Peripatric speciation associated with genome expansion and female-biased sex ratios in the moss genus *Ceratodon*

Marta Nieto-Lugilde[1], Olaf Werner[1], Stuart F. McDaniel[2], Petr Koutecký[3], Jan Kučera[3], Samah Mohamed Rizk[4] & Rosa M. Ros[1]

1 Departamento de Biología Vegetal, Facultad de Biología, Universidad de Murcia, Campus de Espinardo, 30100 Murcia, Spain.

2 Biology Department, University of Florida, Gainesville, Florida 32611, USA.

3 Faculty of Science, University of South Bohemia, Branišovská 31, CZ-370 05 České Budějovice, Czech Republic.

4 Genetics Department, Faculty of Agriculture, Ain Shams University, 68 Hadayek Shubra, 11241 Cairo, Egypt.

Short title: Peripatric speciation in the moss genus *Ceratodon*

[1]Manuscript received _____; revision accepted _____.

[2]Author for correspondence: Marta Nieto-Lugilde, manilu@um.es

ABSTRACT

- PREMISE OF THE STUDY: How cosmopolitan, spore dispersed species diverge and new species arise is unknown. One potentially important mechanism of sympatric speciation in flowering plants is polyploidy, often in combination with hybridization. The main aim of this study is to provide a broad perspective of the possible genetic and genome size diversity inside the moss *C. purpureus* s.l in the Mediterranean area, an important hotspot of biodiversity.
- METHODS: Mosses of the genus *Ceratodon* from mountainous areas and lowlands of the Mediterranean region and some western and central European countries were studied. To reconstruct the phylogenetic relationships five nuclear introns and a chloroplast locus were sequenced. Genome size was estimated using flow cytometry technology with propidium iodide fluorochrome. Sex was determined by a molecular marker.
- KEY RESULTS: Two well differentiated clades with high supports were resolved by the sequence analyses, discriminating two homogeneous groups of specimens: widespread *C. purpureus* and a local group from southern Spain mountains; those that present a mixed genome are interpreted as recombinants, according to a coalescent simulation analysis. The two groups also significantly differ in genome size; moreover, a third group, probably polyploid, has been found. No males were found in samples with the new genotype.

- CONCLUSIONS: A new local species evolved despite significant spore-mediated long-distance gene flow in *Ceratodon* and retains its genetic distinctiveness despite some level of hybridization with sympatric widespread *C. purpureus*. The reproductive isolation may be associated with the decrease of males.

Key words: Bryophyta, cosmopolitan species, DNA sequencing, flow cytometry, hybridization, Mediterranean mountains, phylogenetic data, polyploidy.

INTRODUCTION

The origin of new species represents a major unsolved problem in evolutionary biology (Rieseberg and Willis, 2007; Seehausen et al., 2014; Dev, 2015). Theory shows that the simplest mechanism for generating new species is through allopatric speciation, in which some portion of a species range becomes geographically isolated, allowing natural selection or genetic drift to drive allele frequency changes that ultimately generate additional reproductive barriers (Mayr, 1963; Barraclough and Vogler, 2000; Coyne and Orr, 2004). This is because even modest levels of gene flow can homogenize allele frequencies between populations, retarding divergence (Wright, 1931). While local adaptation can drive peripatric or sympatric divergence in cases where the immigrant rate is less than the intensity of selection (Lenormand, 2002), most empirical studies cannot exclude the possibility that speciation was preceded by a period of allopatry (Nadachowska-Brzyska et al., 2013; Shaner et al., 2015). This presents a paradox in species-rich groups like mosses, where long-distance migration appears to be common: speciation and diversification have occurred in spite of the fact that geographic barriers may not cause a long-term impediment to gene flow (Shaw et al., 2003; Piñeiro et al., 2012; Lewis et al., 2014a; Szövényi et al., 2014; Barbé et al., 2016).

One potential resolution to this paradox is sympatric speciation through polyploidy, which is frequent in flowering plants (Ramsey and Schemske, 1998; Mallet, 2005), and potentially in mosses (McDaniel et al., 2010; Rensing et al., 2013). Polyploidy generates a strong reproductive barrier in a single mutational event (Ramsey and Schemske, 1998; Madlung, 2013). Nevertheless, the homogeneity in bryophyte genome sizes (Voglmayr, 2000) raises the possibility that the role played by polyploidy in moss speciation may be small relative to other speciation mechanisms. The nature of the genomic, demographic, or ecological factors beyond geographic

53 isolation and polyploidy that generate reproductive barriers between nascent species of mosses remain poorly
54 characterized (McDaniel et al., 2010; Yousefi et al., 2017).

55 Within mosses, the genetic basis of reproductive barriers is best characterized among populations of
56 *Ceratodon purpureus* (Hedw.) Brid. (Ditrichaceae) (McDaniel et al., 2007, 2008). Moreover, the developing
57 genomic and laboratory tools make this species a promising model for further ecological genomic study
58 (McDaniel et al., 2016). *C. purpureus* is abundant on every continent, and grows on wide variety of substrates
59 (Crum, 1973). Molecular population genetic analyses indicated that gene flow among northern and even
60 southern hemisphere populations was frequent but tropical populations were more genetically isolated
61 (McDaniel and Shaw, 2005). These observations suggest that the current level of sampling may be insufficient to
62 detect the full scope of population structure among populations in this taxon. Indeed, partial hybrid breakdown
63 was clearly evident in crosses between a temperate and a tropical population, suggesting that reproductive
64 barriers may be in the process of evolving between ecologically distinct regions of the distribution of *C.*
65 *purpureus* (McDaniel et al., 2007, 2008). These barriers did not involve ploidy differences. However, the
66 genome size of *C. purpureus* is well-characterized in only a modest number of European samples (0.39 pg s.d.
67 0.0046, n=10, Voglmayr, 2000), leaving open the possibility that polyploidy contributes to reproductive isolation
68 among isolates from other parts of its broad cosmopolitan distribution.

69 In a previous phylogeographic analysis (McDaniel and Shaw, 2005), the Mediterranean region contained
70 several rare haplotypes that were distantly related to the common haplotypes found throughout the range of *C.*
71 *purpureus*. Here we sought to test for the existence of any relationship between the genetic diversity and DNA
72 content found in the Mediterranean area in the moss genus *Ceratodon*. McDaniel and Shaw (2005) argued that
73 frequent gene flow maintained the genetic homogeneity of the species, at least among the temperate Northern
74 Hemisphere populations, but that the divergent populations were simply outside the main area of spore rain, and
75 therefore had not yet been homogenized. Alternatively, these isolated populations could represent cryptic
76 species, and reproductive isolation evolved in spite of this gene flow (McDaniel et al., 2007, 2008). To
77 distinguish between these alternatives, we evaluated the patterns of polymorphism in five nuclear introns and a
78 single chloroplast locus in plants sampled from mountainous areas of the Mediterranean region and other

mountain regions and lowlands mostly from southern Europe. We also estimated the genome size of these isolates using flow cytometry. These data clearly show that species have evolved within the genus *Ceratodon*, accompanied by both large non-polyploid and allopolyploid changes in genome size, and potentially major changes in sexual system. These insights also highlight the complexity of peripatric speciation mechanisms in bryophytes.

MATERIALS AND METHODS

Plant material— For this study we generated genetic data for a total of 93 samples, 71 (76.4%) from Mediterranean mountain areas (47 from Spanish Sierra Nevada Mountains, 19 from Spanish central mountain ranges, three from Spanish south-eastern mountains, and two from Sicilian Mount Etna). Of the remaining 22 samples, 11 (11.8%) were from other European mountainous systems (eight from the Alps and three from the Pyrenees) and 11 specimens (11.8%) were from lowlands (three from Czech Republic, two from Germany, two from Sweden, two from United Kingdom, and two from South Africa). We collected 84 new samples for this study, all of which are deposited at MUB herbarium, and nine samples were loaned from herbaria, including BOL (Bolus Herbarium, University of Cape Town, South Africa), CBFS (University of South Bohemia, Czech Republic), S (Herbarium of the Swedish Museum of Natural History, Sweden), and two samples were donated from Laura Forrest (at Royal Botanic Garden Edinburgh, United Kingdom). We sequenced four specimens of *Cheilothela chloropus* (Brid.) Lindb. as outgroup (Voucher information and Genbank accession numbers are listed in Appendix 1).

DNA sequencing— To examine the genealogical relationships among the 93 isolates, we sequenced five nuclear exon-primed intron-spanning loci, including *rpL23A* and *TRc1b3.05* (McDaniel et al., 2013a) referenced by EST (accessions AW086590 and AW098560), *hp23.9*, *PPR* and *TBP* (McDaniel et al., 2013a, b), and a single chloroplast locus (*trn*L). We amplified all loci from all individuals in 20 µL polymerase chain reaction using Thermo Scientific DreamTaq DNA Polymerase (Thermo Fisher Scientific Inc.). The cycling conditions were 94ºC for 2 min, then 10 cycles of 94ºC for 15 s, an annealing temperature of 65ºC that dropped one degree each cycle, and 72ºC for 1 min, followed by 20 cycles of 94ºC for 15 s, 56ºC for 30 s, and 72ºC for 1 min, and

terminating with 72ºC for 7 min (McDaniel et al., 2013b). The resulting PCR products were ready to use for sequencing removing unincorporated primers and inactivates unincorporated nucleotides using Exo-AP Clean-up reaction. Sequencing was accomplished on an ABI3730XL DNA Analyzer, Applied Biosystems (Macrogen Europe, The Netherlands, Amsterdam).

Cloning of DNA sequences— In samples where we observed double peaks in the chromatograms, we cloned all loci. PCR products were isolated from agarose gels, and cloned using the CloneJet PCR Cloning Kit (ThermoFisher Scientific, Spain). Cloning efficiency and accuracy were checked using PCR reactions, successful clones then were sequenced using an ABI3730XL DNA Analyzer (Macrogen).

Phylogenetic analyses— We aligned the DNA sequences using CLUSTALW (Larkin et al., 2007) as implemented in Bioedit (Hall, 1999) and manually resolved inconsistencies in the resulting alignment. DnaSP v5 (Librado and Rozas, 2009) was used to observe characteristics such as total length with and without gaps, number of constant positions and number of parsimony-informative variable positions about all loci. We coded gaps as informative with a simple indel coding strategy (Simmons and Ochoterena, 2000) implemented in SeqState (Müller, 2005). We performed phylogenetic analyses using MrBayes v.3.2 (Ronquist et al., 2012). The need for a priori model testing was removed using the substitution model space in the Bayesian MCMC analysis itself (Huelsenbeck et al., 2004) with the option nst=mixed. The sequence and indel data were treated as separate and unlinked partitions. The a priori probabilities supplied were those specified in the default settings of the program. Posterior probability distributions of trees were created using the Metropolis-coupled Markov chain Monte Carlo (MCMCMC) method. Two runs with four chains with 1×10^7 generations were run simultaneously for loci *hp23.9*, *TBP* and *trn*L, with the temperature of the single heated chain set was the default in MrBayes. Eight chains (1×10^6 generations each) were run, with the temperature of the single heated chain set to 2 (*PPR*), 3 (*TRc1b3.05*) and 6 (*rpL23A*). Chains were sampled every 1000 generations and the respective trees were written into a tree file. The first 25% of the total sampled trees of each run were discarded as burnin. Consensus trees and posterior probabilities of clades were calculated by combining the two runs and using the trees sampled after the chains converged and had become stationary. The sump command of MrBayes was used to check whether an appropriate sample from the posterior was obtained. To do so, we first inspected visually the log

likelihood plot, which should not show tendencies to decrease or increase over time and the different runs should show similar values. Then we checked that the effective sampling size (ESS) values for all parameters reached at least 500 and finally that the Potential Scale Reduction Factor (PSRF) for each parameter was close to 1.00. The genealogies were rooted with sequences from *Cheilothela chloropus.* The final trees were edited with TreeGraph2 (Stöver and Müller, 2010). We performed phylogenetic analyses combining the new sequences generated here with other sequences for the *TBP* locus available on GenBank from Antarctica (1), Australia (1), and Eastern North America (54), which were previously reported by McDaniel et al. (2013b).

Low resolution in phylogenetic reconstructions can sometimes be caused by incongruence or conflicts in the molecular datasets that lead to different equally possible solutions (Huson and Bryant, 2006; Draper et al., 2015). To evaluate this possibility, we reconstructed a phylogenetic network based on the neighbor-net method (Bryant and Moulton, 2004) using the program SplitsTree4, version 4.13.1 (Huson and Bryant, 2006) for the six loci together. The calculations were based on uncorrected p-distances. This estimates the mean refined incompatibility score from nearby sites. The significance is then tested using a permutation test. Under the null hypothesis of no recombination, the genealogical correlation of adjacent sites is invariant to permutations of the sites as all sites have the same history. In the case of finite levels of recombination, the order of the sites is important, as distant sites will tend to have less genealogical correlation than adjacent sites (Bruen et al., 2006). To test the hypothesis of recombination in each graph, a pairwise homoplasy index (Phi-test) was calculated, which is a robust and reliable statistic to detect recombination. In accordance with Bruen et al. (2006) for the Phi test of recombination, p-value < 0.05 indicates the presence of recombination signal.DnaSP v5 (Librado and Rozas, 2009) was used to observe characteristics such as total length with and without gaps, number of constant positions and number of parsimony-informative variable positions about all loci.

Coalescent stochasticity analyses— Individual gene trees often differ from each other and from the species tree (Rosenberg, 2002; Mao et al., 2014). In order to assess whether incomplete lineage sorting alone could explain the incongruent topologies of the trees based on different markers, we compared the tree distance of simulated trees with the distance of original gene trees. To do so, we first calculated the effective population size N_e using a mutation rate per generation (μ) in the nuclear regions of $1x10^{-8}$ (McDaniel et al., 2013b). The allelic diversity

(θ_w) for the two well differentiated clades was calculated using DNAsp 5.10 (Librado and Rozas, 2009). N_e can then be calculated using the formula $\theta_w = 2\mu N_e$. Gene trees and species trees in the form of chronograms for the nuclear and *trn*L regions were obtained using BEAST v1.8.0 (Drummond et al., 2012). The clock was set to lognormal relaxed clock, the species tree prior to Yule process. The substitution model was set to HKY, gamma + invariant sites. The MCMC chain was set to 10 000 000 generations and parameters were logged every 1000 generations. The resulting gene trees and species trees were then used to simulate under the coalescent 100 new "gene trees" using the tool "coalescent contained within the current tree" in Mesquite 3.31 (Maddison and Maddison, 2017). The effective population size for the simulations was set to 500,000 based on the maximum of the estimations obtained for individual genetic regions. The tree-to-tree distances (symmetric distance) for each observed gene tree and the corresponding simulated trees (baseline distribution) were calculated with Treedist in Phylip 3.69 (Felsenstein, 2005). These distributions were compared with the distance between the two observed gene trees for each pair of markers. If the distance between the two observed gene trees is larger than the tree-to-tree distance of the gene trees and the corresponding simulated trees, incomplete lineage sorting alone is an unlikely explanation for the incongruence observed among the real gene trees (Maureira-Butler et al. 2008).

Genome size determination— We used flow cytometry (FCM) technology for 75 specimens to estimate nuclear DNA content. One shoot of each sample was chopped with a razor blade together with the internal standard *Carex acutiformis* Ehrh. 1C = 0.41 pg, Lipnerová et al., 2012) or *Bellis perennis* L. (1C = 1.56 pg; our own calibration against *Carex acutiformis*) in 1 ml of LB01 buffer (Doležel et al. 1989). The fluorochrome propidium iodide and RNase IIa (both at final concentration 50 μg/ml) were added immediately; the samples were stained for at least 10 minutes. The samples were analyzed using a Partec CyFlow SL flow cytometer equipped with a 532 nm (green) diode-pumped solid-state laser (100 mW output); the fluorescence intensity of 12000 particles was recorded. We used preferably in vitro cultivated fresh material, but for 47 samples that did not grow satisfactorily in vitro, we used dry material collected in the years 2009-2014. The fluorescence histograms were processed using FlowJo v 10.2 software (TreeStar Inc.).

Sex determination— To determinate sex, one plant per sample was employed. We amplified the *rpS15A* sex-linked locus by PCR and digested the product with HindIII. An intron in the *rpS15A* amplicon contains a cut-site

difference between the male and female products (Norrell et al., 2014) which is clearly observable in the banding patterns which were visualized after electrophoresis in an agarose gel and scored by hand. We identified the sex of 82 samples, 88.17 % of the total, which were from Sierra Nevada Mountains (42), Spanish central mountain ranges (16), Spanish south-eastern mountains (3), Sicilian Mount Etna (2), Alps (7), Pyrenees (3), South Africa (2), Germany (2), Czech Republic (3), and Sweden (2). For the remaining samples we could not unambiguously interpret the pattern in the restriction-site fragment length polymorphism in the *rpS15A* amplicon. We express the results as a proportion of males and computed the 95% confidence interval for this estimate with the *dbinom* function in R (R Development Core Team, 2017).

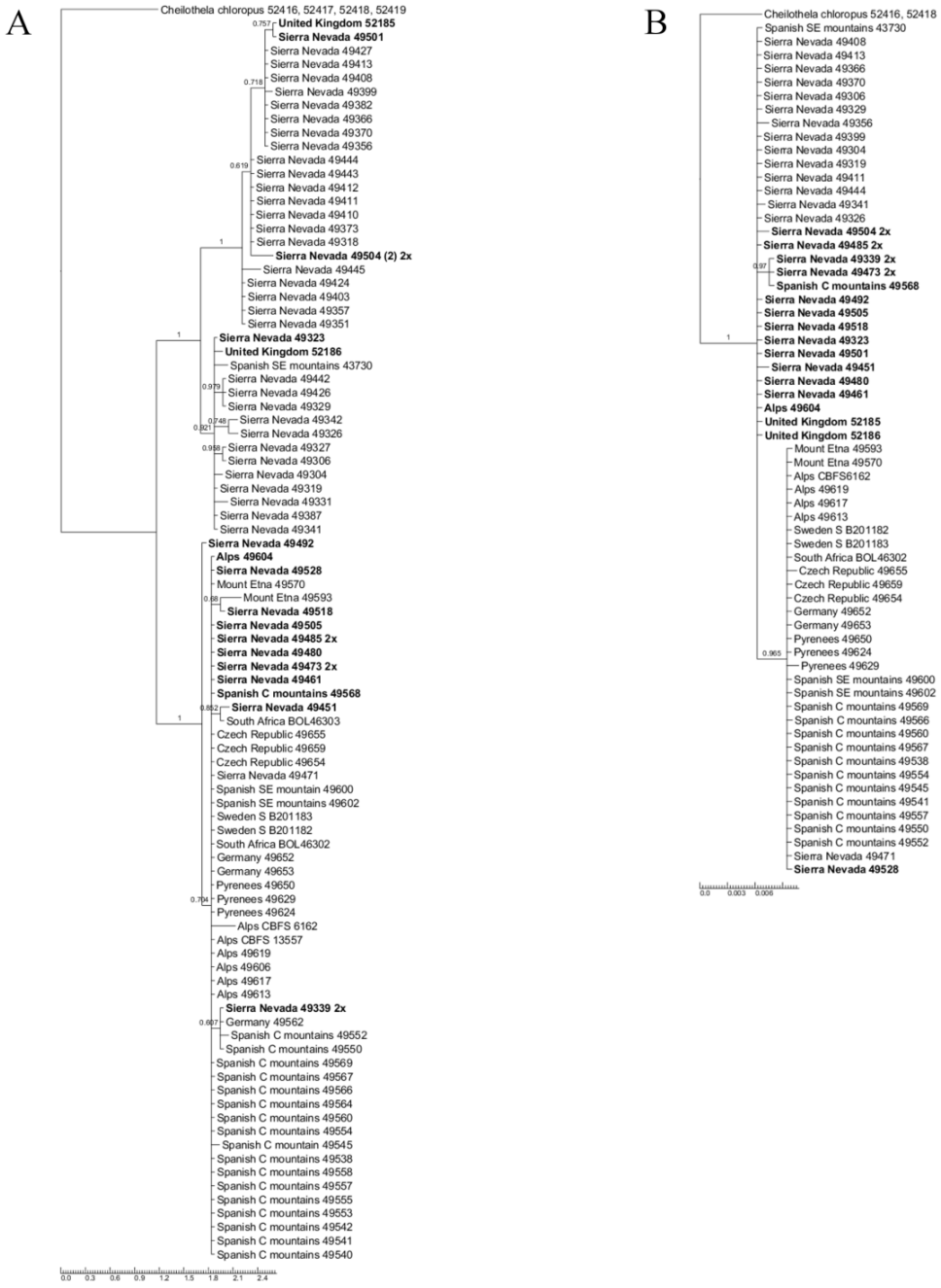
RESULTS

Phylogenetic analyses— The sequence alignments varied in total length between 207 (215 with coded gaps) to 848 (891) positions, for *hp23.9* and *rpL23A* respectively. The number of constant positions was between 186 and 715 for the above mentioned loci and the parsimony-informative variable positions differed between 5 and 95 for *trn*L and *rpL23A* respectively (Table 1). The loci *TRc1b3.05*, *rpL23A*, *TBP*, and *PPR* showed two well differentiated clades with support of 1-1 posterior probability (pp), 1-1pp, 0.956-1pp, 0.866-0.769pp, respectively (Fig. 1, see Supplemental Data with this article, Appendices S1, S2, S3). In the case of *rpL23A*, sequences of *Cheilothela chloropus* were not obtained for use as outgroup, but again two clades were resolved. The *hp23.9* locus had a support for one clade of 1 pp but the other clade had a value of 0.553 pp (Appendix S4). In all the five nuclear loci studied, one of the clades was formed always by 34 Sierra Nevada Mountains samples and one of the Spanish south-eastern mountains; we refer to this as the SN group. The second clade consistently included 42 specimens coming from the rest of the sampled areas, including one from Sierra Nevada and two from Spanish south-eastern mountains; we refer to this as the Worldwide (Ww) group. For one marker (*TBP*) we added sequences available at GenBank, including samples from Antarctica, Australia, and North America. The resulting tree topology shows that our samples give a reasonable good representation of the Ww group and that none of these additional sequences is closely related to the SN samples (Appendix S5). The remaining 17 sequenced samples were strongly resolved in either the SN clade or the Ww clade, depending on the studied

209 locus (they did not present intermediate sequences between both clades, Appendix S6); we considered these
210 samples recombinants. The term "hybrid" applied to bryophytes should strictly be used only for the sporophytic
211 hybrids (2n) (Anderson, 1980); for their gametophytic progeny (n) showing combination of parental alleles after
212 meiosis "recombinants" should be used (Shaw, 1994, 1998) in order not to confuse with hybrids observed among
213 vascular plants. The recombinants derived mainly from SN Mountains, but also from Spanish central mountain
214 ranges, the Alps and the lowlands of the United Kingdom (Fig. 2). The chloroplast locus showed one well
215 supported clade (0.965 pp) and all remaining samples with deeper coalescence ties (Fig. 1). All the samples
216 considered as recombinants based on the nuclear markers were closely related and sister to the rest of the SN
217 samples, with the only exception of one specimen from Sierra Nevada Mountains (MUB 49528), which is a
218 recombinant and belongs to the Ww chloroplast clade.
219
220 **Table 1.** Characteristics of the loci used for molecular evolutionary analyses. The genomic location "nuclear -
221 putative autosomal" is based on unpublished data.

Locus	Genomic location	Sequence length (with gaps)	Invariant sites	Parsimony-informative sites
hp23.9	Nuclear – autosomal	207 (215)	186	15
PPR	Nuclear – U/V	331 (334)	309	8
rpL23A	Nuclear – putative autosomal	848 (891)	715	95
TBP	Nuclear – autosomal	365 (365)	337	11
TRc1b3.05	Nuclear – putative autosomal	402 (417)	362	28
*trn*L	Chloroplast	320 (320)	311	5

222
223 The apparent uncertain position of some individuals is clarified by the result of the Neighbor-Net network (Fig.
224 3). Moreover for the phi-test when the six loci were studied together, a highly significant value (0.0) was
225 obtained, confirming the presence of recombination signal. Graphically two extreme groups can be observed, the
226 SN group and the Ww group, with some individuals in intermediate positions, forming a net.



227

228 **Fig. 1.** Phylogenetic trees inferred from two of the studied loci. For each tip in the trees geographical origin and
229 number of herbarium are given (numbers without letters are from MUB); 2x is used to highlight diploid samples;
230 number of equal sequences obtained by cloning is indicated between parentheses if there was more than one;
231 bold letters indicate recombinant samples. A) From nuclear *TRc1b3.05* locus and B) From chloroplast *trn*L
232 locus.

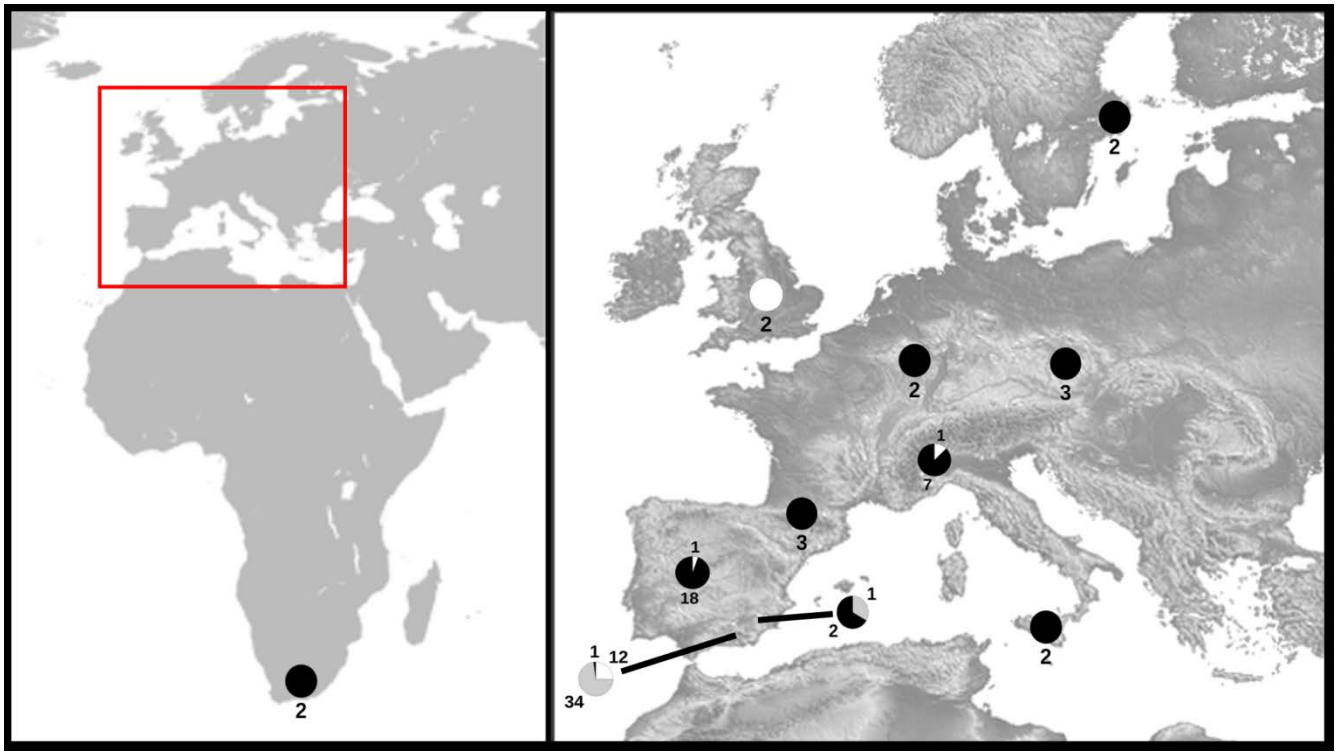


Fig. 2. Geographic location of *Ceratodon* samples included in this study. Pie charts indicate proportion of samples of each genomic group by areas (black: Ww genome group; grey: SN genome group; white: recombinant samples). The number of samples by groups in each area is given.

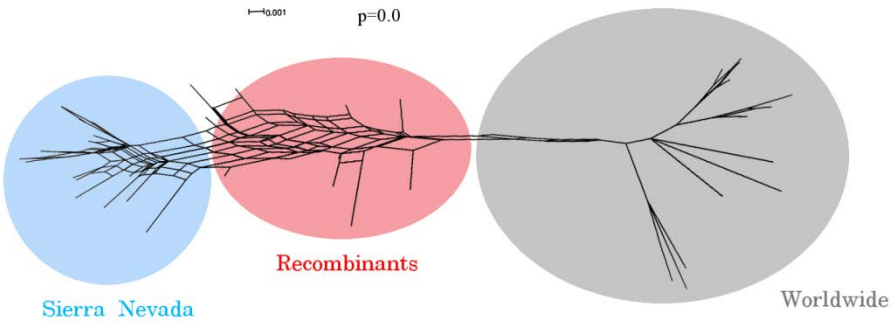


Fig. 3. Neighbor-Net network to test signals of reticulate evolution between the samples. The main groups are highlight by color circles with its names. The p value from the Phi test of recombination is indicated.

Cloning DNA sequences— Loci cloning confirmed that diploid specimens (see Flow cytometry analyses results) present two different copies of the same loci in most cases. The loci *TRc1b3.05*, *PPR* and *rpL23A* presented predominantly a single copy, although some individuals presented the two copies in other loci

(Appendix S6). Some haploid individuals presented two different copies of a locus. This may be due to the possibility of gene redundancy, which can result from unequal crossing over, retroposition or chromosomal (or genome) duplication (Magadum et al., 2013).

Coalescent stochasticity analyses— Although our data suggested the existence of recombinants between the two groups, incomplete lineage sorting and hybridization may result in similar molecular signals. To test our interpretation of the data we compared the distances of simulated trees under the hypothesis of coalescence within the species tree with the differences between gene trees of all marker pairs. In all cases at least 95% of the distances of the simulated trees were smaller than the differences between the original sequence trees (Table 2), indicating that incomplete lineage sorting alone cannot explain the different tree topologies.

Table 2. Tree distances between original and simulated trees in pairwise comparisons. The upper triangular matrix indicates the tree distance between the gene trees of molecular marker pairs. The lower triangular matrix gives the distance values for simulated gene trees using the coalescent contained within the species tree in comparison with the original gene tree. In parenthesis the percentage of the distances of the simulated trees with smaller values than the distances of the two compared original gene trees is given. Values with 100% of smaller distances are given in bold. These results indicate that incomplete lineage sorting alone cannot explain the different tree topologies.

	***trn*L**	***hp23.9***	***TBP***	***TRc1b3.05***	***rpL23A***	***PPR***
***trn*L**	-	142	98	92	94	102
hp23.9	**132-140 (100)**	-	124	110	106	128
TBP	**88-96 (100)**	**116-122 (100)**	-	100	90	48
TRc1b3.05	**84-90 (100)**	**102-106 (100)**	**90-96 (100)**	-	94	100
rpL23A	**86-92 (100)**	98-104 (95)	84-88 (95)	**86-92 (100)**	-	106
PPR	92-100 (99)	**120-124 (100)**	**38-46 (100)**	**92-98 (100)**	**100-104 (100)**	-

Flow cytometry analyses— We obtained three clearly differentiated groups of cytotypes for both fresh and dry material (Table 3, Fig. 4). Measurements from dry material gave higher values (by 18% on average) than those

from fresh material, for this reason a conversion factor (1/1.18 = 0.85) was employed to the former. When fresh and dry materials are considered together, the first cytotype had a mean value of 1C = 0.37 pg, and the second one showed 25.4% more of DNA content (1C = 0.46 pg). The third cytotype had 1C = 0.82 pg mean value of DNA content. All of specimens of Ww group belonged to the smallest cytotype while those of the SN group were categorized in the second cytotype, and the recombinant specimens were found in both the second and the third cytotype (Appendix S6).

Table 3. Nuclear DNA content expressed in pg as measured by flow cytometry. Cytotypes considered, number of samples used in the analyses (N), mean value of DNA, standard deviation and range of values obtained for each cytotype are given (* conversion factor of 0.85 is applied to dry material when fresh and dry material are combined).

	Cytotype	N	Mean (pg)	Standard deviation	Min (pg)	Max (pg)
Fresh material	a	5	0.36	<0.01	0.36	0.37
	b	20	0.46	0.01	0.45	0.48
	c	3	0.81	0.01	0.81	0.82
Dry material	a*	25	0.44	0.01	0.41	0.45
	b*	21	0.54	0.01	0.52	0.57
	c*	1	0.97	--	--	--
Fresh + dry material (*)	a+a*	30	0.37	0.01	0.35	0.38
	b+b*	41	0.46	0.01	0.44	0.48
	c+c*	4	0.82	0.01	0.81	0.82
		75				

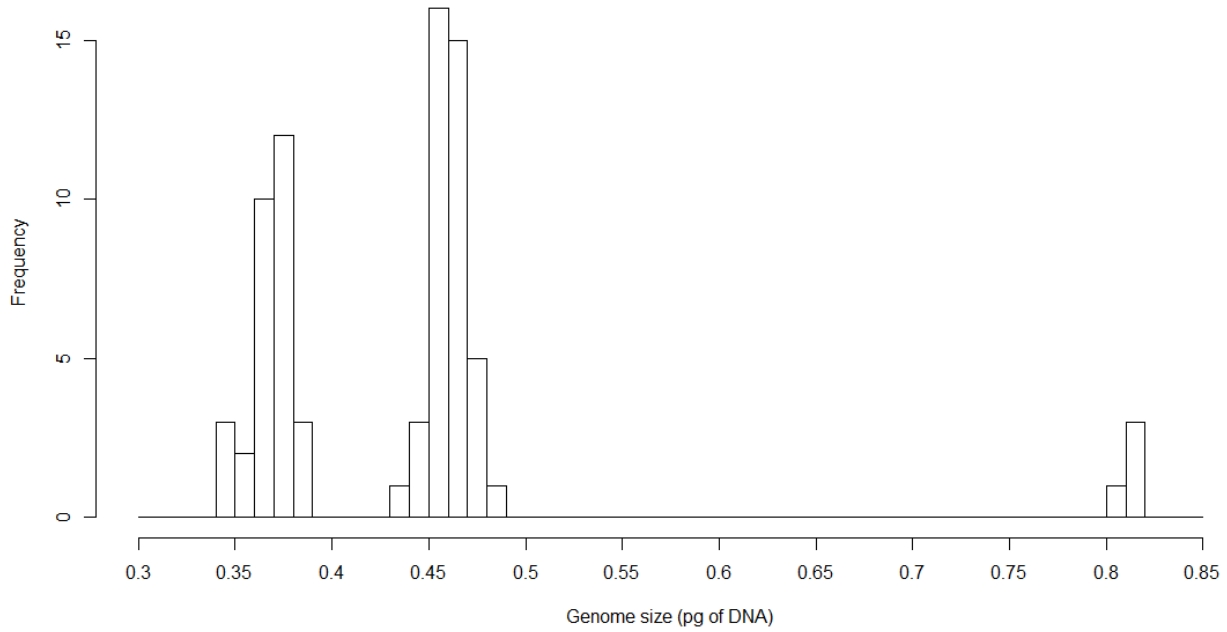


Fig. 4. Histogram of genome sizes of representative samples of *Ceratodon* generated by flow cytometry. A conversion factor of 0.85 was applied to the data obtained from dry material.

***Sex determination*—** All of the samples from SN group (29) and all the recombinant samples (15) were females, while the Ww group (38) consisted mainly of females and only two males (one from Sierra Nevada Mountains and another one from the Alps), see Appendix 1. In the case of the Ww samples, the high proportion of female samples may be due to a strong bias, as moss cushions with the presence of sporophytes were preferentially taken, because in the field the distinction between *Ceratodon* and other genera, even belonging to different orders, is sometimes difficult. This situation is different in Sierra Nevada Mountains, because there we never observed sporophytes and samples were identified in the laboratory using a microscope. If we exclude a possible bias in the case of the Sierra Nevada Mountains samples, we can conclude based on the binominal distribution that with a probability > 95% the proportion of males in the population is below 12% and males might even be completely absent.

DISCUSSION

In most major models of speciation, a period of allopatry is essential to evolve reproductive isolation (Coyne and Orr, 2004). However, in many cosmopolitan species, including many mosses and ferns, the entire habitable range of species is within the range of the dispersal distance of spores (Muñoz et al., 2004; Frahm, 2007; Pisa et al., 2013) making strict allopatry unlikely. Therefore, it is reasonable to propose that speciation mechanisms that either occur in sympatry or accommodate some gene flow contribute to generating the extant diversity in such groups. The two best-studied sympatric speciation mechanisms in plants are polyploidy and the evolution of self-fertilization (Barringer, 2007). Here we show that the evolution of a new species, closely related to the cosmopolitan *Ceratodon purpureus*, was associated with a 25% increase in genome size and a significant decrease in frequency of males (Nieto-Lugilde et al., submitted), at least superficially similar to the evolution of parthenogenetic lineages in animals. Surprisingly, although we have found neither males nor evidence of recent sexual reproduction (i.e., sporophytes) in the new species, the genetic diversity among members of this species is relatively high. Despite the long period of isolation suggested by the sequence divergence between *C. purpureus* and the new species, we have found evidence of interspecific hybridization, suggesting that the new species apparently has retained the capacity for sexual reproduction. We discuss the taxonomic implications of this discovery in Nieto-Lugilde et al., submitted. Here we use genealogical and genome size data to make inferences regarding the genetic architecture of speciation, and the demographic parameters that permit such divergence.

Taxonomists have struggled with species delimitation in the genus *Ceratodon* since the description of the genus. Burley and Pritchard (1990) found references for nearly 50 specific or subspecific taxa within *Ceratodon*, but based on an extensive survey of herbarium specimens recognized only four species, *C. antarcticus* Cardot., *C. conicus* (Hampe) Lindb., *C. heterophyllus* Kindb., and *C. purpureus*, including three infraspecific taxa (subsp. *convolutus* (Reichardt) Burley, subsp. *purpureus*, and subsp. *stenocarpus* (Bruch & Schimp.) Dixon). Previous molecular population genetic analyses indicated that disjunct populations of *C. purpureus* were sometimes very closely related, clearly showing that long distance dispersal, even among

continents, was frequent enough to erase any signal of strong population structure (McDaniel and Shaw, 2005). However, these data did not provide strong genealogical support either for or against the existence of distinct species other than *C. purpureus*. Subsequent classical genetic analyses showed that geographically and ecologically distant populations were partially reproductively isolated from one another (McDaniel et al., 2007, 2008), but these appeared to be somewhat porous reproductive barriers, and it was unclear that the populations represented different species.

McDaniel and Shaw (2005) did find some isolates of *C. purpureus* that were genetically distant from the more common haplotypes found in northern temperate regions. Here we found strong evidence that haplotypes which are distantly related to the typical *C. purpureus* haplotypes are locally abundant in the Sierra Nevada Mountains of southern Spain. We also found populations containing SN haplotypes and recombinants, together with some rare samples with the typical *C. purpureus* haplotypes. To evaluate the possibility that the segregation of these divergent haplotypes in the SN populations represents the retention of ancestral variation in the species (i.e., coalescent stochasticity causing incomplete lineage sorting) we generated coalescent simulations using BEAST and Mesquite. These simulations showed that the divergence between these two haplotypic classes was too great to be explained by coalescent stochasticity. The fact that this polymorphism is found in all of the nuclear loci that we sampled, and is geographically concentrated to the Sierra Nevada region, suggests that balancing selection is also an unlikely explanation. Collectively these data suggest that the SN haplotypes comprise a rare species sister to and partially reproductively isolated from the cosmopolitan *C. purpureus.*

The sympatric occurrence of typical *C. purpureus* haplotypes and SN haplotypes, even at modest frequencies, contradicts the suggestion by McDaniel and Shaw (2005) that the Mediterranean populations were genealogically isolated from the rest of the species as a result of decreased spore rain in peripheral populations separated by prevailing global wind patterns. If we assume that the current dispersal capabilities of *C. purpureus* represent the ancestral condition, this suggests that geography may not have been the primary isolating mechanism between the nascent species. It is certainly possible that an extrinsic factor, like a habitat preference, isolated the two species (Nieto-Lugilde et al., submitted). Remarkably, however, we detected only females in the SN species, implicating some intrinsic isolating mechanisms. Sex in dioecious bryophytes like *C. purpureus* is

determined at meiosis, by the segregation of a UV chromosome pair, meaning that ~50% of the spores produced in a population should be males. Some meiotic sex ratio variation has been observed in this species in natural populations (overall mean of proportion of males was 0.41 (0.17-- 0.72), Norrell et al., 2014) and artificial crosses (male-biased sex ratio = 60%, McDaniel et al., 2008). Even given our sample size (n = 29, with no males), we can conclude that the percentage of males in the SN populations is much lower (95% CI included 0% - 12%; additional samples not included in this study lowers the 95% confidence interval to a range of 0% - 6.7%). We do not know whether the decrease of males coincided with the speciation event, or occurred subsequent to the evolution of reproductive isolation. The evolution of apomixis or obligate selfing from historically outcrossing lineages is a well-documented route to the evolution of new species in plants (Stebbins, 1974; Barrett, 2010; Wright et al., 2013), and parthenogenetic lineages associated with the loss of males are frequent in some animal lineages (Hagimori et al., 2006; Neaves and Baumann, 2011; Montelongo and Gómez-Zurita, 2015). However, we know of no other cases where the loss of males has been associated with speciation in bryophytes.

The presence of recombinants containing both typical *C. purpureus* alleles and alleles from the SN species indicated that rare interspecies hybridization has occurred between individuals of the two species. Most of the recombinants possessed the SN chloroplast type, based on the *trnL* sequence data, suggesting that this species was more often the maternal parent (consistent with the rarity of males). We found one instance of a recombinant plant that had a typical *C. purpureus trn*L sequence, but we cannot determine whether this was a rare case of a hybridization involving a SN male (i.e., a cross in the opposite direction) or whether this resulted from a backcross of a male recombinant to a typical *C. purpureus* female. Intrinsic genetic incompatibilities are often manifest as Dobzhansky-Muller interactions, which result in asymmetric introgression patterns at the causative loci (McDaniel et al., 2008) due to the death of incompatible multi-locus genotypes. Although we sampled only six loci across the genome, the recombinants did have a tendency to have the SN alleles at the *TBP* and *rpL23A* loci. We are currently examining the frequency of polymorphism across the genome of the SN and recombinant genotypes to distinguish among forms of extrinsic and intrinsic isolation between the SN and typical *C. purpureus* populations.

The flow cytometric data also showed that members of the SN species had a genome ~25% larger than typical members of *C. purpureus*. It is possible that the speciation involved a whole genome duplication event followed by rapid genome reduction, the duplication of a large chromosomes (Inoue et al., 2015; Panchy et al., 2016), or the accumulation of transposable elements (TEs), which contribute to the extraordinary variation in genome size within even closely related species in angiosperms (Vitte and Bennetzen, 2006). Although the current data represent the most comprehensive sampling of variation in genome size in *Ceratodon*, we still lack cytological data to determinate if variation in nuclear DNA content is due to an increase in the size of chromosomes or by the increase of number of chromosomes. The variance in genome size is almost equal between the two groups, suggesting that the SN species is fixed for whatever loci underlie the genome size change. Additionally, recombinants between the two groups have the genome size of SN species, not an intermediate value, suggesting that the increase in genome size may come from a single genomic change, rather than many small changes across genome. One hypothesis is that these plants have gained DNA on the sex chromosome which comprises nearly one-third of the genome (Heitz, 1932; Jachimsky, 1935; McDaniel et al., 2007). Sex chromosomes in other organisms are known to accumulate genomic material rapidly, sometimes in large translocations, and potentially generating pronounced evolutionary and ecological consequences (Tennesse et al., 2017). We are now attempting to generate artificial crosses to evaluate the genetic basis of the genome size difference.

We also found a third rare cytotype with a genome size approximately twice that of either SN plants or typical *C. purpureus* plants. These isolates all had mixed haplotypes (i.e., gene sequences from both the SN and typical *C. purpureus* clades) and a genome size very close to the sum of the SN group and Ww group (~1.2 % smaller than the sum of the group means), suggesting that they arose from an allopolyploid event. Without more sequence or cytological data we cannot formally eliminate the possibility that the larger cytotype arose from autopolyploidy followed by hybridization, although this would require the gain of ~10 % or loss (~12 %) of the genomic DNA. Additionally, allopolyploidy is a widely observed mechanism to restore the fertility of F1s hybrids between partially reproductively isolated species with karyotypic differences and exhibit meiotic

abnormalities (De Storme and Mason, 2014). The taxonomic consequences of this third cytotype are further discussed by Nieto-Lugilde et al. (submitted).

Finally, the new SN species apparently maintains levels of genetic diversity nearly equivalent to typical populations of its sister species *C. purpureus* without obviously undergoing sexual reproduction. Moss gametophytes can persist for many years, even in relatively stressful conditions, and easily spread clonally by gametophyte fragmentation. In some cases, such fragments may be dispersed a considerable distance (Frahm, 2007, Lewis et al., 2014b). It is clear that spatially heterogeneous selection (Vrijenhoek, 1978) or frequency-dependent selection (Weeks and Hoffmann, 2008) can maintain high genetic diversity in clonal organisms. Antarctic populations of *C. purpureus*, which similarly lack any sexual reproduction, were also quite variable, although less polymorphic than was observed in the closely related nearby populations from Australia (Clarke et al., 2009). Also similar to the Antarctic studies, we found polymorphic nuclear ITS sequences between samples collected a few meters apart (unpublished data), indicating that these localities were colonized several times independently. However, unlike the Antarctic case, the SN isolates are genetically distinct from any known spore source. It is possible that sexual reproduction in the SN species generated the current variation under a past climate regime, or in undetected localities, although it is clearly far rarer than in *C. purpureus*. Further analyses of the evolutionary history of the SN population are likely to produce a better understanding of the phenomena that generate new species in cosmopolitan taxa.

ACKNOWLEDGEMENTS

The authors thank the curators of herbaria BOL, and S for providing plant material. To Laura Forrest from Royal Botanic Garden Edinburgh, United Kingdom, for donating samples. To the management group of the National Park of Sierra Nevada Natural Space for allowing us to collect samples in the National and Natural Parks. This study has been supported financially by the Spanish Ministry of Science and innovation (Projects CGL2011-22936/BOS and CGL2014-52579-R) and FEDER funds of the E.U., and an NSF grant (DEB 1541005) to SFM. M N.-L. also thanks the Spanish Ministry of Science and innovation, for being received a "Formación de Personal Investigador" fellowship (FPI program 2012) (reference BES-2012-056799).

424

425 LITERATURE CITED

426 Anderson, L. E. 1980. Cytology and reproductive biology of mosses. *In* R. J. Taylor and L. E. Levotin [eds.],
427 The mosses of North America, 37–76. California Academy of Sciences, San Francisco, California, USA.

428 Barbé, M., N. J. Fenton, and Y. Bergeron. 2016. So close and yet so far away: long-distance dispersal events
429 govern bryophyte metacommunity reassembly. *Journal of Ecology* 104: 1707–1719.

430 Barraclough, T. G., and A. P. Vogler. 2000. Detecting the Geographical Pattern of Speciation from Species-
431 Level Phylogenies. *American Naturalist* 155: 419–434.

432 Barrett, S. C. H. 2010. Understanding plant reproductive diversity. *Philosophical Transactions of the Royal*
433 *Society of London, B, Biological Sciences* 365: 99–109.

434 Barringer, B. C. 2007. Polyploidy and self-fertilization in flowering plants. *American Journal of Botany* 94:
435 1527–1533.

436 Bryant, D., and V. Moulton. 2004. Neighbor-Net: An Agglomerative Method for the Construction of
437 Phylogenetic Networks. *Molecular Biology and Evolution* 21: 255–265.

438 Bruen, T. C., H. Philippe, and D. Bryant. 2006. A Simple and Robust Statistical Test for Detecting the Presence
439 of Recombination. *Genetics* 172: 2665–2681.

440 Burley, J. S., and N. M. Pritchard. 1990. Revision of the Genus Ceratodon (Bryophyta). *Harvard Papers in*
441 *Botany* 1: 17–76.

442 Clarke, L. J., D. J. Ayre, and S.A. Robinson. 2009. Genetic structure of East Antarctic populations of the moss
443 Ceratodon purpureus. *Antarctic Science* 21: 51–58.

444 Coyne, J. A., and H. A. Orr. 2004. Speciation. Sinauer Associates, Sunderland, Massachusetts, USA.

445 Crum, H. A. 1973. Mosses of the Great Lakes Forest. University of Michigan Herbarium, Ann Arbor, Michigan,
446 USA.

447 De Storme, N. and A. Mason. 2014. Plant speciation through chromosome instability and ploidy change:
448 Cellular mechanisms, molecular factors and evolutionary relevance. *Current Plant Biology* 1: 10–33.

449 Dev, S. B. 2015. Unsolved problems in biology—The state of current thinking. *Progress in Biophysics and*
450 *Molecular Biology* 117: 232–239.

451 Doležel, J., P. Binárová, and S. Lucretti. 1989. Analysis of nuclear DNA content in plant cells by flow
452 cytometry. *Biologia plantarum*. 31: 113–120.

453 Draper, I., L. Hedenäs, M. Stech, J. Patiño, O. Werner, J. M. González-Mancebo, M. Sim-Sim, et al. 2015. How
454 many species of Isothecium (Lembophyllaceae, Bryophyta) are there in Macaronesia? A survey using
455 integrative taxonomy. *Botanical Journal of the Linnean Society* 177: 418–438.

456 Drummond, A. J., M. A. Suchard, D. Xie, and A. Rambaut. 2012. Bayesian phylogenetics with beauti and the
457 beast 1.7. *Molecular Biology and Evolution* 29: 1969–73.

458 Felsenstein, J. 2005. PHYLIP (Phylogeny Inference Package) version 3.6. Distributed by the author. Department
459 of Genome Sciences, University of Washington, Seatle.

460 Frahm, J. P. 2007. Diversity, dispersal and biogeography of bryophytes (mosses). *In* W. Foissner and D. L.
461 Hawksworth [eds.], Protist Diversity and Geographical Distribution, vol.8, Topics in Biodiversity and
462 Conservation, 43–50. Springer, Dordrecht, Netherlands.

463 Hagimori, T., Y. Abe, S. Date, and K. Miura. 2006. The First Finding of a Rickettsia Bacterium Associated with
464 Parthenogenesis Induction Among Insects. *Current Microbiology* 52: 97–101.

465 Hall, T. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows
466 95/98/NT. *Nucleic Acids Symposium Series* 41: 95–98.

467 Heitz, E., 1932. Geschlectschromosomen bei einem Laubmoss. *Berichte der Deutschen Botanischen*
468 *Gesellschaft* 30: 204–206.

469 Huelsenbeck, J. P., B. Larget, and M. E. Alfaro. 2004. Bayesian phylogenetic model selection using reversible
470 jump Markov chain Monte Carlo. *Molecular Biology and Evolution* 21: 1123–1133.

471 Huson, D. H., and D. Bryant. 2006. Application of phylogenetic networks in evolutionary studies. *Molecular*
472 *Biology and Evolution* 23: 254–267.

473 Inoue, J., Y. Sato, R. Sinclair, K. Tsukamoto, and M. Nishida. 2015. Rapid genome reshaping by multiple-gene
474 loss after whole-genome duplication in teleost fish suggested by mathematical modeling. *Proceedings of*
475 *the National Academy of Sciences of the United States of America* 112: 14918–14923.

476 Jachimsky, H. 1935. Beitrag zur Kenntnis von Geschlechtschromosomen und Heterochromatin bei Moosen.
477 *Jahrbücher für Wissenschaftliche Botanik* 81: 203–238.

478 Larkin, M. A., G. Blackshields, N. P. Brown, R. Chenna, P. A. McGettigan, H. McWilliam, F. Valentin, et al.
479 2007. Clustal W and Clustal X version 2.0. *Bioinformatics* 23: 2947–2948.

480 Lenormand, T. 2002. Gene flow and the limits to natural selection. *Trends in Ecology & Evolution* 17: 183–189.

481 Lewis, L. R., R. Rozzi, and B. Goffinet. 2014a. Direct long-distance dispersal shapes a New World
482 amphitropical disjunction in the dispersal-limited dung moss Tetraplodon (Bryopsida: Splachnaceae).
483 *Journal of Biogeography* 41: 2385–2395.

484 Lewis, L. R., E. Behling, H. Gousse, E. Qian, C. S. Elphick, J.-F. Lamarre, J. Bêty, et al. 2014b. First evidence
485 of bryophyte diaspores in the plumage of transequatorial migrant birds. *PeerJ* 2: e424.

486 Librado, P., and J. Rozas. 2009. DnaSP v5: a software for comprehensive analysis of DNA polymorphism data.
487 *Bioinformatics* 25: 1451–1452.

488 Lipnerová I., P. Bureš, L. Horová, and P. Šmarda. 2012. Evolution of genome size in *Carex* (Cyperaceae) in
489 relation to chromosome number and genomic base composition. *Annals of Botany* 111: 79–94.

490 Maddison, W. P. and D. R. Maddison. 2017. Mesquite: A modular system for evolutionary analysis. Version
491 3.31. http://mesquiteproject.org. Accesed 25/09/2017.

492 Madlung, A. 2013. Polyploidy and its effect on evolutionary success: old questions revisited with new tools.
493 *Heredity* 110: 99–104.

494 Magadum, S., U. Banerjee, P. Murugan, D. Gangapur, and R. Ravikesavan. 2013. Gene duplication as a major
495 force in evolution. *Journal of Genetics* 92: 155–161.

496 Mallet, J. 2005. Hybridization as an invasion of the genome. *Trends in Ecology & Evolution* 20: 229–237.

497 Mao, X., G. Zhu, L. Zhang, S. Zhang, and S. J. Rossiter. 2014. Differential introgression among loci across a
498 hybrid zone of the intermediate horseshoe bat (Rhinolophus affinis). *BMC Evolutionary Biology* 14: 154.

499 Maureira-Butler I. J., B. E. Pfeil, A. Muangprom, T. C. Osborn, and J. J. Doyle. 2008. The reticulate history of
500 *Medicago* (Fabaceae). *Systematic Biology* 57: 466--482.

501 Mayr, E. 1963. Animal species and evolution. Harvard University Press, Cambridge, Massachusetts, USA.

502 McDaniel, S. F., and A. J. Shaw. 2005. Selective sweeps and intercontinental migration in the cosmopolitan
503 moss *Ceratodon purpureus* (Hedw.) Brid. *Molecular Ecology* 14: 1121–1132.

504 McDaniel, S. F., J. H. Willis, and A. J. Shaw. 2007. A Linkage Map Reveals a Complex Basis for Segregation
505 Distortion in an Interpopulation Cross in the Moss *Ceratodon purpureus*. *Genetics* 176: 2489–2500.

506 McDaniel, S. F., J. H. Willis, and A. J. Shaw. 2008. The genetic basis of developmental abnormalities in
507 interpopulation hybrids of the moss *Ceratodon purpureus*. *Genetics* 179: 1425–1435.

508 McDaniel, S. F., M. von Stackelberg, S. Richardt, R. S. Quatrano, R. Reski, and S. A. Rensing. 2010. The
509 speciation history of the Physcomitrium--Physcomitrella species complex. *Evolution* 64: 217–231.

510 McDaniel, S. F., M. J. van Baren, K. S. Jones, A. C. Payton, and R. S. Quatrano. 2013a. Estimating the
511 Nucleotide Diversity in *Ceratodon purpureus* (Ditrichaceae) from 218 Conserved Exon-Primed, Intron-
512 Spanning Nuclear Loci. *Applications in Plant Sciences* 1: 1200387.

513 McDaniel, S. F., K. M. Neubig, A. C. Payton, R. S. Quatrano, and D. J. Cove. 2013b. Recent gene-capture on
514 the UV sex chromosomes of the moss *Ceratodon purpureus*. *Evolution* 67: 2811–2822.

McDaniel, S. F., P. F. Perroud, A. C. Cuming, and P. Szövényi. 2016. The *Ceratodon purpureus* Transcriptome Ushers in the Era of Moss Comparative Genomics. *In* S. A. Rensing, and P. F. Perroud [eds.], Genomes and Evolution of Charophytes, Bryophytes, Lycophytes and Ferns, 141--162. Elsevier Press, Oxford, UK.

Montelongo, T., and J. Gómez-Zurita. 2015. Nonrandom patterns of genetic admixture expose the complex historical hybrid origin of unisexual leaf beetle species in the Genus *Calligrapha*. *The American Naturalist* 185: 113–134.

Müller, K. 2005. SeqState: primer design and sequence statistics for phylogenetic DNA datasets. *Applied Bioinformatics* 4: 65–69.

Muñoz, J., Á. M. Felicísimo, F. Cabezas, A. R. Burgaz, and I. Martínez. 2004. Wind as a long-distance dispersal vehicle in the southern hemisphere. *Science* 304: 1144–1147.

Nadachowska-Brzyska, K., R. Burri, P. I. Olason, T. Kawakami, L. Smeds, and H. Ellegren. 2013. Demographic Divergence History of Pied Flycatcher and Collared Flycatcher Inferred from Whole-Genome Re-sequencing Data. *PLOS Genetics* 9: e1003942.

Neaves, W. B., and P. Baumann. 2011. Unisexual reproduction among vertebrates. *Trends in Genetics* 27: 81–88.

Norrell, T. E, K. S. Jones, A. C. Payton, and S. F. McDaniel. 2014. Meiotic sex ratio variation in natural populations of *Ceratodon purpureus* (Ditrichaceae). *American Journal of Botany* 101: 1572–1576.

Panchy, N., M. Lehti-Shiu, and S. H. Shiu. 2016. Evolution of gene duplication in plants. *Plant Physiology* 171: 2294–2316.

Piñeiro, R., M. Popp, K. Hassel, D. Listl, K. B. Westergaard, K. I. Flatberg, H. K. Stenøien, and C. Brochmann. 2012. Circumarctic dispersal and long-distance colonization of South America: the moss genus Cinclidium. *Journal of Biogeography* 39: 2041–2051.

Pisa, S., O. Werner, A. Vanderpoorten, M. Magdy, and R.M. Ros. 2013. Elevational patterns of genetic variation in the cosmopolitan moss Bryum argenteum (Bryaceae). *American Journal of Botany* 100: 2000–2008.

R Development Core Team (2017). R: A language and environment for statistical computing.

Rensing, S. A., A. K. Beike, and D. Lang. 2013. Evolutionary importance of generative polyploidy for genome evolution of haploid-dominant land plants. *In* J. Greilhuber, J. Dolezel, and J. F. Wendel [eds.], Plant genome diversity, vol. 2, 295–305. Springer, Vienna, Austria.

Ramsey, J., and D. W. Schemske. 1998. Pathways, mechanisms, and rates of polyploid formation in flowering plants. *Annual Review of Ecology and Systematics* 29: 467–501.

Rieseberg, L. H., and J. H. Willis. 2007. Plant Speciation. *Science* 317: 910–914.

Ronquist, F., M. Teslenko, P. van der Mark, D. L. Ayres, A. Darling, S. Höhna, B. Larget, et al. 2012. MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. *Systematic Biology* 61: 539–542.

Rosenberg, N. A. 2002. The probability of topological concordance of gene trees and species trees. *Theoretical Population Biology* 61: 225–247.

Seehausen, O., R. K. Butlin, I. Keller, C. E. Wagner, J. W. Boughman, P. A. Hohenlohe, C. L. Peichel, et al. 2014. Genomics and the origin of species. *Nature Reviews Genetics* 15: 176–192.

Shaner, P. J. L., T. H. Tsao, R. C. Lin, W. Liang, C. F. Yeh, X. J. Yang, F. M. Lei, et al. 2015. Climate niche differentiation between two passerines despite ongoing gene flow. *Journal of Animal Ecology* 84: 829–839.

Shaw, A. J. 1994. Systematics of Mielichhoferia (Bryaceae: Musci). III. Hybridization between M. elongata and M. mielichhoferiana. *American Journal of Botany* 81: 782–790.

558 Shaw, A. J. 1998. Genetic analysis of a hybrid zone in Mielichhoferia (Musci). *In* J. W. Bates, N. W. Ashton,
559 and J. G. Duckett [eds.], Bryology for the twenty-first century, 161–174. Maney Publishing and the
560 Brithish Bryological Society, Leeds, UK.

561 Shaw, A. J., O. Werner, and R. M. Ros. 2003. Intercontinental Mediterranean disjunct mosses: morphological
562 and molecular patterns. *American Journal of Botany* 90: 540–550.

563 Simmons, M. P., and H. Ochoterena. 2000. Gaps as characters in sequence-based phylogenetic analyses.
564 *Systematic Biology* 49: 369–381.

565 Stebbins, G. L. 1974. Flowering plants: Evolution above the species level. Belknap Press of Harvard University
566 Press, Cambridge, Massachusetts, USA.

567 Stöver, B. C., and K. F. Müller. 2010. TreeGraph 2: combining and visualizing evidence from different
568 phylogenetic analyses. *BMC Bioinformatics* 11: 7.

569 Szövényi, P., N. Devos, D. J. Weston, X. Yang, Z. Hock, J. A. Shaw, K. K. Shimizu, et al. 2014. Efficient
570 Purging of Deleterious Mutations in Plants with Haploid Selfing. *Genome Biology and Evolution* 6: 1238–
571 1252.

572 Tennessen, J. A., N. Wei, S. Straub, R. Govindarajulu, A. Liston, T. L. Ashman. 2017. Repeated translocation of
573 a gene cassette drives sex chromosome turnover in strawberries. *bioRxiv* 163808.

574 Vitte, C., and J. L. Bennetzen. 2006. Analysis of retrotransposon structural diversity uncovers properties and
575 propensities in angiosperm genome evolution. *Proceedings of the National Academy of Sciences, USA*
576 103: 17638--17643.

577 Voglmayr, H. 2000. Nuclear DNA Amounts in Mosses (Musci). *Annals of Botany* 85: 531–546.

578 Vrijenhoek, R. C. 1978. Coexistence of clones in a heterogeneous environment. *Science* 199: 549--552.

579 Weeks, A. R., and A. A. Hoffmann. 2008. Frequency-dependent selection maintains clonal diversity in an
580 asexual organism. *Proceedings of the National Academy of Sciences, USA* 105: 17872--17877.

581 Wright, S. 1931. Evolution in Mendelian populations. *Genetics* 16: 97--159.

582 Wright, S. I., S. Kalisz, and T. Slotte. 2013. Evolutionary consequences of self-fertilization in plants.

583 *Proceedings of the Royal Society of London, B, Biological Sciences* 280: 20130133.

584 Yousefi, N., K. Hassel, K. I. Flatberg, P. Kemppainen, E. Trucchi, A. J. Shaw, M. O. Kyrkjeeide, et al. 2017.

585 Divergent evolution and niche differentiation within the common peatmoss *Sphagnum magellanicum*.

586 *American Journal of Botany* 104: 1060–1072.

587 **Appendix 1.** Voucher information for the studied specimens. For each sequenced sample the next information is
588 given: herbarium code; geographical origin, gender if known (F for female, M for male), presence of sporophyte
589 if appropriate, indicated by an asterisk (*), GenBank accession numbers for the six loci studied, given in the next
590 order: *hp23.9*, *PPR*, *rpL23A*, *TBP*, *TRc1b3.05* and *trn*L; sequences obtained by cloning are indicated by their
591 GenBank accession number given in parentheses.

Ingroup

Mediterranean mountain areas

MUB 43730: Spanish south-eastern mountains, F, KP825628, KP826017, KP826181, KP826402, KP826531, KY229001. **MUB 49304:** Sierra Nevada Mountains, F, KP825703, KP826091, KP826265, KP826473, KP826601, MG050779. **MUB 49306:** Sierra Nevada Mountains, F, KP825701, KP826089, KP826263, KP826471, KP826599, KY229023. **MUB 49318:** Sierra Nevada Mountains, KP825698, KP826086, KP826260, KP826468, KP826596, –. **MUB 49319:** Sierra Nevada Mountains, KP825697, KP826085, KP826259, KP826467, KP826595, MG050780. **MUB 49323:** Sierra Nevada Mountains, F, KP825696, KP826084, KP826258, KP826466, KP826594, KY229040. **MUB 49326:** Sierra Nevada Mountains, F, KP825693, KP826081, KP826255, KP826463, KP826591, MG050781. **MUB 49327:** Sierra Nevada Mountains, F, KP825692, KP826080, KP826254, KP826462, KP826590, –. **MUB 49329:** Sierra Nevada Mountains, F, KP825690, KP826078, KP826252, KP826460, KP826588, KY229024. **MUB 49331:** Sierra Nevada Mountains, F, KP825688, KP826076, KP826250, KP826459, KP826586, –. **MUB 49339:** Sierra Nevada Mountains, F, (MG050789, MG050790, MG050791, MG050792, MG050793, MG050794, MG050795, MG050796, MG050797, MG050798, MG050799), (KP826073, MG050748, MG050749, MG050750, MG050751, MG050752), KP826248, (KP826456, MG050761, MG050762, MG050763, MG050764, MG050765), KP826583, KY229035. **MUB 49341:** Sierra Nevada Mountains, F, KP825683, KP826071, KP826246, KP826454, KP826581, MG050782. **MUB 49342:** Sierra Nevada Mountains, F, KP825682, KP826070, KP826245, KP826453, KP826580, –. **MUB 49351:** Sierra Nevada Mountains, F, KP825681, KP826069, KP826244, KP826452, KP826579, –. **MUB 49353:** Sierra Nevada Mountains, F, KP825679, KP826067, KP826242, KP826450, –, –. **MUB 49356:** Sierra Nevada Mountains, KP825677, KP826065, KP826239, KP826448, KP826577, KY229030. **MUB 49357:** Sierra Nevada Mountains, F, KP825676, KP826064, KP826241, KP826447, KP826576, –. **MUB 49366:** Sierra Nevada Mountains, F, KP825670, KP826058, KP826238, KP826442, KP826570, KY229011. **MUB 49370:** Sierra Nevada Mountains, KP825674, KP826062, KP826234, KP826446, KP826574, KY229015. **MUB 49373:** Sierra Nevada Mountains, F, KP825671, KP826059, KP826233, KP826443, KP826571, –. **MUB 49382:** Sierra Nevada Mountains, F, KP825669, KP826057, KP826180, KP826441, KP826569, –. **MUB 49387:** Sierra Nevada Mountains, F, KP825666, KP826054, KP826230, KP826438, KP826565, –. **MUB 49399:** Sierra Nevada Mountains, F, KP825663, KP826051, KP826224, KP826435, KP826563, KY229033. **MUB 49403:** Sierra Nevada Mountains, F, KP825660, KP826048, KP826182, KP826432, KP826560, –. **MUB 49408:** Sierra Nevada Mountains, F, KP825657, KP826045, KP826222, –, KP826557, KY229005. **MUB 49410:** Sierra Nevada Mountains, F, KP825655, KP826043, KP826220, KP826428, KP826555, –. **MUB 49411:** Sierra Nevada Mountains, F, KP825654, KP826042, KP826219, KP826427, KP826554, MG050783. **MUB 49412:** Sierra Nevada Mountains, F, KP825653, KP826041, KP826218, KP826426, KP826553, –. **MUB 49413:** Sierra Nevada Mountains, F, KP825652, KP826040, KP826217, KP826425, KP826552, KY229008. **MUB 49424:** Sierra Nevada Mountains, F, KP825651, KP826039, KP826216, KP826424, KP826551, –. **MUB 49426:** Sierra Nevada Mountains, F, KP825649, KP826037, KP826214, KP826422, KP826549, –. **MUB 49427:** Sierra Nevada Mountains, F, KP825648, KP826036, KP826213, KP826421, KP826548, –. **MUB 49442:** Sierra Nevada Mountains, F, KP825643, KP826031, KP826208, KP826417, KP826544, –. **MUB 49443:** Sierra Nevada Mountains, F, KP825642, KP826030, KP826207, KP826416, KP826543, –. **MUB 49444:** Sierra Nevada Mountains, F, KP825641, KP826029, KP826206, KP826415, KP826542, MG050784. **MUB 49445:** Sierra Nevada Mountains, KP825640, KP826028, KP826209, KP826414, KP826541, –. **MUB 49451:** Sierra Nevada Mountains, F, (KP825639, MG050800, MG050801, MG050802, MG050803, MG050804, MG050805, MG050806, MG050807, MG050808), (KP826027, MG050753), (KP826204, MG050869, MG050870), KP826413, KP826540, KY229045. **MUB 49461:** Sierra Nevada Mountains, F, KP825638, KP826026, KP826203, KP826412, KP826539, KY229052. **MUB 49471:** Sierra Nevada Mountains, M, KP825706, KP826094, KP826201, KP826476, KP826604, KY229043. **MUB 49473:** Sierra Nevada Mountains, F,

(KP825637, MG050809, MG050810, MG050811, MG050812, MG050813, MG050814, MG050815, MG050816, MG050817, MG050818, MG050819), KP826025, (MG050871, MG050872, MG050873, MG050874, MG050875, MG050876), (MG050766, MG050767, MG050768, MG050769, MG050770), KP826538, KY229041. **MUB 49480:** Sierra Nevada Mountains, F, (KP825636, MG050820, MG050821, MG050822, MG050823, MG050824, MG050825, MG050826), KP826024, KP826199, KP826410, KP826537, KY229046. **MUB 49485:** Sierra Nevada Mountains, F, (KP825635, MG050827, MG050828, MG050829, MG050830, MG050831, MG050832, MG050833), (KP826023, MG050754, MG050755, MG050756, MG050757, MG050758), (MG050877, MG050878, MG050879, MG050880, MG050881, MG050882), (KP826409, MG050771, MG050772, MG050773, MG050774, MG050775, MG050776), KP826536, KY229032. **MUB 49492:** Sierra Nevada Mountains, F, (MG050834, MG050835, MG050836, MG050837, MG050838, MG050839, MG050840), KP826022, KP826198, KP826408, –, KY229037. **MUB 49501:** Sierra Nevada Mountains, F, KP825633, –, KP826197, KP826407, KP826535, KY229042. **MUB 49504:** Sierra Nevada Mountains, F, (KP825632, MG050841, MG050842, MG050843, MG050844, MG050845), KP826021, KP826196, KP826406, (MG050867, MG050868), KY229047. **MUB 49505:** Sierra Nevada Mountains, F, KP825631, KP826020, KP826195, KP826405, KP826534, KY229031. **MUB 49518:** Sierra Nevada Mountains, F, (KP825630, MG050846, MG050847, MG050848, MG050849, MG050850, MG050851, MG050852, MG050853, MG050854), (KP826019, MG050759), KP826194, KP826404, KP826533, KY229038. **MUB 49528:** Sierra Nevada Mountains, F, (KP825629, MG050855, MG050856, MG050857, MG050858, MG050859, MG050860), (KP826018, MG050760), KP826193, (MG050777, MG050778), KP826532, KY229027. **MUB 49538:** Spanish central mountain ranges, F, KP825762, KP826150, KP826192, KP826528, KP826659, KY229021. **MUB 49540:** Spanish central mountain ranges, F, KP825760, KP826148, KP826191, KP826526, KP826657, –. **MUB 49541:** Spanish central mountain ranges, F, KP825759, KP826147, KP826190, KP826525, KP826656, MG050785. **MUB 49542:** Spanish central mountain ranges, F, KP825758, KP826146, KP826188, KP826524, KP826655, –. **MUB 49545:** Spanish central mountain ranges, KP825755, KP826143, KP826186, KP826521, KP826652, KY229029. **MUB 49550:** Spanish central mountain ranges, F*, KP825750, KP826138, KP826179, KP826516, KP826647, MG050786. **MUB 49552:** Spanish central mountain ranges, F*, KP825748, KP826136, KP826177, KP826514, KP826645, MG050787. **MUB 49553:** Spanish central mountain ranges, F*, KP825747, KP826135, KP826176, KP826513, KP826644, –. **MUB 49554:** Spanish central mountain ranges, F*, KP825746, KP826134, KP826175, KP826512, KP826643, KY229017. **MUB 49555:** Spanish central mountain ranges, KP825745, KP826133, KP826174, KP826511, KP826642, –. **MUB 49557:** Spanish central mountain ranges, F, KP825743, KP826131, KP826173, KP826509, KP826640, MG050788. **MUB 49558:** Spanish central mountain ranges, F, KP825742, KP826130, KP826172, KP826508, KP826639, –. **MUB 49560:** Spanish central mountain ranges, F*, KP825740, KP826128, KP826170, KP826506, KP826637, KY229013. **MUB 49562:** Spanish central mountain ranges, KP825738, KP826126, –, KP826504, KP826635, –. **MUB 49564:** Spanish central mountain ranges, F*, KP825736, KP826124, KP826168, KP826502, KP826633, –. **MUB 49566:** Spanish central mountain ranges, F, KP825734, KP826122, KP826167, KP826500, KP826631, KY229044. **MUB 49567:** Spanish central mountain ranges, F*, KP825733, KP826121, KP826166, KP826499, KP826630, KY229003. **MUB 49568:** Spanish central mountain ranges, F, KP825732, –, KP826165, –, KP826629, KY229048. **MUB 49569:** Spanish central mountain ranges, F*, KP825731, KP826119, KP826164, KP826497, KP826628, KY229009. **MUB 49570:** Sicilian Mount Etna, F, KP825714, KP826107, –, KP826478, KP826606, KY229016. **MUB 49593:** Sicilian Mount Etna, F, KP825715, KP826106, KP826163, KP826479, KP826607, KY229034. **MUB 49600:** Spanish south-eastern mountains, F*, KP825722, KP826104, KP826159, KP826486, KP826613, KY229022. **MUB 49602:** Spanish south-eastern mountains, F, KP825723, KP826105, KP826160, KP826487, KP826614, KY229050.

Other mountainous systems

CBFS 6159: Alps, KP825712, KP826100, –, KX503294, –, –. **CBFS 6162:** Alps, F, KP825711, KP826099, KP826154, KP826483, KP826611, KY229028. **CBFS 13557:** Alps, F, KP825708, KP826096, KP826151, –, KP826608. **MUB 49604:** Alps, F*, KP825627, KP826016, KP826162, KP826401, KP826530, KY229053. **MUB 49606:** Alps, F*, KP825727, KP826115, KP826161, KP826493, KP826624,--. **MUB 49613:** Alps, F*, KP825726, KP826114, –, KP826492, KP826623, KY229051. **MUB 49617:** Alps, F*, KP825725, KP826113, –, KP826491, KP826622, KY229002. **MUB 49619:** Alps, M, KP825724, KP826112, –, KP826490, KP826621, KY229000. **MUB 49624:** Pyrenees, F*, KP825730, KP826118, –, KP826496, KP826627, KY229007. **MUB 49629:** Pyrenees, F*, KP825729, KP826117, KP826158, KP826495, KP826626, KY229055. **MUB 49650:** Pyrenees, F*, KP825728, KP826116, KP826157, KP826494, KP826625, KY229004.

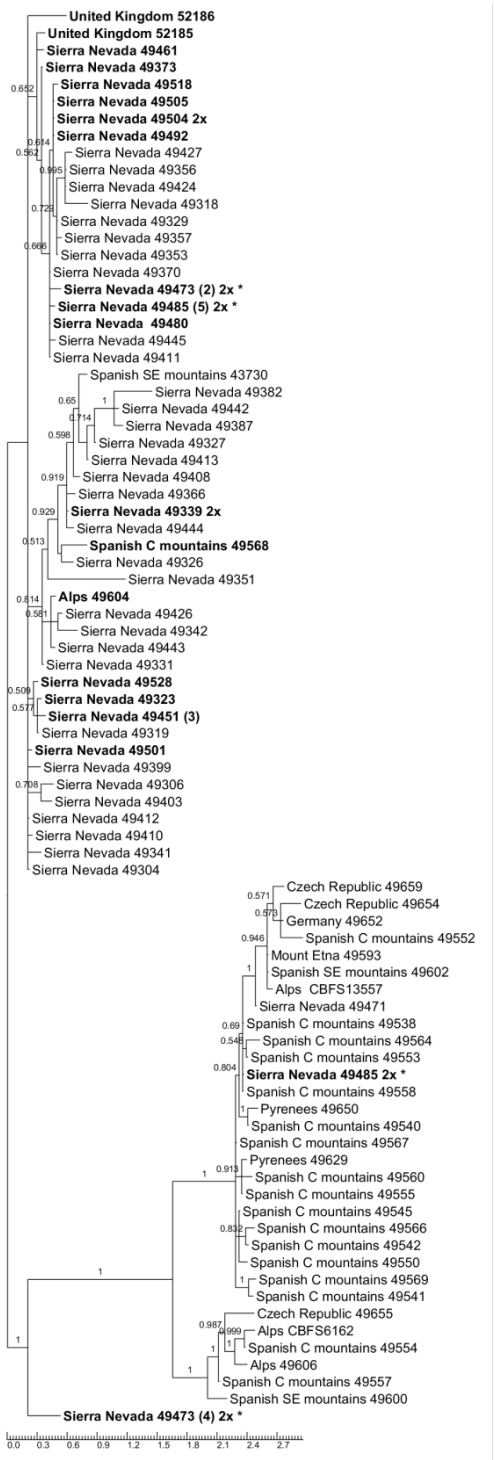
Lowlands

BOL 46302: South Africa, F*, KP825717, KP826109, –, KX503295, KP826618, KY229010. **BOL 46303:** South Africa, F*, KP825716, KP826108, –, –, KP826617, --. **MUB 49652:** Germany, F*, KP825718, KP826110, KP826156, KP826488, KP826619, KY229039. **MUB 49653:** Germany, F*, KP825719, KP826111, –, KP826489, KP826620, KY229020. **MUB 49654:** Czech Republic, F*, KX503276, –, KX503286, KX503291, KX503306, KY229012. **MUB 49655:** Czech Republic, F*, KX503275, –, KX503288, KX503290, KX503305, KY228999. **MUB 49659:** Czech Republic, F*, KX503274, –, KX503287, KX503289, KX503304, KY229006. **MUB 52185:** United Kingdom, KX503277, KX503282, KX503284, KX503292, KX503307, KY229049. **MUB 52186:** United Kingdom, (MG050861, MG050862, MG050863, MG050864, MG050865, MG050866), KX503283, KX503285, KX503293, KX503308, KY229054. **S B201182:** Sweden, F*, KP825721, KP826103, –, KX503296, KP826616, KY229018. **S B201183:** Sweden, F*, KP825720, KP826102, –, KP826485, KP826615, KY229014.

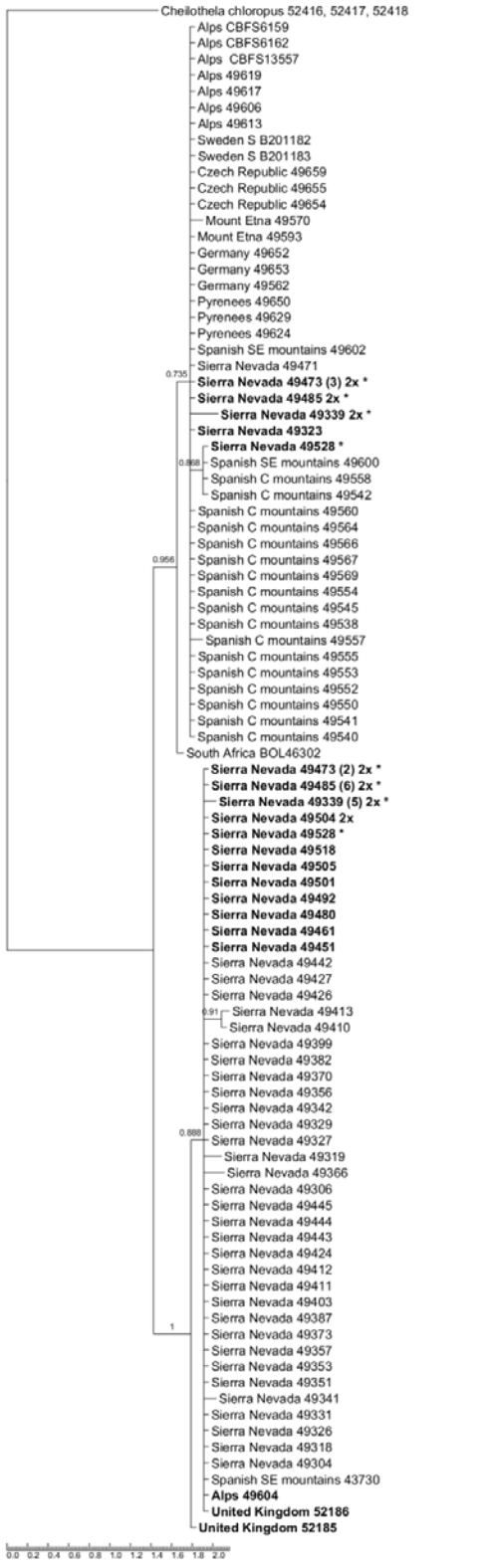
Outgroup: ***Cheilothela chloropus***

MUB52416: Sierra Nevada Mountains, KX503273, KX503281,–, KX503299, KX503303, KY229025. **MUB52417:** Sierra Nevada Mountains, –, KX503280, –, KX503298, KX503302, –. **MUB52418:** Sierra Nevada Mountains, –, KX503279, –, KX503297, KX503301, KY229026. **MUB52419:** Sierra Nevada Mountains, –, KX503278, –, –, KX503300, –.

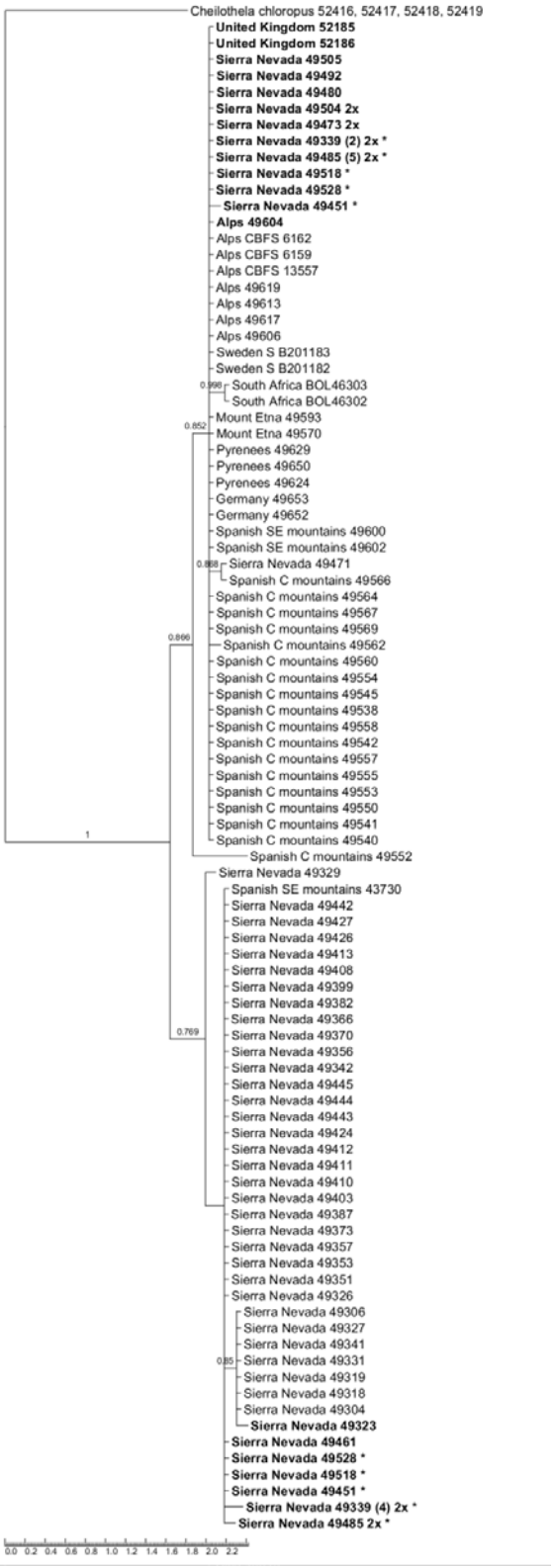
Online Supplementary Materials



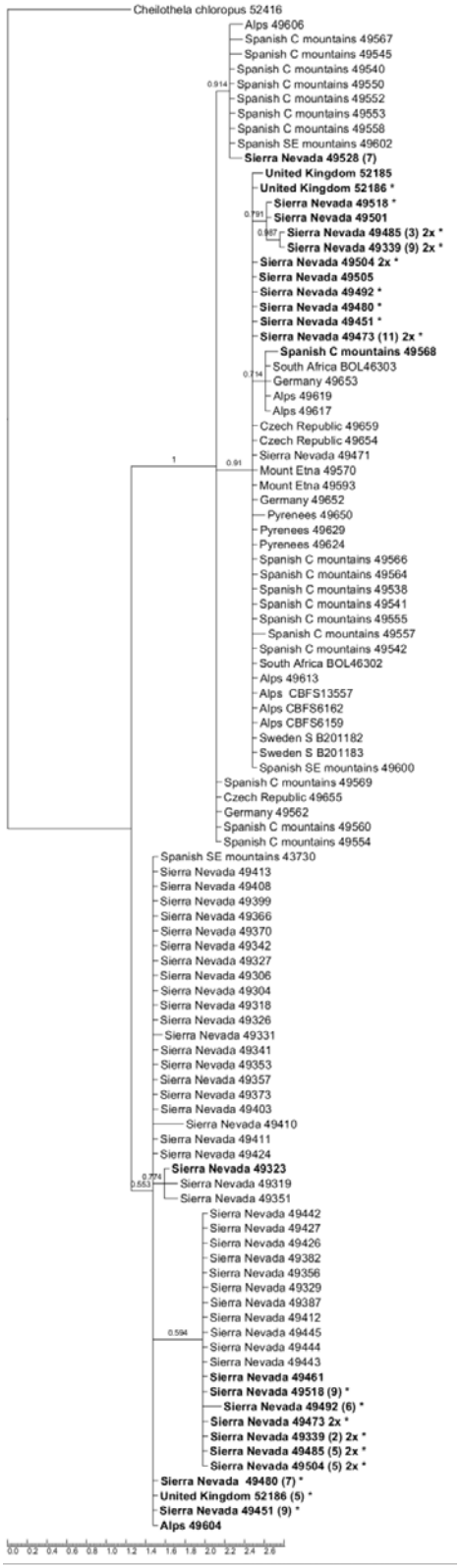
Appendix S1. Phylogenetic tree inferred from the nuclear *rpL23A* locus. For each tip in the trees geographical origin and number of herbarium are given (numbers without letters are from MUB); 2x is used to highlight diploid samples; number of equal sequences obtained by cloning is indicated between parentheses if there was more than one; asterisk (*) is used for indicating samples with more than one copy for the locus; bold letters indicate recombinant samples.



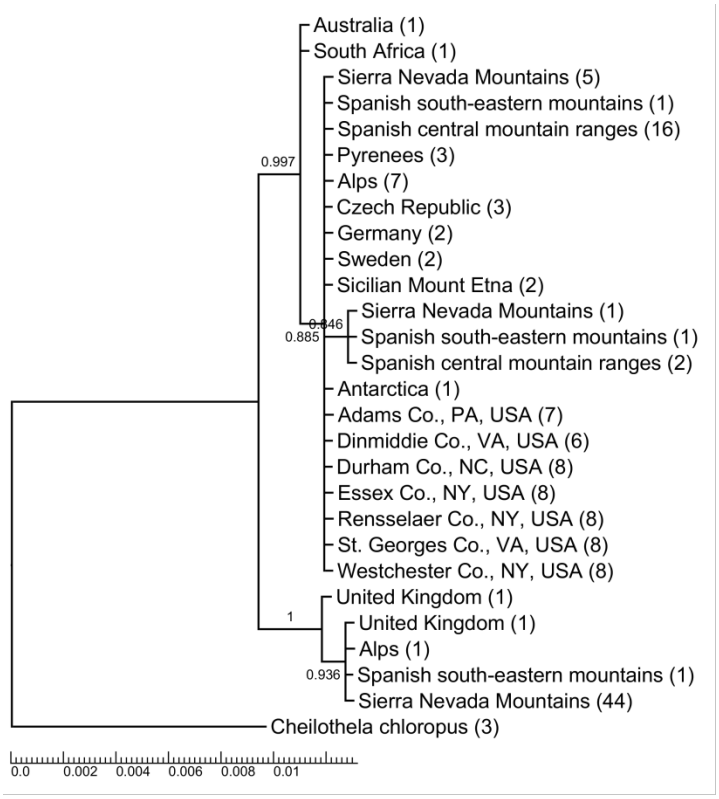
Appendix S2. Phylogenetic tree inferred from the nuclear *TBP* locus. Information about the data given for each tip in the tree as in Appendix S1.



Appendix S3. Phylogenetic tree inferred from the nuclear *PPR* locus. Information about the data given for each tip in the tree as in Appendix S1.



Appendix S4. Phylogenetic tree inferred from the nuclear *hp23.9* locus. Information about the data given for each tip in the tree as in Appendix S1.



Appendix S5. Phylogenetic tree inferred from the nuclear *TBP* locus adding to the samples used in this work other *Ceratodon* samples from Antarctica, Australia, and North America (GenBank accession numbers: KC436690 to KC436698, KC436701 to KC436706 and KC436710 to KC436750); number of similar sequences by area is indicated between parentheses.

Appendix S6. List of samples employed, indicating for each DNA locus analyzed, to which clade obtained in the phylogenetic analysis they belong (blue: SN clade, grey: Ww clade), the state of material used in cytometry analysis, and the amount of DNA (in case of dry material corrected by a factor of 0.85).

Specimen	*hp23.9*	*PPR*	*rpL23A*	*TBP*	*TRc1b3.05*	*Trn*L	State of material used	Amount of DNA (pg)
BOL 46302	Ww	Ww		Ww	Ww	Ww		--
BOL 46303	Ww	Ww			Ww			--
CBFS 13557	Ww	Ww	Ww		Ww		dry	0,36
CBFS 6159	Ww	Ww		Ww				--
CBFS 6162	Ww	Ww	Ww	Ww	Ww	Ww		--
MUB 43730	SN	SN	SN	SN	SN	SN	dry	0,44
MUB 49304	SN	SN	SN	SN	SN	SN	dry	0,47
MUB 49306	SN	SN	SN	SN	SN	SN		--
MUB 49318	SN	SN	SN	SN	SN		fresh	0,47
MUB 49319	SN	SN	SN	SN	SN	SN	fresh	0,45
MUB 49323	SN	SN	SN	Ww	SN	SN	fresh	0,46
MUB 49326	SN	SN	SN	SN	SN	SN	dry	0,46

MUB 49327	SN	SN	SN	SN	SN			--
MUB 49329	SN	SN	SN	SN	SN	SN	dry	0,45
MUB 49331	SN	SN	SN	SN	SN		fresh	0,46
MUB 49339	Ww/SN	Ww/SN	SN	Ww/SN	Ww	SN	fresh	0,82
MUB 49341	SN	SN	SN	SN	SN	SN	fresh	0,45
MUB 49342	SN	SN	SN	SN	SN		fresh	0,46
MUB 49351	SN	SN	SN	SN	SN		fresh	0,47
MUB 49353	SN	SN	SN	SN			dry	0,45
MUB 49356	SN	SN	SN	SN	SN	SN	fresh	0,47
MUB 49357	SN	SN	SN	SN	SN		dry	0,48
MUB 49366	SN	SN	SN	SN	SN	SN	fresh	0,46
MUB 49370	SN	SN	SN	SN	SN	SN	dry	0,46
MUB 49373	SN	SN	SN	SN	SN		dry	0,47
MUB 49382	SN	SN	SN	SN	SN		dry	0,48
MUB 49387	SN	SN	SN	SN	SN		dry	0,47
MUB 49399	SN	SN	SN	SN	SN	SN	fresh	0,47
MUB 49403	SN	SN	SN	SN	SN		fresh	0,47
MUB 49408	SN	SN	SN		SN	SN	dry	0,45
MUB 49410	SN	SN	SN	SN	SN		dry	0,45
MUB 49411	SN	SN	SN	SN	SN	SN	fresh	0,47
MUB 49412	SN	SN	SN	SN	SN		dry	0,48
MUB 49413	SN	SN	SN	SN	SN	SN	dry	0,45
MUB 49424	SN	SN	SN	SN	SN		dry	0,46
MUB 49426	SN	SN	SN	SN	SN		dry	0,44
MUB 49427	SN	SN	SN	SN	SN		fresh	0,46
MUB 49442	SN	SN	SN	SN	SN		dry	0,45
MUB 49443	SN	SN	SN	SN	SN		dry	0,46
MUB 49444	SN	SN	SN	SN	SN	SN	fresh	0,47
MUB 49445	SN	SN	SN	SN	SN		dry	0,47
MUB 49451	Ww/SN	Ww/SN	SN	SN	Ww	SN	fresh	0,46
MUB 49461	SN	SN	SN	SN	Ww	SN	dry	0,47
MUB 49471	Ww	Ww	Ww	Ww	Ww	Ww	fresh	0,37
MUB 49473	Ww/SN	Ww	Ww/SN	Ww/SN	Ww	SN	fresh	0,81
MUB 49480	Ww/SN	Ww	SN	SN	Ww	SN	dry	0,47
MUB 49485	Ww/SN	Ww/SN	Ww/SN	Ww/SN	Ww	SN	dry	0,82
MUB 49492	Ww/SN	Ww	SN	SN	Ww	SN	fresh	0,46
MUB 49501	Ww		SN	SN	SN	SN		--
MUB 49504	Ww/SN	Ww	SN	SN	SN	SN	fresh	0,82
MUB 49505	Ww	Ww	SN	SN	Ww	SN		--
MUB 49518	Ww/SN	Ww/SN	SN	SN	Ww	SN	fresh	0,45
MUB 49528	Ww	Ww/SN	SN	Ww/SN	Ww	Ww	fresh	0,46
MUB 49538	Ww	Ww	Ww	Ww	Ww	Ww	dry	0,35
MUB 49540	Ww	Ww	Ww	Ww	Ww		dry	0,37
MUB 49541	Ww	Ww	Ww	Ww	Ww	Ww	dry	0,37
MUB 49542	Ww	Ww	Ww	Ww	Ww			--
MUB 49545	Ww	Ww	Ww	Ww	Ww	Ww	dry	0,35
MUB 49550	Ww	Ww	Ww	Ww	Ww	Ww	fresh	0,36
MUB 49552	Ww	Ww	Ww	Ww	Ww	Ww	fresh	0,36
MUB 49553	Ww	Ww	Ww	Ww	Ww		dry	0,38
MUB 49554	Ww	Ww	Ww	Ww	Ww	Ww	dry	0,35

MUB 49555	Ww	Ww	Ww	Ww	Ww		dry	0,37
MUB 49557	Ww	Ww	Ww	Ww	Ww	Ww	dry	0,37
MUB 49558	Ww	Ww	Ww	Ww	Ww		dry	0,38
MUB 49560	Ww	Ww	Ww	Ww	Ww	Ww	dry	0,36
MUB 49562	Ww	Ww		Ww	Ww		dry	0,36
MUB 49564	Ww	Ww	Ww	Ww	Ww		dry	0,37
MUB 49566	Ww	Ww	Ww	Ww	Ww	Ww	dry	0,36
MUB 49567	Ww	Ww	Ww	Ww	Ww	Ww		--
MUB 49568	Ww		SN		Ww	SN		--
MUB 49569	Ww	Ww	Ww	Ww	Ww	Ww		--
MUB 49570	Ww	Ww		Ww	Ww	Ww	fresh	0,37
MUB 49593	Ww	Ww	Ww	Ww	Ww	Ww	fresh	0,36
MUB 49600	Ww	Ww	Ww	Ww	Ww	Ww		--
MUB 49602	Ww	Ww	Ww	Ww	Ww	Ww		--
MUB 49604	SN	Ww	SN	SN	Ww	SN	fresh	0,48
MUB 49606	Ww	Ww	Ww	Ww	Ww		dry	0,37
MUB 49613	Ww	Ww		Ww	Ww	Ww	dry	0,38
MUB 49617	Ww	Ww		Ww	Ww	Ww	dry	0,38
MUB 49619	Ww	Ww		Ww	Ww	Ww	dry	0,37
MUB 49624	Ww	Ww		Ww	Ww	Ww	dry	0,37
MUB 49629	Ww	Ww	Ww	Ww	Ww	Ww		--
MUB 49650	Ww	Ww	Ww	Ww	Ww	Ww	dry	0,38
MUB 49652	Ww	Ww	Ww	Ww	Ww	Ww	dry	0,37
MUB 49653	Ww	Ww		Ww	Ww	Ww	dry	0,37
MUB 49654	Ww		Ww	Ww	Ww	Ww	dry	0,37
MUB 49655	Ww		Ww	Ww	Ww	Ww	dry	0,38
MUB 49659	Ww		Ww	Ww	Ww	Ww	dry	0,38
MUB 52185	Ww	Ww	SN	SN	SN	SN		--
MUB 52186	Ww/SN	Ww	SN	SN	SN	SN	fresh	0,47
S B201182	Ww	Ww		Ww	Ww	Ww		--
S B201183	Ww	Ww		Ww	Ww	Ww		--

619