Phytotaxa 677 (3): 251–264
https://www.mapress.com/pt/

Article

ISSN 1179-3155 (print edition)
PHYTOTAXA
ISSN 1179-3163 (online edition)

https://doi.org/10.11646/phytotaxa.677.3.5

Patterns of intraspecific genetic variation of *Andoa berthelotiana* (Myuriaceae, Bryophyta) in the Azores (Macaronesia)

SORAIA MARTINS[1,2,6], MANUELA SIM-SIM[1,2,7], ROSALINA GABRIEL[3,8] & MICHAEL STECH[4,5,9]
[1]*cE3c - Centre for Ecology, Evolution and Environmental Changes & CHANGE - Global Change and Sustainability Institute, Departamento de Biologia Vegetal, Faculdade de Ciências, Universidade de Lisboa, Campo Grande, 1749-016 Lisboa, Portugal*
[2]*MUHNAC - Museu Nacional de História Natural e da Ciência, Universidade de Lisboa, Rua da Escola Politécnica, 58, 1250-102 Lisboa, Portugal*
[3]*University of the Azores, CE3C/GBA - Centre for Ecology, Evolution and Environmental Changes / Azorean Biodiversity Group & CHANGE - Global Change and Sustainability Institute, Departamento de Ciências e Engenharia do Ambiente, Faculdade de Ciências Agrárias e do Ambiente, Universidade dos Açores, Angra do Heroísmo, 9700-042 Lisboa, Portugal*
[4]*Naturalis Biodiversity Center, P.O. Box 9517, 2300 RA Leiden, The Netherlands*
[5]*Leiden University, Leiden, The Netherlands*
[6] *ssmartins@fc.ul.pt; https://orcid.org/0000-0002-3585-3500*
[7] *mmsim-sim@ciencias.ulisboa.pt; https://orcid.org/0000-0003-3467-6538*
[8] *rosalina.ma.gabriel@uac.pt; https://orcid.org/0000-0002-3550-8010*
[9] *Michael.Stech@naturalis.nl; https://orcid.org/0000-0001-9804-0120*
Author for correspondence: Soraia Martins (ssmartins@fc.ul.pt)

Abstract

Macaronesia is home to several endemic bryophytes, such as *Andoa berthelotiana*. Recent genetic studies revealed the existence of two intraspecific lineages within this species, one in Madeira and the Canary Islands and another in the Azores. However, patterns of intraspecific variation within archipelagos, remain a relatively unexplored area of research. In this study we investigate patterns of intraspecific variation of *Andoa berthelotiana* within the Azorean archipelago and potential associations with elevation. Our investigation is based on the analysis of chloroplast DNA sequences and AFLP data.

The dataset includes *trnL-trnF* sequences from 23 samples and AFLP data from 34 samples collected in seven of the nine Azorean islands, encompassing elevations of up to 1200 m. For the DNA sequence data haplotype diversity, nucleotide diversity and a statistical parsimony haplotype network were calculated. The AFLP data were analyzed by AMOVA (Analysis of Molecular Variance) and PCoA (Principal Coordinates Analysis). A Chi-Square test was carried out to assess the significance of the relationship between elevation and AFLP groups or *trnL-trnF* haplotypes, respectively.

The observed intraspecific genetic variation clustered into three *trnL-trnF* haplotypes and two AFLP groups, without a clear geographical structure across the Azorean archipelago. Group AFLP1 and haplotypes trnL_F1 and trnL_F3 only appeared below 600 m elevation, where low-elevation vegetation (coastal woodlands, lowland forests and montane *Laurus azorica* forests) prevails, while group AFLP2 and haplotype trnL_F2 occurring along the whole elevational range, but with most samples above 600 m. Associations between elevation and molecular groups were significant according to the Chi-Square test.

Our study reveals the presence of genetic variation within *Andoa berthelotiana* in the Azorean archipelago. The two main genetic groups do not seem to be related to geographical location of islands nor groups of islands, but rather to elevation. One group exhibits a broader ecological amplitude, whereas the other may be genetically adapted to environmental conditions at lower elevations (below 600 m), which includes the area where the native laurel forests dominate and where anthropogenic activities have altered the majority of natural vegetation. Conservation efforts should therefore not only consider the species *Andoa berthelotiana* itself, which is associated with natural forest, but also intraspecific genetic diversity of *Andoa* populations, in particular genotypes restricted to lower elevations.

Key words: AFLP, conservation, ecology, elevation, haplotypes, *trnL-trnF*

Introduction

Macaronesia (comprising the Azores, Madeira, Selvagens, Canary Islands, and Cape Verde archipelagos) is home to several endemic bryophytes, some of which are present in all but the driest Macaronesian archipelagos Selvagens and

Accepted by Wen Ye: 23 Oct. 2024; published: 18 Dec. 2024

Cape Verde (Vanderpoorten *et al.* 2011). One example is *Andoa*, a monospecific genus of pleurocarpous mosses, which occurs in the Canary Islands (Tenerife, Fuerteventura, La Palma), Madeira Island, and the Azores (all nine islands) (Martins *et al.* 2021 and references therein).

A recent study based on DNA sequences and AFLP data revealed the existence of two intraspecific lineages within *Andoa berthelotiana* Montagne (1840:4) Ochyra (1982:31), one occurring in Madeira and the Canary Islands, and the other in the Azores (Martins *et al.* 2021). The presence of morphological differences corresponding with the molecular lineages, but also considerable morphological overlap, led to the interpretation of both lineages as semi-cryptic species (Martins *et al.* 2021).

Recent studies indicated the ecological and evolutionary importance of intraspecific variation (e.g. Godhe & Rynearson, 2017, Mimura *et al.* 2017, Garcia-Cisneros *et al.* 2018, Des Roches *et al.* 2021). Not incorporating local adaptation in species distribution models hampers our capacity to predict distribution ranges, leading to the concern of whether modelling should be done at the species or intraspecific level (Collart *et al.* 2021). As far as pleurocarpous mosses are concerned, already Cronberg (2004) indicated that alpine and subalpine populations of *Hylocomium splendens* (Hedwig:1801:262) Schimper (1852:173) were genetically differentiated at the molecular level. Désamoré *et al.* (2012) analyzed patterns of intraspecific molecular variation in *Homalothecium sericeum* Hedwig (1801:228) Schimper (1851:93) in Europe in relation to climate change using species distribution modelling. Hedenäs (2016, 2019), based on comparative analysis of several acrocarpous and pleurocarpous species, stated that examining intraspecific variation is vital for biodiversity studies and conservation management, and that each individual species should be investigated instead of relying solely on data from other taxa, due to large differences between species and geographical areas.

Within Macaronesia, Patiño *et al.* (2010) and Sim-Sim *et al.* (2015) investigated patterns of intraspecific genetic diversity in the Canary Islands and Madeira Island, respectively. The latter study explored for the first time relationships between intraspecific genetic variation and elevation in four different bryophyte species, and is still the only such bryological study in Macaronesia.

Since the molecular variation in *Andoa berthelotiana* seemed largest within the Azores (Martins *et al.* 2021), with the current study we aim to provide the first insights into patterns of intraspecific variation within the Azorean archipelago. Specifically, we aim to determine possible relations between genetic variation and geographical (island or group of islands) factors and abiotic (elevation) factors. Although *Andoa berthelotiana* is not currently under threat in the Azores as a whole (cf. Martins *et al.* 2021), this species is considered Vulnerable (Hodgetts *et al.* 2019) in the European IUCN Red List. The results may thus inform future conservation actions to preserve this species and its genetic diversity within the Azorean archipelago.

Material and methods

Study area

The Azores are divided into three island groups. The Eastern group (São Miguel and Santa Maria Islands, and Formigas Islets; Pacheco *et al.* 2013) and the Central group (Faial, Pico, São Jorge, Terceira and Graciosa Islands) are distributed on the Nubia-Eurasia tectonic plate boundary (Mitchell *et al.* 2018, Silva *et al.* 2021). The Western group comprises Flores and Corvo Islands, both located on the North American tectonic plate (Fernandes *et al.* 2006, Mitchell *et al.* 2018, Silva *et al.* 2021). Except for Pico (2350 m a.s.l.), peak elevations of the Azorean islands ranges approximately between 400 and 1100 m a.s.l. (Graciosa 405, Santa Maria 587, Corvo 718, Flores 911, Terceira 1021, Faial 1043, São Jorge 1053, São Miguel 1105 m a.s.l.) (Forjaz *et al.* 2004).

Since the beginning of continuous human settlement in the early 15th century, the natural vegetation in the Azores has been under constant anthropogenic pressure, causing permanent changes in vegetation, according to studies from Flores, Pico and S. Miguel islands (Connor *et al.* 2012, 2013, Rull *et al.* 2017). About 56% of the archipelago territory is occupied by agriculture and pasture, while forests and natural vegetation occupy 35% (Monteiro *et al.* 2008), of which an estimated less than five percent still is original native forest (Triantis *et al.* 2010). Almost one fifth (19%) of the bryophyte species are considered threatened or near threatened (Gabriel & Sérgio 1995, Hodgetts *et al.* 2019). The vast majority of human activities that caused the transformation or degradation of most of the natural vegetation of the Azores, with a larger influence in the laurel forests, occurred in elevations below 600 m, while above 600 m we find the best-preserved vegetation belts (Elias *et al.* 2016).

The laurel forest (Laurissilva) dominated by *Laurus azorica* Seubert (1844:29) Franco (1960:101) and *Myrica* *faya* Aiton (1789:397) seems to be the climax vegetation type up to approximately 600 m a.s.l., above which it is gradually replaced by *Erica azorica* Hochstetter ex Seubert (1844:40) and eventually by *Juniperus brevifolia* (Seubert 1844:26) Antoine (1857:16) (Tutin, 1953). More recently, Elias *et al.* (2016) distinguished eight vegetation types in the Azores based on studies in four islands from the three islands groups (São Miguel, Pico, Terceira and Flores). Below 600 m there are *Erica*-*Morella* coastal woodlands, *Picconia*-*Morella* lowland forests and *Laurus* submontane forests, while above 600 m five vegetation types occur, namely *Juniperus*-*Ilex* montane forests, *Juniperus* montane woodlands, *Calluna*-*Juniperu*s altimontane scrublands, and two high elevation vegetation types not considered here because our study altitudinal limit is 1200 m.

Plant material

A total of 34 Azorean *Andoa* specimens, collected between 2008 and 2011, were analyzed, covering seven Azorean islands, all except Corvo and Graciosa, the two smallest islands, virtually without native forests. Collection sites are shown in Fig. 1. The Azores shape files were supplied by the University of Azores, Azores Biodiversity Group, loaded on QGIS Desktop 3.22.1 and exported to Paint. Voucher information per specimen is given in Table 1.

Molecular analysis

For further details on DNA extraction, PCR, Sanger sequencing, and AFLP analysis see Martins *et al.* (2021). Of the three molecular markers used in that study (*trnL-trnF*, ITS, *nad5*) most sequences from Azorean specimens were obtained for the chloroplast marker *trnL-trnF*; therefore, we compared that marker with the AFLP data in the present study. We included *trnL-trnF* sequences from 23 *Andoa* samples for which also AFLP data were available (Table 2). For the other 11 samples for which AFLP data were present, no properly readable sequences could be obtained. For GenBank accession numbers see Appendix 1 in Martins *et al.* (2021). DNA sequences were manually aligned in PhyDE v0.995 (Müller *et al.* 2006). Absence/presence information of AFLP fragments obtained for two selective primer combinations from 34 specimens (Table 2) was transformed into a binary matrix (0 – fragment absent /1 – fragment present).

Data analysis

A statistical parsimony (TCS) haplotype network (Clement *et al.* 2002) of *trnL-trnF* sequences of 23 *Andoa* samples was carried out with PopArt (Leigh & Bryant, 2015). Haplotype diversity (Hd) and nucleotide diversity (π) were calculated in DNAsp5 (Librado and Rozas 2009).

The AFLP data were analyzed by AMOVA (Analysis of Molecular Variance) and PCoA (Principal Coordinates Analysis), using GenAlex 6.5 (Peakall & Smouse 2012). In PCoA, the data analyzed is a similarity or distance matrix for a set of objects. The similarity or distance matrix is assembled from the data variables (Gower 1966), here the scored AFLP fragments. The AMOVA was carried out to calculate pairwise PhiPT values (Fst analogue for binary data in GenAlex; Bolibok-Brągoszewska *et al.* 2014) to estimate genetic differentiation between two elevational groups (0−600 m, *Picconia*-*Morella* lowland forests and *Laurus* submontane forests; 600−1200 m, *Juniperus*-*Ilex* forests and *Juniperus* woodlands). The number of permutations used to estimate the probability values was 999 (Yamasaki and Ideta 2013). Following Yaacov *et al.* (2012), we considered PhiPT values 0.15–0.25 representing large, 0.05–0.15 moderate, and <0.05 little genetic differentiation with p-value = 0.001.

A Chi-Square test was carried out in Past 4.03 (Hammer 2021) to assess the significance of the relationship between elevation and AFLP groups or *trnL-trnF* haplotypes, respectively. In this analysis, we divided the 34 AFLP samples into four elevational groups (0−300 m, 300−600 m, 600−900 m, 900−1200 m). For *trnL-trnF*, the 23 samples were divided into two elevational groups (0−600 m, 600−1200 m) due to the lower number of samples available, in order to obey the Chi-Square test principles (Zar 2010). Significance levels were considered significant if p-values were below 0.05.

When 20% of the samples are below five, it is required to apply the Chi-Square test by Monte Carlo simulation, which comprises a comparison of the observed data with random samples that are generated in accordance with the tested hypothesis. In the present case, the analyzed p-values are those from Monte Carlo simulation. We used the Bonferroni correction for corrections of p for multiple comparisons, concerning AFLP and *trnL-trnF* samples. The significance level (p=0.05) was divided by the number of samples, respectively eight and six.

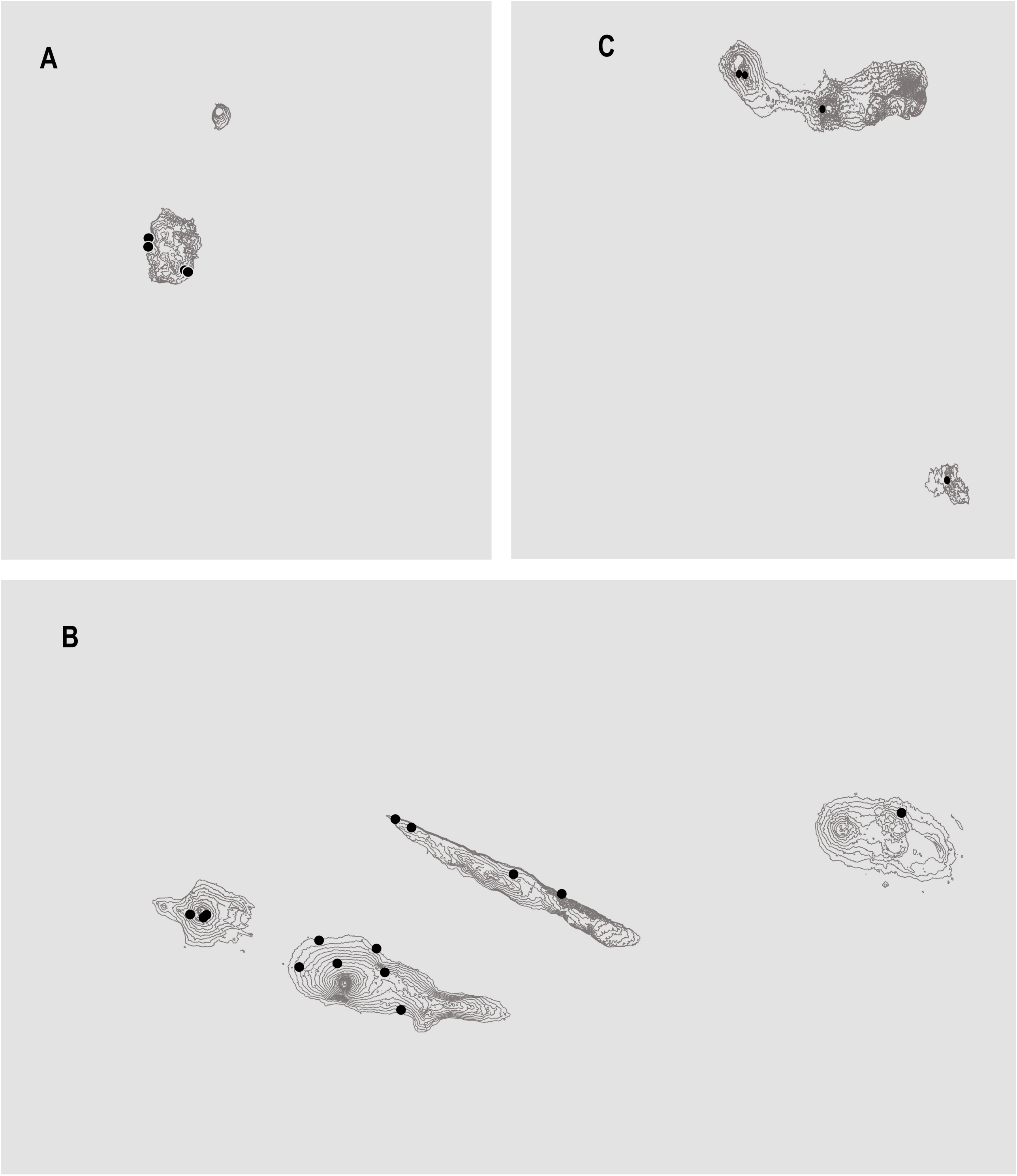


FIGURE 1. Azorean islands with altimetry maps of 100 m altitudinal curves and sampling locations (black dots). A. Western island group (Flores), B. Central island group (Faial, Pico, S. Jorge, Terceira). C. Eastern island group (S. Miguel and Sta Maria).

TABLE 1. Voucher information for the analyzed *Andoa berthelotiana* specimens. CG Central Group, EG Eastern group, WG Western group.

DNA no.	Island group	Island	Collecting site	Elevational group (m)	Main vegetation	Herbarium number
1328	2_CG	Pico	Perto da Furna Henrique Maciel	0–200	Forest with *Pittosporum undulatum*, *Picconia azorica* (Tutin 1933:101) Knoblauch (1934:1128)	267264 (LISU)
1329	2_CG	Pico	Transversal road to Pico	0–200	Forest with *Pittosporum undulatum* ?	267265 (LISU)
1330	2_CG	Pico	Furna da Agostinha	0–200	Vineard in ancient clearing invaded and surrounded by forest with *Pittosporum undulatum*, *Myrica faya* and *Picconia azorica*	267266 (LISU)
1117	1_WG	Flores	Aldeia da Cuada	0–200	Forest with *Laurus azorica* and *Pittosporum undulatum*	267244 (LISU)
1126	1_WG	Flores	Aldeia da Cuada	0–200	Forest with *Laurus azorica* and *Pittosporum undulatum*	267246 (LISU)
1168	1_WG	Flores	Faja Grande - Poço do Bacalhau	0–200	Forest	L.4518907 (L)
1169	1_WG	Flores	near Lombas	0–200	Forest	L.4518940 (L)
1212	1_WG	Flores	near Lombas	0–200	Forest with *Laurus azorica*	267254 (LISU)
1213	1_WG	Flores	Aldeia da Cuada	0–200	Forest with *Laurus azorica* and *Pittosporum undulatum*	267255 (LISU)
1214	1_WG	Flores	Aldeia da Cuada	0–200	Forest with *Laurus azorica* and *Pittosporum undulatum*	267256 (LISU)
1118	2_CG	S. Jorge	Lagoa	0–200		267245 (LISU)
1217	2_CG	S. Jorge	Lagoa	0–200		267259 (LISU)
1327	2_CG	Pico	Gruta das Torres	200–400	*Pittosporum undulatum*, *Juniperus brevifolia*, *Laurus azoria*	267263 (LISU)
1170	2_CG	S. Jorge	Near to the road, close to Pico dos Cutelos	200–400	Forest	L.4518981 (L)
1116	2_CG	Terceira	Escaleira	200–400	Forest with *Laurus azorica*	267243 (LISU)
1127	2_CG	Terceira	Escaleira	200–400	Forest with *Laurus azorica*	267247 (LISU)
1215	2_CG	Terceira	Escaleira	200–400	Forest with *Laurus azorica*	267257 (LISU)
1216	2_CG	Terceira	Escaleira	200–400	Forest with *Laurus azorica*, *Erica azorica* and *Pittosporum undulatum*.	267258 (LISU)
1219	2_CG	Terceira	Escaleira	200–400	Forest with *Laurus azorica*	267261 (LISU)
1220	2_CG	Terceira	Escaleira	200–400	Forest with *Laurus azorica*, *Erica azorica* and *Pittosporum undulatum*.	267262 (LISU)
1218	2_CG	S. Jorge	Sete Fontes Park	400–600		267260 (LISU)
1529	3_EG	S. Miguel	Forest track on Caldeira das Sete Cidades	400–600	Forest with *Cryptomeria japonica* (Thumberlet ex. Linnaeus 1782:421) D.Don (1839:167)	GM Dirkse 27212
1149	3_EG	Sta Maria	Pico Alto	400–600		267250 (LISU)
1128	2_CG	S. Jorge	Ribeira d'Areias. Perímetro Florestal.	400–600	Forest	267248 (LISU)
1159	2_CG	Faial	Forest very close to Road Cabeço dos 30	600–800	Forest - *Laurus azorica*?	267252 (LISU)

...continued on the next page

TABLE 1. (Continued)

DNA no.	Island group	Island	Collecting site	Elevational group (m)	Main vegetation	Herbarium number
1542	2_CG	Faial	Forest very close to Road Cabeço dos 30	600–800	Forest - *Laurus azorica*?	L267270 (LISU)
1343	2_CG	Pico	Mistério de Santa Luzia	600–800	Forest with *Erica azorica*	267267 (LISU)
1359	2_CG	Pico	Close to Lagoa do Capitao	600–800	Pasture	267268 (LISU)
1148	3_EG	S. Miguel	Lagoa do Canário	600–800		267249 (LISU)
1158	2_CG	Faial	Caldeira	800–1000		267251 (LISU)
1541	2_CG	Faial	Caldeira	800–1000		267269 (LISU)
1528	3_EG	S. Miguel	Mountain area along the road from Lagoa to Ribeira Grande	800–1000	Forest with *Laurus azorica*, *Ilex canariensis* Poir (1813:67), *Calluna vulgaris* (Linnaeus 1753:352) Hull (1808:114)	GM Dirkse 27259
1160	2_CG	Faial	Geodesic mark	1000–1200	Pasture area	267253 (LISU)
1543	2_CG	Faial	Geodesic mark	1000–1200	Pasture area	267271 (LISU)

TABLE 2. DNA numbers of samples analyzed for AFLP (all) and *trnL-trnF* haplotypes (gray shade), respectively, per 200 m a.s.l. interval. For further specimen information see Table 1.

0−200 m	200−400 m	400−600 m	600−800 m	800−1000 m	1000−1200 m
1117	1116	1128	1148	1158	1160
1118	1127	1149	1159	1528	1543
1126	1170	1218	1343	1541	
1168	1215	1529	1359		
1169	1216		1542		
1212	1219				
1213	1220				
1214	1327				
1217					
1328					
1329					
1330					

Results

Intraspecific variation of *Andoa berthelotiana* within the Azorean archipelago

Three *trnL-trnF* haplotypes were detected (Fig. 2), haplotype trnL-F1 with 11 samples from islands Flores, Terceira, São Jorge, Pico and Santa Maria, haplotype trnL-F2 with eight samples from São Jorge, Faial, Terceira, Pico and São Miguel islands, and haplotype trnL-F3 with four samples from Flores, Terceira and Pico islands. Overall Azorean haplotype diversity (Hd) and nucleotide diversity (π) were 0.642 and 0.00202, respectively.

According to the PCoA analysis of the AFLP results, two main groups can be distinguished (AFLP1 and AFLP2; Fig. 3), of which group AFLP1 was found on Flores, Pico, São Jorge, Santa Maria, São Miguel and Terceira islands and group AFLP2 on Faial, Flores, Pico, São Jorge, São Miguel and Terceira islands. AFLP group 2 includes all samples with *trnL-trnF* haplotypes trnL-F1 and trnL-F3, except for sample 1168 from Flores (haplotype trnL-F1) that belongs to group AFLP2. All samples with haplotype trnL-F2 correspond to group AFLP2.

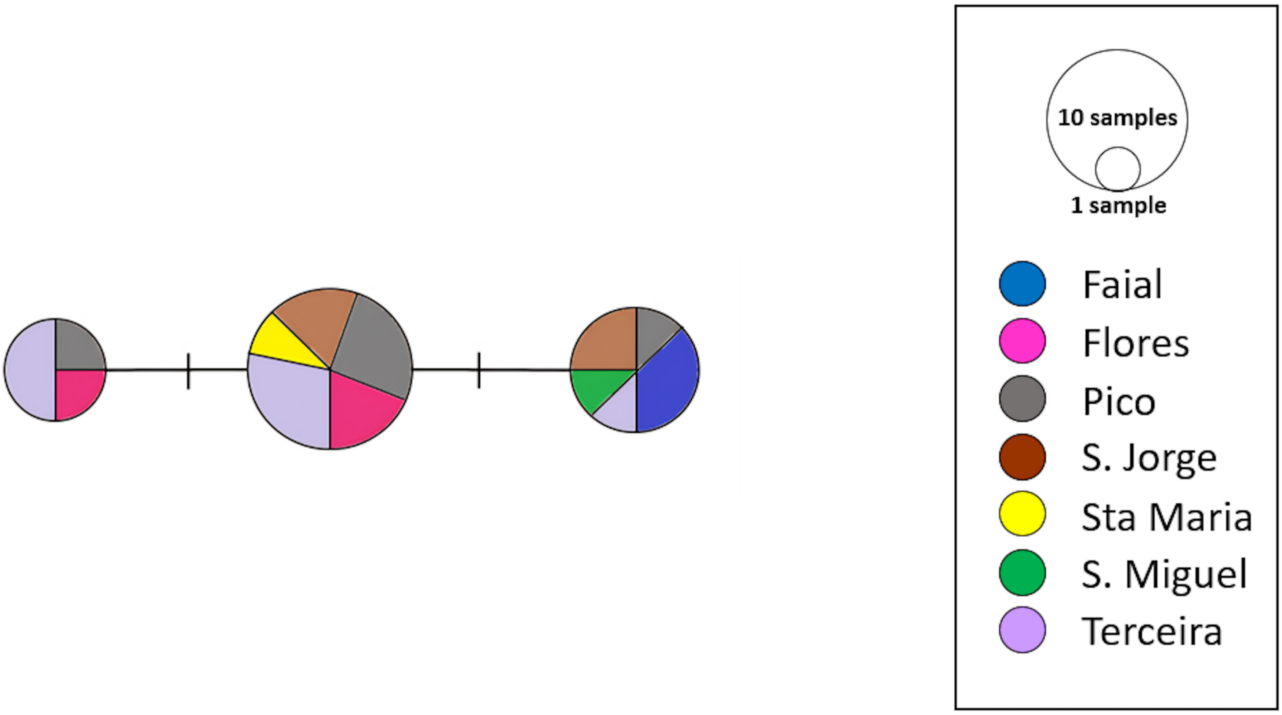


FIGURE 2. Haplotype network resulting from an alignment of *trnL-trnF* sequences of 23 specimens. Center: haplotype trnL-F1, right: haplotype trnL-F2, left: haplotype trnL-F3.

Relations between genetic variation and elevation

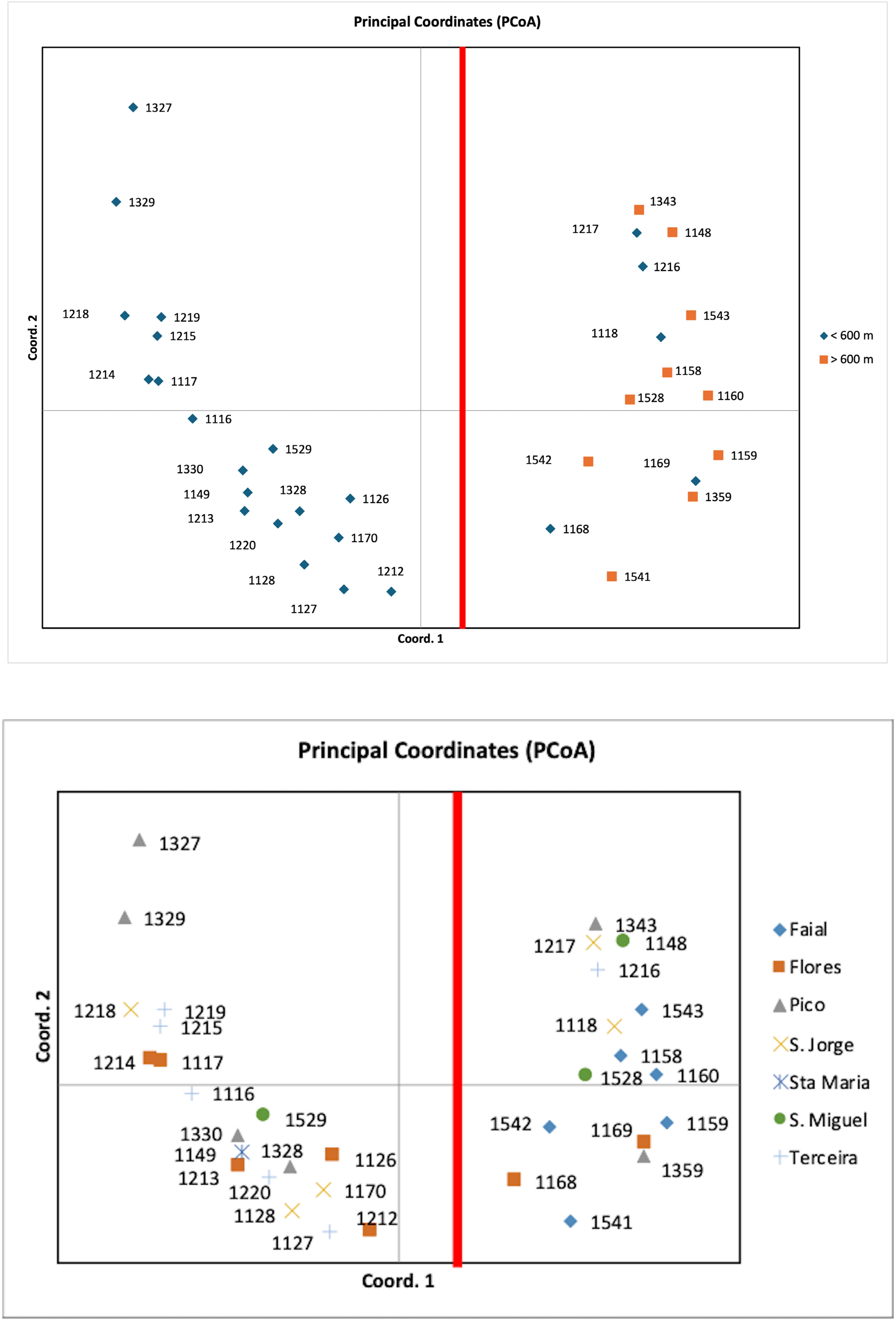
Of the three *trnL-trnF* haplotypes, trnL-F1 is only present below 600 m, while trnL-F3 is only present below 400 m. TrnL-F2 is the most widespread haplotype, occurring along the whole elevational range but with most samples above 600 m (Fig. 4). When grouping all samples from each haplotype into two intervals (0−600 m and 600−1200 m), the Chi-Square test indicated a significant difference between both elevation levels (Chi-Square: 11.979; Degrees of freedom: 2; p= 0.0019).

Group AFLP1 is formed exclusively by samples located below 600 m (Fig. 3 and Fig. 5), while group AFLP2 spans the whole elevational range but is observed mainly above 600 m. The PhiPT value between samples located between 0−600 m and 600−1200 m was 0.0151, indicating large genetic differentiation between these two altitudinal intervals. When grouping all samples from each AFLP group into four intervals (0−300 m, 300−600 m, 600−900 m, 900−1200 m; Table 3), the Chi-Square test indicated a significant difference between the frequencies of AFLP1 and AFLP2 among the four altitudinal levels (Chi-Square: 19.08; Degrees of freedom: 3; p=0.0001).

Using the Bonferroni correction, the corrected significance level is 0.05/6=0.0083 for *trnL-trnF* and 0.05/8=0.00625 for AFLP (both superior to the p-values obtained for *trnL-trnF* and AFLP, respectively 0.0019 and 0.0001), again confirming significant difference between both elevation levels for *trnL-trnF* haplotypes and indicating a significant difference between the presence of AFLP1 and AFLP2 considering the four altitudinal levels.

Discussion

In addition to the intraspecific variation across Macaronesia (Martins *et al.* 2021), the current study explores the intraspecific variation within *Andoa berthelotiana* in the Azorean archipelago. Across Macaronesia the genetic variation is clearly geographically structured, leading to the presence of two semi-cryptic species, one in Canary Islands plus Madeira and another in the Azores (Martins *et al.* 2021). This differentiation, together with evidence at species level (e.g. *Echinodium spinosum* Mitten (1865:7) Juratzka (1866:178) in Madeira plus Canary Islands versus *Echinodium renauldii* (Cardot 1897:69) Brotherus (1909:1217) in the Azores; Stech *et al.* 2008) and comparison of whole bryophyte flora's (González-Mancebo *et al.* 2008), support the classification of the Macaronesian archipelagos into different biogeographic regions. For example, Rivaz-Martinéz *et al.* (2014) considered the Azores to belong to the Atlantic Central European sub-region, while Madeira belongs to Canarian-Madeirense biogeographic sub-region.



a)

b)

FIGURE 3. PCoA analysis of *Andoa berthelotiana* samples from Azores archipelago: Faial, Flores, Pico, São Jorge, Santa Maria, São Miguel, Terceira islands, for the first two axes based on AFLP distance matrices. On the left and right sides samples cluster together, seemingly forming two groups, respectively AFLP1 and AFLP2. In a) symbols indicate elevation lower and higher than 600 m, in b) symbols indicate the different islands.

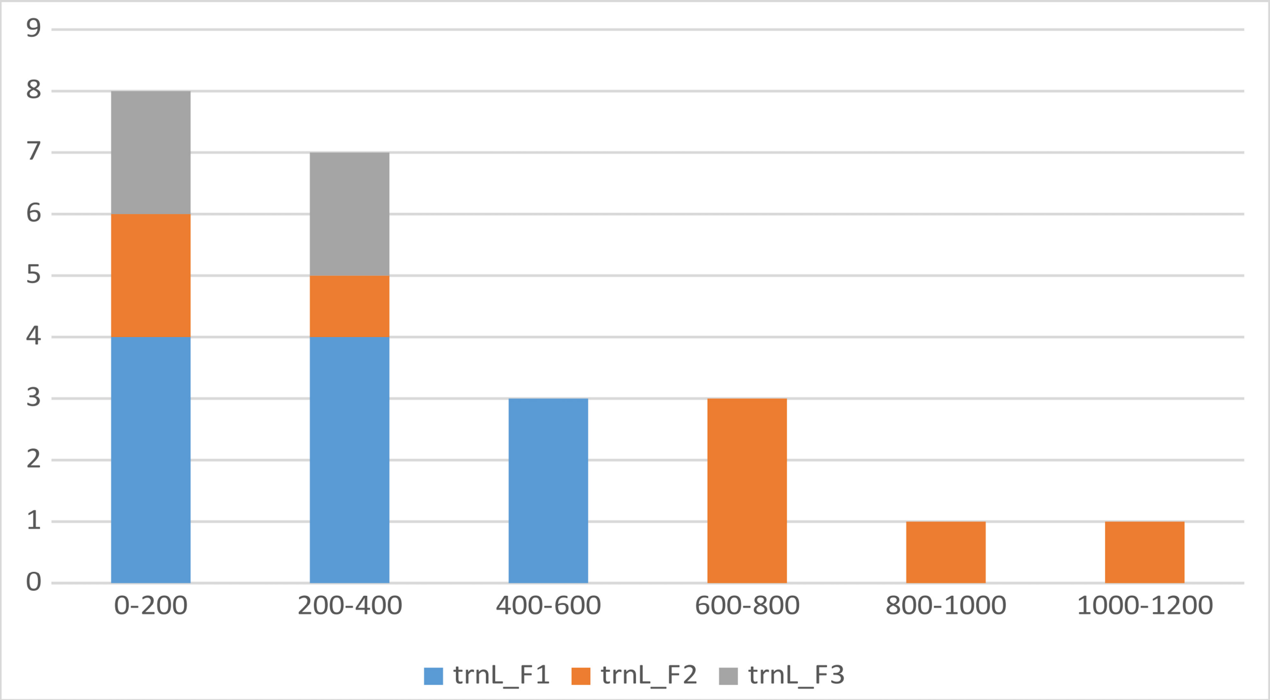


FIGURE 4. Elevational gradient with 200 m intervals, between haplotypes trnL-F1, trnL-F2 and trnL-F3. 200 m intervals were considered to see if there were if there was any haplotype differentiation below or above 600 m.

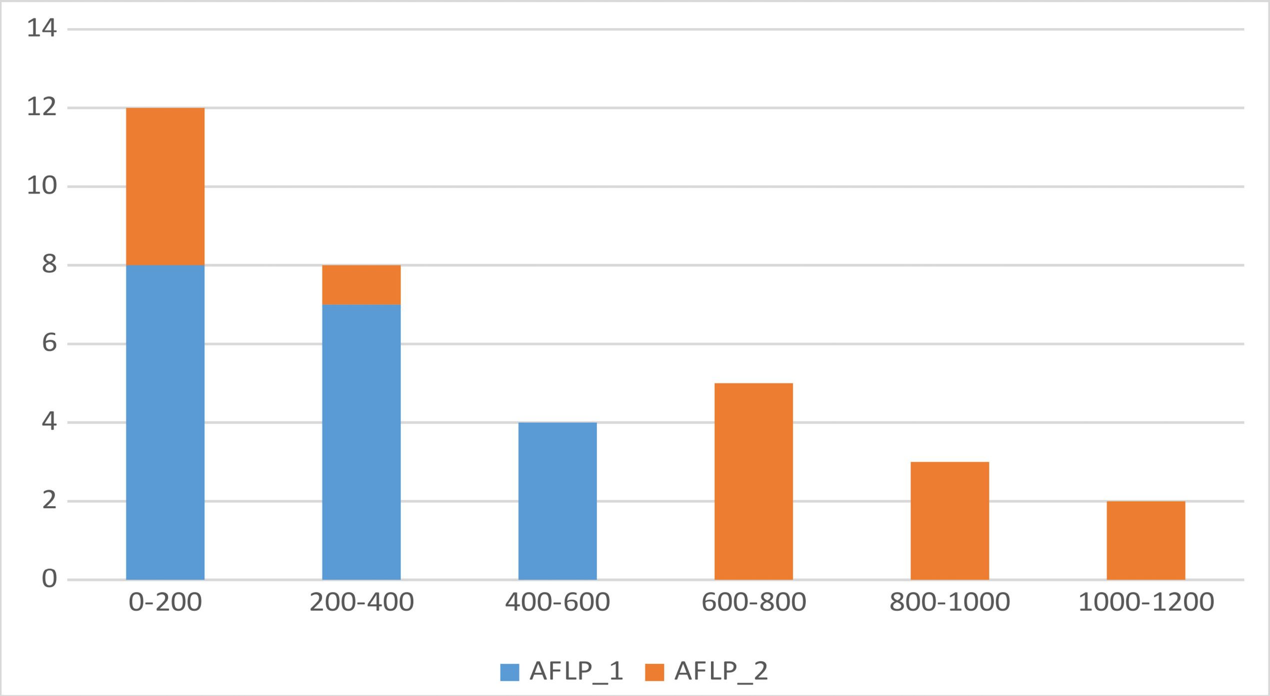


FIGURE 5. Elevational gradient with 200 m intervals, between groups AFLP1 and AFLP2. 200 m intervals were considered to see if there were any AFLP group differentiation below or above 600 m.

Within the Azores, the present data do not show a clear geographical structure of genetic variation with *Andoa berthelotiana*. There seems to be a slight shift in *trnL-trnF* haplotype distribution from the Western to the Eastern island groups as well as some differentiation in frequency of occurrence of AFLP groups 1 and 2 (e.g. AFLP1 more frequent on Flores and Pico, and AFLP2 on Faial), but a higher number of samples needs to be analyzed to substantiate this observation. Due to the shorter distances between the islands of the Azores archipelago than between the Azores *versus* Madeira and Canary Islands, assuming more frequent dispersal events and genetic exchange in *Andoa* populations within the Azorean islands might be plausible. Though haplotype diversity is relatively high within the Azores, the low nucleotide diversity reveals small variation amid haplotypes.

TABLE 3. Distribution of samples from both AFLP groups between 300 m a.s.l. intervals, with pairwise significant differences. The column (Significance) indicates the significance letters assigned (a – b) to the altitude intervals based on the post-hoc test. Intervals that share the same letters do not significantly differ from each other, whereas intervals with different letters indicate significant differences in the AFLP distribution of *Andoa berthelotiana* across the four elevation intervals ($p < 0.05$).

Intervals	AFLP1	AFLP2	Significance
0−300	9	4	a
300−600	10	1	a
600−900	0	6	b
900−1200	0	4	b

In contrast to a relationship with geographical location of islands or groups of islands, intraspecific variation within the Azores is related to the elevation at which the samples were collected. Elevation is well known as a propeller of species diversity and genetic diversity, also in bryophytes (Cronberg 2004, Sim-Sim *et al.* 2015), given that it influences temperature and precipitation, which in turn impact on plant species distribution and possibly intraspecific genetic variation (Sim-Sim *et al.* 2015). *Andoa* specimens with genotypes restricted to elevations below 600 m (AFLP group 1, haplotypes trnL-F1 and trnL-F3) may be genetically adapted to environmental conditions that prevail in those areas. There, most samples were collected in forests with *Laurus azorica* (endemic) and/or *Pittosporum undulatum* Ventenat (1802:76) (introduced invasive species) and sometimes also with *Erica azorica* (endemic). The other genotypes (AFLP group 2, haplotype trnL-F2), in contrast, seem to have a wider ecological amplitude, ranging from low- to high-elevation vegetation.

Within the Azores archipelago, anthropogenic activities have altered the majority of natural vegetation, in particular the laurel forests between 300 and 600 m a.s.l. (Elias *et al.* 2016). Beyond 600 m until about 900 m a.s.l., the best conserved vegetation belts are found, which include *Juniperus*-*Ilex* forests and *Juniperus* woodlands (Elias *et al.* 2016). Generally, bryophyte species richness in the Azores is highest at around 600 m a.s.l. (Gabriel & Bates, 2005, Gabriel *et al.* 2011, Henriques *et al.* 2017), with exception of the highest island Pico, where bryophyte richness peaks from 600 m to 1000 m a.s.l. (Coelho *et al.* 2021). Conservation of the remains of vegetation types below 600 m a.s.l. (*Erica*-*Morella* coastal woodlands, *Picconia*-*Morella* lowland forests and *Laurus* submontane forests) is thus crucial for the conservation of bryophyte diversity. As our data show, this does not only hold for the species *Andoa berthelotiana* itself, which is associated with natural forest (Gabriel *et al.* 2011), but also for the intraspecific genetic diversity of *Andoa* populations, i.e. the genotypes restricted to lower elevations. AFLP group 1 and haplotypes trnL-F1 and trnL-F3 might be related to habitats contained in *Picconia-Morella* lowland forests (generally between 100–300 m a.s.l., also down to coastal ares) and particularly *Laurus* submontane forests (frequently between 300 and 600 m a.s.l.). This observation was taken from the data concerning the main vegetation present at the collection site and the elevation. Other authors have found that environmental determinants, namely orographic mist layer, were important to predict bryophyte richness in the Macaronesian Islands (cf. Aranda *et al.* 2014), and these environmental factors may also be related to intraspecific variation. More research is required to infer whether the different genotypes correlate with environmental conditions and specific habitat types.

Hedenäs (2016) already concluded that intraspecific diversity may significantly vary in different regions and that reducing regional frequencies of bryophyte species could cause an important reduction of genetic variation. Population genetic diversity is usually not given proper consideration in conservation priorities (Kahilainen *et al.* 2014), despite the fact that genetic diversity can increase the probability of species survival and lessen fast anthropogenic environmental changes (Hughes and Tillman, 2004; Jump *et al.* 2009; Spagnuolo *et al.* 2009). For *Andoa*, the present data indicates that, in parallel to conserving populations across its distribution range, in particular the few remaining populations on Tenerife, Fuerteventura and La Palma, Canary Islands (González-Mancebo *et al.* 2008, 2009, 2019), efforts are required to create priority conservation areas or to improve extant ones that sustain the current genetic variation within *Andoa* on the Azores, mainly below 600 m.

Conclusions

Together with the earlier study by Sim-Sim *et al.* (2015), the present study shows an avenue for the study of intraspecific variation within Macaronesian endemic bryophyte species in relation to elevation, where the preservation of one or few populations with unique genotypes might be of importance to protect the future existence of species in a context of possible continuous anthropogenic environmental changes. Our analyses on the elevational distribution of intraspecific molecular variation in the endemic *Andoa berthelotiana* furthermore support the view that the conservation of vegetation types below 600 m is essential for the conservation of bryophyte diversity in the Azores.

Acknowledgements

Heartfelt thanks are due to Foundation of Science and Technology for the PhD scholarship SFRH/BD/46274/2008, Direção Regional dos Açores and Eng. Carlos Pimentel for permission to collect material and assistance with the field work, Eduarda Goulart, Eng. José Costa and co-worker forest guard for support with logistics and field work, Drª. Cecília Sérgio for information and advice regarding *Andoa*, Dr. César Garcia for helping with field work on Pico Island, Profª. Juana González-Mancebo for supplying samples from Canary Islands, Dr. Gerard M. Dirkse for supplying samples from S. Miguel Island, Anabela Martins for providing advice concerning SIG Software, Enésima Pereira for supplying the Azores shapefiles, the Collection department of Naturalis Biodiversity Center for assistance with collection work, and the technicians of the laboratories of Naturalis Biodiversity Center and Institute of Biology, Leiden University, for assistance with the molecular lab work.

References

Aiton, W. (1789) *Hortus Kewensis; or, a catalogue of the plants cultivated in the Royal Botanic Garden at Kew*, vol. 3. George Nicol, London, 547 pp.

Antoine, F. (1857) *Die Cupressineen-Gattungen: Arceuthos, Juniperus und Sabina.* Friedrich Beck'sche Universitäts-Buchhandlung, Wien, 72 pp., 92 pls.

Aranda, S.C., Gabriel, R., Borges, P.A., Santos, A.M., de Azevedo, E.B., Patiño, J., Hortal, J. & Lobo, J.M. (2014) Geographical, temporal and environmental determinants of bryophyte species richness in the Macaronesian Islands. *PLoS One* 9 (7): e101786. https://doi.org/10.1371/journal.pone.0101786

Bolibok-Brągoszewska, H., Targońska, M., Bolibok, L., Kilian, A. & Rakoczy-Trojanowska, M. (2014) Genome-wide characterization of genetic diversity and population structure in *Secale*. *BMC Plant Biology* 14: 184. https://doi.org/10.1186/1471-2229-14-184

Brotherus, V.F. (1909) Bryales. *In*: Engler, H.G.A. & Prantl, K. (Eds.) *Die Natürlichen Pflanzenfamilien.* Part. I (3). Engelmann, Leipzig. pp. 1217.

Cardot, J. (1897) The Mosses of the Azores. *Annual Report of the Missouri Botanical Garden* 8: 51–72.

Clement, M., Snell, Q., Walker, P., Posada, D. & Crandall, K. (2002) TCS: Estimating gene genealogies. Parallel and Distributed Processing Symposium, International Proceedings 2: 184.

Coelho, M.C.M., Gabriel, R., Hespanhol, H., Borges, P.A.V. & Ah-Peng, C. (2021) Bryophyte Diversity along an Elevational Gradient on Pico Island (Azores, Portugal). *Diversity* 13 (4): 162. https://doi.org/10.3390/d13040162

Collart, F., Hedenäs, L., Broennimann, O., Guisan, A. & Vanderpoorten, A. (2021) Intraspecific differentiation: Implications for niche and distribution modelling. *Journal of Biogeography* 48 (2): 415−426. https://doi.org/10.1111/jbi.14009

Connor, S.E., van Leeuwen, J.F., Rittenour, T.M., van der Knaap, W.O., Ammann, B. & Björck, S. (2012) The ecological impact of oceanic island colonization – a palaeoecological perspective from the Azores. *Journal of Biogeography* 39: 1007–1023. https://doi.org/10.1111/j.1365-2699.2011.02671.x

Connor, S.E., van Leeuwen, J.F. & Kuneš, P. (2013) Holocene palaeoclimate and palaeovegetation of the islands of Flores and Pico. *In:* Fernández-Palacios Carmona, J.M. & Fernández-Palacios, J.M. (Eds.) *Climate Change Perspectives from the Atlantic: Past, Present and Future.* Servicio de Publicaciones de la Universidad de La Laguna, San Cristobal de La Laguna, pp. 149–162.

Cronberg, N. (2004) Genetic differentiation between populations of the moss *Hylocomium splendens* from low versus high elevation in the Scandinavian mountain range. *Lindbergia* 29 (1): 64−72.

Don, D. (1839) VIII. Descriptions of two new genera of the natural family of plants called Coniferae. *Transactions of the Linnean Society of London* 18 (2): 163−179.

Des Roches, S., Pendleton, L.H., Shapiro, B. & Palkovacs, E.P. (2021) Conserving intraspecific variation for nature's contributions to

people. *Nature Ecology & Evolution* 5 (5): 574−582.
https://doi.org/10.1038/s41559-021-01403-5

Désamoré, A., Laenen, B., Stech, M., Papp, B., Hedenäs, L., Mateo, R. & Vanderpoorten, A. (2012) How do temperate bryophytes face the challenge of a changing environment? Lessons from the past and predictions for the future. *Global Change Biology* 18: 2915−2924.
https://doi.org/10.1111/j.1365-2486.2012.02752.x

Elias, R.B., Gil, A., Silva, L., Fernández-Palacios, J.M., Azevedo, E.B. & Reis, F. (2016) Natural zonal vegetation of the Azores Islands: characterization and potential distribution. *Phytocoenologia* 46 (2): 107–123.
https://doi.org/10.1127/PHYTO/2016/0132

Forjaz, V.H., Tavares, J.M., Azevedo, E.M.V.B., Nunes, J.C., Santos, R.S., Barreiros, J.P., Gallagher, L., Barcelos, P.J.M., Silva, P.H., Cardigos, F., França, Z.T.M., Dentinho, T., Costa, M,P., Magalhães, L., Rodrigues, M.C., Gonçalves, J.F., Silva, V. & Serpa, V. (2004) The Azores Basic Atlas. *In:* Forjaz, V.H. (Ed,) *Observatório Vulcanológico e Geotérmico dos Açores*. Ponta Delgada, Azores, Portugal, 112 pp.

Fernandes, R.M.S., Bastos, L., Miranda, J.M., Lourenço, N., Ambrosius, B.A.C., Noomen, R. & Simons, W. (2006) Defining the plate boundaries in the Azores region. *Journal of Volcanology and Geothermal Research* 156 (1–2): 1−9.
https://doi.org/10.1016/j.jvolgeores.2006.03.019

Franco, J.A. (1960) Lauráceas. Macaronésia. *Anais Instituto Superior de Agronomia* V–XXIII: 89–104.

Gabriel, R. & Bates, J.W. (2005) Bryophyte community composition and habitat specificity in the natural forests of Terceira, Azores. *Plant ecology* 177: 125–144.
https://doi.org/10.1007/s11258-005-2243-6

Gabriel, R. & Sérgio, C. (1995) Bryophyte survey for a first planning of conservation areas in Terceira (Açores). *Cryptogamica Helvetica* 18: 35−41.

Gabriel, R., Homem, N., Couto, A.B., Aranda, S.C. & Borges, P.A. (2011) Azorean bryophytes: a preliminary review of rarity patterns. *Açoreana, Supl.* 7: 149−206.

Garcia-Cisneros, A., Palacin, C., Ventura, C.R.R., Feital, B., Paiva, P.C. & Pérez-Portela, R. (2018) Intraspecific genetic structure, divergence and high rates of clonality in an amphi-Atlantic starfish. *Molecular Ecology* 27 (3): 752−772.
https://doi.org/10.1111/mec.14454

Godhe, A. & Rynearson, T. (2017) The role of intraspecific variation in the ecological and evolutionary success of diatoms in changing environments. Philosophical Transactions of the Royal Society B: *Biological Sciences* 372 (1728): 20160399.
https://doi.org/10.1098/rstb.2016.0399

González-Mancebo, J., Draper, I., Lara, F., Marrero, J.D., Muñoz, J., Patiño, J., Romaguera, F. & Vanderpoorten, A. (2009) Amendments to the bryophyte flora of the Cape Verde and Canary Islands. *Cryptogamie, Bryologie* 30 (4): 433−441.

González-Mancebo, J., Romaguera, F., Ros, R.M., Patiño, J. & Werner, O. (2008) Bryophyte flora of the Canary Islands: an updated compilation of the species list with an analysis of island distribution patterns in the context of the Macaronesian Region. *Cryptogamie, Bryologie* 29 (4): 315−357.

González-Mancebo, J., Sim-Sim, M., Gabriel, R., Hodgetts, N. & Martins, A. (2019) *Andoa berthelotiana*. *The IUCN Red List of Threatened Species* 2019: e.T84711905A87713954.
https://doi.org/10.2305/IUCN.UK.2019-2.RLTS.T84711905A87713954.en

Gower, J.C. (1966) Some distance properties of latent root and vector methods used in multivariate analysis. *Biometrika* 53 (3−4): 325−338.
https://doi.org/10.1093/biomet/53.3-4.325

Hammer, Ø. (2021) PAST—Paleontological Statistics, Version 4.03—Reference Manual. Natural History Museum, University of Oslo, Oslo. Available from: https://www.nhm.uio.no./english/research/resources/past (accessed 17 December 2024)

Hedenäs, L. (2016) Intraspecific diversity matters in bryophyte conservation–internal transcribed spacer and rpl16 G2 intron variation in some European mosses. *Journal of Bryology* 38 (3): 173−182.
https://doi.org/10.1080/03736687.2016.1145522

Hedenäs, L. (2019) On the frequency of northern and mountain genetic variants of widespread species: essential biodiversity information in a warmer world. *Botanical Journal of the Linnean Society* 191 (4): 440−474.
https://doi.org/10.1093/botlinnean/boz061

Hedwig, J. (1801) Species muscorum frondosorum: descriptae et tabulis aeneis lxxvii coloratis illustratae. Opus posthumum editum a Friderico Schwaegrichen. Joannis Ambrosii Barthii, Lipsiae, 353 pp. + 77 pls.

Henriques, D.S.G., Rigal, F., Borges, P.A.V., Ah-Peng, C. & Gabriel, R. (2017) Functional diversity and composition of bryophyte water-related traits in Azorean native vegetation. *Plant Ecology & Diversity* 10 (2−3): 127−137.
https://doi.org/10.1080/17550874.2017.1315839

Hodgetts, N., Cálix, M., Englefie, E., Fett, N., Criado, M.G., Patin, L., Nieto, A., Bergamini, A., Bisang, I., Baisheva, E., Campisi, P., Cogo, A., Hallingbäck, T., Konstantinova, N., Lockhart, N., Sabovljevic, M., Schnyder, N., Schröck, C., Sérgio, C., Sim-Sim, M., Vrba, J., Ferreira, C.C., Afonina, O., Blockeel, T., Blom, H., Caspari, S., Gabriel, R., Garcia, C., Garilleti, R., González-Mancebo, J., Goldberg, I., Hedenäs, L., Holyoak, D., Hugonnot, V., Huttunen, S., Ignatov, M., Ignatova, E., Infante, M., Juutinen, R., Kiebacher, T., Köckinger, H., Kučera, J., Lonnell, N., Lüth, M,, Martins, A., Maslovsky, O., Papp, B., Porley, R., Rothero, G., Söderström, L., Ştefănuţ, S., Syrjänen, K., Untereiner, A., Váňa, J., Vanderpoorten, A., Vellak, K., Aleffi, M., Bates, J., Bell, N., Brugués, M., Cronberg, N., Denyer, J., Duckett, J., During, H., Enroth, J., Fedosov, V., Flatberg, K.I., Ganeva, A., Gorski, P., Gunnarsson, U., Hassel, K., Hespanhol, H., Hill, M., Hodd, R., Hylander, K., Ingerpuu, N., Laaka-Lindberg, S., Lara, F., Mazimpaka, V., Mezaka, A., Müller, F., Orgaz, J.D., Patiño, J., Pilkington, S., Puche, F., Ros, R.M., Rumsey, F., Segarra-Moragues, J., Seneca, A., Stebel, A., Virtanen, R., Weibull, H., Wilbraham, J. & Zarnowiec, J. (2019) *A miniature world in decline. European Red List of Mosses, B. Liverworts and Hornworts*. IUCN. Available from: https://portals.iucn.org/library/sites/library/files/documents/RL-4-027-En.pdf (accessed 17 December 2024)

Hughes, A.R. & Tilman, D. (2004) Genetic diversity enhances the resistance of a seagrass ecosystem to disturbance. *Proceedings of the National Academy of Sciences* 101: 8998–9002.
https://doi.org/10.1073/pnas.0402642101

Hull, J. (1808) *The British Flora, or a Linnaean Arrangement of British Plants*. 2nd ed, vol. 1. Manchester & London.

Juratzka, J. (1866) Bryologisches. *Botanische Zeitung (Berlin)* 24 (23): 177–179.

Jump, A.S., Marchant, R. & Peñuelas, J. (2009) Environmental change and the option value of genetic diversity. *Trends in Plant Science* 14 (1): 51–58.
https://doi.org/10.1016/j.tplants.2008.10.002

Kahilainen, A., Puurtinen, M. & Kotiaho, J.S. (2014) Conservation implications of species–genetic diversity correlations. *Global Ecology and Conservation* 2: 315–323.
https://doi.org/10.1016/j.gecco.2014.10.013

Knoblauch, E. (1934) Neue und seltene Oleaceen aus dem tropischen Afrika, Neu-Guinea, China und Indo-China. *Notizblatt des Botanischen Gartens und Museums zu Berlin-Dahlem* 11: 1027−1031.
https://doi.org/10.2307/3994607

Librado, P. & Rozas, J. (2009) DnaSP v5: A software for comprehensive analysis of DNA polymorphism data. *Bioinformatics* 25: 1451−1452.
https://doi.org/10.1093/bioinformatics/btp187

Leigh, J.W. & Bryant, D. (2015) PopART: Full-feature software for haplotype network construction. *Methods in Ecology and Evolution* 6 (9): 1110–1116.

Linnaeus, C. (1753) *Species Plantarum*, vol. 1. Laurentii Salvii, Holmiae, 560 pp.

Linnaeus, C. (1782) *Supplementum Plantarum Systematis Vegetabilium Editionis decimae tertiae, Generum Plantarum Editiones sextae, et Specierum Plantarum Editionis secundae.* Editum a Carolo a Linné. Orphanotrophei, Brunsvigae, 468 pp.

Martins, S., Sim-Sim, M. & Stech, M. (2021) The Macaronesian endemic moss *Andoa berthelotiana* (Myuriaceae, Bryophyta): Phylogenetic relationships and cryptic speciation. *Nova Hedwigia* 112 (3−4): 335−357.
https://doi.org/10.1127/nova_hedwigia/2021/0629

Mimura, M., Yahara, T., Faith, D.P., Vázquez-Domínguez, E., Colautti, R.I., Araki, H., Javadi, F.,Núñez-Farfán, J., Mori, A.S., Zhou, S., Hollingsworth, P.M., Neaves, L.E., Fukano, Y., Smith, G.F., Sato, Y.-I., Tachida, H. & Hendry, A.P. (2017) Understanding and monitoring the consequences of human impacts on intraspecific variation. *Evolutionary applications* 10 (2): 121−139.
https://doi.org/10.1111/eva.12436

Mitchell, N., Stretch, R., Tempera, F. & Ligi, M. (2018) Volcanoes in the Azores: A Marine Geophysical Perspective. *In*: Kueppers, U. & Beier, C. (Eds.) *Volcanos of the Azores. Revealing the Geological Secrets of the Central Northern Atlantic Islands*. Springer, Berlin, Germany, pp. 101−126.

Mitten, W. (1865) Contribution to the Cryptogamic flora of the Atlantic Islands. *Journal of the Linean Botanical Society* 8: 1−10.

Montagne, C. (1840) *Hypnum berthelotianum*. *In*: Webb, M.B. & Berthelot, S. (Eds.) *Histoire Naturelle des Iles Canaries*, vol. 3 (2.4). pp. 4−6.

Ochyra, R. (1982) New names for genera of mosses. *Journal of Bryology* 12: 31−32.

Monteiro, R., Furtado, S., Rocha, M., Freitas, M., Medeiros, R. & Cruz, J.V. (2008) *O ordenamento do território nos Açores. Política e instrumentos.* Ed. SRAM, Ponta Delgada, Azores, Portugal, 159 pp.

Müller, K., Müller, J., Neinhuis, C. & Quandt, D. (2006) PhyDE: Phylogenetic Data Editor, v0.995. Program distributed by the authors. Available from: http://www.phyde.de (accessed 17 December 2024)

Pacheco, J.M., Ferreira, T., Queiroz, G., Wallenstein, N., Coutinho, R., Cruz, J.V., Pimentel, A., Silva, R., Gaspar, J.L. & Goulart, C. (2013) Notas sobre a geologia do arquipélago dos Açores. *In*: Dias, R., Araújo, A., Terrinha, P. & Kullberg, J.C. (Eds.) *Geologia de*

Portugal, vol. 2. Escolar Editora, pp. 595−690.

Patiño, J., Werner, O. & González-Mancebo, J.M. (2010) The impact of forest disturbance on the genetic diversity and population structure of a late-successional moss. *Journal of Bryology* 32: 220–231. https://doi.org/10.1179/037366810X12735734836133

Peakall, R. & Smouse, P.E. (2012) GenAlEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research-an update. *Bioinformatics* 28: 2537−2539. https://doi.org/10.1093/bioinformatics/bts460

Poir, J.L.M. (1813) *Encyclopédie Méthodique.* Botanique, Supplément, vol. 3. H. Agasse, Paris, 780 pp.

Rivas-Martinez, S., Penas, A., Diaz-González, T.E., Río, S.D., Cantó, P., Herrero, L., Pinto Gomes, C. & Costa, J.C. (2014) Biogeography of Spain and Portugal. Preliminary typological synopsis. *International Journal of Geobotanical Research* 4: 1−64.

Rull, V., Lara, A., Rubio-Inglés, M.J., Giralt, S., Gonçalves, V., Raposeiro, P., Hernández, A., Sánchez-López, G., Vásquez-Loureiro, D., Bao, R., Masqué, P. & Sáez, A. (2017) Vegetation and landscape dynamics under natural and anthropogenic forcing on the Azores islands: A 700-year pollen record from the São Miguel Island. *Quaternary Science Reviews* 159: 155−168. https://doi.org/10.1016/j.quascirev.2017.01.021

Schimper, W.P. (1851−1853) Bryologia Europaea, seu genera muscorum Europaeorum monographice illustrata vol. 5. Schweizerbart, Stuttgart.

Seubert, M. (1844) *Flora Azorica*. Adolphus Marcus, Bonnae, 50 pp. + 15 pls.

Sim-Sim, M., Lopes, T., Ruas, S. & Stech, M. (2015) Does altitude shape molecular diversity and richness of bryophytes in Madeira's natural forest? *Plant Ecology and Evolution* 148: 171−180.

Silva, R., Carmo, R. & Marques, R. (2021) Characterization of the tectonic origins of historical and modern seismic events and their societal impact on the Azores Archipelago, Portugal. *In*: Dilek, Y., Ogawa, Y. & Okubo, Y. (Eds.) *Characterization of Modern and Historical Seismic–Tsunamic Events, and Their Global–Societal Impacts.* The Geological Society of London, London, pp. 245−267.

Spagnuolo, V., Terracciano, S. & Giordano, S. (2009) Intra-Specific Genetic Variation in Mosses: A Novel Approach to Detect Environmental Changes. *In*: Mahoney, C.L. & Springer, D.A. (Eds.) *Genetic Diversity.* Nova Science Publishers, New York, U.S.A., pp. 271−283.

Stech, M., Sim-Sim, M., Esquível, M.G., Fontinha, S., Tangney, R., Lobo, C., Gabriel, R. & Quandt, D. (2008) Explaining the 'anomalous' distribution of *Echinodium* (Bryopsida: Echinodiaceae): independent evolution in Macaronesia and Australasia. *Organisms Diversity & Evolution* 8 (4): 282–292. https://doi.org/10.1016/j.ode.2008.02.001

Triantis, K.A., Borges, P.A.V., Ladle, R.J., Hortal, J., Cardoso, P., Gaspar, C., Dinis, F., Mendonca, E., Silveira, L.M.A., Gabriel, R., Melo, C., Santos, A.M.C., Amorim, I.R., Ribeiro, S.P., Serrano, A.R.M., Quartau, J.A. & Whittaker, R.J. (2010) Extinction debt on oceanic islands. *Ecography* 33 (2): 285−294. https://doi.org/10.1111/j.1600-0587.2010.06203.x

Tutin, T.G. (1933) Notes from the University Herbarium, Cambridge. On *Ilex perado* Ait. and *Notelaea excelsa*. *Journal of Botany, British and Foreign* 71: 99−101.

Tutin, T.G. (1953) The vegetation of the Azores. *Journal of Ecology* 41: 53–61.

Vanderpoorten, A., Laenen, B., Gabriel, R., González-Mancebo, J.M., Rumsey, F. & Carine, M.A. (2011) Dispersal, diversity and evolution of the Macaronesian cryptogamic floras. *In*: Bramwell, D. & Caujapé-Castells, J. (Eds.) *The Biology of Island Floras.* Cambridge University Press, Cambridge, UK, pp. 338−364.

Ventenat, E.P. (1802) *Description des plantes nouvelles et peu connues, cultivées dans le jardin de J. M. Cels.* Crapelet, Paris, 100 pp.

Yaacov, D.B., Arbel-Thau, K., Zilka, Y., Ovadia, O., Bouskila, A. & Mishmar, D. (2012) Mitochondrial DNA Variation, but Not Nuclear DNA, Sharply Divides Morphologically Identical Chameleons along an Ancient Geographic Barrier. *PLoS ONE* 7 (3): e31372. https://doi.org/10.1371/journal.pone.0031372

Yamasaki, M. & Ideta, O. (2013) Population structure in Japanese rice population. *Breeding Science* 63 (1): 49−57. https://doi.org/10.1270/jsbbs.63.49

Zar, J.H. (2010) *Biostatistical analysis*. Pearson Prentice-Hall, Upper Saddle River, NJ, 944 pp.