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REGULAR PAPER

Does altitude shape molecular diversity and richness of bryophytes in Madeira's natural forest? A case study with four bryophyte species at two altitudinal levels

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Background and aims – The importance of altitude as a driver of both species and genetic diversity has been widely acknowledged, since it affects other environmental variables such as temperature and precipitation, also influencing the distribution of plant species and, potentially, intraspecific genetic variation. In this study, we hypothesize that molecular (haplotype) variation within four Macaronesian bryophyte species co-varies with other diversity variables for two altitudinal levels.
Methods – Samples with molecular and floristic data were grouped into two altitudinal levels for mean comparisons. We measured the genetic diversity (haplotype diversity) of four bryophytes (*Exsertotheca intermedia*, *Isothecium prolixum*, *Frullania polysticta* and *Porella canariensis*) and determined floristic richness variables as well as each species cover using the data collected in 92 plots across the natural Laurel forest of Madeira Island. Molecular analyses included the sequencing of ITS1-5.8S-ITS2 and the chloroplast DNA *rps*4-*trn*T-*trn*L region. A haplotype diversity index, based on haplotype frequencies, was also calculated and compared with the percentage of sporophytes, for each studied species.
Key results – The results obtained by the mean comparisons revealed that bryophyte species richness and endemic species richness are higher for high altitudes levels in the natural forest. A coincident pattern between the species richness and the genetic diversity was observed for the mosses *I. prolixum* and *E. intermedia*, in which a higher species cover and genetic diversity occurred at high altitudes. However, *F. polysticta* displayed an inverse pattern, and *P. canariensis* did not present any significant differences in cover and haplotype frequency means.
Conclusion – From a conservation and management perspective, our findings highlight the importance of maintaining large patches of Macaronesian bryophyte species, since our results indicate a possible effect of altitude on species cover, affecting species genetic diversity. This should be considered in management plans, especially for endemics and red-listed species.

Key words – Bryophytes, molecular diversity, richness, Laurel forest, altitude.

INTRODUCTION

The definitions of biodiversity usually refer to three main levels, namely, ecosystem variation, species variation, and genetic variation. The latter is generally not included in conservation strategies and environmental policies (Laikre et al. 2010, Taberlet et al. 2012). However, the significance of genetic diversity within species (Papageorgiou & Kasimiadis 2013) and the importance of maintaining it to reduce extinction risk are recognised by the IUCN and the scientific community (Frankham et al. 2002, Hobbs et al. 2013). In fact, intraspecific variation provides the material for long term evolutionary adaptation and short term adaptation to seasonal and rapid fluctuations of environmental factors (Ramel 1998), being of great importance for the ability of species to survive to environmental changes (Frankham 1996, Hughes et al. 1997, Bolnick et al. 2011).

Knowledge of species genetic structure is therefore important to evaluate species viability and to help establishing

significant units for conservation (Crozier 1997, Hitchings & Beebee 1997, Frankham 2005a, He et al. 2008). This is especially relevant for biodiversity hotspots such as the Macaronesian Laurel forest belonging to the Mediterranean Basin region (Myers et al. 2000).

Important variables referred in literature that affect both species richness and genetic diversity are altitude and habitat disturbance by human activities (e.g. fragmentation, canopy structure alteration) (Pausas & Austin 2001, Escudero et al. 2003, Dixo et al. 2009). With the decrease of habitat suitability, population sizes normally decline and inbreeding may increase, leading to lower genetic diversity (Young et al. 1996, Hahn et al. 2012).

In mountain landscapes and islands, altitude is one of the variables considered to be a principal driver of species distribution patterns (Wei & Jiang 2012). Numerous studies proved that altitude affects plant diversity in general (e.g. Lomolino 2001, Kreft & Jetz 2007, Trigas et al. 2013) and bryophytes in particular (e.g. Frahm & Ohlemüller 2001, Ah-Peng et al. 2012, Sun et al. 2013). In fact, as Lomolino (2001) and Hahn et al. (2012) refer, altitudinal gradients are correlated with a group of environmental variables, such as temperature and precipitation (Pausas & Austin 2001), which influences the distribution of plant species and, potentially, population genetic variation.

Most studies in the literature describing relationships between species richness and elevation show mainly decreasing and hump-shaped patterns (Rahbek 1995). According to Wei & Jiang (2012) the hump-shaped patterns in relation to altitude are typical for both species diversity and genetic diversity. For example, Oshawa et al. (2007; for a review see Oshawa & Ide 2008) found that populations of *Quercus crispula* Blume at intermediate altitudes have greater genetic diversity than populations at lower and higher altitudes. Grau et al. (2007) also found a clear hump-shaped pattern for bryophyte species richness in Nepal (for liverworts and mosses separately). However, as Bruun et al. (2006) verified, the total plant richness peaked at intermediate altitudes, but the richness of mosses and liverworts separately showed an increasing trend, revealing a considerable variation over functional groups for a range of altitudes and study areas (see also Dorji et al. 2014).

Several authors have indicated the existence of a positive correlation between species diversity and genetic diversity, denominated the species-genetic diversity correlation (SGDC) (Vellend & Geber 2005, Finn & Poff 2001, Papadopoulou et al. 2011). This is hypothesised since, according to Vellend & Geber (2005) and Taberlet et al. (2012), both measures of diversity should, in theory, react to the same environmental conditions, and one level may influence the other level of biodiversity. Species richness could thus be used as a proxy of genetic diversity in conservation planning (Gugerli et al. 2008).

Allied to environmental variations and conditions, each species characteristics such as fecundity or mode of dispersal will also have an influence on genetic variation and, thus, on the ability of species to respond to environmental changes (Bolnick et al. 2011). As Hassel et al. (2005) highlighted, the frequency of sexual reproduction is likely to affect the degree of genetic variation. Sexual reproduction can also be affected by environmental factors, (since it is dependent on species fitness) and it has been reported that species at high altitudes invest more in growth than in reproduction, while species at low altitude tend to invest more in reproduction (von Arx et al. 2006, Hautier et al. 2009). However, in tropical areas, with less severe climatic conditions, this pattern was not observed at higher altitudes (Maciel-Silva et al. 2012). Benassi et al. (2011) observed that a decrease in water availability in the Mojave Desert (USA) at lower elevations influenced the growth of female-only individuals, diminishing sexual reproduction. According to Eppley et al. (2011) there is a decrease in sexual reproduction of mosses in extreme-stress conditions, but an increase at more moderate stress levels.

In this study, we hypothesize that the molecular (haplotype) variation within four Macaronesian bryophyte species co-varies with other diversity variables for two altitudinal levels. For this, samples with molecular and floristic data were grouped into meaningful environmental groups of altitude. In our study we used a large unit, namely the natural forest of Madeira Island, to measure genetic diversity.

MATERIALS AND METHODS

Study area and sampling design

This study was conducted in Madeira's natural forest (more accurately, the Laurel forest, including its ecological frontiers), between 102 and 1496 m a.s.l., which occupies about 20% of the island area (approximately 15 000 hectares) (fig. 1).

A grid with a mesh size of 500 m was placed all over the area delineated as the forest sampling area, to guarantee randomness in the selection of the sampled plots. At each intersection a plot was placed (denominated *centroid*) after adding a random variation of ± 50 m. A total of 92 plots were sampled between 2011 and 2013.

The sampling of all bryophytes for the determination of species richness and each taxon's cover was done in a circular area of 100 m^2 (with a radius of 5.64 m from the centre of the plot), in four 1 m^2 ground square-plots and in two different trees. A larger area of 400 m^2, with a radius of 11.28 m from the centre of the plot (including the 100 m^2 plot), was considered for the inventory of vascular plants diversity.

On each tree considered we assessed bryophytes on two rectangle subplots of 160 × 10 cm placed NNW (30°) and SSE (210°).

The cover of each taxon was registered in the four ground plots and in the sampled trees. We registered each taxon cover in the ground plots and in the trees according to the following logarithmic scale: 1 = < 0.3% cover; 2 = between 0.3 and 2.9% cover; 3 = between 3 and 29% cover; 4 = > 30% cover. To be selected, the two trees had to have a perimeter of at least 20 cm at 1.6 m height. When possible, this selection also considered the trees belonging to two out of the following three groups: group 1: typical trees of the laurel forest (e.g. *Laurus novocanariensis* Rivas Mart. et al., *Clethra arborea* Aiton, *Ocotea foetens* Benth. & Hook.f.); group 2: typical trees of the heather forest (*Erica platycodon* subsp.

maderincola (D.C.McClint.) Rivas Mart. et al., *Erica arborea* L., *Vaccinium padifolium* Sm.) or *Myrica faya* Dryand; group 3: non-native species (e.g. *Pinus* spp., *Pittosporum undulatum* Vent., *Acacia* spp.). For cover values of the bryophytes species under study we determined the weighted average of the values registered on the four one square meter subplots and on the trees.

Different environmental variables were registered in the field, such as altitude, tree cover or the presence of human intervention (detectable changes caused by human activities) and afterwards, using the GPS coordinates, additional variables were extracted from GIS maps. In the electronic appendix 1 there is a complete list of the variables obtained for each sampled plot.

Two Macaronesian endemic moss species – *Isothecium prolixum* (Mitt.) M.Stech, Sim-Sim, Tangney & D.Quandt and *Exsertotheca intermedia* (Brid.) S.Olsson, Enroth & D.Quandt – and two liverworts – *Frullania polysticta* Lindenb. (also a Macaronesian endemic) and *Porella canariensis* (F.Weber) Underw. (mainly occurring in Macaronesia) (Ros et al. 2007, 2013) – were selected to test the formulated hypothesis and, thus, subjected to molecular analysis. All four species have their main distribution in the Laurel forest s. lat. and are relatively widely distributed on Madeira Island (especially *P. canariensis*). For the molecular analyses, the sampling unit of to 400 m^2 was used to raise the likelihood of encountering the respective species. One specimen of each target species was collected per plot. According to Gugerli et al. (2008) genetic structures can be reliably detected if a low sample number *per* location is counterbalanced by a large number of sampling locations. However, it was still not possible to collect all species under study in all the plots and in some plots none of the species was present, even in the 400 m^2 plot. In addition, in some cases although a species was present in a plot, the material was not sufficient for molecular analyses (e.g., *P. canariensis*).

Presence or absence of sporophytes as indicator for successful fertilization was registered for each sample used in the molecular analysis to determine the extent of sexual reproduction. We determined the percentage of sporophytes for each species *per* altitudinal level to understand if the patterns displayed by the studied species were coincident, revealing a common trend.

DNA extraction, PCR and sequencing

The distal portions of the upper young shoots of the four selected bryophyte species were thoroughly cleaned with distilled water and ultrasonic treatment under a magnifying glass. Total genomic DNA was extracted using the DNeasy® Plant Mini Kit (Qiagen, GmBh, Germany) according to the manufacturer's instructions. Protocols for PCR amplification were carried out as described in previous publications: the ITS1-5.8S-ITS2 region from Draper et al. (2007) and the chloroplast DNA *rps*4-*trn*T-*trn*L region from Hernández-Maqueda et al. (2008). Amplification products were puri-

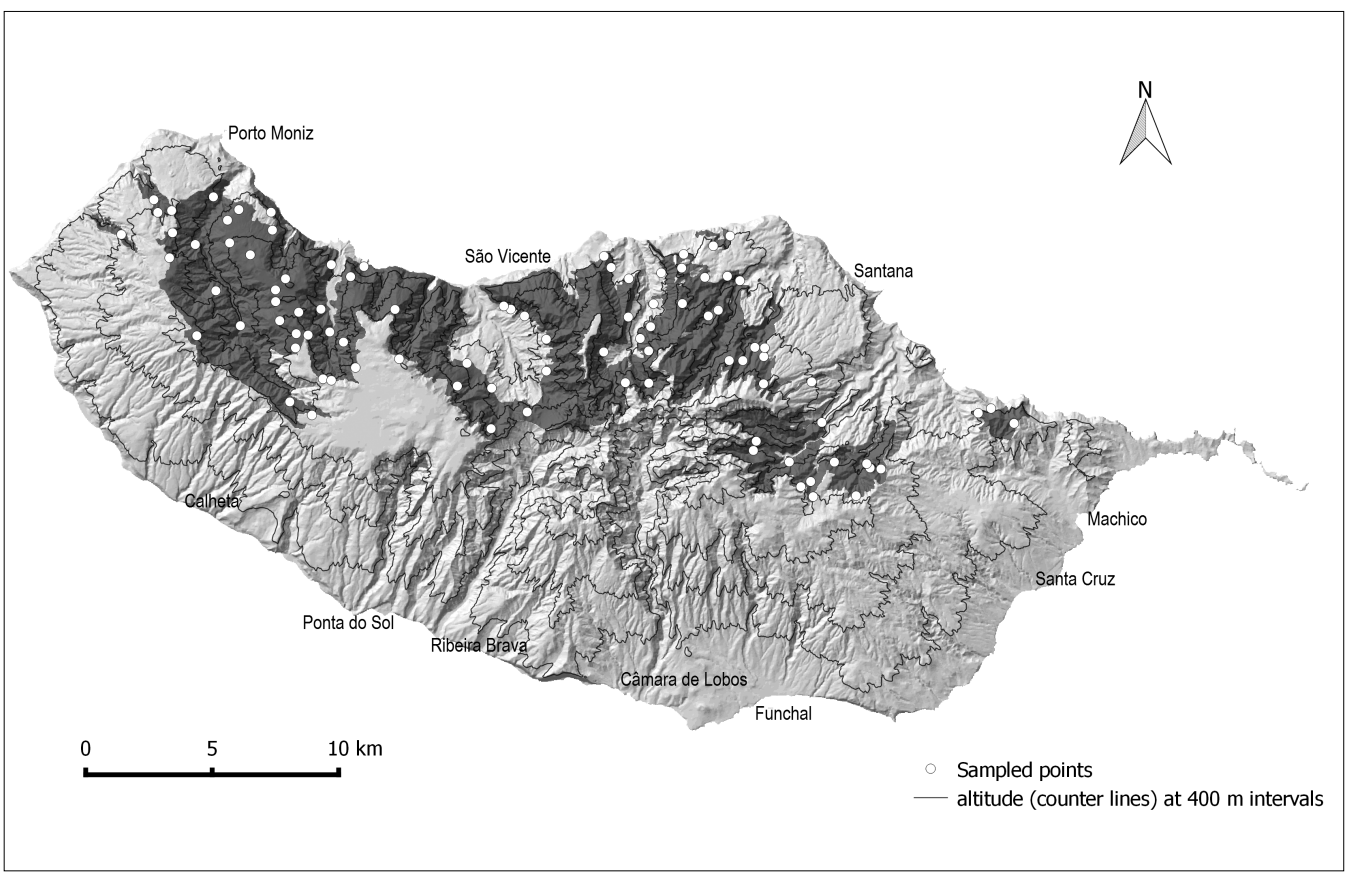

Figure 1 – Delimitation of Madeira's natural forest considered as sampling area with the distribution of the 92 sampled plots.

fied by the Exo-Sap enzymatic method (ExoSAP-It, Amersham USB Corp.). Purified PCR products were sequenced by STABVIDA Lda. (www.stabvida.com/) and bi-directional sequences were generated using the Big Dye® Terminator v3.1 method and the amplification primers. Sequences were edited using Geneious 7.1 (www.geneious.com/) and aligned manually with the help of the MAFFT algorithm v. 7 (Katoh & Standley 2013).

Data analyses

The number of plastid and ITS haplotypes in the species analysed was determined using TCS v.1.21 (Clement et al. 2000). TCS implements the estimation of gene genealogies from DNA sequences as described by Templeton et al. (1992). The plastid marker *rps*4-*trn*T-*trn*L revealed no variation among the *E. intermedia* specimens analysed and was, therefore, not included in the analyses.

Vouchers of the 157 newly sequenced specimens are deposited in LISU. Voucher information is available upon request. The 337 sequences generated in this study were deposited in GenBank (accession numbers: nrITS KM589551 – KM589599, KM604761 – KM604784, KF648790, KF648792 – KF648801, KM676255 – KM676288, KM656055 – KM656057, KM506913 – KM506933, KM506935 – KM506936; *rps*4-*trn*T-*trn*L KM604712 – KM604760, KM655983 – KM656054, KM823616 – KM823654, KM676221 – KM676254, KM582721 – KM582734, KM582736 – KM582741, KM582743 – KM582744).

For each species, only complete sequences without any ambiguous positions were included and analyses were performed with indels coded as missing data.

For each variable, groups for comparison of the means were established based on altitude. A first analysis revealed a hump-shape of species richness distribution along altitude, however we verified that for higher altitudes species richness displayed higher values than for lower altitudes (fig. 2). Therefore in the present study, only two altitudinal groups were considered for all the studied variables: 'Low altitudes' and 'High altitudes'. The altitudinal groups were defined based on the mean of each variable and each species since not all the species were collected in the same plots revealing different ecological preferences.

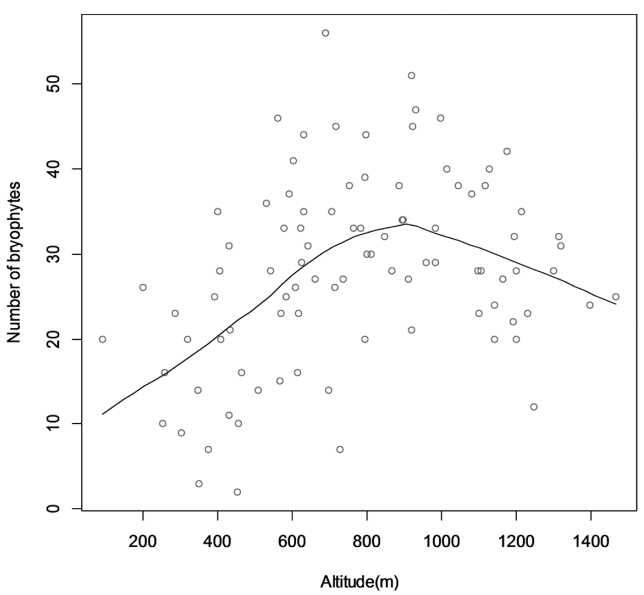

Figure 2 – Distribution of bryophytes richness for the sampled plots per altitude.

To test whether the mean values for the altitude levels defined for each variable were statistically different, we used the *t*-test (95% confidence interval), by applying the function t.test (function that assumes unequal variance and applies the Welch degrees of freedom modification) in R v. 2.15.2 (R Core Team 2012). The variables used for this approach are summarized in table 1 and are separated into 'richness variables', and 'molecular variables'.

A preliminary analysis was performed for each species cover in the sampled plots (*E. intermedia*, *I. prolixum*, *F. polysticta*, *P. canariensis*) to test the influence of the altitude, using regression models (see electronic appendices 2 & 3). Thus, it was possible to understand the relevance of using altitude on the variables under study. The models were performed after doing pairwise correlations (Spearman correlations) between all environmental variables. Only the ones which had a more direct ecological effect were kept, taking in consideration a threshold of $|r| > 0.7$ (Dormann et al. 2013) (results not shown). For instance, temperature and precipitation show a very strong correlation with altitude ($r = -0.96$ and $r = 0.94$, respectively) and therefore were not considered as explanatory variables. Linear models were fitted for each species cover *per* plot using the lm() function in R.

A measure of gene diversity (haplotype diversity index) was applied for each species (*per* altitudinal level). The haplotype diversity index describes the relative diversity of haplotypes considering their frequencies.

The haplotype diversity index (H) was calculated using the formula given below:

$$H = \frac{n}{n-1}\left(1 - \sum_{i=1}^{k} pi^2\right) \text{ ((Nei \& Tajima 1981, Nei 1987)}$$

where
n = number of samples
k = number of haplotypes
pi = haplotype frequency

This measure is analogous to the heterozygosity at a single locus reaching its maximum when haplotypes observed in the sampling group occur at equal frequencies (Beaty et al. 2005).

RESULTS

From the models fitted to each species cover, altitude was a significant explanatory variable ($p < 0.05$) for '*E. intermedia* cover', '*I. prolixum* cover', and '*P. canariensis* cover' (electronic appendix 3) giving an indication on the effect expected by this variable on these species abundance.

From a total of 538 bryophyte taxa present in Madeira and Selvagens Archipelagos we recorded 202, of which 21 were endemics, in the 92 plots sampled in this study (Sim-Sim et al. 2014). The most frequent liverwort species were

Table 1 – Variables designation, altitudinal ranges and sample sizes (N) per species and per altitudinal group.

Variable group	Variable designation	Altitudinal range	Mean altitude	N (total)	N Low altitudes	N High altitudes
Richness variables	Bryophyte species richness	102–1496	776.0	92	48	44
	Endemic bryophyte richness	102–1496	776.0	92	48	44
	E. intermedia cover	220–1327	866.0	48	22	26
	I. prolixum cover	576–1496	989.0	39	18	21
	F. polysticta cover	429–1299	824.0	33	18	15
	P. canariensis cover	291–1327	853.0	63	31	32
Molecular variables	*E. intermedia* haplotype frequency nrITS	425–1496	909.3	49	27	22
	E. intermedia haplotype frequency cpDNA	425–1496	909.0	48	25	23
	I. prolixum haplotype frequency nrITS	674–1496	1036.4	44	21	23
	I. prolixum haplotype frequency cpDNA	674–1496	1049.4	39	18	21
	F. polysticta haplotype frequency nrITS	441–1299	829.6	24	13	11
	F. polysticta haplotype frequency cpDNA	425–1299	852.3	36	19	17
	P. canariensis haplotype frequency nrITS	220–1404	877.7	26	11	15
	P. canariensis haplotype frequency cpDNA	220–1404	838.0	28	14	14

Table 2 – Mean values of 'richness variables' and 'molecular variables' for altitude groups.
T-test results for means comparison are shown by the p-values. Bold type font for higher values of cover and higher haplotype diversity (lower haplotype frequency values). *significant difference between the means (*t*-test).

	Low altitudes	High altitudes	*t*-test (p-value)
Bryophyte species richness	25.26	**30.82**	< 0.05*
Endemic bryophyte richness	2.55	**3.00**	> 0.05
E. intermedia cover	0.39	**0.44**	> 0.05
E. intermedia haplotype frequency - nrITS	0.63	**0.33**	< 0.05*
E. intermedia haplotype frequency - cpDNA	1	1	-
I. prolixum cover	0.34	**0.82**	< 0.05*
I. prolixum haplotype frequency - nrITS	0.76	**0.65**	> 0.05
I. prolixum haplotype frequency - cpDNA	0.65	**0.47**	< 0.05*
F. polysticta cover	**0.43**	0.39	> 0.05
F. polysticta haplotype frequency - nrITS	**0.29**	0.84	< 0.05*
F. polysticta haplotype frequency - cpDNA	**0.50**	0.89	< 0.05*
P. canariensis cover	0.75	**1.03**	> 0.05
P. canariensis haplotype frequency - nrITS	**0.49**	0.44	> 0.05
P. canariensis haplotype frequency - cpDNA	0.87	0.87	> 0.05

Microlejeunea ulicina (Taylor) A.Evans, *Lejeunea lamacerina* (Steph.) Schiffn. and *Saccogyna viticulosa* (L.) Dumort. and, the most frequent moss species were *Fissidens serrulatus* Brid., *Andoa berthelotiana* (Mont.) Ochyra and *Hypnum cupressiforme* Hedw.

Table 2 shows the results of the *t*-test comparison of the means (significant differences of the mean for $p < 0.05$) for each variable ('richness variables' and 'molecular variables'). We grouped the variables by species (cover and haplotype frequencies) to better identify the existence of coinci-

dent patterns. Electronic appendix 4 displays the boxplots for the significant results of the mean comparisons, for different variables.

Bryophyte species richness ranged from 3 to 56 at low altitudes and from 12 to 51 at high altitudes. The mean values of species richness were significantly different ($p < 0.05$), with 25.26 for low altitudes and 30.82 for high altitudes (table 2). Endemic species richness varied from 0 to 11 at low altitudes and from 1 to 8 at high altitudes. The mean values of endemics richness were not significantly different, with 2.55 for low altitudes and 3.00 for high altitudes (table 2).

Bryophyte species cover at low altitudes varied from a minimum of 0.34 for *I. prolixum* to a maximum of 0.75 in *P. canariensis*. At high altitudes, species cover ranged from a minimum of 0.39 in *F. polysticta* to a maximum of 1.03 in *P. canariensis*.

The graphs in fig. 3 show the results of the haplotype diversity index (*H*) calculated for each species and molecular marker as well as the percentage of sporophytes for both altitudinal levels.

Overall, the index of haplotype diversity (*H*) ranged from 0.12 in *F. polysticta* (cpDNA) at high altitudes to 0.77 also in *F. polysticta* (nrITS) but at low altitudes.

The abundance of sporophytes (%) at low altitudes varied from 10.5, in *I. prolixum*, to 75.0 in *P. canariensis*. At high altitudes, it ranged from a minimum of 22.7 in *E. intermedia* to a maximum of 80.0 in *P. canariensis*.

DISCUSSION

This is one of the first studies that relate environmental factors as drivers of genetic diversity for the Macaronesian region. The other known study is that of Patiño et al. (2010) who investigated the genetic diversity and spatial population structure of the moss *Isothecium myosuroides* Brid. in the subtropical cloud forests of La Gomera, Canary Islands, relating genetic diversity with the availability of suitable microhabitats in anthropogenically disturbed forest stands.

With the results obtained by the mean comparisons, bryophyte species richness and endemic species richness are higher for high altitudes in the Laurel forest, although for endemics richness this difference is not statistically significant. This pattern is also observed in the moss species *I. prolixum* and *E. intermedia*, in which a higher species cover and a higher genetic diversity (lower values of haplotypic frequencies) occur at high altitudes. A coincident pattern between the species richness and the genetic diversity was obtained by several authors, such as Vellend (2004), He et al. (2008) and Wei & Jiang (2012) for other plant taxa. For instance, Vellend (2004) demonstrated a long-term legacy of land-use history at different levels of biodiversity, the author found that land-use history and the size of communities influenced the correlations between species diversity and genetic diversity of *Trillium grandiflorum* (Michx.) Salisb. Similar results were obtained by Wei & Jiang (2012) for *Euptelea pleiospermum* Hook.f. & Thomson along an altitudinal gradient

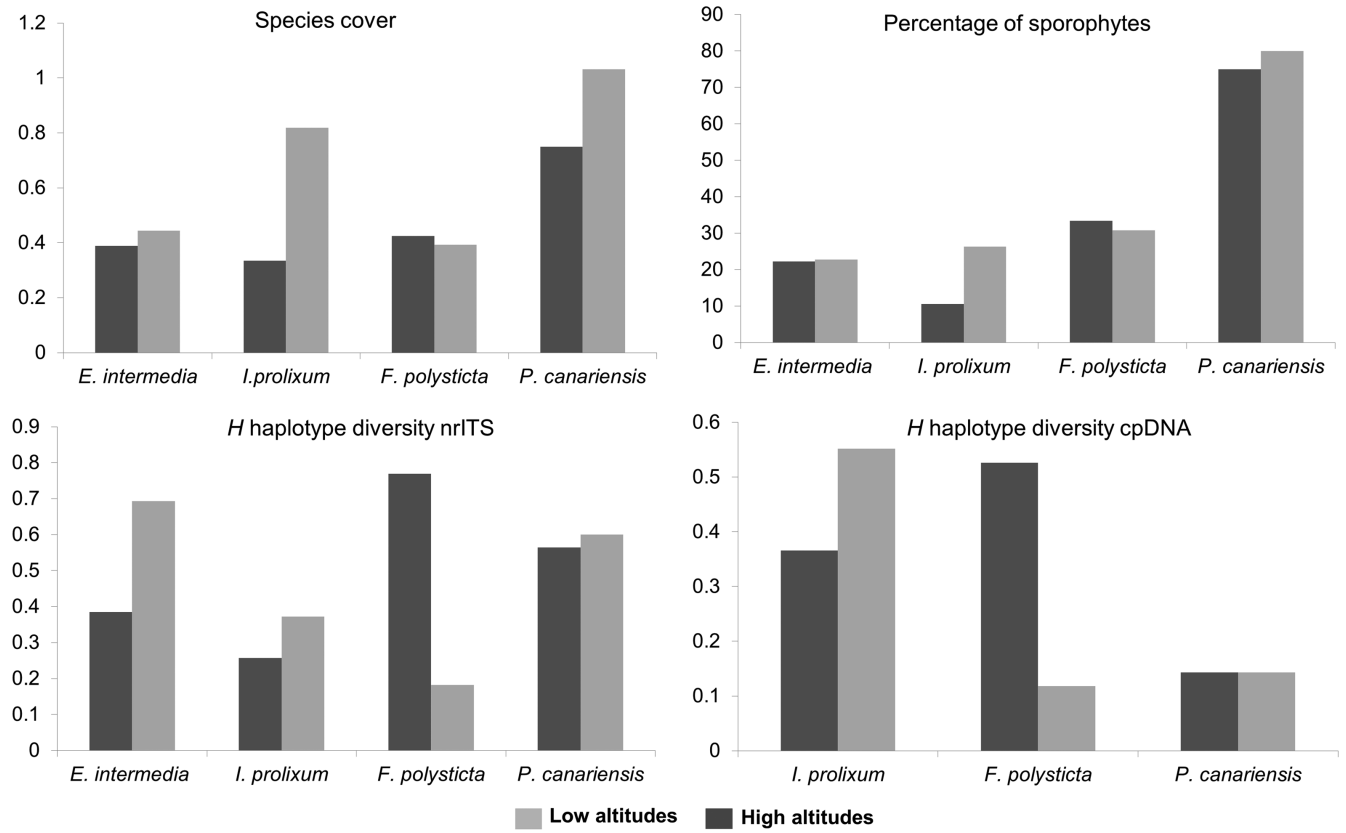

Figure 3 – Species cover, abundance of sporophytes (%) and haplotype diversity index values (nrITS and cpDNA) for the studied taxa (*Exsertotheca intermedia*, *Isothecium prolixum*, *Frullania polysticta* and *Porella canariensis*) in the two altitudinal levels.

in natural forests of China, where a positive correlation between species diversity and genetic diversity was found. This pattern of higher species richness matching a higher genetic diversity is commonly referred in literature – Antonovics (1976) hypothesised that the drivers that maintain species diversity and genetic diversity are similar and this can, to some extent, be shown by our results.

However, this concordance is not followed by the liverwort species. In fact, *F. polysticta* displays an inverse pattern both at the species cover and at the genetic diversity levels in relation to species richness. This is not surprising as this species has a preference for habitats at low altitudes in the Laurel forest (Sim-Sim 1999).

The other liverwort species, *P. canariensis* does not present any significant difference in cover and haplotype frequency means, suggesting that altitude does not affect this species distribution in the study area. Hahn et al. (2012) and Perronne et al. (2014) had similar results for grassland plant species referring that altitude does not affect its genetic diversity. *Porella canariensis* is known for its ecological plasticity occurring frequently in the 'Til' Laurel forest (*Clethro arboreae-Ocotea foetensis*), being also found in the 'Barbusano' natural forest (*Semele androgynae-Apollonietum barbujana*e) and more rarely in the high altitude heathlands (*Vaccinio padifoli-Ericetum maderinicolae* and *Polysticho falcinelli-Erico arboreae*) (Fontinha et al. 2010).

Our results revealed that there is not a general concordance between haplotype diversity and bryophyte species richness, in similarity to Finn & Poff's (2011) results for Chironomidae species in stream habitats. Conversely, our data appear to show a convergence between each species cover and its correspondent mean haplotype diversity. This pattern is, as expected, further highlighted, by the haplotype diversity index (fig. 3) for *I. prolixum*, *E. intermedia, F. polysticta* and *P. canariensis* is superior for higher values of cover.

Odat et al. (2010) referred that the observed relationship between species diversity and genetic diversity appears to be indirect rather than direct (related to habitat quality), after verifying that the abundance of *Plantago lanceolata* L. at a site co-varied positively with the increase in species number.

The cover of *E. intermedia* and *I. prolixum* is higher at high altitudes, where bryophyte species richness is also higher. An inverse pattern is shown by *F. polysticta* that presents higher cover and genetic diversity values at low altitudes. Therefore, and according to Odat et al. (2010), negative relationships between species richness and genetic diversity of some species are to be expected. Our results also appear to indicate that a species genetic diversity is more closely linked to its abundance, and probably habitat suitability.

The pattern of sporophytes abundance (percentage) also matches cover and genetic diversity. In fact, according to some authors this measure of sexual expression is related to the species cover – Huttunen (2003) referred that the biomass of shoots seems to have an effect on the production of perichaetia and Sundberg (2000) verified that the patch size of some *Sphagnum* species appeared to be important for sporophyte production. The author demonstrated that for the majority of the species, sporophytes were produced in good years (the year after a wet summer), as long as the species were relatively abundant at a site. Furthermore, Stark et al. (2005) verified that *Syntrichia caninervis* Mitten decreased sporophyte production as stress increased and Eppley et al. (2011) referred that physiological stress constrains sexual reproduction in the extremophile system for *Pohlia nutans* (Hedw.) Lindb.

As shown in fig. 3, *P. canariensis* is the species that produces more sporophytes having simultaneously higher cover values, which indicates a possible relation between a species cover and its ability to reproduce sexually, thus affecting species genetic diversity due to outcrossing possibilities (Sundberg & Rydin 2000). Further studies with other dioecious species are necessary in order to understand how habitat suitability, species cover, genetic diversity and sexual reproduction are linked.

According to our results, altitude seems to have an effect on genetic diversity of species in addition to the effect on bryophyte species richness and, in some of the selected species, cover.

The importance of including genetic diversity in biodiversity perceptions is highlighted by several authors (e.g. Boshier & Young 2000, Frankham 2005b) since species lacking substantial genetic variation are thought to be more vulnerable to extinction from natural or human-caused environmental changes (Colwell 2009). However, some authors consider the conservation of the genetic component of biodiversity impractical arguing that the assessment of all possible genes of all populations of a species is technically impossible (e.g. Papageorgiou & Kasimiadis 2013). The finding of surrogate measures of genetic diversity in Madeira's Laurel forest such as species cover or species diversity is an important strategy to avoid the constraints (time and money) associated to genetic diversity measurements. Nevertheless, as shown by this study, a genetic diversity study is necessary to confirm patterns of biodiversity. Future work should, therefore, include the monitoring of haplotypic diversity along with species diversity and abundance in order to: (i) confirm the trends observed by using additional bryophyte taxa and other molecular methods such as fingerprinting and (ii) understand if climate change will affect the relation between altitude and diversity patterns.

From a conservation and management perspective, our results highlight the importance of maintaining large patches of bryophyte species, which should be considered in management plans, especially for endemics and red-listed species.

SUPPLEMENTARY DATA

Supplementary data are available in pdf at *Plant Ecology and Evolution*, Supplementary Data Site (http://www.ingentaconnect.com/content/botbel/plecevo/supp-data), and consist of: (1) environmental variables registered in the field and extracted from GIS layers for each sampled plot; (2) liverwort and moss species cover along the altitude values for the 92 sampled plots; (3) results from the models fitted to each species cover; and (4) boxplots of significant results of means differences for the two altitudinal levels.

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