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RESEARCH PAPER

Climate-driven vicariance and long-distance dispersal explain the Rand Flora pattern in the liverwort *Exormotheca pustulosa* (Marchantiophyta)

Running title: Biogeography of *Exormotheca pustulosa*

Rodrigues, Ana Sofia Bartolomeu[1*+]; Martins, Anabela[1+]; Garcia, César Augusto[1]; Sérgio, Cecília [1]; Porley, Ron[2]; Fontinha, Susana[3,4]; González-Mancebo, Juana[5]; Gabriel, Rosalina[6]; Phephu, Nonkululo[7,8]; Van Rooy, Jacques[8,9]; Dirkse, Gerard[10]; Long, David[11]; Stech, Michael[10,12]; Patiño, Jairo[13,14$]; Sim-Sim, Manuela[1,3$]

[1]cE3c - Centre for Ecology, Evolution and Environmental Changes, Natural History and Systematics (NHS) Research Group /MUHNAC - Museu Nacional de História Natural e da Ciência, Universidade de Lisboa, Rua da Escola Politécnica, 58, 1250-102 Lisboa, Portugal

[2]Cerca dos Pomares, CxP 409M, 8670-052 Aljezur, Portugal

[3]cE3c - Centre for Ecology, Evolution and Environmental Changes, Departamento de Biologia Vegetal, Faculdade de Ciências, Universidade de Lisboa, Campo Grande, 1749-016 Lisboa, Portugal

[4]Banco de Germoplasma ISOPlexis, Universidade da Madeira, Funchal, Madeira, Portugal

[5]Department of Botany, University of La Laguna, Tenerife, Canary Islands, Spain

[6]cE3c/ABG – Centre for Ecology, Evolution and Environmental Changes/Azorean Biodiversity Group and University of Azores, 9700-402 Angra do Heroísmo, Azores, Portugal

[7]Department of Nature Conservation, Tshwane University of Technology, Pretoria, South Africa

[8]School of Animal, Plant and Environmental Sciences, University of the Witwatersrand, Johannesburg, PO WITS 2050, South Africa

[9]National Herbarium, South African National Biodiversity Institute (SANBI), Private Bag X101, Pretoria 0001, South Africa

[10]Naturalis Biodiversity Center, P.O. Box 9517, 2300 RA Leiden, The Netherlands

[11]Royal Botanic Garden, Edinburgh EH3 5LR, United Kingdom

[12]Leiden University, Leiden, The Netherlands

[13]Plant Conservation and Biogeography Group. Departamento de Botánica, Ecología y Fisiología Vegetal. Facultad de Ciencias. Apartado 456, CP 38200, Universidad de La Laguna. La Laguna, Tenerife, Islas Canarias, Spain

[14]Island Ecology and Evolution Research Group, Instituto de Productos Naturales & Agrobiología (IPNA-CSIC), La Laguna, Tenerife, Spain

*** Corresponding author:**
Ana S. B. Rodrigues
E-mail: ana87bartolomeu@gmail.com

[+]These authors contributed equally to this paper

[$]The two last authors share the senior position

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Abstract

The Rand flora is a biogeographical disjunction which refers to plant lineages occurring at the margins of the African continent and neighbouring oceanic archipelagos. Here, we tested whether the phylogeographic pattern of *Exormotheca pustulosa* Mitt. was the result of vicariance induced by the past climatic changes or the outcome of a series of recent long-distance dispersal events. Two DNA chloroplast and one nuclear marker were analysed. Phylogenetic and phylogeographical relationships were inferred as well as divergence time estimates and ancestral areas. *Exormotheca* originated in Eastern Africa during the Late Oligocene/Early Miocene while *Exormotheca putulosa* diversified during the Late Miocene. Three main *E. pustulosa* groups were found: the northern Macaronesia/Western-Mediterranean; the South Africa/Saint Helena and the Cape Verde. The major split events among these groups occurred during the Late Miocene/Pliocene; diversification is recent, dating back to the Pleistocene. Climate-driven vicariance and subsequent long-distance dispersal events may have shaped the current disjunct distribution of *E. pustulosa* that corresponds to the Rand Flora pattern. Colonisation of Macaronesia seems to have occurred twice by two independent lineages. The evolutionary history of *E. pustulosa* populations of Cape Verde warrants further study.

Keywords: ancestral area estimation – bryophytes – continental disjunction – dating analyses – dispersal – spore-producing plants

Introduction

Biogeographical disjunctions are common in many organisms and their patterns and underlying mechanisms have long intrigued scientists. Transcontinental distributions are well known and explained either by vicariance resulting from plate tectonics (i.e. continental drift; Givnish & Renner, 2004; Sanmartín & Ronquist, 2004) or by long-distance dispersal events (Renner, 2004). Within-continent disjunctions, in which related *taxa* are distributed across geographically isolated regions within the same continent, have received less attention. Contrary to transoceanic disjunctions, within-continent disjunction patterns seem to have been shaped by vicariance phenomena caused by macroclimatic events, such as global climate cooling or aridification, thus originating from the fragmentation of a once widespread continental macroflora (e.g. Crisp & Cook, 2007). Climatic changes and geological events appear to have played a key role in shaping the disjunct distributions of many animals and plants.

The African Rand Flora is a typical example of a within-continent disjunction (Christ, 1892). This floristic pattern is based on the existence of plant lineages that share similar disjunctions at the margins of the African continent and nearby archipelagos, forming "a ring and leaving the center of the continent hollow" (Pokorny *et al.*, 2015; Mairal *et al.*, 2017). The regions that typically define Rand Flora distributions are Macaronesia (Azores, Madeira, Selvagens, Canaries and Cape Verde archipelagos), northwest Africa, southern Arabia and western Asia, along with floras from the east and south of Africa. The climatic changes that occurred during the Miocene (23.0 – 5.3 Ma) and Pliocene (5.3 – 2.6 Ma) are thought to be consequential in shaping the biogeography of African plant lineages (Pokorny *et al.*, 2015). During these periods, increasing aridification of Africa and the formation of the Sahara Desert (~4.8 – 0.4 Ma; Muhs *et al.*, 2019) caused the fragmentation of a large number of African plant lineage distributions, with the subsequent restriction of persisting geographically disjunct sister clades to these peripheral African regions (Sanmartín *et al.*, 2010; Mairal & Sanchez-Meseguer, 2012; Pokorny *et al.*, 2015). Most of these Rand flora lineages are adapted to temperate or (sub)tropical montane humid climates and the tropical lowlands of Central Africa, the Sahara and Sino-Arabic Deserts in the north and the Kalahari Desert in the south, are major environmental barriers to dispersal (Mairal *et al.*, 2017). The vicariance scenario is thus referred to as one of the hypotheses for the origin of the Rand Flora pattern. A second but non-exclusive hypothesis to explain this disjunct distribution proposes long-distance dispersal events between geographically isolated parts of Africa and adjacent islands, followed by local *in situ* diversification of the surviving lineages (Sanmartín *et al*., 2010; Barres *et al.*, 2013; Mairal *et al.*, 2015).

Despite the prevalent Mediterranean origin of the Macaronesian angiosperm flora (Carine *et al.*, 2004; Romeiras *et al.*, 2016), sister relationships between Macaronesian clades and southern/eastern African and southern Arabian clades were also reported in several families including Asteraceae (Pelser *et al.*, 2007; Barres *et al.*, 2013; Calleja *et al.*, 2016) and Campanulaceae (Alarcón *et al.*, 2013; Mairal *et al.*, 2015), and genera such as *Hypericum* (Meseguer *et al.*, 2013) and *Euphorbia* (Riina *et al.*, 2013), conforming to the Rand Flora pattern. Within the Macaronesian region, Cape Verde contrasts with the northern archipelagos by its distinct native flora showing affinities with the arid/semi-arid Saharo-Arabian regions, but also with the tropical Sudano-Zambesian area and the archipelagos of the Canaries and Madeira (Duarte & Romeiras, 2009; Francisco-Ortega *et al.*, 2009). In the context of the Rand Flora pattern, phylogeographic studies have indeed shown that some Macaronesian *taxa* are relicts of a once widespread mainland flora with, to some extent, vicariance. This is shown in the palaeotropical plant genus *Campylanthus* (Thiv *et al.*, 2010) and *Campanula jacobaea,* a species endemic to Cape Verde that diverged from a north African species/ancestor during the Pleistocene (Alarcón *et al.*, 2013) following climate-induced vicariance.

Contrary to vascular plants, the type of geographical disjunction that corresponds with the Rand flora pattern and its underlying processes remain poorly understood in bryophytes, a group of spore-producing plants with high dispersal capabilities. They are thought to have distribution ranges explained largely by long-distance dispersal events, the tiny spores being easily carried by winds

(Patiño & Vanderpoorten, 2018), but it has also been shown that it is possible for bryophyte diaspores to be distributed over long distances by migratory birds (Lewis *et al*, 2014). Long-distance dispersal capability is reflected in the postglacial history of several European bryophyte populations, with a complex mixture of origins (Patiño & Vanderpoorten, 2018; Ledent *et al*., 2019), with recent divergence times being incongruent with potential vicariance scenarios. The moss *Bryoxiphium* and the pantropical leafy liverwort *Ceratolejeunea* (Patiño *et al.*, 2016; Scheben *et al.*, 2016) exemplify these trends. The historical biogeography of broadly distributed groups such as the liverwort family Schistochilaceae (Sun *et al.,* 2014), the liverwort genus *Frullania* (Carter *et al.,* 2017), and the moss genus *Homalothecium* (Huttunen *et al.,* 2008) are also best explained by long-distance dispersal events. Despite mounting evidence pointing to the role of dispersal in shaping current distribution ranges of widely distributed bryophytes, a few case studies have provided support for the vicariance hypothesis in bryophytes in determining their distribution ranges (e.g. McDaniel & Shaw, 2003; Heinrichs *et al.*, 2006; Patiño *et al.*, 2017).

The liverwort genus *Exormotheca* Mitt. belongs to the family Corsiniaceae (Long *et al.,* 2016) and it is represented by five valid species and four *Incertae sedis* species (Long *et al.,* 2016; Söderström *et al.,* 2016). *Exormotheca pustulosa* is the most widely distributed species of the genus and its biogeographical range conforms to the Rand Flora distribution pattern, occurring in Macaronesia (Azores, Madeira, Canary Islands and Cape Verde) and the Mediterranean region (Portugal, Spain, France and Sicily) and extending to tropical and southern/eastern Africa and to the south Atlantic island of Saint Helena and the western Indian island of La Réunion (Bischler *et al.,* 2005; Hodgetts, 2015; Ros *et al.,* 2007). Reports of its presence in mainland Italy and Morocco are relatively old (pre-1962) and its present occurrence in these regions could not be confirmed by recent surveys (Ros *et al.,* 2007). *Exormotheca pustulosa* is also reported from Central America (Mexico), where it is considered a human-induced introduction (Bischler *et al.,* 2005).

Based on phylogenetic analyses, molecular dating and estimates of ancestral ranges of multiple datasets (nuclear ITS and chloroplast regions), we aimed to reconstruct the historical biogeography of the widespread *E. pustulosa* and its relationships with other *Exormotheca* species. Specifically, we tested (i) whether accessions of *E. pustulosa* constitute a monophyletic clade, (ii) whether the current disjunct distribution pattern of *E. pustulosa* is a result of either the fragmentation of a once continuous range (vicariance) that correlates with the most important aridification climatic events in Africa or from long distance dispersal events, and (iii) whether Cape Verde, given its geographical location and biogeographical affinities, could have acted as a crossroads between northern Macaronesia archipelagos and Africa.

Material and Methods

Taxon and specimen sampling

Morphologically, the distinctive liverwort genus *Exormotheca* is characterised by a small to medium-sized thallus with dorsal conical air chambers basally filled with chlorophyllose filaments. Ventral scales are small to medium-sized, obliquely triangular, hyaline to purple. The gynoecial receptacles are stalked or sessile. *Exormotheca* species have a short-lived sporophyte and commonly produce spores (60-140 µm) and specialised vegetative propagules are not reported (Perold, 1999; Frey *et al.*, 2006).

For the molecular analyses, a total of 48 specimens of *E. pustulosa* were investigated, 23 from herbarium collections and 25 collected in the field during 2016/2017. Voucher specimens were obtained from herbaria LISU, E, PRE and from the private collection of G. Dirkse. Field samples were collected in Macaronesia (1 from the Azores, 6 from Madeira, 8 from the Canary Islands and 15 from Cape Verde), southern Portugal (4 specimens), Oman (1 specimen), South Africa (7 specimens), Tanzania (1 specimen), Saint Helena island (4 specimens) and La Réunion island (1 specimen) (Fig.1 & Table S2.1). The sampling covered the known distribution range of *E. pustulosa*. The specimen-level sampling within *E. pustulosa* was completed with other species in the genus *Exormotheca* including *E. welwitschii* Steph., *E. holstii* Steph., *E. bischlerae* Furuki & Higuchi and a sample identified as *Exormotheca* sp. nov. (D. Long pers. comm. 2019). *Corsinia* *coriandrina* (Spreng.) Lindb., *Targionia lorbeeriana* Müll.Frib. and *Mannia androgyna* (L.) A.Evans (Fig. 1 & Table S2.1) were used as outgroups based on large-scale phylogenetic analyses of liverworts (Laenen *et al.*, 2014).

DNA extraction, amplification and sequencing

The specimens were washed with distilled water under a dissection microscope for mechanical removal of contaminants, and the cleaned plant material was dried in an incubator at 37ºC. Following agitation at 30 Hz for 2x 1 min in a TissueLyser (Qiagen), DNA was extracted from the resulting plant powder using the DNeasy Plant Mini Kit (Qiagen, Crawley, UK) or the plant kit (Analytic Gene) following the manufacturer's protocol. Three plastid fragments (*rps*4-*trn*L, *trn*L-F and *psb*A-*trn*H) and one nuclear region (nuclear ribosomal internal transcribed spacer 2 - ITS2) were amplified by polymerase chain reaction (PCR). See Appendix S1 for primer sequences and PCR protocols. PCR products were purified with SureClean (Bioline) following the manufacturer's protocol and sequenced at Macrogen (Spain).

Phylogenetic analyses

Sequences were verified and edited using GENEIOUS v. 6.1.5 (Biomatters Ltd.) and PhyDE v. 0.9971 (Müller *et al.*, 2006), then aligned using MAFFT v. 7.409 (Katoh & Standley, 2013) and finally manually edited and trimmed at the ends in PhyDE. Indels were scored using the simple index coding (SIC) (Simmons & Ochoterena, 2000) as implemented in the SeqState v. 1.4.1 (Müller, 2005). GenBank accession numbers are presented in Table S2.1.

Phylogenetic analyses were performed for the plastid (cpDNA dataset) and nuclear (nDNA dataset) markers separately to check for phylogenetic incongruences. Incongruence was defined as the presence of incompatible groups with a branch support > 95%, which ensures that strongly conflicting signals between markers are eliminated (Patiño *et al.*, 2017). Because our findings provide supported incongruent signatures, the nucleo-cytoplasmic regions (cpDNA + nDNA dataset; see results) were not concatenated. The GTR+G model was selected as the best-fit substitution model for both nucleotide partitions based on the corrected Akaike Information Criterion (AICc; Sugiura, 1978) and using jModelTest2 (Darriba *et al.*, 2012). A Maximum-Likelihood (ML) analysis was conducted in RAxML v. 8.2.4 (Stamatakis, 2014) using the GTRCGAMMA nucleotide substitution model. Branch support was estimated by performing 1000 bootstrap replicates. A Bayesian Inference (BI) analysis was conducted in MrBayes v. 3.2.6 (Ronquist *et al.*, 2012), using the settings nst=6 and rates=gamma. Two runs using Monte Carlo Markov Chain (MCMC) method were performed with four chains for 4 million generations and with trees sampled every 4000th generation. Convergence of the chains was assessed using TRACER v. 1.6 (Rambaut *et al.*, 2014). The first 25% of trees were discarded as burn-in and a 50% majority rule consensus tree obtained. FIGTREE v. 1.4.3 (available at http://tree.bio.ed.ac.uk/software/figtree/) was used to visualise the trees. Haplotype networks for cpDNA and nDNA datasets were obtained using the median-joining algorithm in POPART v. 1.7 (Bandelt *et al.,* 1999), with gaps coded as SIC.

Divergence time estimates

In order to produce ultrametric dated trees suitable for the subsequent biogeographical analyses, we ran strict clock and uncorrelated lognormal relaxed clock analyses of our two molecular datasets (cpDNA, nDNA) using BEAST v.1.8.4 (Drummond *et al.*, 2012). Because the inclusion of identical sequences results in many zero-length branches at the tip of the tree and can cause the model to over-partition the dataset (Reid & Carstens, 2012), we pruned these sequences from the datasets by reducing our list of specimens to haplotypes using TriFusion (http://odiogosilva.github.io/TriFusion/) and rechecked by eye in PhyDE. We ran BEAST analyses for two independent chains of 100 million generations each, sampling every $10E^4$ generations under a birth-death and a Yule speciation model, respectively, and assessed convergence by checking that all parameters had reached stationarity and sufficient (> 200) effective sample sizes using TRACER v.1.6 (Rambaut *et al.*, 2014). The runs were combined with LOGCOMBINER v.1.8.4 and the resulting maximum clade credibility (MCC) tree was summarised in TREEANNOTATOR v.1.8.4 and viewed in FIGTREE. The best model was selected through marginal likelihood estimates (MLEs) that were assessed using path-sampling (PS, Lartillot *et al.*, 2006) and stepping-stone (SS, Xie *et al.*, 2011) methods. The resulting MLEs were averaged across replicate runs to generate a single PS and SS value for each model. The uncorrelated lognormal relaxed clock under a Yule model was thus selected for the cp (Yule model = PS MLE: -8811.7; SS MLE: -8812.6 versus Birth-Death model = PS MLE: -8817.1; SS MLE: -8817.8) and for the ITS2 (Yule model = PS MLE: -8811.7; SS MLE: -8812.6 versus Birth-Death model = PS MLE: -8817.1; SS MLE: -8817.8) datasets, respectively, and therefore employed in subsequent analyses. In the absence of fossil records of *Exormotheca*, we used a normal prior distribution on the substitution rate ('ucld.mean' parameter) with a mean of 5×10^{-4} and a standard deviation (SD) of 1×10^{-4} substitutions/site/Myr for the cpDNA dataset (Palmer, 1991; Villarreal & Renner, 2012). A normal prior distribution on the substitution rate ('ucld.mean' parameter) with a mean of 1.35×10^{-3} and SD of 5×10^{-3} substitutions/site/Myr (Les *et al.*, 2003), in combination with the truncate option and upper and lower bounds of $0.4–8.3 \times 10^{-3}$ substitutions/site/Myr (see Kay *et al.*, 2006; Villarreal & Renner, 2014), was adopted for the ITS2 dataset. This calibration approach has been successfully applied to groups of liverworts (e.g. Bechteler *et al.*, 2017) and mosses (e.g. Vigalondo *et al.*, 2019).

Ancestral area estimation analyses

Ancestral area estimations were performed in the R package BioGeoBEARS (Matzke, 2013a, 2014). Seven geographic regions were based on the distribution of *E. pustulosa* and of the other *Exormotheca* species included in the study: Iberian Peninsula/north Africa; Azores; Madeira/Canary Islands; Cape Verde; western Africa; southern/eastern Africa and Asia. The time-calibrated tree (the maximum-credibility tree) obtained from BEAST, removing the outgroups for each cpDNA and nDNA dataset were performed separately to provide ancestral area estimations and each haplotype assigned to the geographic region/s where it occurs. BioGeoBEARS allows the use of the Lagrange DEC model (Dispersal-Extinction-Cladogenesis), which includes dispersal (d) and extinction (e) as free parameters, and a model (DEC+J) that includes an additional parameter J taking founder-event speciation into account (Matzke *et al.*, 2014 and references therein). Since different ancestral area reconstructions are based on different assumptions, one can compare these two versions of the DEC model with a likelihood version of the Dispersal-Vicariance Analysis (DIVALIKE) and a likelihood version of the range evolution model of the Bayesian Binary Model (BAYAREA), with the option of also adding founder-event speciation to either of these two alternative models. However, in a recent study, Ree & Sanmartín (2018) proposed that DEC+J might be a poor model of founder-event speciation and statistical comparisons of its likelihood with a pure DEC model may be inappropriate. Consequently, we refrained from implementing the DEC+J in the present study and focused on the classical versions of the three biogeographical models implemented in BioGeoBEARS (DEC, DIVALIKE, BAYAREA). These three models were estimated under a maximum likelihood framework and compared in terms of how well they fitted the data using the Akaike Information Criterion corrected for small sample size (AICc) (Matzke 2013b, 2014).

Results

Phylogenetic analyses

The final cpDNA and nDNA matrices had 57 and 62 sequences each, with an aligned length of 2858 bp and 835 bp, respectively. Among the aligned sites, we inferred 478 and 206 parsimony-informative sites (PICs) in the cpDNA and nDNA datasets, respectively, but without indels included.

Phylogenetic analyses based on ML and BI approaches for cpDNA and nDNA show well-supported groups that correspond to the present taxonomical *Exormotheca* species described ($BS_{ML} < 100$; $PP_{BI} = 1.0/1.0$), except for *E. pustulosa* (Fig. S3.1 & Fig. S3.2). A conflicting signal is found between cpDNA and nDNA markers. In the nDNA tree, *Corsinia coriandrina* is nested within the genus *Exormotheca* and *E. pustulosa* appears as a monophyletic group (Fig. S3.1). An exception is the Oman specimen which does not group with the remaining *E. pustulosa* samples. The cpDNA tree (Fig. S3.2) shows a different topology inferring *C. coriandrina* as sister to the *Exormotheca* genus. *Exormotheca bischlerae* (China, Asia) and *E.* sp. nov. (Bhutan, Asia) cluster within *E. pustulosa*, but this grouping lacks support. Within *E. pustulosa,* presumable incongruences are observed with respect to the position of the Cape Verde (CV) group (Fig. S3.1 & Fig. S3.2). In the nDNA tree, the Cape Verde specimens are sister to a clade comprising populations from western Mediterranean and northern Macaronesian (MED-MACN) but without full support ($BS_{ML} < 50$; $PP_{BI} = 0.87/0.98$, Fig. S3.1). On the contrary, the CV clade is sister to Oman and Tanzania specimens in the cpDNA tree ($BS_{ML}=96$; $PP_{BI} = 1.0/1.0$, Fig. S3.2). Apart from the two groups described within *E. pustulosa*, a third group formed by specimens from South Africa and Saint Helena (SAF-STH) was consistently inferred ($BS_{ML} > 85$; $PP_{BI} > 0.95$/-). The specimen from La Réunion appears to be basal to the clade comprising the MED-MACN and SAF-STH groups (Fig. S3.1 & Fig. S3.2). In the cpDNA tree, the specimens from Tanzania and Oman are sister to the CV clade ($BS_{ML} = 93$; $PP_{BI} = 1.0/1.0$), Fig. S3.2). Limited levels of diversification are found within the SAF-STH clade, in the nDNA tree: (i) three well-supported sub-groups are inferred within the

SAF ($BS_{ML} \geq 84$; $PP_{BI} \geq 0.98/1.0$; Fig. S3.1) and (ii) two sub-groups are also identified for the STH ($BS_{ML} \geq 97$; $PP_{BI} \geq 0.96/0.96$; Fig. S3.1).

Neighbour-Net analyses

Both cpDNA and nDNA median-joining networks corroborate the general phylogeographical pattern observed with three main groups (MED-MACN; SAF-STH; CV) and show differences in the relationship of Cape Verde with the other groups (Fig. S3.3). In the cpDNA network, Cape Verde haplotypes are distant from MED-MACN and SAF-STH haplotypes. In turn, they appear to be connected to the MED-MACN and SAF-STH haplotypes in the nDNA network. In summary, network relationships elucidated by each dataset were predominantly tree-like and highly consistent with their respective tree-based phylogenetic inferences.

Time estimates and ancestral area reconstruction

Time estimates and 95% HPD intervals are presented in Table 1. BEAST analyses indicate that the genus *Exormotheca* originated approximately 22.42 Ma (95% HPD: 34.05 – 13.93 Ma) and 17.63Ma (95% HPD: 58.61 – 8.22 Ma), according to cpDNA and nDNA datasets respectively (Fig. 2 & Fig.3). From all species analysed, *E. pustulosa* originated most recently. According to nDNA time estimates, the crown age for the origin of the species dates back to 7.58 Ma (95% HPD: 26.05 – 3.08 Ma). cpDNA marker indicates two different time origins for the cpDNA lineages: one lineage differentiated at approximately 5.75 Ma (95% HPD: 10.38 – 2.60 Ma) while the other is younger originated around 3.40 Ma (95% HPD: 5.89 – 1.57 Ma) (Fig. 3). Within *E. pustulosa*, the split between MED-MACN and SAF-STH groups was at 3.71 Ma (95% HPD: 6.42 – 1.73 Ma) and 2.86 Ma (95% HPD: 5.46 – 0.49Ma), for cpDNA and nDNA respectively. The main diversification within MED-MACN group occurred around 1.50 Ma (95% HPD: 2.80 – 0.60 Ma) (Fig. 2) and 1.0 Ma (95% HPD: 3.56 – 0.32 Ma) (Fig. 3). For nDNA marker, the differentiation of CV lineage was estimated around 0.39Ma (95% HPD: 1.55 – 0.03 Ma) (Fig.2). For cpDNA, the split between eastern Africa (Tanzania) and CV occurred at approximately 2.38Ma (95% HPD: 4.22 – 1.03 Ma) and the differentiation of the CV lineage around 1.24 Ma (95% HPD: 2.34 – 0.51 Ma) (Fig. 3). BEAST analyses also revealed that most of the genetic differentiation within *E. pustulosa* occurred recently, less than 2.5 Ma (Fig. 2 & Fig. 3).

The best-fit model of ancestral area estimations, the DIVALIKE (Table S2.2), suggests a southern/eastern African origin for the *Exormotheca* genus (Fig. 4 & Fig. 5). The reconstruction analyses also indicate an southern/eastern African ancestor of *E. pustulosa* that dispersed to La Réunion and towards South Africa (Fig. 4 & Fig. 5). Data also support a south African ancestor for Saint Helena populations. For cpDNA reconstruction analysis (Fig. 5), Cape Verde colonisation was inferred to have occurred independently of the remaining Macaronesia archipelagos, via southern/eastern Africa, from an Asiatic (Arabian Peninsula) ancestor (Fig. 5). The nDNA data however suggests colonisation from Madeira and/or Canary Islands to Cape Verde (Fig. 4). The origin of the most recent common ancestor of populations of northern Macaronesia is uncertain. Nevertheless, dispersal from Madeira and/or Canary Islands to Azores seems to have occurred. According to ancestral area analyses, the western-Mediterranean (southern Portugal) region seems also to have been colonised from northern Macaronesia (Fig. 4).

Discussion

Phylogenetic relationships and topological incongruence

Our phylogenetic inferences support the species identity of all the *Exormotheca* species analysed, but we failed to corroborate the monophyly of *E. pustulosa*. Although *E. bischlerae* and *E.* sp. nov. cluster within *E. pustulosa*, these species are morphologically different from *E. pustulosa* specimens (Sim-Sim, unpublished results). Therefore, the relationships within *Exormotheca* remain unresolved, and further phylogenetic and taxonomical studies are needed to address species delimitations.

The conflicting phylogenetic signal between cpDNA and nDNA datasets, at first glance, is only found at the population level, affecting the clade of *E. pustulosa* restricted to Cape Verde. Because this conflicting node is well supported by each locus partition, it seems unlikely that shortcomings in the methods for phylogenetic inference would explain the incongruence observed for the position of the Cape Verdean clade. Some biological processes that are not necessarily exclusive could thus help to explain the nucleo-cytoplasmic discordance signature here observed, as differentiating mechanisms can generate very similar patterns (see Meng & Kubatko, 2009). First, incomplete lineage sorting of ancestral polymorphisms (Piñeiro *et al.*, 2012), particularly in the cpDNA, may explain the incongruence. Incomplete lineage sorting can often yield random patterns of interspecific and intraspecific relationships, which might lead to supported incongruence among gene trees (e.g. Bräuchler & Meimberg, 2010; Drábková & Vlček, 2010; Gurushidze *et al.*, 2010). However, since incomplete lineage sorting should yield a random phylogenetic pattern due to the stochastic nature of coalescence (Avise, 2004), this process cannot account for the observed incongruence in *E. pustulosa*; the species clusters displayed a strong non-random geographical signature (e.g. supporting the monophyly of the Cape Verde clade). Alternatively, the pattern observed may be explained by secondary contact and subsequent chloroplast capture, i.e. the plastid introgression from one plant clade into another following hybridization. Introgression-mediated chloroplast capture events have been inferred in sympatry (Folk *et al.*, 2017; Hojjati *et al.*, 2019), and often occur in the absence of detectable nuclear introgression (Fehrer *et al.*, 2007), as it appears to be the case in *Exormotheca*. In plants chloroplast introgression is more likely than in the nuclear genome due to a number of possible reasons: it is generally uniparentally inherited, free (or almost free) of recombination, and suffer lower influence of selection (Dong *et al.*, 2012). As additional evidence, a younger origin of the putative hybrid lineage with respect to the two parental lineages was found for the two partitions (see section below), which has been postulated as an explanation for inconsistency among gene trees resulting from hybridization (de Sousa et al. 2016). In this context, the most plausible scenario is that ancestors of *E. pustulosa* species colonised the archipelago of Cape Verde from two different origins, northern Macaronesia and eastern Africa (Tanzania) and Middle East (Oman), then underwent introgression and subsequent hybridization which may have rapidly resulted in most of CV offspring containing the whole chloroplast genome of one species as well as most nuclear DNA of the other species. We discuss the biogeographical implications of this hybridization event in the two following sections.

Biogeographic origin of *Exormotheca pustulosa* and the Rand Flora pattern

The genus *Exormotheca* originated in Africa during the Late Oligocene/Early Miocene, between 22.42 Ma (95% HPD: 34.05 – 13.93 Ma) and 17.63Ma (95% HPD: 58.61 – 8.22 Ma), according to the cpDNA and nDNA datasets, respectively. *Exormotheca pustulosa* is more recent and southern and eastern Africa seems to have been the centre of origin of this species. During the Late Miocene/Pliocene, *E. pustulosa* diversified in three main lineages (Fig. 2 & Fig. 3). One of these lineages occurs in northern Macaronesia and the western-Mediterranean region (MED-MACN clade), whereas the other two are restricted to south Africa and Saint Helena (SAF-STH) and to Cape Verde (CV), respectively. This is corroborated by both cpDNA and nDNA markers. According to nDNA time estimates, the crown age of the species dates back to the Late Miocene around 7.58 Ma (95% HPD: 26.05 – 3.08 Ma). The cpDNA time estimates suggests a more recent origin at approximately 5.75 Ma (95% HPD: 10.38 – 2.60 Ma), while the putative Cape Verde hybrid lineage was inferred to be even younger and originated around 3.40 Ma (95% HPD: 5.89 – 1.57 Ma). While dispersal events from mainland regions explain the current distribution of *E. pustulosa* on the different oceanic archipelagos, the major split events might have resulted from the fragmentation of a once continuous and widespread *E. pustulosa* population (vicariance). For example, the separation of northern Macaronesian and western Mediterranean populations from South Africa and Saint Helena populations occurs in the Pliocene, and this coincides with recent estimates for the formation of the Sahara Desert (~4.8 – 0.4 Ma; Muhs *et al.*, 2019). Prior to the formation of Sahara, a significant portion of north Africa was covered by lowland rainforest, which was gradually replaced by woodland savannah (Plana, 2004; Senut *et al.*, 2009) allowing the connection between the Macaronesian and Mediterranean regions with eastern Africa. The emergence of a physical barrier such as the Sahara Desert may have caused a disruption between regions and the subsequent isolation of MED-MACN with respect to eastern and southern African populations. Moreover, it has been proposed that the gradual aridification of the northern African continent created a dispersal route from west and central Asia to Africa, mostly inferred for non-tropical lineages (Fiz *et al.,* 2008; Barres *et al.,* 2013; Meseguer *et al.,* 2013; see also Popp *et al.,* 2008). This route would allow a secondary contact between Asiatic/Arabian and *E. pustulosa* lineages from northern Macaronesia and may explain the origin of the putative hybrid *E. pustulosa* lineage that dispersed to Eastern Africa and colonised Cape Verde (see above). The current Rand Flora disjunct distribution pattern of *E. pustulosa* can therefore be explained by both the fragmentation of a once-continuous eastern African population (vicariance) driven by the emergence of environmental and physical barriers (formation of the Sahara) and by trans-oceanic wind dispersal of *E. pustulosa* diaspores to Macaronesia.

Colonisation of Macaronesia by two independent lineages

Based on the cpDNA and nDNA datasets, Macaronesia seems to have been colonised during the Pleistocene at least twice by two independent lineages. One of the lineages probably originated in south-eastern Africa, dispersed across the northern Macaronesian archipelagos while the other, with an origin in the Middle East/eastern Africa, colonised and remained in Cape Verde. The most recent common ancestor of the northern Macaronesian lineage is dated back to 1.50 Ma (95% HPD: 2.80-0.60 Ma) while the ancestor of the Cape Verde lineage is dated back to 1.24 Ma (95% HPD: 2.34-0.51 Ma) (Table 1). Our ancestral area reconstruction analyses indicate dispersal from the Canary Islands and/or Madeira to the Azores as well as dispersal from the islands to the western-Mediterranean continental regions. This phenomenon of back colonisation from islands to continent or continental colonisation *de novo* has also been inferred in other bryophyte species such as the liverwort *Radula lindenbergiana* (Laenen *et al.*, 2011) and the moss *Rhynchostegium riparoides* (Hutsemékers *et al.,* 2011). Our results therefore corroborate the hypothesis of Patiño *et al.* (2015) that the Macaronesian archipelagos may have acted as stepping stones for transoceanic migrants, contributing to the colonisation of continental areas. Although *E. pustulosa* spores are relatively large (60 – 75 μm), there is evidence that such spores can be dispersed by wind over long distances (Frahm 2008; Zanatta *et al.*, 2016). In addition, Macaronesia and the western Mediterranean fall within the East Atlantic Flyway, one of eight major global flyways for migratory land and waterbirds (Boere & Stroud, 2006). Water- or shorebirds use sites along this flyway to migrate from breeding grounds in northern Europe to non-breeding, overwintering sites in western Europe and along the western seaboard of Africa, and back. Some of the migratory birds stop over or spend the winter in Macaronesia (Garcia-del-Rey, 2011, Becker *et al*, 2016). Moreover, the affinities found between MACN and the MED (southern Portugal) matches with the dominant pattern in terms of floristic and phylogenetic affinities within the Macaronesian angiosperm flora (e.g. Francisco-Ortega et al., 2001; Carine et al., 2004; Navarro-Pérez e*t al.,* 2015).

As mentioned earlier, an eastern African origin for Cape Verde populations of *E. pustulosa* is also supported by cpDNA (Fig. 3) but an earlier Asiatic (Arabian Peninsula) origin is suggested by reconstruction analysis (Fig. 5). Such a pattern is not new, since affinities between the Cape Verde flora and those of the arid/semi-arid Saharo-Arabian regions and of the tropical Sudano-Zambesian area have already been reported in angiosperms (Duarte & Romeiras, 2009; Francisco-Ortega *et al.*, 2009; see also Pokorny *et al.*, 2015) for genera such as *Canarina* (Campanulaceae*;* Mairal *et al.*, 2015) and *Volutaria* (Asteraceae; Calleja *et al.*, 2016). Our findings thus support the view that the Cape Verde archipelago constitutes a distinct biogeographical region for bryophytes (Vanderpoorten *et al.*, 2007). Based on the present results, the colonisation of the archipelago may have been mediated by long-distance wind dispersal of individuals from an eastern tropical African population, through an arid corridor that extended from south-west to north-east arid regions in Africa (Winterbottom, 1967; Verdcourt, 1969; de Winter, 1971) and through the Sahel/Sudanian region localised between the Sahara and the Sudanian savannah. However, colonisation via north Africa cannot be ruled out. The ancestor of the current populations of Cape Verde could have migrated northward from eastern to north-west Africa from where they then colonised Cape Verde. These ancestral populations may have become extinct in this region, as proposed for the plant angiosperm genus *Canarina* (Mairal *et al.*, 2015), or may persist today undetected in North Africa. *Exormotheca pustulosa* has been reported for North Africa (Morocco), although the record dates back to 1941 (Trabut, 1941; Ros *et al.*, 2007) and there is no recent evidence of its occurrence in the region.

Evolutionary history of *E. pustulosa* populations of South Africa and Saint Helena

The present data infers a consistent close relationship between South Africa and Saint Helena populations, with the most recent common ancestor of the SAF-STH clade dating back to the Pleistocene (less than 2.0 Ma). Our ancestral area estimation approach suggests a probable south African origin, via long-distance dispersal, of the Saint Helena populations. An affinity between

Saint Helena and a southern African flora has already been reported for angiosperms (Cronk, 1987; Eastwood *et al.*, 2004) but is here reported for the first time in bryophytes. At the nuclear level, south African populations were found to be genetically structured, with three sub-groups indicating the existence of barriers to gene flow; the Cape Fold mountains of the southwestern Cape could act as a physical barrier as suggested for other species (e.g Daniels *et al.*, 2006; Visser *et al.*, 2018). The existence of different climatic zones in southern Africa may also cause an asynchrony in the times of fertilisation and dispersal of *E. pustulosa*, contributing to the genetic isolation of these populations. Indeed, the Western Cape has a Mediterranean-type climate with the rainfall occurring mainly in winter months. This contrasts with the rest of southern Africa area where the rainfall is distributed more evenly throughout the year or occurs mainly in summer (Manning & Goldblatt, 2012).

Conclusions

The present study is a contribution to understanding how Miocene and Pleistocene climatic changes have possibly shaped the current distribution of the liverwort *E. pustulosa*. Our findings have revealed that the genus originated in southern/eastern Africa during the Late Oligocene/Early Miocene, while *E. pustulosa* diverged in the same region during the Late Miocene. Climate-driven vicariance (due to aridification and orogenic movements in Africa) and long-distance dispersal events appear to have most likely driven this disjunction, which exhibits a close affinity to the Rand Flora pattern described for many angiosperm *taxa.* To our knowledge, this is the first case of Rand Flora pattern reported in bryophytes. Our data also strongly supports affinities between northern Macaronesia and western-Mediterranean, and between southern Africa and Saint Helena. The colonisation history of Cape Verde is more difficult to explain, and the role of this archipelago as a possible crossroads and/or meeting point between northern Macaronesia and southern and eastern Africa needs further investigation.

References

Alarcón M, Roquet C, García-Fernández A, Vargas P, Aldasoro JJ. **2013**. Phylogenetic and phylogeographic evidence for a Pleistocene disjunction between *Campanula jacobaea* (Cape Verde Islands) and C. balfourii (Socotra). *Molecular Phylogenetics and Evolution* **69**: 828–836.

Avise JC. 2004. Molecular Markers, Natural History, and Evolution, second eds. *Sinauer Associates*, Sunderland.

Bandelt HJ, Forster P, Rohl A. **1999**. Median-joining networks for inferring intraspecific phylogenies. *Molecular Biology and Evolution* **16**: 37–48.

Barres L, Sanmartin I, Anderson CL, Susanna A, Buerki S, Galbany-Casals M, Vilatersana R. **2013**. Reconstructing the evolution and biogeographic history of tribe Cardueae (Compositae). *American Journal of Botany* **100**: 867–882.

Bechteler J, Schäfer-Verwimp A, Lee GE, Feldberg K, Pérez-Escobar OA, Pócs T, Peralta DF, Renner MA, Heinrichs J. **2017**. Geographical structure, narrow species ranges, and Cenozoic diversification in a pantropical clade of epiphyllous leafy liverworts. *Ecology and Evolution* **7**: 638–653.

Becker PH, Schmaljohann H, Riechert J, Wagenknecht G, Zajková Z, González-Solí J. 2016. Common Terns on the East Atlantic Flyway: temporal–spatial distribution during the non-breeding period. *Journal of Ornithology*. DOI 10.1007/s10336-016-1346-2.

Bischler-Causse, H, Gradstein, SR, Jovet-Ast, S, Long, DG, Allen, NS. 2005. Marchantiidae. *Flora Neotropica Monograph* **97:** 1-262.

Boere GC, Stroud DA. 2006. The flyway concept: what it is and what it isn't. In Boere G, Galbraith CA, Stroud DA, eds, *Waterbirds around the world.* The Stationery Office, Edinburgh, UK. pp. 40-47.

Bräuchler C, Meimberg H, Heubl G. **2010**. Molecular phylogeny of Menthinae (Lamiaceae, Nepetoideae, Mentheae) – Taxonomy, biogeography and conflicts. *Molecular Phylogenetics and Evolution* **55**: 501–523.

Calleja JA, Garcia-Jacas N, Roquet C, Susanna A. **2016**. Beyond the Rand Flora pattern: Phylogeny and biogeographical history of Volutaria (Compositae). *Taxon* **65**: 315–332.

Carine MA, Russell SJ, Santos-Guerra A, Francisco-Ortega J. **2004**. Relationships of the Macaronesian and Mediterranean floras: molecular evidence for multiple colonizations into Macaronesia and back-colonization of the continent in Convolvulus (Convolvulaceae). *American Journal of Botany* **91**: 1070–1085.

Carter BE, Larraín J, Manukjanová A, Shaw B, Shaw AJ, Heinrichs J, de Lange P, Suleiman M, Thouvenot L, von Konrat M. **2017**. Species delimitation and biogeography of a southern hemisphere liverwort clade, Frullania subgenus Microfrullania (Frullaniaceae, Marchantiophyta). *Molecular Phylogenetics and Evolution* **107**: 16–26.

Christ H. 1892. Exposé sur le rôle que joue dans le domaine de nos flores la flore dite ancienne africaine. *Archives des Sciences Physiques et Naturelles*, **3:** 369–374.

Crisp MD, Cook LG. **2007**. A congruent molecular signature of vicariance across multiple plant lineages. *Molecular Phylogenetics and Evolution* **43**: 1106–1117.

Cronk QCB. **1987**. The history of endemic flora of St Helena: a relictual series. *New Phytologist* **105**: 509–520.

Daniels SR, Gouws G, Crandall KA. **2006**. Phylogeographic patterning in a freshwater crab species (Decapoda: Potamonautidae: Potamonautes) reveals the signature of historical climatic oscillations. *Journal of Biogeography* **33**: 1538–1549.

Darriba D, Taboada GL, Doallo R, Posada D. **2012**. jModelTest 2: more models, new heuristics and parallel computing. *Nature Methods* **9**: 772.

de Sousa F, Bertrand YJK, Pfeil BE. **2016**. Patterns of phylogenetic incongruence in Medicago found among six loci. *Plant Systematics and Evolution* **302**: 493–513.

de Winter B. 1971. Floristic relationships between the northern and southern arid areas in Africa. *Mitteilungen der Botanischen Staatssammlung München* **10:** 424–437.

Dong W, Liu J, Yu J, Wang L, Zhou S. **2012**. Highly Variable Chloroplast Markers for Evaluating Plant Phylogeny at Low Taxonomic Levels and for DNA Barcoding (A Moustafa, Ed.). *PLOS ONE* 7: e35071.

Drábková LZ, Vlček Č. **2010**. Molecular phylogeny of the genus Luzula DC. (Juncaceae, Monocotyledones) based on plastome and nuclear ribosomal regions: A case of incongruence, incomplete lineage sorting and hybridisation. *Molecular Phylogenetics and Evolution* **57**: 536–551.

Drummond AJ, Suchard MA, Xie D, Rambaut A. 2012. Bayesian Phylogenetics with BEAUti and the BEAST 1.7. *Molecular Biology and Evolution* **29:** 1969–1973.

Duarte MC, Romeiras MM. **2009**. Cape Verde Islands. In: Gillespie RG, Clague DA, eds. *Encyclopedia of Islands*. University of California Press, 143–148.

Eastwood A, Gibby M, Cronk QCB. **2004**. Evolution of St Helena arborescent Astereae (Asteraceae): relationships of the genera *Commidendrum* and *Melanodendron*. *Botanical Journal of the Linnean Society* **144**: 69–83.

Fehrer J, Gemeinholzer B, Chrtek J & Bräutigam S. **2007**. Incongruent plastid and nuclear DNA phylogenies reveal ancient intergeneric hybridization in *Pilosella* hawkweeds (Hieracium, Cichorieae, Asteraceae). *Molecular Phylogenetics and Evolution* **42**: 347–361.

Fiz O, Vargas P, Alarcón M, Aedo C, García JL, Aldasoro JJ. **2008**. Phylogeny and Historical Biogeography of Geraniaceae in Relation to Climate Changes and Pollination Ecology. *Systematic Botany* **33**: 326–342.

Folk RA, Mandel JR, Freudenstein JV. 2017. Ancestral Gene Flow and Parallel Organellar Genome Capture Result in Extreme Phylogenomic Discord in a Lineage of Angiosperms. *Systematic Biology* **66**: syw083.

Frahm JP. **2008**. Diversity, dispersal and biogeography of bryophytes (mosses). *Biodiversity and Conservation* **17**: 277–284.

Francisco-Ortega J, Barber JC, Santos-Guerra A, Febles-Hernández R, Jansen RK. **2001**. Origin and evolution of the endemic genera of Gonosperminae (Asteraceae: Anthemideae) from the Canary Islands: evidence from nucleotide sequences of the internal transcribed spacers of the nuclear ribosomal DNA. *American journal of botany* **88**: 161–169.

Francisco-Ortega J, Santos-Guerra A, Bacallado JJ. **2009**. Canary islands, biology. In: Gillespie RG, Clague DA, eds. *Encyclopedia of Islands*. University of California Press, 127–133.

Frey W, Frahm J-P, Fischer E, Lobin W. **2006.** The liverworts, mosses and ferns of Europe. Colchester, Harley Books, 1-512.

Garcia-del-Rey E. 2011. *Field Guide to the Birds of Macaronesia*. Barcelona: Lynx Edicions. 341 pp.

Givnish TJ, Renner SS. **2004**. Tropical Intercontinental Disjunctions: Gondwana Breakup, Immigration from the Boreotropics, and Transoceanic Dispersal. *International Journal of Plant Sciences* **165**: S1–S6.

Gurushidze M, Fritsch RM, Blattner FR. **2010**. Species-level phylogeny of *Allium* subgenus Melanocrommyum: Incomplete lineage sorting, hybridization and trnF gene duplication. *Taxon* **59**: 829–840.

Heinrichs J, Lindner M, Groth H, Hentschel J, Feldberg K, Renker C, Engel JJ, Von Konrat M, Long DG, Schneider H. **2006**. Goodbye or welcome Gondwana? - Insights into the phylogenetic biogeography of the leafy liverwort *Plagiochila* with a description of *Proskauera*, gen. nov. (Plagiochilaceae, Jungermanniales). *Plant Systematics and Evolution* **258**: 227–250.

Hodgetts NG. 2015. Checklist and country status of European bryophytes – towards a new Red List for Europe. *Irish Wildlife Manuals*, No. 84. National Parks and Wildlife Service, Department of the Arts, Heritage and the Gaeltacht, Ireland.

Hojjati F, Adams RP, Terry RG. **2019**. Discovery of chloroplast capture in *Juniperus excelsa* complex by multi- locus phylogeny. *Phytotaxa* **413**: 11–26.

Hutsemekers V, Szovenyi P, Shaw AJ, Gonzalez-Mancebo JM, Munoz J & Vanderpoorten A. **2011**. Oceanic islands are not sinks of biodiversity in spore-producing plants. *Proceedings of the National Academy of Sciences* **108**: 18989–18994.

Huttunen S, Hedenäs L, Ignatov MS, Devos N, Vanderpoorten A. **2008**. Origin and evolution of the northern hemisphere disjunction in the moss genus *Homalothecium* (Brachytheciaceae). *American Journal of Botany* **95**: 720–730.

Katoh K, Standley DM. **2013**. MAFFT multiple sequence alignment software version 7: improvements in performance and usability. *Molecular biology and evolution* **30**: 772–80.

Kay KM, Whittall JB, Hodges SA. 2006. A survey of nuclear ribosomal internal transcribed spacer substitution rates across angiosperms: An approximate molecular clock with life history effects. *BMC Evolutionary Biology* **6**: 36.

Laenen B, Désamoré A, Devos N, Shaw AJ, González-Mancebo JM, Carine MA, Vanderpoorten A. **2011**. Macaronesia: a source of hidden genetic diversity for post-glacial recolonization of western Europe in the leafy liverwort *Radula lindenbergiana*. *Journal of Biogeography* **38**: 631–639.

Laenen B, Shaw B, Schneider H, Goffinet B, Paradis E, Désamoré A, Heinrichs J, Villarreal JC, Gradstein SR, McDaniel SF, Long DG, Forrest LL, Hollingsworth ML, Crandall-Stotler B, Davis EC, Engel J, Von Konrat M, Cooper ED, Patiño J, Cox CJ, Vanderpoorten A, Shaw AJ. **2014**. Extant diversity of bryophytes emerged from successive post-Mesozoic diversification bursts. *Nature Communications* **5**: 5134.

Lartillot N, Philippe H, Lewis P. 2006. Computing Bayes Factors Using Thermodynamic Integration. *Systematic Biology* **55**: 195–207.

Ledent A, Désamoré A, Laenen B, Mardulyn P, McDaniel SF, Zanatta F, Patiño J, Vanderpoorten A. **2019**. No borders during the post-glacial assembly of European bryophytes (F Lloret, Ed.). *Ecology Letters* **22**: 973–986.

Les DH, Crawford DJ, Kimball RT, Moody ML, Landolt E. 2003. Biogeography of discontinuously distributed hydrophytes, a molecular appraisal of intercontinental disjunctions. *International Journal of Plant Sciences* **164**: 917–932.

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

Long DG, Forrest LL, Villarreal JC, Crandall-Stotler BJ. **2016**. Taxonomic changes in Marchantiaceae, Corsiniaceae and Cleveaceae (Marchantiidae, Marchantiophyta). *Phytotaxa* **252**: 077–080.

Mairal M, Sánchez-Meseguer A. **2012**. Resolviendo una inógnita biogeográfica, eñ caso de la Rand Flora afro-mediterránea . *Chronica Naturae* **2**: 53–63.

Mairal M, Pokorny L, Aldasoro JJ, Alarcón M, Sanmartín I. **2015**. Ancient vicariance and climate-driven extinction explain continental-wide disjunctions in Africa: the case of the Rand Flora genus *Canarina* (Campanulaceae). *Molecular Ecology* **24**: 1335–1354.

Mairal M, Sanmartín I, Pellissier L. **2017**. Lineage-specific climatic niche drives the tempo of vicariance in the Rand Flora. *Journal of Biogeography* **44**: 911–923.

Manning J, Goldblatt P. 2012. Plants of the Greater Cape Floristic Region 1: the Core Cape flora, Strelitzia 29. South African National Biodiversity Institute, Pretoria.

Matzke NJ. 2013a. BioGeoBEARS: BioGeography with Bayesian (and Likelihood) Evolutionary Analysis in R Scripts. R package, version 0.2.1, published July 27, 2013 at: http://CRAN.R-project.org/package=BioGeoBEARS.

Matzke NJ. 2013b. Probabilistic historical biogeography: new models for founder-event speciation, imperfect detection, and fossils allow improved accuracy and model-testing. *Frontiers of Biogeography* **5**: 242-248.

Matzke NJ. 2014. Model Selection in Historical Biogeography Reveals that Founder-event Speciation is a Crucial Process in Island Clades. *Systematic Biology* **63**: 951-970.

McDaniel SF, Shaw AJ. **2003**. Phylogeographic structure and cryptic speciation in the trans-antarctic moss *Pyrrhobryum mnioides*. *Evolution* **57**: 205–215.

Meng C, Kubatko LS. **2009**. Detecting hybrid speciation in the presence of incomplete lineage sorting using gene tree incongruence: A model. *Theoretical Population Biology* **75**: 35–45.

Meseguer AS, Aldasoro JJ, Sanmartín I. **2013**. Bayesian inference of phylogeny, morphology and range evolution reveals a complex evolutionary history in St. John's wort (*Hypericum*). *Molecular Phylogenetics and Evolution* **67**: 379–403.

Muhs DR, Meco J, Budahn JR, Skipp GL, Betancort JF, Lomoschitz A. **2019**. The antiquity of the Sahara Desert: New evidence from the mineralogy and geochemistry of Pliocene paleosols on the Canary Islands, Spain. *Palaeogeography, Palaeoclimatology, Palaeoecology* **533**: 109245.

Müller K. **2005**. Seqstate: Primer Design and Sequence Statistics for Phylogenetic DNA Datasets. *Applied Bioinformatics* **4**: 65–69.

Müller K, Müller J, Neinhuis C, Quandt D. 2006. PhyDE – phylogenetic data editor, v0.995. Program distributed by the authors at: http://www.phyde.de

Navarro-Pérez ML, Vargas P, Fernández-Mazuecos M, López J, Valtueña FJ, Ortega-Olivencia A. **2015**. Multiple windows of colonization to Macaronesia by the dispersal-unspecialized *Scrophularia* since the Late Miocene. *Perspectives in Plant Ecology, Evolution and Systematics* **17**: 263–273.

Palmer J.D. 1991. Plastid chromosome, structure and evolution. In L. Bogorad, & I. K. Vasil, eds. *The Molecular Biology of Plastids*. San Diego, CA: Academic Press, 5–53.

Patiño J, Carine M, Mardulyn P, Devos N, Mateo RG, González-Mancebo JM, Shaw AJ & Vanderpoorten A. 2015. Approximate Bayesian Computation Reveals the Crucial Role of Oceanic Islands for the Assembly of Continental Biodiversity. Systematic Biology 64: 579–589.

Patiño J, Vanderpoorten A. **2018**. Bryophyte Biogeography. *Critical Reviews in Plant Sciences* **37**: 175–209.

Patiño J, Goffinet B, Sim-Sim M, Vanderpoorten A. **2016**. Is the sword moss (*Bryoxiphium*) a preglacial Tertiary relict? *Molecular Phylogenetics and Evolution* **96**: 200–206.

Patiño J, Wang J, Renner MAM, Gradstein SR, Laenen B, Devos N, Shaw AJ, Vanderpoorten A. **2017**. Range size heritability and diversification patterns in the liverwort genus *Radula*. *Molecular Phylogenetics and Evolution* **106**: 73–85.

Pelser PB, Nordenstam B, Kadereit JW & Watson LE. **2007**. An ITS phylogeny of tribe Senecioneae (Asteraceae) and a new delimitation of *Senecio* L. *TAXON* **56**: 1077–1104.

Perold S.M. 1999. Flora of Southern Africa Hepatophyta. Part 1. Marchantiopsida, Fascicle 1: Marchantiidae. Pretoria: National Botanical institute, 1-512.

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

Plana V. **2004**. Mechanisms and tempo of evolution in the African Guineo–Congolian rainforest (PT Pennington, QCB Cronk, and JA Richardson, Eds.). *Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences* **359**: 1585–1594.

Pokorny L, Riina R, Mairal M, Meseguer AS, Culshaw V, Cendoya J, Serrano M, Carbajal R, Ortiz S, Heuertz M, Sanmartín I. **2015**. Living on the edge: timing of Rand Flora disjunctions congruent with ongoing aridification in Africa. *Frontiers in Genetics* **6**: 1–15.

Popp M, Gizaw A, Nemomissa S, Suda J, Brochmann C. **2008**. ORIGINAL ARTICLE: Colonization and diversification in the African 'sky islands' by Eurasian *Lychnis* L. (Caryophyllaceae). *Journal of Biogeography* **35**: 1016–1029.

Rambaut A, Suchard MA, Xie D, Drummond AJ. 2014. Tracer v1.6, Available from http://beast.bio.ed.ac.uk/Tracer

Ree RH & Sanmartín I. **2018**. Conceptual and statistical problems with the DEC+J model of founder-event speciation and its comparison with DEC via model selection. *Journal of Biogeography* **45**: 741–749.

Reid NM, Carstens BC. **2012**. Phylogenetic estimation error can decrease the accuracy of species delimitation: a Bayesian implementation of the general mixed Yule-coalescent model. *BMC Evolutionary Biology* **12**: 196.

Renner S. **2004**. Plant Dispersal across the Tropical Atlantic by Wind and Sea Currents. *International Journal of Plant Sciences* **165**: S23–S33.

Riina R, Peirson JA, Geltman D V, Molero J, Frajman B, Pahlevani A, Barres L, Morawetz JJ, Salmaki Y, Zarre S, Kryukov A, Bruyns PV, Berry PE. **2013**. A worldwide molecular phylogeny and classification of the leafy spurges, *Euphorbia* subgenus Esula (Euphorbiaceae). *Taxon* **62**: 316–342.

Romeiras MM, Vieira A, Silva DN, Moura M, Santos-Guerra A, Batista D, Duarte MC, Paulo OS. **2016**. Evolutionary and Biogeographic Insights on the Macaronesian Beta-Patellifolia Species (Amaranthaceae) from a Time-Scaled Molecular Phylogeny (T Robillard, Ed.). *PLOS ONE* **11**: e0152456.

Ronquist F, Teslenko M, van der Mark P, Ayres DL, Darling A, Höhna S, Larget B, Liu L, Suchard MA, Huelsenbeck JP. **2012**. MrBayes 3.2: Efficient Bayesian Phylogenetic Inference and Model Choice Across a Large Model Space. *Systematic Biology* **61**: 539–542.

Ros RM, Mazimpaka V, Abou-Salam U, Aleffi M, Blockeel TL, Brugués M, Cano MJ, Cros RM, Dia MG, Dirkse GM, El Saadawi W, Erdağ A, Ganeva A, González-Mancebo JM, Herrnstadt I, Khalil K, Kürschner H, Lanfranco E, Losada-Lima A, Refai MS, Rodríguez-Nuñez S, Sabovljević M, Sérgio C, Shabbara H, Sim-Sim M, Söderström L. 2007. Hepatics and Anthocerotes of the Mediterranean, an annotated checklist. *Cryptogamie, Bryologie* **28**: 351–437.

Sanmartín I, Ronquist F. **2004**. Southern Hemisphere Biogeography Inferred by Event-Based Models: Plant versus Animal Patterns (C Cunningham, Ed.). *Systematic Biology* **53**: 216–243.

Sanmartín I, Anderson CL, Alarcon M, Ronquist F, Aldasoro JJ. **2010**. Bayesian island biogeography in a continental setting: the Rand Flora case. *Biology Letters* **6**: 703–707.

Scheben A, Bechteler J, Lee GE, Pócs T, Schäfer-Verwimp A, Heinrichs J. **2016**. Multiple transoceanic dispersals and geographical structure in the pantropical leafy liverwort *Ceratolejeunea* (Lejeuneaceae, Porellales). *Journal of Biogeography* **43**: 1739–1749.

Senut B, Pickford M, Ségalen L. **2009**. Neogene desertification of Africa. *Comptes Rendus - Geoscience* **341**: 591–602.

Simmons MP, Ochoterena H. **2000**. Gaps as characters in sequence-based phylogenetic analyses. *Systematic Biology* **49**: 369–381.

Söderström L, Hagborg A, von Konrat M, Bartholomew-Began S, Bell D, Briscoe L, Brown E, Cargill DC, Costa DP, Crandall-Stotler BJ, Cooper ED, Dauphin G, Engel JJ, Feldberg K, Glenny D, Gradstein SR, He X, Heinrichs J, Hentschel J, Ilkiu-Borges AL, Katagiri T, Konstantinova NA, Larraín J, Long DG, Nebel M, Pócs T, Puche F, Reiner-Drehwald E, Renner MAM, Sass-Gyarmati A, Schäfer-Verwimp A, Moragues JGS, Stotler RE, Sukkharak P, Thiers BM, Uribe J, Váňa J, Villarreal JC, Wigginton M, Zhang L, Zhu RL. **2016**. World checklist of hornworts and liverworts. *PhytoKeys* **59**: 1–828.

Stamatakis A. **2014**. RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics* **30**: 1312–1313.

Sugiura N. **1978**. Further analysts of the data by akaike's information criterion and the finite corrections. *Communications in Statistics - Theory and Methods* **7**: 13–26.

Sun Y, He X, Glenny D. **2014**. Transantarctic disjunctions in Schistochilaceae (Marchantiophyta) explained by early extinction events, post-Gondwanan radiations and palaeoclimatic changes. *Molecular Phylogenetics and Evolution* **76**: 189–201.

Thiv M, Thulin M, Hjertson M, Kropf M, Linder HP. **2010**. Evidence for a vicariant origin of Macaronesian–Eritreo/Arabian disjunctions in *Campylanthus* Roth (Plantaginaceae). *Molecular Phylogenetics and Evolution* **54**: 607–616.

Trabut L. 1941. Flore des Hépatiques de l'Afrique du Nord. *Revue bryologique et lichénologique 12* (=Mélanges bryologiques et lihénologiques [1942]) 1–42.

Vanderpoorten A, Rumsey FJ, Carine MA. **2007**. Does Macaronesia exist? Conflicting signal in the bryophyte and pteridophyte floras. *American Journal of Botany* **94**: 625–639.

Verdcourt B. 1969. The arid corridor between the northeast and southwest areas of Africa. *Palaeoecology of Africa* **4:** 140–144.

Vigalondo B, Patiño J, Draper I, Mazimpaka V, Shevock JR, Losada-Lima A, González-Mancebo JM, Garilleti R, Lara F. 2019. The long journey of *Orthotrichum shevockii* (Orthotrichaceae, Bryopsida): From California to Macaronesia. *PLOS ONE* **14**: e0211017.

Villarreal JC, Renner SS. 2012. Hornwort pyrenoids, a carbon- concentrating mechanism, evolved and were lost at least five times during the last 100 million years. *Proceedings of the National Academy of Sciences of the United States of America*, **109**: 18873–18878.

Villarreal JC, Renner SS. 2014. A review of molecular-clock calibrations and substitution rates in liverworts, mosses, and hornworts, and a timeframe for a taxonomically cleaned-up genus *Nothoceros*. *Molecular Phylogenetics and Evolution*, **78**: 25–35.

Visser JH, Bennett NC, Van Vuuren BJ. **2018**. Spatial genetic diversity in the Cape mole-rat, *Georychus capensis*: Extreme isolation of populations in a subterranean environment. *PLOS ONE* **13**: 1–18.

Winterbottom JM. **1967**. Climatological implications ofavifaunal resemblances between South Western Africa and Somaliland. *Palaeoecology of Africa* **2**: 77–79.

Xie W, Lewis PO, Fan Y, Kuo L, Chen MH. 2011. Improving Marginal Likelihood Estimation for Bayesian Phylogenetic Model Selection. *Systematic Biology*, **60**: 150–160.

Zanatta F, Patiño J, Lebeau F, Massinon M, Hylander K, De Haan M, Ballings P, Degreef J, Vanderpoorten A. **2016**. Measuring spore settling velocity for an improved assessment of dispersal rates in mosses. *Annals of Botany* **118**: 197–206.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Appendix S1 Supplementary Material & Methods

Appendix S2 Supplementary Tables

Appendix S3 Supplementary Figures

Author contributions: M.S.S. and J.P. conceived the idea. A.S.B.R., A.M., J.P. and M.S.S. designed the study and wrote the manuscript. A.S.B.R carried out the laboratory work. A.S.B.R. and J.P analysed the data. A.M, C.A.G, C.S., R.P., S.F., J.G.M., R.G., N.P., J.V.R., G.D., D.L.,

M.S.S. collected and contributed with plant material. M.S. assisted with the manuscript revision. J.P. and M.S.S. contributed equally for this paper as senior authors, while A.S.B.R. and A.M. contributed equally as junior authors. All authors contributed and approved the final version of the manuscript.

FIGURES

Figure 1: Sampling locations of *Exormotheca pustulosa* populations and 4 other species according to the different symbols. 1 – Southern Portugal; 2 – Azores; 3 – Madeira; 4 – Canary Islands; 5 – Cape Verde; 6 – Saint Helena; 7 – South Africa & Lesotho; 8 – Tanzania; 9 – La Réunion Island; 10 – Oman; 11 – Bhutan; 12 – China. Photo of the liverwort *E. pustulosa*.

Figure 2: Maximum clade credibility (MCC) tree obtained from molecular clock analysis of nDNA (ITS2) in BEAST. Posterior probabilities are indicated above the branches.

Figure 3: Maximum clade credibility (MCC) tree obtained from molecular clock analysis of combined cpDNA (*rps*4-*trn*F + *psb*A-*trn*H) in BEAST. Posterior probabilities are indicated above the branches.

Figure 4: Ancestral area reconstruction under DIVALIKE model implemented in BioGeoBEARS using the BEAST-derived nDNA chronogram (see Fig. 2) run on *Exormotheca* group.

Figure 5: Ancestral area reconstruction under DIVALIKE model implemented in BioGeoBEARS using the BEAST-derived cpDNA chronogram (see Fig. 3) run on *Exormotheca* group.

TABLES

Table 1: Mean age estimates in million years ago (Mya) and 95% highest probability density intervals of *Exormotheca* genus and *E. pustulosa* clades for the cpDNA and nDNA datasets.