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Long-distance dispersal explains the bipolar disjunction in *Carex macloviana*[1]

José I. Márquez-Corro[2,5], Marcial Escudero[3], Santiago Martín-Bravo[2], Tamara Villaverde[2,4], and Modesto Luceño[2]

PREMISE OF THE STUDY: The sedge *Carex macloviana* d'Urv presents a bipolar distribution. To clarify the origin of its distribution, we consider the four main hypotheses: long-distance dispersal (either by mountain hopping or by direct dispersal), vicariance, parallel evolution, and human introduction.

METHODS: Phylogenetic, phylogeographic, and divergence time estimation analyses were carried out based on two nuclear ribosomal (ETS and ITS) regions, one nuclear single copy gene (CATP), and three plastid DNA regions (*rps*16 and 5′*trn*K introns, and *psb*A-*trn*H spacer), using Bayesian inference, maximum likelihood, and statistical parsimony. Bioclimatic data were used to characterize the climatic niche of *C. macloviana*.

KEY RESULTS: *Carex macloviana* constitutes a paraphyletic species, dating back to the Pleistocene (0.62 Mya, 95% highest posterior density: 0.29–1.00 Mya). This species displays strong genetic structure between hemispheres, with two different lineages in the Southern Hemisphere and limited genetic differentiation in Northern Hemisphere populations. Also, populations from the Southern Hemisphere show a narrower climatic niche with regards to the Northern Hemisphere populations.

CONCLUSIONS: *Carex macloviana* reached its bipolar distribution by long-distance dispersal, although it was not possible to determine whether it was caused by mountain hopping or by direct dispersal. While there is some support that *Carex macloviana* might have colonized the Northern Hemisphere by south-to-north transhemisphere dispersal during the Pleistocene, unlike the southwards dispersal pattern inferred for other bipolar *Carex* L. species, we cannot entirely rule out north-to-south dispersion.

KEY WORDS Bipolar distribution; *Carex macloviana*; divergence time estimation; long-distance dispersal; *Ovales*; phylogeography; *Vignea*

Bipolar distributions are exhibited by taxa inhabiting high latitudes in both hemispheres. Bipolar species have attracted the attention of multiple authors, such as Humboldt (1817) and Darwin (1859), since the 19th century. Moore and Chater (1971) defined bipolar species as those reaching latitudes near Alaska or the European Arctic (approximately 55°N lat.) in the Northern Hemisphere, and the Strait of Magellan (approximately 52°S lat.) in the Southern Hemisphere. Based on these criteria, they listed 30 vascular plant species with this type of disjunction, of which six belong to the genus *Carex* L. in the monocotyledon plant family Cyperaceae Juss. (*C. canescens* L., *C. macloviana*, *C. maritima* Gunn., *C. arctogena* Harry Sm., *C. microglochin* Wahlenb., and *C. magellanica* Lam.). *Carex* is the genus with the most species with such a distribution pattern, even though Poaceae Barnhart is the family with more bipolar species (eight).

Bipolar disjunctions could be explained by four different hypotheses. The first one proposes long-distance dispersal, either by a gradual latitudinal colonization through mountain ranges, or by direct long-distance dispersal. Since the Miocene (approximately 24 million years ago [Mya]; Hoorn et al., 2010), the uplift of the Andes, together with several glacial periods, provided favorable habitats for the Arctic–alpine species at high elevations of the mountain ranges to cross the Equator (Raven, 1963; Moore and Chater, 1971; Heide, 2002; Vollan et al., 2006). Direct long-distance dispersal could have involved one vector such as birds, wind, or ocean currents (Cruden, 1966; Muñoz et al., 2004; Nathan et al., 2008) or a combination of these. The long-distance dispersal hypothesis, either by mountain hopping or direct jump, could have been more plausible during Pleistocene glacial periods, when Arctic and Antarctic species could have had a broader distribution (Darwin, 1859). The second hypothesis proposes parallel evolution of the disjunct populations, which implies populations that are

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[2] Department of Molecular Biology and Biochemical Engineering, Universidad Pablo de Olavide, Carretera de Utrera km 1, ES-41013, Seville, Spain;

[3] Department of Plant Biology and Ecology, Universidad de Sevilla, Reina Mercedes sn, ES-41012, Seville, Spain; and

[4] Real Jardín Botánico, CSIC, Plaza de Murillo 2, ES-28014, Madrid, Spain

[5] Author for correspondence (e-mail: jimarcorr@gmail.com)

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phenotypically very similar but, genetically, distantly related (Humboldt, 1817; Losos, 2011; Scotland, 2011). This was demonstrated by Escudero et al. (2010) for *Carex microglochin* and *C. camptoglochin* V. I. Krecz, but not for any other bipolar *Carex* species. The third hypothesis suggests a vicariant event (Du Rietz, 1940) to explain the bipolar disjunction during the transtropical breakup between Laurasia and Gondwana during the Early Jurassic period (195–200 Mya; Raven and Axelrod, 1974; Ross and Scotese, 1988; Scotese, 2004). This hypothesis can be rejected because the divergence of Cyperaceae is estimated to approximately 76 Mya (Janssen and Bremer, 2004). Finally, the last hypothesis contemplates the possibility of a human introduction of the populations in one of the hemispheres.

Carex is one of the most diverse angiosperm genera in the temperate and cold regions of the world (Escudero et al., 2012), with about 2000 species and an almost cosmopolitan distribution (Kükenthal, 1909; Egorova, 1999; Mastrogiuseppe et al., 2002). Within genus *Carex*, the subgenus *Vignea* comprises half of the bipolar species (*C. canescens*, *C. maritima* and *C. macloviana*). This clade is composed of about 320 species, and only a small number of species from this group occur in the Southern Hemisphere (Egorova, 1999). *Carex macloviana* is included in the section *Ovales* Kunth, the largest section within *Vignea*, which contains about 90 species (Egorova, 1999; Mastrogiuseppe et al., 2002; Hipp et al., 2006), and it is mainly distributed in North America with several species in South America, Europe, and Asia (Reznicek, 1993; Egorova, 1999; Mastrogiuseppe et al., 2002). It includes about 15 species endemic to South and Central America (Reznicek, 1993).

Recent molecular studies of the six bipolar *Carex* species have recurrently suggested long-distance dispersal from the Northern to the Southern Hemisphere as the well-supported hypothesis to explain the bipolar disjunction, either by one or more dispersal events (Vollan et al., 2006; Escudero et al., 2010; Villaverde et al., 2015a, b, 2017). Escudero et al. (2010) also proposed a southwards long-distance dispersal to explain the *C. macloviana* disjunction. Nonetheless, their analyses were based only on two DNA regions (ITS and *rps*16) and on a very limited sampling, which only included four *C. macloviana* populations (one from the Southern Hemisphere).

The aim of this study is to test the main hypotheses to explain the bipolar distribution of *C. macloviana* using an extensive population sampling, four molecular DNA regions from nuclear and plastid genomes, and climatic data. Specific aims are to (1) clarify the relationship among populations of both hemispheres using phylogenetic-phylogeographic analyses, (2) estimate divergence time among populations, and (3) to study the ecological niche of the populations from both hemispheres. These analyses have proved to be a successful approach to test the hypotheses described above in other bipolar *Carex* species (Escudero et al., 2010; Villaverde et al., 2015a, b, 2017).

MATERIALS AND METHODS

Sampling—Twenty-seven populations and 28 individuals of *Carex macloviana* were sampled to represent the geographical range of the species (Fig. 1). At least one individual from each population was sampled for every "botanical country" (see level 3 in Brummitt, 2001) of its distribution area according to the range indicated by Govaerts et al. (2016; Fig. 1), except for some regions where material was not available (Appendix S1, see the Supplementary Data with this article). Also, 10 closely related species from *Carex* section *Ovales* were sampled to represent its main phylogenetic lineages (Hipp et al., 2006): three populations of *C. pachystachya* Cham. ex Steud. and one population of *C. harfordii* Mack., *C. gracilior* Mack., *C. haydeniana* Olney, *C. bonplandii* Kunth, *C. mandoniana* Boeckeler, *C. merritt-fernaldii* Mack., *C. longii* subsp. *meridionalis* (Kük.) Luceño & M.Alves, *C. scoparia* Schkuhr ex Willd., and *C. mariposana* L.H.Bailey ex Mack. Samples were obtained from leaf material collected in the field and dried in silica gel or from herbarium specimens (Appendix S1) provided by CAN, MA, MICH, MOR, UPOS, and WIS herbaria (abbreviations according to *Index Herbariorum*; Thiers, 2015). One individual of *C. maritima* from section *Foetidae* (Tuckerm. ex L. H. Bailey) Kük. (Ford et al., 2012) was set as outgroup (Appendix S1).

Extraction, PCR amplification and sequencing—Leaf material was homogenized with a TissueLyser RESCH M-301 bead mill (Qiagen, California) and DNA was extracted using the DNeasy Plant Mini Kit (Qiagen, Valencia, California). Six DNA regions (Appendix S2) were amplified using the polymerase chain reaction (PCR) method, two corresponding to the nuclear ribosomal DNA region (nrDNA), one to coding nuclear DNA gene (nDNA), and three to plastid DNA regions (ptDNA). The nuclear DNA regions were the internal transcribed spacer (ITS, primers ITS-A and ITS-4; White et al., 1990; Blattner, 1999), the external transcribed spacer (ETS, primers ETS-1f, and 18S-R; Starr et al., 2003), and the CATP gene (primers CATP11F and CATP13R; E. Maguilla, UPO, personal communication). The plastid DNA regions were the *rps*16 intron (primers rps16F, rps16R; Shaw et al., 2005), the 5′*trn*K intron (primers 5′trnK*Carex*F, 5′trnK*Carex*R; Escudero and Luceño, 2009), and the *psb*A-*trn*H intergenic spacer (primers psbAF, trnH2; Sang et al., 1997; Tate and Simpson, 2003). A total of 28 individuals from 27 populations of *C. macloviana* were successfully sequenced for all six loci. Most of these loci were also successfully sequenced for the remaining species of *Ovales* and the outgroup stated above, or were taken from the GenBank database: http://ncbi.nlm.nih.gov/genbank.

After amplification (Appendix S3), PCR products were sent to Stab Vida (Lisbon, Portugal) or Macrogen (Amsterdam, Netherlands) for Sanger sequencing.

All the sequences were edited, aligned with Muscle 3.8.425 (Edgar, 2004) and revised using Geneious 6.1.7 (Biomatters, Auckland, New Zealand). Gaps corresponding to indels (except those due to mononucleotide repetitions regions; poly A and poly T) were codified based on the 'simple indel coding' method (Simmons and Ochoterena, 2000).

Phylogenetic analysis—To study the evolutionary relationships between populations of *C. macloviana*, two methods for inferring phylogenies were used. Six gene trees from the six DNA regions were reconstructed independently, as well as trees from nuclear, plastid, and combined regions. The combined matrix with the six concatenated DNA regions (ITS, ETS, CATP, *rps*16, 5′*trn*K, and *psb*A-*trn*H) consisted of 41 combined sequences and 4027 sites (Appendix S4).

Two different analyses were conducted, maximum likelihood (ML) and Bayesian inference (BI). The ML analyses were executed using RAxML 7.2.6 (Stamatakis, 2006) with 10,000 bootstrap (BS) runs to assess clade support and a general time-reversible (GTR) model with gamma-distributed rate variation. Bayesian phylogenetic inference analyses were performed in MrBayes 3.2.5

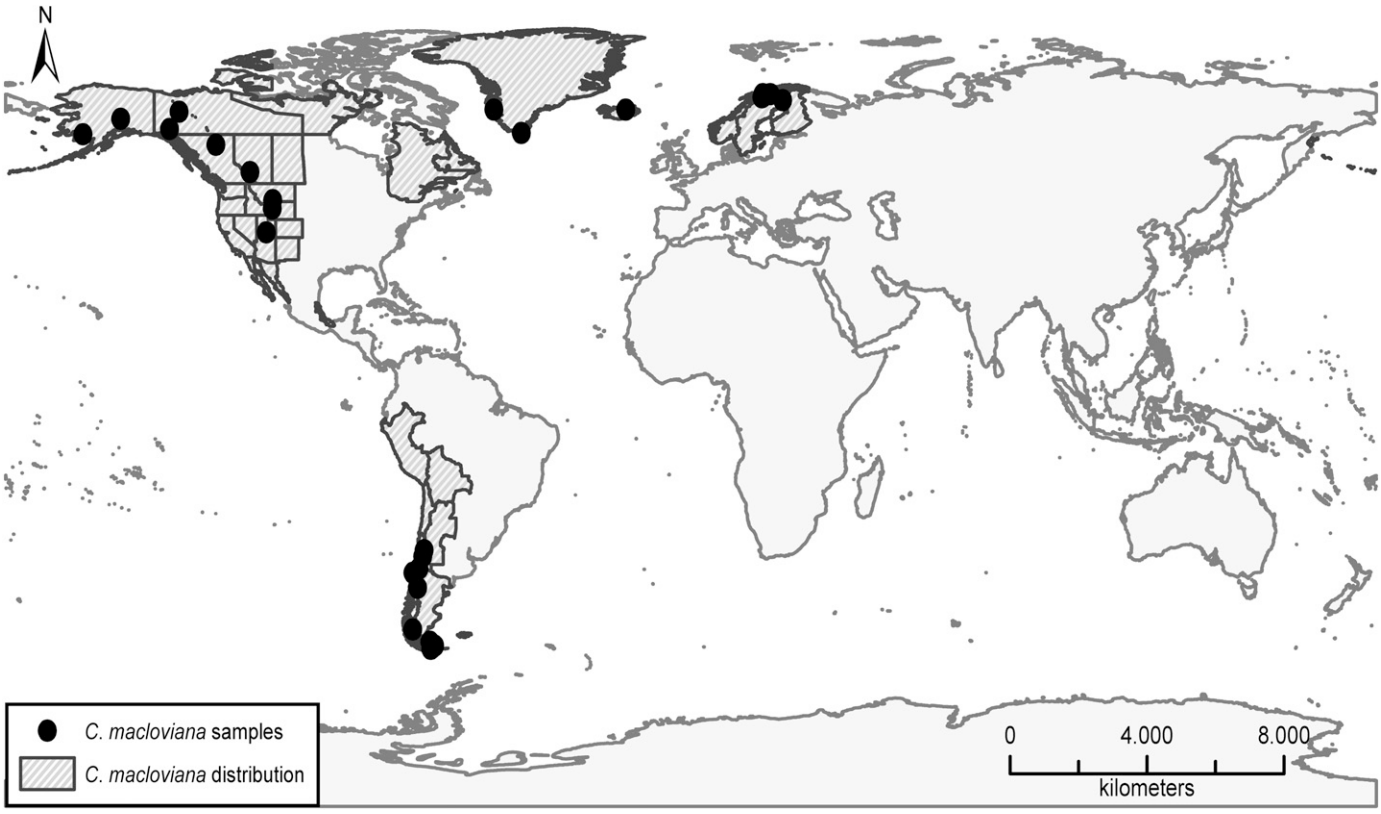

FIGURE 1 Distribution of *Carex macloviana* (in dashed gray) based on the World Monocot Checklist (Govaerts et al., 2016; botanical countries, level 3, Brummitt, 2001) and the sampled populations for this study (black circles).

(Ronquist et al., 2012), and optimal nucleotide substitution models of the loci were obtained through the Akaike information criterion (AIC, Akaike, 1974) in jModelTest (Posada, 2008), where the number of substitution schemes was set to 3 to check among the 24 simplest models. The nucleotide substitution model applied to coded indels was F81, following the manual of MrBayes (Ronquist et al., 2012). Two sets (Nruns = 2) of four Markov chains (Nchains = 4) with 5 million generations were run for every locus and the combined matrices, sampling every 1000 generations, discarding 20% of trees as burn-in. Trees were summarized by constructing 50% majority rule consensus trees. Clade supports below 70% in bootstrap or 95% in posterior probability were not considered statistically significant. Trees were edited using FigTree 1.4.0 (http://tree.bio.ed.ac.uk/software/figtree/).

Haplotype network—Matrices containing combined ptDNA sequences (*rps*16, 5′*trn*K, and *psb*A-*trn*H) from 28 individuals of *C. macloviana* and its most closely related species (see results), *C. pachystachya* (three individuals), *C. gracilior* (one individual) and *C. merritt-fernaldii* (one individual), were analyzed with and without coded indels. *Carex harfordii* was removed from this analysis because the *trn*K intron was not successfully sequenced.

The TCS 1.21 computer program (Clement et al., 2000) was used to calculate a haplotype network based on statistical parsimony, with a probability higher than 95% and the minimum number of mutational steps required for differentiating the observed haplotypes. Gaps were considered as missing data. This analysis also showed intermediate haplotypes, either extinct or not sampled. The posterior probability distribution of the total number of haplotypes was calculated by the Dixon (2006) method to estimate the haplotype sampling completeness.

Divergence time estimation—The analysis was configured on beauti 1.7.2 (Drummond et al., 2012) using an uncorrelated log-normal relaxed clock method and a tree prior with a constant size coalescent process, which is appropriate for population-level studies. There are no known fossil records for *C. macloviana* or the whole section *Ovales* (Jiménez-Mejías et al., 2016); therefore, we had to resort to secondary calibrations. A calibration of 3.53 ± 1.02 Mya (E. Maguilla, UPO, personal communication) was assigned to the crown node of section *Ovales*, and a calibration of 14.82 ± 2.69 Mya (Escudero et al., 2012) to the divergence between section *Foetidae* and section *Ovales*. A normal distribution prior was applied as recommended for secondary calibrations (Drummond et al., 2012). Finally, four independent Markov chain Monte Carlo (MCMC) simulations were run for 150 million generations and the parameters were sampled every 1000 generations in beast 1.8.2 (Drummond et al., 2012), as implemented in the CIPRES Science Gateway (Miller et al., 2010).

Posterior estimates from each analysis were examined and compared in Tracer 1.6 (Rambaut and Drummond, 2014), which also enabled checking for the stationary plateau in the estimates. A burn-in of 10% was set in trees files coming from each of the four runs, which were subsequently joined in the same file using LogCombiner 1.7.2 (Drummond et al., 2012). A consensus of the trees (maximum clade credibility tree) was calculated with

TreeAnnotator 1.7.2 (Drummond et al., 2012) with a posterior probability threshold of 0.95 and the mean heights option for node heights.

Climatic environment—Climatic niche analysis was based on recorded populations throughout the distribution of *C. macloviana*, split into two groups, Northern vs. Southern Hemisphere populations. Two data sets were generated: (1) one including the populations of *C. macloviana* sampled for the molecular analysis ('subset matrix' henceforth, 27 populations); (2) the other data set contained the subset matrix plus populations recorded in the Global Biodiversity Information Facility data portal (GBIF, http://www.gbif.org/, downloaded 08 October 2014) from year 1950 onwards. This 'complete matrix' was pruned by species, selecting exclusively *C. macloviana* and excluding populations that were likely erroneously georeferenced (i.e., individuals occurring outside its distribution range –in other land regions or in oceans–; Govaerts et al., 2016), using the "extract by mask" tool in ArcGis (ESRI, 2010). Then, individuals inside plots of 20 km^2 were eliminated—together with those samples considered as duplicates—to reduce the undesirable effects of spatial autocorrelation, keeping a single individual. After this pruning and cleaning process, the 'complete matrix' consisted of a total of 226 populations (199 from GBIF and 28 from the 'subset matrix'; Appendix S1).

Nineteen bioclimatic variables (Appendix S5) were downloaded from the WorldClim database (Hijmans et al., 2005) with a resolution of 2.5 arc-minutes, using the 'Raster' (Hijmans, 2015) and 'Dismo' (Hijmans et al., 2015) R packages. Bioclimatic information of every layer corresponding to each studied sample of the mentioned data sets were taken and used to perform principal components analyses (PCA) using the 'prcomp' function in R (retx, center, and scale options were set as "true").

RESULTS

Phylogenetic relationships—Both ML and BI analyses rendered the same tree topology. Phylogenetic relationships obtained from analyses of the individual matrices were different from those of the combined matrix (Fig. 2), although all trees showed *C. macloviana* as paraphyletic (see Appendices S6–S13). Trees based on ptDNA (Appendix S6) showed that the *C. macloviana* clade includes *C. pachystachya*, *C. gracilior*, *C. merritt-fernaldii*, and *C. harfordii*. While the combined tree including all loci showed the same clade, with *C. merritt-fernaldii* outside, the tree based on nDNA only included *C. mariposana* within the *C. macloviana* clade (Appendix S12). The combined matrix showed a clade (0.99 PP) of *C. macloviana*, which included all but one Northern Hemisphere populations. A population from Montana (USA) was nested in a clade with *C. harfordii*, *C. gracilior*, and *C. pachystachya* (0.96 PP). This latter clade appeared within a well-supported clade (0.99 PP) that included the Southern Hemisphere populations. Most populations of the Southern Hemisphere are grouped within a strongly supported clade (1.00 PP / 96% BS). Within this last clade, a group of two populations from central Chile and two belonging to southern Argentina (1.00 PP / 89% BS) appeared in a clade (1.00 PP / 83% BS) that was nested within another group of southern Argentina populations (1.00 PP / 96% BS).

Haplotype network—The ptDNA haplotype network with coded indels (Fig. 3) showed 7 sampled haplotypes and 11 not sampled or extinct haplotypes. The central haplotype (H2) corresponded to three populations of *C. macloviana* from Argentina and Chile, from which the haplotype from Montana (H3) and the remaining Northern Hemisphere haplotype (H1; 15 *C. macloviana* populations), *C. pachystachya* (H5), *C. merritt-fernaldii* (H6), and *C. gracilior* (H7) are differentiated. Haplotype 4 included the remaining nine *C. macloviana* populations from the Southern Hemisphere. The number of observations and number of distinct haplotypes encountered were set as 33 and 7, respectively, in the Dixon (2006) method. In this network, the probability that all haplotypes were observed was 90%.

The haplotype network, excluding coded indels (Appendix S14), showed five sampled haplotypes and seven not sampled or extinct haplotypes. The topology was similar to the network with coded indels, except that this network showed a single haplotype for most of the Southern Hemisphere populations and other haplotypes for Northern Hemisphere populations and three Southern Hemisphere populations. In this network, the probability that all haplotypes were observed was 98% (Dixon, 2006).

Divergence time estimation—The dated tree (Fig. 4, Table 1) showed an incongruent topology with respect to the phylogenetic tree retrieved from the Bayesian Inference and Maximum Likelihood analyses (Fig. 2); *C. harfordii*, *C. gracilior*, and *C. pachystachya* appeared inside the *C. macloviana* clade, while in the dated tree, *C. mariposana* was the only nested species. This is probably explained by the fact that the coded indels partition was not included in the BEAST analysis. The population of *C. macloviana* from Montana was differently located as sister to the remaining populations. The age of the clade that includes all the sampled species from the section *Ovales* (crown node) was estimated to be 4.40 Mya (95% HPD: 2.74–6.05 Mya), during the Pliocene. The mean of the diversification age of the *C. macloviana* complex was dated to the Pleistocene (0.62 Mya, 95% HPD: 0.29–1.00 Mya). Within this group, part of the Northern Hemisphere populations, together with a population from Argentina, constituted a recently diverged, well-supported clade (0.19 Mya, 95% HPD: 0.05–0.36 Mya), while the remaining Northern Hemisphere populations appeared together in a polytomy with two others from the Southern Hemisphere (central Chile and southern Argentina), as well as the population of *C. mariposana*. The diversification time for the remaining populations of the Southern Hemisphere was estimated as 0.27 Mya (95% HPD: 0.09–0.48 Mya). A subset of these Southern Hemisphere populations grouped in a clade dated back to 0.17 Mya (95% HPD: 0.05–0.32 Mya). This last clade was divided in two clades. The first one grouped two populations from southern Argentina (0.05 Mya, 95% HPD: 0.00–0.14 Mya). And the second one clustered the remaining populations from southern Argentina and central Chile and was dated back to 0.06 Mya (95% HPD: 0.01–0.14 Mya; Table 1).

Climatic environment—The principal component analysis (PCA) resulting from the complete data matrix (Fig. 5) showed that the first principal component (PC1) explained 51.26% of the variance observed, while 18.69% corresponded to PC2 (Appendix S15). Bioclimatic variables with high loadings for PC1 were temperature seasonality (BIO 4), temperature range (BIO 7), and minimum temperature of the warmest month (BIO 6), while maximum temperature of the coldest month (BIO 5), mean temperature of the warmest quarter (BIO 10), and mean diurnal temperature range

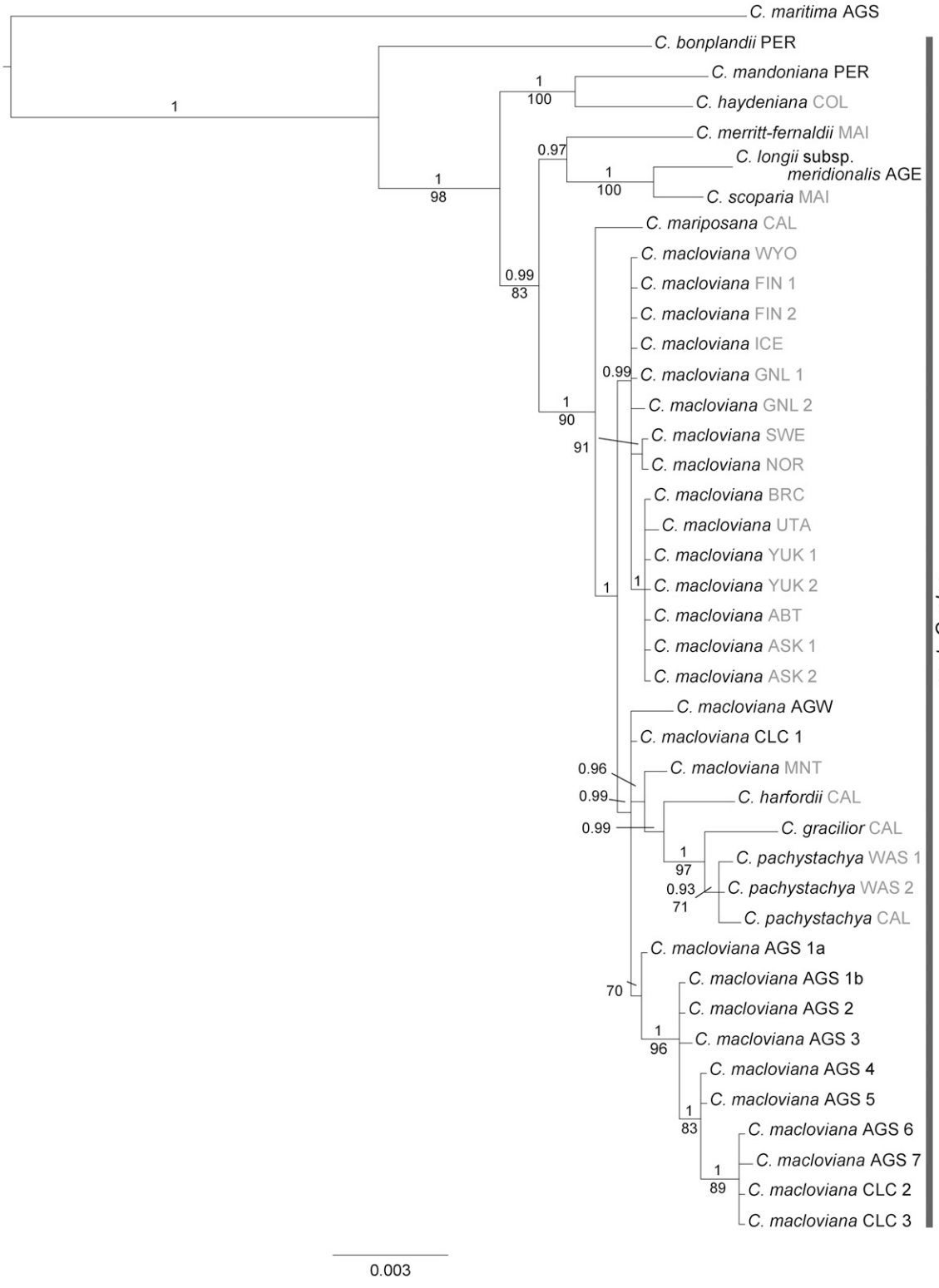

FIGURE 2 Majority rule (50%) consensus tree resulting from the Bayesian Inference analysis of the six loci (ITS, ETS, CATP, *rps*16, 5′*trn*K, and *psb*A-*trn*H) in the combined nDNA-ptDNA matrix. Numbers above the branches represent Bayesian posterior probability clade support (>0.95 PP) and, below the branches, maximum likelihood bootstrap support (>70% BS). The scale bar indicates substitutions per site. Contractions after the species represent southern populations (black) and northern populations (grey).

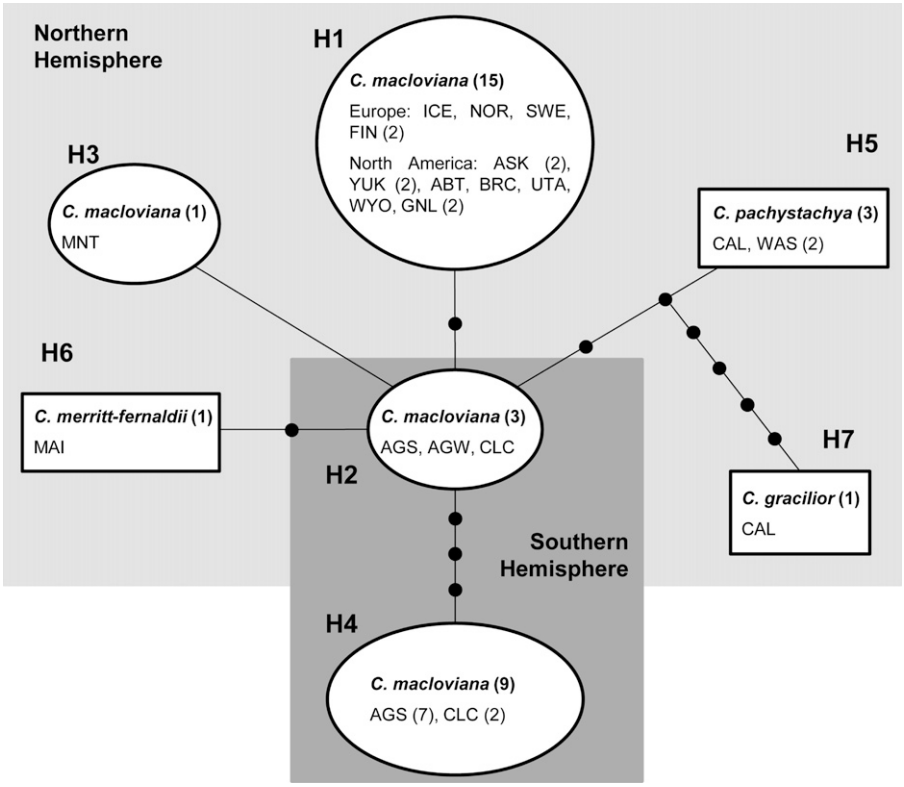

FIGURE 3 Plastid DNA haplotype network (*rps*16, 5′*trn*K, and *psb*A-*trn*H) with coded indels. Circles represent *C. macloviana* sampled haplotypes (H1–H4), squares represent ingroup sampled haplotypes (H5–H7), lines depict single mutational steps, and black circles correspond to not sampled or extinct haplotypes.

(BIO 2) were the most relevant for PC2. Some overlapping from Northern and Southern Hemisphere populations was found in the representation of PC1 against PC2 of this complete matrix, although Southern Hemisphere populations formed a partially differentiated group (Fig. 5). The boxplots of the different variables of PC1 showed narrower values for Southern vs. Northern Hemisphere populations (Table 2, Fig. 6). These results should be taken with caution because of the possibility of errors made in identifying the records taken from GBIF, but it could be useful as an approach for general ecological patterns for the species.

The results for the 'subset matrix' PCA (Appendix S16) were similar to those for the 'complete matrix', although the bioclimatic differentiation between populations from both hemispheres was more evident in the results from the former. The proportion of variation explained by PC1 was 43.13%, while 24.66% corresponded to PC2 (Appendix S17). Bioclimatic variables with higher loadings for PC1 were mean temperature of the driest quarter (BIO 9), mean temperature of the coldest quarter (BIO 11), and temperature seasonality (BIO 4). Precipitation of the driest month (BIO 14), maximum temperature of the coldest month (BIO 5), and mean temperature of the warmest quarter (BIO 10) had the highest loadings for PC2. There were two clearly distinct clusters on the scatterplot of PC1 and PC2 (Appendix S16). The boxplots of the PC1 most important variables showed clear differences between populations from both hemispheres (Appendix S18). For both 'complete matrix' and the 'subset matrix' (Appendices S5 and S15), PC2 did not show significant differences between hemispheres.

DISCUSSION

***Evolutionary history of* Carex macloviana**—Our phylogenetic analyses reveal that all the samples of *C. macloviana* form a paraphyletic group that diverged 0.62 ± 0.2 Mya (Table 1), although the species that appear inside this group differ, depending on whether the tree is inferred using matrices with coded indels or not (Figs. 2 and 4, respectively). Populations from both hemispheres had well-supported genetic differences, and in addition, there was phylogenetic structure in the Southern Hemisphere (Fig. 2). In addition to genetic differentiation, there was a certain degree of ecological differentiation between populations from both hemispheres (Figs. 5–6, Appendices S16 and S18). Thus, Southern Hemisphere populations inhabit regions with warmer temperatures and a lower seasonality. This ecological difference between the hemispheres was already found in previous studies of other bipolar species (*C. arctogena*, *C. maritima, C. canescens*; Villaverde et al., 2015a, b, 2017). The haplotype network (Fig. 3) shows an interesting pattern in the distribution of lineages, revealing the presence of an intermediate haplotype shared by three populations of the Southern Hemisphere (southern Chile, as well as southern and western Argentina) when coded indels are included. Interestingly, this haplotype network suggests that the origin of *C. macloviana* might have occurred in the Southern Hemisphere, rather than in the Northern Hemisphere, where the species distribution is larger (Egorova, 1999; Mastrogiuseppe et al., 2002; Govaerts et al., 2016). According to coalescent theory (Posada and Crandall, 2001), currently common haplotypes are likely to have more connections in networks because they represent the older haplotypes. In *C. macloviana*, we found that the most connected haplotype is placed in an intermediate position in the network (H2, Fig. 3); the haplotype belongs to three populations from the Southern Hemisphere. This Southern Hemisphere haplotype not only connects the remaining *C. macloviana* haplotypes, but also the haplotypes of its most closely related species (Fig. 3). This is a particularity of *C. macloviana* that has not been found in other bipolar species of *Carex* (Villaverde et al., 2015a, b, 2017), in which the haplotype with the greater number of connections was from the Northern Hemisphere, and it was also the one represented by a large number of populations. The fact that only three populations of the species share this ancestral haplotype might be due to the low number of populations sampled in the Southern Hemisphere vs. the Northern Hemisphere (sampling in each hemisphere is proportional to the distribution of the species; see Material and Methods). In addition, our data also suggest a stronger differentiation process among Southern Hemisphere populations than among Northern Hemisphere populations (Fig. 2), that could also be pointing to an ancient presence of this species in the Southern Hemisphere. The Northern Hemisphere populations do not reflect such strong patterns of genetic differentiation with all populations

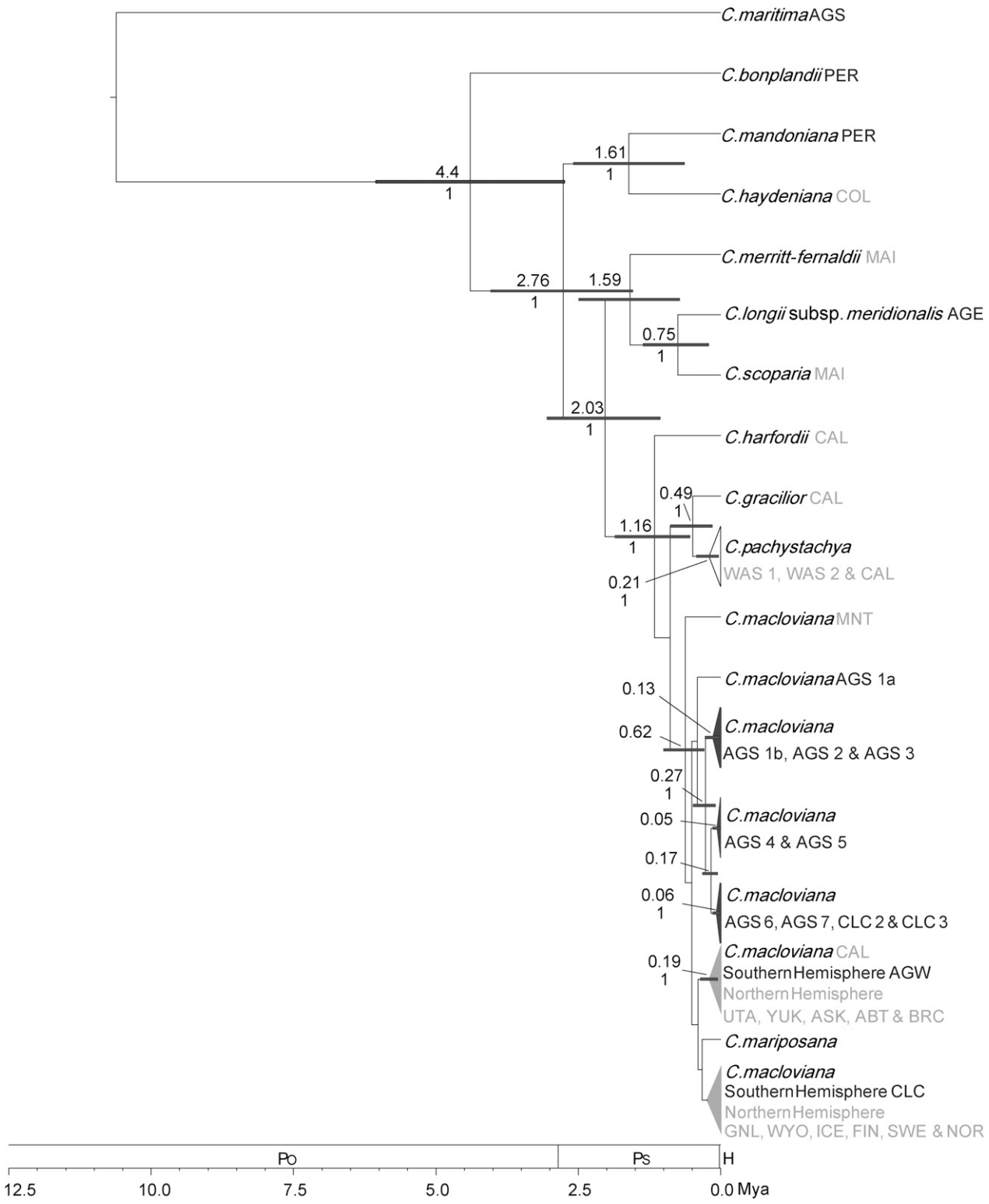

FIGURE 4 Maximum credibility clade tree of the divergence time estimation analysis with Bayesian inference using the combined nDNA-ptDNA matrix (ITS, ETS, CATP, *rps*16, 5′*trn*K, and *psb*A-*trn*H). Node ages are shown above the branches, together with the 95% highest posterior density displayed as node bars in nodes with a posterior probability (displayed below node ages) above 0.95. Contractions after the species represent southern populations (black) and northern populations (grey). Po: Pliocene, Ps: Pleistocene, H: Holocene.

TABLE 1. Divergence time estimation for *Carex* section *Ovales* with every clade posterior probability (PP), mean estimation of the millions of years ago (Mya) to the last common ancestor and 95% interval of the highest posterior density (HPD).

Clade	PP	Mean (Mya)	95% HPD interval (Mya)	
C. macloviana + *C. mariposana*	0.91	0.62	0.29	1.00
C. macloviana Argentina South and Chile Central (without AGS 1a and CLC 1)	1	0.27	0.09	0.48
C. macloviana AGS 4, AGS 5, AGS 6, AGS 7, CLC 2 and CLC 3	0.91	0.17	0.05	0.32
C. macloviana AGS 4 and AGS 5	0.92	0.05	0.00	0.14
C. macloviana AGS 6, AGS 7, CLC 2 and CLC 3	1	0.06	0.01	0.14
C. macloviana (AGW and part of Northern Hemisphere)	1	0.19	0.05	0.36

sharing the same haplotype across a comparatively much larger area (with the exception of a population from Montana, USA) that could suggest a more recent origin (Hewitt, 2000). Nevertheless, this last conclusion of south-to-north migration needs additional research because of the incongruence derived from different DNA regions (plastid vs. nuclear, Appendices S6 and S7), and additional incongruence from the inclusion or exclusion of indel information (Figs. 2 and 4).

Tackling hypothesis on the species' current distribution—As already stated, vicariance and parallel evolution hypotheses were rejected by Escudero et al. (2010). Our results confirmed the rejection of these two hypotheses. Taking into account the diversification time of the clade—including the paraphyletic *C. macloviana* (0.62 Mya, 95% HPD: 0.29–1.00 Mya) and the diversification of subgroups from the Southern and Northern Hemispheres (Table 1, Fig. 4)—we can reject a human-mediated origin of the bipolar disjunction, for which the migration from North America to South America is dated to 14.5–18.5 Kya (Dillehay et al., 2015).

The only hypothesis that cannot be rejected is long-distance dispersal. The remaining question is the type of long-distance dispersal: direct (Cruden, 1966; Muñoz et al., 2004; Nathan et al., 2008) or stepwise (Raven, 1963; Moore and Chater, 1971; Heide, 2002; Vollan et al., 2006). In a scenario of direct long-distance dispersal (Cruden, 1966; Muñoz et al., 2004; Nathan et al., 2008), we would not expect to find genetically intermediate populations between the Northern and Southern Hemispheres. In a scenario of mountain hopping (Raven, 1963; Moore and Chater, 1971; Heide, 2002; Vollan et al., 2006), we would expect to find intermediate populations that are geographically closer—unless those hypothetical intermediate populations are extinct, in which case, we could only find the remaining fossil (Popp et al., 2011). In addition, they would also be genetically more similar, and in this specific case, we would expect that populations are genetically more similar the closer they are. Our results do not allow us to discern between both hypotheses even though we have found genetic structure within both hemispheres (although genetic structuring was limited in the Northern Hemisphere). As already pointed out by Darwin (1859), the long-distance dispersal hypothesis, either by mountain hopping or direct jump, could have been more plausible during the glacial period when Arctic and Antarctic species could have had a broader distribution. The inferred times of divergence for *C. macloviana* and its main clades were during the Pleistocene, a period characterized by its cycling climate. This result appears to support Darwin's hypothesis. Thus, long-distance dispersal could have been more plausible during Pleistocene glacial periods.

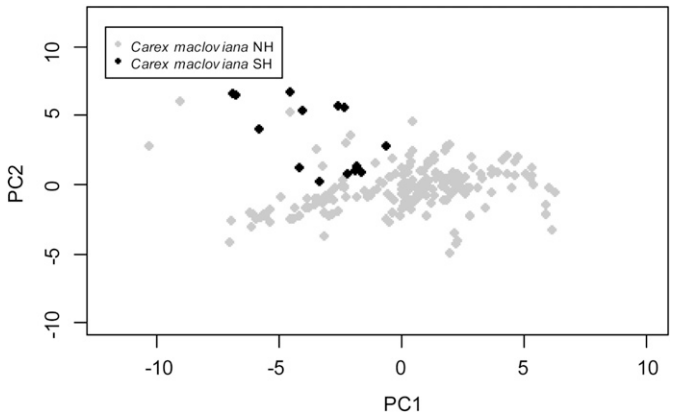

FIGURE 5 Scatter plots of principal components PC1 and PC2 based on the 'complete matrix' data set of Northern and Southern *Carex macloviana* 226 populations, representing our sampling sites and GBIF data.

In terms of dispersal direction, north-to-south long-distance dispersal was clearly inferred in previous studies of bipolar *Carex* species (Vollan et al., 2006; Escudero et al., 2010; Villaverde et al., 2015a, b, 2017), as well as in many other examples in angiosperms (Vargas et al., 1998; Vijverberg et al., 1999; Yokoyama et al., 2000; Clayton et al., 2009; Escudero et al., 2009; Schaefer et al., 2009; Wen and Ickert-Bond, 2009; Emadzade et al., 2011; Popp et al., 2011; Banasiak et al., 2013; see also Lewis et al., 2014 work on bryophytes). In our particular case, long-distance dispersal might have happened from the Southern to Northern Hemisphere (Fig. 3). This is supported by the haplotype network structure (when indels are codified) and the lower differentiation among haplotypes from the Northern Hemisphere. Although uncommon, south-to-north transhemispheric dispersal has been proven in some angiosperms, such as species of the genera *Larrea* (Zygophyllaceae, Lia et al., 2001) and *Hoggmannseggia* (Fabaceae, Simpson et al., 2005). However, the haplotype network structure of our data does not support south-to-north dispersal when indel information is not coded (Appendix S14). Without coded indels (Appendix S14), there was a single haplotype for most of the Southern Hemisphere populations, and another haplotype for Northern Hemisphere populations and three Southern Hemisphere populations. This alternative network structure with less resolution (although not incongruent with the one based on coded indels) does not suggest south-to-north dispersal. Although phylogenetic inferences based on indels are expected to be less homoplasious than those based on substitutions (Rokas and Holland, 2000), indels also have been shown to be affected by homoplasy (especially poly A and poly T; Golenberg et al., 1993). In addition, indels are inferred from the alignment, and thus sensitive to alignment errors (Ashkenazy et al., 2014). Despite having into account these issues, we have found incongruent phylogenetic topology when including or not including indels

TABLE 2. Maximum and minimum values of bioclimatic variables with higher loadings for the Principal Component 1 (PC1) resulting from the full and the subset matrices.

		C. macloviana Northern Hemisphere		*C. macloviana* Southern Hemisphere	
		Min	Max	Min	Max
Complete matrix	Temperature seasonality (SD, °C) BIO 4	3.776	15.128	2.760	4.104
	Minimum temperature of the warmest month (°C) BIO 6	−33.8	1.0	−10.4	0.0
	Temperature range (BIO 6–BIO 5; °C) BIO 7	15.7	53.1	15.0	26.7
Subset matrix	Mean temperature of the driest quarter (°C) BIO 9	−10.9	3.3	3.5	12.1
	Mean temperature of the coldest quarter (°C) BIO 11	−24.4	−2.6	−4.6	4
	Temperature seasonality (SD, °C) BIO 4	4.135	14.355	2.760	4.104

(Fig. 2 vs. Fig. 4) and alternative (but not incongruent) haplotype networks when including or not including indels (Fig. 3 vs. Appendix S14). To reach further conclusions on this issue, additional informative characters based on DNA base substitutions and indels might be helpful.

Long-distance dispersal vectors—This peculiar dispersal between extremely disjunct areas at high latitudes of both hemispheres inferred for the present data set could have happened by "wind highways", ocean currents, or bird migrations (Cruden, 1966; Muñoz et al., 2004; Nathan et al., 2008). Although *C. macloviana* has winged utricles (Mastrogiuseppe et al., 2002) that could facilitate hydrochory or anemochory, such dispersal is unlikely to have happened by means of wind or ocean currents (Wilkinson et al., 2012), principally because of the opposite current directions where the Equator separates current systems between hemispheres. Because this would affect both aforementioned kinds of vectors, this hypothesis is very unlikely, so dispersal is expected to be mediated by other vectors. Some birds are capable of migrating over thousands of kilometers, ranging six to nine days of nonstop flight (Gill et al., 2009), so the dispersal could have taken place by means of birds. The genus *Carex* has silica bodies in the achenes' pericarp (Graven et al., 1996; Prychid et al., 2003). These bodies could allow achene survival throughout the digestive tract, or could make these achenes useful as gastroliths (Alexander et al., 1996). Long-distance dispersal by endozoochory has been shown in many other angiosperms (e.g., Proctor, 1968; Costea et al., 2016). Interestingly, a recent review of dispersal by water birds reported that *Carex* seeds are frequently found in bird digestive tracts (seeds from 36 different species of *Carex* were found in digestive tracts of eight dabbling duck species in Europe), including seeds from bipolar *Carex* species (*C. canescens* and *C. magellanica*; Green et al., 2016). This fact makes dispersal by birds a plausible explanation for bipolar disjunctions. Additionally, it is also possible that the seeds have been carried in a mud crust on bird's feet (Carlquist, 1967). Experimental results using *C. bohemica* show that ectozoochory is also a very powerful mechanism for long-distance dispersal in angiosperms and specifically in *Carex* (Hohensee and Frey, 2001); it could also explain the observed bipolar disjunction.

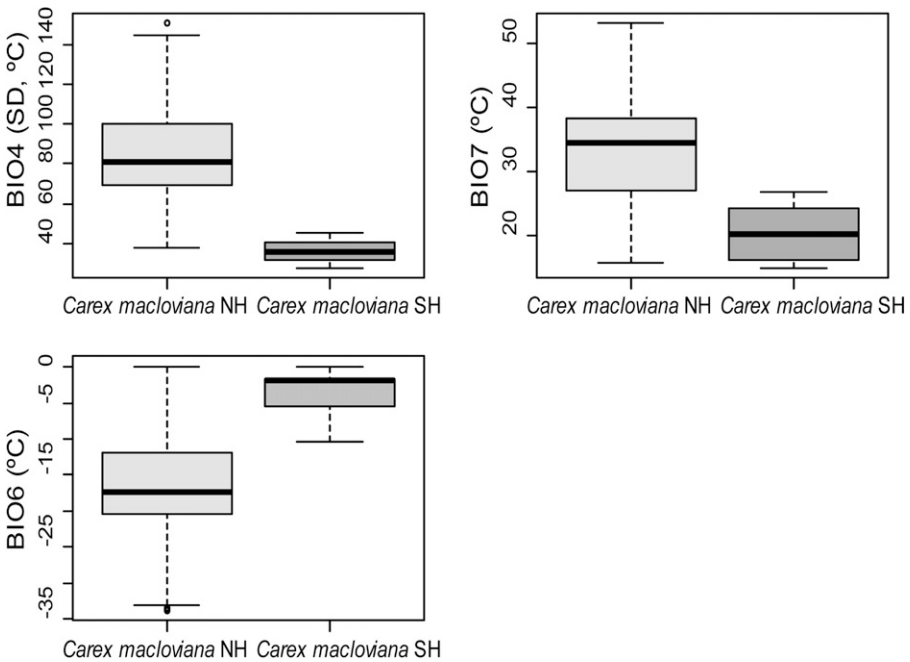

FIGURE 6 Boxplots of the bioclimatic variables with the highest loadings from the PC1 of the 'complete matrix' data set of the Northern and Southern *Carex macloviana* populations. All temperatures are in degrees Celsius, °C. (A) Temperature seasonality (standard deviation of monthly temperature), (B) minimum temperature of the warmest month, and (C) temperature range.

CONCLUSIONS

This study reveals that the paraphyletic species *Carex macloviana* is dated back to the Pleistocene. It includes two lineages in the Southern Hemisphere differentiated from those in the Northern Hemisphere, where we could not detect any genetic difference among populations, with the exception of a population from Montana. The genetic structure across the species range matches with a long-distance dispersal between hemispheres. It remains uncertain whether the dispersal was due to mountain hopping or one or more direct dispersal events. Also, populations from the Southern Hemisphere show a certain ecological differentiation from their northern counterparts. Specifically, they show a narrower range of ecological regimes from the northern populations in bioclimatic variables such as annual mean temperature or minimum temperature of the warmest month, probably because of the differences in overall area extension and because of the presence of a wider range of possible niches.

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