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Long-distance dispersal during the middle–late Pleistocene explains the bipolar disjunction of *Carex maritima* (Cyperaceae)

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ABSTRACT

Aim We set out to explain the bipolar distribution of *Carex maritima*, clarifying the direction and timing of dispersal. We also tested mountain-hopping and direct long-distance dispersal hypotheses, as well as the relationship of *C. maritima* with biotic and abiotic factors that could explain the bipolar distribution.

Location Arctic/boreal latitudes of both hemispheres.

Methods Molecular and bioclimatic data were obtained for *C. maritima* and related species from section *Foetidae*. We sequenced two (*rps16* and *5'trnK* intron) plastid DNA regions (cpDNA) and the external and internal transcribed spacers (ETS and ITS) of the nuclear ribosomal gene region (nrDNA) and inferred phylogenetic relationships, divergence time estimates and biogeographical patterns using maximum likelihood, statistical parsimony, Bayesian inference and ecological niche modelling.

Results *Carex maritima* populations from the Southern Hemisphere were genetically and ecologically differentiated from their northern counterparts and formed a monophyletic group nested within a paraphyletic *C. maritima*. Divergence time analysis estimated a middle–late Pleistocene divergence of the southern lineage (0.23 Ma; 95% highest posterior density: 0.03–0.51 Ma). Southern Hemisphere populations are more stenotopic than the Northern Hemisphere ones, which tolerate harsher conditions.

Main conclusions Our results point to a middle–late Pleistocene migration of *C. maritima* by long-distance dispersal, either directly or via mountain-hopping, from the Northern Hemisphere to the Southern Hemisphere.

Keywords

Biogeography, bipolar distribution, *Carex*, climatic niche, Cyperaceae, divergence time estimation, *Foetidae*, long-distance dispersal.

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INTRODUCTION

Darwin (1872) studied some potential mechanisms underlying the disjunctions of arctic–alpine plant species to refute the idea of multiple creations (Gmelin, 1747) in favour of the hypothesis of a single origin and subsequent migrations. He compiled evidence about the time in which these plants could have initiated their migrations, together with the means and directions of colonization (e.g. seed survival in oceans or seed dispersal by birds), making use of data to explain the similarities between the floras of very distant

mountain ranges spread throughout the world. He invoked signs of an Ice Age in the high latitudes of the Northern Hemisphere to argue that these plants could have migrated southwards and descended from the mountain summits during the glacial epoch. As the climate subsequently warmed up in the high latitudes of the Northern Hemisphere, plants would have recolonized northwards, as well as moved to higher elevations in the mountainous regions of the lower latitudes of the Northern Hemisphere. When the Southern Hemisphere experienced a glacial period, these isolated populations would have been able to spread, in time reaching

the scattered locations in the high latitudes of the Southern Hemisphere where they persist. Nearly a century and a half later, the ideas and hypotheses proposed by Darwin to explain bipolar disjunctions are still admired as having been well ahead of their time (Donoghue, 2011). Nowadays, a combination of bioclimatic and molecular data can help us to better assess the possible evolutionary, climatic and geological changes at the origin of the biogeographical patterns (Crisp *et al.*, 2011).

Historical biogeography has been dominated over the past few decades by investigations of shared distribution patterns among taxa (e.g. Sanmartín *et al.*, 2001; Posadas *et al.*, 2006). Organism distributions in the Southern Hemisphere, together with the underlying causes, have long been analysed (e.g. Raven, 1963; Raven & Axelrod, 1974; Wen & Ickert-Bond, 2009; reviewed in Sanmartín & Ronquist, 2004).

One of the most fascinating plant distribution patterns concerning the Southern Hemisphere encompasses the bipolar disjunction ($> 55^\circ \text{N}$ and $> 52^\circ \text{S}$), achieved only by *c.* 30 vascular plant species (Moore & Chater, 1971). Four hypotheses have historically been put forward to account for bipolar disjunctions: (1) convergent or parallel evolution of the disjunct populations (Scotland, 2011); (2) vicariance (Du Rietz, 1940), which implies a continuous distribution fragmentation dating back to the trans-tropical highland bridges during the Mesozoic Era (from the early Jurassic, 195 Ma; Scotese *et al.*, 1988); (3) stepwise long-distance dispersal across the equator and via mountain ranges ('mountain-hopping'; Raven, 1963; Moore & Chater, 1971; Ball, 1990; Heide, 2002; Vollan *et al.*, 2006); and lastly (4) direct long-distance seed dispersal by birds, wind and/or ocean currents (Cruden, 1966; Muñoz *et al.*, 2004; Nathan *et al.*, 2008).

Six out of the *c.* 30 bipolar vascular plant species known belong to the genus *Carex* L. (Moore & Chater, 1971), a species-rich genus (> 2000 species) found especially in the temperate and cold regions of the Northern Hemisphere (Reznicek, 1990). Molecular studies focused on bipolar *Carex* species (Vollan *et al.*, 2006; Escudero *et al.*, 2010a; Villaverde *et al.*, 2015) determined low levels of genetic differentiation between the disjunct populations, suggesting either mountain-hopping or direct long-distance dispersal, yet none of these studies could determine which hypothesis best explained the observed distributions of the bipolar species. *Carex maritima* Gunn. [sect. *Foetidae* (Tuckerm. ex L.H. Bailey) Kük.] is an arctic–alpine species with a circumboreal distribution including the European Alps and the Himalayas in the Northern Hemisphere, while in the Southern Hemisphere it is distributed from Ecuador to Patagonia (Govaerts *et al.*, 2014; see Fig. 1). It is a wind-pollinated herbaceous hemi-cryptophyte or rhizome geophyte, which generally colonizes water-influenced habitats (e.g. lake, river, ocean shores or snowmelt water areas) and hydromorphic soils (e.g. beaches, fens, alluviums). Recent morphological and taxonomical studies of *C. maritima* (Moore & Chater, 1971; Reznicek, 2002) did not reveal any infraspecific taxa. Although Escudero *et al.* (2010a) detected some degree of genetic differen-

tiation between Northern and Southern Hemisphere populations of *C. maritima*, no North American populations were included in their analyses.

Although the vicariance hypothesis (Du Rietz, 1940) has traditionally been considered in explanation of *Carex* bipolar distribution (e.g. Villaverde *et al.*, 2015), it can now easily be rejected for *Carex* bipolar species as the age of the diversification of the Cyperaceae family is younger than the trans-tropical highland bridges (82.6 Ma, 95% highest posterior density, HPD: 75.9–85.6 Ma; Escudero & Hipp, 2013). We can also discard parallel evolution for *C. maritima* because Escudero *et al.* (2010a) showed that populations from the Northern Hemisphere and the Southern Hemisphere were part of the same clade.

The aim of the present study was to explain the bipolar distribution of *C. maritima*. Specifically, our aims were: (1) to clarify the direction of the dispersal (north-to-south or south-to-north); (2) in the case of genetic structure, to estimate the timing of dispersal; and (3) to test mountain-hopping and direct long-distance dispersal hypotheses, as well as the relationship of *C. maritima* with biotic and abiotic factors that could explain the bipolar distribution. In order to accomplish this task we combined a wide sampling of the species' range with data from nuclear and plastid molecular markers and bioclimatic data. We analysed the phylogenetic and phylogeographical relationships of *C. maritima* populations and compared its ecological niche throughout its distribution.

MATERIALS AND METHODS

Sampling

We obtained plant material representing the geographical range of *C. maritima* (42 populations) as circumscribed by Egorova (1999). Samples used for the molecular study were obtained from fresh leaf material collected in the field and dried in silica gel, and from herbarium specimens (see Appendix S1 in Supporting Information). Vouchers for new collections are deposited in CAN, COLO, SI and UPOS herbaria (abbreviations according to *Index Herbariorum*; <http://sciweb.nybg.org/science2/IndexHerbariorum.asp>). We emphasized the sampling of the most northern Southern Hemisphere populations and we were able to obtain material from northern parts of Argentina. We were not, however, able to sample other more northerly populations in the Southern Hemisphere, from Ecuador and Bolivia (Govaerts *et al.*, 2014). We also included four other species from *Carex* sect. *Foetidae* [10–15 species in total, including *C. maritima*; Reznicek, 2002; eMonocot Cyperaceae (<http://cyperaceae.e-monocot.org>, accessed 3 December 2014)]: *C. incurviformis* Mack. (two populations), *C. pseudofoetida* Kük. (two populations), *C. sajanensis* V. I. Krecz. (four populations) and *C. vernacula* L. H. Bailey (three populations). As outgroups, we included taxa from the subgenus *Vignea* (P. Beauv. ex Lestib. f.) Perterm. (Hendrichs *et al.*, 2004;

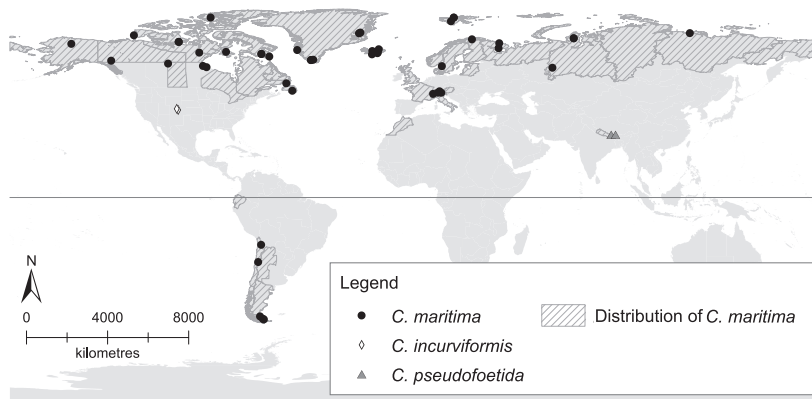


Figure 1 Distribution map of the sampled populations of *Carex maritima*, *C. incurviformis* and *C. pseudofoetida*. *Carex maritima* populations (46; some populations overlap in the map, see Appendix S1 for more details) are depicted by black circles, white diamonds represent *C. incurviformis* (two populations) and grey triangles indicate *C. pseudofoetida* samples (two populations). The dashed region denotes the distribution of *C. maritima*, obtained from the *World Checklist of Cyperaceae* (Govaerts *et al.*, 2014).

Escudero & Hipp, 2013): *C. stenophylla* Wahlenb. from section *Divisae* H. Christ ex Kük. (three populations), *C. remota* L. from section *Remotae* (Ascherson) C. B. Clarke (one population), *C. canescens* L. from section *Glareosae* G. Don (one population) and *C. paniculata* L. from section *Heleoglochis* Dumort. (one population; see Fig. 1 and Appendix S1). For all species, one individual per population was sampled except for four populations of *C. maritima*, for which two individuals were included (Yukon, Nunavut, Iceland and Argentina; see Appendix S1).

PCR amplification and sequencing

Total DNA was extracted using DNeasy Plant Mini Kit (Qiagen, Valencia, CA, USA). Forward and reverse primers were used for amplifications of the internal transcribed spacer (ITS) region (ITS-A, ITS-4; White *et al.*, 1990; Blattner, 1999), external transcribed spacer (ETS) region (ETS-1f, 18S-R; Starr *et al.*, 2003), 5'*trnK* intron (5'*trnK*CarexF, 5'*trnK*CarexR; Escudero & Luceño, 2009) and *rps16* intron (*rps16-rps16R*; Shaw *et al.*, 2005). Thermal cycling was carried out in a Perkin Elmer PCR-system 9700 (Foster City, CA) under the conditions specified by Escudero *et al.* (2010a) for ITS and *rps16*; and Starr *et al.* (2003) and Escudero & Luceño (2009) for the ETS region and 5'*trnK* intron, respectively. Polymerase chain reaction (PCR) products were cleaned and sequenced following Escudero *et al.* (2008). Sequences were edited, automatically aligned with MUSCLE (Edgar, 2004) and manually adjusted using GENEIOUS 6.1.7 (Biomatters, Auckland, New Zealand).

Phylogenetic and haplotype analyses

We used a total of 44 sequences of ITS (six from GenBank), 43 of ETS (one from GenBank), 48 of 5'*trnK* (one from GenBank) and 51 of *rps16* (two from GenBank; see Appendix S1). Each locus was analysed independently and in combination using maximum likelihood (ML) and Bayesian inference (BI). The combined nuclear and plastid aligned matrix consisted of 197 sequences from 64 individuals and 2699 sites (Appendix S1).

There are two main strategies for inferring phylogenies from multiple DNA regions: (1) the total evidence approach, in which phylogeny is reconstructed from as much data as possible to obtain the dominant signal (Kluge, 1989); (2) gene-by-gene strategy, in which it is often possible to identify and explain gene tree incongruences (Rannala & Yang, 2008). In the last few years, coalescent species tree methods have been used to reconcile population history with incongruent phylogenies derived from different DNA regions (Degnan & Rosenberg, 2009). Because of the absence of incongruences between DNA regions (results not shown) and the little genetic variation found in them, we have used the total evidence strategy in the current study.

Maximum likelihood analyses of the unpartitioned combined matrix were performed using RAXML 7.2.6 (Stamatakis, 2006) with a GTR-GAMMA model of sequence evolution and node support assessed with 1000 bootstrap (BS) replicates. Bayesian inference analyses were executed in MRBAYES 3.2 (Ronquist *et al.*, 2012). The most appropriate nucleotide substitution model for each partition was chosen using the Akaike information criterion (AIC) in jMODELTEST (Posada, 2008). Selected nucleotide substitution models were GTR+I, HKY and GTR+G for ITS1, 5.8S and ITS2, respectively; HKY+I for ETS; F81 + I for 5'*trnK* and GTR for *rps16* (Appendix S1). The Markov chain Monte Carlo (MCMC) search was run for five million generations with a tree sampled every 1000 generations and two simultaneous analyses started from different random trees ($N_{\text{runs}} = 2$), each with four Markov chains ($N_{\text{chains}} = 4$). The first 20% of the trees were discarded from each run as the burn-in. A Bayesian majority-rule consensus tree was calculated in MRBAYES with posterior probability (PP) values as a measure for clade support.

We estimated the genealogical relationships among the two cpDNA haplotypes using the plastid 5'*trnK-rps16* matrix and statistical parsimony as implemented in *tcs* 1.21 (Clement *et al.*, 2000). Owing to the polyphyly of the section (see Results), this analysis was only performed for the core *Foetidae*, which comprises all sampled members of section *Foetidae* except for *C. vernacula*. The maximum number of differences resulting from single substitutions among

haplotypes was calculated with 95% confidence limits. Two informative indels in *5'trnK* and one in *rps16* were coded as a presence–absence character for analysis. Gaps due to mononucleotide repeat units (poly-T and poly-A), which are considered to be highly homoplastic (Kelchner, 2000), were treated as missing data. We estimated completeness of haplotype (*5'trnK*–*rps16*) sampling using a Stirling probability distribution as described by Dixon (2006), which calculates a posterior probability distribution of the total number of haplotypes (sampled or not).

Divergence time estimation

Dated phylogenies were estimated for the combined nuclear and plastid matrix in BEAST 1.7.5 (Drummond *et al.*, 2012). Phylogeny was estimated using an uncorrelated lognormal relaxed clock model. A normal age prior with a mean of 14.82 Ma \pm 2.5 Myr was applied to the root of the tree based on the previous estimate for the divergence of the subgenus *Vignea* (Escudero & Hipp, 2013). Analyses were conducted using two independent MCMC runs of 60 million generations each, assuming the birth–death tree prior with a mean substitution rate set at 1.0. Run convergence and burn-in were assessed in TRACER 1.5 (Rambaut & Drummond, 2009). Maximum clade credibility trees were calculated with TREEANNOTATOR 1.7.2 (Drummond & Rambaut, 2007) using a posterior probability limit of 0.7 and the mean heights option.

Climatic environment – ecological niche

We obtained bioclimatic data for the localities of our molecular sampling (56 samples of core *Foetidae*: 47 of *C. maritima*, two each of *C. incurviformis* and *C. pseudofaletida*, and five of *C. sajanensis*; ‘reduced data set’ from here on) in order to study the ecological factors influencing species’ range. We compiled a new data set (‘full data set’) and also obtained bioclimatic data. This new data set was completed by adding: (1) three additional populations from Austria, Italy and Sweden for which we failed to amplify any loci (Appendix S1); and (2) species occurrence data between 1950 and 2000 downloaded from the Global Biodiversity Information Facility data portal (<http://www.gbif.org/>, downloaded 22 December 2014) after pruning for likely incorrect identification or georeferencing (e.g. occurrences in oceans) and removing duplicate records from the same locality to reduce the effects of spatial autocorrelation (847 new presence data from preserved specimens of *C. maritima*). Finally, our full second data set included 894 populations in total (see Appendices S1 and S2). For each sampled population in our data sets we obtained values for 19 bioclimatic variables (Appendix S1) as described by Escudero *et al.* (2013). We ran principal components analyses (PCA) using the full and reduced climatic data sets, as Villaverde *et al.* (2015). The phylogenetic size-correction was performed in our reduced data set for non-independence among the observations for

lineages. We represented data associated with the most important bioclimatic variables retained in the phylogenetic PCA for *C. maritima* in boxplots. In order to compare climate regime similarities and differences of the species of core *Foetidae* we included the samples of *C. incurviformis*, *C. pseudofaletida* and *C. sajanensis* in the PCA of the climatic environment.

Past and present distribution under climatic change scenarios

Species distribution modelling was performed to reconstruct the potential ranges of *C. maritima* under present climatic conditions and for two historical periods, the Last Interglacial (LIG; 120–140 ka) and the Last Glacial Maximum (LGM; 21 ka), with MAXENT 3.3.3k (Phillips *et al.*, 2006). Neither *C. incurviformis* nor *C. pseudofaletida* was included in the model with *C. maritima* because their different ecological requirements may confound *C. maritima* distribution modelling. *Carex incurviformis* is distributed only in North America and its ecology differs from that of *C. maritima* in this region (Reznicek, 2002). Moreover, the partial molecular differentiation (see Results) and their distinctive morphology (Reznicek, 2002) also support this decision. Settings were established following Blanco-Pastor *et al.* (2013). We performed a correlation analysis with the variance inflation factor (VIF) using the ‘vif’ function in the usdm package in R (R Core Team, 2014) and a correlation threshold of 0.7. Only three variables were uncorrelated and consequently included in the analyses: BIO1 (annual mean temperature), BIO6 (minimum temperature of the warmest month) and BIO12 (annual precipitation). Replicate runs (500) were performed by using the bootstrap run type. All 19 of these variables have a grid size of 30 arc seconds for present and LGM conditions but 2.5 arc minutes for LIG scenarios. These grid size differences required us to omit 82 data points from the *C. maritima* full data set from the model, leaving a total of 812 points. Analyses were performed for all populations of *C. maritima* and separately for Northern and Southern Hemisphere populations of *C. maritima*. We partitioned all the locality data into training and testing data sets (75% vs. 25%, respectively) in order to build niche models and to evaluate the quality of the model. Nonetheless, projections to past scenarios have to be interpreted with caution due to the absence of fossils to validate the model and the low number of existing localities in the Southern Hemisphere. Similar results were obtained when modelling the climatic niche of *C. maritima* using the reduced data set (results not shown).

RESULTS

Phylogenetic reconstruction

BI and ML analyses revealed a lack of monophyly both for *Carex* sect. *Foetidae* and *C. maritima*. Strong support (100% BS/1 PP; Fig. 2) was obtained for the core *Foetidae*, including

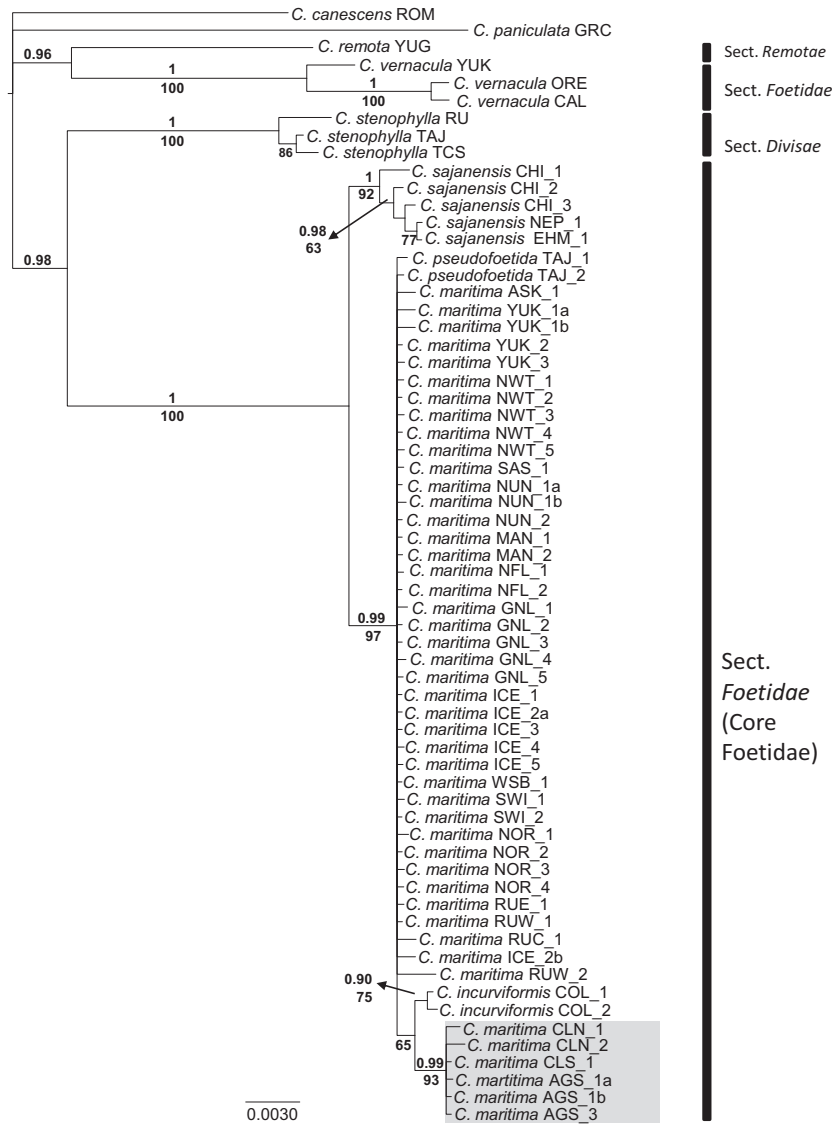


Figure 2 Majority rule (50%) consensus tree derived from the Bayesian analysis of *Carex maritima* and the related species in section *Foetidae* inferred from the combined nuclear (ITS and ETS) and chloroplast (*5'trnK* and *rps16*) matrix; *C. remota*, *C. canescens* and *C. paniculata* were used as outgroups. Numbers above and below the branches represent the Bayesian posterior probability (> 0.9 PP) and bootstrap (> 60% BS) values of the maximum likelihood analysis, respectively. A grey rectangle highlights the *C. maritima* samples of the Southern Hemisphere. Vertical bars indicate supraspecific taxa from the same taxonomic group. Abbreviations after the names correspond to the geographical regions of the world (Brummitt, 2001) and to the population number. The scale bar indicates substitutions per site.

all sampled section *Foetidae* species except *C. vernacula*. Nevertheless, several species from section *Foetidae*, not sampled in the current study, could potentially also be part of the core *Foetidae*. A strongly supported (100% BS/1 PP) monophyletic *C. stenophylla* (sect. *Divisae*) was sister to the core *Foetidae* (100% BS/1 PP). Within the latter, *C. sajanensis* was retrieved as monophyletic (92% BS/1 PP) and sister to a strongly supported clade (97% BS/0.99 PP), including *C. pseudofoetida*, *C. incurviformis* and *C. maritima*. Two different subclades were detected: (1) a strongly supported lineage comprising all *C. maritima* samples from South America (93% BS/0.99 PP); and (2) *C. incurviformis* (75% BS/0.90 PP).

Haplotype network

The cpDNA haplotype network obtained for the core *Foetidae* (Fig. 3) revealed seven haplotypes and five missing haplotypes. A probability of 81% that all haplotypes have been sampled is given by Dixon's (2006) method. Five haplotypes

were found within the sampled *C. maritima* populations. Two of them were widely distributed but geographically overlapping in part: one of them was shared by 10 samples of *C. maritima* from Russia, Canada and Greenland (H1), and the other by 26 samples of *C. maritima* from northern North America and Europe (H2). In addition, H1 was also shared with one population of *C. incurviformis* and two of *C. pseudofoetida*. Interestingly, all *C. maritima* samples from South America (5) shared the same exclusive haplotype (H3). We found unique haplotypes for *C. maritima* samples from Norway (H4; one sample) and for samples from eastern Russia (H5; two samples). Finally, *C. sajanensis* displayed two haplotypes (H5–H6) separated by four mutational steps from *C. maritima* haplotypes.

Estimation of divergence times

The dating analyses produced a congruent topology with respect to BI and ML analyses presented above (Fig. 4,

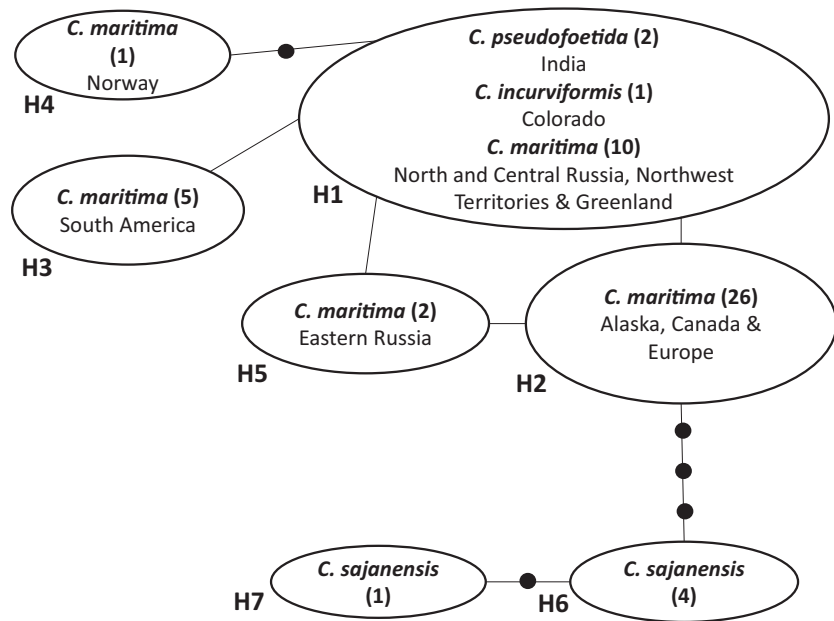


Figure 3 Haplotype network of concatenated cpDNA sequences of *Carex maritima*, *C. pseudofoetida*, *C. incurviformis* and *C. sajanensis*. Circles represent the seven haplotypes found (H1–H7), lines represent single mutational steps, and small black circles represent missing haplotypes. Numbers of samples per haplotype are indicated in parentheses.

Table 1). The divergence time of the clade comprising core *Foetidae* was 2.85 Ma (95% HPD: 0.93–5.01 Ma), which falls in the late Pliocene to middle Pleistocene. The diversification of the clade consisting of *C. maritima*, *C. incurviformis* and *C. pseudofoetida* could have occurred during the middle–late Pliocene to early–middle Pleistocene (1.61 Ma; 95% HPD: 0.61–2.96 Ma). Finally, the divergence of the clade consisting of *C. maritima* samples from South America could have begun during the middle–late Pleistocene (0.23 Ma; 95% HPD: 0.03–0.51 Ma). While the age of the core *Foetidae* could experience some variation after including some of the missing species from section *Foetidae*, the estimated age for *C. maritima* clade (the clade including all *C. maritima* samples) is reliable and should experience little or no variation after including unsampled species even if one or several of those fell nested within the *C. maritima* clade.

Climatic environment

The phylogenetic PCA of the reduced climatic data set showed that PC1 explained 50.35% of the variance whereas PC2 explained 22.29% (see Fig. 5). The variables with the highest loadings in PC1 were temperature seasonality (BIO4), temperature range (BIO7) and minimum temperature of the coldest month (BIO6; see Appendix S1). Maximum and minimum values for each variable are shown by groups (Table 2). Similar results were obtained when the analysis is not corrected with the phylogeny (results not shown). Northern and Southern Hemisphere samples of *C. maritima* were clearly separated into two groups, probably revealing some degree of ecological differentiation. The box-plots of the variables with the highest loadings revealed that *C. maritima* populations from the Northern Hemisphere occur in localities with greater temperature oscillations through the year and a wider range of minimum tempera-

tures during the coldest month than populations from the Southern Hemisphere (Table 2, Fig. 6). The PCA of the full climatic data set showed that PC1 explained 65.9% of the variance whereas PC2 and PC3 explained 14.7% and 9.4%, respectively (Appendix S1). A clear separation between Northern and Southern Hemisphere samples of *C. maritima* is also obtained when plotting PC1 and PC3 or PC2 and PC3 (Appendix S2).

Past and present distribution under climatic change scenarios

Current conditions

Our results show that the modelled ecological niche of *C. maritima*, including Northern and Southern Hemisphere samples, predicts suitable areas in both hemispheres. Values for AUC were all above 0.9, which indicate a good fit of the models. The average AUC values for each group and the most important environmental variables detected in each analysis are reported in Appendix S1. Scatter diagrams of the variables used in MAXENT analyses also depicted clear differences between Northern and Southern Hemisphere populations of *C. maritima* (Appendix S2). The modelled ecological niche of *C. maritima* including only the Northern Hemisphere populations predicts suitable habitats also in the Southern Hemisphere (Appendix S2). By contrast, the modelled ecological niche of *C. maritima* including only Southern Hemisphere populations does not predict suitable habitats in the Northern Hemisphere.

Past conditions

The projection of suitable environments to past conditions in all lineages revealed a wider distribution range in LGM

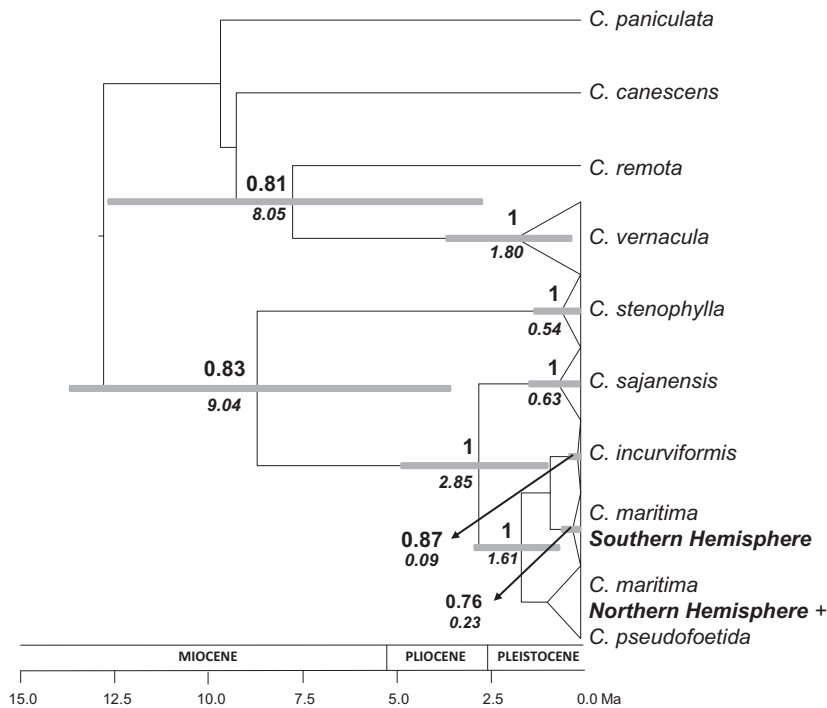


Figure 4 Maximum credibility cladogram of the Bayesian divergence time analysis considering *Carex maritima* and other related species in section *Foetidae* carried out on a combined matrix of nuclear (ITS and ETS) and plastid (*5' trnK* and *rps16*) sequences. *Carex remota*, *C. canescens*, *C. paniculata* and *C. stenophylla* were used as outgroups. Node bars represent the 95% highest posterior density intervals of the divergence time estimates linked to nodes with posterior probabilities above 0.75 (values above branches) with mean ages inferred for clades in million years (below branches).

scenarios than at the present time and in the LIG scenarios (Appendix S2). Although suitable environments are predicted in the Southern Hemisphere for *C. maritima* populations from the Northern Hemisphere, when Southern Hemisphere populations are analysed alone these areas are considerably reduced in LGM scenarios and absent in the LIG scenario (Appendix S2). These results have to be interpreted with caution due to the absence of fossils to validate the model.

DISCUSSION

Pleistocene north-to-south long-distance dispersal

Haplotypes H1 and H2 are widely distributed throughout the Northern Hemisphere (North America, Europe and Asia) and comprise the highest number of haplotype connections (Fig. 3), implying under the coalescent theory that they amount to the ancestral haplotypes (Posada & Crandall, 2001). South American *C. maritima* populations are monophyletic and nested with a strong statistical support within the Northern Hemisphere accessions (Figs 2 & 3). In addition, the haploid genotype diversity pattern consisting of four different haplotypes found in relation to the *C. maritima* populations of the Northern Hemisphere (H1, H2, H4, H5), whilst a single haplotype was detected for the southern populations (H3; Fig. 3), suggests a migration event from the Northern Hemisphere to the Southern Hemisphere as the most plausible explanation. This evidence, together with the fact that 11 out of the 15 species in *Carex* sect. *Foetidae* (eMonocot Cyperaceae; <http://cyperaceae.e-monocot.org>, accessed 3 December 2014) are also distributed in the Northern Hemisphere, supports the hypothesis that *C. maritima* originated in the Northern Hemisphere, according to our

analysis during the middle–late Pliocene and early–middle Pleistocene (Table 1, Fig. 4).

The other five bipolar *Carex* species manifest most of their distribution in the Northern Hemisphere (Govaerts *et al.*, 2014). At least *C. arctogena*, *C. macloviana* and *C. maritima* display a higher morphological variation in North America than in South America (Moore & Chater, 1971), which could also support the idea that the bipolar species generally migrated southwards (Raven, 1963; Moore & Chater, 1971). Studies of other bipolar taxa have also suggested a north-to-south dispersal as the most plausible migration direction (e.g. Moore & Chater, 1971; Vollen *et al.*, 2006; Popp *et al.*, 2011). The *C. maritima* biogeographical history elucidated in our study appears to be congruent with the predominantly inferred pattern, and it seems that Northern Hemisphere to South Hemisphere dispersal is predominant in plant dispersals (reviewed in Wen & Ickert-Bond, 2009). However, other plant genera present the opposite direction of dispersal. For example, the centre of origin of the genus *Larrea* (Zygophyllaceae) is located in South America and this genus was inferred to have migrated to North America during the late Neogene by long-distance dispersal, using way stations in Peru and Bolivia and probably mediated by birds (Lia *et al.*, 2001). Likewise, the Rubiaceae family was inferred to have migrated from South to North America during the late Palaeocene–early Eocene using land bridges (Antonelli *et al.*, 2009), as was *Hoffmannseggia glauca* (Fabaceae) via birds during the late Miocene or later (Simpson *et al.*, 2005).

Mountain-hopping or direct long-distance dispersal?

The subsequent question arises of how the inferred north-to-south middle–late Pleistocene long-distance dispersal

Table 1 Divergence times of clades in *Carex* sect. *Foetidae* and outgroups presented as the posterior probability followed by the mean time to the most recent common ancestor in million years and the 95% highest posterior density (HPD) interval obtained from the divergence time analysis of the combined nuclear (ITS and ETS) and plastid (*5' trnK* intron and *rps16*) regions. *Carex maritima* populations of the Northern Hemisphere (NH) and Southern Hemisphere (SH), respectively, are indicated.

Clade	Posterior probability	Mean (Ma)	95% HPD interval (Ma)	
<i>C. remota</i> + <i>C. vernacula</i>	0.81	8.0522	2.7638	13.1959
<i>C. vernacula</i>	1	1.8038	0.2665	3.7436
<i>C. stenophylla</i> + Core <i>Foetidae</i> (<i>C. sajanensis</i> + <i>C. incurviformis</i> + <i>C. maritima</i> + <i>C. pseudofloetida</i>)	0.83	9.0452	3.6505	14.2733
<i>C. stenophylla</i>	1	0.5365	0.0291	1.2872
Core <i>Foetidae</i> (<i>C. sajanensis</i> + <i>C. incurviformis</i> + <i>C. maritima</i> + <i>C. pseudofloetida</i>)	1	2.8456	0.9327	5.0077
<i>C. sajanensis</i>	1	0.6275	0.0440	1.4295
<i>C. incurviformis</i> + <i>C. maritima</i> NH + <i>C. maritima</i> SH + <i>C. pseudofloetida</i>	1	1.6110	0.6062	2.9603
<i>C. incurviformis</i>	0.87	0.0884	0	0.3099
<i>C. maritima</i> SH	0.76	0.2266	0.0286	0.5092

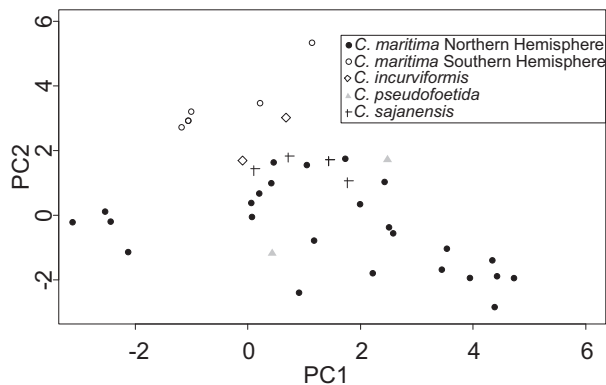


Figure 5 Scatter plot of the first two components explaining up to 72.64% of the observed variance, derived from the principal components analysis as corrected by phylogeny and depicting the position in a climate-niche space of Northern and Southern Hemisphere samples of *Carex maritima* (black and white circles, respectively), *C. pseudofloetida* (triangles), *C. incurviformis* (diamonds) and *C. sajanensis* (crosses).

Table 2 Maximum and minimum values of the variables with the highest loadings for principal component 1 for the Northern Hemisphere populations (NH) and Southern Hemisphere populations (SH) of *Carex maritima*. Bioclimatic variables correspond to temperature seasonality (BIO4, SD), the temperature range (BIO7 = BIO6 – BIO5, °C), the minimum temperature during the coldest month (BIO6, °C) and the maximum temperature during the coldest month (BIO5).

		BIO4	BIO7	BIO6
<i>C. maritima</i> HN	min.	35.33	14.2	-44.9
	max.	177.28	58	-3.5
<i>C. maritima</i> SH	min.	24	15.4	-9.6
	max.	32.78	25.4	-0.6

could have occurred. According to our data, the current *C. maritima* distribution can be explained by either of two hypotheses: (1) Northern Hemisphere populations could

have migrated stepwise by mountain-hopping all the way through the Andes, with a posterior extinction of most of the intermediate populations; or (2) Northern Hemisphere populations could have been disseminated by a direct long-distance dispersal to South America, where they subsequently colonized northwards or southwards until reaching their current distribution (Fig. 1).

The mountain-hopping hypothesis (Ball, 1990) proposes a long-distance, stepwise migration of arctic and temperate taxa using mountains peaks as stepping-stones to cross the tropics. A route connecting North and South America through the American cordillera has been in place since the late Miocene epoch (Smith, 1986). Then, a gradual uplift of the cordillera during the late Pliocene created the high mountainous environment with a much colder climate later on during the Pleistocene compared to that occurring today at the same latitudes and elevations (van der Hammen, 1974). According to our results, from the Last Interglacial (LIG; c. 120 ka) to the present time similar ecological niches could have existed that were suitable for *C. maritima* in South America (although these results should be taken with caution, see Results; Appendix S2). Therefore, we cannot rule out the mountain-hopping migration since the early Pleistocene, with a subsequent extinction of most of the northern South American intermediate populations. In addition, the sister relationship between *C. maritima* of the Southern Hemisphere and *C. incurviformis* (present in western North America yet reaching southern latitudes) could also support this hypothesis (nonetheless see lack of clade support; Fig. 2). As already demonstrated by Heide (2002), changes in flowering requirements would not have been necessary for the other bipolar *Carex* species to migrate across of the tropical belt, still making mountain-hopping a plausible premise.

Alternatively, *C. maritima* could have reached the Southern Cone by a direct long-distance dispersal event, with a subsequent genetic differentiation in the South American continent and a northward or southward colonization along

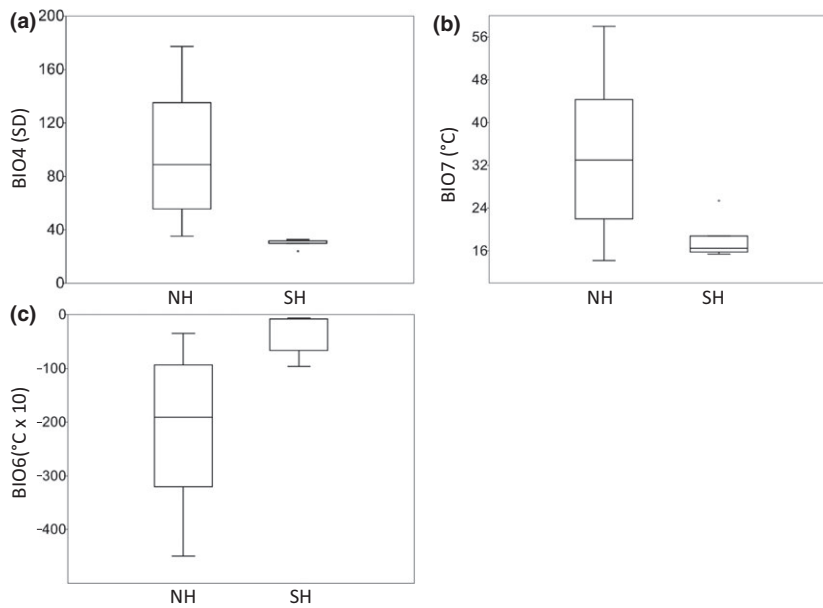


Figure 6 *Carex maritima* boxplots comparing the three bioclimatic variables with the highest loadings regarding the first component of the bioclimatic principal components analysis (PCA) taking into account the Northern and Southern Hemisphere samples (NH and SH). (a) Temperature seasonality (BIO4), (b) annual temperature range (BIO7, i.e. minimum temperature during the warmest month minus maximum temperature during the coldest month, BIO6 – BIO5), and (c) minimum temperature during the warmest month (BIO6). Each box represents the interquartile range which contains 50% of the values and the median (horizontal line across the box); the whiskers are the lines that extend from the box to the highest and lowest values, excluding outliers (o).

the Andes, as suggested for the bipolar *C. arctogena* (Villaverde *et al.*, 2015). The molecular data in this study, without genetic structure between northern and southern populations within each hemisphere, but strong genetic structure between both hemispheres, could support the direct long-distance dispersal hypothesis and subsequent genetic differentiation. Nevertheless, more information is still needed to confirm either the mountain-hopping or the direct long-distance dispersal hypothesis.

Breeding system, dispersal syndrome and the bipolar disjunction

Some self-fertilization is a reproductive characteristic displayed by many species with disjunct populations in the temperate zones (Carlquist, 1983). This attribute could favour local survival and establishment after long-distance dispersal events, given that a single propagule of self-compatible individuals could in principle be sufficient to start a sexually reproducing colony (Baker, 1955). In congruence, *Carex* species are predominantly monoecious and in general highly self-pollinated (Friedman & Barrett, 2009), which has been inferred from studies based on hand pollinations, isozyme work (e.g. Ohkawa *et al.*, 2000; Friedman & Barrett, 2009) and microsatellite data (e.g. Escudero *et al.*, 2010b, 2013). This characteristic could explain, at least in part, the often successful colonization of *Carex* species after a long-distance dispersal event (Moore & Chater, 1971; Ball, 1990; Escudero *et al.*, 2009).

Carex maritima inhabits water-influenced areas (e.g. lakes, river, ocean shores or snowmelt water areas) or else populates hydromorphic soils (e.g. beaches, fens, alluviums), and seed dispersal of *C. maritima* could be mediated by birds, wind or ocean currents. Except for their small size, fruits of *C. maritima* lack the evident morphological features for a

long-distance dispersal, unlike other bipolar species (*C. microglochin*; Savile, 1972). On the one hand, species with small seeds and from water-influenced habitats are often highly dispersible taxa (McGlone *et al.*, 2001). On the other hand, the long-distance dispersal of seeds might not necessarily be driven by standard dispersal vectors inferred from plant morphology (as described by Higgins *et al.*, 2003) or by regular events; in fact, great long-distance dispersals (> 100 km) are usually associated with stochastic events (unusual behaviour of regular events or a combination of vectors; Nathan *et al.*, 2008). Thus, arctic species have been demonstrated to migrate enormous distances despite the lack of specific syndromes (Abbott & Brochmann, 2003).

Considering the extreme dispersal distance, together with the shape and structure of the *C. maritima* propagules, we consider that its dispersal was more likely to have been mediated by migratory animals than by wind or ocean currents, which seem insufficient for such an enormous task. Some birds which migrate from North America to temperate zones of South America have already been pointed out as the most likely dispersal agents of the several disjunct plant groups (Cruden, 1966, and references therein; Popp *et al.*, 2011). Moreover, arrivals of *Carex* species to newly formed islands have predominantly been reported to happen when seeds were embedded in mud attached to birds' feet or else when eaten and carried inside by birds (Carlquist, 1967). Therefore, we consider it plausible that *C. maritima* could have acquired its bipolar distribution by means of bird-mediated dispersal.

CONCLUSIONS

This study contributes to the general knowledge regarding biogeographical patterns of bipolar taxa whilst presenting a combination of multiple approaches (phylogenetic and

phylogeographical analyses, together with divergence time estimates and bioclimatic data) to test the traditional hypotheses used to understand the distribution of bipolar species. *Carex maritima* populations of the Southern Hemisphere were retrieved as a monophyletic lineage within a paraphyletic *C. maritima*. The phylogeographical structure found within *C. maritima* suggests that the bipolar disjunction could be explained by a middle–late Pleistocene long-distance dispersal derived from the Northern Hemisphere. Our study highlights the importance of long-distance dispersal mechanisms to explain this fascinating plant distribution pattern.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Appendix S1 Supplementary tables. Studied material of *Carex maritima*, related species and outgroups (Table S1); molecular characteristics of the amplified regions (Table S2); results from the principal components analysis of 19 bioclimatic variables from the WorldClim database, uncorrected (on full and reduced data sets) and corrected for phylogeny (Tables S3–S5); description of bioclimatic variables (Table S6); and AUC values from MAXENT analyses (Table S7).

Appendix S2 Supplementary figures. Distribution map of *Carex maritima* (Fig. S1); ecological niche models of *C. maritima* groups fitted to current climatic conditions from MAXENT analyses (Fig. S2), and projections of the models to Last Glacial Maximum (18–21 ka; MIROC and CCSM models) and Last Interglacial Period (c. 120–140 ka) (Fig. S3); scatter plots of the uncorrelated bioclimatic variables used in MAXENT analyses (Fig. S4); and scatter plot of the three first components from the principal components analysis of the full data set (Fig. S5).

BIOSKETCH

Tamara Villaverde is a PhD student at Pablo de Olavide University, Seville (Spain). Her research is focused on the evolution and phylogeography of angiosperms, with special interest in the systematics and biogeography of the genus *Carex* (Cyperaceae).

Author contributions: M.E. and M.L. conceived the idea; T.V., S.M-B and M.L. collected the plant material; T.V., M.E. and S.M-B, carried out the lab work and analysed the data; T.V., M.E. and S.M-B led the writing and drafted the manuscript, although all authors contributed to its preparation.

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