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What is the origin of the Scottish populations of the European endemic *Cherleria sedoides* L. (Caryophyllaceae)?

Cherleria sedoides L. (*Minuartia sedoides* (L.) Hiern) is a montane perennial which, with some species in *Minuartia* sect. *Spectabiles*, is more closely related to *Scleranthus* than to other *Minuartia* species and is therefore best restored to the reinstated and redefined genus *Cherleria*. Reconstruction of the ancestral area of the clade containing *C. sedoides* suggests that it evolved in the Alps or the Balkan peninsula. The species now has an unusual distribution, being present in the mountains of southern Europe and Scotland but absent from the Arctic. Three historical scenarios which might have led to the presence of the species in Scotland are outlined and tested by a molecular analysis comparing Scottish populations with populations from the Pyrenees and the Alps. The sampled populations show little variation in ITS/ETS but much more in cpDNA. The latter reveals a major division between some Alpine material and the other Alpine, Pyrenean and Scottish plants. Once the anomalous Alpine haplotypes are excluded, Scottish populations are at least as variable as those from the Alps and Pyrenees, and are closely related to both. We conclude that they have not undergone a long period of isolation, nor have they originated by recent, long-distance dispersal from the Alps or Pyrenees. They appear to be derived from a metapopulation which was probably widespread at the Last Glacial Maximum and gave rise to the Alpine, Pyrenean and Scottish plants.

Keywords: Arctic, Arctic-montane, Alps, cpDNA, *Minuartia*, Pyrenees

Introduction

The Arctic-montane or Arctic-alpine plant species in Britain and Ireland have long been a source of fascination. Many field botanists have enjoyed tracking down the rarer species in their remote, montane habitats, whilst phytogeographers have relished the equally difficult if more cerebral problem of explaining their disjunct distributions (Raven & Walters, 1956; Ratcliffe, 1977). Edward Forbes (1846) was the first to propose that many species of the ‘alpine flora’ or ‘Scandinavian type’ had formerly occupied wider ranges in the ‘Glacial epoch’ (as it was then conceived), ranges which had become fragmented as they retreated northwards or upwards onto high ground in the face of a warming climate. This brilliant piece of speculation was later confirmed for some species, such as *Dryas octopetala* and *Salix herbacea*, by the detailed study of macrofossil and pollen remains in the 20th century, work which was summarised from a British perspective by Godwin (1956, 1975) and West (2000).

Many different lines of evidence have contributed to our current knowledge of the environmental changes in the Pleistocene and Holocene (Roberts 2014), changes which repeatedly disrupted the ranges of the fauna and flora of the northern hemisphere. The Pleistocene period (which started 2.5 million years ago) was marked by repeated glacial-interglacial oscillations. The last glacial period began over 100,000 years ago and the ice sheets reached their maximum extent between 25,000 and 18,000 years ago. At this time, the Last Glacial Maximum, an immensely thick ice sheet was present in northern Europe, with smaller ice caps in the mountains to the south. These excluded the current Arctic-montane species from most if not all the areas in which they currently grow. Fossil evidence indicated that many of the more wide-ranging species (including *Dryas octopetala*, *Salix herbacea*, *S. reticulata* and *Thalictrum alpinum*) were able to grow in tundra-like vegetation south of the northern ice sheet, both in southern England and at similar latitudes elsewhere in Europe (Tralau, 1963; Godwin, 1975). Strictly Alpine species had less available habitat at this period, being restricted by the growth of trees to the south and, in the case of some species, by the cold and dry steppe-like

vegetation to the north. Molecular evidence combined with geological and palaeo-environmental data indicates that many current Alpine species persisted in refugia along the south-western, southern, eastern and northern borders of the Alps; in addition some high-altitude species may have persisted on ice-free mountain tops (Schönswetter *et al.*, 2005). The retreat of the ice in the Late-glacial and early Holocene allowed these species to expand northwards and upwards, until their distribution was again restricted by the expansion of other vegetation, notably woodland and peat bog.

In recent years molecular studies have begun to provide a detailed picture of the genetic relationships of the Arctic-montane flora. Eidensen *et al.* (2013) included Scottish samples of 15 species in their study of 17 'widespread arctic-alpine species', although they interpret the term arctic-alpine very broadly; the species they studied are variously classified by Preston & Hill (1997) as Arctic-montane, Boreo-arctic Montane, Boreal-montane and Boreo-temperate. Their results show the affinity of Scottish populations with populations of the same species occurring in Iceland and Fennoscandia. This is clear both for individual species such as *Arabis alpina*, *Deschampsia (Avenella) flexuosa* and *Kalmia (Loiseleuria) procumbens*, and from the synthesis of all the results as a 'metamap' illustrating both areas of genetic association and the barriers between these areas. The lowlands of northern Europe represent a barrier between the area of genetic affinity in Iceland-Scotland-Fennoscandia and the populations in the Alps. This study supports the conclusions reached in an earlier review by Westergaard *et al.* (2008), based in part on the same datasets, which also highlights the affinities of the Scottish populations with northern European or Eurasian populations, and the absence from Scotland of genetic groups found in southern Europe. It is also clear from this review by Westergaard *et al.* that Scottish populations have a low level of genetic diversity and distinctiveness when compared with other members of the same genetic groups elsewhere in northern Europe. Westergaard *et al.* conclude that the Arctic-montane species in Scotland are most likely to have colonised the deglaciated areas of Britain from unglaciated tundra south and east of the northern ice sheets, and they suggest that their lack of genetic diversity is a result of recolonisation by cohorts with limited genetic diversity, the so-called 'founder effect'.

These conclusions, based on intraspecific variation, are consistent with the evidence provided by the distribution of species. Of the 79 Arctic-montane species recognised by Preston & Hill (1997) in the British Isles, 56 are found in both the Arctic further north and in the mountains of the Temperate zone further south in Europe, 18 are found in the Arctic but are absent from the European mountains further south and just three are found in the mountains and in Britain or Ireland but not in the Arctic. The three species which clearly indicate a link between the central European mountains and Britain or Ireland are *Cherleria (Minuartia) sedoides*, *Gagea (Lloydia) serotina* and *Minuartia recurva*. Two more species, *Arabis ciliata* and *Cochlearia pyrenaica*, are members of taxonomically critical complexes in which there is currently no consensus about species limits; if narrowly defined they may represent further species which are present in the mountains of mainland Europe and Britain or Ireland but not in the Arctic.

This paper concerns *Cherleria sedoides* L., Cyphel, which is confined in the British Isles to Scotland. We first describe the distribution and habitat of the species, and then outline alternative (but not mutually exclusive) historical scenarios which might have led to the presence of this species in Scotland. We then attempt to provide evidence which will allow these hypotheses to be tested, initially by drawing upon a study of *Minuartia sensu lato* to establish the ancestral area of the species and then by describing a more detailed study of the molecular variation within this species.

Nomenclature of British taxa follows Stace (2010) except for the name *Cherleria sedoides*, which is preferred to *Minuartia sedoides*. Dillenberger & Kadereit (2014) have shown that the genus *Minuartia* as traditionally defined is highly polyphyletic. *Minuartia sedoides*, together with some members of *Minuartia* sect. *Spectabiles*, form one of the clades which cannot be retained as part of *Minuartia*; this clade is in fact sister to *Scleranthus*. *Cherleria* is the oldest available generic name for this group. Since a revision of this newly circumscribed *Cherleria* (by A.J.M.) is in progress, Dillenberger & Kadereit did not combine the additional species into *Cherleria*. The other species of *Cherleria* are therefore still given their old *Minuartia* names in this paper; they include *M. biflora* and *M. doerfleri*.

Distribution, habitat, altitudinal range and floral biology of *Cherleria sedoides*

Cherleria sedoides is found in the central and eastern Pyrenees, the Alps, the Dinaric Alps and the Carpathians in mainland Europe, and in central and northern Scotland (Jalas & Suominen 1983; Fig. 1). For brevity, the populations in mainland Europe are referred to in this paper as ‘Continental European’.

Cherleria sedoides is usually found as densely caespitose plants in exposed, rocky mountain habitats or in rock fissures (Fig. 2a-b). Although usually found in dry habitats, it can also grow on wetter soils in flushed grassland, by streams and, exceptionally, in areas with longer periods of snow cover than characterise its usual, exposed sites (Lippert, 1966; Favarger & Montserrat, 1990; Villar *et al.*, 1997; Lusby & Wright, 2001). In these moister sites it can grow with a laxer habit (Fig. 2c-d). Its soil preferences vary across its European range. In the Pyrenees it always grows on siliceous substrates (mainly on granite), and Favarger & Montserrat (1990) describe it as a strict calcifuge in Spain. However it is found over a greater range of soils in the Alps, although it is most frequent in base-poor sites (Ellenberg, 1996; Aeschmann *et al.*, 2004). In its more southerly Scottish sites it is limited to base-rich rocks, especially calcareous schists, but further north it is also found on base-poor substrates (Rothero, 1994). Its altitudinal ranges in the Pyrenees (2000-3200 m) and the Alps (1700-3800 m) are similar (Favarger & Montserrat, 1990; Ellenberg, 1996; M.S.D., pers. obs.). In Scotland it is usually found between 600 and 1200 m but it grows at lower altitudes in the north-west, especially in the Inner Hebrides (Rum, Skye), descending to 165 m on ultrabasic rocks on Rum (Pearman *et al.*, 2008). There are modern records from three sites on the north coast of Scotland, all in West Sutherland: between Port Vasgo and Achiniver, NC56 (M. McCallum Webster, 1970), Bettyhill, NC76 (J. Grant Roger, 1951) and Strathy East, NC842661 (J.K. Butler, 1971, 1972).

According to Knuth (1908), who reports the observations of Müller (1881) and Schulz (1890) from the Alps, the flowers of *Cherleria* have half-concealed nectar secreted between the bases of the stamens, and are visited by small flies. There are both hermaphrodite and female flowers, distributed gynodioeciously (i.e. there are some hermaphrodite and some functionally female plants) or gynomonoeiously (i.e. with both hermaphrodite and functionally female flowers on the same plants). The flowers are described as homogamous or slightly protandrous (Schulz) or distinctly protandrous, so that automatic self-pollination is usually prevented (Müller). There must, therefore, be a degree of outcrossing in this species, despite its small, usually apetalous flowers.

Historical scenarios which would lead to the occurrence of *C. sedoides* in Scotland

The current areas in which *C. sedoides* grows in Scotland and the Alps would have been largely covered by ice at the Last Glacial Maximum (LGM) at c. 20,000 BP (Siegert, 2001). A number of scenarios can be suggested which might have led to the presence of *C. sedoides* in Scotland, including the following:

- 1) the Scottish and Continental European populations were separate at the LGM, growing in different areas, and colonised their current range without coming into contact with each other;
- 2) the core populations of *C. sedoides* are those in Continental European mountains, and the species spread to Scotland in the post-glacial period from this area;
- 3) the Scottish and Continental European populations are relicts of populations which were not always distinct, and might even be regarded as one large metapopulation in space and time, which retreated northwards to Scotland and upwards to the current montane localities in Continental Europe after the LGM.

Scenario 1 suggests that the Scottish and Continental European populations were derived from an ancestral range which became fragmented long before the LGM. This scenario has also been suggested as an explanation for the genetic divergence between the Welsh populations of the temperate herb *Meconopsis cambrica* and those in the mountains of France and Spain (Preston *et al.*, 2012; Valtueña *et al.*, 2012). Scenario 2 is the ‘out of the Alps hypothesis’ of Schönswetter *et al.* (2003) and is exemplified by *Ranunculus glacialis*, which appears to have colonised northern Europe from source populations in the eastern Alps in postglacial times. One variant of Scenario 2 is the possibility that *Cherleria sedoides* reached Scotland by long-distance dispersal on one or more occasion. Long-distance dispersal of vascular plant seeds is inherently less likely than that of smaller cryptogam spores, and molecular studies of the Alpine flora have provided only a few examples of long-distance dispersal even within the Alpine massif (Schönswetter *et al.*, 2005). However, disjunct populations of *Adenostyles alpina* in the mountains of southern Europe appear to result from long-distance dispersal from the Alps and Apennines (Dillenberger & Kadereit, 2013) and, despite the fact that the European lowlands represent a barrier to gene exchange, there is evidence from *Carex bigelowii* of ‘multiple migration events between central and northern Europe’ (Schönswetter *et al.*, 2008). There is much evidence for both trans-Atlantic dispersal of the Arctic flora (Abbott & Brochmann, 2003) and of long-distance dispersal within the Arctic (Alsos *et al.*, 2007). Scenario 3 is suggested by the fossil evidence for the persistence of many wide-ranging Arctic-montane species south of the northern ice sheet. Under this scenario plants from Alpine refugia may have joined the source population, although we note that Schönswetter *et al.* (2004, 2006) have suggested that some species such as *Minuartia biflora* and *Oxytropis campestris* may have failed to persist in the vicinity of the Alps but colonised them after the retreat of the glaciers from the lowlands between the northern and Alpine ice sheets.

There are clearly scenarios other than the three listed above. One might, for example, suggest that the Scottish populations were the core populations and the species reached the Continental European mountains by long-distance dispersal. However, the above three appear to us to be the most likely. They are summarised in Table 1, where we outline the consequences each would have for the genetic divergence between the Scottish and the Continental European populations, and for the genetic variability in Scotland. Each of the scenarios is of necessity a simplification. Scenario 3, in particular, must if true over-simplify a much more complicated series of migrations and interactions between populations. Furthermore, the scenarios are not

mutually exclusive, and the Scottish populations might conceivably have originated as a combination of any two scenarios, or even all three.

Material and methods for the molecular studies

Sampling strategy

Twenty-seven populations of *Cherleria sedoides* were sampled from the Pyrenees, Alps and Scotland (Fig. 1, Table 2). In Scotland, we targeted populations where plants were known to be numerous. The Scottish material includes samples from northern populations on acidic substrates (Beinn Dearg, Ben Hope, Ben Klibreck), a relatively low-altitude population on Tertiary volcanic rocks in the Hebrides (The Storr, Skye) and a southern, calcicole population (Ben Oss). Two Scottish and two Pyrenean populations (S3, S4; P4, P5) were sampled on the same mountain, but at different altitudes. In each population, 12-20 individuals were collected, although only 1 and 5 individuals were used in the analysis of Continental European and Scottish populations, respectively. Samples were kept in paper envelopes and dried in silica gel until they were analysed.

DNA extraction and sequencing

Genomic DNA was extracted using the Qiagen DNeasy Plant Mini Kit (Qiagen GmbH, Hilden, Germany), following the manufacturer's protocol. Two nuclear and 6 chloroplast (cp) regions were amplified and sequenced using the following primers: for nuclear internal transcribed spacer (ITS), ITS5 and ITS 4 (White *et al.*, 1990); for nuclear external transcribed spacer (ETS), 18S-2L (Linder *et al.*, 2000) and ETS-Lar (Moore & Kadereit, 2013); for the cp spacer *trnQ-rps16*, *trnQ*(UUG) and *rps16x1* (Shaw *et al.*, 2007); for the cp spacer *psbD-trnT*, *psbD* and *trnT*(GGU)-R (Shaw *et al.*, 2007); for the cp 3'*trnK* intron, *trnK* 39F and *trnK* 546R (Pelsler *et al.*, 2002); for the cp spacer *ndhF-rpl32*, *rpl32*-R and *ndhF* (Shaw *et al.*, 2007); for the cp spacer *ndhJ-trnF*, *ndhJ* (Shaw *et al.*, 2007) and *TabE* (Taberlet *et al.*, 1991); for the cp *trnL* intron, *TabC* and *TabD* (Taberlet *et al.*, 1991). Amplification was carried out as described by Moore & Kadereit (2013) for nuclear regions and by Zhang *et al.* (2007) for chloroplast regions.

Sequences were manually checked and edited using Sequencher version 4.10 (GeneCodes, Ann Arbor, Michigan, USA) and were aligned by eye with MacClade version 4.08 (Maddison & Maddison, 2005). All sequences were submitted to GenBank (Table 1).

Phylogenetic analysis

Analyses of phylogenetic relationships were conducted to assess the monophyly of *C. sedoides* and to investigate the possibility of hybridisation or introgression in its lineage. To do this, a concatenated matrix with ITS and ETS sequences was analysed including 17 taxa belonging to *Minuartia* sect. *Spectabiles* and two species of *Scleranthus* as the outgroup (Supplementary Data Table S1). Three different approaches were used: maximum parsimony (MP), maximum likelihood (ML) and Bayesian inference (BI). In the last approach, two different matrices were used in the same analysis, one with the nuclear markers (ITS+ETS) and the other with *trnQ-rps16* sequences.

MP analyses were performed in PAUP version 4.0b10 (Swofford, 2002) with 1000 random taxon addition replicates, tree bisection-reconnection (TBR) branch swapping, gaps treated as missing data and MulTrees off. Branch support was assessed by parsimony bootstrap search with 1000 bootstrap replicates, random taxon addition and MulTrees off.

ML analyses were performed in RAxML version 7.6.3 (Stamatakis *et al.*, 2008) or version 8.0.24 (Stamatakis *et al.*, 2014) on XSEDE in the Cipres Portal (Miller *et al.*, 2012), with automatic termination of bootstrapping by RAxML. MODELTEST v.3.5 (Posada & Crandall, 1998) was used to find the appropriate substitution rate model, and the GTR+G+I model was used in RAxML.

BI analyses were performed with Bayesian Evolutionary Analysis by Sampling Trees (BEAST) version 1.8.0 (Drummond & Rambaut, 2007). The substitution model used was GTR+G with base frequencies estimated and a gamma model for site heterogeneity with 10 categories. A relaxed, uncorrelated, lognormal clock was used, and a birth and death prior was set for branch lengths. Other priors were at their default settings, and the Monte Carlo Markov Chain (MCMC; Drummond *et al.*, 2002) was initiated on a random starting tree. Runs were performed with 20 000 000 iterations and a sample frequency of 1000. Two BEAST runs were performed and combined with LogCombiner version 1.8 (Drummond & Rambaut, 2007) with a burn-in of 10% to reach satisfactory effective sample sizes (ESS), after assessing convergence in Tracer version 1.5 (Rambaut & Drummond, 2007). Finally, the maximum clade credibility tree was summarised in TreeAnnotator version 1.4.8 (Drummond & Rambaut, 2007) with a posterior probability (PP) limit of 0.9.

To study the phylogenetic relationships between the different sampled populations of *C. sedoides* two matrices were created with all the sequences belonging to *C. sedoides* plus one sequence from *M. doerfleri* as the outgroup. The first matrix included only nuclear sequences (ITS+ETS) and the second matrix only chloroplast sequences (the six regions indicated above). Both matrices were analysed by MP, ML and BI as described above.

Finally, a haplotype network including the six cpDNA sequences together was obtained by statistical parsimony in TCS 1.31 (Clement *et al.*, 2000) for all the *C. sedoides* samples and with *M. doerfleri* as outgroup. Unambiguous indels were coded as single characters.

RASP version 2.1b (build 20121119; Yu *et al.*, 2013) was used for reconstructing the evolution of biogeography on the maximum likelihood tree of sect. *Spectabiles* from RAxML. Twelve different areas were used: Alps, Apennines, Arctic, Balkan Peninsula, Carpathians, Caucasus, Europe north of the Alps, Jura, Lebanon, non-Arctic North America, Pyrenees and Scotland. In all cases, areas were coded according to the collection locality of the individual. Both Statistical Dispersal Vicariance Analysis (S-DIVA; Yu *et al.*, 2010) and the Bayesian Binary method were used to reconstruct ancestral areas on the maximum likelihood tree. For S-DIVA, ranges were allowed to encompass all possible combinations of areas (i.e., an ancestor could potentially have occurred throughout the range of the entire clade), but only three different ranges were allowed per node. For the Bayesian Binary method, 10 chains were run for 50,000 generations each, with sampling every 100 generations, and *Scleranthus perennis* was used as the outgroup. Ancestral ranges were allowed to encompass at most six areas and state frequencies were fixed.

Analyses of haplotype diversity were run in Arlequin version 3.5.1.2 (Excoffier & Lischer, 2010). AMOVA was run with 1,000 permutations and using pairwise distances among haplotypes. Significance of the pairwise F_{ST} was assessed with 100 permutations. Transitions, transversions, and indels were given equal weight. Two sets of analyses were run, one with the populations divided into three groups (Pyrenees, Alps, and Scotland) and one with the populations divided into two groups (Continental Europe and Scotland). Two analyses were

run for each set: one including all sequences and the other excluding the four divergent Alpine haplotypes (found in five populations).

Results

Ancestral area of C. sedoides

The ancestral area of the clade formed by *C. sedoides* and *M. doerfleri* was reconstructed as some combination of the Alps and Balkan Peninsula, depending on the analysis (S-DIVA: 0.50 Alps + Balkan Peninsula, 0.5 Alps; Bayesian Binary: 0.47 Balkan Peninsula, 0.29 Alps, 0.23 Alps + Balkan Peninsula, and 0.01 other). The values represent the probabilities of the different ancestral areas, with the probability divided evenly between all equally parsimonious ancestral areas in S-DIVA and the probability assigned according to how often each of the ancestral areas was visited by the Markov Chain in the Bayesian Binary reconstruction. Although the relationships among the various *C. sedoides* accessions were not well-supported, the earliest-diverging branches within *C. sedoides* were all Alpine, causing the clade formed by *C. sedoides* except for Alps 8, which was sister to *M. doerfleri*, to be reconstructed as Alpine (S-DIVA: 1.00 Alps; Bayesian Binary: 0.90 Alps, 0.08 Alps + Balkan Peninsula, 0.2 other). The ancestral area of ser. *Laricifoliae*, the larger clade that contains both *C. sedoides* and *M. doerfleri*, was reconstructed as Balkan, congruent with the results of Moore and Kadereit (2013).

Relationship of the Scottish and Continental European populations

Although the ITS/ETS tree (Fig. 3) shows very little variation within *C. sedoides*, cpDNA is much more variable. The cpDNA results, which are summarised as a tree (Fig. 4) and a diagram illustrating the relationships between the haplotypes (Fig. 5), therefore provide much more insight into the relationships between the Scottish populations and those in the two nearest mountain ranges on which it grows in mainland Europe.

The main division between the populations is that between five of the Alpine populations (Alps 2, 6-9) and the remaining Alpine, Pyrenean and Scottish populations. The former share a group of very distinct haplotypes (U, V, W, X) which form a well-supported clade on the cpDNA tree (Fig. 4), although there are appreciable differences between the haplotypes within this group. The populations with these distinctive haplotypes are widespread in the Alps, occurring throughout the sampled range of the species (Table 2). As we have analysed only one plant from each Alpine population, we cannot say whether any of the Alpine populations possess a mixture of haplotypes from each of the two groups or whether they all only have haplotypes from one group or the other.

The remaining populations form a group in which the cpDNA variation bears relatively little relationship to geographical origin. Two of the more frequent haplotypes, A and M, which differ by only one mutation, are shared between Scotland and the Alps and one haplotype, F, is shared between Scotland and the Pyrenees. There are no haplotypes shared between all three areas, or between the Alps and Pyrenees, but there is one group of closely related haplotypes, I, J, K, and L, which is found in all three areas and which forms a clade in the cpDNA tree (Alps 1, Pyrenees 3, 4; Scotland 4).

The cpDNA tree divides the Scottish haplotypes into five groups. Haplotype I (Beinn Dearg, below summit) forms the clade with the mainland Pyrenean haplotypes J, K and the Alpine haplotype L discussed above. Haplotype B (Beinn Dearg, summit and below summit) forms a purely Scottish clade with no obvious relationships to the rest. Haplotypes M (Ben Hope, Ben Klibreck; shared with two Alpine populations) and N (Ben Hope) form a third clade. Another

purely Scottish clade is formed by haplotypes P (Ben Hope), Q, R and S (all from The Storr); an Alpine population is sister to this clade. The fifth group consists of haplotype F (Ben Oss), which is shared with a Pyrenean population and forms a clade to which another Pyrenean population is sister. One Scottish haplotype, A (Beinn Dearg, below summit), is shared with three Alpine populations but individuals with this haplotype do not form a clade.

Diversity of Scottish and Continental European populations

The cpDNA diversity within the three main areas, Scotland, Pyrenees and Alps, measured by mean pairwise differences, is summarised in Table 3; the Scottish populations are also compared to the combined Continental European populations. When all sequences are included, the plants from the Alps (and hence those from Continental Europe) have about one order of magnitude greater sequence divergence than the other group(s). However, if the divergent Alpine haplotypes are excluded, the pairwise sequence differences within the groups are of the same order of magnitude. When the pairwise differences are calculated using one sequence per population (which is the most appropriate, given that only one individual was sequenced for all Alpine and most Pyrenean populations), the Scottish populations have the highest sequence diversity, although this difference is not statistically significant.

Discussion

Origin of the Scottish populations

With the exception of the divergent Alpine populations, the populations of *Cherleria sedoides* in Scotland are closely related to those in the Alps and the Pyrenees. This relationship is sufficient to rule out Scenario 1, outlined above, the derivation of the Scottish plants from a long-isolated population which was separate from the source of the extant Continental European populations at the LGM.

The broadly comparable level of diversity in the Scottish populations and the Continental European populations (once the divergent Alpine haplotypes are excluded) suggests that Scenario 2, the ‘out of the Alps’ (or ‘out of the Pyrenees’) hypothesis, is also unlikely. It is true that the pairwise sequence divergence in Scotland is somewhat lower than that in the Alps or Pyrenees (Table 3), but, as variation within populations is less than that between populations, this is attributable to the fact that in Scotland five individuals per population were sampled. If only one sequence from each of the six Scottish populations (and from the single Pyrenees population from which we obtained more than one sequence) is included, the pairwise sequence divergence of the Scottish populations is higher than that in the Pyrenees, and in the Alps when the divergent Alpine haplotypes are excluded.

The rejection of Scenario 2 is also consistent with the fossil record of *C. sedoides*, meagre though this is. We know of only one fossil record, from a mid Weichselian deposit at Barnwell, Cambridgeshire. The seeds were first reported by Chandler (1921) and the identity of the material was accepted by C.A. Dickson, although she considered that one seed attributed by Chandler to *C. sedoides* was from another species, cf. *Stellaria crassifolia* (Bell & Dickson 1971, Godwin 1975). Seeds of *C. sedoides* were frequent in the Barnwell deposit, and were associated with fossils of the Arctic species *Ranunculus hyperboreus* and *Salix arbuscula* and the Arctic-montane *Dryas octopetala*, *Salix herbacea*, *S. reticulata*, *Saxifraga oppositifolia* and *Thalictrum alpinum*, as well as species with other ecological and phytogeographical affinities in a characteristically diverse Weichselian assemblage (cf. West 2000). A radiocarbon date of 19,500 BP was obtained for this site (Godwin & Willis 1964), confirming that it was indeed a mid Weichselian deposit, as the flora had indicated. Although a single fossil occurrence

contributes little to our understanding of the history of *C. sedoides*, the presence of the species in Cambridgeshire, south of the northern ice sheet, at this date suggests that Scenario 2 is unlikely to be the sole explanation of the presence of the species in Scotland.

One variant of Scenario 2 is that the Scottish populations represent a contact zone between distinct populations in the Pyrenees and the Alps. We have rejected this interpretation for three reasons. The Pyrenean and Alpine populations include closely related haplotypes, and thus do not appear as distinct clades in Figs 4 and 5; levels of diversity in the Scottish and continental populations are broadly comparable, whereas higher diversity would be expected in Scottish populations if they represented a contact zone (Petit *et al.* 2003) and there are haplotypes in Scotland which we have not found in the Alpine or Pyrenean samples.

Elimination of the first two scenarios leaves us with Scenario 3, the suggestion that both the Scottish and Continental European populations were derived from populations which do not have a long history of separation, and might even be regarded as a single metapopulation in time and space. This suggestion is supported by the pattern of divergence of the haplotypes shown in Fig. 5, with the Pyrenean, Scottish and typical (as opposed to divergent) Alpine haplotypes all radiating from haplotype A, possibly an ancestral haplotype and one shared between Scotland and the Alps. The relationship of the Scottish plants to both the Pyrenean and the Alpine populations is also consistent with Scenario 3.

Comparison with studies of other species

It is, unfortunately, impossible to make quantitative comparisons of the variation in *C. sedoides* with that in other Arctic-montane species studied in Britain and mainland Europe. Other studies have different sampling strategies and have examined different marker systems (usually AFLPs). Westergaard *et al.* (2008) found that the average within-population variation is generally lower in Scotland than it is in the “main genetic group” (the group that contained the largest number of populations). However, it is higher for the Scottish group than the main group in four of 17 species, and in several others it is comparable. Even at this very general level, our data are not comparable with those of Westergaard *et al.*, as they did not calculate between-population variation because they had considerably fewer populations from Scotland than from the “main group”, and we cannot calculate within-population variation for non-Scottish populations as we sampled only a single individual per population.

Although it is difficult to compare the data of Westergaard *et al.* (2008) with ours, it is important to note that in none of the species they sampled were the Scottish populations more closely related to Alpine or non-Arctic European plants than to the Arctic plants. Although *C. sedoides* clearly cannot be more closely related to Arctic than to Alpine plants, the close relationship of the Scottish and Alpine populations is notable.

Minuartia recurva, one of the other species known from the mountains of mainland Europe and from Britain or Ireland but not from the Arctic, was studied by Deng (2012). He found little variation in the cpDNA of Irish, Pyrenean and Alpine populations, although there was more variation in the Balkans. He therefore concluded that this species probably spread from Balkan refugia relatively recently. Two haplotypes were identified from Ireland, one of which was widespread in the Alps and Pyrenees and the other recorded from the Pyrenees.

*Variation of *Cherleria sedoides* within Scotland*

Our sampling was designed to facilitate the comparison of the Scottish and Continental European populations; it is not sufficiently intensive to reveal patterns of differentiation within

Scotland. The striking contrast between the two populations sampled on Beinn Dearg suggests the possibility of small-scale variation which our sampling strategy was not designed to detect. The five plants sampled from the edge of stone stripes in the summit area (Fig. 2) all share haplotype B, whereas the five growing in turf between rocks above a stream 250 m below have three different haplotypes (A, B, I). If this is more than just a chance effect, it may reflect a relationship between environmental selection pressure and genetic diversity. Any relationships between the Scottish populations can therefore only be sketched tentatively and may well be confounded by further sampling.

Despite these cautionary remarks, the results do hint at the presence of geographically related patterns of variation, and suggest that a more detailed study of the Scottish populations would be worthwhile. Beinn Dearg, almost certainly the largest population we sampled, is also the most variable. The northern populations (Ben Hope, Ben Klibreck) are closely related to each other and the affinity of the population on Skye seems to lie with these rather than the geographically closer Beinn Dearg plants. Finally, the southern, calcicole population on Ben Oss is rather distinctive in being the one Scottish population to share a haplotype with the Pyrenees (where, however, the species grows on siliceous substrates). It would be interesting to investigate the relationship of this population to the other southern populations, such as those on Ben Lawers and other sites in the Breadalbanes.

Need for further study of the Alpine populations

Our results suggest that a more detailed study of molecular variation in the Alpine populations of *Cherleria sedoides* would be worthwhile. The divergent Alpine haplotypes clearly require further investigation. One has to assume that they were not part of the ancestral 'metapopulation' from which we suggest the other Alpine, Pyrenean and Scottish populations are derived. They are presumably derived from populations of *Cherleria sedoides* that were isolated from our putative metapopulation at the LGM, unless the haplotypes have been incorporated into the genome of that species in some other way (e.g., by later hybridisation with related taxa). In investigating their origin and history, it will clearly be necessary to sample the populations of *C. sedoides* in the Dinaric Alps and the Carpathians. We have not studied these eastern populations, as the Scottish populations can be interpreted in the light of the results from the Pyrenees and the Alps alone, but it would be interesting to know how they relate to the Alpine populations. Another question which requires further work is the absence of *Cherleria sedoides* from the Arctic. If the Scottish and Continental European populations were derived from a common source, there seems to be no obvious reason why plants from this source failed to reach the Arctic. Further research is required to establish whether their absence might have an ecological rather than an historical explanation. Climate envelope modelling would be one way of testing whether there might be a climatic explanation for its absence.

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Table 1. Historical scenarios leading to presence of *C. sedoides* in Scotland, and the expected genetic consequences of each.

	Scenario	Expected divergence between Scottish and Continental European populations	Expected variation within Scottish populations
1	Continental European and Scottish populations separate since before the LGM	High	Low to high
2	Scottish populations derived by dispersal from Continental Europe after the LGM	Low	Low
3	Continental European and Scottish populations derived from one large metapopulation	Low	Low to high

Table 2. Samples of *Cherleria sedoides* analysed. Alpine populations have codes beginning with A, Pyrenean populations with P and Scottish populations with S. GB indicates GenBank accession number.

Code	Locality	Grid reference (Scotland) or lat./long. (Continental Europe)	Altitude (m)	cpDNA haplotype	GB
A1	Italy. Aosta, Col d'Olen	45°51.76'N / 7°52.08'E	2480	L	
A2	Italy. Trentino, Passo del Paradiso	46°14.37'N / 10°34.86'E	2573	U	
A3	Austria. Tyrol, Gornegrat	46°59.50'N / 12°35.88'E	2436	A	
A4	Slovenia. Tolmin, Triglav	46°25.61'N / 13°45.12'E	1720	A	
A5	Austria. Salzburg, Schneibstein	47°34.19'N / 13°2.81'E	1833	C	
A6	Austria. Styria, Kalbling	47°32.87'N / 14°31.30'E	2179	X	
A7	Austria. Tyrol, Ötztal	46°56.69'N / 10°56.00'E	2681	V	
A8	France. Alpes-de-Haute-Provence, Barcelonnette	approx 44°24'N / 6°42'E	c. 2380	W	
A9	Italy. Trentino, Passo del Paradiso	46°14.18'N / 10°34.72'E	2634	U	
A10	Italy. Alto Adige, Sciliar	46°30.49'N / 11°34.57'E	2455	M	
A11	Switzerland. Valais, Zermatt	46°0.9'N / 7°48.05'E	2444	D	
A12	Italy. Alto Adige, Rosengarten	46°26.56'N / 11°36.76'E	2558	M	
A13	Austria. Salzburg, Speiereck	47°7.91'N / 13°38.30'E	2146	T	
A14	Switzerland. Monte Moropass	46°0.60'N / 7°58.07'E	2400	A	
P1	Spain. Ibón de Botornás	42°35.42'N / 0°40.89'E	2336	O	
P2	Andorra. Comapadrosa, Estany Negre	42°35.08'N / 1°26.11'E	2589	H	
P3	Andorra. Soldeu, Les Pessons	42°30.86'N / 1°39.92'E	2559	K	
P4	Spain. Coll de la Marrana	42°25.21'N / 2°14.92'E	2390	J	
P5	Spain. Coll de la Marrana	42°25.04'N / 2°14.57'E	2526	G	
P6	Spain. Vall de Nuria, Coma del Clot	42°23.70'N / 2°10.46'E	2240	E	
P7	Spain. Vall de Nuria, Puigmal	42°23.78'N / 2°08.02'E	2310	F	
S1	Ben Klibreck, v.c. 108	NC579298- NC580300	790	M	
S2	Ben Hope, v.c. 108	NC475497	890	M, N, P	
S3	Beinn Dearg, summit area, v.c. 105	NH259813	1050	B	

S4	Beinn Dearg, below summit, v.c. 105	NH258820	800	A,B,I
S5	The Storr, Skye, v.c. 104	NG499545	530	Q, R, S
S6	Coire Buidhe, Ben Oss, v.c. 88	NN295261	750	F

LEGENDS FOR FIGURES

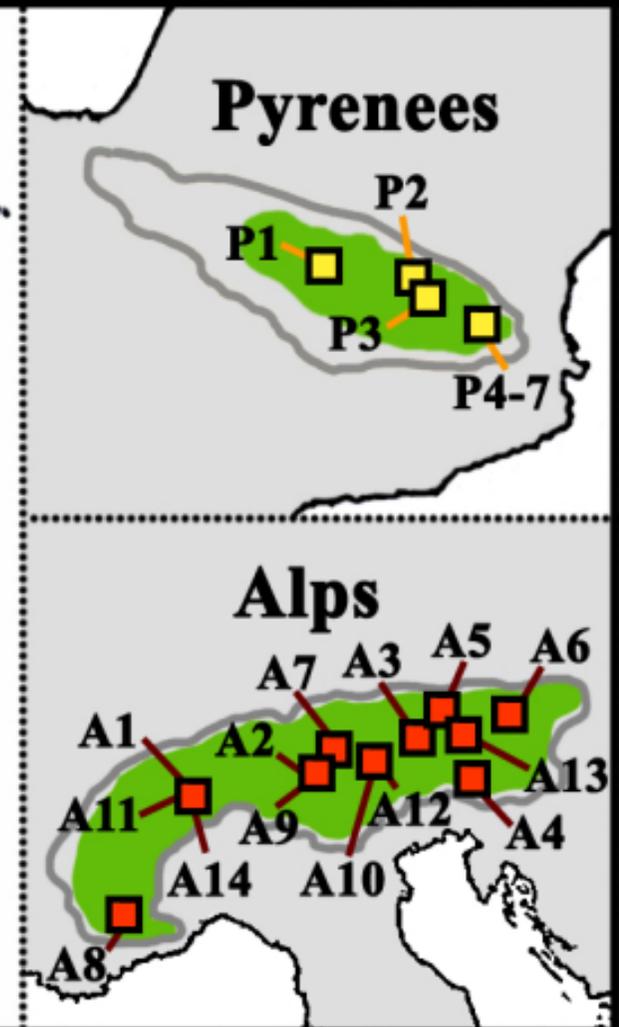
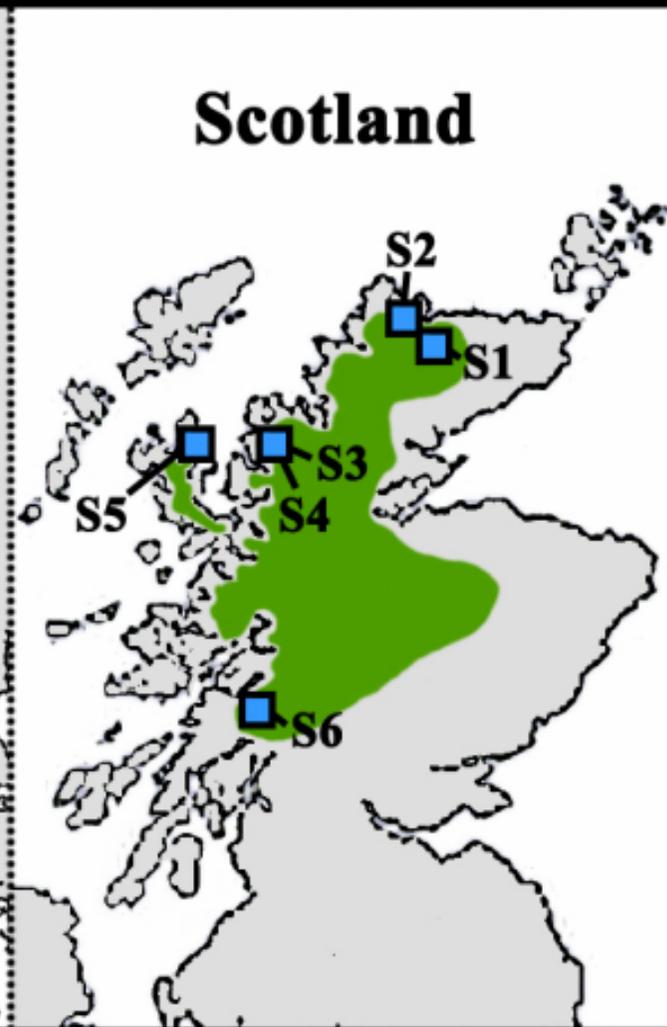
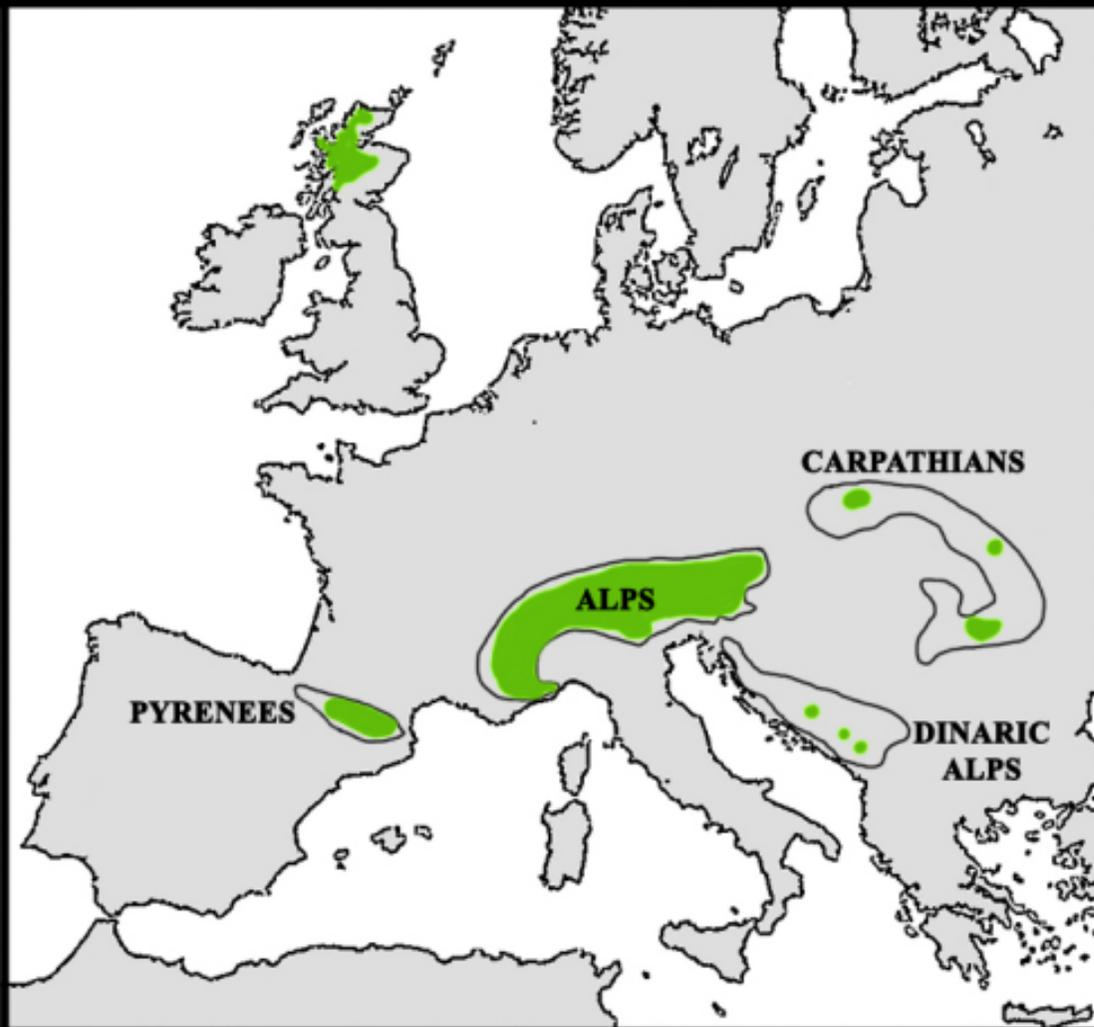
Figure 1 Distribution map of *Cherleria sedoides* and location of studied populations in the three areas sampled (Scotland, Alps and Pyrenees). The main mountain ranges are indicated by grey lines. Population codes as in Table 1.

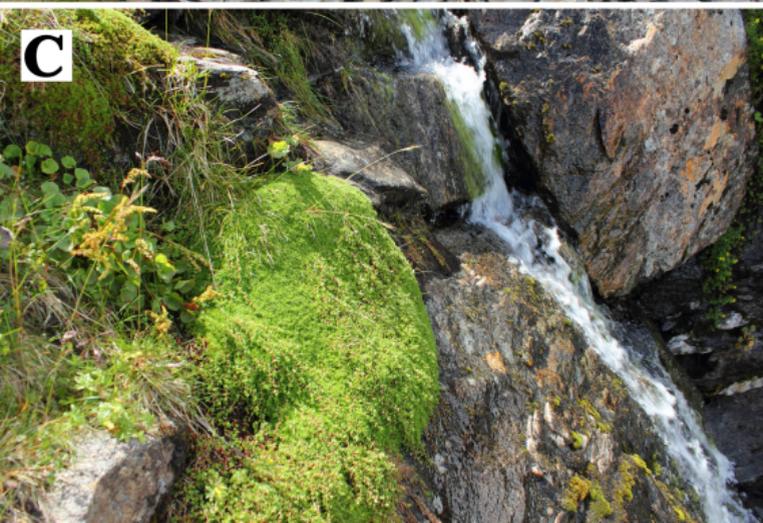
Figure 2 A, Habitat of *C. sedoides*, which grows at the edge of the stone stripes in the summit area of Beinn Dearg, v.c. 105 and (B) densely caespitose plants at this site; C, streamside habitat at Coire Buidhe, Ben Oss, v.c. 88, a site where the population includes some plants with a laxer habit (D). All photos F.J.V., 2012.

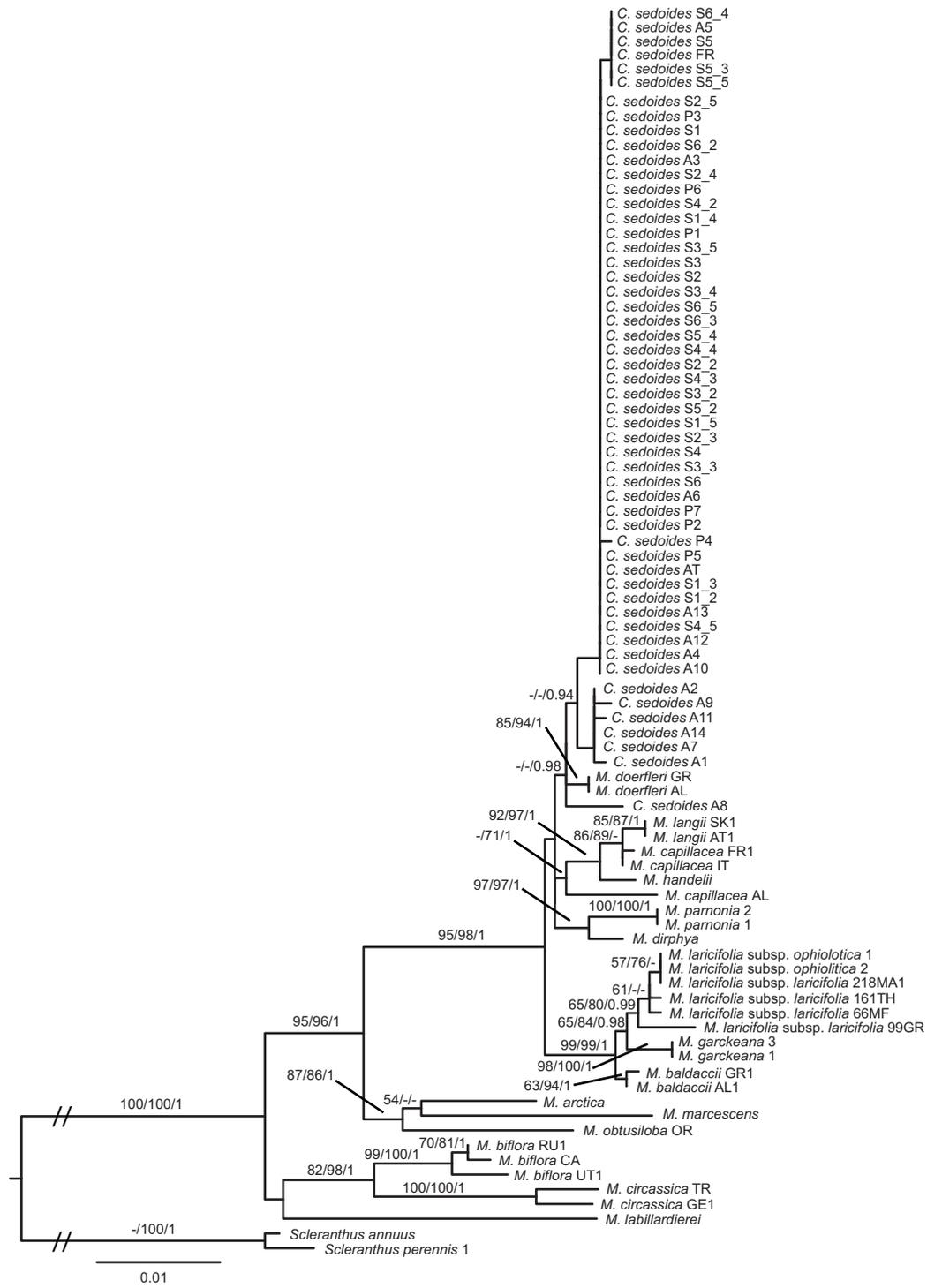
Figure 3 Phylogeny of *Cherleria*. ML phylogeny of the ITS/ETS dataset obtained with RAxML. Values above branches are maximum parsimony (MP)/maximum likelihood (ML) bootstrap values (BS)/posterior probability values (PP). Only MP BS > 50, ML BS > 70 and PP > 0.9 are shown. Branches with “/” are longer than shown. *C.*, *Cherleria*; *M.*, *Minuartia*.

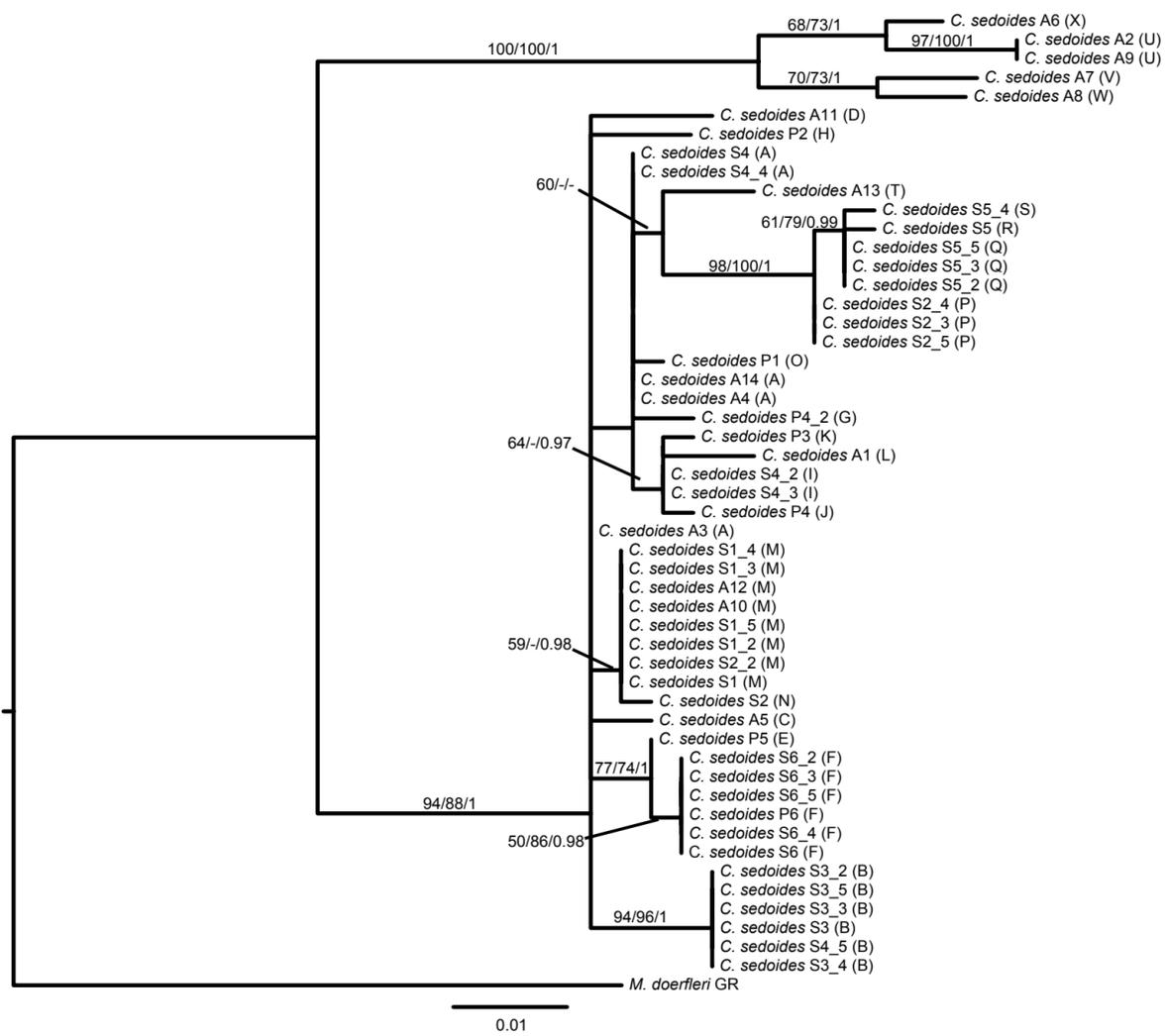
Figure 4 Chloroplast phylogeny of *Cherleria sedoides* using all six markers. ML phylogeny obtained with RAxML. Values above branches are maximum parsimony (MP)/maximum likelihood (ML) bootstrap values (BS)/posterior probability values (PP). Only MP BS > 50, ML BS > 70 and PP > 0.9 are shown. *C.*, *Cherleria*; *M.*, *Minuartia*. The haplotype letters are in parentheses following the accession names.

Figure 5 TCS statistical parsimony network of cpDNA haplotypes found in *Cherleria sedoides*. Small circles represent inferred mutational steps (open, one step; grey, more than one step, as indicated). The size of the haplotype symbol indicates the number of populations where it has been found, as shown. The regions in which the haplotypes have been found are indicated by yellow (Pyrenees), red (Alps) and blue (Scotland); their precise localities are given in Table 2.



A**B****C****D**





*Minuartia
doerfleri*

