

Dramatic impact of future climate change on the genetic diversity and distribution of ecologically relevant Western Mediterranean *Carex* (Cyperaceae)

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Anticipating the evolutionary responses of species to ongoing climate change is essential to propose effective management and conservation measures. The Western Mediterranean Basin constitutes one of the hotspots of biodiversity where the effects of climate change are expected to be more dramatic. Plant species with ecological relevance constitute ideal models to evaluate and predict the impact of climate change on ecosystems. Here we investigate these impacts through the spatio-temporal comparison of genetic diversity/structure (AFLPs), potential distribution under different future scenarios of climate change, and ecological space in two Western Mediterranean sister species of genus *Carex*. Both species are ecologically key in their riparian habitats, but display contrasting distribution patterns, with one widespread in the Iberian Peninsula and North Africa (*C. reuteriana*), while the other (*C. panormitana*) is a restricted, probably endangered, Central Mediterranean endemic. At present, we found a strong genetic structure driven by geography in both species, and lower values of genetic diversity and a narrower ecological space in *C. panormitana* than in *C. reuteriana*, although the allelic rarity was higher in the former. Future projections predict an overall dramatic reduction of suitable areas for both species under all climate change scenarios, which could be almost total for *C. panormitana*. In addition, gene diversity was inferred to decrease in all taxa, with genetic structure reinforcing in *C. reuteriana* by the loss of admixture among populations. Our findings stress the need for a reassessment of *C. panormitana* conservation status under IUCN Red List criteria and the implementation of conservation measures.

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2 **ecologically relevant Western Mediterranean *Carex* (Cyperaceae)**

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10 Short title: Impact of climate change in *Carex*

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14

15 **Abstract**

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17 propose effective management and conservation measures. The Western Mediterranean Basin
18 constitutes one of the hotspots of biodiversity where the effects of climate change are expected to
19 be more dramatic. Plant species with ecological relevance constitute ideal models to evaluate and
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23 Mediterranean sister species of genus *Carex*. Both species are ecologically key in their riparian
24 habitats, but display contrasting distribution patterns, with one widespread in the Iberian
25 Peninsula and North Africa (*C. reuteriana*), while the other (*C. panormitana*) is a restricted,
26 probably endangered, Central Mediterranean endemic. At present, we found a strong genetic

27 structure driven by geography in both species, and lower values of genetic diversity and a
28 narrower ecological space in *C. panormitana* than in *C. reuteriana*, although the allelic rarity
29 was higher in the former. Future projections predict an overall dramatic reduction of suitable
30 areas for both species under all climate change scenarios, which could be almost total for *C.*
31 *panormitana*. In addition, gene diversity was inferred to decrease in all taxa, with genetic
32 structure reinforcing in *C. reuteriana* by the loss of admixture among populations. Our findings
33 stress the need for a reassessment of *C. panormitana* conservation status under IUCN Red List
34 criteria and the implementation of conservation measures.

35 **Keywords:** conservation genetics, ecological niche, global climate change, habitat loss,
36 Mediterranean Basin, restricted endemic, species distribution modeling

37 **1. Introduction**

38 Nowadays, anthropogenically-driven global climate change (GCC herein) is one of the main
39 threats to life on our planet. The main conclusion from the Intergovernmental Panel on Climate
40 Change (IPCC, 2014) is that contemporary warming of land and ocean is irrefutably taking
41 place, and since the 1980s each successive decade has been warmer than any preceding one.
42 Projections of change in global mean temperature for the period 2016-2035 point to an increase
43 of 0.3-0.7°C compared to the 1986-2005 period. By the end of this century (2081-2100),
44 estimates predict a higher frequency of extreme weather events and an increase of mean global
45 surface temperature of 0.3°C-4.8°C, depending on the emission and climate variability scenario
46 considered (IPCC 2013, 2014).

47 Many studies confirm that GCC has already affected the biota and ecosystems
48 (Parmesan, 2006; Johnson et al., 2017; Pecl et al., 2017; Ripple et al., 2017; García and Jordano,
49 2021). The responses of species populations to climate change can be categorized into three main
50 types: migration, extinction, and in situ adaptation (Christmas et al., 2016). Specifically, ongoing
51 GCC is inducing changes in the distribution of plant species with greater capacity for migration
52 to areas of higher latitude and/or altitude that may provide suitable environmental conditions
53 (niche conservatism, e.g., Jump and Peñuelas, 2005; Wiens et al., 2010; Morueta-Holme et al.,
54 2015; Christmas et al., 2016). However, migration rates for most species could be insufficient to
55 match the pace and magnitude of predicted climate changes (e.g., Loarie et al., 2009; Freeman

56 and Freeman, 2014; Morueta-Holme et al., 2015; González-Varo et al., 2017), leaving in situ
57 adaptation as the only alternative to extinction for these species. In species with restricted ranges,
58 low genetic variability and/or narrow ecological tolerance, genetic and phenotypic variation may
59 not provide enough adaptability to the new local conditions (Jump and Peñuelas, 2005; Aitken et
60 al., 2008; Aubin et al., 2016; Christmas et al., 2016; Gray, 2018).

61 Species distribution models (SDM, Guisan and Thuiller, 2005) are useful tools to predict
62 and compare present and future potential distribution of species (e.g., Blanco-Pastor et al., 2013;
63 Velásquez-Tibatá et al., 2013). This helps to assess species vulnerability under different GCC
64 future scenarios (e.g., Beaumont et al., 2008; Forester et al., 2013). While these methods do not
65 take into account population evolutionary potential, which may enable in situ adaptation
66 (Soberon and Peterson, 2005; Razgour et al., 2019), they are still useful to evaluate GCC impact
67 on extant populations under hypotheses of niche conservatism (Ackerly, 2003; Wiens and
68 Graham, 2005), which is the most common evolutionary response of lineages (Liu et al., 2012;
69 Lososová et al., 2020; Sanz-Arnal et al., 2021). They do not either consider habitat destruction
70 and fragmentation directly produced by human action. Furthermore, several studies have
71 projected current genetic diversity to future times, assuming the extinction of populations falling
72 outside the inferred potential distribution (Espíndola et al., 2012; Lima et al., 2017; Rizvanovic
73 et al., 2019), while others have projected future genetic structure based on molecular and
74 climatic data (Jay et al., 2012, 2015).

75 The Mediterranean Basin constitutes one of the Earth's hotspots of biodiversity and
76 harbors an exceptional plant diversity, featuring a high level of endemism (Myers et al., 2000;
77 Mittermeier et al., 2011; Vargas, 2020). Unfortunately, this will likely be one of the regions most
78 affected by GCC (Fletcher and Zielhofer, 2013; IPCC, 2013; Guiot and Cramer, 2016; Lionello
79 and Scarascia, 2018; Cramer et al., 2018; Fenu et al., 2020; García and Jordano, 2021). As a
80 result of this, about 30% of the Mediterranean Basin hotspot could be losing its current climate
81 and turning into a non-analogous one, which could threaten about 30% of its endemic species,
82 assuming loss of analogue climate as a proxy for habitat loss (Bellard et al., 2014). Specifically,
83 the Western Mediterranean includes numerous areas that have been considered climatic refugia
84 during historical climatic oscillations from Miocene to Pleistocene (Médail and Diadema, 2009).
85 These refugia are reservoirs of unique genetic diversity and evolutionary potential due to the

86 long-term persistence of species, and therefore have high conservation priority (Médail and
87 Quézel, 1999; Médail and Diadema, 2009).

88 *Carex* L. (Cyperaceae) is a megadiverse angiosperm genus with several characteristics
89 that makes it an ideal model for the study of the effects of GCC on its species, populations and in
90 the ecosystems where it lives. With c. 2000 species it ranks among the three largest angiosperm
91 genera in the world (POWO, 2020; Roalson et al., 2021). It has an almost cosmopolitan
92 distribution although higher species diversity in temperate and cold areas of both hemispheres.
93 *Carex* diversification seems to have been historically favoured by global cooling periods
94 (Martín-Bravo et al., 2019), which lead to think that GCC could be negative for its species at
95 least from an evolutionary perspective. In the Western Mediterranean, as in many other world
96 regions, *Carex* species dominate a variety of plant communities, from wetland and river shores to
97 peat bogs and high mountain meadows. Some of these habitats are considered of special interest
98 and have conservation priority in the Directive 2013/32/EU. Therefore, responses of ecologically
99 important *Carex* species to climate changes could be probably decisive for the future persistence
100 of these habitats.

101 *Carex reuteriana* Boiss. and *C. panormitana* Guss. (sect. *Phacocystis*), are two endemic
102 sister species disjunctly distributed in the Western Mediterranean Basin (Luceño and Jiménez-
103 Mejías, 2008; Jiménez-Mejías et al., 2011), growing in creeks and river shores at medium
104 altitudes (Benítez-Benítez et al., 2018). *Carex reuteriana* comprises two subspecies: *C.*
105 *reuteriana* subsp. *reuteriana* from the centre to NW Iberian Peninsula and *C. reuteriana* subsp.
106 *mauritanica* (Boiss. & Reut.) Jim.-Mejías & Luceño from NW Africa (Morocco) to the S Iberian
107 Peninsula with populations on both sides of the Guadalquivir Valley (Sierra Morena and Betic
108 ranges; Jiménez-Mejías et al., 2011). The conservation status of *C. reuteriana* has not been
109 evaluated, although it is seemingly not endangered due to its relatively large distribution range
110 and high numbers of populations and individuals in many of them. On the other hand, *C.*
111 *panormitana* is a restricted Tyrrhenian endemic from Sardinia with a few additional small
112 populations in Sicily and Tunisia (Pignatti, 1982; Urbani et al., 1995; Gianguzzi et al., 2013;
113 Jiménez-Mejías et al., 2014). Regarding its conservation status, it has been listed under different
114 conservation categories according to a variety of regional and global red lists (Bilz et al., 2011;
115 Domina, 2011; Rossi et al., 2013; Urbani et al., 2013). Both species are conspicuous, tussock-

116 forming plants, which are ecologically relevant as they are dominant and characteristic taxa in
117 the plant communities (phytosociological ranks; Molina, 1996; Navarro et al., 2001; Rufo Nieto
118 and de la Fuente, 2011; Gianguzzi et al., 2013; Rodríguez-Gutián et al., 2017). Previous studies
119 revealed a strong genetic structure in both species driven by geography, with distinct genetic
120 clusters corresponding to disjunct populations (Jiménez-Mejías et al., 2011; Benítez-Benítez et
121 al., 2018).

122 In the present study, we analyse the genetic makeup and potential distribution in these
123 two ecologically relevant *Carex* sister species endemic to the Western Mediterranean Basin. We
124 examine the relationship between these factors in a comparative and geographic-temporal
125 framework in order to forecast the effects of GCC on these species. Thus, the main aims of this
126 study are to: (1) estimate and compare the genetic diversity/structure and potential distribution
127 within and between the species in the present and future; (2) tackle the hypothesis that pose a
128 negative relationship between range size, genetic diversity and ecological niche breadth on one
129 side, and vulnerability to environmental changes on the other; (3) relate the current and future
130 projected situation with the conservation status of both species.

131 **2. Materials and methods**

132 ***2.1 Analyses of Present Population Genetics***

133 We reanalysed the AFLP sampling that included 182 polymorphic loci used by Benítez-Benítez
134 et al. (2018), which consisted in 130 individuals from 18 different locations (69 individuals from
135 12 populations of *C. reuteriana* and 61 individuals from 6 populations of *C. panormitana*; Table
136 1), that representatively cover the range of both species fairly well.

137 Present gene diversity (Nei's diversity; Nei, 1978) at taxa and population level was
138 computed with AFLP_{DAT} (Ehrich, 2006) as implemented in R v. 3.2.1 (R Core Development
139 Team, 2020). Since many *Carex* species often propagate vegetatively, including the two study
140 species (Luceño and Jiménez-Mejías, 2008; P. Jiménez-Mejías and M. Urbani, pers. comm.), we
141 tried to minimize sampling ramets from the same clone (genet) by selecting individuals at a
142 distance of at least 2m. The distribution of the number of pairwise genetic distance comparisons
143 among phenotypes and individuals within a species representing putative clones were inferred
144 with the clone function also in AFLP_{DAT}. The Arlequin function from AFLP_{DAT} was used to

145 calculate gene diversity of populations at the ramet and genet levels before and after removing
146 putative clones, respectively. These clones were identified as phenotypes that differed in a
147 number of bands below the frequency of the error rate (Bonin et al., 2004). Frequency-down-
148 weighted marker values (DW) were also estimated as a measure of allele rarity (Schönswetter
149 and Tribsch, 2005).

150 To further investigate the genetic structure found by Benítez-Benítez et al. (2018), a
151 phylogenetic tree of AFLP phenotypes was obtained with a Neighbor-Joining (NJ) analysis
152 based on Nei-Li distances including 1000 replicates to assess bootstrap support, as implemented
153 in PAUP v. 4.0b10 (Swofford, 2002). An analysis of molecular variance (AMOVA) was
154 conducted using ARLEQUIN v. 3.5.2.2 (Excoffier and Lischer, 2010) in which we also
155 calculated the fixation index F_{st} (Wright, 1965). We conducted two partitions in order to examine
156 in detail the variation of genetic diversity, one considering the two species (*C. reuteriana* and *C.*
157 *panormitana*), and the other partition considering the four main genetic clusters previously found
158 by Benítez-Benítez et al. (2018): (1) *C. reuteriana* subsp. *reuteriana*, (2) *C. reuteriana* subsp.
159 *mauritanica*, (3) Sicilian-Tunisian populations of *C. panormitana*, and (4) Sardinian populations
160 of *C. panormitana*. We also used BAPS v. 6.0 (Corander et al., 2003) to look for possible
161 underlying fine structure, estimating the number of genetic clusters (K) by assigning individuals
162 and populations in undefined mixture clusters under a Bayesian framework. We ran the analysis
163 with a predefined 10 replicates from K=5 to K=8. Finally, Discriminant Analysis of Principal
164 Components (DAPC; Jombart, 2008) is a multivariate method that, unlike Principal Component
165 Analysis (PCA) and Discriminant Analysis (DA), maximizes the separation between groups
166 while minimizing variation within groups to identify genetic clusters (Jombart et al., 2010). It
167 was used to obtain a visual spatial assessment of genetic structure using the four main genetic
168 clusters previously defined as priors. In view of the weak genetic structure found within *C.*
169 *reuteriana* subsp. *reuteriana* (see results), a Mantel test was performed with GenAlEx v. 6.5
170 (Peakall and Smouse, 2012) to evaluate the correlation between genetic (Nei, 1972) and
171 geographic distances between its populations.

172 In order to enable the comparison of present genetic structure with that inferred for the
173 future for the taxonomically-driven partition considering three groups (*C. reuteriana* subsp.
174 *reuteriana*, *C. reuteriana* subsp. *mauritanica*, and *C. panormitana*), we conducted an analysis

175 with Structure v. 2.3.4 (Pritchard et al., 2000). For each taxon, we performed 10 independent
176 runs of 100,000 iterations each one, with a burn-in period of 1,000 for each value of K from 1 to
177 3. The best K was chosen comparing the probabilities for the K values inferred with Structure
178 Harvester (Earl and VonHoldt, 2012) and the graphic was performed with Structure Plot v. 2.0
179 (Ramasamy et al., 2014).

180 ***2.2 Potential species distribution and ecological niche modeling for present times***

181 We used the same occurrence dataset (316 georeferenced records of *C. reuteriana* subsp.
182 *reuteriana*, 118 of subsp. *mauritanica*, and 29 (all known records) of *C. panormitana*; Table S1)
183 and uncorrelated bioclimatic variables used in Benítez-Benítez et al. (2018): bio2 (mean diurnal
184 range), bio4 (temperature seasonality), bio15 (precipitation seasonality) and bio16 (precipitation
185 of wettest quarter). Distribution models were obtained for the three studied taxa: *C. reuteriana*
186 subsp. *reuteriana*, *C. reuteriana* subsp. *mauritanica*, and *C. panormitana*. We used three groups
187 (taxonomically-driven partition) rather than four (see genetic analyses) because of the low
188 number of occurrences for *C. panormitana*.

189 Biomod2 (Thuiller et al., 2009) implemented in R was used for SDM, testing six different
190 implemented modeling algorithms: classification tree analysis (CTA), Generalized Additive
191 Model (GAM), Generalized Boosted Regression Model (GBM), Generalized Linear Model
192 (GLM), Maximum Entropy Algorithm (MaxEnt), and Random Forest (RF). Thereafter, an
193 ensemble modeling was run including all algorithms in order to build more accurate projections
194 (Araújo and New, 2007; Forester et al., 2013). We randomly built three sets of pseudo-absences
195 and generated a data splitting (80% training data and 20% burn-in) to assess the models by cross-
196 validation, with two independent runs. We used True Skill Statistics (TSS; Allouche et al., 2006)
197 and Area Under the Curve (AUC; Swets, 1988) with a threshold > 0.7 as evaluation metrics for
198 building models.

199 Finally, we conducted a Principal Component Analysis (PCA; Janžekovič and Novak,
200 2012) of the retained bioclimatic variables using the `prcomp` function in the package `ggplot2` in
201 R (Wickham, 2016) for visualizing the ecological niche occupied by each taxon (*C. reuteriana*
202 subsp. *reuteriana*, *C. reuteriana* subsp. *mauritanica*, and *C. panormitana*) under present
203 conditions.

204 **2.3 Future projections of potential distribution, genetic diversity/structure, and environmental**
205 **niche**

206 Future potential distribution was modeled with the same methodology as for the present (see
207 above). Future climate can be projected with different general circulation models (GCMs) which
208 are the primary source for studying the dynamics and components of the global climate system.
209 GCMs were selected following Parding et al. (2020): BCC-CMS2-MR, CanESM5, CNRM-
210 CM6-1, CNRM-ESM2-1, MIROC6 and MRI-ESM2-0 (data available in
211 <https://www.worldclim.org/>; Fick and Hijmans, 2017). These GCMs include four different
212 Representative Concentration Pathways (RCPs) addressed in the IPCC Fifth Assessment Report
213 (IPCC, 2014). We selected RCP2.6 and RCP8.5 to represent two extreme scenarios (an
214 optimistic with lowest greenhouse gas atmospheric concentration vs. a pessimistic with highest
215 concentration). In addition, two future temporal ranges (2041-2060, 2081-2100) were used to
216 project the set of GCMs. We have averaged the six different GCMs per each of the two scenarios
217 resulting from modeling analyses in a single raster layer for each of the two future temporal
218 ranges. This assembly accounts for uncertainty of future climatic conditions across the potential
219 distribution range of species, as shown by Wróblewska and Mirski (2018). We overlapped
220 present and future projections of potential distribution in a single map using QGIS v. 3.4.15
221 (QGIS Development Team, 2021), enabling the visualization of the potential loss and/or gain of
222 climatic suitable areas under different GCC scenarios. Thus, SDM have been used to show how
223 the different Mediterranean regions where *C. reuteriana* and *C. panormitana* inhabit are
224 responding to the GCC comparing current distribution models against the future ones in order to
225 visualize the proportion of area loss/gain. It is important to note that this methodological
226 approach assumes no migration and that species ecological requirement would remain the same
227 (niche conservatism) in the future.

228 To estimate future gene diversity, we recalculated the values after removing those
229 sampled populations placed within the predicted lost range according to the inferred potential
230 distribution under the optimistic (RCP2.6) climate change scenario for 2081-2100. Therefore, we
231 assumed the extinction of populations located in unsuitable areas by future projections of SDM
232 (Espíndola et al., 2012; Lima et al., 2017; Rizvanovic et al., 2019). We did not obtain genetic

233 diversity values for the pessimistic (RCP8.5) 2081-2100 scenario, since all sampled populations
234 of *C. panormitana* and *C. reuteriana* subsp. *reuteriana* were predicted to go extinct (see results).

235 We used the spatial Bayesian clustering algorithm implemented in POPS v. 1.2 (Jay et
236 al., 2012, 2015) to infer population genetic structure in the future (2081-2100) also based on the
237 optimistic climate change scenario. We performed analyses for each taxon (*C. reuteriana* subsp.
238 *reuteriana*, *C. reuteriana* subsp. *mauritanica*, and *C. panormitana*) with and without admixture.
239 Two independent replicates were run for the best number of clusters (K=2) obtained in Structure
240 analyses for present times (see above), using genetic data from AFLPs, as well as spatial
241 coordinates of each population and quantitative bioclimatic variables as covariates. We selected
242 the same bioclimatic variables utilized in the future projections of SDM described above. For the
243 simulated data, we used 10,000 sweeps with a burn-in of 2,000 sweeps to run POPS. The
244 resulting output files were introduced in CLUMPP v. 1.1.2 (Jakobsson and Rosenberg, 2007) in
245 order to average two runs per each K. Subsequently, the outputs from CLUMPP were imported
246 in Distruct v. 1.1 (Rosenberg, 2004) to visualize the genetic structure for each independent
247 genetic cluster analysed.

248 Finally, we projected the future environmental space of each taxon. A PCA was
249 performed in the same way as for contemporary conditions, but removing those occurrences
250 located in areas of lost habitat as explained for the estimation of future genetic diversity
251 (optimistic scenario in 2081-2100).

252 **3. Results**

253 ***3.1 Present genetic structure and diversity***

254 NJ analysis of AFLP phenotypes recovered three moderately to well-supported clades (Fig. 1a):
255 (1) *C. reuteriana* (88% Bootstrap [BS]), (2) Sicilian-Tunisian *C. panormitana* populations (75%
256 BS), and (3) Sardinian *C. panormitana* populations (100% BS). In turn, Sicilian and Tunisian
257 populations were recovered as sisters in well-supported distinct clades (98% BS Tunisia, 92%
258 BS Sicily). On the contrary, subspecies and populations of *C. reuteriana* were unresolved in a
259 polytomy.

260 AMOVA analysis results are shown in Table 2. The complete dataset considering the two
261 species revealed that 28.06% of the variation of genetic diversity is explained by differences

262 between the species, 37.22% by differences among populations within species, and 34.73% by
263 differences within populations. Considering each species separately, variation among
264 populations in *C. reuteriana* was considerably lower (39.82%) than in *C. panormitana* (68.76%),
265 so variation within populations showed the opposite pattern (60.18% vs. 31.24%, respectively).
266 When partitioning the four main genetic clusters previously found by Benítez-Benítez et al.
267 (2018), AMOVA analysis revealed 42.13% of the variation of genetic diversity is explained by
268 differences between groups, 21.46% by differences among populations within groups, and
269 36.42% by differences within populations. Regarding *C. reuteriana*, the differentiation between
270 populations was higher in subspecies *mauritanica* (37.02%) than in subspecies *reuteriana*
271 (20.36%). In *C. panormitana*, there was a stronger difference between Sicilian-Tunisian
272 populations (53.51%) than among Sardinian ones (41.4%).

273 DAPC analysis also confirmed the clear differentiation between the four genetic clusters
274 previously commented (Fig. 1b), and a perfect assignment of each individual to its a priori group.
275 However, BAPS analyses found a certain degree of additional, underlying genetic structure in *C.*
276 *reuteriana* subsp. *mauritanica* and *C. panormitana*, and suggested that six is the most accurate
277 number of genetic clusters (K=6; Fig. 1c). Three of these clusters corresponded to *C. reuteriana*:
278 (1) all six *C. reuteriana* subsp. *reuteriana* sampled populations; (2) four populations of *C.*
279 *reuteriana* subsp. *mauritanica*, two located in northern Africa (Morocco) and the other two from
280 the Betic ranges south of Guadalquivir Valley in southern Spain; and (3) the remaining two
281 subsp. *mauritanica* populations from Sierra Morena (north of Guadalquivir Valley). *Carex*
282 *panormitana* was also split into three clusters: (4) the four populations from Sardinia, (5) the
283 population from Sicily and (6) the population from Tunisia.

284 STRUCTURE retrieved two as the optimal number of genetic clusters for the three taxa
285 (K=2; Fig. 2a). *Carex reuteriana* subsp. *reuteriana* clusters lacked geographic structure and
286 presented admixture in all sampled populations. The Mantel test was not significant for this
287 subspecies (p-value = 0.46; R² = 0.001; Fig. S2 in Supplementary Material). On the contrary, *C.*
288 *reuteriana* subsp. *mauritanica* presented a clearer genetic structure, with the two clusters mostly
289 corresponding to populations from north of the Guadalquivir Valley and south of the valley
290 (Betic ranges) plus North Africa, respectively. Finally, the two genetic clusters in *C.*

291 *panormitana* corresponded, almost without admixture, to Sardinian populations and Sicilian-
292 Tunisian ones, respectively.

293 The distribution of the number of pairwise differences among AFLP individual
294 phenotypes in both species was unimodal for *C. reuteriana* while bimodal for *C. panormitana*
295 (Fig. S1). The latter indicated the existence of clones in this species. In congruence, three of the
296 four sampled Sardinian *C. panormitana* populations showed a significant presence of putative
297 ramets (18 of 38 sampled individuals), according to the clone function in AFLP_{DAT}. The final
298 AFLP error rate was 1.44% when clones were removed.

299 Finally, the analysis of the 130 sampled individuals from 18 populations revealed a high
300 genetic differentiation between *C. reuteriana* and *C. panormitana* with an F_{st} value of 0.653.
301 Values were also high when considering *C. reuteriana* subspecies ($F_{st}=0.46$) and Sicilian-
302 Tunisian vs Sardinian populations of *C. panormitana* ($F_{st}=0.765$).

303 Values of present genetic (Nei's) diversity for each taxon and sampled population are
304 shown in Table 1. *Carex reuteriana* s.l. (0.184), as well as both its subspecies (subsp. *reuteriana*:
305 0.142; subsp. *mauritanica*: 0.170), have higher genetic diversity than *C. panormitana*. For the
306 latter, the presence of putative clones was reflected in slightly different values at the genet
307 (0.133) and ramet (0.128) levels. These clones were only detected for Sardinian populations,
308 which was reflected in a very low genetic diversity in these populations (0.037 at genet and
309 0.032 at ramet level) in comparison with Sicilian-Tunisian counterparts (0.115). *Carex*
310 *panormitana* as a whole presented higher DW value (436.028) in comparison to both subspecies
311 of *C. reuteriana* (subsp. *reuteriana*: 154.041; subsp. *mauritanica*: 324.723), whilst *C. reuteriana*
312 s.l. retrieved the highest DW value (905.394). In addition, *C. reuteriana* subsp. *reuteriana*
313 displayed lower DW values than subsp. *mauritanica* in most of its populations (Table 1).

314 **3.2 Future genetic structure and diversity**

315 The POPS analyses projecting the future genetic structure did not detect any new genetic clusters
316 with respect to STRUCTURE analyses for present times (Fig. 2b), either implementing
317 admixture or non-admixture analyses. However, it revealed substantial changes in the genetic
318 structure of both subspecies of *C. reuteriana*. Thus, *C. reuteriana* subsp. *reuteriana* was
319 projected to almost completely lose one of its two current genetic clusters (except for one

320 individual). In *C. reuteriana* subsp. *mauritanica*, all Iberian sampled populations were assigned
321 to the same genetic cluster, while the other cluster would remain exclusively for Moroccan
322 populations, in both cases with no admixture between clusters. In contrast, *C. panormitana* was
323 projected to retain the same strong genetic structure found for present times, which was even
324 reinforced by the loss of the marginal admixture.

325 Future projections of genetic diversity removing populations affected by habitat loss
326 according to SDM results (see below; RCP2.6 for 2081-2100 in Fig. 3) yielded lower values of
327 genetic diversity when compared to present for all taxa, although only slightly for *C. reuteriana*
328 s.l. (*C. reuteriana* subsp. *reuteriana*: 0.107; *C. reuteriana* subsp. *mauritanica*: 0.164; *C.*
329 *panormitana*: 0.118 (genet), 0.100 (ramet)). DW values were considerably higher in the Sicilian,
330 and, to a lesser extent, in the Tunisian population of *C. panormitana* than in the Sardinian ones
331 and both subspecies from *C. reuteriana* (Table 1).

332 **3.3 Species Distribution Modeling**

333 Projected potential distribution ranges of *C. reuteriana* and *C. panormitana* shifted according to
334 six GCMs in different time periods (2041-2060 and 2081-2100) and two RCPs scenarios
335 (RCP2.6 and RCP8.5). Both species were inferred to gain and lose suitable areas in response to
336 GCC under different scenarios (see projections for 2041-2060 in Fig. S2 in Supplementary
337 Material and Fig. 3b or 2081-2100). Herein, we will mainly comment on the results from 2081-
338 2100 since this scenario represents more severe climatic conditions in comparison with current
339 ones (Fig. 3b).

340 Future SDM revealed important losses of suitable areas for all taxa, which was reinforced
341 in the RCP8.5 scenario, especially for *C. panormitana*. Likewise, currently suitable areas
342 inferred to remain stable (current potential range overlapping with that inferred by future
343 models) were always reduced in the RCP8.5 with respect to RCP2.6. In *C. reuteriana* subsp.
344 *reuteriana* (Figs. 3a, 3b) climate change was predicted to produce the loss of around the half of
345 its currently suitable areas (41% in the RCP2.6 scenario and 57% in the RCP8.5 scenario),
346 including all the Central Iberian range, and, in the RCP8.5, also the NW Iberian quadrant. The
347 most stable areas (40% RCP2.6, 10% RCP8.5) would be located in the NW Iberian Peninsula.
348 Future suitable conditions (22% RCP2.6, 33% RCP8.5) could spread northwards of its current
349 distribution (Atlantic coast of North Spain and Western France; Fig. 3a). *Carex reuteriana* subsp.

350 *mauritanica* (Figs. 3c, 3d) displayed a predicted loss of potential area in its current range in
351 Sierra Morena (34% RCP2.6, 66% RCP8.5), while stable areas (53% RCP2.6, 24% RCP8.5)
352 could persist south of Guadalquivir Valley and in the Tingitan Peninsula. Future suitable areas
353 also extended to the north Atlantic coast of Portugal for both scenarios (13% RCP2.6, 10%
354 RCP8.5). *Carex panormitana* (Figs. 3e, 3f) yielded a remarkable reduction of its potential
355 distribution area under the two future climate scenarios (66% RCP2.6, 95% RCP8.5) with
356 respect to its current distribution range. The potentially future suitable areas were very low (8%
357 RCP2.6, 3% RCP8.5), mostly recovered in Balearic Islands (Fig. 3e, Fig. S3). A 26% of stable
358 areas were found in the coast of Tyrrhenian Islands (Corsica, Sardinia, and Sicily) for the
359 RCP2.6, whilst the RCP8.5 scenario only showed 1%.

360 PCAs showed (see Fig. S4) a similar environmental space both for present and future in
361 2081-2100 under RCP2.6 scenario, with also analogous percentages for the explained variances
362 along their PC1s (54.2% and 56.2%, respectively) and PC2s (27.8% and 23.5%, respectively).
363 *Carex reuteriana*, including its subspecies, displayed a wider environmental niche than *C.*
364 *panormitana* both in the present and the future.

365 4. Discussion

366 4.1 Geographically-driven strong genetic structure and contrasting patterns of genetic 367 diversity

368 The strong genetic structure for the present time (Figs. 1, 2a) supports the genetic clusters
369 previously obtained by Jiménez-Mejías et al. (2011) and Benítez-Benítez et al. (2018). AFLPs
370 revealed a slight differentiation between the two subspecies of *Carex reuteriana*, in congruence
371 with their allopatric distribution (Fig. 1), suggesting the importance of geographic speciation
372 during their differentiation (Cabej, 2012; Sobel, 2016). For the subspecies *reuteriana*, DAPC and
373 BAPS analyses recovered a single genetic cluster likely due to gene flow between its
374 populations, whilst for subspecies *mauritanica* two genetic clusters with geographical
375 congruence were identified (Figs. 1, 2a). Thus, the latter subspecies seems to have been
376 influenced by the role of Guadalquivir Valley as a barrier to dispersal since there exists a clear
377 genetic differentiation between Sierra Morena and Betic populations (Figs. 1c, 2a, Table 2). This
378 pattern was previously pointed out in other studies about the sect. *Phacocystis* (Jiménez-Mejías

379 et al., 2011; Benítez-Benítez et al., 2018), as well as in other Mediterranean plant groups (Ortiz
380 et al., 2008; Casimiro-Soriguer et al., 2010; Tremetsberger et al., 2016; Fernández i Marti et al.,
381 2018). By contrast, populations at both sides of the Strait of Gibraltar display a higher genetic
382 similarity (Figs. 1c, 2a), supporting this area as a single connected refuge (Marañón et al., 1999;
383 Arroyo et al., 2008; Rodríguez-Sánchez et al., 2008; Molina-Venegas, 2015). The higher DW
384 value and genetic diversity in the subspecies *mauritanica* (324.723, 0.170; see Table 1) than
385 subspecies *reuteriana* (154.041, 0.142; Table 1) seems to correspond with the higher genetic
386 structure found across the populations of the first subspecies. This higher allelic rarity identified
387 in the subspecies *mauritanica* from these southernmost, rear-edge populations of the Iberian
388 Peninsula could be a reflect of their long-term isolation and persistence in refugia during
389 Pleistocene glaciations (Médail and Diadema, 2009; Provan and Maggs, 2011; see Last Glacial
390 Maximum projections in Benítez-Benítez et al., 2018).

391 On the other hand, *C. panormitana* displayed a significant genetic structure between
392 Sicilian-Tunisian and Sardinian populations for all genetic analyses (Figs. 1, 2a), as shown in
393 other Tyrrhenian endemics (e.g., Bittkau and Comes, 2005; Molins et al., 2018). The genetic
394 similarity found across the Strait of Sicily in different group of plants (e.g., Fernández-Mazuecos
395 and Vargas, 2011; Lo Presti et al., 2011; De Castro et al., 2015; Tremetsberger et al., 2016), also
396 identified in *C. panormitana* (Fig. 1), suggests an important dispersal route between Sicily and
397 North of Africa. The genetic diversity in the Tyrrhenian *C. panormitana* is much lower than in
398 its mainland sister *C. reuteriana* (0.133 and 0.184 respectively, Table 1), supporting the finding
399 that species with wider distribution ranges combined with larger population sizes frequently
400 display higher levels of genetic diversity than restricted endemic plants (López-Pujol et al., 2009,
401 2013; García-Verdugo et al., 2015; Fernández-Mazuecos et al., 2016). Nonetheless, high genetic
402 diversity has been reported in some endemic species with narrow distributions from the
403 Mediterranean region (e.g., Mameli et al., 2008; Mayol et al., 2012; Fernández-Mazuecos et al.,
404 2016; Jiménez-Mejías et al., 2015). Specifically, *C. panormitana* displays striking contrasting
405 patterns between its disjunct populations, with lower genetic diversity but larger distribution
406 range in Sardinia, while higher diversity and distinctiveness but extremely reduced distribution
407 in Sicily and Tunisia (only one population each; Urbani et al., 1995, 2013). These higher DW
408 values could be due to a long-term persistence and isolation of Sicilian-Tunisian populations.
409 This agrees with Sicily and NE Tunisia serving as glacial refugia during the Last Glacial

410 Maximum (e.g., Schönswetter et al., 2003; Magri et al., 2006; Médail and Diadema, 2009;
411 Jiménez-Mejías et al., 2012; see Last Glacial Maximum projections in Benítez-Benítez et al.,
412 2018). Sardinian populations were the only that displayed a significant number of clones.
413 Therefore, the clonality detected within them could be probably responsible at least in part for its
414 low genetic diversity. This low genetic diversity in Sardinian populations could have been also
415 caused by a population bottleneck (see below).

416 ***4.2 Future reduction of potential distribution and genetic diversity under GCC***

417 Our climatic predictions show that at the end of the century the loss of distribution range
418 will be clearly higher than the range gain for both Mediterranean species (Fig. 3; e.g., Casazza et
419 al., 2014; Al-Qaddi et al., 2017; Vessella et al., 2017; Kougioumoutzis et al., 2020). This could
420 convey ecological consequences because *C. reuteriana* and *C. panormitana* play important roles
421 within the ecosystem functioning of rivershores where they inhabit (Rodríguez-Gutián et al.,
422 2017). Although the more restricted *C. panormitana* seems to be more negatively affected by
423 climate change effects (Fig. 3e, 3f) than the more widespread *C. reuteriana* (Fig. 3a-d), their
424 potential distribution range will respond in similar ways under future GCC in terms of habitat
425 loss. Given the ecological dominance of these two species in their respective ranges, their
426 disappearance could even alter the communities and boundaries of habitats that these species
427 help define (see Benítez-Benítez et al., 2021).

428 *Carex reuteriana* would maintain a larger distribution area (Fig. 3) and environmental
429 space (Fig. S4), as well as greater genetic diversity (Table 1) than *C. panormitana*, despite many
430 of its populations could be potentially wiped out in response to the GCC (Fig. 3a-d; Fig. S3).
431 Whether these populations would disappear, the dominant phytosociological associations (*Carici*
432 *reuterianae-Betulum celtiberica*, Rodríguez-Gutián et al., 2017 and *Caricetum tartessianae*,
433 Molina, 1996; Navarro et al., 2001; Rufo Nieto and de la Fuente, 2011) that forms part of the
434 Iberian riparian forests will be affected. *Carex reuteriana* subsp. *reuteriana* might be able to
435 migrate and change its distribution range (Bussotti et al., 2015) whilst conserve its ecological
436 niche according to the displacement of climatic suitability to northwards of its current
437 distribution range (e.g., Alsos et al., 2012; Wróblewska and Mirski, 2018). The relatively high
438 levels of genetic diversity and ecological width of species may also enable in situ local

439 adaptations, triggering high resilience to environmental changes and in turn buffering the loss of
440 its genetic diversity (Aubin et al., 2016; Bussotti and Pollastrini, 2017; Lima et al., 2017).
441 However, it has been reported that species might not be able to shift their distribution range
442 toward suitable conditions as fast as the GCC is taking place (Loarie et al., 2009) and thus their
443 survival could also depend on other factors (e.g., phenotypic plasticity, adaptive capacity,
444 dispersal or colonization ability; Hoffmann and Sgrò, 2011; Razgour et al., 2019). A significant
445 loss of suitable potential areas is predicted for both subspecies of *C. reuteriana*, although with
446 slight differences between them (Fig. 3a-d), whilst their genetic structure also could suffer
447 important changes in the future (2081-2100; Fig. 2). Specifically, the subspecies *mauritanica*
448 could undergo gene flow limitation across the Strait of Gibraltar, strengthening genetic structure
449 between Morocco and southernmost Iberian Peninsula populations (Fig. 2b; e.g., Escudero et al.,
450 2008; Ortiz et al., 2008; Terrab et al., 2008). In contrast, populations at both sides of the
451 Guadalquivir Valley could increase their genetic admixture.

452 Otherwise, the more restricted *C. panormitana* seems to recover very little available
453 potential habitat and great habitat loss as well as reduction in its genetic diversity in the future
454 (Fig. 3; Table 1; Fig. S3). On the contrary, the current genetic structure of this Tyrrhenian
455 endemism (Sicilian-Tunisian vs. Sardinian populations, Fig. 2) could remain stable over time.
456 Predictions are especially alarming under the pessimist emission scenario (95% RCP8.5, Fig. 3f),
457 which indicate that this species could almost disappear in the future as a direct consequence of
458 the GCC. The extinction of *C. panormitana* will probably affect the functioning of the
459 ecosystem, as it dominates the riparian communities with *Carex pendula* forming a
460 phytosociological association (*Caricetum pendulo-panormitanae*, Gianguzzi et al., 2013). Our
461 findings support the hypothesis that local extinction rates appear to be higher in species with
462 restricted distribution, although it could also depend on their specific niche characteristics
463 (Lavergne et al., 2006; Gray, 2018). Specifically, the low genetic diversity found in *Carex*
464 *panormitana*, which would likely decrease in the future, foretells little capacity to adapt to the
465 new climatic conditions and therefore to modify its niche, which may contribute to its extinction
466 (Dagnino et al., 2020; Olave et al., 2019; Fig. 3e, 3f; Table 1; Fig. S4). However, a niche shift
467 during the Pliocene has been proposed for this lineage of plants (Benítez-Benítez et al., 2018),
468 which suggests that it may retain certain adaptation capacity when facing future climatic
469 changes.

470 Although distribution models do not incorporate factors such as biotic interaction,
471 dispersal or adaptation into future projections, our results demonstrate the usefulness of
472 combining SDM and molecular genetic analysis to approach the future of species. Specifically,
473 the Mediterranean Basin is considered a vulnerable hotspot, where plant extinction is already
474 taking place (Médail, 2017; Orsenigo et al., 2018; IUCN, 2021) and as much as 3.000 species
475 (Malcolm et al., 2006) have been predicted to become extinct in the future under GCC scenarios
476 (Bellard et al., 2014). Likewise, the threat by desertification and expansion of arid regions
477 (IPCC, 2014) are also being induced by global warming. Those habitats which have been
478 inferred to remain mostly stable under ongoing GCC (e.g., parts of southernmost areas of Iberian
479 Peninsula, northern Africa, and Tyrrhenian Islands; Médail and Diadema, 2009) have been
480 considered as important refugia of current diversity (Temunovic et al., 2013). Nevertheless, the
481 permanence of species in these areas will depend not only on the future climatic conditions but
482 also the species ability to resist other threats like anthropogenic fragmentation or habitat loss
483 (Thomas et al., 2004; Malcolm et al., 2006; Newbold et al., 2015; Hoffmann et al., 2018).

484 ***4.3 Conservation and survival issues in C. panormitana***

485 Our results express great concern about the conservation perspectives and future viability
486 of *Carex panormitana*. A review of different assessments of its conservation status clearly points
487 to a conflicting situation. Assessments at different geographical scales frequently result in the
488 application of different criteria and conservation categories due to considering different parts of
489 the taxon range. However, in the case of the restricted endemic *C. panormitana*, the whole range
490 was considered. On the one hand, it has been listed as a species with conservation priority for the
491 European Union in the Annex II of the Habitat Directive 92/43/CEE, and it has also been
492 catalogued as threatened in different regional red lists from Italy (“critically endangered (CR)”
493 by Conti et al., 1997; and “endangered (EN)” by Rossi et al., 2013; Urbani et al., 2013). On the
494 other hand, it has been classified as “least concern (LC)” in the European Red List of Vascular
495 Plants (Bilz et al., 2011) and at the global level in the IUCN Red List (Domina, 2011).
496 Importantly, the IUCN global assessment is based on a wrong distribution of the species
497 (Domina, 2011), which includes regions of Greece as part of the distribution range of *C.*
498 *panormitana*, whereas it is absent there (Jiménez-Mejías et al., 2014). This was probably due to a
499 misleading identification with the closely related *C. acuta* or *C. kurdica*, which is present in the

500 Balkan Peninsula (Jiménez-Mejías et al., 2014). Thus, *C. panormitana* requires, in view of our
501 results, a reassessment of its conservation status at the global level applying the IUCN criteria
502 and guidelines (IUCN, 2012, 2019; respectively).

503 The only known Sicilian population is located in the outskirts of Palermo city (Urbani et
504 al., 1995; 2013) along the shores of the Oreto River (Gianguzzi et al., 2013). This population
505 displays high genetic distinctiveness (see DW values; Table 1) and has important historical
506 value, as it is the type locality of the species (Gussone, 1844; see Jiménez-Mejías et al., 2014),
507 but its habitat is considered especially sensitive to disturbances (Thiébaud, 2006). The proximity
508 of this population to Palermo increases human pressure through different threats (e.g., urban
509 expansion, pollution of river water by agricultural and domestic activities, invasive species, etc.)
510 leading to habitat degradation and fragmentation (Gianguzzi et al., 2013). Moreover, a
511 fragmentation of this population and a decline in its size have been detected in recent years
512 (Gianguzzi et al., 2013). Habitat fragmentation and small population size could produce loss of
513 genetic diversity (Table 2) through processes like interruption of gene flow between
514 subpopulations, inbreeding and genetic drift. The latter process could also entail the loss of rare
515 alleles.

516 In view of our results, conservation measures should be urgently implemented to
517 safeguard the future of *C. panormitana* and the habitats where it lives. The reinforcement of
518 natural populations through the translocation of individuals between populations should be
519 avoided due to the strong genetic differentiation and distinctiveness found between Sicilian-
520 Tunisian and Sardinian populations. Accordingly, a specific conservation program should be
521 designed for *C. panormitana* which should include collection of seeds (and propagules) and ex-
522 situ storage in germplasm banks. Seed collecting (and ex situ growing of living individuals)
523 should be conducted separately for each of the three regions where this species inhabits
524 (Sardinia-Sicily-Tunisia), that should constitute different management units, to adequately
525 represent its genetic diversity and maintain its genetic structure. *Carex panormitana* displays
526 high levels of genetic diversity in Sicily and Tunisia, representing an important reservoir for
527 conservation of genetic resources, whilst Sardinian populations show an important genetic
528 impoverishment (Table 1). In some of the latter, the number of individuals could have severely
529 decreased due to river floods (M. Urbani, pers. comm.), perhaps producing bottlenecks that

530 would help to explain the low genetic diversity. Likewise, there are also at least one population
531 (Cantoniera Pirae' onni) in which overgrazing by cattle seems to be hindering flowering of
532 individuals (M. Urbani, pers. comm.), which could reduce sexual reproduction and therefore
533 perhaps affecting genetic diversity (e.g., Wu et al., 2010; Fernández-Mazuecos et al., 2016;
534 Souto and Tadey, 2019). Pursuant to the foregoing, it would be of paramount importance
535 investing on projects for biodiversity conservation, maintenance of the structure and ecosystem
536 functions, as well as decreasing the degree of disturbance in the habitat to guarantee its long-term
537 survival.

538 On the other side, the distribution range of *C. panormitana* is poorly known in Tunisia,
539 where its presence was not noted until a few years ago due to the confusion with other species of
540 *Carex* sect. *Phacocystis* (Jiménez-Mejías et al., 2014). Prospections would be also desirable in
541 NE Algeria, as one of the recently discovered Tunisian populations (Jiménez-Mejías et al., 2014)
542 is fairly close to the border (see Benítez-Benítez et al., 2018). Therefore, further field and/or
543 herbarium surveys are needed for this area of NW Africa to search for new populations which
544 may influence its conservation status.

545 **5. Conclusions**

546 Our results establish a clear genetic differentiation and strong structure both between and within
547 *C. panormitana* and *C. reuteriana* (including both its subspecies *reuteriana* and *mauritanica*).
548 The finding of genetic clusters according to disjunct areas suggests restricted gene flow among
549 populations and a significant role of geographical barriers. *Carex reuteriana* showed higher
550 levels of genetic diversity than *C. panormitana*, which presented the lowest values in Sardinia
551 probably due to the importance of vegetative reproduction. On the contrary, *C. panormitana*
552 displayed a greater genetic distinctiveness than both subspecies of *C. reuteriana*. Our future
553 scenarios of climate change forecast a reduction in the genetic admixture and diversity in most
554 populations of both species. In addition, SDM infer an overall loss of potential area for the three
555 taxa, especially for *C. panormitana* which could lose almost its entire distribution range and even
556 disappear under the most severe GCC scenario in the further future (RCP8.5 in 2081-2100).
557 These results, combined with the conflicting conservation assessments previously proposed for
558 *C. panormitana* and the extant threats to its persistence, support the urgent need to reassess

559 globally the conservation status of this Tyrrhenian restricted endemic and implement ex-situ/in-
560 situ conservation measures.

561 To sum up, this work displays how SDM in conjunction with molecular data can be used
562 to forecast the effects of GCC on the potential distribution and future dynamics of genetic
563 diversity and structure of species in the future. Therefore, this could be a useful approach for
564 conservation management and planning, helping the allocation of resources for priority species
565 and/or populations.

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Figure 1

Figure 1

Main results of the analyses of present genetic structure in *C. reuteriana*-*C.*

***panormitana*.** (a) Neighbor-joining tree based on Nei-Li genetic distances obtained from AFLP phenotype for all sampled individuals; numbers above branches indicate bootstrap values (>50%); (b) DAPC scatter plot showing the discriminant function (axis x) and density (axis y) across two principal components; (c) BAPS admixture bars obtained for all sampled individuals, each represented by a bar. Colours represent the taxonomic or geographic adscription of samples: *C. reuteriana* subsp. *reuteriana* (blue), *C. reuteriana* subsp. *mauritanica* (green), and *C. panormitana* (red -Sardinia-, pink -Sicily-, and purple -Tunisia-). In DAPC (b), purple represents both Sicilian and Tunisian populations of *C. panormitana*; and lines below each density plot represent individuals. In BAPS (c), two shades of green are used to represent underlying geographic structure found within *C. reuteriana* subsp. *mauritanica* (dark green -Iberian Peninsula-, light green-North Africa-).

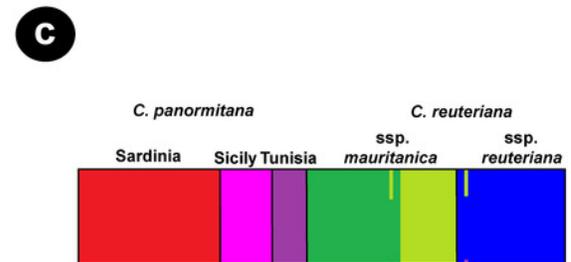
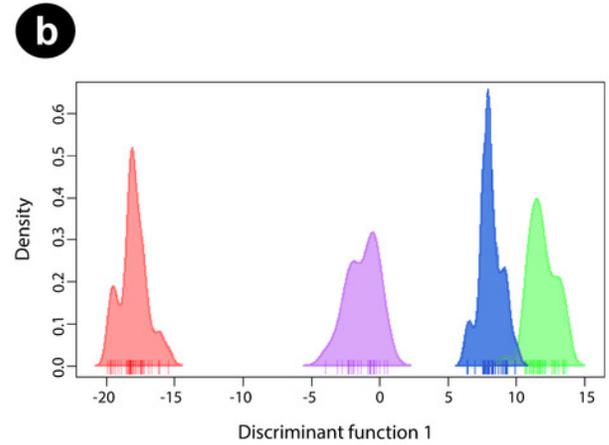
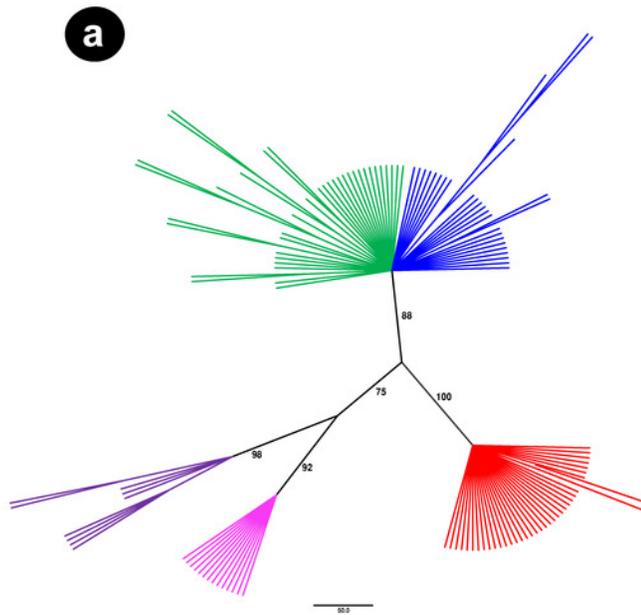


Figure 2

Figure 2

Population genetic structure analyses. (a) Population genetic structure for present times inferred by STRUCTURE, and (b) for future conditions (2081-2100) under RCP2.6 climate scenario projected with POPS. Circles indicate sampled populations of *C. reuteriana* subsp. *reuteriana*, *C. reuteriana* subsp. *mauritanica*, and *C. panormitana*. Colours are as in Fig.1 and represent the identified genetic groups (K=2 for the three taxa). Pie charts show the proportion of individuals in each population assigned to each of the two genetic groups.

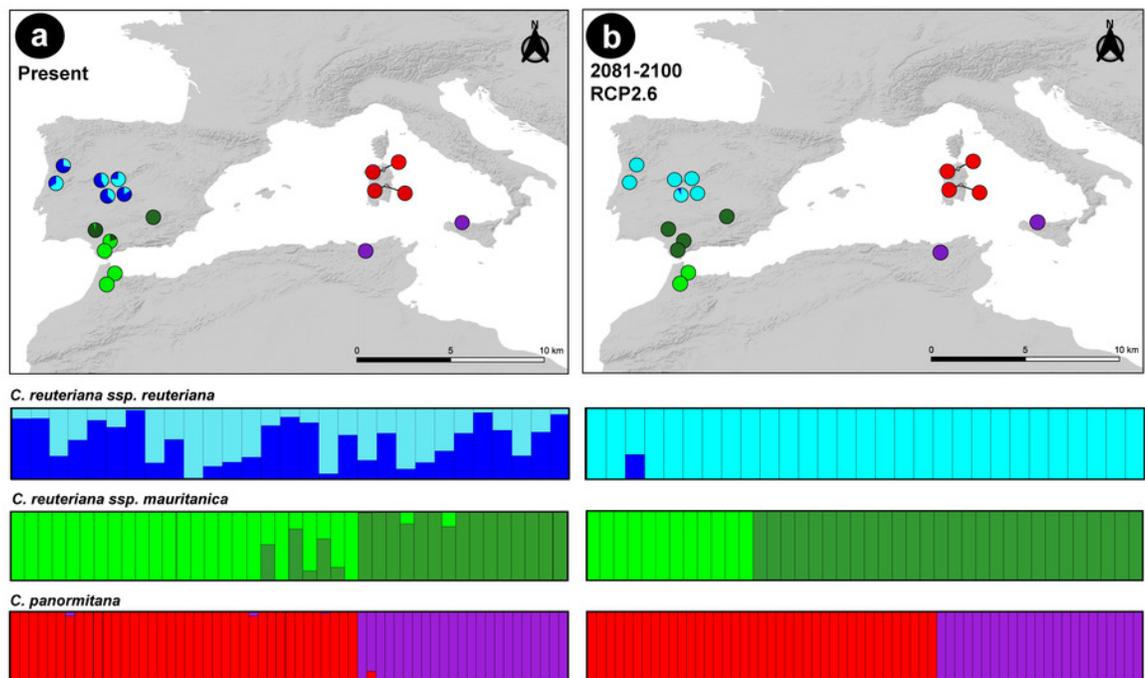


Table 1 (on next page)

Table 1

Geographic location and voucher of each sampled population of *C. reuteriana* (two subspecies) and *C. panormitana*, average gene diversity at ramet and genet level for present and future (2081-2100) under RCP2.6 GCC scenario, and allelic rarity levels (DW). Labelling of the populations specifies the taxa (REU = *Carex reuteriana* subsp. *reuteriana*; MAU = *Carex reuteriana* subsp. *mauritanica*; PAN = *Carex panormitana*), and the TDWG botanical country abbreviation (Brummitt, 2001) [MOR = Morocco, POR = Portugal, SAR = Sardinia, SIC = Sicily, SPA = Spain, TUN = Tunisia]. Herbarium acronyms are according to Index Herbariorum (Thiers, 2020). Populations inferred as extinct (IAE) under RCP2.6 scenario for 2081-2100 are not included in the genetic diversity calculation.

Taxon/ Population	Locality	Voucher/ Herbarium	Number of individuals	Longitude/ Latitude	DW present (genet)	Mean gene diversity ± SD (present; ramet level)	Mean gene diversity ±SD (present; genet level)	Mean gene diversity ± SD (future; ramet level)	Mean gene diversity ±SD (future; genet level)
<i>C. reuteriana</i>			69		905.394	0.184		0.176	
<i>C. reuteriana</i> ssp. <i>reuteriana</i>			29		154.041	0.142		0.107	
REU_POR- TM_1	Portugal, Tras os Montes, Lamego, Bigorne, Petrouca	M. Escudero <i>et</i> <i>al.</i> , 37ME07 (UPOS- 7374)	6	-7.88/41.03	13.558	0.106 ± 0.064			
REU_POR- BL_2	Portugal, Beira Litoral, Coimbra, Lousã	M. Escudero <i>et</i> <i>al.</i> , 60ME07 (UPOS- 7373)	7	-8.23/40.10	11.873	0.083 ± 0.048			
REU_SPA- Av_3	Spain, Ávila, Sierra de Gredos, Las Chorreras del Tormes	J.M. Marín, 5504JMM (UPOS- 1004)	5	-5.16/40.34	25.219	0.110 ± 0.069		IAE	
REU_SPA- CcN_4	Spain, Cáceres, Valley of Jerte	P. Jiménez- Mejías & I. Pulgar, 24PJM13 (UPOS-	4	-5.75/40.22	9.278	0.114 ± 0.077		IAE	

			5449)					
REU_SPA-CcS_5	Spain, Cáceres, Ibor river	P. Jiménez-Mejías <i>et al.</i> , 24PJM13 (UPOS-5449)	4	-5.44/39.62	21.162	0.183 ± 0.122		IAE
REU_SPA-To_6	Spain, Toledo, Navalucillos	P. Jiménez-Mejías <i>et al.</i> , 60PJM13 (UPOS-5479)	3	-4.66/39.64	9.189	0.158 ± 0.120		IAE
<i>C. reuteriana</i> ssp. <i>mauritanica</i>			40		324.723	0.170		0.164
MAU_SPA-Se_7	Spain, Sevilla, El Ronquillo, Rivera de Huelva	P. Jiménez-Mejías, 35PJM07 (UPOS-7372)	7	-6.17/37.67	21.125	0.155 ± 0.089		
MAU_SPA-CaGu_8	Spain, Cádiz, El Gastor, Guadalete river	P. Jiménez-Mejías, 34PJM07 (UPOS-7371)	5	-5.45/36.88	8.625	0.100 ± 0.063		

MAU_SPA-CaAl_9	Spain, Cádiz, Alcornocales Natural Park,	P. Jiménez-Mejías & I. Pulgar, 17PJM07 (UPOS)	8	-5.59/36.55	24.108	0.084 ± 0.048	
MAU_SPA-J_10	Spain, Jaén, Despeñaperros	P. Jiménez-Mejías & L. Reina, 67PJM09 (UPOS)	8	-3.06/38.39	25.558	0.103 ± 0.058	IAE
MAU_MOR-Lao_11	Morocco, Tanger, Rif, Oued Laou	A.J. Chaparro <i>et al.</i> , 8AJC05 (UPOS-1637)	5	-5.30/35.14	13.032	0.131 ± 0.081	
MAU_MOR-Lou_12	Morocco, Tanger, Rif, Oued Loukos	A.J. Chaparro <i>et al.</i> , 3AJC05 (UPOS-1630)	7	-5.44/35.03	24.417	0.114 ± 0.066	
C. panormitana			61		436.028	0.128	0.133
Tunisia-Sicily			23		114.057	0.115	
PAN_TUN_13	Tunisia, Jendouba, El Feija National Park	P. Jiménez-Mejías & J.E. Rodríguez, 132PJM13 (UPOS-6636)	9	8.31/ 36.49	36.949	0.067 ± 0.038	IAE
PAN_SIC_15	Italy, Sicily, Fiume Oreto	D. Cusimano s.n. (SS)	14	13.34/38.09	51.900	0.077 ± 0.041	

Sardinia			38		110.234	0.032	0.037		
PAN_SAR-Bau_16	Italy, Sardinia, Bau Mela river, Villagrande	M. Urbani, 2013 (SS)	9	9.42/39.98	11.239	0.024 ± 0.015	0.033 ±0.022	0.024 ± 0.015	0.033 ±0.022
PAN_SAR-Pira_17	Italy, Sardinia, Cantoniera, Pirae'omni, Villagrande	M. Urbani, 2013 (SS)	10	9.40/ 40.02	7.471	0.026 ± 0.016	0.031±0.021	0.026 ± 0.016	0.031±0.021
PAN_SAR-Ber_18	Italy, Sardinia, Ramacaso river, Berchidda	M. Urbani, 2013 (SS)	8	9.24/ 40.82	7.934	0.023 ± 0.015			
PAN_SAR-Cal_19	Italy, Sardinia, Miriacheddu river, Calangianus	M. Urbani, 2013 (SS)	11	9.26/ 40.89	7.577	0.012 ± 0.008	0.028±0.030	0.012 ± 0.008	0.028±0.030

Table 2 (on next page)

Table 2

AMOVA analyses for AFLPs data. The first group includes for comparisons the *Carex reuteriana*-*C. panormitana* complex and for each species separately. The second group compares the four AFLPs groups found.

1

2

Grouping compared and source of variation	d.f.	Sum of Squares	Variance components	Percentage of variation
1 - Whole dataset (2 groups: <i>C. reuteriana</i> s.l. vs. <i>C. panormitana</i>)				
Among groups	2	622.957	6.24105	28.06%
Among pops.	15	800.836	6.46433	37.22%
Within pops.	112	798.007	7.12506	34.73%
<i>C. reuteriana</i> s.l.				
Among pops.	10	369.104	4.65780	39.82%
Within pops.	57	593.411	10.41071	60.18%
<i>C. panormitana</i>				
Among pops.	3	44.146	1.16277	68.76%
Within pops.	55	204.596	3.71993	31.24%
2- Whole dataset (4 groups: <i>C. reuteriana</i> ssp. <i>reuteriana</i> vs. <i>C. reuteriana</i> spp. <i>mauritanica</i> vs. <i>C. panormitana</i> (Sicily + Tunisia) vs. <i>C. panormitana</i> (Sardinia))				
Among groups	3	920.170	8.24129	42.13%
Among pops.	14	503.623	4.19747	21.46%
Within pops.	112	798.007	7.12506	36.42%
<i>C. reuteriana</i> ssp. <i>reuteriana</i>				
Among pops.	5	117.297	2.70605	20.36%
Within pops.	23	243.393	10.58230	79.64%
<i>C. reuteriana</i> ssp. <i>mauritanica</i>				
Among pops.	5	251.807	6.05238	37.02%
Within pops.	34	350.018	10.29464	62.98%
<i>C. panormitana</i> (Sicilian-Tunisian populations)				
Among pops.	1	90.374	7.64231	53.51%
Within pops.	21	139.452	6.64059	46.49%
<i>C. panormitana</i> (Sardinian populations)				
Among pops.	3	44.146	1.35354	41.40%
Within pops.	34	65.144	1.91599	58.60%

3

Figure 3

Figure 3

Predicted changes of potential distribution inferred by Biomod. Projections comparing present and future times (2081-2100) under RCP2.6 (top maps) and RCP8.5 (bottom maps) climatic change scenarios for (a, b) *C. reuteriana* subsp. *reuteriana*, (c, d) *C. reuteriana* subsp. *mauritanica*, and (e, f) *C. panormitana*. Future projections represent the consensus SDM averaged across six GCMs. Percentages indicate the proportion of potential range inferred to be gained, lost, or to remain stable in the future in comparison with the present, according to the following colour scheme: red areas are currently suitable areas predicted to be lost, orange areas are currently suitable areas projected to remain stable, and green areas are currently unsuitable areas projected to become suitable. Black dots represent current occurrences of each taxon used for SDM.

2081-2100

