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RESEARCH PAPER

How to define nativeness in vagile organisms: lessons from the cosmopolitan moss *Bryum argenteum* on the island of Tenerife (Canary Islands)

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ABSTRACT

The distinction between native and introduced biotas presents unique challenges that culminate in organisms with high long-distance dispersal capacities in a rapidly changing world. Bryophytes, in particular, exhibit large distribution ranges, and some species can truly be qualified as cosmopolitan. Cosmopolitan species, however, typically occur in disturbed environments, raising the question of their nativeness throughout their range. Here, we employ genetic data to address the question of the origin of the cosmopolitan, weedy moss *Bryum argenteum* on the island of Tenerife. The genetic diversity of *B. argenteum* on Tenerife was comparable to that found in continental areas due to recurrent colonisation events, erasing any signature of a bottleneck that would be expected in the case of a recent colonisation event. The molecular dating analyses indicated that the first colonisation of the island took place more than 100,000 years ago, *i.e.* well before the first human settlements. Furthermore, the significant signal for isolation-by-distance found in *B. argenteum* within Tenerife points to the substantial role of genetic drift in establishing the observed patterns of genetic variation. Together, the results support the hypothesis that *B. argenteum* is native on Tenerife; although the existence of haplotypes shared between Tenerife and continental areas suggests that more recent, potentially man-mediated introduction also took place. While defining nativeness in organisms that are not deliberately introduced, and wherein the fossil record is extremely scarce, is an exceedingly challenging task, our results suggest that population genetic analyses can represent a useful tool to help distinguish native from alien populations.

INTRODUCTION

Comparative analyses of alien and native species are a crucial step for describing global patterns of invasions and making hypotheses about the ecological and evolutionary mechanisms promoting invasion (Pysek 2003). The distinction between native and introduced biota presents, however, unique challenges (Jewell *et al.* 2012). This is especially true in organisms with high long-distance dispersal capacities (Bean 2007) in a rapidly changing world, wherein fast species migrations in response to climate change substantially impact on their distribution patterns and, consequently, our ability to trace back source and target geographic areas (Webber & Scott 2012).

Bryophyte species typically exhibit broader distribution ranges than angiosperms, and some species are truly cosmopolitan (Medina *et al.* 2011). Cosmopolitan species in particular exhibit a low genetic structure among continents (Werner & Guerra 2004 in *Tortula muralis* Hedw.; McDaniel & Shaw 2005 in *Ceratodon purpureus* (Hedw.) Brid.; Pisa *et al.* 2014 in *Bryum argenteum* Hedw.), pointing to the importance of transcontinental migrations in the group. Moreover, the bulk of cosmopolitan species are confined to open or disturbed sites, potentially showing an increase in their local abundance and distribution range due to anthropogenic activity (Schuster 1983). This is reminiscent of invasive species' behaviour (Essl *et al.* 2013) and raises the question of whether cosmopolitan bryophyte species are native throughout their range. This is especially true on oceanic islands, which are particularly exposed to biological invasions (Denslow *et al.* 2009; Essl *et al.* 2014).

It is, however, extremely difficult to determine whether bryophyte species are native to an area because of the scarcity of their fossil record and strong long-distance dispersal capabilities. Traditionally, three lines of evidence were used to identify alien bryophytes (Söderström 1992; Essl *et al.* 2013). First, species distributions were analysed to detect potential range expansion and/or anomalous geographic distributions. For example, *Orthodontium lineare* Schwägr. and *Campylopus introflexus* (Hedw.) Brid. exhibit a primarily circum-sub-Antarctic distribution, and invaded Europe from the beginning of the 20th century onwards (Hassel & Söderstöm 2005). Second, alien species are thought to be associated with some means of introduction, *e.g.* ports, botanical gardens and other gardening activities. For example, the southern hemisphere liverwort

Lophocolea semiteres (Lehm.) Mitt. is now present and locally abundant in the UK, the Netherlands and Belgium. It was initially found in gardens on the isles of Scilly off southwest England, where it is thought to have been introduced in the early 1900s with garden plants from Australia (Stieperaere 1994). Third, alien species are typically associated with open, disturbed or temporary sites. For example, *Pseudoscleropodium purum* (Hedw.) M. Fleisch., a moss native to Central and Western Europe, is well established in New York State, where its restricted occurrence to lawns of cemeteries suggests that the spread of the species has been achieved through horticultural practices such as mowing, racking and planting (Miller & Trigoboff 2001).

All these factors provide, however, inconclusive indication of introduction. Range expansion is, for instance, not a decisive criterion in itself because native species can also exhibit similar trends. For example, the moss *Pogonatum dentatum* (Menzies ex Brid.) Brid. used to exhibit a montane range in Scandinavia but started to spread southwards during the second half of the 20th century, probably using forest roads as dispersal corridors over long distances (Hassel *et al.* 2005). It is therefore extremely difficult to distinguish between natural long-distance dispersal and accidental human-induced introduction events. For example, *Atrichum crispum* (James) Sull., a common species in eastern North America, was first detected in Wales in 1848, where it grows on sand or gravel near water, a substratum characterised by a natural disturbance regime. All British populations are male, which suggests a single or very low number of successful introductions of this dioecious moss. Whether the species was naturally introduced from wind-borne spores or human transportation is, however, unknown (Söderström 1992).

The history of populations can even conceal a complex mix of native and alien origins. In the aquatic moss *Rhynchostegium riparioides* (Hedw.) Cardot., for instance, population genetic studies identified at a regional scale two pools of populations with different histories, one of them being characterised by a fast recolonisation following massive extinctions during the pollution peaks in the 1970s from populations outside of that area (Hutsemekers *et al.* 2010). In this context, genetic diversity, genetic structure and estimated time since divergence derived from the analysis of neutral genetic markers have increasingly been employed to seek evidence as to the native status of populations of uncertain origin (May & Beebee 2010; Fussi *et al.* 2012; Fuentes-Utrilla *et al.* 2014).

Here, we address the question of the origin of the cosmopolitan weedy moss *Bryum argenteum* on the island of Tenerife (Canary Islands), where the species is most abundant in anthropogenic habitats, but also occurs on seasonally wet soils in open natural habitats. In the context of the molecular phylogeography of *B. argenteum* worldwide (Pisa *et al.* 2014) and characterising its local genetic structure and diversity, we test the hypotheses that: (i) the species is native on the island and subsequently expanded into human-made habitats (H1); (ii) the species is native, but local populations on Tenerife were outcompeted or introgressed by expanding alien populations (H2); and (iii) the species is of recent, alien origin and colonised natural habitats secondarily (H3). If H1 applies, we expect the timing of the colonisation of the island by *B. argenteum* to predate human colonisation of the archipelago, 2500 years before present (BP). We further expect population genetic diversity to be shaped by local processes, *i.e.* isolation-by-distance and, possibly, environmental variation. If H2 applies, we expect to discover a significant signal of a bottleneck that corresponds to the extirpation of the local populations and a founding effect in the alien ones. If H3 applies, we expect that the origin of Tenerife populations dates back to either the first settlement by the native 'guanches', about 2500 years BP, or the European colonisation, 500 years BP. Given this recent timeframe, we expect that all of the genetic diversity of the species on the island originates from external recruitment and is shared with the source areas. Populations might originate from a single introduction event, in which case low genetic diversity and genetic structure are expected. In the case of multiple colonisation events, we expect that any signal of isolation-by-distance would be erased by recurrent long-distance dispersal events.

MATERIAL AND METHODS

Sampling design and molecular protocols

A total of 220 accessions of *B. argenteum* were used for this study. Detailed information is listed in Appendix S1. From these, 74 specimens were collected from 17 localities in the island of Tenerife at an elevation ranging between 322 and 2151 m a.s.l. Those samples were taken in both human-made habitats (roadsides) and natural habitats (*e.g.* along temporary pond margins in the National Park of Teide). A total of 133 accessions were taken from a previously published worldwide phylogeography of *B. argenteum* (Pisa *et al.* 2014), representing the entire distribution range of the species. Additionally, five accessions from Africa and eight from America were sequenced for this study.

Each accession was sequenced at the ribosomal nuclear ITS locus. The use of ITS for phylogenetic reconstruction has been questioned because of the potential presence of divergent paralogous copies and pseudogenes (see Nieto Feliner & Rosselló 2007; for review), which, although not the rule, have also been discovered in mosses (Košnar *et al.* 2012). Nevertheless, ITS remains the most widely used source of genetic variation at the species level in plants and fungi (reviewed in Nagy *et al.* 2012). In *B. argenteum*, its use was justified for two reasons. First, the 5.8S gene was invariant, an indication that the obtained sequences do not correspond to pseudogenes. Second, no conflicting base calls during direct sequencing were observed, suggesting that the presence of intragenomic paralogous copies is unlikely. DNA extraction, amplification and sequencing procedures followed Pisa *et al.* (2013).

Forward and reverse sequence fragments for both ITS1 and ITS2 were edited and assembled using BioEdit 7.05 (Hall 1999) and MEGA5 (Tamura *et al.* 2011), and every polymorphism was checked from the chromatograms. The sequences were aligned by eye, allowing gaps where necessary to conserve homology among sequences. Accessions sharing identical ITS sequences were identified with DnaSP (Librado & Rozas 2009) and assigned to respective genotypes.

Phylogenetic analyses, molecular dating and ancestral area reconstructions

Phylogenetic analysis among genotypes was performed by Bayesian inference using MrBayes 3.1.2 (Ronquist & Huelsenbeck

2003). Indels were coded with SeqState 1.25 (Müller 2005) using simple coding (Simmons & Ochoterena 2000) and added to a separate binary character matrix. A model implementing identical forward and backward transition rates was applied to the indel matrix. The nucleotide substitution model HKY+G was selected based upon both the Akaike information (AIC) and Bayesian information (BIC) criteria, as implemented in JModeltest 2 (Darriba *et al.* 2012). Two Metropolis-coupled Markov Chain Monte Carlo (MCMC) analyses, including three hot chains and one cold chain, were run for 20,000,000 generations and sampled every 5000 generations in MrBayes. Stationarity and convergence of the chains were determined by (i) graphical inspection of the values of the log-likelihoods of the two MCMC analyses; (ii) confirmation that the SD of split frequencies was below 0.01 at the completion of the analysis; and (iii) verification that the potential scale reduction factor for each of the parameters shown in the summary statistics of the analyses was close to 1. The first 800 trees for each of the two runs were discarded as burn-in.

The trees sampled from the posterior probability (PP) distribution of the MrBayes analysis were used to reconstruct ancestral distribution areas to determine the geographic origin of the genotypes sampled in Tenerife. Each genotype was assigned to one or several of the following geographic regions: Tenerife, Africa, Europe, America, Asia, Australasia and Antarctica. Because the internal nodes of the present phylogeography represent the divergence among specimens/genotypes rather than the divergence of monophyletic groups or species, challenging the implementation of explicit biogeographic models assuming cladogenetic events at those nodes (Matzke 2014), we applied the continuous-time model implemented in BayesTraits 2.0 (Pagel *et al.* 2004), wherein branch lengths, and not cladogenetic events, determine the probability of change, and which can therefore be applied to a specimen tree. A model implementing a forward and backward transition rate between each pair of regions was implemented with a MCMC of 25,000,000 generations that was sampled every 10,000 generations. At each iteration the chain proposes a new combination of rate parameters and randomly selects a new tree from the Bayesian sample. The likelihood of the new combination is calculated and this new state of the chain is accepted or rejected following evaluation based on the Metropolis–Hastings term. The trees and rate parameters sampled from the PP distribution were used to reconstruct, at each node, the probability of occurrence within each of the geographic areas. In order to circumvent issues associated with the fact that not all of the trees necessarily contain the internal nodes of interest, reconstructions were performed using a 'most recent common ancestor' (MRCA) approach that identifies, for each tree, the MRCA of a group of haplotypes and reconstructs the state at the node, then combines this information across trees (Pagel *et al.* 2004).

Divergence time among distinct genotypes from the Tenerife and worldwide accessions was investigated with a relaxed clock model as implemented in BEAST version 1.8.0 (Drummond *et al.* 2012). In the absence of fossil records in *Bryum*, a prior on the absolute rate of molecular evolution was used, as described in Huttunen *et al.* (2008) and Aigoin *et al.* (2009). We used a prior distribution of rates with a mean of 4.125e-3 and SD of 1.807e-3 substitutions per site per Ma, which covers the entire range of absolute substitution rates of ITS across a wide range of herbaceous species (Kay *et al.* 2006). The performance of five tree models (coalescent with constant size population, coalescent under an extended Bayesian skyline including the two linear and step-wise models, speciation under a birth–death process and speciation under Yule process) was compared to test which tree model performed best. Comparisons were carried out using a model selection procedure based on Bayes factors (BF) calculated in Tracer version 1.5 (Rambaut & Drummond 2009). Overall, the model using the coalescent under a step-wise extended Bayesian skyline model (Heled & Drummond 2008) performed best and was used for the final dating analysis. Four independent MCMC analyses were each run for 100,000,000 generations. Parameter values were sampled every 10,000 generations and convergence and acceptable mixing of the samples were checked using Tracer version 1.5. After discarding the burn-in steps (first 2000 trees), the runs were combined to obtain an estimate of the PP distributions of the dates of divergence.

The hypothesis of a monophyletic origin of the specimens sampled from Tenerife was explicitly tested by contrasting the likelihood of two competing topologies, one that resulted from the unconstrained analyses described above, and the other produced under the constraint that all Tenerife accessions are included within the same clade, following the approach described in Bergsten *et al.* (2013). Model likelihoods were estimated with the stepping-stone method (Xie *et al.* 2011) using 196,000 MCMC steps sampled every 2500th generations for each of 50 b-values between 1 (posterior) and 0 (prior) after discarding the first 196,000 generations as initial burn-in set by default. Analyses were run for two independent MCMC chains of 5,000,000 steps, from which the arithmetic mean of marginal likelihoods was estimated for each model to calculate BF.

Population genetics analyses

Haploid genetic diversity values and frequency of endemic genotypes were calculated with GENALEX 6.5 (Peakall & Smouse 2006) for the geographic regions defined above and splitting Tenerife into anthropogenic and natural habitats. Genetic divergence between Tenerife and each of the geographic regions was assessed through F_{ST} and N_{ST} as implemented in SPAGeDi 1.3 (Hardy & Vekemans 2002). Both F_{ST} and N_{ST} measure the genetic differentiation among populations, but while F_{ST} is only based on the difference of genotype frequencies among regions, N_{ST} takes in addition the phylogenetic relationships among alleles into account, here computed from a matrix of pair-wise distance among genotypes obtained with Mega 5. A significantly higher value of N_{ST} compared to F_{ST} provides evidence for a phylogeographic signal in the data, *i.e.* indicates that the genotypes sampled within geographic regions are, on average, phylogenetically closer than genotypes sampled among regions, meaning that mutation rates exceed dispersal rates (Pons & Petit 1996). Significant departure of F_{ST} from 0 was tested by means of 9999 random permutations of individuals among regions. Significance of the phylogeographic signal ($N_{ST} > F_{ST}$) was tested by 9999 random permutations of the matrix of phylogenetic distance among genotypes. A signature of demographic changes, such as a bottleneck and founder effect, was sought in Tenerife and for each anthropogenic and natural habitat of Tenerife using Tajima's D (Tajima 1989) and Fu's Fs (Fu 1996) as implemented in Arlequin 3.5 (Excoffier *et al.* 2005). The genetic structure within the island was investigated through the computation of Kinship coefficients (*Nij*), which measure genetic distances among individuals. More specifically, we assessed the correlation between kinship coefficients, geographic distance and altitudinal difference (used as a measure of local environmental variation) to seek a signal of isolation-by-distance. *Nij* is analogous to J. Nason's *Fij* estimator as defined by Loiselle *et al.* (1995), but takes the phylogenetic relationship among alleles into account (Vanderpoorten *et al.* 2011). The significance of the slope of the regression of *Nij* on the logarithm of spatial distance between individuals, ln (*dij*), was tested by means of 9999 random permutations of population locations in SPAGeDi 1.3 (Hardy & Vekemans 2002). The same test was employed between the matrices of *Nij* and of altitudinal distance among pairs of individuals. Partial Mantel test was used to remove the effect of geographic distance in the matrix of altitudinal distance. In particular, we tested the correlation between matrices of kinship coefficients and of altitudinal distance between individuals, whilst controlling the information held in the geographic distance matrix. Partial Mantel test was calculated in ZT (Bonnet & Van de Peer 2002), and the significance of the correlations was tested by means of 9999 randomisations.

RESULTS

There were 131 polymorphic positions in the matrix, resolving a total of 24 genotypes sampled in Tenerife, 18 of which were endemic to this island (Table 1). Sample size, number of genotypes, genetic diversity and frequency of endemic genotypes for each geographic region are shown in Table 1. The Tenerife accessions exhibited similar genetic diversity values when compared to continental regions. The frequency of endemic genotypes found in Tenerife was comparable to those found in Europe, although lower than the remaining continental groups.

The consensus tree of MrBayes (Fig. 1) showed that all genotypes from Tenerife were found in a large clade with a PP of 0.95 that also comprised haplotypes from all continents except Antarctica. The estimated node age corresponding to that clade was 1641 Ka (Fig. 2; 942 – 2227 Ka highest posterior density interval, HPD). Within this large clade, the Tenerife accessions were scattered among six clades (clade I to VI in Fig. 1b) and eight genotypes occupying an unresolved position at a large polytomy. Constraining all accessions from Tenerife to monophyly resulted in a significant decrease in log-likelihood. Indeed, the marginal likelihood estimates resulted in values of $\ln L = -5673.14$ for the backbone constrained topology (informed prior) and $\ln L = -5699.54$ for the constrained topology (backbone + samples from Tenerife monophyletic). We found a difference of 26.40 ln units, strongly supporting the rejection of the hypothesis of a monophyletic origin of the Tenerife specimens.

Table 1. Sample size, number of genotypes, haploid diversity, haploid diversity unbiased by population and frequency of endemic genotypes for each defined geographic region and a subdivision of Tenerife into anthropogenic and natural habitats for a sample of 220 accessions of the moss *Bryum argenteum* based on the nuclear ITS locus.

region	sample size	number of haplotypes	haploid diversity	haploid diversity unbiased by population	frequency of endemic haplotypes
Tenerife	74	24	0.890	0.903	0.750
Tenerife anthropogenic	64	20	0.865	0.878	0.650
Tenerife natural	10	6	0.700	0.778	0.667
Africa	12	12	0.917	1.000	0.917
America	35	27	0.952	0.980	0.889
Antarctica	16	9	0.852	0.908	1.000
Asia	20	19	0.945	0.995	0.895
Australasia	7	7	0.857	1.000	0.857
Europe	56	28	0.902	0.918	0.786

The molecular dating analyses indicated that multiple colonisation of Tenerife took place during a period of time ranging between the recent past for the six genotypes shared with continental areas to >100 Ka BP for the others (Table 2). Pair-wise F_{ST} values among Tenerife and each continental region of the world were consistently significant ($F_{ST} > 0$; Table 3). Pair-wise N_{ST} values were significantly higher than F_{ST} among Tenerife and most continents, except for Tenerife and Africa, and Tenerife and Europe (Table 3). Within Tenerife, the F_{ST} between populations from natural and secondary habitats was not significantly different from 0.

The slope of the regression between *Nij* and geographic distance (isolation-by-distance test) among the Tenerife accessions was significant ($P = 0.005$). Mantel's test between *Nij* and altitudinal distance among Tenerife individuals was also significant ($P = 0.0001$). This relationship remained significant after removing the geographic component of the matrix of altitudinal distance (partial Mantel test, $P = 0.0001$).

Neutrality tests (Tajima's *D* and Fu's *Fs*) for the Tenerife accessions as a whole and for each habitat type (natural *versus* disturbed) showed that the Tenerife populations in anthropogenic and natural habitats do not significantly depart from 0 (Table 4), failing to provide evidence for recent demographic changes such as population bottleneck and/or founding event in any of the habitats.

DISCUSSION

The genetic diversity of *B. argenteum* on Tenerife was comparable to that found in continental areas, weakening the hypothesis that the island was recently colonised by the species. In fact, Tajima's *D* and Fu *Fs* statistics computed at the scale of Tenerife did not significantly depart from 0, indicating that no signature of a bottleneck associated with a recent colonisation event was present in extant patterns of genetic diversity. Such patterns could be interpreted in terms of an ancient origin of the species on the island, or in terms of several colonisation events erasing the signal of a recent founding event (Dlugosch & Parker 2008).

In line with the second interpretation, numerous sister group relationships observed among Tenerife genotypes and the surrounding continental masses, confirmed by the significant rejection of a monophyletic origin of *B. argenteum* on Tenerife, indicates that the island has been colonised multiple times independently. This observation parallels recurrent colonisation patterns reported in island bryophytes (Grundmann *et al.* 2007; Vanderpoorten *et al.* 2008; Hutsemékers *et al.* 2011;

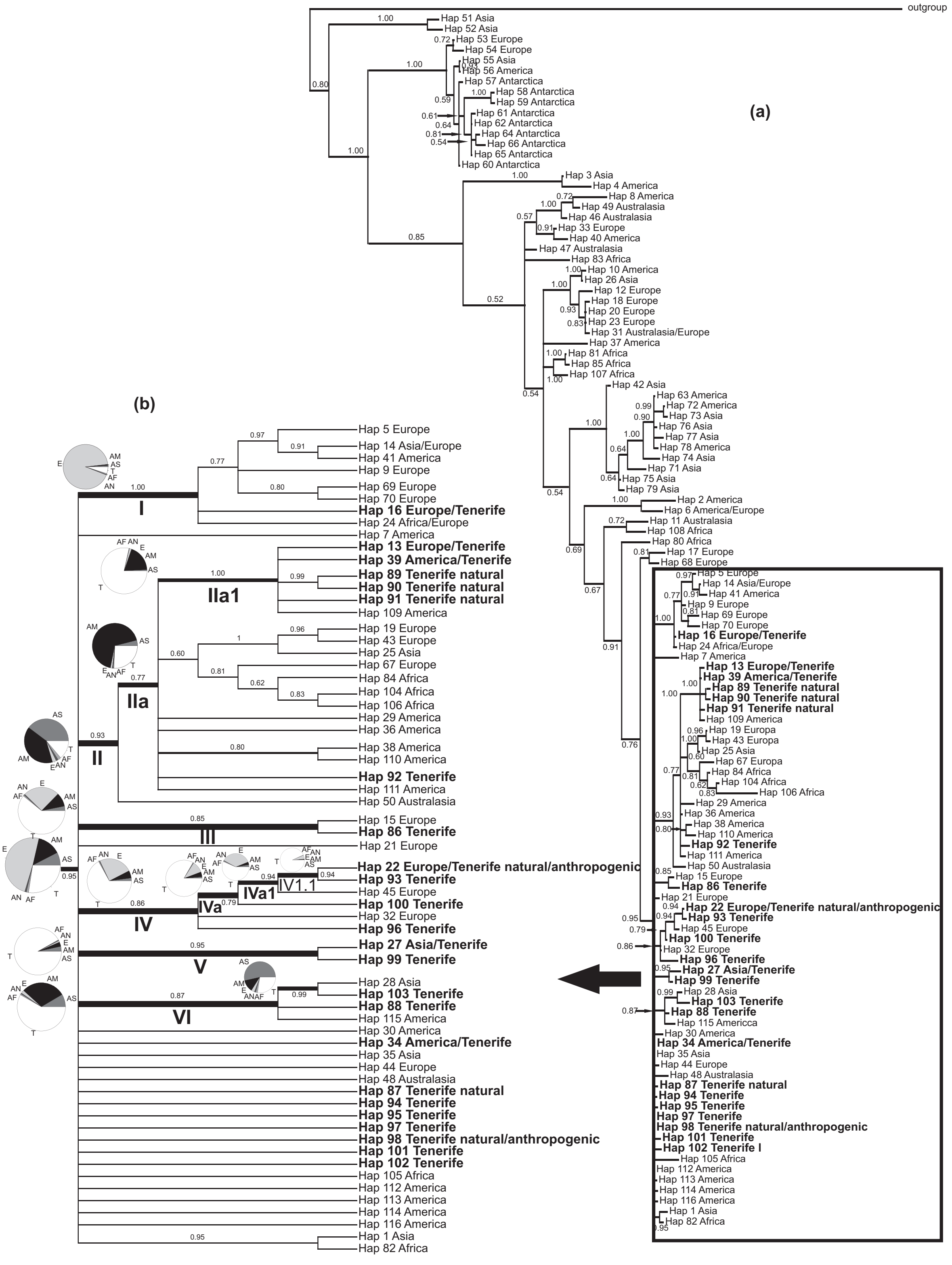

Fig. 1. Phylogenetic relationships among *Bryum argenteum* ITS genotypes estimated by Bayesian inference: (a) 50% majority-rule consensus with branch lengths averaged across the trees of the posterior probability distribution from a Bayesian analysis of ITS sequences of *B. argenteum* genotypes sampled worldwide. The genotype number and the region of origin are indicated. Genotypes from Tenerife are marked in bold. Samples found at natural sites are indicated. Other samples from Tenerife come from anthropogenic sites. The clade marked with a box is amplified in part (b) is a cladogram view of the subclade of (a) that includes all samples from Tenerife. The clades are labelled from I–VI, as mentioned in the text. The pie charts show the probabilities of geographic occurrence at internal nodes derived from the reconstruction of ancestral areas (AF = Africa, AM = America, AN = Antarctica, AS = Asia + Australasia, E = Europe and T = Tenerife).

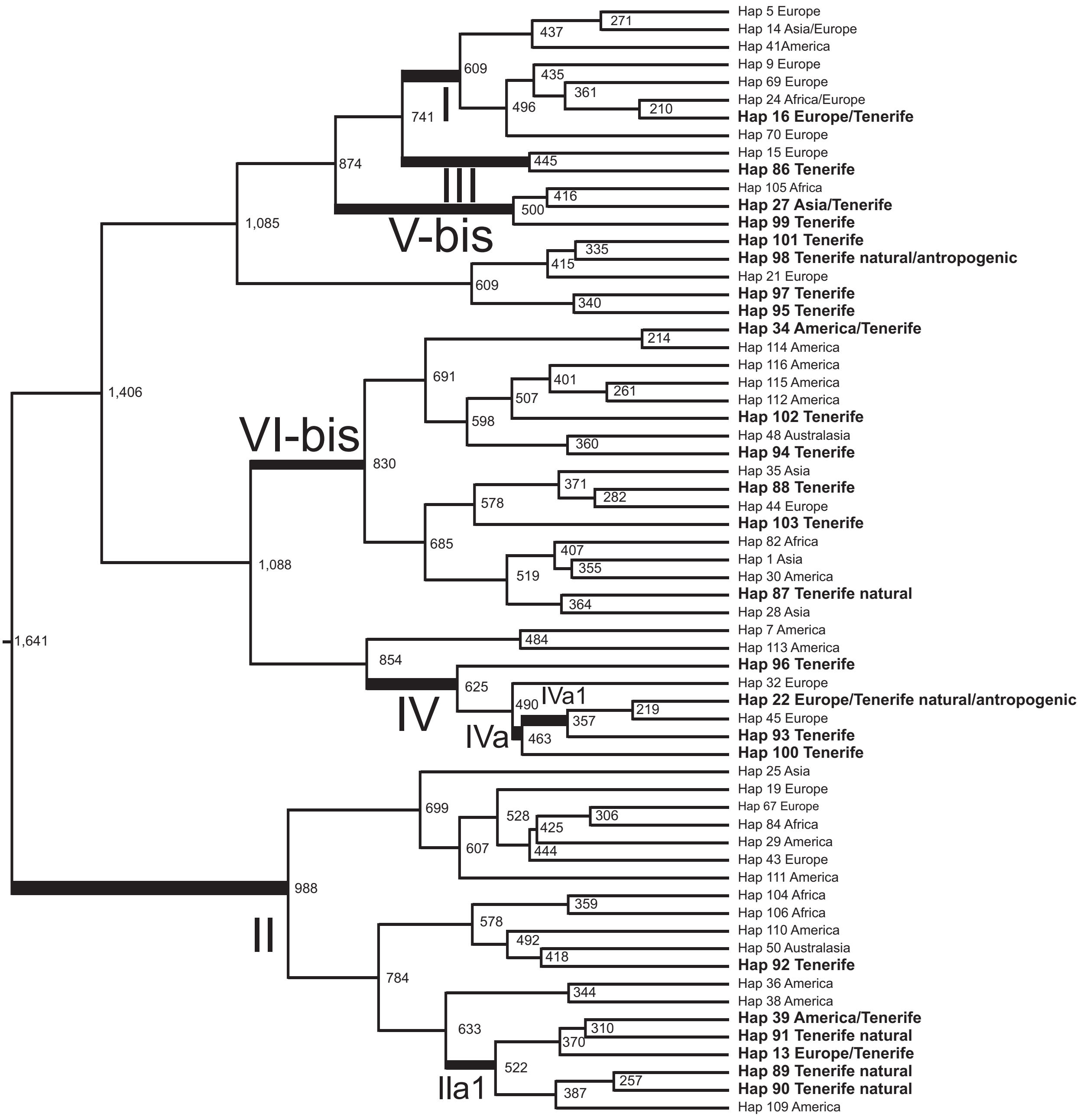

Fig. 2. Chronogram of the phylogeographic relationships among genotypes in the moss *Bryum argenteum* derived from a molecular dating analysis. Numbers at internal nodes indicate the estimated divergence time in Ka. See (b) for clade labels.

Laenen *et al.* 2011). The absence of a significant phylogeographic signal between Tenerife and both Europe and Africa, whereas N_{ST} was significantly higher than F_{ST} in all other pairwise comparisons, points to these two continents as main sources for the island. Nevertheless, even in a weed like *B. argenteum*, which is characterised by a dual mating system involving both sexual (spores) and asexual (production of specialised asexual diaspores) reproduction, and despite the comparatively close proximity of the African coasts (<300 km), there was a significant geographic partitioning of allele frequencies ($F_{ST} > 0$) between Tenerife, Europe and Africa. This suggests that migration rates between oceanic islands and continents are not sufficient to prevent the effects of genetic drift.

Table 2. Probability of ancestral area and divergence time of the most recent common ancestor of genotypes of *Bryum argenteum* sampled in Tenerife.

clade name[a]	probability of an ancestral area in Tenerife	divergence time (Ka BP) HPD values in brackets
IIa1	0.78	522 (284–761)
III	0.60	445 (136–769)
IV	0.67	625 (305–1054)
IVa	0.83	463 (174–763)
V/ V-bis	0.92	500 (216–901)
VI/ VI-bis	0.58	830 (595–1095)

[a]clade names as shown in Figs 1 and 2.

Table 3. Pair-wise F_{ST} and N_{ST} values among Tenerife and the six continental regions of the worldwide distributed moss *Bryum argenteum* based on the nuclear ITS locus. The *P*-values are associated with the null hypotheses that $F_{ST} = 0$ and that $F_{ST} = N_{ST}$, respectively.

population indices	geographic regions	Africa	America	Antarctica	Asia	Australasia	Europe
F_{ST}	Tenerife	0.054*	0.052***	0.095***	0.054***	0.057*	0.065***
N_{ST}		n.s	0.119*	0.828***	0.157*	0.249**	n.s

n.s. = not significant, $P > 0.05$; $*P < 0.05$; $**P < 0.01$; $***P < 0.001$.

Table 4. Tajima's *D* and Fu's *Fs* statistics (and associated *P*-values) in Tenerife and a subdivision of the accessions into anthropogenic and natural habitats for a sample of 74 accessions of the moss *Bryum argenteum* based on the nuclear ITS locus.

region	Tajima's *D*	Fu's *Fs*
Tenerife	−0.03 (0.57)	0.79 (0.68)
Tenerife anthropogenic	−0.02 (0.53)	2.5 (0.86)
Tenerife natural	−0.03 (0.56)	1.3 (0.70)

Such an observation is consistent with the existence of differences in life-history traits between island and continental bryophyte populations (Patiño *et al.* 2013a) and contradicts the view that the sea is not a major impediment for migration in bryophytes (Grundmann *et al.* 2007).

Nevertheless, the results of the molecular dating analyses and the ancestral area reconstructions suggested that the earliest events of colonisation of Tenerife dates back to more than 100,000 years BP, *i.e.* well before the first human settlements, about 2500 years BP (Rando *et al.* 1999). The absolute nucleotide substitution rate of Kay *et al.* (2006) that was used in the present study is, however, based on studies of annual herbaceous species, and is likely to be much higher than in mosses. A study on relative substitution rates amongst major plant groups indeed showed that, on average, the substitution rate of 18s rDNA, the neighbouring region of ITS, is more than twice as high in vascular plants compared to mosses (Stenøien 2008). This suggests that the presence of *B. argenteum* on Tenerife may be considerably more ancient than estimated here. Despite the fact that *B. argenteum* is mostly restricted to man-made and disturbed habitats, a feature that is typical for introduced and invasive species (Chytrý *et al.* 2008; Williamson *et al.* 2009; Essl *et al.* 2014), our results thus suggest that *B. argenteum* is native in Tenerife, although the existence of genotypes shared between Tenerife and continental areas suggests that more recent, potentially man-mediated introduction, also took place.

This interpretation is further supported by local patterns of genetic structure. Indeed, our analyses revealed a significant signal of isolation-by-distance in Tenerife, confirming earlier evidence pointing to dispersal limitations of bryophytes at a local scale (Hutsemékers *et al.* 2013; Korpelainen *et al.* 2013; Patiño *et al.* 2013b). Furthermore, significant correlations were found between genetic variation and altitude after controlling for geographic distance (partial Mantel test), pointing to the role of environmental variation in shaping the spatial genetic structure of *B. argenteum*. This structure might have emerged either through the evolution of reproductive barriers and progressive divergence of lineages that evolved *in situ* or the recruitment of pre-adapted lineages from other areas. These observations, along with recent evidence from fine-scale population genetic analyses (Hutsemekers *et al.* 2010; Horsley *et al.* 2011; Korpelainen *et al.* 2012; Pisa *et al.* 2013), suggest that moss species develop ecotypes to adapt to a wide range of environmental conditions. Most importantly, the strong spatial genetic structure found in *B. argenteum* within Tenerife points to the substantial role of genetic drift in establishing the observed patterns of genetic variation. Such patterns contrast with those reported in recently introduced alien species, wherein the founder effect, coupled with a rapid spread, erases any spatial patterns of genetic variation (St. Quinton *et al.* 2011; Fussi *et al.* 2012), and therefore, also points to the long history of *B. argenteum* on Tenerife, where the species opportunistically diversified in both natural and human-made environments.

Our results suggest that, although *B. argenteum* is a weedy cosmopolitan species mostly restricted to disturbed environments, it is native to the island of Tenerife. They also suggest that even in organisms with high dispersal capacities like bryophytes, genetic data can provide cumulative evidence to retrace the geographic origin and assess the timing of colonisation patterns of target populations. While defining nativeness in taxa that are not deliberately introduced, and wherein the fossil record is extremely scarce, is an exceedingly challenging task, this suggests that population genetic analyses can represent a useful tool based on a series of criteria such as the timing of founding events, genetic diversity and local patterns of genetic structure in relation to geographic distance and environmental variation, to help distinguishing native from alien populations. We suggest that such an approach would represent a useful test of hypotheses regarding patterns of invasion inferred from distribution data, which have most recently been proposed for long-neglected groups in invasion biology such as bryophytes (Essl *et al.* 2013, 2014).

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SUPPORTING INFORMATION

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

Appendix S1. Accessions used in this study. For each sample the following information is given: *Bryum argenteum* genotype based on nrITS sequences; geographic origin; herbarium where it is retained or publication source if published previously; geographic coordinates; GenBank accession numbers for ITS1 and ITS2 separately, or for the whole ITS region where the number is given in the middle of ITS1 and ITS2 columns. For each Tenerife accession, the altitude (m a.s.l.) and the habitat (anthropogenic *versus* natural) are also provided.

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