

RESEARCH ARTICLE



Are Cenozoic relict species also climatic relicts? Insights from the macroecological evolution of the giant sedges of *Carex* sect. *Rhynchocystis* (Cyperaceae)

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Abstract

Premise: Most of the Paleotropical flora widely distributed in the Western Palearctic became extinct during the Mio-Pliocene as a result of global geoclimatic changes. A few elements from this Cenozoic flora are believed to remain as relicts in Macaronesia, forming part of the laurel forests. Although the origins of the present species assembly are known to be heterogeneous, it is unclear whether some species should be considered climatic relicts with conserved niches. An ideal group for studying such relict characteristics is a Miocene lineage of *Carex* sect. *Rhynchocystis* (Cyperaceae), which comprises four species distributed in mainland Palearctic and Macaronesia.

Methods: We reconstructed the current and past environmental spaces for extant mainland and Macaronesian species, as well as for Pliocene fossils. We also studied the bioclimatic niche evolution. Species distribution modeling and ensemble small modeling were performed to assess the potential distribution over time.

Results: Climatic niche analyses and distribution modeling revealed that the ecological requirements of Macaronesian species did not overlap with those of either mainland species or with the Pliocene fossils. Conversely, the niches of mainland species displayed significant similarity and equivalence.

Conclusions: Macaronesian species are not climatic relicts from the Paleotropical flora, but instead seem to have changed the ecological niche of their ancestors. By contrast, despite their ancient divergence (Late Miocene), mainland *C. pendula* and *C. agastachys* show conserved niches, with competitive exclusion likely shaping their mostly allopatric ranges.

KEYWORDS

fossil records, laurisilva, niche conservatism, niche shift, null model, projection

The distribution patterns of species are strongly influenced by geoclimatic changes (Taberlet et al., 1998; Valiente-Banuet et al., 2006; Koecke et al., 2013). The responses of species to these changes include adaptation (Evans et al., 2009), migration (Wen and Ickert-Bond, 2009), and extinction (Robles et al., 2013). In particular, the latter two processes have been suggested as the principal drivers of current species distribution in the Western Palearctic (WP;

Fernández-Palacios et al., 2011). Some taxa in this region have been postulated to originate from the Paleotropical flora that occupied middle and low latitudes of Eurasia from the Late Cretaceous–Early Paleocene to the Late Miocene (65–5.3 million years ago [mya]), which was dominated by evergreen and lauroid forests (Mai, 1991; Barrón, 2003; Kovar-Eder et al., 2006). This flora was strongly affected by climatic oscillations that occurred from the Early Oligocene

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to the present (e.g., Rodríguez-Sánchez et al., 2010; Valtueña et al., 2012; Chen et al., 2014), forcing its gradual disappearance. This began because of climatic cooling and continentalization (Mai, 1989), continued with the increase in aridity during the Messinian salinity crisis (Late Miocene, 5.96–5.33 mya), and culminated with the onset of the Mediterranean climate during the Pliocene (3.4–2.8 mya; Krijgsman et al., 1999). Thus, the progressively harsher conditions resulted in the disappearance of most of the Paleotropical vegetation from the WP before the beginning of the Pleistocene glaciation cycles (Mai, 1989; Milne and Abbott, 2002; Kovar-Eder et al., 2006). Nonetheless, some Paleotropical elements were able to survive by retreating to refugia in southeastern Asia (Ye et al., 2016; Chen and Lou, 2019) and southwestern Europe (Valcárcel et al., 2017; Vitelli et al., 2017; Benítez-Benítez et al., 2018), including locations in the Mediterranean and Black Sea basins (Rodríguez-Sánchez and Arroyo, 2008; Médail and Diadema, 2009; Miguez et al., 2017) and the Macaronesian archipelagos (Valiente-Banuet et al., 2006; Alarcón et al., 2013; Chen et al., 2014).

Macaronesian laurel forests (MLFs, commonly known as laurisilva) constitute an emblematic vegetation unit of high conservation and biogeographic value (Vanderpoorten et al., 2007) restricted to Madeira, the Azores, and the Canary Islands—three of the five Atlantic Ocean archipelagos that form Macaronesia (Fernández-Palacios and Dias, 2001). These archipelagos, located off the coast of the Iberian Peninsula and North Africa, are characterized by a wet and temperate climate with a strong oceanic influence (Salas-Pascual and Naranjo-Cigala, 2015), which has been related to their role as climatic refugia (Carine et al., 2010; Mairal et al., 2017). MLFs have traditionally been considered a remnant of the Paleotropical flora (Takhtajan, 1969; Bramwell, 1972; Cronk, 1992) because they harbor species whose fossils have been found only in the WP (e.g., *Laurus*; Arroyo-García et al., 2001; Rodríguez-Sánchez and Arroyo, 2008). This idea is reinforced by the fact that the dominant species in these forests commonly have laurophyllous leaves (entire, elongated, and evergreen; Fernández-Palacios, 2009), which are morphologically similar to those of the Paleotropical flora. Specifically, some Macaronesian lineages have been reported to be older than the disappearance of the Paleotropical flora from the mainland (Late Pliocene–Early Pleistocene), and thus are considered relict taxa—for example, *Picconia azorica* (Tutin) Knobl. (Ferreira et al., 2011), *Davallia canariensis* (L.) Sm. (Liu and Schneider, 2013), *Visnea mocanera* L.f., and *Geranium* L. (Schüßler, 2020). By contrast, Kondrakov et al. (2015) proposed that MLFs could be a heterogeneous assembly composed of species of more recent origin, resulting from migration and colonization from different source areas during the Pleistocene (Vargas, 2007; Schüßler et al., 2019).

While the relative influence on MLF flora of migration and extinction in response to climatic changes has been well documented (Chanderbali et al., 2001; Rodríguez-Sánchez

et al., 2009; Fernández-Palacios et al., 2011, 2017; Schüßler et al., 2019), the role of ecological adaptation has remained underexplored in the Azores and Madeira compared with the Canary Islands (e.g., García-Maroto et al., 2009; Curto et al., 2018; Ganivet et al., 2019; Schüßler et al., 2019). Species inhabiting laurisilva forests could have changed their ancestral environmental requirements (niche shift; Ackerly, 2003) to adapt to the current Macaronesian climate, or have retained the niche of their putative Paleotropical flora ancestors (niche conservatism; Wiens and Graham, 2005; Wiens et al., 2010), thus remaining as climatic relicts (e.g., Pulido et al., 2008; Hampe and Jump, 2011). The development of ecological niche modeling approaches has promoted the study of ecological responses of species (i.e., adaptation vs. niche conservation) by relating environmental requirements to distribution range. Species distribution modeling (SDM; Guisan and Thuiller, 2005) and related methods have provided insights into the ecological evolution of species (Rodríguez-Sánchez and Arroyo, 2008). Thus, many studies have demonstrated the relative overlap of environmental niches for related species (Warren et al., 2008; Edwards and Donoghue, 2013), revealing a relationship between ecological requirements and lineage diversification (e.g., Ahmadzadeh et al., 2013; López-Alvarez et al., 2015; Benítez-Benítez et al., 2018; Maia-Carvalho et al., 2018).

One ideal group with which to study the ecological evolution of allegedly Paleotropical relict elements is *Carex* section *Rhynchocystis* Dumort. (Cyperaceae), a small and well-supported monophyletic group formed by seven species distributed in the WP and sub-Saharan Africa (Miguez et al., 2018, 2021). It includes a clade (lineage B) dating back to the Late Miocene (~11.6 mya; 95% highest posterior density [HPD] 16.0–6.15 mya; Miguez et al., 2021) and is composed of four species (*C. agastachys* L.f.; *C. leviosa* Miguez, Jim.-Mejías, H. Schaeff. and Martín-Bravo; *C. pendula* Huds.; and *C. sequeirae* Miguez, Jim.-Mejías, Benítez-Benítez and Martín-Bravo). All these species had traditionally been subsumed under a broad *C. pendula* concept until the recent recognition of three cryptic species based on morphological and molecular phylogenetic data (Miguez et al., 2018, 2021). *Carex pendula* and *C. agastachys* grow in riparian forests and habitats in Europe, southwestern Asia, and North Africa, with mostly allopatric ranges (Figure 1), though with a narrow contact zone across central and southeastern Europe (e.g., Miguez et al., 2018; Meierott, 2019; Dimopoulos et al., 2020; Sutorý and Řepka, 2020; Martín-Bravo et al., in press). The other two species, which constitute a monophyletic group with *C. pendula* (referred to here as *C. pendula* clade; Miguez et al., 2021), are endemic to two Macaronesian archipelagos (*C. sequeirae* from Madeira and *C. leviosa* from Azores) where the plants inhabit laurisilva forests (Figure 1). These Macaronesian endemics have been related to independent colonization of each archipelago following two different long-distance dispersal events from the WP dated back to the Plio-Pleistocene (~2.22 mya; 95% HPD 0.39–4.75 mya;

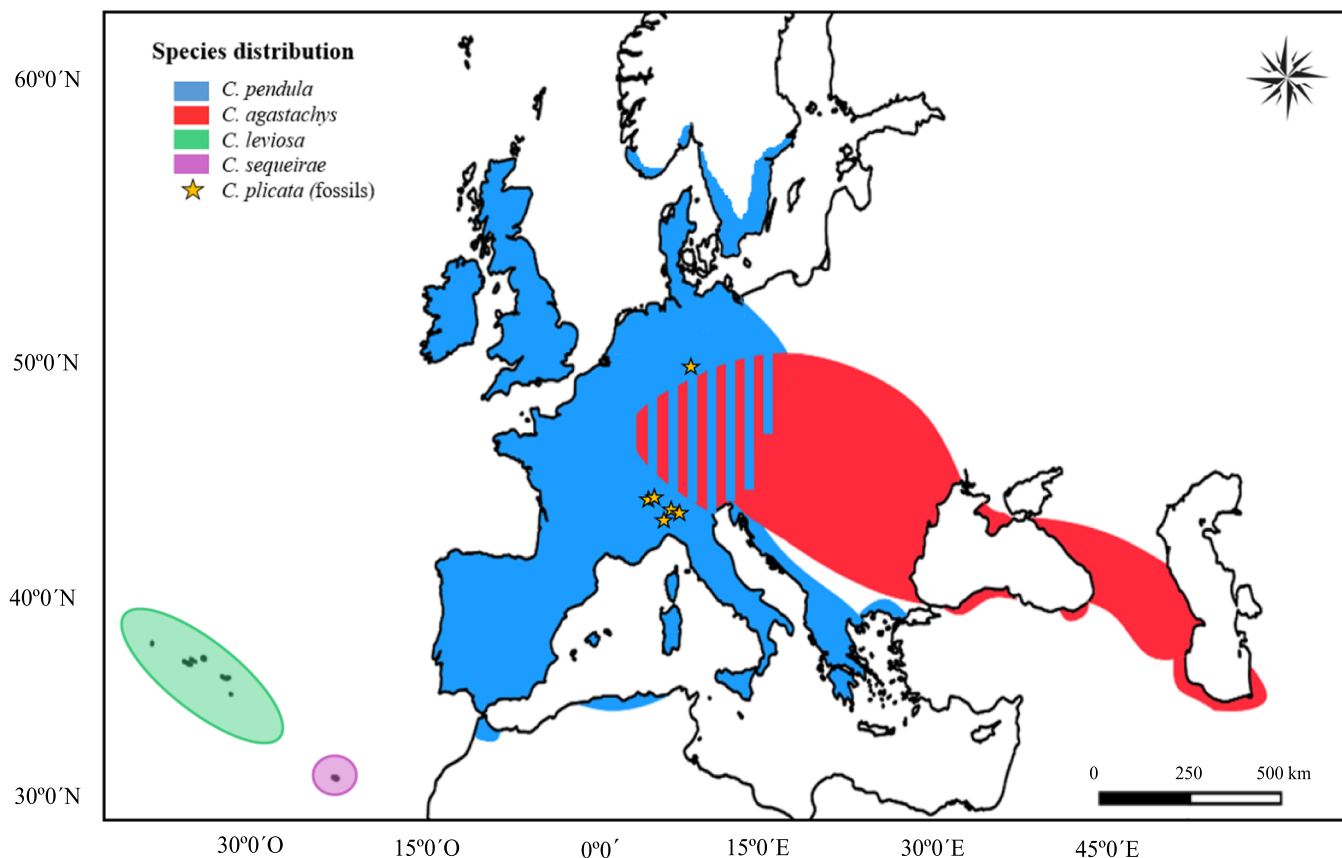


FIGURE 1 Approximate distribution map of lineage B of *Carex* sect. *Rhynchocystis* (*C. pendula*, *C. agastachys*, and Macaronesian species *C. leviosa* and *C. sequeirae*). Occurrences of Pliocene fossil species *C. plicata* are denoted by stars

Miguez et al., 2021). All the species belonging to sect. *Rhynchocystis* have very broad non-coriaceous leaves (often > 1 cm width; Miguez et al., 2018), resembling those of other laurisilva-dwelling *Carex* species such as *C. hochstetteriana* Seub. (Azores), *C. lowei* Bech. (Madeira), and *C. perraudieriana* J.Gay (Canary Islands). Furthermore, the sect. *Rhynchocystis* has a relatively rich and carefully assessed fossil record (Jiménez-Mejías et al., 2016) to which the fossil species *C. limosioides* Negru (Early Miocene–Late Miocene) and *C. plicata* Lañc.-Środ. (Early Miocene–Late Pliocene), both known from central and eastern Europe, have been assigned. Given the old divergence times inferred for *C. pendula* and *C. agastachys* and the strong morphological affinities between the achene of *C. plicata* and that of *C. pendula* (Jiménez-Mejías et al., 2016)—one of the main diagnostic traits distinguishing extant mainland species (Miguez et al., 2018)—it suggests that *C. plicata* is part of the stem group of the *C. pendula* clade. The main aim of this study is to evaluate whether lineage B of sect. *Rhynchocystis*, especially the Macaronesian species, are climatic relict elements retaining the niche of ancestors that putatively formed part of the Paleotropical flora. We selected this group for three reasons: (1) its origin has been dated back to the Late Miocene, with a reliable fossil record from the Early Miocene to the present that indicates a

long-term presence in the WP predating the extinction of the Paleotropical flora; (2) Macaronesian species have a divergence dated to the Plio-Pleistocene, which places its origin when the Paleotropical vegetation in WP had been almost wiped out; and (3) mainland and Macaronesian species are present in apparently similar habitats, which allows for the comparison of niches between both land-masses. According to the principle of phylogenetic niche conservatism (PNC; Wiens and Graham, 2005; Losos, 2008), we would expect that closely related species like those comprising the *C. pendula* clade would retain bioclimatic requirements similar to those of each other and their ancestors (Pliocene *C. plicata*), rather than to those of *C. agastachys*. To address these questions, we will explore the evolution of the bioclimatic niche and distribution range over time within lineage B of sect. *Rhynchocystis*.

MATERIALS AND METHODS

Species distribution data

The occurrences of the extant species were collected from GBIF (GBIF.org, 2019), and revised and complemented with bibliographic references (Schäfer, 2003; Miguez et al., 2018, 2021)

and herbarium specimens from BM, E, MA, MW, P, UPOS, and WU (acronyms following Thiers, 2016; Appendix S1). A fieldwork trip to Madeira in 2018 was undertaken by C.B.-B. and M.M. to locate *C. sequeirae* populations, which are extremely rare. We removed unreliable records that had incorrect georeferencing (e.g., populations outside the known distribution range or points in the sea), and also occurrences before 1960. We also cleaned the resulting database by removing duplicates. Given that until very recently the name *C. pendula* has been used to refer to both *C. pendula* and *C. agastachys* (see Miguez et al., 2018), we reassigned points to *C. agastachys* according to their geographic placement. Because both taxa are mostly allopatric, a large number of occurrences were easily reassigned to *C. agastachys*. However, *C. pendula* and *C. agastachys* display an overlapping distribution in central and southeastern Europe (Figure 1). Thus, whenever we could not confirm the identity of specimens, points located in the contact zone were removed. Because of the large number of occurrences obtained for mainland *C. pendula*, we randomly reduced the number of data points, retaining only those that were not duplicated, using an accuracy of two decimal points. The final data set for the studied extant groups was composed of 4859 occurrences for *C. pendula* and 69 for *C. agastachys*. Macaronesian species were represented by 558 localities, including 553 for *C. leviosa* from the Azores archipelago, and only five for *C. sequeirae* from Madeira, including all the known existing populations (for more details, see Miguez et al., 2021). The fossil ancestor data set of the *C. pendula* clade was composed of six Pliocene records identified as *C. plicata* that remained after discarding *C. limosioides* records dating from the Miocene (Jiménez-Mejías et al., 2016), a period for which environmental layers are not available.

Environmental data analyses

We retrieved 19 bioclimatic variables from WorldClim (<https://www.worldclim.org/version1>; Hijmans et al., 2005) for current conditions (spatial resolution 2.5 min) and projections to the Late Quaternary period: Mid-Holocene (~6 ka), Last Glacial Maximum (LGM, ~21 ka; spatial resolution 0.5 min), and Last Interglacial (LIG, about 120–140 ka; 30 arc-seconds resolution). In addition, 19 variables for projections to the Middle Pliocene (3 mya) were downloaded from ecoClimate (spatial resolution 0.5 min; <http://ecoclimate.org/>; Lima-Ribeiro et al., 2015). Following the method used by Benítez-Benítez et al. (2018) to avoid using highly correlated variables, we computed a correlation matrix and transformed it into a matrix of distances represented as a dendrogram using the R package stats (R Core Team, 2020). We subsequently selected one variable per clade when the branch longitude was <0.5. We also applied the variance inflation factor (VIF), an estimation of the multicollinearity effect on model variance, using the package HH (Heiberger, 2017) in R; variables with

VIF > 5 were excluded. Finally, we selected the five bioclimatic variables that contributed most to our models: three representing temperature data (bio1: annual mean temperature [AMT]; bio7: temperature annual range [TAR]; bio8: mean temperature of wettest quarter [MTWQ]) and two representing precipitation (bio12: annual precipitation [AP]; and bio15: precipitation seasonality [PS]).

We conducted a principal component analysis (PCA; Janžekovič and Novak, 2012) of the retained variables using the package ggplot2 (Wickham, 2016) and the “prcomp” function in R to visualize the ecological niche occupied by extant populations (*C. pendula*, *C. agastachys*, and Macaronesian species) and Pliocene fossil records. We also used the “NiceOverPlot” function (Fernández-López and Villa-Machío, 2017) implemented in the package ecospat (Di Cola et al., 2017) for pairwise comparisons of bioclimatic variables implicated in niche overlapping. Since *C. plicata* is the putative ancestor of the *C. pendula* clade, we did not compare the fossils with *C. agastachys*. Hence, the following groups were analyzed: (1) *C. pendula* vs. *C. agastachys*; (2) both mainland species vs. Macaronesian ones; (3) *C. pendula* vs. *C. plicata*; and (4) Macaronesian species vs. *C. plicata*. Further, we implemented density plots using the “sm.density.compare” function in R to compare the frequency distribution per each bioclimatic variable using response curves. We also measured the niche overlap calculating Schoener's *D* index (Schoener, 1968), as well as niche similarity and equivalency tests (Warren et al., 2008; Broennimann et al., 2012) implemented in the package ecospat (Di Cola et al., 2017). These pairwise comparisons were performed only for extant species because the environmental space (e-space) of fossils was likely incompletely recovered as a result of their scarce sampling (see above). Furthermore, the non-analogous climates of the present and the Pliocene would not allow us to determine whether our results indicated a niche shift or, by contrast, an ecological conservatism as a consequence of an expansion toward a preadapted niche not available during the Pliocene (Petitpierre et al., 2012). Therefore, the pairwise statistical comparisons were (1) *C. pendula* vs. *C. agastachys*; (2) *C. pendula* vs. Macaronesian species; (3) *C. agastachys* vs. Macaronesian species; and (4) *C. leviosa* vs. *C. sequeirae*. Because we are studying closely related taxa, we tested the PNC hypothesis (that niche overlap is more similar than expected by chance) for each pairwise comparison, and we used the alternative “greater” in the “ecospat.niche.similarity.test” function to test whether the niche has been preserved. When there was no reference niche, for example with *C. pendula* vs. *C. agastachys* and *C. agastachys* vs. Macaronesian species, we assumed that both niches could be simultaneously shifted between the two areas (rand.type = 1). However, because the Macaronesian species are derived from *C. pendula* (Miguez et al., 2021), we assigned the niche of the latter species as reference (rand.type = 2).

We retained the same five uncorrelated variables for the present in order to reconstruct ancestral bioclimatic requirements over the lineage B phylogeny (modified from

Miguez et al., 2021). For this estimation, we used the mean value for each variable with all occurrences per species and the ultrametric dated tree. The dataset including the mean value per variable for extant species was used to construct a PCA. We used the values per species obtained for the first principal component (PC1) as a proxy of its climatic niche because this axis explained a high percentage of the total variance of the climatic data (>70%; see below) in this group, as shown in Albaladejo et al. (2020). Finally, we assessed the maximum likelihood (ML) of ancestral state reconstruction under two models of continuous trait evolution (the random drift of Brownian motion [BM] and the constant stabilizing selection of the Ornstein-Uhlenbeck [OU] process) in the R package phytools (Revell, 2012). Both models were compared using the Akaike information criterion (AIC) as implemented in the package geiger (Harmon et al., 2008). The best model of evolution was selected for ML ancestral reconstruction of climatic values along the phylogeny (including lineage B species) using the “anc.ML” function, also implemented in phytools.

Ecological niche modeling

SDM was used to evaluate the potential distribution of our set of extant species by projecting the suitable bioclimatic conditions toward (1) the current time and (2) the Late Quaternary (including Holocene, LGM, and LIG). For present and past projections, we considered the two sets of mainland populations (*C. pendula* and *C. agastachys*) separately, and the Macaronesian species as a single group. We took two different approaches to the same method, using four different algorithms: MaxEnt (Phillips et al., 2006), generalized linear models (GLM), generalized additive models (GAM), and random forest (RF). In the first approach, we calculated the contribution of the environmental variables individually for each algorithm and retained the most explanatory variables for projections at different time periods (see above). Thereafter, we ran an ensemble model that included all algorithms with the “calc” function in the package raster (Hijmans, 2020) to reduce the variance of the predictions and create a more accurate projection than with single models (Araújo and New, 2007; Forester et al., 2013). We evaluated all models by comparing the area under the curve (AUC; Swets, 1988), retaining those with the highest AUC scores. As an alternative, we performed a second approach applying SDM using the package biomod2 (Thuiller et al., 2009), to calculate directly the assembly of all algorithms. The relative importance of bioclimatic variables was calculated by default. We built three sets of 10,000 pseudo-absences randomly selected from the complete area, and generated a data split (80% training data and 20% test data) to assess the models in turn by cross-validation, each with four independent runs. We also used AUC as an evaluation metric for these models with a threshold >0.7 (Guisan et al., 2017). More methodological information about the SDM approaches is described in Appendix S2.

Carex plicata projections to the Pliocene (fossil records) were performed using ensemble small modeling (ESM; Breiner et al., 2015, 2018), a novel and powerful predictive approach to reduce overfitting for modeling with a small number of occurrences for comparison with standard SDM results (results not shown). We implemented ESM in biomod2 (Thuiller et al., 2009) using the same modeling algorithms as SDM (see above). For each algorithm, simple models were generated with a subset of two bioclimatic variables (i.e., bivariate models) for all possible combinations within our five selected variables. Each bivariate model was evaluated with Somers’ *D* index (Newson, 2006). Models with Somers’ $D \geq 0$ ($AUC \geq 0.5$) were retained and used to build the assembly projection as described in Breiner et al. (2015). We used the same set of parameters to evaluate ESM and SDM (80% training data and 20% test data), but ESM was implemented in the R package ecospat (Broennimann et al., 2017; Di Cola et al., 2017). We implemented the Mann-Whitney-Wilcoxon nonparametric test (Nachar, 2008) in R to assess whether there was a significant difference in the mean AUC values obtained using these both modeling strategies (SDM vs. ESM) for the fossil data set. Previously, we had also used the Fligner-Killeen test to explore homoscedasticity (Donnelly and Kramer, 1999) because equal variance between groups is a requirement for applying the Mann-Whitney-Wilcoxon test.

The use of AUC in data sets with low numbers of occurrences has been controversial because of the inclusion of many pseudo-absences environmentally distant from the species’ presence, which can trigger higher AUC values (Lobo et al., 2008; Jiménez-Valverde, 2012). For this reason, we implemented the assessment method using null models (with the same algorithms mentioned above) carried out by Collart et al. (2021) in order to explore the robustness of the ESM. We used the average suitability values for each fossil presence ($n=6$) combined with the pseudo-absences to compute AUC values (100 replicates) implemented in the packages biomod2 (Thuiller et al., 2009), ecospat (Broennimann et al., 2017; Di Cola et al., 2017), and dismo (Hijmans et al., 2017). However, we did not achieve the minimum requirement sampling number; therefore, the use of our data set with few occurrences should be handled with caution.

RESULTS

Environmental niche evolution

We obtained PCA plots that included the most important bioclimatic variables and pairwise comparisons of e-space (Figure 2), as well as the response curves of each group per variable (Figure 3A). The plot with the complete data set (Figure 2A) revealed that the e-space occupied by extant species, despite being much wider, did not overlap with that of *C. plicata* fossils; the latter primarily occupied an e-space with lower values for temperature variables (AMT, MTWQ,

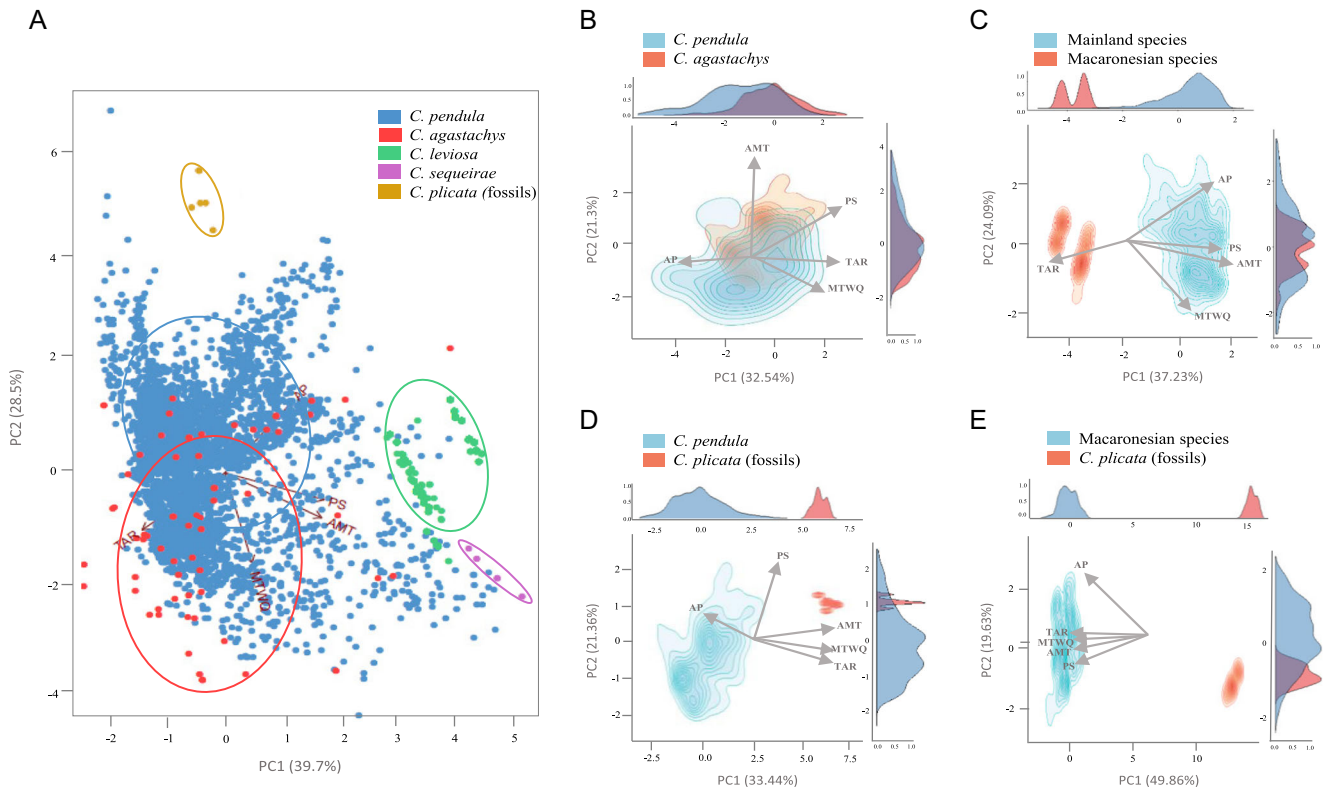


FIGURE 2 Representation of the e-space of extant *Carex* species (*C. pendula*, *C. agastachys*, *C. leviosa*, and *C. sequeirae*) and Pliocene fossils (*C. plicata*). Each plot displays values for five bioclimatic variables (see main text for more information) as retrieved by principal component analysis (PCA) and their contributions in both axes. Arrows indicate the bioclimatic variables' contribution to the PCAs. The centroids represent the overall mean of the points of each plotted group. Comparisons of (A) extant species and Pliocene fossils; (B) *C. pendula* vs. *C. agastachys*; (C) Macaronesian species (*C. leviosa*–*C. sequeirae*) vs. mainland species (*C. pendula*–*C. agastachys*); (D) *C. pendula* vs. fossils; and (E) Macaronesian species vs. fossils. Density plots represent whether the distribution of variables is overlapping or non-overlapping for the different pairwise groups in each axis of the PCA. AMT, annual mean temperature; TAR, temperature annual range; MTWQ, mean temperature of wettest quarter; AP, annual precipitation; PS, precipitation seasonality

and TAR; Figure 3A) than for precipitation variables (Figure 3A). In addition, the ancestor of the *C. pendula* clade showed more ecological affinity with *C. pendula* than with Macaronesian species (Figure 2A, D, E). However, given the limited number of fossils studied, we cannot entirely rule out that the ancestral e-space of the *C. pendula* clade was actually larger and overlapped that of present *C. pendula*. For the extant species, there was a large e-space overlap between the mainland species (*C. pendula* and *C. agastachys*; Figures 2A, B and 3A). Macaronesian species were placed peripheral to the mainland ones (Figure 2C) or marginally overlapping with *C. pendula* (when the fossil records were also introduced to the e-space; Figure 2A), with some bioclimatic variables (AMT and TAR; Figure 3A) slightly differentiated with respect to the mainland species, and others definitely overlapping (MTWQ and PS; Figure 3A). When comparisons were performed between the Macaronesian and the mainland species (Figure 2C), the e-space of the two groups did not overlap along PC1, mainly because of differences in some temperature variables (AMT and TAR; Figure 3A). Interestingly, Macaronesian species displayed small differences in their ecological requirements between archipelagos (Azores and Madeira; Figure 2A).

Further, *C. leviosa* populations were arranged in three non-overlapping clusters (see Appendix S3), each corresponding to a different set of Azorean islands: (1) the western island of Flores; (2) the central islands of Faial, São Jorge, Pico, and Terceira; and (3) Santa Maria and São Miguel, the easternmost islands of the archipelago.

The results from Schoener's *D* index and niche similarity-equivalency tests are reported in Table 1. The largest niche overlap occurred between the two mainland species *C. pendula* and *C. agastachys* ($D = 0.642$), which also showed significantly more similar and equivalent niches than expected by chance ($p < 0.05$). On the other side, Macaronesian and mainland species displayed very little niche overlap ($D = 0.087$ with *C. pendula*; $D = 0.003$ with *C. agastachys*). In these latter pairwise comparisons, the niche similarity test did not show higher similarities than expected by chance. Lastly, there was no niche overlap ($D = 0$) or similarity of niches among the species of both Macaronesian archipelagos.

The PCA performed for the extant lineage B displayed a clear trend toward higher weights of temperature and precipitation variables (except for TAR) along PC1, which explained 71.5% of the total variance (results not shown).

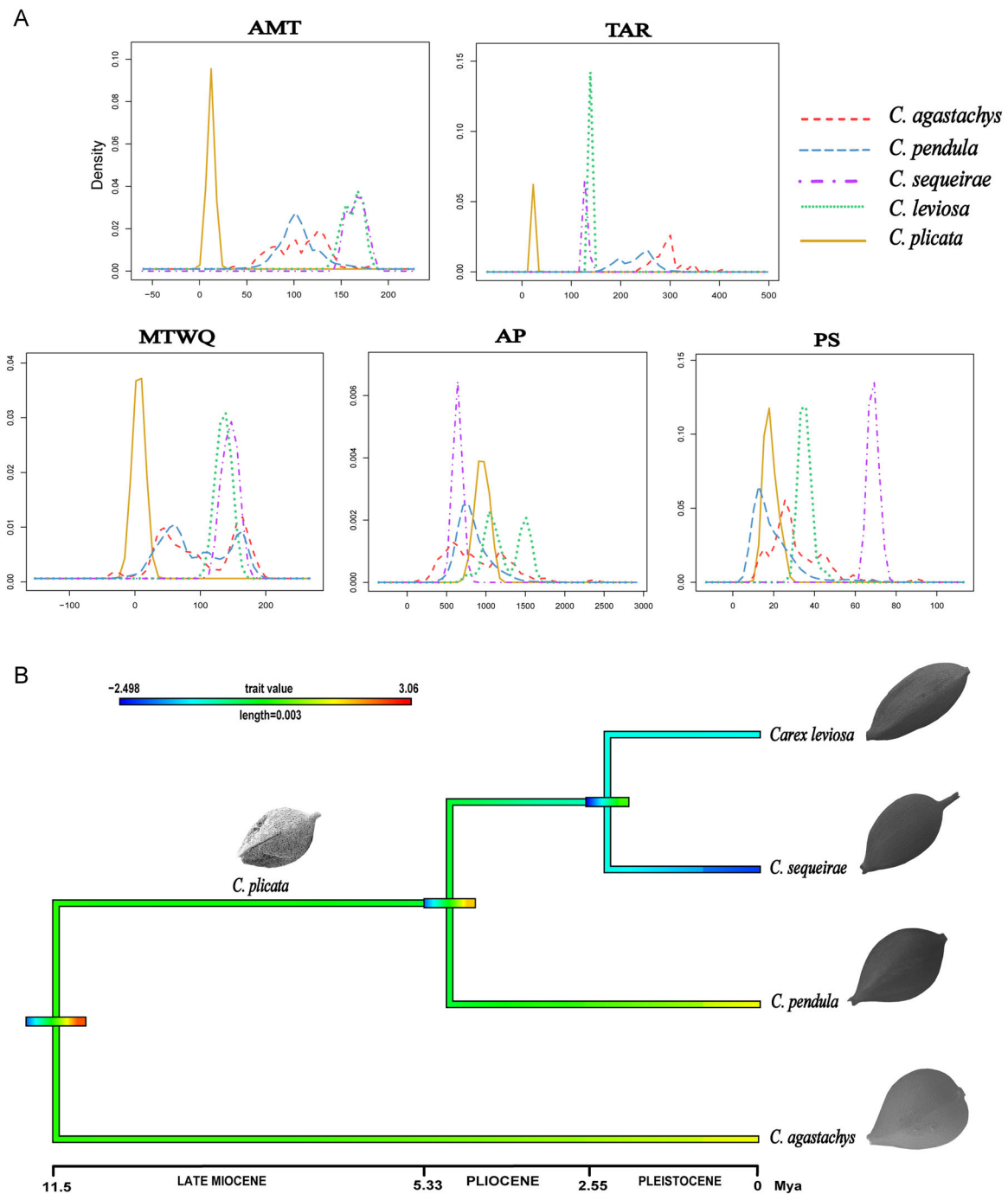


FIGURE 3 (A) Comparison of response curves of the five selected bioclimatic variables for the extant species *Carex pendula*, *C. agastachys*, *C. leviosa*, and *C. sequeirae* and for *C. plicata* fossils. (B) Ancestral climatic niche reconstruction for lineage B of *Carex* sect. *Rhynchocystis*, according to the PC1 values of the PCA for five continuous bioclimatic variables. AMT, annual mean temperature; TAR, temperature annual range; MTWQ, mean temperature of wettest quarter; AP, annual precipitation; PS, precipitation seasonality. The dated tree used was obtained with BEAST (Miguez et al., 2021). The maximum likelihood reconstruction is represented as gradational colors along the branches (warmer and drier conditions in red, colder and wetter in blue, and intermediate values in green). The lengths of the horizontal bars at the nodes along the phylogeny represent the 95% confidence intervals of the ancestral niche estimates (error range)

According to AIC values, the best evolutionary model for ancestral bioclimatic reconstruction over the phylogeny was the Ornstein-Uhlenbeck model (OU = -8.38, BM = -10.35; Figure 3B). The bioclimatic niche evolution along the phylogeny (Figure 3B) displayed medium-high PC1 values

for mainland species in contrast to the extreme low values for Macaronesian species. Specifically, *C. sequeirae* occupied the extreme of variation (value = -2.494), although with slight changes in relation to its sister species from Azores (*C. leviosa*).

TABLE 1 Pairwise statistical tests for comparison of ecological niche overlap between mainland and Macaronesian extant species

Hypothesis: "The niche overlap is more similar/equivalent than at random" "rand-type = 1"	Number of populations (<i>n</i>)	Schoener's <i>D</i>	Niche similarity (1→2) <i>p</i>	Niche similarity (2→1) <i>p</i>	Niche equivalency <i>p</i>
Comparisons among groups					
<i>Carex pendula</i> vs. <i>C. agastachys</i>	4869/69	0.642	0.0099*	0.0099*	0.0099*
Macaronesian species vs. <i>C. agastachys</i>	558/69	0.003	0.1287	0.1188	–
Azores vs. Madeira	553/5	0	1	1	–
"rand-type = 2"	Number of populations (<i>n</i>)	Schoener's <i>D</i>	Niche similarity <i>p</i>		Niche equivalency <i>p</i>
Macaronesian species vs. <i>C. pendula</i>	558/4869	0.087	0.069		–

Note: Significant differences ($p < 0.05$) are marked with an asterisk.

Species distribution modeling

Although we consider that the first methodological approach more accurately explores the relative weight per variable within each algorithm to select the fittest models, our results did not differ with either of the two approaches (see above). Accordingly, we selected the Biomod approach (Figure 4; Appendix S4) because it involved less computational effort and retained higher (though not remarkably differentiated) AUC scores than the first. Thus, we decided to rely on this method for the subsequent explanation of the results, but SDM results from all time periods using the first methodological approach are also shown in Appendix S5.

With respect to fossil modeling, we recorded higher AUC values using ESM than with SDM for each algorithm, except for MaxEnt (Appendix S6). When we studied the homoscedasticity of the mean AUC value for each algorithm per modeling strategy, we did not detect significant evidence against the equality of the variance ($p = 0.09$), and for this reason we performed Mann-Whitney-Wilcoxon testing. A marginally significant difference between the two modeling strategies ($p = 0.057$) was obtained. However, when MaxEnt was excluded from the data set (Appendix S6) because of the similarity between AUC values from both modeling strategies, the differences between ESM and SDM were significant ($p = 0.05$). Thus, we inferred that applying ESM to a low number of occurrences, such as our *C. plicata* data set, is better than SDM as shown by our results for three of the four algorithms implemented. Hereinafter, we explain Pliocene projections via ESM only.

The Biomod modeling approach used for SDM (extant species) and ESM (fossils) retrieved AUC values between 0.8 and 1 from different models following a cross-validation assessment, which indicated good predictive ability. By contrast, ESM evaluation from null model procedure showed that the resulting AUC values (mean = 0.6) were always lower than observed in all bivariate models evaluated (Appendix S6), revealing that Pliocene projection should be cautiously interpreted. Potential suitable conditions for the

extant groups (*C. pendula*, *C. agastachys*, and Macaronesian species) for the present day, LGM, and Pliocene are represented in Figure 4, as is the Pliocene projection using fossil occurrences (Figure 4D).

Projections for the present time (Figure 4A) revealed climatic suitability similar to the current distribution ranges of species (Figure 1). On the one hand, higher potential distribution probabilities for *C. pendula* were retrieved in Atlantic Europe, and lower probabilities in the Azores and Madeira. On the other hand, *C. agastachys* showed higher occurrence probabilities in eastern-southeastern Europe and southwestern Asia. Remarkably, a potential overlapping area for both species was identified across Germany, Austria, and northern Italy (Figure 4A). Projections to the present time for Macaronesian species displayed potential distributions in their respective archipelagos (Figure 4A).

During LGM times (Figure 4B), the potential areas retrieved were considerably reduced for *C. pendula* and *C. agastachys*, with the former mostly limited to coastal areas of southern Europe and the Black Sea basin, while the latter displayed scattered, small isolated regions in eastern Europe and the Black and Adriatic Sea basins as potential suitability areas. Notably, the Azores and Madeira archipelagos continued to be recovered as suitable regions during LGM for Macaronesian projections. Additionally, LIG (about 120–140 ka) and Holocene (~6 ka) projections (Appendix S4) displayed very similar potential distributions to the present day (Figure 4A).

In the Pliocene (3 mya), the potential distribution retrieved for *C. pendula* (Figure 4C) was very similar to the model for present times (Figure 4A). However, *C. agastachys* displayed areas of potential distribution in northern Europe and regions surrounding the Black Sea (Figure 4C), while *C. pendula* showed higher suitability conditions in central Europe. The Macaronesian archipelagos were also recovered as potential areas in *C. pendula* projections for that period, but with lower probabilities. Pliocene projection for the Macaronesian species recovered the Azores and Madeira. Finally, the projection to the Pliocene using *C. plicata* occurrences (using ESM because of the poor fossil record) showed a potential distribution clearly centered in central Europe and northern Italy (Figure 4D).

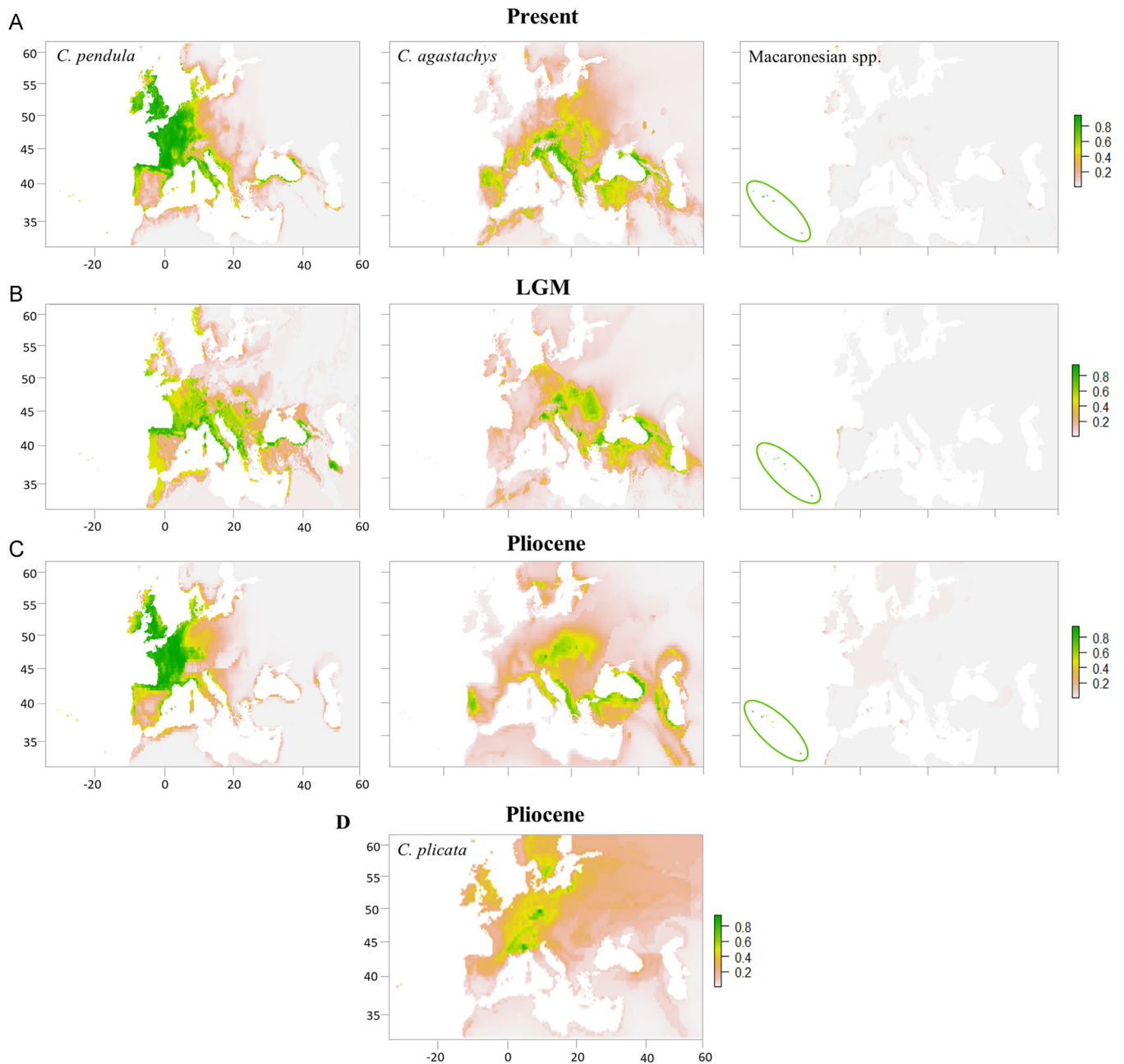


FIGURE 4 Climatic suitability and potential distributions predicted by Biomod at (A) present, (B) Last Glacial Maximum (LGM, 21 ka), and (C) Pliocene (3 mya) for *Carex pendula* (maps on left), *C. agastachys* (maps in center), and Macaronesian species (maps on right); and (D) Pliocene times for *C. plicata* occurrences performed with ensemble small modeling. Pliocene and LGM are represented with current coastlines. A green circle is used for better visibility when Macaronesian archipelagos showed climatically suitable areas

DISCUSSION

Carex sect. *Rhynchocystis* is not a Macaronesian laurel forest climatic relict

After geoclimatic changes during the Mio-Pliocene, many taxa of the Paleotropical flora have been reported to have become extinct in mainland Europe but to persist in certain refugia of the Mediterranean basin (Médail and Diadema, 2009; Linares, 2011; Santiso et al., 2016; García-Aloy et al., 2017; Villa-Machío

et al., 2018), as well as in the laurisilva restricted to the Macaronesian archipelagos (Liu and Schneider, 2013; Mairal et al., 2015). The persistence of these allegedly relict forests was believed to be related to the buffering of the climatic deterioration in the WP (Cronk, 1992; Fernández-Palacios, 2009; Ferreira et al., 2011). Specifically, Miguez et al. (2017) suggested that the long-term residence of sect. *Rhynchocystis* in the WP since the Miocene and its species' morphological-ecological traits resembling that of the Paleotropical flora (large size, broad leaves, shady and wet habitats) could be associated with it being a

possible climatic relict. However, our results from e-space and distribution modeling (Figures 2 and 4) support a niche shift for the Macaronesian species, because they show different bioclimatic requirements not only from the Pliocene ancestor of the *C. pendula* clade (Figures 2A, E and 3), but also from their extant mainland counterparts (Figures 2A, C and 3; Table 1). Accordingly, both geography and subsequent ecological changes have played an important role in the differentiation of Macaronesian species. Furthermore, *C. leviosa* and *C. sequeirae* recovered low suitability in the continental areas inhabited by *C. plicata* (Figure 4C), suggesting that the niche of the Macaronesian species evolved in response to climatic conditions far different from those that occurred in the mainland during the Late Cenozoic. This relatively recent shaping of the climatic niche has also been reported for other MLF species—for example, *Bencomia caudata* (Aiton) Webb and Berthel., *Echium pininana* Webb and Berthel., *Isoplexis sceptrum* (L.f.) Steud., and *Gesnouinia arborea* (Schüßler et al., 2019). This would reject the PNC hypothesis (Wiens and Graham, 2005) for Macaronesian sect. *Rhynchocystis* species and their consideration as climatic relicts. Furthermore, their niches in each archipelago (Azores and Madeira) are not similar (Figures 2A and 3A; Table 1). This implies that *C. leviosa* and *C. sequeirae* have undergone local adaptation processes (e.g., Díaz-Pérez et al., 2006). Even conspecific populations from different islands within the same archipelago (Azores) displayed non-overlapping e-spaces (Appendix S3), as shown for other Macaronesian islands (Canary Islands: *Helianthemum* [Albaladejo et al., 2020], *Micromeria* [Meimberg et al., 2006], *Daucus* [Frankiewicz et al., 2020]; Cape Verde: *Echium* [Romeiras et al., 2018]). Remarkably, the different bioclimatic niches between Macaronesian species and their mainland counterparts (Figure 2A, C) have provided evidence of their ecological differentiation (Figure 3A), further supporting their taxonomic recognition at the species level, as recently proposed by Miguez et al. (2021) on the basis of molecular and morphological data.

Niche conservatism shaped the current distribution of mainland species

Carex pendula and *C. agastachys* showed similar ecological preferences, despite being mostly allopatric species, although with a poorly delimited contact zone across central Europe (Figure 1; Meierott, 2019; Sutorý and Řepka, 2020). Accordingly, two alternative evolutionary processes could explain their currently overlapping niche (Figures 2A, B and 3B): (1) a possible niche conservatism since their Miocene divergence (11.6 mya; Miguez et al., 2021), rarely reported for WP (Liu et al., 2012; Romdal et al., 2012; Wang et al., 2018), which would constitute a remarkable case of long-term retention of ecological preferences; and (2) a similarity of ecological preferences (niche homoplasy), which would have been independently acquired rather than inherited from their common ancestor. Homoplasy has been frequently inferred through genetic and morphological studies (Dorris et al., 1999; Parra-Olea and Wake, 2001), but not from bioclimatic data.

Ancestral reconstruction of climatic data indicates that the niche between *C. pendula* and *C. agastachys* is relatively conserved, even more so when taking into account that uncertainty about the phylogeny is not considered (mean bioclimatic PC1 values; Figure 3B). This is also supported by the SDM, because the *C. pendula* projection to the Pliocene largely overlaps with that of its ancestor *C. plicata* at that time (Figure 4D). In addition, PCA displays overlapping e-spaces for *C. pendula* and *C. agastachys* (Figure 2B), and significant similarity/equivalency of niches was detected (Table 1). By contrast, there was also partial differentiation between *C. pendula* and *C. plicata* in the PCA (Figure 2D), which would point to a homoplastic assembly of the niches of *C. pendula* and *C. agastachys*, because at least the ancestor of *C. pendula* would present different macroecological requirements. However, we should interpret such potential evidence of homoplasy from PCA very cautiously, because (1) PCAs using fossils rely on few points, which may represent only part of the actual niche of *C. plicata*; (2) pairwise comparison between *C. pendula* and *C. plicata* shows overlap over PC2, which still explains a high amount of niche variation with respect to PC1 (33.4% vs. 21.4%; Figure 2D); and (3) the potential niche of *C. plicata* in the Pliocene may have included that of *C. pendula* (Figure 4C, D), which may not be displayed in the PCA plots because *C. pendula*-like niches may not have existed at that time (Figure 2A, D; Petitpierre et al., 2012). Niche conservatism would not imply any niche shifts along the sect. *Rhynchocystis* phylogeny, while at least one must be invoked in a homoplastic scenario. Pursuant to the foregoing, and given that niche conservatism seems to be the most parsimonious explanation, we favor it as the most plausible evolutionary scenario for the mainland species.

As explained above, the main evolutionary pattern retrieved in the continent appears to involve a long-term niche conservatism for *C. pendula* and *C. agastachys* since the Late Miocene (11.6 mya; Miguez et al., 2021). This could be consistent with previous studies that invoke niche conservatism in WP Cenozoic plant relicts (Milne and Abbott, 2002; Rodríguez-Sánchez and Arroyo, 2008; Lososová et al., 2020). The similarity and equivalence of the bioclimatic niches retrieved for both mainland species (Table 1) is congruent with their occurrence in comparable habitats (temperate riparian forests; Miguez et al., 2018). This points to the lesser importance of ecology at macroevolutionary scales and specifically regarding the mainland species, as also found in other *Carex* sections (Benítez-Benítez et al., 2021). Remarkably, although SDM identified some areas as suitable for both *C. pendula* and *C. agastachys* in the present time (Figure 4A), these species are almost entirely allopatric (Figure 1; see also Miguez et al., 2018; Meierott, 2019). This could point to their reciprocal competitive exclusion: *C. pendula* could be preventing *C. agastachys* from establishing in its native range and vice versa (e.g., Hardin, 1960; for a further example in sedges, see Slingsby and Verboom, 2006).

During the LGM, *C. agastachys* displayed a greater contraction in its potential distribution than *C. pendula*. Thus, we found that *C. agastachys* refugia were restricted to southwestern Asia and central-eastern Europe (Figure 4B),

as already pointed out by Miguez et al. (2017), while *C. pendula* was more broadly distributed in western Europe and the Mediterranean. The smaller refugial areas available for *C. agastachys* could explain the much lower genetic diversity displayed by this species compared with *C. pendula* (Miguez et al., 2017). LIG and Holocene projections recovered distributions of *C. pendula* and *C. agastachys* very similar to their current distributions, supporting their postglacial recolonization (Appendix S4A, B).

CONCLUSIONS

Although the origin of Macaronesian species of *Carex* sect. *Rhynchocystis* dates back to the Plio-Pleistocene, and these species' habitats (MLFs, or laurisilva) have been considered remnants of the Paleotropical flora in the WP, their ecological requirements differ from those of their ancestors and extant mainland counterparts. This finding supports the conclusion that niches of both *C. leviosa* (Azores) and *C. sequeirae* (Madeira) are of relatively recent formation, and hence that they do not represent climatic relicts from the Late Cenozoic. By contrast, the high niche similarity between *C. pendula* and *C. agastachys* seems to be due to long-term niche conservatism. The latter species' allopatric distributions across mainland WP seems to be related to reciprocal competitive exclusion. Nonetheless, a microevolutionary study focused on their contact zone could shed light on the ecological processes shaping their current distribution.

Ecological niche comparison and distribution modeling in combination with the study of fossils allows for assessment of whether historical environmental changes have affected ecological requirements and how these have also shaped the biogeographic patterns and evolutionary history of species. Our results also show the importance of exploring bioclimatic data using different methodologies (SDM, ESM, PCA, ancestral state reconstruction, similarity/equivalence niche tests) to tackle alternative hypotheses of ecological evolution. Ultimately, our macroevolutionary research may facilitate understanding of evolutionary and taxonomic questions related to other groups or regions, especially under current climate-change scenarios.

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AUTHOR CONTRIBUTIONS

C.B.-B., P.J.-M., and S.M.-B. conceived and supervised the study. C.B.-B. and M.S.-A. analyzed the data and drafted the manuscript. C.B.-B., M.S.-A., M.M., P.J.-M., and S.M.-B. reviewed the manuscript. C.B.-B. and M.M. conducted fieldwork in Madeira, and S.M.-B. in Azores. The authors have no conflicts of interest to declare.

DATA AVAILABILITY STATEMENT

Supplementary data files are available online in Zenodo (<https://doi.org/10.5281/zenodo.5552343>). They include information about the herbaria and accession numbers for specimens used in this study; point localities employed in the environmental data and distribution modeling analyses obtained from GBIF, bibliographic references, and herbarium specimens; as well as occurrences of Pliocene fossils of *Carex plicata*.

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SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

Appendix S1. Occurrence map of extant species (*C. pendula*, *C. agastachys*, *C. leviosa*, and *C. sequeirae*) and Pliocene fossil ancestors.

Appendix S2. Additional methodological details on species distribution modeling.

Appendix S3. Principal component analysis displaying the geographical structure of the e-space for the Azores archipelago.

Appendix S4. Biomod projections of climatic suitability and potential distribution for extant species in LIG and Holocene periods.

Appendix S5. Projections of climatic suitability and potential distributions performed with our first modeling approach (see main text), implemented for extant species at present, Late Quaternary, and Pliocene periods.

Appendix S6. Mean AUC values from both modeling strategies (species distribution modeling [SDM] vs. ensemble small modeling [ESM]), including all algorithms for Pliocene fossils.

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