
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

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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; Sanmartin & 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 (Sanmartin 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 *Ceratojeunea* (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 chlorophylllose 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 37°C. Following agitation at 30 Hz for 2 x 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 (*rps4-trnL*, *trnL-F* and *psbA-trnH*) 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 10E4 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 $5 \times 10^{-4}$ and a standard deviation (SD) of $1 \times 10^{-4}$ 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 $1.35 \times 10^{-3}$ and SD of $5 \times 10^{-3}$ 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\textsubscript{ML} < 100; PP\textsubscript{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\textsubscript{ML} < 50; PP\textsubscript{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\textsubscript{ML} = 96; PP\textsubscript{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\textsubscript{ML} > 85; PP\textsubscript{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\textsubscript{ML} = 93; PP\textsubscript{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 17.63 Ma (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 – 0.49 Ma), 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 0.39 Ma (95% HPD: 1.55 – 0.03 Ma) (Fig. 2). For cpDNA, the split between
eastern Africa (Tanzania) and CV occurred at approximately 2.38 Ma (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 et 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.
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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

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 (*rps4-trnF + psbA-trnH*) 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.