Bipolar disjunctions in *Carex*: Long-distance dispersal, vicariance, or parallel evolution?

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ABSTRACT

The long-standing fascination of naturalists and scientists in the evolutionary and biogeographical causes behind the pattern of distribution of bipolar plants has lead to an intense debate around the three well-known biogeographical hypotheses of vicariance, long-distance dispersal, and parallel evolution. Genus *Carex*, despite lacking any general long-distance dispersal devices, represents six of the 30 plant species with bipolar distribution. We aimed to evaluate the role of the three alternative mechanisms mentioned above in the origin and evolution of five bipolar *Carex* species. Phylogenetic and phylogeographical reconstructions using Bayesian Inference, maximum parsimony, and statistical parsimony were performed with plastid (*rps*16 intron) and nuclear (ITS) DNA sequences. As a result, five cases of long-distance dispersal are proposed (*C. canescens*, *C. macloviana*, *C. magellanica*, *C. maritima*, *C. microglochin* s.str.) with an inferred southward migration from Northern to Southern Hemisphere for three of them. On the other hand, parallel evolution seems to be the most plausible explanation to understand the particular case of the bipolar species *C. microglochin* s.l.

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Introduction

The striking fact that many species from different plant families display similar bipolar distributions (Moore and Chater, 1971) has fascinated scientists since the beginning of the nineteenth century (Darwin, 1859; Humboldt, 1817). Moore and Chater (1971) compiled 30 bipolar species considering those present at high latitudes, reaching at least the Strait of Magellan in the southern hemisphere and Alaska or Arctic Europe in the northern and irrespective of their presence at lower latitudes. Six of these 30 species are *Carex* species (*C. canescens* L., *C. capitata* L., *C. macloviana* D' Urv., *C. magellanica* Lam., *C. maritima* Gunn., *C. microglochin* Wahlenb.). More recently, Vollan et al. (2006) considered two additional *Carex* species (*C. echinata* Murray, *C. lachenalii* Schkuhr) as examples of bipolar plants, although these plants do not reach such high latitudes in the southern hemisphere. Many studies have focused on elucidating the origin of such disjunctions (Ball, 1990; Darwin, 1859; Du Rietz, 1940; Heide, 2002; Humboldt, 1817; Moore, 1972; Moore and Chater, 1971; Smith, 1986; Steffen, 1939; Vollan et al., 2006; Wilson, 1986). As a result, three main hypotheses have been proposed to explain the origin of present-day bipolar disjunctions: parallel evolution, vicariance, and long-distance dispersal. Historically,

parallel evolution was proposed as the most likely mechanism behind bipolar disjunctions (Humboldt, 1817); however, this hypothesis is nowadays discarded by most authors. Vicariance, which implies the disruption of a previous continuous distribution, has also been proposed as the origin of bipolar disjunctions (Du Rietz, 1940). In particular, this hypothesis placed the origin of disjunctions in the Mesozoic (65–250 Myr B.P.), when trans-tropical highland bridges disappeared. Finally, the third and most popular hypothesis currently proposed for bipolar species is long-distance dispersal, either direct (Van Steenis, 1962) or over stepping stones (mountain hopping; Moore and Chater, 1971; Vollan et al., 2006).

Morphological and ecological studies have historically prevailed in the investigation of bipolar plants (see authors cited above). Improved molecular techniques during the last three decades have provided the suitable new tools for tackling with some evolutionary questions such as the origin of disjunctions. To our knowledge, only the AFLP fingerprinting technique has been used for elucidating the origin of *Carex* bipolar disjunctions (Vollan et al., 2006). In this paper we sequenced genome regions in an attempt to elucidate the origin of five of the six bipolar *Carex* species. Particularly, we analysed the variability of the nuclear ribosomal ITS region and the plastid *rps*16 intron. The ITS region has a level of variation suitable for evolutionary studies at the species level (review: Nieto Feliner and Roselló, 2007) and has been successfully used in studies of *Carex* with biogeographical conclusions (Escudero et al., 2008a, b; Hipp et al., 2006; Roalson

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and Friar, 2004). Plastid DNA sequences have been widely used to reconstruct phylogenetic and phylogeographical patterns (Taberlet et al., 1998). In particular, the *rps*16 intron has been proven to be one of the most variable plastid regions in *Carex* (Escudero et al., 2008b) and was used for phylogeographical studies (Schönswetter et al., 2006).

In this study, we analysed nuclear and plastid sequences in order to investigate the origin of disjunctions in five bipolar *Carex* species and evaluate the role of the three main biogeographical hypotheses for these taxa, parallel evolution, vicariance, and long-distance dispersal.

Material and methods

Study species

Five *Carex* species (*C. canescens, C. macloviana, C. magellanica, C. maritima, C. microglochin*) were investigated in the northern and southern hemispheres. We did not include the sixth bipolar *Carex* species, *C. capitata*, as we were unable to obtain recent material of this species from the southern hemisphere. Outgroup taxa for each *Carex* species were selected based on previous phylogenetic information (Waterway and Starr, 2007). There are four main clades in the tribe *Cariceae* related to these five studied species (Starr and Ford, 2009; Starr et al., 2008; Waterway and Starr, 2007). Clade 1: most species of the subgenera *Carex* and *Vigneastra* (group 1, including *C. magellanica*). Clade 2: most species of the subgenus *Vignea* (group 2, including *C. canescens, C. macloviana, C. maritima*). Clades 3 and 4: the unispicate clade (most species of subgenus *Psyllophora*, plus a few atypical *Carex* species such as *C. curvula*, and the genera *Cymophyllus, Kobresia,* and *Uncinia*) and the *Schoenoxiphium* clade (genus *Schoenoxiphium* and some species of *Psyllophora*), respectively (group 3, including *C. microglochin*).

Sample for ITS sequencing

A total of 28 samples of the five study species were analysed. Five samples were additionally taken from the GenBank database (http://www.ncbi.nlm.nih.gov/): one sample of *C. magellanica* (Waterway and Starr, 2007), three samples of *C. canescens* (Hipp et al., 2006; Roalson et al., 2001; Waterway and Starr, 2007) and one sample of *C. macloviana* (Hipp et al., 2006). The remaining 23 samples were obtained in the field or from herbarium species and sequenced for this study: five samples of *C. magellanica*, seven samples of *C. microglochin*, four samples of *C. canescens*, three samples of *C. macloviana* and four samples of *C. maritima* (Table 1). In group 1, nine species closely related to *C. magellanica* were included, together with *C. tuckermanii* and *C. trichocarpa* (outgroup). In group 2, four sequences of *C. macloviana*, seven of *C. canescens* and four of *C. maritima* were analysed together because of their close phylogenetic relationship, together with 31 closely related species and *C. pensylvanica, C. curvula* and *Cymophyllus fraserianus* as an outgroup. In group 3, sequences of seven samples of *C. microglochin* and 19 samples of 19 closely related species, *C. bromoides* and *C. deweyana* (outgroup) were analysed. Sequences of closely related species and outgroups were obtained from Waterway and Starr (2007).

Sample for plastid rps16 sequencing

A total of 23 samples of five species obtained in this study were analysed: five samples of *C. magellanica*, seven samples of *C. microglochin*, four samples of *C. canescens*, three samples of *C. macloviana*, and four samples of *C. maritima* (Table 1). No *rps*16

sequences of the five species and closely related species were deposited in the GenBank. In group 1, three species of the subgenus *Carex* (two sequences got in the present study, (Table 1); one species from Escudero et al., 2008b) were included together with *C. furva* (outgroup) (Table 1). In group 2, *C. macloviana, C. canescens,* and *C. maritima* were analysed together with four subgenus *Vignea* species plus *C. microglochin* and *C. binervis* (outgroup) (Table 1; Escudero et al., 2008b). In group 3, *C. microglochin*, five closely related species (five samples) and *C. canescens* and *C. maritima* (outgroup) were analysed (Table 1).

Molecular analysis

PCR amplification and sequencing

Total DNA was extracted from silica-dried material collected in the field as well as from herbarium specimens (UPOS, SI, TRH; abbreviation according to *Index Herbariorum*) using DNeasy Plant Mini Kit (Qiagen, California). Direct and reversal primers were used for amplifications of the ITS region (ITS-A, ITS-4; Blattner, 1999; White et al., 1990) and *rps*16 intron (rpsF, rps2R2; Oxelman et al., 1997). Amplifications were obtained in a Perkin Elmer PCR-system 9700 (California) under the conditions specified by Escudero et al. (2008b) for the ITS and Schönswetter et al. (2006) for *rps*16. PCR products were cleaned using spin filter columns (PCR Clean-up kit, MoBio Laboratories, California). Cleaned products were sequenced using dye terminators (Big Dye Terminator v. 2.0, Applied Biosystems, California) and run in polyacrylamide electrophoresis gels (7%) using an Applied Biosystems Prism Model 3700 automated sequencer. Sequences were edited using the program Seqed (Applied Biosystems). Limits of the ITS region and *rps*16 intron were determined following Starr et al. (1999) and Schönswetter et al. (2006), respectively.

Sequence analyses

Six matrices were manually aligned, three for the ITS and three for the *rps*16 analyses: group-1 matrices (17 of ITS/nine of *rps*16 sequences); group-2 matrices (49/18 sequences); group-3 matrices (28/14 sequences). In light of the first results, the *rps*16 matrices were rebuilt and reanalysed when length mutations were found in the ingroup. The gaps were coded as a simple base (A/T, presence/absence) in the *rps*16 matrices.

Each of the three ITS matrices was split into three different matrices (ITS-1, ITS-2, 5.8S). The resulting nine ITS and three *rps*16 matrices were additionally analysed to determine the simplest model of sequence evolution that best fits the data, under the Akaike Information Criterion (AIC), as implemented in MrModeltest 1.1b (Nylander, 2002). The selected models were used to (1) perform Bayesian inference (BI), and (2) calculate pairwise distances. When gaps were found in the *rps*16 matrices, the selected model for coded gaps was F81 following the manual of MrBayes v3.0b4 (Ronquist and Huelsenbeck, 2003). The six matrices (three ITS/three *rps*16) were analysed using MrBayes v3.0b4 (Ronquist and Huelsenbeck, 2003). Four Markov chain Monte Carlo runs were performed simultaneously in each BI analysis for 5,000,000 generations with an interval of 100 generations. Burn-in was evaluated over generations. After discarding trees yielded before the Likelihood stationary point was reached, the remaining trees were compiled in a majority rule consensus tree, using posterior probability (pp) as a measure of clade support (Alfaro et al., 2000). ITS-1 and ITS-2 pairwise genetic distances were calculated together with PAUP (Swofford, 2003).

For the six same matrices (three ITS/three *rps*16), maximum parsimony (MP) analyses were conducted under Fitch parsimony, as implemented in TNT (Goloboff et al., 2003) with equal

Table 1
List of studied materials including population, locality, voucher, and sequence number.

	Population	Locality	Voucher	Sequence number	
				ITS	*rps*16
INGROUP					
Group 1					
Carex magellanica	1	Finland, between Pöntsö and Rauhala	M. Luceño and M. Guzmán, 605ML (UPOS 300)	EU541814	EU541849
	2	Finland, Pallas-Ounastunturi N.P.	M. Luceño and M. Guzmán, 1205ML (UPOS306)	EU541817	EU541850
	3	Finland, Sotkamo	Sotkamo E. Kemppanen, 649 (MA378289)	EU541816	EU541852
	4	Denmark, SW Greenland, Tugtutoq Island	M. Luceño and M. Guzmán, 5407ML	EU541818	EU541853
	5	Canada, Quebec, Schefferville region	Waterway, 97090 (MTMG)	–	AY757594
	6	Chile, XII region, Punta Arenas, Parrillas Lake	M. Luceño and R. Álvarez, 606ML (UPOS1816)	EU541815	EU541851
Group 2					
Carex canescens	1	Finland, Pallas-Ounastunturi N.P.	M. Luceño and M. Guzmán, 2005ML (UPOS314)	EU541833	EU541864
	2	Russia, Siberia	Murray et al., 256	–	AF284990
	3	Iceland, between Storaborg and Mosfell	M. Luceño, 3206ML (UPOS1941)	EU541836	EU541867
	4	Spain, Burgos, Neila	M. Luceño, 44000ML (UPOS87)	EU541835	EU541866
	5	Canada, Quebec, Mont Tremblat	Bond, s.n. (MTMG)	–	AY757406
	6	USA, Wyoming, Albany	Hipp et al., 587 (WIS)	–	AY779078
	7	Chile, XII region, Punta Arenas, Seno Otway	M. Luceño and R. Álvarez, 17905ML (UPOS1797)	EU541834	EU541865
Carex macloviana	1	Finland, Muonio (Kittila Lapland), Tiurajärvi village ca 500 m NW from the eastern end of the lake	J. Nurmi, 97-39 (MA692658)	EU541842	EU541861
	2	Denmark, SW Greenland, Tasermiut, Nalumsortoq	M. Luceño and M. Guzmán, 7607ML	EU541844	EU541863
	3	USA, Wisconsin, Jackson	Hipp, 1893 (WIS)	–	AY779117
	4	Chile, XII region, between Punta Arenas and Puerto Natales, Km.125	M. Luceño and R. Álvarez, 18605ML (UPOS1804)	EU541843	EU541862
Carex maritima	1	Norway, Stabbursnes	M. Luceño and M. Guzmán, 7305ML (UPOS370)	EU541846	EU541873
	2	Iceland, Skaftafell N.P.	M. Guzmán *s.n.* (UPOS706)	EU541847	EU541874
	3	Denmark, SW Greenland, Oaleragdlit	M. Luceño and M. Guzmán, 4707ML	EU541848	EU541876
	4	Chile, XII region, Tierra de Fuego, between Cullen y Cerro Sombrero	M. Luceño and R, Álvarez, 1806ML (UPOS1830)	EU541845	EU541875
Group 3					
Carex microglochin	1	Norway, Hedmark, Folldal	E. Fremstad (TRH151800)	EU541823	EU541858
	2	Norway, Oppland, Dovre	E. Fremstad (TRH153220)	EU541826	–
	3	Iceland, between Hvolsvöllur and Vik	M. Luceño, 4906ML (UPOS1960)	EU541824	EU541855
	4	Iceland, Gullfoss	M. Luceño, 4006ML (UPOS1950)	EU541825	EU541857
	5	Denmark, SW Greenland, Igaliko	M. Luceño and M. Guzmán, 8507ML	EU541827	EU541860
	6	Argentina, Andes	Kiesling, 8707 (SI10171)	–	EU541859
	7	Chile, XII region, Punta Arenas, Parrillas Lake	M. Luceño and R. Álvarez, 506ML (UPOS1814)	EU541821	EU541854
	8	Chile, XII region, Punta Arenas, Parrillas Lake	M. Luceño and R. Álvarez, 1906ML (UPOS1831)	EU541822	EU541856
SISTERGROUP AND OUTGROUP					
Carex furva	1	Spain, Granada, Sierra Nevada	P. Jiménez-Mejías et al., 161PJM06(1)	EU541838	EU541868
Carex lachenalli	1	Spain, Lérida, Aran Valley	M. Luceño, 16898ML (UPOS128)	EU541841	EU541870
	2	Norway, Kvaenangsfjellet	M Luceño and M. Guzmán, 5305ML (UPOS353)	EU541840	EU541869
Carex glareosa	1	Iceland, Djúpivogur, Berufjördur	M. Luceño 7206ML (UPOS1983)	EU541839	EU541871
Carex brunescens	1	Finland, Rovaniemi	M. Luceño and M. Guzmán, 105ML (386UPOS)	EU541837	EU541872
Carex pulicaris	1	Iceland, Nisjahverti-Djúpivogur, Hvalnes	M. Luceño, 5806ML (UPOS1970)	EU541829	–
Carex macrostyla	1	Spain, Lérida, Aran Valley	J.M. Marín, 7204JMM	EU541831	–
Carex pauciflora	1	Finland, between Huuoniomi and E75 road	M. Luceño, 7905ML (UPOS375)	EU541832	–
Carex curvula	1	Spain, Huesca, Benasque	P. Jiménez et al., 429PJM05 (UPOS2109)	EU541828	–
Uncinia lechleriana	1	Chile, Punta Arenas, Magallanes Forestal Reserve	M. Luceño and R. Álvarez, 18405ML (375UPOS)	EU541830	–
Carex rostrata	1	Spain, Lérida, Aran Valley	M. Luceño et al., 90ML05B (UPOS1723)	EU541820	–
Carex acutiformis	1	South Africa, Trasvaal, Gauteng	C. Reid, 1367 (UPOS3084)	EU541819	–

weighting of all characters, and transitions : transversions. Heuristic searches were replicated 10,000 times retaining a maximum of two trees in each replicate, with tree bisection–reconnection (TBR) branch swapping. A second heuristic search set was made based on the trees retained in RAM memory in the first heuristic search set. The trees were obtained from the strict consensus of all trees obtained in the heuristic searches. Clade supports were assessed by bootstrapping with 10,000 re-samplings based on the conditions of the first heuristic search set.

ITS and *rps*16 intron were not analysed together in a combined matrix because there are missing *rps*16 sequences for many species.

Finally, plastid haplotypes of the groups 1 and 2 (excluding outgroups) were analysed under statistical parsimony following the algorithm described by Templeton et al. (1992), as implemented in the program TCS 1.20 (Clement et al., 2000). Gaps between population sequences of bipolar *Carex* species were considered as missing data but recoded in the matrix as a new character.

Results

Characterization of ITS sequences

Length variation of ITS sequences in the three study groups ranged as follows: 613–614 base pairs (bp) in *C. magellanica* (group 1); 607–608 bp in *C. canescens*, 608 bp in *C. macloviana*, and 607 bp in *C. maritima* (group 2); and 607–608 in *C. microglochin* (group 3). Variable/informative characters in ITS sequences were found as follows: 4/1 (3/1 in ITS-1; 0/0 in 5.8S; 1/0 in ITS-2) in *C. magellanica* (group 1); 4/3 (2/1 in ITS-1; 0/0 in 5.8S; 2/2 in ITS-2) in *C. canescens*; 1/0 (in ITS-1) in *C. macloviana*; 7/0 (6/0 in ITS-1; 0/0 in 5.8S region, and 1/0 in the ITS-2) in *C. maritima* (group 2); and 47/47 (25/25 in ITS-1; 3/3 in 5.8S; 19/19 in ITS-2) in *C. microglochin* (group 3).

The simplest model sequence evolution that best fit the data was GTR+G for the ITS-1 and ITS-2 data sets of groups 1, 2 and 3; for 5.8S, the best-fitting models were K80 (group 1) and K80+I (groups 2 and 3). Sequence pairwise distances of the ITS-1 and ITS-2 spacers using these models had the following ranges: 0–0.69% in *C. magellanica* (group 1), 0–0.95% in *C. canescens*, 0–0.23% in *C. macloviana*, 0.00–0.17% in *C. maritima* (group 2), and 0–17.98% in *C. microglochin* (group 3).

Characterization of rps16 sequences

Length variation of *rps*16 sequences within ingroup species had the following ranges: 756 bp in *C. magellanica* (group 1), 794–795 bp in *C. canescens*, 796 bp in *C. macloviana*, 785–873 bp in *C. maritima* (group 2), and 777–787 bp in *C. microglochin* (group 3).

The ratios of variable/informative characters were as follows: 1/0 for *C. magellanica* sequences (group 1), 2/0 for *C. macloviana*, 1/0 for *C. canescens*, 0/0 for *C. maritima* (group 2), and 20/19 for *C. microglochin* (group 3).

The simplest models of sequence evolution that best fit the data were GTR in the data set of group 1, GTR+I in the data set of the group 2 and GTR+G in the data set of group 3.

Sequence pairwise distances of the *rps*16 intron using these models had the following ranges: 0–0.13% in *C. magellanica* (group 1), 0–0.13% in *C. canescens*, 0–0.25% in *C. macloviana*, 0.00% in *C. maritima* (group 2), and 0–2.76% in *C. microglochin* (group 3).

ITS phylogenetic analyses

The BI analyses of ITS sequences of group 1 reached a stationary point of the Likelihood scores before 100,000 generations. Accordingly, the first 1000 trees were discarded. The heuristic search found the six most parsimonious trees. The topology of MP consensus tree is congruent with BI consensus. BI consensus tree and bootstrap analysis revealed one highly supported clade (100% pp; 99% bs) including all samples of *C. magellanica* (Fig. 1A). Within this clade, a subclade (99% pp; 63% bs) with the five accessions from the northern hemisphere was retrieved. European and Greenland accessions clustered together in a third, less-supported subclade (72% pp; < 50% bs).

In group 2, the stationary point of the Likelihood scores in the BI analysis was reached before 150,000 generations. Accordingly, the first 1500 trees were discarded. The heuristic search found the 32 most parsimonious trees. The topology of MP consensus tree is congruent with BI consensus. Monophyly was highly supported for each of the three species (Fig. 1B; *C. macloviana*, 98% pp, 63% bs; *C. canescens*, 100% pp, 95% bs; and *C. maritima*, 100% pp, 99% bs). Within the *C. canescens* clade, only one well -supported subclade was retrieved (98% pp, 63% bs), which included samples from Russia and Finland. Finally, within the *C. maritima* clade, samples from the northern hemisphere formed a well -supported subclade (100% pp, 91% bs), while the accession of Chile is the sister group of the remaining samples of *C. maritima*.

In group 3, the stationary point of the Likelihood scores was reached before 100,000 generations. Accordingly, the first 1000 trees were discarded. The heuristic search found two most parsimonious trees. The topology of MP consensus tree is congruent with BI consensus. Accessions of *C. microglochin* do not form a monophyletic group (Fig. 1C) and appeared in two distant clades. The two samples from Chile cluster together in a well supported (95% pp, < 50% bs) clade with *Schoenoxiphium* species (*S. sparteum*, *S. filiforme*, *S. lehmannii*). In contrast, the five accessions from the northern hemisphere (Iceland, Norway, Greenland) plus one from Argentina form a well -supported group (100% pp, 100% bs) in an distant clade.

Phylogenetic analysis of rps16

The BI analysis of the *rps*16 sequences of group 1 reached the stationary point of the Likelihood scores before 100,000 generations. Accordingly, the first 1000 trees were discarded. The heuristic search found a single most parsimonious tree. The topology of the most parsimonious tree is congruent with BI consensus. *Carex magellanica* is a monophyletic species with all the samples grouped in a well -supported clade (100% pp, 100% bs; Fig. 2A). In the haplotypes network, the southern hemisphere haplotypes of *C. magellanica* are in tip positions while the northern hemisphere haplotypes are in a more central position (Fig. 3A).

In group 2, we found length mutations. A remarkable 93 bp-long duplication in the *C. maritima* sequence of the southern hemisphere and a 5 bp-long deletion in all northern hemisphere *C. maritima* sequences were detected. The coded and non-coded gaps matrices were analysed. The BI analyses of the *rps*16 sequences reached the stationary point of the Likelihood scores before 100,000 generations. Accordingly, the first 1000 trees were discarded. The heuristic search found five most parsimonious trees. The topology of MP consensus tree is congruent with BI consensus. The results of the analyses of the code and no coded length mutations matrices were similar. Only the internal resolution of *C. maritima* showed changes. The results of the analysis of the coded gaps matrix are shown in Fig. 2B. Monophyly
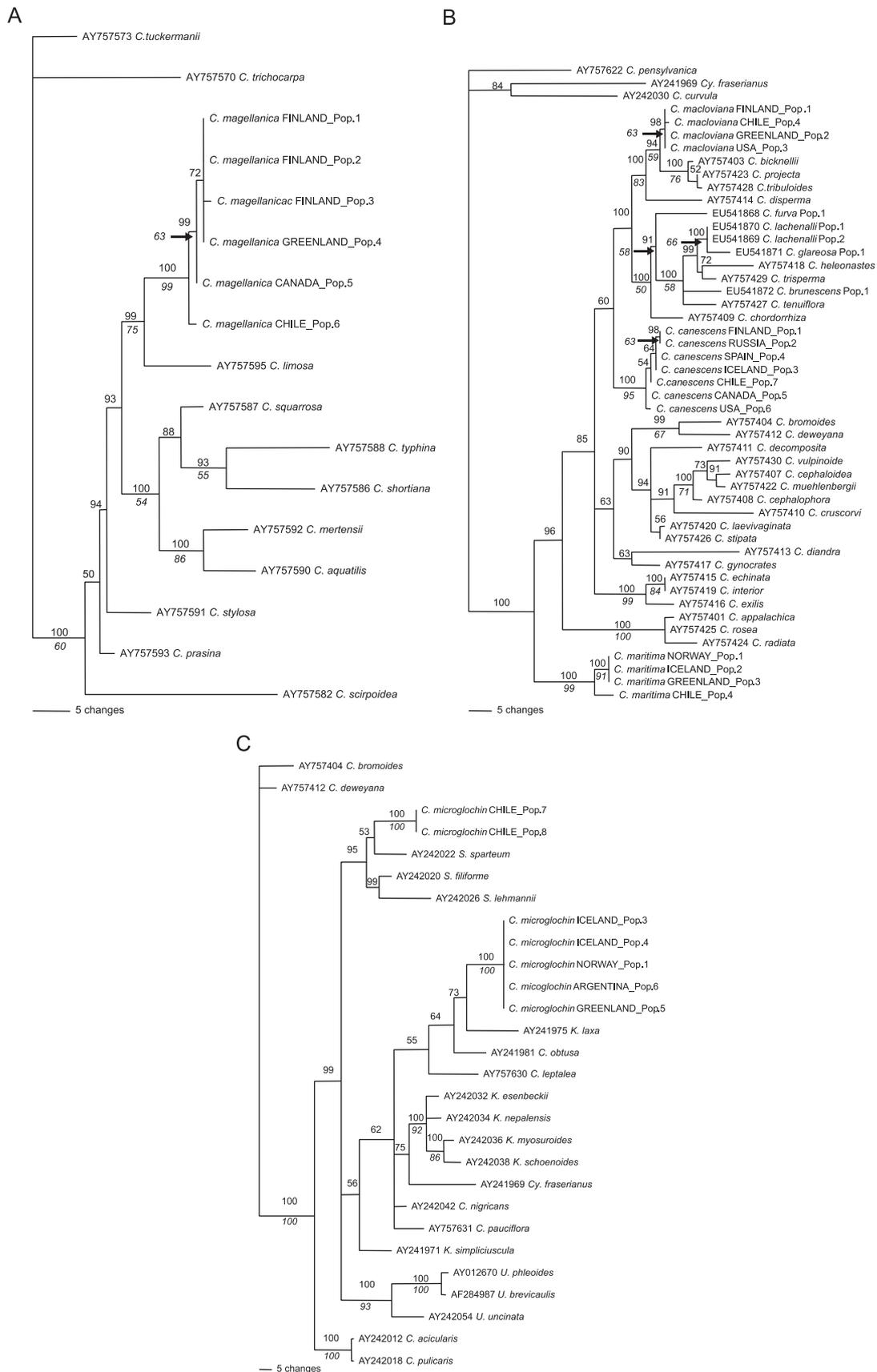
Fig. 1. (A) Majority rule consensus tree of the 49,000 trees retained in the Bayesian inference analysis of the 17 ITS sequences of subgenus *Carex*, and two of *C. trichocarpa* —*C. tuckermanii* as the outgroup. (B) Majority rule consensus tree of the 48,500 trees retained in the Bayesian inference analysis of the 46 ITS sequences of subgenus *Vignea*, and three species *Carex curvula*—*Cymophillus fraserianus*—*Carex pensylvanica* as the outgroup. (C) Majority rule consensus tree of the 49,000 trees retained in the Bayesian inference analysis of the 26 ITS sequences, and two of *C. bromoides*—*C. deweyana* as the outgroup. Posterior probabilities and bootstrap supports are given above and below branches respectively.
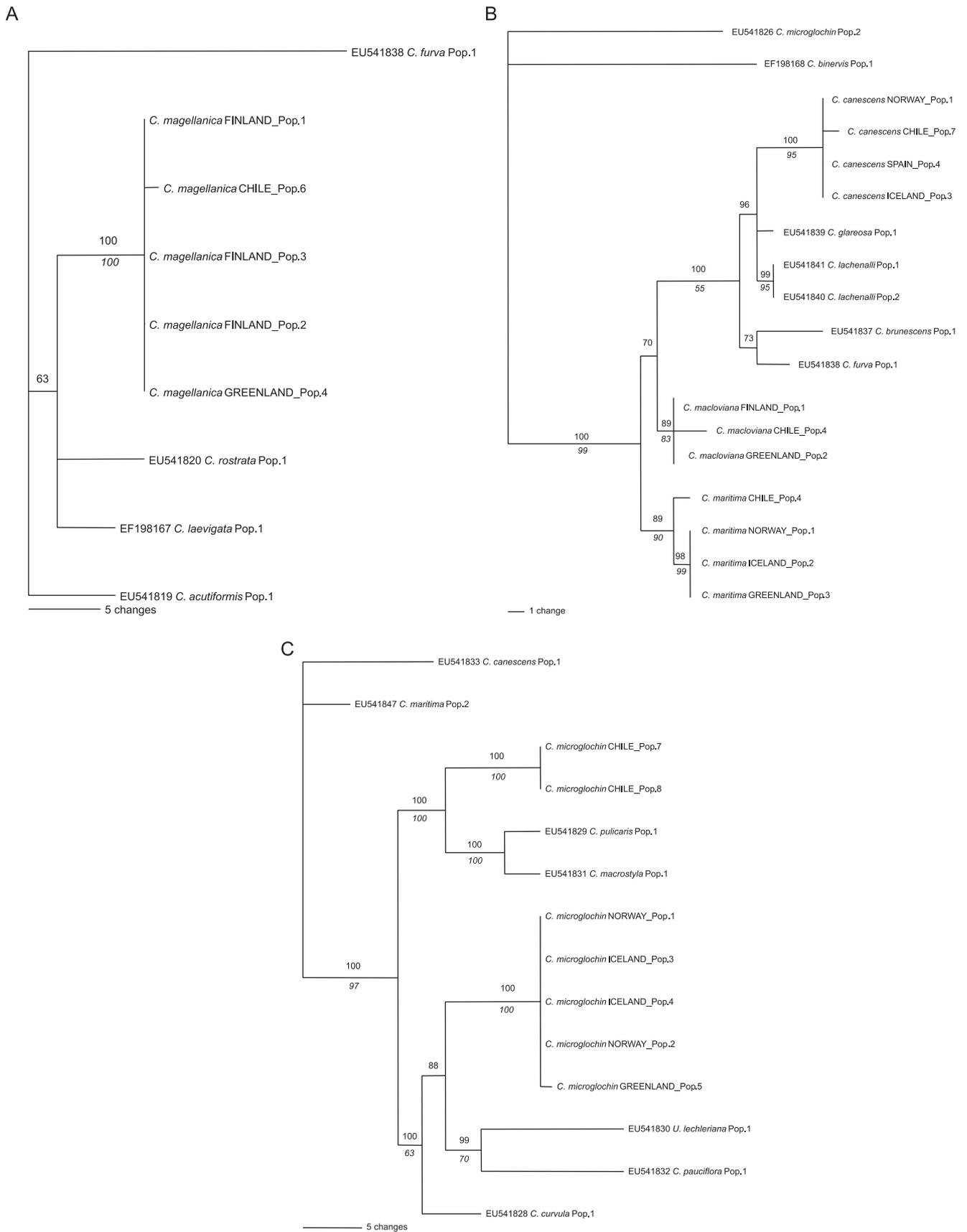
Fig. 2. (A) Majority rule consensus tree of the 49,000 trees retained in the Bayesian inference analysis of the 8 *rps*16 sequences of subgenus *Carex*, and one of *C. furva* as the outgroup. (B) Majority rule consensus tree of the 49,000 trees retained in the Bayesian inference analysis of the 16 *rps*16 sequences, and two of *C. binervis—C. microglochin* as the outgroup. (C) Majority rule consensus tree of the 49,000 trees retained in the Bayesian inference analysis of the 12 *rps*16 sequences, and two of *C. canescens—C. maritima* as the outgroup. Posterior probabilities and bootstrap supports are given above and below branches, respectively.

A

B
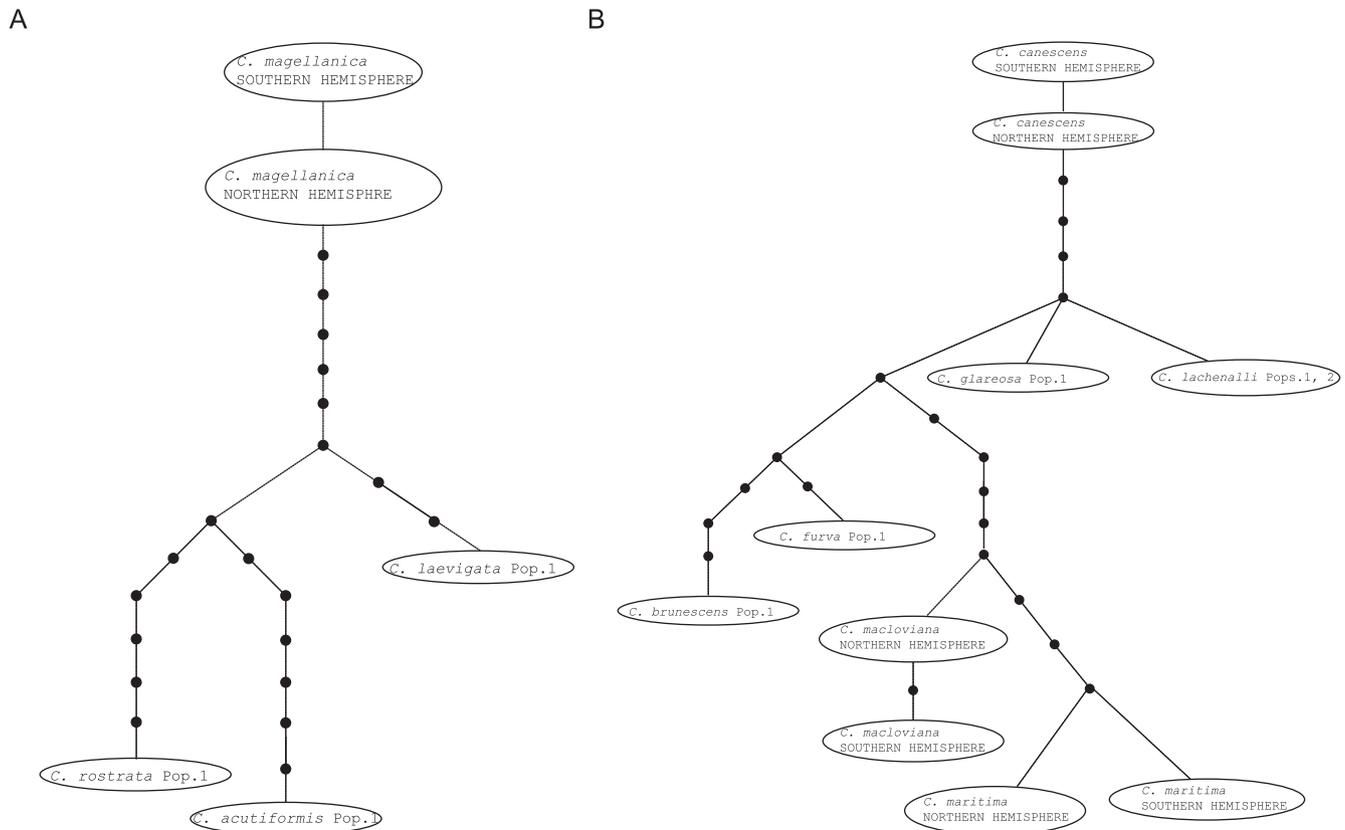
Fig. 3. (A) A statistical parsimony network of 5 cpDNA haplotypes, including the two detected in *C. magellanica* and three haplotypes detected in the remaining species. White ovals indicate the 5 haplotypes, black circles haplotypes extinct or not found, and each line between haplotypes sequence mutation steps. (B) A statistical parsimony network of 10 cpDNA haplotypes, including the two detected in *C. canescens*, the two detected in *C. macloviana* and the two detected in *C. maritima* and four haplotypes detected in the remaining species. White ovals indicate the 10 haplotypes, black circles haplotypes extinct or not found, and each line between haplotypes sequence mutation steps.

of *C. macloviana*, *C. canescens* and *C. maritima* were supported (89% pp, 83% bs; 100% pp, 95% bs; and 89% pp, 90% bs, respectively; Fig. 2B). In the haplotypes network, the southern hemisphere haplotypes of *C. macloviana* and *C. canescens* are in tip positions while the northern hemisphere haplotypes are in a more central position. All haplotypes of *C. maritima* are in tip positions (Fig. 3B).

In group 3, the BI analysis of the *rps*16 sequences reached the stationary point of the Likelihood scores before 100,000 generations. Accordingly, the first 1000 trees were discarded. The heuristic search found a single most parsimonious tree. The topology of the single tree is congruent with BI consensus. Lack of monophyly of *C. microglochin* was retrieved (Fig. 2C) in congruence with ITS results. The two samples from Chile appeared together with *C. pulicaris* and *C. macrostyla* in a well supported (100% pp; 100% bs) and independent clade from the remaining five samples (Iceland, Norway, Greenland), which grouped with *Uncinia lechleriana*, *C. pauciflora*, and *C. curvula* (100% pp; 63% bs).

Discussion

Species circumscription

Four of the five species analysed seemed to be monophyletic (*C. magellanica*, *C. canescens*, *C. macloviana*, *C. maritima*), whereas *C. microglochin* was not. Although naturalness of *C. magellanica* is out of any doubt, two subspecies have been recognized based on morphology and geography: subspecies *magellanica* Lam.

(Patagonia, Tierra de Fuego) and subspecies *irrigua* (Wahlenb.) Hultén (occurs in cold-temperate regions of the northern hemisphere; see maps in: Ball, 2002; Hultén, 1962; Moore and Chater, 1971). This subdivision is also molecularly supported by our ITS phylogeny (Fig. 1A). *Carex canescens* L. reaches low latitudes, the Iberian Peninsula in the northern hemisphere and New Guinea in the southern hemisphere (see maps in: Hultén, 1962; Moore and Chater, 1971; Toivonen, 2002). It is highly variable from a morphological point of view and several infraspecific taxa have been proposed for this species (Hultén, 1962; Kükenthal, 1909). Our phylogenetic results revealed low clade support for considering populations of both hemispheres as different taxa, consistent with the lack of any clear geographical pattern of morphological variation between both hemispheres. *Carex macloviana* is a highly variable species that occurs in meadows of cold regions in both hemispheres (see maps in: Mastrogiuseppe et al., 2002; Moore and Chater, 1971). The scarce molecular differences between samples from both hemispheres (Figs. 1B and 2B) and the unclear morphological differences (Moore and Chater, 1971) lead us to be cautious for supporting any taxonomical recognition within *C. macloviana* (see Kükenthal, 1909). *Carex maritima* Gunn. is a highly polymorphic and widespread species distributed in cold regions of both hemispheres (see maps in: Hultén, 1962; Moore and Chater, 1971; Reznicek, 2002). Five varieties were recognized by Kükenthal (1909): three taxa in the northern hemisphere and two taxa in the southern hemisphere. However, the most recent taxonomical study did not recognize any infraspecific taxa despite the variability (Reznicek, 2002). Our phylogenetic results revealed some degree of differentiation between northern and southern

hemisphere populations, although no populations were included from North America. In both nuclear and plastid phylogenies, the Chilean accession is sister to a well-supported subclade that contains all northern populations (Figs. 1B and 2B). Although there are no nucleotide substitutions in the rps16 sequences, two consistent indels differentiate rps16 sequences from northern and southern populations.

Carex microglochin is a widespread species distributed throughout cold regions and mountains of both hemispheres (see maps in: Cochrane, 2002; Moore and Chater, 1971; Wheeler and Guaglianone, 2003). Most of the taxonomical disagreement regarding this species affects southern hemisphere populations. Some authors considered all material from South America as belonging to the same species as the one from the northern hemisphere (*C. microglochin*; Moore, 1983). Other authors suggested that southern hemisphere populations constitute a different variety (var. *oligantha* (Both) Kük., Kükenthal, 1909), subspecies (ssp. *fuegina* Kük., Barros, 1935), or species (*C. microglochin* in the northern and *C. camptoglochin* Krecz. (=*C. oligantha* Boott); (Boott, 1867; Kreczetowicz, 1937). Finally, Roivainen (1954) and more recently Wheeler and Guaglianone (2003) detected the two taxa in South America, *C. microglochin* in montane wet *Sphagnum*-free meadows, and *C. camptoglochin* in *Sphagnum magellanicum* peat bogs (Wheeler and Guaglianone, 2003). In our ITS tree, *C. microglochin* appears segregated in two of the four major clades detected in the phylogeny of the tribe Cariceae (Starr et al., 2008; Waterway and Starr, 2007). On the one hand, the southern samples of *C. microglochin* growing in *S. magellanicum* peat bogs and characterized by some morphological features (see Wheeler and Guaglianone, 2003) appeared within the *Schoenoxiphium* clade (Waterway and Starr, 2007). On the other hand, the northern and southern plants of *C. microglochin* living in wet meadows without or with a very low *Sphagnum* density and displaying unique morphological characteristics nest in the unispicate clade together with *Cymophyllus*, *Kobresia*, and *Uncinia* (Waterway and Starr, 2007). These results strongly support the separation between *C. microglochin*, widespread in the northern hemisphere and locally distributed in the wet meadows of the Andes, Tierra del Fuego and Patagonia, and *C. camptoglochin*, endemic to the *Sphagnum* peat bogs of the Andes, Tierra del Fuego, and Patagonia.

Parallel evolution and long-distance dispersal

Vicariance was the main accepted hypothesis in the past and placed the origin of bipolar disjunctions in the Mesozoic (65–250 Myr B.P.; Du Rietz, 1940) as a result of the build-up of equatorial and tropical barriers for polar plants. This interpretation cannot be ruled out for higher taxonomical categories (families) within angiosperms, given that multiple lineages split in the Jurassic (Wikström et al., 2001). However, for species levels it is unlikely to place the origin of such disjunctions before tropical and equatorial barriers built up (see molecular-clock estimates at species level: Kay et al., 2005; Klak et al., 2003; Richardson et al., 2001a, b). In the absence of particular molecular-clock estimates, low genetic distances between northern and southern hemisphere populations of the study species support a recent evolutionary history, except for the case of *C. microglochin* and *C. camptoglochin* (see below). Indeed, divergence time estimates (76 ± 16 Myr B.P.; Janssen and Bremer, 2004) for the Cyperaceae diversification (5000 species) and dating of the single known fossil record of Cariceae (beginning of Paleocene, 59 Myr ago, approx.; Egorova, 1999) are incongruent with the hypothesis of vicariance (Mesozoic 65–250 Myr B.P.) to account for bipolar disjunctions in *Carex*.

As supported by Humboldt (1817), parallel evolution could explain some distributional patterns found in angiosperms. As far as we know, there is no documented *Carex* species fitting into this evolutionary model. Interestingly, our phylogenetic results demonstrate the existence of two distantly related lineages: the group of populations from both hemispheres of *C. microglochin* and the group from the southern hemisphere of *C. camptoglochin* (see above). These lineages are morphologically very similar but highly divergent molecularly (*Schoenoxiphium* and unispicate clades, see Waterway and Starr, 2007). We consider parallel evolution as the most plausible explanation for these species, considering the great morphological similarity between the two lineages, lack of any evidence of recent or ancestral hybridization and their distant placement in our phylogenies (Figs. 1C and 2C).

The most accepted biogeographical hypothesis for plant bipolar disjunctions is long-distance dispersal (Moore and Chater, 1971; Raven, 1963; Van Steenis, 1962; Vollan et al., 2006). Raven (1963) suggested that such plants could have migrated at the end of the Pliocene or in Pleistocene, during some of the last cold periods that expanded the polar regions in both hemispheres. Graham (1987) and Ball (1990) agree with Raven (1963) in chronological estimations of migrations, but differ in the proposed mechanism of dispersal. Raven (1963) proposed a direct jump between the adjacent regions of both hemispheres, whereas Graham (1987) and Ball (1990), following Guppy's (1917) hypothesis, suggested a migration pathway through mountain chains (mountain hopping) across tropical regions where climatic conditions were very similar to the polar zones. The same hypothesis has recently been documented on the basis of AFLP results in *Carex canescens*, *C. echinata*, *C. lachenalii*, and *C. magellanica* (Vollan et al., 2006). In addition, Heide (2002) established for the same four bipolar species that no ecophysiological changes or adaptations in the flowering requirements would have been necessary for these plants to migrate across the tropics and penetrate into the southern hemisphere by mountain hopping. The current occurrence of two high-latitude species (*C. microglochin*, *C. camptoglochin*) in tropical mountains (Wheeler and Guaglianone, 2003) is congruent with Heide's hypothesis (2002) and supports a dispersal pattern through mountain hopping. An alternative hypothesis is the allochthonous origin of bipolar *Carex* species. However, the differentiation between southern and northern hemisphere populations together with the presence of their populations in undisturbed habitats pleads for the autochthonous character of the bipolar *Carex* species in both hemispheres. In conclusion, the low genetic differentiation found between northern and southern hemisphere populations of *C. magellanica*, *C. canescens*, *C. macloviana*, *C. maritima*, and *C. microglochin* sensu stricto indicates that the most likely hypothesis explaining their migrations is recent long-distance dispersal sensu lato. Despite documented examples of direct long-distance migrations (Europe to South America) that have been detected in *Carex* species (Escudero et al., 2008a) there is a higher probability of migration success between geographically close areas. The South American populations of C. *magellanica* and *C. canescens* are genetically closer to populations from North America than to other northern hemisphere populations supporting the mountain hopping hypothesis across the Rocky Mountains and the Andes mountain ranges. In addition, the low levels of genetic variation between the northern and southern hemisphere populations are compatible with the recent uplift of the Andes and the appearance of cold upland habitats (2–4 Myr ago; Gregory-Wodzicki, 2000). However, the present results are not enough to narrow down between the two possibilities: direct long-distance dispersal vs. mountain hopping.

The genus *Carex*, is worldwide distributed (Reznicek, 1990). despite only some species display long distance dispersal devices.

In the last decade, *Carex* dispersal mechanisms and syndromes have been described (reviewed by Allessio Leck and Schütz, 2005). These studies revealed the presence of several dispersal syndromes in the genus such as anemochory, autochory, endozoochory, epizoochory, hydrochory, and myrmecochory. Only some of them are associated with special devices in utricles and flowering stems such as inflated or roundish utricles, limp flowering stems, coloured seeds, acute beaks, corky pericarps, and elaiosomes. Two of five studied bipolar *Carex* species (*C. macloviana*, *C. microglochin*) display two additional dispersal devices. *Carex macloviana* shows winged utricles (Mastrogiuseppe et al., 2002) that could be related with hydrochory or anemochory, and *C. microglochin s.s.* displays hooked utricles associated with zoochory (Savile, 1972). However, the distribution patterns are hard to be interpreted simply based on dispersal peculiarities, given the lack of peculiar dispersal devices in some of the taxa or even when considering the above-mentioned dispersal mechanisms. Another question is the direction of dispersal. The bipolar *Carex* species studied present wider distributions in the northern hemisphere than in the southern hemisphere. Based on these patterns of distribution, dispersals from the northern to the southern hemisphere were proposed as the most likely scenario (Ball, 1990; Moore, 1972; Smith, 1986), although no explicit tests were performed. Our results show that the southern hemisphere plastid haplotypes of *C. magellanica*, *C. canescens*, and *C. macloviana* contain tip haplotypes in the network (Fig. 3A and B). These haplotypes are assumed to be derived according to the coalescent theory (Avise, 2000). Our haplotype network supports the direction of dispersal from north to south in these cases. However, this pattern of haplotype relationship was not found for *C. maritima*. All results herein presented agree with recent northern-southern connections. In particular, the low genetic distance measured between populations of *C. canescens* from North America and South America is lower than between some populations from the northern hemisphere (North America and Finland), perhaps indicating a more recent migration between North America and Patagonia than among some areas of the northern hemisphere.

Acknowledgements

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