al*., 2014). More specifically, our aims are as follows: (1) to test whether the species traditionally named *F. desertorum* is monophyletic; (2) to assess the taxonomic identity of those *Filago* from north-western Morocco and south-eastern Spain with hairs at the margin of the internal paleae.

MATERIAL AND METHODS

Plant material, sampling strategy and outgroup selection

An exhaustive review of herbarium material previously identified as *F. desertorum* (hereafter *F. desertorum s.l.*) was conducted. In total, 290 sheets deposited in the herbaria ABH, ALME, B, BC, BCN, BM, COA, COI, G, GDA, GOET, JACA, K, MA, MAF, MJG, MPU, P, SALA, SEV, VAL, W and WU (listed by their acronyms according to Thiers, 2014, continuously updated) were studied. To complete the information obtained, 30 populations in their natural habitats were visited and at least one sheet from each was lodged at the herbarium of the University of Salamanca, Spain (SALA).

In addition, leaf material from 71 individuals from 19 populations from North Africa, the Iberian Peninsula and the Canary Islands, identified as *F. desertorum s.l.* (17 populations) and *F. mareotica* (two populations), was collected and dried in silica gel (see Appendix). Three to five individuals per population were included, except for two populations of *F. desertorum s.l.* from the Iberian Peninsula, because, in these cases, only one or two individuals were available. Each sampling site was geo-referenced with a GARMIN GPSMAP 60 and voucher specimens were deposited at SALA. Five additional samples from the same population of *F. lutescens* Jord. were selected to be used as outgroup in the neighbour-joining (NJ) analysis. The selection of this outgroup was based on the results of Galbany-Casals *et al*. (2010).

DNA isolation and AFLP fingerprinting

Total genomic DNA was isolated from crushed dried leaf material (*c*. 25 mg) following the 2 × cetyl trimethyl ammonium bromide (CTAB) protocol (Doyle & Doyle, 1987) with modifications. The quality of the extracted DNA was checked in 1% tris acetate ethyl-

enediaminetetraacetic acid (TAE) agarose gel. A negative control sample was consistently included to test for contamination, and five random chosen samples were replicated to test for reproducibility. Total isolated DNA was deposited at the Plant DNA Bank of the University of Salamanca.

AFLP profiles were drawn for the 76 sampled plants following established protocols (Vos *et al*., 1995) with minor modifications. An initial screening of selective primers was performed using 19 primer combinations. The three finally selected primer combinations (fluorescent dye in parentheses), *Eco*RI-AGA(6-FAM)/*Mse*I-CC, *Eco*RI-ACT(6-FAM)/*Mse*I-CTC and *Eco*RI-AGG(VIC)/*Mse*I-CTC (hereafter, primer combinations 1, 2 and 3, respectively), were used for the selective polymerase chain reaction. These combinations were selected because they generated clear and reproducible bands for which homology was easy to ensure. They also showed appropriate levels of variation among the taxa included in the *F. desertorum* clade and within and among populations. Samples (3 μL) of the fluorescently labelled selective amplification products were combined and separated on a capillary electrophoresis sequencer (ABI 3730 DNA Analyser; Applied Biosystems, Foster City, CA, USA) with GenScan ROX (Applied Biosystems) as an internal size standard.

AFLP data analysis

Raw AFLP data with amplified fragments from 100 to 500 base pairs (bp) were scored and exported as a presence/absence matrix using the software GeneMapper 4.0 (Applied Biosystems). Ambiguous peaks were manually removed. The data matrix used for further analyses is available on request from the corresponding author.

As a first approach to the global genetic relationships among the analysed individuals and possible structure of the data, an NJ analysis including 10 000 bootstrap (BS) pseudoreplicates (Felsenstein, 1985) based on a matrix of Nei & Li (1979) distances was conducted with the software PAUP 4.0b10 (Swofford, 2003). To show reticulate relationships among putative taxa, an unrooted NeighborNet (NNet) was produced using the program SplitsTree 4.13.1 (Huson & Bryant, 2006), with the distance uncorrected *P*. The outgroup *F. lutescens* was excluded from this analysis.

In addition, a principal coordinate analysis (PCoA) based on a matrix of Dice's coefficient, which is suitable for multilocus dominant genetic data (Dice, 1945; Lowe, Harris & Ashton, 2004), among individuals was performed in NTSYS-pc 2.21c (Applied Biostatistics, Inc.; Rohlf, 2009).

Population genetic structure was additionally investigated using a Bayesian clustering method implemented in STRUCTURE v. 2.3.4 (Pritchard, Stephens & Donnelly, 2000) following the approach described by Falush, Stephens & Pritchard (2007) for dominant markers. This method uses a Markov chain Monte Carlo simulation approach to group samples into an optimal number of genetic clusters (*K*) and does not assume the a priori assignment of individuals to populations or to clusters. Analyses were based on an admixture ancestral model with correlated allele frequencies among populations. The proportion of membership of each individual and population to the *K* clusters was calculated, performing five runs for each *K* value between two and 19 (equal to the number of populations plus one and excluding the outgroup *F. lutescens*) with a run length of the Markov chain Monte Carlo and a burn-in period of 1×10^6 and 1×10^6 iterations, respectively. *K* was estimated using the *ad hoc* parameter (ΔK statistic) of Evanno, Regnaut & Goudet (2005), as implemented in the online application of Structure Harvester software (v0.63; Earl & von Holdt, 2012).

Analysis of molecular variance (AMOVA; Excoffier, Smouse & Quattro, 1992) was carried out with Arlequin 3.5.1.2 (Excoffier & Lischer, 2010). This analysis was used to distribute genetic variation into portions assignable to differences among predefined groups (F_{CT}), among populations within these groups (F_{SC}) and within populations across the entire dataset (F_{ST}) (Turner *et al*., 2000). From a taxonomic perspective, this could be translated into differences between species and allows us to test the taxonomic hypothesis (Knijff *et al*., 2001). The AMOVA was performed, arranging all populations of the *F. desertorum* clade into three of the four taxonomic groups found in NJ, NNet and PCoA excluding *F. lutescens* (outgroup).

RESULTS

Morphology

The identification of *F. mareotica* from *F. desertorum s.l.* is relatively easy on the basis of morphological characters. *Filago mareotica* has solitary capitula, generally arranged as a monochasium, whereas *F. desertorum s.l.* has clusters of capitula, usually arranged as a dichasium or pleochasium. The number of paleae per capitulum is 15–25 in *F. mareotica* and 25–30 in *F. desertorum s.l.*, and the abaxial face of the internal paleae is completely glabrous in *F. mareotica* and only has hairs near the margin in *F. desertorum s.l.*

Furthermore, the study of herbarium material allowed the identification of two morphotypes within the variation of *F. desertorum s.l.* First, those samples hereafter named as *F. desertorum s.s.* have villose–tomentose external paleae, capitula and clusters

Table 1. Number of samples, populations, fragments, polymorphic fragments and their percentage per species

Taxon	No. samples	No. populations	No. fragments	No. polymorphic fragments	% polymorphic fragments
Filago lutescens	5	1	78	33	0.42
F. mareotica	8	2	117	80	0.68
F. desertorum	24	7	197	166	0.84
F. castroviejoi	39	10	147	124	0.84
Total	76	20			

(because the abaxial face of the paleae is villose–tomentose) and ovate capitula with five slightly marked angles. They are present in south-western Asia (from the Middle East to India), North Africa (from Egypt to Morocco, but here restricted to the south of the Atlas mountain range) and the Canary Islands.

In contrast, those individuals assigned to 'morphotype *castroviejoi*' are characterized by glabrous to subglabrous external paleae, capitula and clusters (the abaxial face of the external paleae is glabrous or has a few hairs near the margin in this case) and pyramidal capitula with five strongly marked angles. These plants grow in the Iberian Peninsula and north-eastern Morocco (north of the Atlas range).

AFLP DATA ANALYSES

The three primer combinations applied to 76 selected plants representative of the variation of the *F. desertorum* clade plus *F. lutescens* produced 255 polymorphic and reproducible fragments for which homology was easy to ensure (primer combinations 1, 2 and 3 generated 104, 80 and 71 fragments, respectively). The final error rate was not significant. Table 1 summarizes data on the total number of alleles, number and percentage of polymorphic fragments for each species or morphotype.

Both the NJ analysis (Fig. 1A) and PCoA (Fig. 1B) showed that the genetic variation of the study group was organized into four groups, which correspond to well-established species (i.e. *F. mareotica*, *F. lutescens*) and to the morphotypes previously described within the variation of *F. desertorum s.l.* The NNet (Fig. 1C) diagram conducted on all individuals, excluding the outgroup, revealed an overall structure of genetic variation within the ingroup into three main groups that was congruent with NJ analysis and PCoA. NNet, in contrast with the NJ-derived results, showed a position of *F. desertorum s.s.* closer to *F. mareotica* than to 'morphotype *castroviejoi*'.

BS values calculated for the NJ topology (Fig. 1A) provided strong support for the monophyly of the *F. desertorum* clade (100% BS) and for *F. mareotica* (100% BS) and for 'morphotype *castroviejoi*' (90% BS). However, neither *F. desertorum s.s.* nor the suggested close phylogenetic affinity between 'morphotype *castroviejoi*' and *F. mareotica* shown by the NJ analysis received strong BS support.

The first three axes of the PCoA accounted for 17.53%, 11.48% and 8.95%, respectively (37.98% in total), of the total variance of the model.

The samples belonging to *F. desertorum s.s.* formed a less discrete group than those corresponding to 'morphotype *castroviejoi*' or to *F. mareotica* in both the NNet and PCoA diagrams. Also, the branch lengths were longer for these samples in the NJ analysis.

The Bayesian analysis of population structure showed a maximum $\Delta K = 975.842032$ value for the optimal value $K = 3$. In this analysis, the individuals identified as *F. mareotica* were detected as a single group (cluster 1; Fig. 1D, green), whereas those initially determined as *F. desertorum s.l.* were mainly grouped into two different major clusters, corresponding to *F. desertorum s.s.* (cluster 2; Fig. 1D, blue) and 'morphotype *castroviejoi*' (cluster 3; Fig. 1D, red). A low proportion of membership to clusters 1 and 3 was detected in particular individuals from populations 7 and 9, and clusters 1 and 2 were almost insignificantly represented in populations 10, 12, 14, 17 and 18.

The AMOVA showed that 35.89% of the total genetic variability of the dataset could be attributed to genetic differentiation within all populations ($F_{ST} = 0.6411$), 34.37% to variation among populations within taxonomic groups ($F_{SC} = 0.4891$) and 29.75% to differences among taxonomic groups ($F_{CT} = 0.2974$).

DISCUSSION

DETERMINATION OF SPECIES BOUNDARIES WITHIN THE *F. DESERTORUM* CLADE

Our results demonstrate the capacity of AFLP markers to determine species boundaries in *Filago*, a genus that lacks sufficient morphological traits relevant to provide satisfactory infrageneric taxonomic treatments (Wagenitz, 1969; Galbany-Casals *et al.*, 2010; Andrés-Sánchez *et al.*, 2011, 2013c).

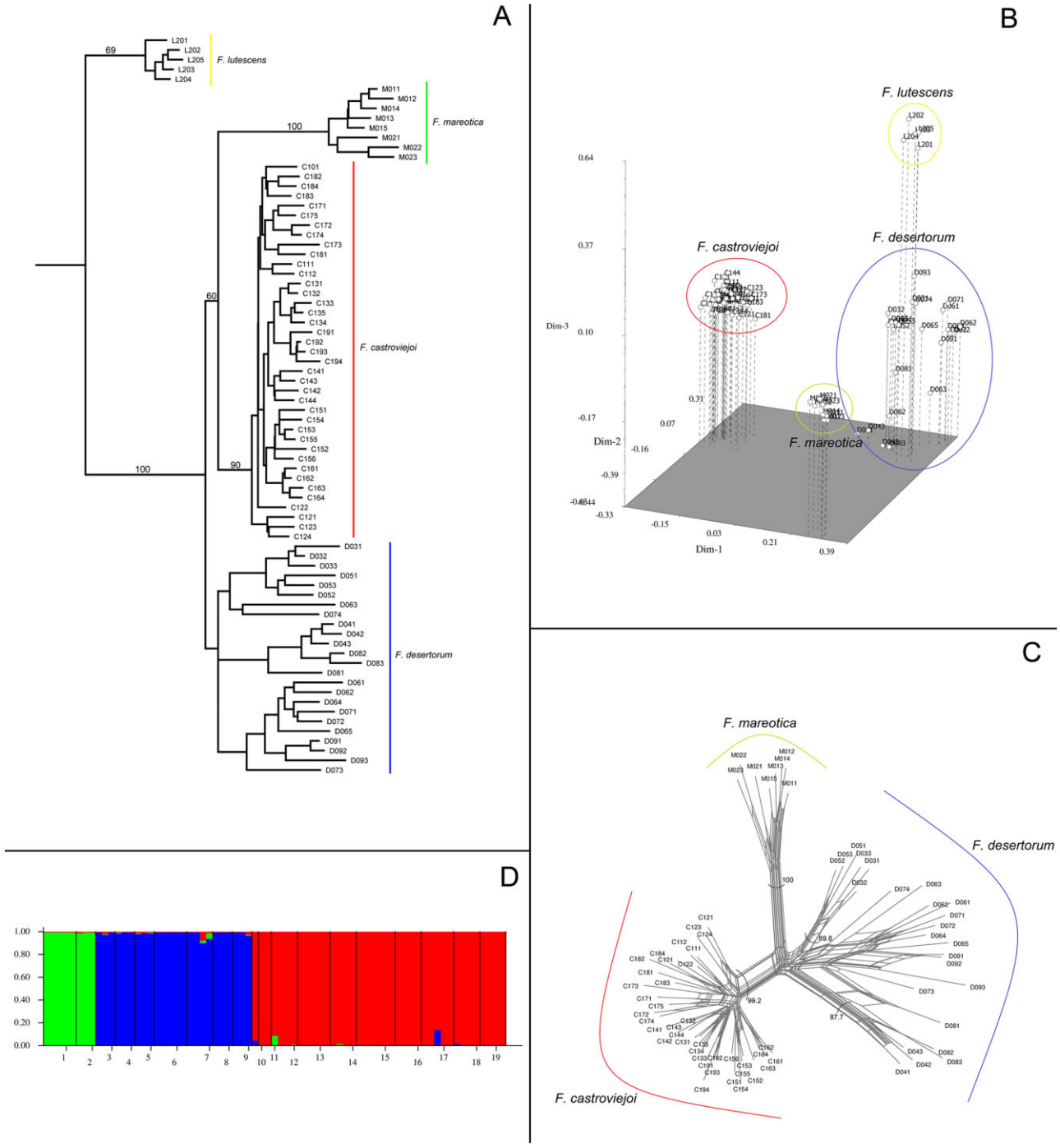

Figure 1. A, Amplified fragment length polymorphism (AFLP) phylograms of a neighbour-joining (NJ) analysis of pairwise Nei & Li distances among individuals of the *Filago desertorum* complex; bootstrap values are shown. B, Principal coordinate analysis (PCoA) of all investigated individuals of the *F. desertorum* complex. C, NeighborNet (NNet) derived from AFLP data of the *F. desertorum* complex. In A, B and C, the names of all the individuals are coded to allow identification: the first letter indicates the species name (C for ***F. castroviejoi***; D for *F. desertorum*; L for *F. lutescens* and M for *F. mareotica*); the first two numbers correspond to the population number (01 to 20); the last number indicates the individual within a particular population. D, Bayesian analysis of population structure of the studied *F. desertorum* complex. AFLP genotypes were arranged into three clusters using the software STRUCTURE v. 2.3.4; green, *F. mareotica*; blue, *F. desertorum*; red, ***F. castroviejoi***.

Filago desertorum s.l. has been traditionally considered to be a widely distributed and morphologically variable species (Wagenitz, 1968). Both our AFLP genetic data and the intraspecific levels of sequence variation detected by Galbany-Casals *et al*. (2010) between two samples identified as *F. desertorum s.l.* congruently suggest that this species is not monophyletic.

The distance-based phenograms (Fig. 1A, C) and Bayesian analysis of population structure (Fig. 1D) resulted in good resolution levels for the recognition of three taxonomic entities in the *F. desertorum* clade. In addition, AMOVA suggested that a significant part of the total genetic variability of the dataset could be attributed to genetic differentiation among these taxonomic entities.

These genetic data support the recognition of at least two independent taxa in the variation of those *Filago* that have hairs on the margin of the internal paleae. In a superficial analysis, these two taxonomic entities are morphologically similar, but the exhaustive revision of herbarium specimens and the closer examination of morphological traits have allowed the recognition of two morphotypes, each characterized by a set of morphological characters, which correspond to the groups identified using genetic markers. Furthermore, these morphotypes show non-sympatric distribution areas: *F. desertorum s.s.* has a Saharo-Sindican, Irano-Turanian and Macaronesian distribution, whereas 'morphotype *castroviejoi*' is restricted to the south-eastern Iberian Peninsula and north-eastern Morocco north of the Atlas range. Based on all this evidence, we consider that 'morphotype *castroviejoi*' should be recognized at the specific rank as *Filago castroviejoi* Andrés-Sánchez, D.Gut.Larr., E.Rico & M.M.Mart.Ort.

The third taxonomic entity identified in the '*Filago desertorum* clade' corresponds to the traditionally recognized and well-characterized species *F. mareotica*. Its distribution area roughly coincides with that of *F. desertorum s.l.* (Andrés-Sánchez, Martínez-Ortega & Rico, 2013b), but it shows differences in morphology (Galbany-Casals *et al*., 2010), genome size (Andrés-Sánchez *et al*., 2013c) and ecological preferences (Galbany-Casals *et al*., 2010).

Phylogenetic relationships within the *F. desertorum* clade

The genetic markers used in this study have provided additional independent data to explore the phylogenetic relationships among those taxa belonging to the *F. desertorum* clade. *Filago mareotica* was traditionally considered to be an isolated taxon in *Filago*. Pomel (1888), for example, considered that this species should be recognized as a different genus, *Gifolaria* (Coss. & Kral.) Pomel., and other authors (Cosson & Kralik, 1857; Wagenitz, 1969) included it in *Filago* section *Gifolaria* Coss. & Kral. DNA sequence data from nuclear and plastid genomes suggested, for the first time, a phylogenetic relationship between *F. mareotica* and *F. desertorum s.l.* (Galbany-Casals *et al*., 2010). In particular, these authors identified a close affinity between a sample from Israel identified as *F. desertorum s.s.* (sub '*F. desertorum* 1') and *F. mareotica*, a group that was recovered as monophyletic (99% BS), with *F. castroviejoi* (sub '*F. desertorum* 2') as sister to it (98% BS). AFLP data generated in the present study confirmed the monophyly of the *F. desertorum* clade (100% BS, Fig. 1A), including the three species *F. castroviejoi*, *F. desertorum s.s.* and *F. mareotica*. However, these data did not shed light on the relationships among the species, as the phylogenetic relationships between *F. mareotica*, *F. castroviejoi* and *F. desertorum s.s.* were not congruent between the NJ and NNet analyses (Fig. 1A, C). The first analysis showed a close relationship between *F. mareotica* and *F. castroviejoi*, whereas the positions of *F. mareotica* and *F. desertorum s.s.* in NNet [congruent with the internal transcribed spacer (ITS) tree; Galbany-Casals *et al*., 2010] suggested a close relationship between these two taxa (Fig. 1C). This incongruence may be a result of the fact that we used an outgroup in the first analysis, but not in the second. Moreover, the phylogenetic affinity between *F. castroviejoi* and *F. mareotica* suggested by the NJ analysis received low BS support (60%). This low statistical support is probably a result of the lack of congruence among the reconstructions based on each selective primer combination separately (Supporting Information, Figs S1–S3), as one suggests a connection between *F. castroviejoi* and *F. desertorum s.s.* (Fig. S1, primer combination *Eco*RI-AGA(6-FAM)/*Mse*I-CC), whereas primer combinations 2 and 3 (Figs S2 and S3, corresponding to primers combinations *Eco*RI-ACT(6-FAM)/*Mse*I-CTC and *Eco*RI-AGG(VIC)/*Mse*I-CTC) show a relationship between *F. mareotica* and *F. castroviejoi*.

Incongruence between AFLP data and nuclear DNA sequences (e.g. Hodkinson *et al*., 2000; El-Rabey *et al*., 2002; Semerikov *et al*., 2003; Koopman, 2005) are less frequent than between AFLP and plastid DNA (Koopman, 2005). Semerikov *et al*. (2003) and Koopman (2005) suggested that these incongruences could be artefactual or reflect some peculiarities in the evolution of the genome. According to these authors, AFLP markers are quickly evolving and might be less reliable than ITS for phylogenetic inference. For this reason, and according to the results obtained by Galbany-Casals *et al*. (2010), *F. mareotica* and *F. desertorum s.s.* could have a closer affinity than either of these species with *F. castroviejoi*.

High genetic polymorphism in *F. desertorum s.s.*

Although all the samples identified as *F. desertorum s.s.* were grouped in the same cluster in NJ analysis, NNet and PCoA, this cluster did not receive significant BS support. Moreover, these samples defined a less discrete group than those corresponding to *F. castroviejoi* or to *F. mareotica* in all of these analyses, and the branch lengths in NJ were higher. In addition, the total number of alleles and the number of polymorphic alleles were relatively high, in comparison with those found for the species *F. castroviejoi*.

High levels of intraspecific genetic variation have been found previously in some genera of tribe Gnaphalieae [e.g. *Helichrysum* Mill. (Galbany-Casals *et al*., 2011), *Raoulia* alliance (Smissen, Breitwieser & Ward, 2004) and *Leucogenes* Beauverd (Smissen & Breitwieser, 2008)], and in some *Filago* spp. [e.g. *F. argentea* (Pomel) Chrtek & Holub and *F. pygmaea* L. (Galbany-Casals *et al*., 2010) and *F. aegaea* Wagenitz (Andrés-Sánchez *et al*., 2015)]. According to Galbany-Casals *et al*. (2010) and Andrés-Sánchez *et al*. (2015), the intraspecific variation found in some *Filago* spp. could suggest hybridization or incomplete lineage sorting of ancestral polymorphism. Hybridization among extant taxa seems unlikely in *Filago* as, after more than a decade of field and herbarium observations, we have never found plants showing intermediate morphologies between well-established species, not even in areas in which species from the same clade grow nearby, but ancient hybridization cannot be excluded as an important mechanism that might have acted in the evolution of the *F. desertorum* clade. These evolutionary mechanisms could also be the cause of the relatively high genetic variation found in *F. desertorum s.s.*

The high genetic polymorphism could also be related to the fact that *F. desertorum s.s.* is a morphologically variable taxon (Wagenitz, 1968; Authors, pers. observ.), and the possibility of identifying hidden unrecognized taxa within it exists, particularly considering the small number of morphological characters with taxonomic value traditionally used in *Filago*.

Last, it should also be considered that *F. desertorum s.s.* shows a wide distribution area (Wagenitz, 1968, 1969; Andrés-Sánchez *et al*., 2013b) and, although the samples selected for this study constitute a rough representation of the distribution area of the species (Tunisia, Algeria and the Canary Islands), they may not represent well its complete geographical range and variation. This could also be a reason why apparent high levels of genetic polymorphism have been found within *F. desertorum s.s*. Further studies are needed in order to try to give a satisfactory explanation to all of these issues.

Taxonomic treatment

A relatively small number of morphological characters have been traditionally used for the characterization and identification of *F. desertorum s.l. Filago castroviejoi* shares states of these morphological characters (namely abaxial face of the internal paleae glabrous with hairs near the margin) with *F. desertorum s.s.*, and this is probably the reason why the first taxonomic entity has been overlooked for so long.

Given that the lectotype of *F. desertorum* is a specimen (MPU004840; Wagenitz, 1968) collected by Pomel in Algeria, it corresponds well with the Saharo-Sindican, Irano-Turanian and Macaronesian morphotype and, according to the *Internacional Code of Nomenclature for Algae, Fungi and Plants* (McNeill *et al*., 2012), the name *F. desertorum* Pomel should be retained.

A complete description of the new south-eastern Iberian/north-eastern Moroccan species is provided here, with a complete taxonomic treatment for *F. desertorum* and *F. mareotica*.

Filago castroviejoi Andrés-Sánchez, D.Gut.Larr., E.Rico & M.M.Mart.Ort. **sp. nov.** (Figs 2, 3)

Diagnosis: A propiore specie, *F. desertorum*, differt propter externis receptacularibus paleis glabris ad subglabris in abaxialis faciei tergo exhibere et pyramidalia capitula cum quinque angulis fortiter notatis.

Identification key

1. Internal paleae glabrous; capitula solitary; paleae 15–25 per capitula ..... *F. mareotica*
–. Internal paleae with hairs near the margin; capitula arranged in subglobose clusters; paleae 25–30 per capitula 2
2. Clusters, capitula and external paleae glabrous or subglabrous; capitula pyramidal with five strongly marked angles ..... *F. castroviejoi*
–. Clusters, capitula and external paleae villose–tomentose; capitula ovate with five slightly marked angles ..... *F. desertorum*

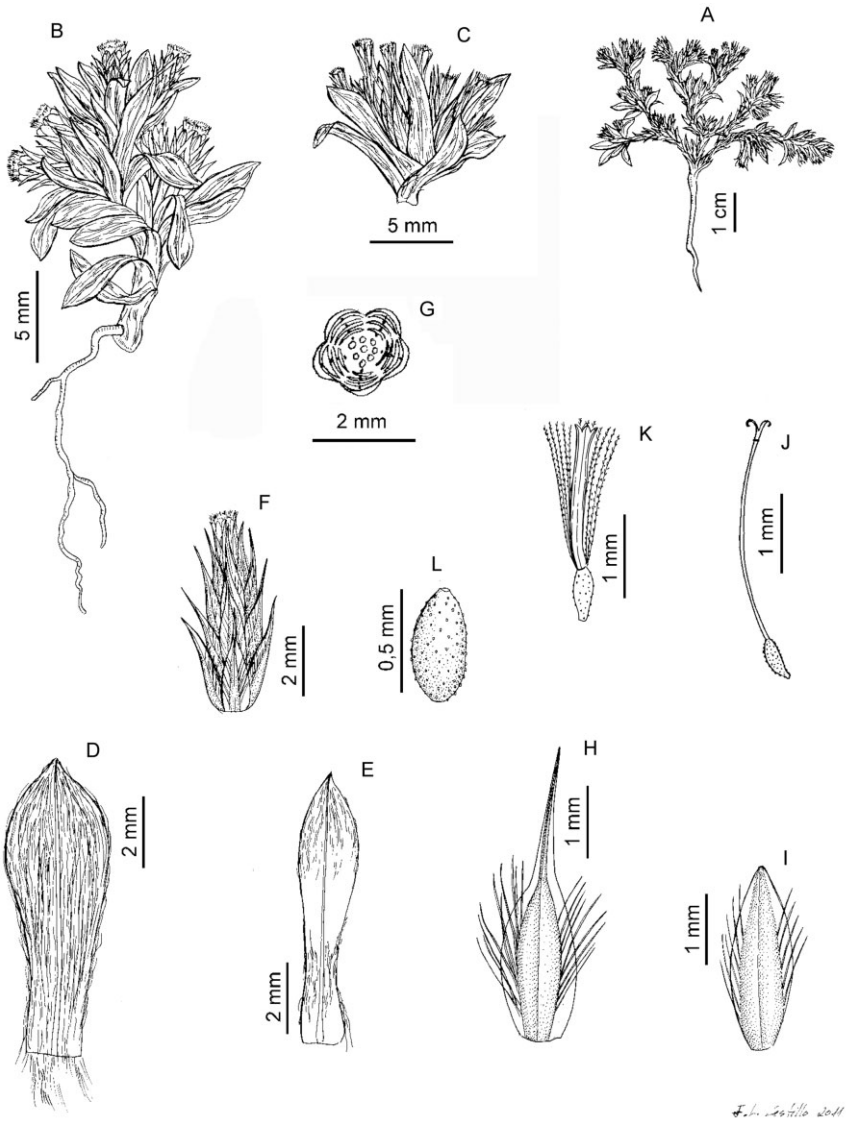

Figure 2. ***Filago castroviejoi*** from S. Andrés-Sánchez SA278 (SALA139218) (holotype): A, B, plant habit; C, cluster of capitula, D, stem leaf; E, involucrant leaf; F, capitulum; G, diagram of transverse section of the capitulum; H, external palea; I, internal paleae; J, external female floret; K, hermaphrodite floret; L, achene. Drawn by Juan Castillo.

Type: Holotype: Spain, Murcia, Monteagudo, castle, 3 802 196N–109 747W, 76 m, 25.iv.2009, *S. Andrés-Sánchez SA278* SALA139218!; *isotype*: MA854757!

Description: Annual herbs, densely hairy, with eglandular, villose–tomentose, greyish to grey–whitish, adpressed indumentum. STEMS 0.7–16.5 cm, ascending to erect, unbranched or branched in the inflorescence, in this case arranged like to cymes: dichasium or pleochasium, rarely monochasium, sometimes branched from the base. LEAVES of the stem 5–24 × 1–4 mm, alternate, ±adpressed, oblanceolate to spathulate, entire, acute rarely obtuse, with a small scarious brownish mucro, flat to slightly undulate in the margin, sessile; involucrant leaves seven to 13, 5–14 × 1–4 mm, similar length or longer than the capitula, oblanceolate to spathulate, entire, acute rarely obtuse, with a small scarious brownish mucro, margin straight to slightly undulate and flat to slightly involute. INFLORESCENCE in clusters, arranged like to cymes: dichasium or pleochasium, rarely monochasium, lax; clusters with four to 12 capitula, 8–13 mm in diameter, subglobose, contracted. CAPITULA 3–6 × 2–4 mm, disciform, heterogamous, sessile, pyramidal, with five strongly marked angles, glabrous or subglabrous. INVOLUCRE null RECEPTACLE clavate. PALEAE 25–30, 4–5 × 1–1.5 mm, arranged in five vertical rows with five or six paleae

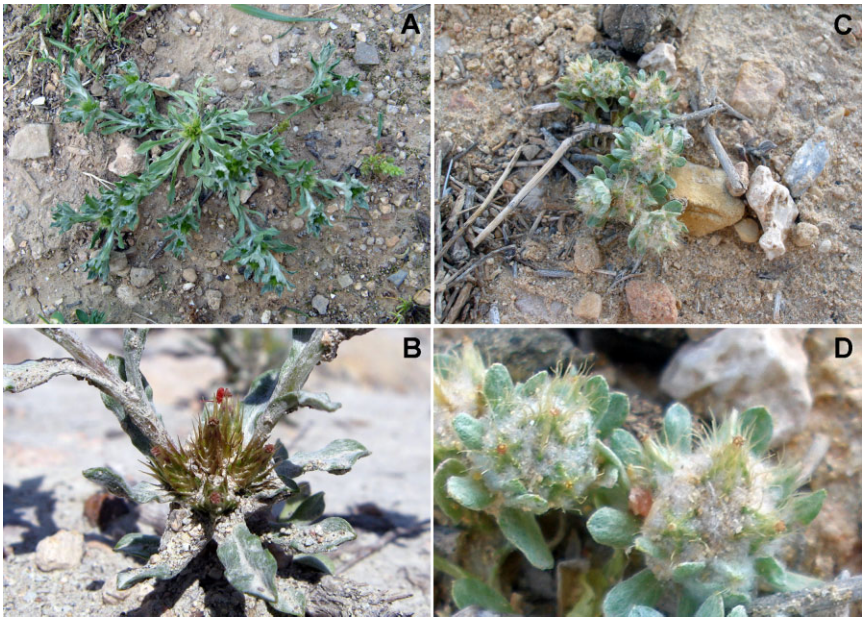

Figure 3. A, Habit of ***Filago castroviejoi*** in the field *S. Andrés-Sánchez et al. SA149* (SALA134391). B, Cluster of capitula of ***F. castroviejoi*** *S. Andrés-Sánchez et al. SA95* (SALA134350). C, Habit of *F. desertorum A. Herrero et al. AH3933* (SALA 110201). D, Cluster of capitula of *F. desertorum A. Herrero et al. AH3933* (SALA 110201).

per vertical row, patent in fruit, slightly concave, green with hyaline margin, sometimes purple at the tip, herbaceous in flower and scarious in fruit; external and medium ones, lanceolate to ovate, not enclosing a female floret placed in this axil, aristate with arista yellowish of 1–2 mm, with the abaxial face glabrous or subglabrous with few long hairs in the margin and the adaxial face glabrous; internal elliptic, surrounding together the internal florets, obtuse, green with hyaline margin, sometimes purple at the tip, with the abaxial face glabrous with hairs near the margin and the adaxial face glabrous. FLORETS heteromorphic; female florets, external in the axil of the paleae or internal together with the hermaphrodite ones surrounded by the internal paleae, corolla 3.0–3.5 mm long, filiform, whitish–yellowish, entire or slightly lacerated and reddish to brownish at the tip; external 20–25 eppapose; internal three to six pappose; hermaphrodite florets all internal, four to eight, fully fertile, corolla 2.5–3.5 mm long, tubular, whitish–yellowish, with four reddish teeth. ANTHERS with short basal appendages. ACHENES homomorphic, 0.8–1.0 × 0.3–0.4 mm, ellipsoid to cylindrical, slightly compressed dorsiventrally, with short clavate twin hairs. PAPPUS absent in external female florets; internal female florets and hermaphrodite ones with 12–16 white, scabrid, free bristles.

Iconography: The plant is illustrated in Figure 2; the habit and cluster of the capitula are shown in Figure 3A, B.

Genome size: Andrés-Sánchez *et al*. (2013c) estimated the genome size of *F. castroviejoi* (sub *F. desertorum*) as 2C = 1.3852 ± 0.0074 pg.

Phenology: Flowering from March to May (rarely in February).

Distribution: Filago castroviejoi is known from the south-eastern Iberian Peninsula (provinces of Albacete, Alicante, Murcia and Almería) and north-eastern Morocco (regions of Taza-Al Hoceima-Taounate and Oriental) (Fig. 4).

Ecology: The plants grow in open xerophytic or subdesert areas, terophytic pastures generally ruderalized, fallow lands, road margins, cultures, salt marshes and dry riverbeds, preferably on basic (limestone or chalk) substrates. See also Andrés-Sánchez *et al*. (2013b) under *F. desertorum*.

Etymology: The species is posthumously dedicated to our friend and colleague Professor Santiago Castroviejo Bolibar, who was the coordinator of *Flora iberica* for a long time.

Observations: Filago castroviejoi has been frequently confused with *F. pyramidata* L. They usually grow together in the Iberian Peninsula and Morocco. In a superficial observation, they show a high proportion of morphological similarities, but *F. castroviejoi* has

Figure 4. Distribution map of ***Filago castroviejoi***.

hairs near the margin of the internal paleae, whereas, in *F. pyramidata*, these paleae are glabrous. In addition, Galbany-Casals *et al*. (2010) demonstrated that they are not phylogenetically related.

Selected specimens examined: See Supporting Information, Fig. S4.

FILAGO DESERTORUM POMEL (FIG. 3B, C)

Filago desertorum Pomel, Nouv. Mat. Fl. Atlant. 1: 46 (1874). *Filago spathulata* var *desertorum* (Pomel) Batt. in Batt. & Trab., Fl. Algérie (Dicot.): 441 (1889). *Filago germanica* var *desertorum* (Pomel) Maire in Jahand. & Maire, Cat. Pl. Maroc. 3: 745 (1934).

Lectotype (designated by Wagenitz, 1968): ALGERIE. 'Djebel Amour, El Abiod, Metlil, Pomel' (MPU-MAIRE MPU004840!). Syntypes: P00084151 photograph!, MPU004838!, MPU004839!.

Filago spathulata var. *alexandrina* Bornm. in Feddes Repert. 18: 41 (1922). Lectotype (**designated here**): EGYPT. 'Alexandria, in arenosis maritimis ad Sidi-Gaber, 7.iv.1908, *Bornmüller 10768* (B100094036 photograph!). Syntypes: B100094037 photograph!; P!; LD, E.

Filago spathulata f. *evaciformis* Bornm. in Feddes Repert. 18: 42 (1922). Lectotype (**designated here**): EGYPT. 'Alexandria, El Meks, *Petry 14* (B100094039 photograph!). Syntype: B100094038 photograph!

Distribution: Filago desertorum is present in south-western Asia (from the Middle East to India), North Africa (from Egypt to Morocco, but here restricted to the south of the Atlas mountain range) and the Canary Islands.

Ecology: The plants grow in open desert or sub-desert areas, ruderalized terophytic pastures, fallow lands, road margins, salt marshes and dry riverbeds, rocky outcrops, sandy places, preferably on basic (limestone or chalk) substrates.

Selected specimens examined: See Fig. S4.

FILAGO MAREOTICA DELILE

Filago mareotica Delile, Descr. Égypte, Hist. Nat. 2: 274 (1813). *Micropus mareoticus* (Delile) Spreng., Syst. Veg. 3: 499 (1826). *Evax mareotica* (Delile) D.C., Prodr. 5: 459 (1836). *Evacopsis mareotica* (Delile) Pomel in Bull. Soc. Bot. France 35: 334 (1888). *Gifolaria mareotica* (Delile) Chrtek & Holub in Preslia 35: 10 (1963).

Lectotype (designated by Andrés-Sánchez *et al*., 2011): figure 2, table 47 in Delile, Descr. Égypte, Hist. Nat. 2 (1813). Epitype (designated by Andrés-Sánchez *et al*., 2011): EGYPT. 'Alexandrie et du lac Mareotis' (MPU-DELILE MPU007025!).

Gifolaria floribunda Pomel in Bull. Soc. Bot. France 35: 335 (1888). *Filago floribunda* Coss. & Kralik in L. Kralik, Pl. Tunetanae Exsiccatae (1854) nom. inval. *Filago mareotica* var. *floribunda* (Pomel) Maire in Bull Soc. Hist. Nat. Afrique N. 26: 209 (1935). Lectotype (**designated here**): TUNISIA. 'In alluvie exsic-

cate Oued Gabes', 21.iv.1854, *L. Kralik Pl. Tunetanae* (MPU-MAIRE!). Syntype: K274246!.

Filago mareotica var. *murcica* Maire in Bull. Soc. Hist. Nat. Afrique N. 26: 209 (1935). Lectotype (**designated here**): SPAIN. 'in Hispaniae regno Murcico pr. Punta de Galindo, Guirao' (COI00035427!). Syntpes: W! (Halácsy collection), UPS photograph!, B!, COI-Willk COI00035426!, COI00035427!.

Distribution: South-eastern Spain, Cyprus and North Africa. See also Andrés-Sánchez *et al*. (2013b).

Ecology: Margins and open areas in salt marshes, loamy–salty hills and coastal dunes.

Selected specimens examined: See Fig. S4.

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APPENDIX

Information on the samples of the species from the '*Filago desertorum* clade' included in the AFLP analysis. Voucher information is listed as follows: taxon name, country, locality, collector name and number, (herbarium). The names used in the analyses of the individuals are included in parentheses: the first letter indicates the species name (C for *F. castroviejoi*; D for *F. desertorum*; L for *F. lutescens* and M for *F. mareotica*); the first two numbers correspond to the population number (01 to 20); the last number indicates the individual within a particular population.

Filago castroviejoi Andrés-Sánchez, D. Gut. Larr., E. Rico & M. M. Mart. Ort. MOROCCO Oriental, Taurirt, north slopes of Narguechoum, Za river, Andrés-Sánchez *et al*. SA12 (SALA1353659!) (C101); Oriental, between Guercif and Saka, S. Andrés-Sánchez *et al*. SA157 (SALA134396!) (C121, C122, C123, C124); Oriental, between Oujda and the bordier with Algeria, S. Andrés-Sánchez *et al*. SA153 (SALA134395!) (C111, C112); Taza-Al Hoceima-Taounate, close to Tarhilest, S. Andrés-Sánchez *et al*. SA36 (SALA134366!) (C181, C182, C183, C184). SPAIN Alicante, Crevillente, road to Albacete, reservoir to Crevillente, S. Andrés Sánchez SA280 (SALA139167!) (C161, C162, C163, C164); Alicante, Aspe, Casa de la Monfortera, S. Andrés-Sánchez SA282 (SALA139168!) (C171, C172, C173, C174, C175); Almería, southern base of Filabres range, S. Andrés-Sánchez *et al*. SA95 (SALA134350!) (C191, C192, C193, C194); Almería, Tabernas, Llanos de Tabernas, close to Hospedería del Desierto, Carril de Juan, S. Andrés-Sánchez SA270 (SALA139169!) (C131, C132, C133, C134, C135); Murcia, Puerto Lumbreras, road to Almendricos, S. Andrés Sánchez SA271 (SALA139170!) (C141, C142, C143, C144); Murcia, Monteagudo, castle, S. Andrés Sánchez SA278 (SALA139218!) (C151, C152, C153, C154, C155, C156).

Filago desertorum Pomel ALGERIA Bougtob, close to the village, M. M. Martínez Ortega *et al*. MO5508 (SALA110256!) (D081, D082, D083). SPAIN Canary Islands, Fuerteventura, between Tetir and La Matilla, M. M. Martínez Ortega *et al*. MO6010 (SALA142104!) (D061, D062, D063, D064, D065); Canary Islands, Fuerteventura, Tiscamanita, close to the village, S. Barrios *et al*. SB6 (SALA142102!) (D091, D092, D093); Canary Islands, Lanzarote, between Tías and Masdache, M. M. Martínez Ortega *et al*. MO6015 (SALA142099!) (D071, D072, D073, D074). TUNISIA Gabès, Matmata Mountains, road Matmata-Gabès, *c*. 1 km Matmata, C. Aedo *et al*. AH3767B (SALA110200!) (D031, D032, D033); Medenine, south of Zarzis, Slab el Gharbi, C. Aedo *et al*. CA16276 (SALA110189!) (D051, D052, D053); Jerid, Celada, Sidi Ben Arbes-Sidi Bouhlel, C. Aedo *et al*. AH3933 (SALA110201!) (D041, D042, D043).

Filago lutescens Jordan SPAIN Tarragona, La Conca de Barberá, Vimbodi-Poblet, Prade Mountains, S. Andrés-Sánchez *et al*. SA312 (SALA110199!) (L201, L202, L203, L204, L205).

Filago mareotica Delile SPAIN Alicante, Santa Pola, close Playa Lisa, M. Santos Vicente *et al*. MS487 (SALA134371!) (M021, M022, M023). TUNISIA Gabès, close Aouinette, C. Aedo *et al*. CA16179 (SALA139165!) (M011, M012, M013, M014, M015).

SUPPORTING INFORMATION

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

Figure S1. Amplified fragment length polymorphism (AFLP) phylograms of a neighbour-joining (NJ) analysis of pairwise Nei & Li distances among individuals of the *Filago desertorum* complex for the primer pair *Eco*RI-AGA(6-FAM)/*Mse*I-CC; bootstrap values are shown.

Figure S2. Amplified fragment length polymorphism (AFLP) phylograms of a neighbour-joining (NJ) analysis of pairwise Nei & Li distances among individuals of the *Filago desertorum* complex for the primer pair *Eco*RI-ACT(6-FAM)/*Mse*I-CTC; bootstrap values are shown.

Figure S3. Amplified fragment length polymorphism (AFLP) phylograms of a neighbour-joining (NJ) analysis of pairwise Nei & Li distances among individuals of the *Filago desertorum* complex for the primer pair *Eco*RI-AGG(VIC)/*Mse*I-CTC; bootstrap values are shown.

Figure S4. Selected specimens examined of *Filago castroviejoi*, *F. desertorum* and *F. mareotica*. Voucher information is listed as follows: taxon name, country, locality, collector name and number (herbarium).