UNIVERSIDAD DE MURCIA

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Long Distance Dispersal, Local Adaptation and Long Term Persistence in Bryophytes: Studies in the Moss *Bryum argenteum*

Dispersión a Larga Distancia, Adaptación Local y Persistencia a Largo Plazo en Briófitos: Estudios en el Musgo *Bryum argenteum*

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A Silvia,

mi compañera de viaje,

madre de mi hijo

Ever tried. Ever failed. No matter. Try again

Fail again. Fail better.

Samuel Beckett.

Every act of conscious learning requires the willingness

to suffer an injury to one's self-esteem.

Thomas Szasz

That which does not kill me makes me stronger

Nietsche

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RESUMEN

Las especies de briófitos tienen, generalmente, unos rangos de distribución más amplios que las plantas vasculares y es común que abarquen dos o más continentes. Las distribuciones de la mayoría de las familias de briófitos son tan vastas que se extienden por los dos hemisferios del planeta. Algunas especies de briófitos tienen una distribución cosmopolita, aunque las especies que se distribuyen en todos los continentes y en una amplia variedad de hábitats, son muy raras. Entre éstas se encuentra el musgo *Bryum argenteum* Hedw., que es una de las plantas con mayor rango de distribución del planeta. Por otro lado, en comparación con las plantas vasculares, los briófitos exhiben una proporción significativamente menor de endemismos y presentan mayores rangos geográficos. Por ejemplo, el 40% de la flora de angiospermas en Macaronesia es endémica, mientras que sólo son endémicos el 10% de los briófitos. Además, las especies endémicas de briófitos en archipiélagos están generalmente ubicadas en dos o más islas, lo que contrasta con la situación de las angiospermas, donde la mayoría de endemismos ocupan una sola isla.

Los amplios rangos de distribución y reducida proporción de endemismos de los briófitos pueden ser debidos a algunas de sus características intrínsecas como a su amplitud ecológica, tolerancia a la desecación, modo dual (asexual – sexual) de reproducción y capacidad de dispersión a largas distancias. Las especies de briófitos generalmente tienen mayor amplitud ecológica que las plantas con semilla y aparentemente, tienden a no desarrollar ecotipos. Su estrategia es, por tanto, más generalista, predominando en las especies la plasticidad frente a la diversificación

ecológica. Los briófitos desarrollaron la estrategia de tolerar la desecación a través del mantenimiento de la vida en forma latente y una vez rehidratados, volver a la actividad normal. La propagación asexual es, en muchas especies, usada frecuentemente para mantener las poblaciones a un nivel local e incluso regional y para expandirse rápidamente tras un proceso de colonización. La reproducción sexual produce decenas de miles de esporas en cada esporófito, a veces hasta millones, que, comúnmente, se dispersan por el aire. Las esporas, al ser mucho más pequeñas que las semillas pueden dispersarse más lejos. El minúsculo tamaño de las esporas de muchos especies de briófitos les permite elevarse en la atmósfera y dispersarse a largas distancias a través de corrientes de aire, mientras que su fuerte resistencia a las temperaturas extremas y a la radiación les permite mantener su viabilidad. De hecho, hay evidencias de que las esporas se establecen a kilómetros de distancia del origen y que un porcentaje, aunque reducido, de la lluvia de esporas regional tiene un origen trans- o intercontinental. Recientes estudios filogeográficos indican que la dispersión a larga distancia es un proceso relativamente frecuente.

Dos hipótesis se han discutido tradicionalmente para explicar los amplios y disyuntos rangos geográficos de los briófitos. La primera interpreta dichas distribuciones como resultado de la fragmentación de un rango continuo ancestral debido a procesos geológicos como la deriva continental (vicarianza). La segunda explica dichas distribuciones como consecuencia de la dispersión intercontinental a larga distancia.

Muchas especies de briófitos exhiben las mismas disyunciones geográficas que se encuentran a nivel de género en las plantas vasculares y en otros organismos. Estos rangos son congruentes con la hipótesis de la deriva continental y, por tanto, apoyan la hipótesis de vicarianza. Por otro lado, algunas distribuciones disyuntas son difíciles de interpretar bajo la hipótesis de la deriva continental. Por ejemplo, muchas de las especies de briófitos boreales tienen una distribución circumpolar que abarca el norte de Europa, Asia y Norte América. En otros casos, las especies presentes en la Antártida tienen una distribución disyunta bipolar que incluye regiones boreales y templadas en ambos hemisferios. Dichos patrones se explican mejor por la hipótesis de dispersión a larga distancia que por vicarianza.

Muchos estudios genéticos indican que las distribuciones disyuntas e intercontinentales de muchos briófitos no pueden explicarse meramente por una de las dos hipótesis, sino que la dispersión a larga distancia entre continentes y la vicarianza se complementan. En general, los estudios genéticos indican que la dispersión entre continentes ha ocurrido varias veces, en más o menos ocasiones, durante la diversificación de los briófitos, aunque no ha sido suficiente como para evitar la diferenciación alopátrica. En particular, las especies cosmopolitas de briófitos exhiben una baja, quizás insignificante, estructura filogeográfica entre continentes, lo que sugiere que la dispersión a larga distancia intercontinental podría no tener límites y las diferentes variantes dentro de una especie cosmopolita se distribuirían por todas partes.

El principio de Baas Becking (propone que “todo está en todas partes, pero el ambiente selecciona” para explicar las distribuciones cosmopolitas de especies sin aparentes restricciones de dispersión. Recientemente, este principio se ha debatido en diferentes taxones. Muchos de éstos comparten características con briófitos, tales como: (1) la dispersión pasiva por todas partes, (2) la reproducción por medios asexuales (o por partenogénesis) incrementando eficientemente el número de individuos a un ritmo rápido, y (3) la inclusión de etapas de descanso en su ciclo de vida que les permite subsistir en hábitats con condiciones ambientales adversas. Estas características, junto con aquellas distintivas de los briófitos y, en particular, con los que tienen distribuciones cosmopolitas, como su alta plasticidad, sugieren que son candidatos ideales para poner a prueba el principio de Baas Becking.

La dispersión a larga distancia podría ser lo suficientemente recurrente como para homogeneizar una determinada especie cosmopolita a lo largo de su rango de distribución intercontinental. En este caso, las secuencias moleculares de poblaciones disyuntas deberían ser muy similares y no se identificaría ninguna estructura biogeográfica, por lo que la hipótesis del principio de Baas Becking sería aceptada. Por otro lado, si se encontraran límites a la dispersión y por tanto se rechazara la hipótesis del principio de Baas Becking, los briófitos cosmopolitas podrían haber logrado su distribución global con la ayuda de actividades antropogénicas. Esta hipótesis podría verse reforzada por la afinidad de los briófitos cosmopolitas a los hábitats perturbados, que recuerda la conducta de especies invasoras.

El objetivo general de esta tesis es abordar diferentes hipótesis concernientes a la ecología, distribución, historia y potencial invasivo de plantas con capacidad para dispersarse a larga distancia y más en concreto, de briófitos cosmopolitas. A este respecto, el musgo *Bryum argenteum* fue usado como especie modelo. En relación directa con el objetivo general, esta tesis trata del principio de Baas Becking tanto a nivel local como a escala mundial bajo un contexto biogeográfico y ecológico. Además, otras hipótesis fueron testadas en base a los resultados preliminares. Más específicamente, esta tesis tiene como objetivos:

- Testar a escala local y a lo largo de gradientes altitudinales, en las montañas de Sierra Nevada (España) y en la isla de Tenerife, si la diversidad genética está homogeneizada en *B. argenteum* y, por tanto, no hay ninguna estructura genética debido a las altas capacidades de dispersión y la falta de diferenciación ecológica atribuidas a los briofitos cosmopolitas.

- Reconstruir la filogeografía mundial de *B. argenteum* con el objetivo de descifrar si la dispersión a larga distancia intercontinental es ubicua o por el contrario, si la vicarianza juega algún papel en la explicación de los patrones globales de la variación genética.
- Estimar el tiempo y el origen de la colonización de *B. argenteum* en la Antártida y poner a prueba las hipótesis de si la especie persistió en el continente a lo largo de los últimos ciclos glaciales o si ha (re)colonizado el continente recientemente, después del último máximo glacial.
- Estimar el tiempo y el origen de la colonización de *B. argenteum* en la isla de Tenerife con el fin de evaluar el papel de los seres humanos en la colonización de la especie en Tenerife.
- Comprobar si las poblaciones a escala local, en Sierra Nevada y Tenerife y a escala continental, en la Antártida, se mantuvieron estables o, por el contrario, hay evidencia de un reciente cambio demográfico.

Para abordar estos objetivos, se utilizaron métodos de genética molecular y los datos se analizaron con herramientas estadísticas de los campos de genética de poblaciones y filogeografía. En concreto se usaron secuencias de ADN, de marcadores genéticos ampliamente usados, de numerosas muestras de *B. argenteum* recolectadas en Sierra Nevada (España), la isla de Tenerife y en cada masa continental de la Tierra. Dichas secuencias se analizaron usando una batería de técnicas tales como: análisis comparativos, estimadores de diversidad genética, tests de correlación (tests de Mantel), estimadores de estructura genética entre poblaciones, reconstrucciones de áreas ancestrales, datación de reloj molecular y tests de neutralidad.

Las conclusiones generales derivadas de la investigación llevada a cabo en esta tesis doctoral son las siguientes:

1. Se encuentran patrones de estructura genética y diversidad en *B. argenteum* a lo largo de un gradiente altitudinal en las montañas de Sierra Nevada. Dichos patrones apoyan la hipótesis del principio de Baas Becking. La falta de pruebas de aislamiento por distancia sugiere que la primera mitad del principio, "Todo está en todas partes", se aplica plenamente (a nivel local) en Sierra Nevada. La segunda mitad del principio, "pero el ambiente selecciona", es apoyado por la evidencia de un patrón de diferenciación genética controlado por el medio ambiente que sugiere divergencia adaptativa.
2. La mayor diversidad genética de Sierra Nevada se encuentra en la zona más alta de las montañas, por encima de 1.900 m s.n.m. y sugiere que *B. argenteum* persistió durante el Pleistoceno tardío en una área glacial.
3. La reconstrucción mundial de la filogeografía de *B. argenteum* sugiere que los patrones de distribución genética se explican mejor por la dispersión intercontinental que por vicarianza.
4. La fuerte señal filogeográfica entre la Antártida ($N_{ST} > F_{ST}$) y el resto de masas continentales y la ausencia de una señal filogeográfica significativa entre cualquier otra pareja de continentes ($N_{ST} = F_{ST}$) sugieren que la Antártida es, con mucho, el continente más aislado de todos en términos de la dispersión de briófitos.

5. La hipótesis de la dispersión por todas partes, y por lo tanto el principio de Baas Becking a escala global, es rechazada por el aislamiento genético de la Antártida y por la partición geográfica de las frecuencias alélicas ($F_{ST} > 0$) entre parejas de continentes.

6. Los análisis genéticos de población, la filogenia, las reconstrucciones de áreas ancestrales y los análisis de datación de reloj molecular indican en conjunto que *B. argenteum* colonizó la Antártida al menos en tres ocasiones y que esos eventos tuvieron lugar en escalas de tiempo del orden de millones a cientos de miles de años. Por lo tanto, *B. argenteum* persistió con éxito *in situ* en la Antártida durante varios ciclos glaciales en el Pleistoceno, Plioceno y quizás el Mioceno tardío. Por lo tanto, se rechaza la hipótesis de que la especie (re)colonizara la Antártida después del último máximo glacial.

7. Los análisis genéticos de población, la filogenia, las reconstrucciones de áreas ancestrales y los análisis de datación de reloj molecular indican en conjunto que: (1) *B. argenteum* colonizó la isla de Tenerife en múltiples ocasiones, (2) Los primeros eventos de la colonización de la isla provenían principalmente de África y Europa, y (3) tuvieron lugar en escalas de tiempo de cientos de miles de años. Es decir, mucho antes de los primeros asentamientos humanos. Por lo tanto, se rechaza la hipótesis de que los seres humanos introdujeron por primera vez la especie en la isla de Tenerife.

8. Los patrones de diferenciación genética a lo largo de un gradiente altitudinal en Tenerife sugieren que la variación ambiental juega un papel clave en la conformación de la estructura genética, como ya se ha sugerido en el estudio de las montañas de Sierra Nevada. Sin embargo, la deriva genética también juega

un papel en el establecimiento de los patrones de variación genética observados en la isla.

9. No se detectan cambios demográficos en las poblaciones de *B. argenteum* en Sierra Nevada, la Antártida ni Tenerife.

Los resultados y las conclusiones derivadas de la investigación científica de esta tesis contribuyen a una mejor comprensión de la estructura biogeográfica de las especies de briófitos cosmopolitas, lo que será útil para una variedad de disciplinas como la biogeografía, la biología de briófitos, la biología evolutiva, la ecología y la gestión de la conservación.

ABSTRACT

Bryophyte species generally display wider distribution ranges than vascular plants and it is common that they span two or more continents. Most of the bryophyte families are so widespread that their distribution spans the North and South hemispheres. Some species of bryophytes have a cosmopolitan distribution, although cosmopolitan species that are broadly distributed in all continents in a wide variety of habitats are very rare. The moss *Bryum argenteum* Hedw. is included among those and it is one of the plants with the widest distribution on the planet. On the other hand, bryophytes display significantly lower proportion of endemism than vascular plants and those exhibit broader geographical ranges. For instance, in Macaronesia, endemism account for 40% of the angiosperm flora and only less than 10% of bryophytes. Moreover, bryophytes endemics in archipelagos are usually located in more than one island in contrast with angiosperms, where single island endemics predominate.

The wide distribution ranges and low proportion of endemism of bryophytes is probably due to some of their intrinsic characteristics such as their broad ecological amplitude, their tolerance to desiccation, their dual (asexual – sexual) mode of reproduction and their dispersal abilities. Bryophyte species tend to display much broader ecological amplitude than seed plants and apparently do not develop ecotypes. Their strategy is therefore more generalist where plasticity predominates over ecological diversification. Bryophytes evolved the strategy of tolerating desiccation by being capable of maintaining latent life and resume normal metabolic activity when rehydrated. Asexual propagation is used in many species to maintain the populations at

a local and even regional level and to fast expansion after colonization. Sexual reproduction produces tens of thousands to even millions of spores within each sporophyte that are, commonly, dispersed by air. The spores are much smaller than seeds and are likely to be dispersed over longer distances. In many bryophytes their small size allows them to be lifted into the atmosphere and be dispersed by air currents to far distances, while their strong resistance to temperature extremes and radiation allows them to keep their viability. In fact, there are evidence indicating that regular establishment of spores takes place at the km-scale and that a proportion, although small, of the regional spore rain have a trans- or intercontinental origin. Recent phylogeographic studies point that long distance dispersal is a relative frequent process.

Two hypotheses have been traditionally discussed to explain the generally broad and disjunctive geographical distributions of bryophytes. The first hypothesis interprets it as a result of a fragmentation of ancient continuous distributions (i.e. vicariance). The second hypothesis explains it as a consequence of intercontinental, long distance dispersal. The patters of disjunction in many bryophytes are similar to those found at a genre scale in vascular plants and other groups of organisms. Those disjunctive ranges are highly congruent with the continental drift hypothesis and, therefore, support the vicariance hypothesis. On the other hand, some disjunctive distributions are difficult to interpret under the Earth's history of continental drift. For example, most boreal bryophytes have a circumpolar distribution that spans the northern parts of Europe, Asia and North America. In another example, many bryophytes present in Antarctica have a bipolar disjunctive range that includes boreal and temperate regions in both hemispheres Those patterns are best explained by recent long distance dispersal events rather than vicariance.

Many genetic studies indicate that the disjunctive and intercontinental distributions in many bryophytes cannot be merely explained by one of the two hypotheses and that intercontinental dispersal and vicariance complement each other. In general, genetic studies suggest that dispersal among continents has occurred repeatedly during bryophyte diversification, although it has not been recurrent enough to prevent allopatric differentiation. In particular, cosmopolitan bryophyte species exhibit a low, potentially negligible, structure among continents, suggesting that intercontinental long distance dispersal may be ubiquitous in cosmopolitan bryophytes and the variants of a given species are distributed everywhere.

The Baas Becking tenet posits that "Everything is everywhere, but the environment selects" to explain the apparent cosmopolitan distribution of some vagile taxa by assuming a lack of dispersal limitations. This tenet has been recently debated among different taxa. Most of those shared characteristics with bryophytes such as: (1) passive dispersal everywhere, (2) reproduction by asexual or parthenogenic means efficiently increasing their numbers at a fast rate and (3) the inclusion of resting stages in their life cycle that allows them to subsist in habitats with adverse environmental conditions. These characteristics together with the distinctive features of bryophytes and, in particular, of bryophytes with cosmopolitan distributions, such as their high plasticity, suggest that they are ideal candidates to test the Baas Becking tenet. Recurrent long distance dispersal and establishment, if strong enough, may homogenize a given species along its distribution range. If this is the case, the molecular sequences of disjunctive populations should be rather similar and no biogeographic structure would be expected, supporting the Baas Becking tenet. On the other hand, if there are limits to dispersal at a global scale and, thus, the Baas Becking tenet is rejected, cosmopolitan bryophytes may have achieved their global distribution aided by anthropogenic activities. This hypothesis is reinforced by the affinity of cosmopolitan bryophytes to disturbed habitats, which is reminiscent of invasive species' behaviour.

The general objective of this thesis is to address different hypotheses concerning the ecology, distribution, history and potential invasiveness of vagile plants and more specifically, of cosmopolitan bryophytes. The moss *Bryum argenteum* was used in this respect as a model species. In direct relationship with the general objective above mentioned, this thesis deals with the EiE tenet both at a local and at global scale, under a biogeographical and an ecological framework. Additionally, based on the preliminary findings, further hypotheses were tested. More specifically, this thesis aimed to:

- Test at a local scale and along altitudinal gradients, in Sierra Nevada Mountains (Spain) and Tenerife Island, if genetic diversity is homogenized in *B. argenteum* and there is no genetic structure due to the high dispersal capabilities and the lack of ecological differentiation attributed to cosmopolitan bryophytes.
- Reconstruct the worldwide phylogeography of *B. argenteum* to decipher whether intercontinental long distance dispersal approaches to ubiquitous dispersal or vicariance plays a role in explaining the global patterns of genetic variation.
- Estimate the time and origin of colonization *of B. argenteum* in Antarctica and test the hypotheses of whether the species persisted within the continent throughout glacial cycles or recently (re-)colonize it after the last glacial maximum.

- Estimate the time and origin of the colonization of *B. argenteum* on the island of Tenerife in order to evaluate the potential role of humans in the introduction of the species.
- Test whether the populations at a local level, in Sierra Nevada and Tenerife, and at a continental level, in Antarctica, were stable or, on the contrary, there is evidence for a recent demographic shift.

To address these objectives we use methods of molecular genetics and the data was analyzed with statistical tools from the fields of population genetics and phylogeography. More specifically, we use DNA sequences of widely used genetic markers from numerous accessions of *B. argenteum* collected on Sierra Nevada (Spain), the island of Tenerife and from each continental mass on Earth. The sequences were analyzed using a battery of techniques such as comparative analyses, genetic diversity estimators, correlation tests (Mantel tests), estimators of genetic differentiation among populations, ancestral area reconstructions, molecular dating and tests of neutrality.

The general conclusions derived from the research of this thesis are as followed:

1. There were patterns of genetic structure and diversity in *B. argenteum* along an altitudinal gradient in Sierra Nevada Mountains of Spain. Those patterns completely support the EiE tenet. The lack of evidence for isolation by distance suggests that the first half of the tenet, “Everything is everywhere”, fully applies (at a local level) in Sierra Nevada. The last part of the tenet, “but the environment selects”, is supported by evidence for an environmentally-driven pattern of genetic differentiation that suggests adaptive divergence.

2. Genetic diversity on the mountains of Sierra Nevada peaks above 1,900 m a.s.l. and suggest that *B. argenteum* persisted in a range that was extensively glaciated during the late Pleistocene.
3. The worldwide reconstruction of the phylogeography of *B. argenteum* suggests that the extant patterns of genetic distribution are best explained by intercontinental dispersal rather than vicariance.
4. The strong phylogeographic signal between Antarctica and any other continental mass and the absence of a significant phylogeographic signal among any other pair of continents suggest that Antarctica is by far the most isolated continent of all in terms of bryophyte dispersal.
5. The hypothesis of ubiquitous dispersal, and therefore the EiE tenet at a global scale, is rejected by the significant genetic isolation of Antarctica and by the significant geographic partitioning of allele frequencies between pairs of continents.
6. Population genetic analyses, phylogeny, ancestral area reconstructions and molecular dating analyses indicate altogether that *B. argenteum* colonized Antarctica on at least three occasions and those events took place in time scales in the order of millions to hundred thousands of years. Thus, *B. argenteum* successfully persisted *in situ* during several glacial cycles within the Pleistocene, Pliocene and perhaps late Miocene. Therefore, the hypothesis that the species (re-)colonize Antarctica after the last glacial maximum is rejected by the data.
7. Population genetic analyses, phylogeny, ancestral area reconstructions and molecular dating analyses indicate altogether that *B. argenteum* colonized the island of Tenerife on multiple occasions. Earlier events of colonization on

the island took place in time-scales of hundred thousand years, well before the first human settlements and mainly came from Africa and Europe. Therefore, the hypothesis that humans introduced for the first time the species onto the island is rejected.

8. The patterns of genetic differentiation along altitudinal gradients in Tenerife suggest that environmental variation plays a key role in shaping the genetic structure of *B. argenteum*, as already suggested in the study from the mountains of Sierra Nevada. Nevertheless, genetic drift also plays a role in establishing the observed patterns of genetic variation in the island.
9. No signature of demographic shifts was detected in the populations of *B. argenteum* on Sierra Nevada, Antarctica or Tenerife.

The results and conclusions derived from the scientific research of this thesis contribute to a better understanding of the present and past biogeographic structure of cosmopolitan bryophyte species, which will be useful to an array of disciplines such as biogeography, bryophyte biology, evolutionary biology, ecology, and conservation management.

GENERAL INTRODUCTION

General aspects of bryophyte biology

Bryophytes are the second largest group of land plants (embryophytes) after the flowering plants (angiosperms) and consist of around 18,000 species, although estimates range from 14,000 to 25,000 (Medina *et al*. 2011). They have a basal phylogenetic position among the extant embryophytes and include three distinct lineages: mosses (Bryophyta), liverworts (Marchantiophyta) and hornworts (Anthocerotophyta) (Mishler 2001; Qiu *et al*. 2006).

Bryophytes in general are highly successful plants that practically colonized all land ecosystems, including the toughest environments such as freezing and hot deserts (Vanderpoorten & Goffinet 2009). In global terms, bryophytes play a key role in the world's climate as they significantly contribute to the production of biomass. They are the dominant vegetation element in the peatlands, which cover about 3% of the Earth's land surface and store about 15-30% of the world' soil carbon (Limpens *et al*. 2008).

Embryophytes evolved different strategies in response to drought. Bryophytes lack the structures and mechanisms to prevent desiccation. They are therefore, poikilohydric, which means that their water content is rapidly determined by the water potential of the environment. Most bryophytes evolved the strategy of tolerating desiccation by being capable of maintaining latent life (quiescence) and resume normal metabolic activity when rehydrated. The degree of desiccation tolerance, however, varies greatly among species. Additionally, many bryophytes, as well as many other taxonomically diverse groups, are able to escape drought through desiccation tolerant diaspores (Proctor & Tuba 2002).

The life cycle of bryophytes differs from that of all other extant embryophytes in the predominance of the gametophyte (haploid) generation. The sporophyte (diploid) in bryophytes is, in fact, never detached to the maternal gametophyte and nutritionally depends on it (Vanderpoorten & Goffinet 2009).

Most bryophytes are able to reproduce both, sexually and asexually. To achieve sexual reproduction, an aquatic medium is required between the two sex organs since the motile sperm cells must swim to the egg. Fertilization ranges are typically on the order of tens of centimetres and hardly reach more than one meter (Longton 1997). This implies, in dioicous species, that male and female plants must grow very near to each other, which is a severe constraint in some cases. To cover the time span in between sexual reproduction, many bryophytes propagate asexually. Reproduction in the absence of sex is used in many taxa, including monoicous species, to maintain the populations at a local and even regional level and to fast expansion after colonization. The importance of asexual reproduction in bryophytes may be denoted by the evolution of a remarkable variety of more or less specialized vegetative structures for asexual propagation such as rhizoidal gemmae, axillary gemmae, brood bodies, detaching leaves or buds, leaf fragments, etc. (Frahm 2008; Vanderpoorten & Goffinet 2009).

The success of bryophytes in colonizing almost all available habitats is mainly due to a high turnover of specialist species rather than the wide occurrence of ubiquitous generalist species such as the moss *Bryum argenteum* Hedw., the species studied in this thesis. On the other hand, bryophytes, in contrast with most seed plants (spermatophytes), do not tend to develop ecotypes and exhibit an inherent ability to cope with environmental variation. For instance, the ability of bryophytes to survive cold and dry environments seems to outrange any other group of embryophytes (Glime

2007). In other words, they tend to display a more generalist strategy and exhibit much broader ecological amplitude than spermatophytes (Vanderpoorten & Goffinet 2009). For example, mosses in continental salt meadows do not display the striking zonation pattern exhibited by vascular plants along a salinity gradient (Zechmeister 2005). In another example, morphological variability along an environmental gradient in the moss *Syntrichia caninervis* Mitt., results from plasticity (Reynolds & McLetchie 2011). On the other hand, recent, yet scant evidence, supports the formation of ecotypes to adapt to a wide range of ecological conditions and accomplished large distribution ranges (e.g. Hutsemekers *et al.* 2010; Horsley *et al*. 2011; Dong *et al*. 2012).

Biogeography of bryophytes

Bryophyte dispersal

Dispersal is of critical importance to determine the dynamics of range distributions and the genetic structure of a species as well as to shape the tempo and mode of diversification (Shaw *et al.* 2011). Bryophyte diaspores are commonly dispersed by wind, although there are other mechanisms such as water or animal transportation. In contrast with spermatophytes, the spores of bryophytes are much smaller than seeds and are likely to be dispersed over longer distances (Wilkinson *et al.* 2012). Nevertheless, dispersal of spores by air has a leptokurtic pattern and most bryophyte spores are dispersed within meters of the source (Miles & Longton 1992). On the other hand, the enormous amount of spores contained in the sporangia, from tens of thousands to millions, suggests that dispersal over longer distances may be significant (reviewed in Shaw 2001). Indeed, regular establishment of spores takes place at the km-scale (Lönnel *et al*. 2012) and about 1% of the regional spore rain is thought to have a transor intercontinental origin (Sundberg 2013). The smaller spores (< 25µm) can easily be lifted into the atmosphere by warm air and dispersed by air currents to far distances (van Zanten 1978; Wilkinson *et al*. 2012). The spores of widely distributed species survive long periods of desiccation, high UV radiation levels and temperature extremes, which make them suitable for efficient long distance dispersal (van Zanten 1978; van Zanten & Pócs 1981). Recurrent long distance dispersal in bryophytes is also consistent with phylogeographic evidence (e.g. Werner & Guerra 2004; Devos & Vanderpoorten 2009; Szövényi *et al*. 2012). Bryophytes, indeed, exhibit a high potential for generative and vegetative propagation. It should be noted, however, that only few species of bryophytes are ubiquitous at regional, let alone global scales and that some bryophytes species have narrow ranges. Therefore, successful dispersal and establishment are generally limited (Frahm 2008; Shaw *et al.* 2011).

Wide distribution ranges

Bryophytes display wider distribution ranges than angiosperms. This is probably due to their modes of dispersal, tolerance to desiccation, broad ecological amplitude and dual mode of reproduction. In fact, it is common that the geographic distribution of bryophyte species span two or more continents (Schofield & Crum 1972). Many bryophyte species exhibit the same disjunctions that are found, at the generic level, in angiosperms (Shaw 2001). Some species of bryophytes, termed as cosmopolitan, are widespread across all continents. Although the number of cosmopolitan species depends on the definition of the concept, they are rare in any case and their percentage might be far below 1% (Medina *et al*. 2011). In fact, only few “weedy” species are considered ubiquitous such as *B. argenteum*. Ubiquity among bryophytes is therefore, extremely rare (Frahm 2008). Nevertheless, more than 75% of the families of bryophytes are widespread across the North and South hemispheres (Tan & Pócs 2000).

Endemism

In contrast to vascular plants, bryophytes display significantly lower proportion of endemism. For example, in Macaronesia, endemisms account for 40% of the angiosperm flora (Carine *et al.* 2004) and only less than 10% of bryophytes (Vanderpoorten *et al.* 2007). In New Zealand, 86% of the angiosperms are endemic but only 28% of the mosses (van Zanten & Pócs 1981). Moreover, bryophytes endemics in archipelagos are usually located in more than one island in contrast with angiosperms, where single island endemics predominate (Patiño *et al.* 2013). Examples of narrow endemics restricted to single islands in bryophytes are the liverwort *Vandiemenia ratkowskiana* Hewson that is only known from two localities on the island of Tasmania (Furuki and Dalton 2008) or the moss *Orthotrichum handiense* F. Lara, Garilleti & Mazimpaka (Lara *et al.* 1999), restricted to a small area on the island of Fuerteventura (Canary Islands). Possible reasons for restricted ranges in some bryophyte species are their preference for a highly specialized habitat, lack of spore production, lack of ability for successful dispersal or establishment, a combination of some of these factors, etc. (Frahm 2008). The greatest concentrations of bryophyte endemic species are apparently found in microenvironments where wind dispersal may be prevented (Medina *et al.* 2011). Thus, limits to dispersal may play a key role.

Vicariance versus dispersal

To explain the generally broad and disjunctive geographical distributions of bryophytes, two hypotheses have been traditionally discussed. The first and long held hypothesis interprets it as a result of a fragmentation of ancient continuous distributions (i.e. vicariance) (Schofield & Crum 1972; Schuster 1983). The second hypothesis explains it as a consequence of intercontinental, long distance dispersal (reviewed in Heinrichs *et al.* 2009; Shaw *et al.* 2011).

The patters of disjunction in many bryophytes are similar to those found in many other groups of organisms. Those disjunctive ranges are highly congruent with the continental drift hypothesis and, therefore, support the vicariance hypothesis (Schofield & Crum 1972; Schuster 1983). On the other hand, some disjunctive distributions are difficult to interpret under the Earth's history of continental drift. For example, most boreal bryophytes, including most of the dominant peatmosses (*Sphagnum*), have a more or less continuous circumpolar distribution, including the northern parts of Europe, Asia and North America (Shaw 2001). Most remarkable, there are 18 species of bryophytes present in Antarctica with a bipolar disjunctive range that include boreal and temperate regions in both hemispheres (Ochyra *et al.* 2008). Those patterns are best explained by recent long distance dispersal events rather than vicariance (Shaw 2001).

A combination of recently developed statistical techniques with molecular data and distributions of extant taxa provide powerful tools to assess phylogenetic relationships. These are useful to disentangle vicariance from dispersal and help us to retrace geographical origins, migration routes, and assess the timing of colonization. These tools were the methods of research used in this thesis. Based on this type of research, many recent studies indicate that long distance dispersal is more common than previously expected in many bryophyte lineages (reviewed in Heinrichs *et al.* 2009). However, many distributions cannot be merely explained as one alternative over the other, i.e. either continental drift or long distance dispersal, but continental drift as well as long distance dispersal (Frahm 2008). Thus, the apparently competing hypotheses of dispersal *versus* vicariance complement each other. In general, genetic studies suggest that dispersal among continents has occurred repeatedly during bryophyte diversification, although it has not been recurrent enough to prevent allopatric differentiation (Shaw *et al.* 2011). In particular, cosmopolitan bryophyte species exhibit a low, potentially negligible, structure among continents (Werner & Guerra 2004 in *Tortula muralis* Hedw.; McDaniel & Shaw 2005 in *Ceratodon purpureus* (Hedw.) Brid.) favouring the long distance dispersal hypothesis over vicariance. This raises the question of whether dispersal is ubiquitous in cosmopolitan bryophytes and the variants of a given species are distributed everywhere.

The Baas Becking tenet

The "Everything is everywhere, but the environment selects" tenet (EiE) (Baas Becking 1934), also referred as the ubiquity hypothesis, has been recently resurrected and debated for vagile taxa (e.g. Heino *et al*. 2010 in diatoms; Aguilar & Ladó 2012 in amoebae; Faurby *et al*. 2012 in tardigrades; Rout & Callaway 2012 in soil microbes; Baltanás & Danielopol 2013 in crustaceae; Hazard *et al*. 2013 in arbuscular fungi; Sul *et al*. 2013 in marine bacteria). The EiE tenet explains the apparent cosmopolitan distribution of some vagile taxa by assuming a lack of dispersal limitations (Sul *et al*. 2013). Many of these taxa and spore-bearing plants, including the bryophytes, shared characteristics such as: (1) passive dispersal everywhere, (2) reproduction by asexual or parthenogenic means efficiently increasing their numbers at a fast rate and (3) the inclusion of resting stages in their life cycle that allows them to subsist in habitats with adverse environmental conditions (Fontaneto & Brodie 2011). These characteristics together with the distinctive features of bryophytes and, in particular, of bryophytes with cosmopolitan distributions, such as their high plasticity, suggest that they are ideal candidates to test the EiE tenet. Yet, the EiE hypothesis has remained untested in bryophytes until now.

Recurrent long distance dispersal and establishment, if strong enough, may homogenize a given species along its distribution range (Vanderpoorten *et al*. 2008). If this is the case, we would expect that the molecular sequences of disjunctive populations were rather similar. Thus, the observation on the historical effects of dispersal limitation would be denied (Locey 2010) and no biogeographic structure would be expected (Vanderpoorten *et al.* 2010) supporting the EiE hypothesis. On the other hand, if there are limits to dispersal at a global scale and, thus, the EiE hypothesis is rejected, the continental populations would have different evolutionary histories and we would expect a biogeographic structure providing evidence for vicariance (Shaw *et al.* 2011). If this is the case, and there are natural limitations to dispersal, cosmopolitan bryophytes may have achieved their global distribution aided by anthropogenic activities. This hypothesis is reinforced by the affinity of cosmopolitan bryophytes to disturbed habitats, which is reminiscent of invasive species' behaviour.

Human contribution to the dispersion of bryophytes

Human activities are causing range distributional changes in many species at an unprecedented scale (Ellis *et al.* 2012). Biological invasions can alter the structure and function of colonized ecosystems, species interactions and community composition. These invasions may impact the population dynamics and fitness of native species (Lau 2008). However, just a small fraction of invasive species have had significant impact in the ecosystems (Williamson & Fitter 1996).

Invasion patterns of bryophytes, in contrast with vascular plants, have not attracted significant attention (Essl & Lambdon 2009; Miller 2009; Essl *et al*. 2013, 2014). The negligence of not studying more in depth bryophyte invasions may be due to: (1) bryophytes are almost never deliberately introduced and have little socio-economic importance, (2) their effects on native species as well as on ecosystem functions and services are hardly known (Essl *et al.* 2013), and (3) it is extremely difficult to determine that a bryophyte species is alien because of the scarcity of their fossil record and strong long-distance dispersal capabilities.

Bryum argenteum is thought to be accidentally dispersed by humans as well as to small animals (Glime 2007). Asexual propagules of the species may be attached to shoes and other clothing and become established elsewhere, as experimentally demonstrated by Clare & Terry (1960). Accidental propagation of this species and other cosmopolitan species by anthropogenic activities may be potentiated by their affinity for disturbed soils, which may increase their local abundance (Schuster 1983). However, there seems to be no study which claims that *B. argenteum* is alien to a particular region of the world, with the exception of Essl *et al.* (2013) that identified *B. argenteum* as alien species in one out of the 82 areas studied by the authors (the island of St Helena). Although the authors acknowledged that the presence of the species was reported from the 19th century they considered it as alien since it was only found in anthropogenically disturbed habitats. This claim is, however, problematical since: (1) *B. argenteum* is far more conspicuous in disturbed rather than in natural habitats and to spot the species in the latter may be a very difficult task, (2) although the species was indeed only present in anthropogenically disturbed habitats, this alone should not be a sufficient reason to classify the species as alien, (3) *B. argenteum* is present in the most pristine areas of the world such as sub-Antarctic islands and the Antarctic continent and its presence was reported since the earliest botanical records (Ochyra *et al.* 2008; Cannone *et al.* 2013). These regions are far more distant to potential source areas than the island of St Helena. Nevertheless, the criteria used in Essl *et al.* (2013) to identify alien species have been recently questioned by Patiño & Vanderpoorten (2015).

The moss Bryum argenteum

The short turf forming moss *B. argenteum* is one of the plant species with the widest distribution on Earth. It is is broadly distributed in all continents, in highly contrasted climates from equatorial environments up to polar and alpine habitats. The species thrives in disturbed habitats but also occupies native and landscaped environments (Stark *et al.* 2010; Horsley *et al.* 2011). Diaspores of this species have been found in soil banks of very different habitats including various types of woodlands, shrublands, heath, mobile dunes (Biggs & Wittkuhn 2006) and open grasslands (Hock *et al.* 2004). Reproduction in this dioicous species is achieved by both, sexual and asexual means. The spores' size, number and the ability to survive harsh environmental conditions make it suitable for efficient long distance dispersal (van Zanten 1978). Asexual reproduction is achieved by producing large numbers of deciduous bulbils and branchlets that detach and serve as a primary mode of local dispersal (Selkirk *et al.* 1998; Horsley *et al.* 2011). It is desiccation tolerant and dehydrated gametophytes can survive at extremely high temperatures (Mertens *et al.* 2008) In addition, it is able to grow at 5/-5°C (Longton 1981) and it is capable to persist in the ice cover of a glacier for centuries and regenerate thereafter (La Farge *et al.* 2013). Its sexual/asexual propagation system along with its affinity for disturbed soils makes the species a typical colonist (*sensu* During 1992) whose high dispersal capacity is able to efficiently track ephemeral habitats at both short and long distances.

The species is morphologically very variable (Wijk *et al.* 1959; Ochyra *et al.* 2008). Longton (1981) observed high morphological variation even between clones cultivated *in vitro* at homogenized environmental conditions. Ecophysiological experiments *in vitro* of specimens derived from natural populations of temperate, tropical and polar populations (Longton 1981) and of specimens derived from clean and heavily polluted environments (Shaw *et al.* 1989; Shaw & Albright 1990) suggest that *B. argenteum* is a highly plastic, broadly adapted species. On the other hand, the research of Horsley *et al.* (2011) suggested that sexual reproduction investment differs among male and female organisms and that there may be different ecotypes in the degree of sexual versus asexual reproductive investment. In addition, male individuals have not been observed for this species in environments with harsh environmental conditions such as Antarctica (Longton 1988) and the Mojave Desert (Stark *et al.* 2010) involving that sexual reproduction is rare or absent in those habitats.

Several studies on the genetic diversity of *B. argenteum* have been reported (Skotnicki *et al.* 1998; Longton & Hedderson 2000; Hills *et al.* 2010). Longton and Hedderson (2000) analyzed the genetic distances of some geographically diverse populations of *B. argenteum* and compared them with other species of the Bryaceae. Results indicated that genetic distances among *B. argenteum* were greater than distances between closely related species. Finally, Hills *et al.* (2010) found that populations of Antarctica and Australasia were genetically structured.

Objectives

The general objective of this thesis is to address different hypotheses concerning the ecology, distribution, history and potential invasiveness of vagile plants and more specifically, of cosmopolitan bryophytes. The moss *Bryum argenteum* was used in this respect as a model species. In direct relationship with the general objective above mentioned, this thesis deals with the EiE tenet both at a local and at global scale, under a biogeographical and an ecological framework. Additionally, based on the preliminary findings, further hypotheses were tested. More specifically, this thesis aimed to:

- Test at a local scale and along altitudinal gradients, in Sierra Nevada Mountains (Spain) and Tenerife Island, if genetic diversity is homogenized in *B. argenteum* and there is no genetic structure due to the high dispersal capabilities and the lack of ecological differentiation attributed to cosmopolitan bryophytes.
- Reconstruct the worldwide phylogeography of *B. argenteum* to decipher whether intercontinental long distance dispersal approaches to ubiquitous dispersal or vicariance plays a role in explaining the global patterns of genetic variation.
- Estimate the time and origin of colonization *of B. argenteum* in Antarctica and test the hypotheses of whether the species persisted within the continent throughout glacial cycles or recently (re-)colonize it after the last glacial maximum.
- Estimate the time and origin of the colonization of *B. argenteum* on the island of Tenerife in order to evaluate the potential role of humans in the introduction of the species.
- Test whether the populations at a local level, in Sierra Nevada and Tenerife, and at a continental level, in Antarctica, were stable or, on the contrary, there is evidence for a recent demographic shift.

Thesis structure

The bulk of this thesis is structured in three chapters (chapters I to III) corresponding to three scientific articles that have already been published in international peer-reviewed journals. The three articles are:

Chapter I. Pisa S., Werner O., Vanderpoorten A., Magdy M., Ros R.M. (2013) Elevational patterns of genetic variation in the cosmopolitan moss *Bryum argenteum* (Bryaceae). *American Journal of Botany*, **100**, 2000-2008.

Chapter II. Pisa S., Biersma E., Convey P., Patiño J., Vanderpoorten A., Werner O., Ros R.M. (2014) The cosmopolitan moss *Bryum argenteum* in Antarctica: recent colonisation or in situ survival? *Polar Biology*, **37**, 469-1477.

Chapter III. Pisa S., Vanderpoorten A., Patiño J., Werner O., González-Mancebo J.M., Ros R.M. (2015) How to define nativeness in vagile organisms: Lessons from the cosmopolitan moss *Bryum argenteum* on the island of Tenerife. *Plant Biology*, **17**, 1057-1065.

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CHAPTER I

ELEVATIONAL PATTERNS OF GENETIC VARIATION IN THE COSMOPOLITAN MOSS *BRYUM ARGENTEUM* (BRYACEAE)

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Abstract

- *Premise of the study:* The Baas Becking tenet posits that ‘everything is everywhere, but the environment selects’ to explain cosmopolitan distributions in highly vagile taxa. Bryophyte species show wider distributions than vascular plants and include examples of truly cosmopolitan ranges, which have been interpreted as a result of high dispersal capacities and ecological plasticity. In the present study, we documented patterns of genetic structure and diversity in the cosmopolitan moss *Bryum argenteum* along an elevational gradient to determine if genetic diversity and structure is homogenized by intense migrations in the lack of ecological differentiation.
- *Methods:* 60 specimens were collected in Sierra Nevada (Spain) between 100 and 2,870 m and sequenced for ITS and *rps4*. Comparative analyses, genetic diversity estimators, and Mantel tests were employed to determine the relationship between genetic variation, elevation and geographic distance and seek for signatures of demographic shifts.
- *Key results:* Genetic diversity peaked above 1,900 m and no signature of demographic shifts was detected at any elevation. There was a strong phylogenetic component in elevational variation. Genetic variation was significantly correlated with elevation, but not with geographic distance.
- *Conclusions:* The results point to the long-term persistence of *B. argenteum* in a range that was glaciated during the late Pleistocene. Evidence for an environmentally-driven pattern of genetic differentiation suggests adaptive divergence. This supports the Baas Becking tenet and indicates that ecological specialization might play a key role in explaining patterns of genetic structure in cosmopolitan mosses.

Key words: adaptive divergence; Baas Becking hypothesis; bryophytes; *Bryum argenteum*; cosmopolitan species; elevational gradients; genetic diversity; mountains.

Introduction

Elevational gradients offer many characteristics that make them extremely suitable for uncovering the underlying causes of spatial variation in diversity because of the dramatic changes in environmental conditions across comparatively short distances (Sanders & Rahbek 2012). Alpine ecosystems have in particular become an increasing area of research since climate change impacts on alpine and nival vegetation may be more pronounced than on vegetation at lower elevation, with an upward shift of treelines and range reduction in alpine and nival plant species preceding massive extinctions (Randin *et al*. 2009).

Bryophytes are among the last land plants to persist in snow beds and other extreme high-elevation habitats up to 5,800 m (Mordaunt 1998; Frey & Kürschner 2012). In fact, a feature common among most bryophytes is their ability to grow at low temperature. More than half of the 40 mid-European species investigated by Furness and Grime (1982) showed a growth reduction of less than 50% at 5°C compared to growth at their optimal temperature. Most species, including tropical ones, seem to be pre-adapted to cold and survive temperatures ranging from -10 to -27°C (Glime 2007). As a result, bryophytes generally have much broader elevation ranges than vascular plants (Vittoz *et al*. 2010). Previous studies reported partitioning of morphological (Benassi *et al*. 2011; Pereira *et al*. 2013) and genetic (Korpelainen *et al*. 2012a) variation across elevational gradients, but whether this reflects adaptation or dispersal limitations and genetic drift remains ambiguous.

Little evidence of ecotypic differentiation was found in bryophytes (Shaw 1991). Their cold tolerance varies seasonally (Rütten & Santarius 1992; 1993), suggesting that they develop tolerance in response to changes in environmental conditions. In fact, it was experimentally shown that incubation at low, but > 0°C, temperatures significantly increases survival rates upon subsequent exposure to negative temperatures (Minami *et al*. 2005). In the desert moss *Syntrichia caninervis* Mitt., morphological variation of populations from extreme micro-habitats results from plasticity (Reynolds & McLetchie 2011). In *Bryum argenteum* Hedw., plants from clean and heavily polluted environments exhibit indistinguishable growth responses to media supplemented with heavy metals. It may be that this inherent, relatively high level of tolerance makes the evolution of specialized races unnecessary (Shaw *et al*. 1989; Shaw & Albright 1990). Although bryophytes are not genetically depauperate and, in fact, display amounts of genetic diversity comparable with angiosperms (Shaw 2000a), this genetic variation does not appear to be adaptive to specific environments (Shaw & Bartow 1992). Thus, by contrast with flowering plants, physiological acclimatization is much more important for bryophytes than is genetic specialization.

In addition, mosses are traditionally perceived as extremely good dispersers (see Shaw *et al*. 2011 for review). Regular establishment occurs at the km-scale (Lönnel *et al*. 2012), while 1% of the regional spore rain is assumed to have a trans- or intercontinental origin (Sundberg 2013), which is consistent with phylogeographic evidence for recurrent long-distance dispersal in the group (Werner & Guerra 2004; Devos & Vanderpoorten 2009; Szövényi *et al*. 2012). The high dispersal ability of bryophytes appears as a strong homogenizing force that may prevent local differentiation and adaptation and explain the wide distribution of mosses and their low rates of endemism as compared to angiosperms (Vanderpoorten *et al*. 2010). The high dispersal ability of bryophytes supports the first half of the Baas-Becking tenet (Baas Becking 1934) ‘everything is everywhere’, which explains the seemingly cosmopolitan distributions typically observed in micro-organisms by invoking a lack of dispersal limitation (see Sul *et al*. 2013, for review). Meanwhile, the apparent failure of bryophytes to develop ecotypes because of their inherently high ecological plasticity suggests that the second part of the tenet, ‘but the environment selects’, does not apply.

In the present paper, we revisit the Baas-Becking tenet, taking the cosmopolitan *B. argenteum* as a model. *Bryum argenteum* has one of the widest distributions of all plants on Earth. It is found in all continents in contrasting climates from the equator up to polar and alpine habitats. In addition to sexual reproduction, *B. argenteum* can produce large numbers of deciduous bulbils and branchlets that detach and serve as a primary mode of local dispersal (Selkirk *et al*. 1998). The dual mating system of *B. argenteum*, along with its preference for disturbed soils, makes the species a typical colonist (sensu During 1992) whose high dispersal capacity makes it capable of efficiently tracking ephemeral habitats at both short and long distances. It is morphologically extremely variable, but this variation does not appear to be adaptive (Longton 1981; Shaw *et al*. 1989; Shaw & Albright 1990). Consequently, patterns of genetic diversity and variation are expected to show no geographic structure among populations sharing the same evolutionary history. This hypothesis is tested here along an elevational gradient in the Sierra Nevada Mountains of southern Europe. If this hypothesis is rejected, we attempt to (i) determine whether populations from low, mid and high elevation experienced contrasting demographic histories and (ii) disentangle the roles of dispersal limitations and ecological specialization in spatial patterns of genetic variation.

Materials and Methods

Sampling design and molecular protocols

Sixty specimens of *Bryum argenteum* were collected from 15 localities in the mountains of Sierra Nevada, Spain, along an elevational gradient from 100 to 2,870 m a.s.l. All accessions but two were sterile at the time of collection. Voucher information including geographic coordinates, elevation of each locality and GenBank accession numbers are given in appendix 1. Samples within each locality were separated from each other by at least 1 m. The maximum distance between any two localities was 52 km. Samples were air dried and stored until DNA extraction. Total genomic DNA was extracted using a modified version of the NaOH extraction protocol (Werner *et al*. 2002) in which 5µl of crude NaOH extract were diluted by the addition of 45 µl of 100mM Tris - 1mM EDTA (pH 8.3), stored frozen at -18°C and used as template for PCR. The ITS1- 5.8S rDNA - ITS2 nuclear genomic fragment was amplified by PCR using primers (5´-CCGATTGAATGGTCCGGTGAAGTTTTCG and 5´-GCTGGGCTCTTTCCGGTTCG-CTCGCCGTTAC) specifically redesigned for *B. argenteum* from sequences obtained with universal primers. The *rps4* cpDNA was amplified using the primers rps5 (Nadot *et al*. 1995) and trnas (Buck *et al*. 2000). PCR reactions were performed in a thermo cycler using 2 µl of the DNA solution in a 50 µl final volume. The reaction mix contained 1.5 µl of each primer (10 µM), 5ul 10X reaction buffer with $MgCl_2$, 2 µl (1 U μl^{-1}) DNA polymerase (Biotools, Madrid, Spain), 2 µl 10 mM dNTP mix, 1µl 10% skimmed milk powder in water and 35 µl nuclease free water. ITS amplification included the following steps: initial denaturation for 3 min at 95ºC; 40 cycles each of 94ºC for 30 s, 60ºC for 30 s and 72ºC for 75 s; and a final extension at 72ºC for 5 min. Amplification for the *rps4* marker started with 3 min denaturation at 95ºC; 40 cycles each of 94ºC for 30 s, 55ºC for 30 s and 72ºC for 60 s; and a final extension at 72ºC for 5 min. We used agarose electrophoresis to test for amplification of single fragments before cleaning PCR products with the GenElute PCR Clean-Up Kit (Sigma-Aldrich Co., St Louis, MO, USA). Forward and reverse sequence fragments for both ITS1 and ITS2 and for *rps4* were generated using BigDye®Terminator v3.1 and separated on an ABI-Prism®3730 sequencing machine (Applied Biosystems, Foster City, CA, USA). In addition to the amplification primers, the primers 5.8R and 5.8F (Stech & Frahm 1999) were used in the sequencing reactions of the ITS region. Forward and reverse sequence fragments were edited and assembled using Bioedit ver.7.0.5 (Hall 1999) and every polymorphism was checked from the chromatograms. The sequences were aligned by eye, allowing gaps where necessary to conserve homology among sequences. No polymorphism was observed in the 5.8S gene, which was excluded from further analyses.

Phylogenetic analyses

Indels were coded with SeqState 1.25 (Müller 2005) using simple coding (Simmons & Ochoterena, 2000) and added to a separate binary character matrix. The nucleotide substitution models HKY+G and TPM3uf were selected based upon the AIC and BIC criteria as implemented by JModeltest2 (Darriba *et al*. 2012) for the ITS and *rps4* partitions, respectively. A model implementing identical forward and backward transition rates was applied to the indel matrix. Independent phylogenetic analyses of each cpDNA and nrDNA dataset were performed in MrBayes 3.1.2 (Ronquist & Huelsenbeck 2003). For each dataset, two Metropolis-coupled Markov Chain Monte Carlo (MCMC) analyses, including three hot chains and one cold chain, were run for 10^7 generations and sampled every 10^4 generations. To confirm that the chains had reached stationarity and converged, we (i) graphically inspected the values of the log-likelihoods of the two MCMC analyses; (ii) made sure that the standard deviation of split frequencies was below 0.01 at the completion of the analysis; and (iii) made sure 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 200 trees were discarded as burn-in and the remaining trees were used to construct a 50 % majority rule consensus tree. The *rsp4* analysis resolved two haplotypes that correspond to the two main clades identified in the ITS analysis (see below). The partitions were therefore congruent and combined within a heterogeneous Bayesian analysis employing the nucleotide substitution models indicated above for each partition. The other settings were identical as those described above.

The generalized least square models implemented by the Continuous option of BayesTraits (Pagel 1997) were used to investigate the phylogenetic component of elevational variation through the scaling parameter lambda (λ). A value of $\lambda = 1$ indicates that the tree correctly predicts the patterns of elevational variation observed, whereas $\lambda = 0$ points to the phylogenetic independence of trait evolution (Freckleton *et al*. 2002). We employed an MCMC analysis to sample values of λ depending on their posterior probability. At each iteration, the chain selects a tree and a value of λ, and the combination is assessed though the Metropolis-Hastings term. We then re-ran the analysis, setting λ to 0, and determined whether imposing complete phylogenetic independence in elevational variation ($\lambda = 0$) significantly decreases the log-likelihood by computing the Bayes factors. The latter were measured as twice the difference in the harmonic means of the log-likelihood of the two analyses, and differences of 2, 5, and

10 were considered as evidence, strong evidence, and very strong evidence for differences of fit between the models, respectively (Pagel *et al*. 2004).

Population genetics analyses

Haploid diversity corrected for sample size (uh) and nucleotide diversity (pi) were calculated with GENEALEX 6.5 (Peakall & Smouse 2006) along the elevational gradient partitioned into three elevational belts that correspond to vegetation zones in the Sierra Nevada of southern Europe: low elevation, < 800 m; mid-elevation, 800-1900 m; and high elevation > 1900 m (Anderson *et al*. 2011). Tajima's *D* (Tajima 1989) and Fu's *Fs* (Fu 1996) were calculated in Arlequin 3.5 (Excoffier *et al*. 2005) for each of the three elevation levels to seek for a signature of demographic changes in patterns of genetic diversity. Both statistics measure whether variation at the locus considered is consistent with the hypothesis that the populations are at neutral mutation-drift equilibrium. If *D* and *Fs* do not significantly depart from 0, there is no evidence for changes in population size or for selection at the locus. Significantly negative *D* and *Fs* point to purifying selection or expansion, while a positive value of those statistics is suggestive of bottleneck or dominant selection.

Mantel tests were used to measure the correlation between genetic distances on the one hand, and geographical distance and elevational gradients on the other. We therefore computed kinship coefficient among individuals, *Nij*, which 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). Phylogenetic distance among alleles was measured from a Tamura 3-parameter model distance matrix with Mega 5 (Tamura *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 10^4 random permutations of population locations in SPAGeDi 1.3 (Hardy & Vekemans 2002). The same test was then applied between the matrix of *Nij* and of the elevational difference among pairs of individuals. To obtain a graphic representation of the change in genetic similarity with increasing elevational difference among individuals, the mean *Nij* values were computed over *i*, *j* pairs separated by predefined elevational intervals. Threshold distance separating intervals were 0, 250, 670, 1,300, 2,000 and 2,600 m, the first interval corresponding to pairs of individuals from the same population. In order to remove the potentially confounded signal of geographic distance in the matrix of elevational differences, partial Mantel tests were used. The latter were employed to test the correlation between matrices of kinship coefficients and of elevational difference among individuals, while controlling for the information present in the geographic distance matrix. Partial Mantel tests were performed with ZT (Bonnet & Van de Peer 2002), and the significance of the correlations was tested by means of 10^4 randomization runs.

Results

There were 112 (80 indels and 32 mutations) and 6 polymorphic positions (no indels) in the ITS and *rps4* matrices, respectively. Two clades, hereafter labeled as A and B, were resolved with full support in the rps4 tree. These two clades also corresponded to the first dichotomy in the ITS phylogeny (Fig. 1). In the combined analysis, these two clades were supported with posterior probabilities of 0.97 and 1.00, respectively. Specimens from clade A are restricted to localities above 1,900 m, whereas specimens from clade B were sampled from the whole elevational range (Fig. 1). There was a strong phylogenetic component in elevational variation.

In fact, the posterior probability of lambda (λ = 0.87, min = 0.54, max = 0.99) did not encompass 0, and constraining λ to 0 resulted in a Bayes factors of 100.7, unambiguously indicating that a phylogenetically dependent model describes patterns of elevational variation significantly better than a phylogenetically independent one.

Table 1. Unbiased haploid diversity ± standard deviation (uh ± S.D.), nucleotide diversity ± standard deviation (pi ± S.D.), Tajima's *D* and Fu's *Fs* statistics and associated *P*-values, sample size (N) and number of haplotypes (*n*) in the moss *Bryum argenteum* along an elevation gradient in the Sierra Nevada of southern Europe.

Diversity and Neutral test statistics	**All**	**< 800 m**	**800-1900 m**	**> 1900 m**
uh ± s.d.	0.177 ± 0.011	0.068 ± 0.012	0.082 ± 0.015	0.381 ± 0.017
pi ± s.d.	0.008 ± 0,004	0.001 ± 0,001	0.002 ± 0.001	0.010 ± 0.005
Tajima's *D* (*P*-value)	0.397 (0.74)	-0.805 (0.24)	0.472 (0.70)	1.424 (0.95)
Fu's *FS* (*P*-value)	14.601 (1.00)	5.610 (0.98)	6.249 (0.99)	16.380 (1.00)
N(n)	59(16)	16(5)	11(4)	29(9)

Both haploid diversity and nucleotide diversity were lowest at low elevation and highest at >1,900 m (Table 1). Tajima's *D* and Fu's *Fs* were not significant at any of the elevation belts. The slope of the regression between *Nij* and geographic distance (isolation-by-distance test) was not significant ($P > 0.05$). Mantel tests between *Nij* and elevation difference among individuals were significant. On average, mean *Nij* per class of altitudinal difference decreased with increasing altitudinal difference among individuals (Fig. 2). This relationship remained significant after removal of the geographical component of the matrix of elevational difference among individuals (partial Mantel test, r = 0.055, $P < 0.01$).

Fig. 1. Fifty per cent majority-rule consensus with branch length averaged across the trees sampled from the posterior probability distribution from the Bayesian analysis of ITS and *rps4* sequences in the moss *Bryum argenteum* along an elevation gradient in the Sierra Nevada of southern Europe. Numbers below the branches are the posterior probabilities.

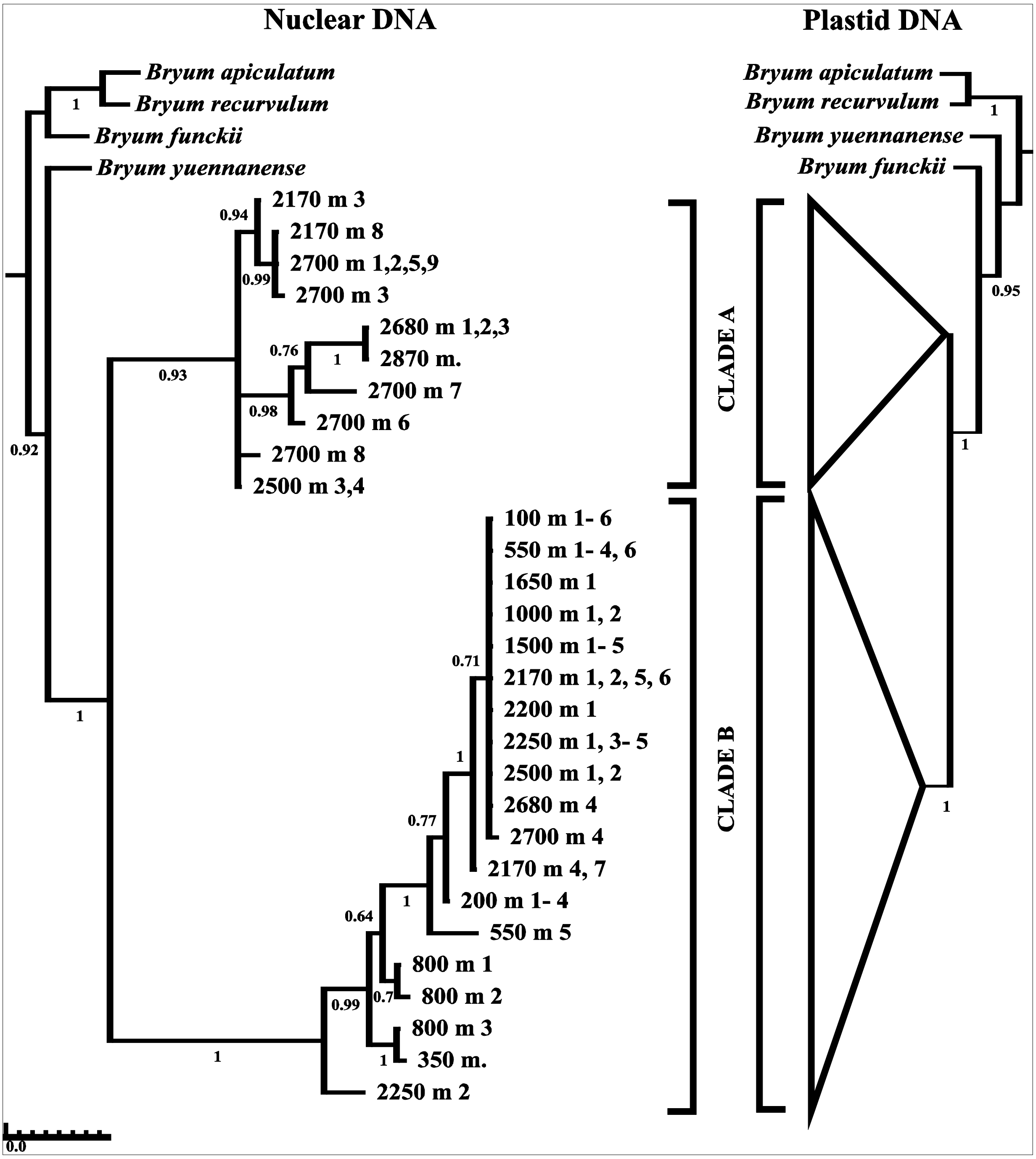
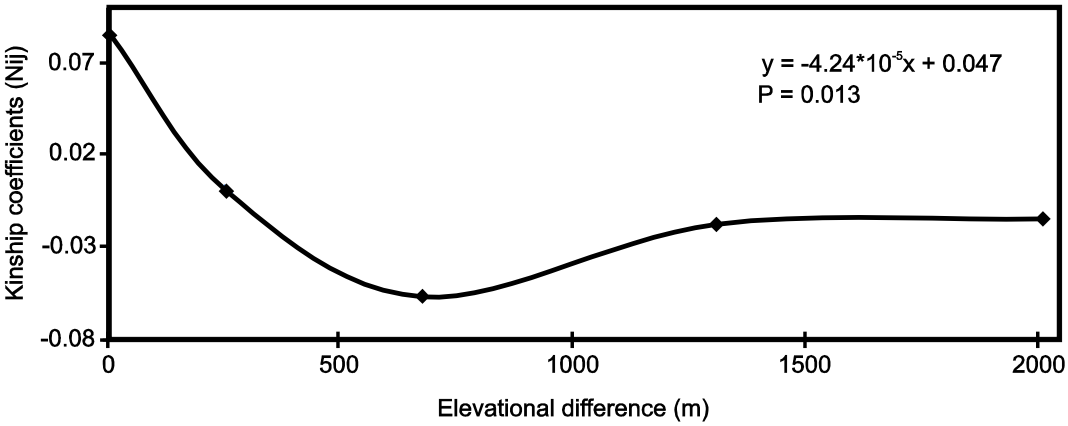


Fig. 2. Mean kinship coefficients *Nij* between pairs of individuals of the moss *Bryum argenteum* based on their sequence variation at ITS and *rps4* depending on the elevational difference among them in the Sierra Nevada. The slope of the regression between pairwise *Nij* and elevational difference and its *P*-value are indicated in the upper right corner.



Discussion

Striking levels of genetic diversity, similar to the highest levels of ITS divergence reported within moss species (e.g., Shaw 2000b), were found in ITS sequences of *B. argenteum* along an elevation gradient in Sierra Nevada, confirming previous reports of high ITS diversity in the species (Longton & Hedderson 2000). The ITS region has been and remains one of the most widely exploited sources of molecular variation at the species level (e.g., Nagy *et al*. 2012; Pettigrew *et al*. 2012; Kučera *et al*. 2013), but there has been an increasing concern about its reliability for phylogenetic reconstruction, especially due to the existence of paralogs and pseudogenes (see Nieto Feliner & Rosselló 2007 for review). The 5.8S gene was completely invariant among *B. argenteum* accessions, rendering the pseudogene hypothesis unlikely. The hypothesis that several paralogous copies were sequenced is also weakened by two lines of evidence. First, no conflicting base calls during sequencing were observed. Second, although the levels of polymorphisms in *rps4* were low, there was a congruent phylogenetic signal between *rps4* and ITS. Altogether, these observations suggest that the variation observed reflects actual diversification of orthologous ITS sequences.

There was a substantially higher genetic diversity at high elevation as compared to that observed at mid and low elevation, ruling out our primary hypothesis that everything is everywhere in the absence of dispersal limitations and ecological differentiation along the elevational gradient. Similar patterns were reported in previous studies and have been interpreted as evidence of adaptation to severe conditions at high elevation, human factors, or demographical shifts associated with climate change (see Ohsawa & Ide 2008 for review). Since Tajima's *D* and Fu *Fs* statistics did not significantly depart from 0, there was no evidence for changes in population size, weakening the latter hypothesis. Intense migration from lower areas could potentially erase any signature of bottlenecks associated with founding events and lead to non-significant *Fs* and *D* statistics (Busch *et al*. 2007), but this hypothesis is not compatible with the observed partitioning of genetic diversity. The results thus suggest that *B. argenteum* successfully persisted in a high-elevation range that was extensively glaciated during the late Pleistocene (Anderson *et al*. 2011). This parallels molecular support for Pleistocene persistence of the species in continental Antarctica (Hills *et al*. 2010) and, more generally, of temperate bryophyte species in micro-refugia across largely glaciated landscapes (Désamoré *et al*. 2012).

Bryophytes are almost never deliberately introduced and *B. argenteum* is not a species that is harvested for commercial purpose, so that the high levels of genetic diversity found at the highest elevations cannot be attributed to human factors, such as lower collection or exploitation intensity at high elevation, which has been reported for some commercially valuable taxa (Wen & Hsiao 2001; Maghuly *et al*. 2006). Human factors could potentially play a role in generating higher disturbance levels at low elevation, but recent evidence suggests that moss populations from natural and disturbed areas display similar levels of genetic diversity (Korpelainen *et al*. 2012b, but see Patiño *et al*. 2010), especially in a colonist species (sensu During 1992) such as *B. argenteum* with a life-history typically adapted to highly disturbed environments. Altogether, this suggests that although the influence of human factors in the patterns of genetic diversity of *B. argenteum* along the elevation gradient cannot be ruled out, it is unlikely to account for the substantially higher diversities observed at high elevation.

A third possibility to explain the peak of genetic diversity at high elevation is that the severe conditions at high elevation trigger adaptation, and in particular, adaptive divergence fostering genetic diversity (Porter & Rice 2013). In *B. argenteum*, in fact, genetic variation was significantly correlated with elevation. Furthermore, the elevational range displayed by the species has a significant phylogenetic component, pointing to heritable elevational preferences. Such a genetic differentiation along a steep ecological gradient was unexpected, because in bryophytes in general (Shaw 2000a), and in *B. argenteum* in particular (Longton 1981; Shaw *et al.* 1989; Shaw & Albright 1990), physiological plasticity rather than ecotypic differentiation is thought to account for the ability to occur in contrasting ecological conditions. The results presented here hence support yet scant evidence (Shaw & Beer 1999; Hutsemekers *et al*. 2010; Horsley *et al*. 2011) that although ecologically highly plastic, moss species also develop ecotypes to adapt to a wide range of environmental conditions and achieve large distribution ranges. As in other organisms, such as tardigrades (Faurby *et al*. 2012), the present results fully support the Baas Becking tenet and indicate that ecological specialization might play a much more important role than dispersal limitation in explaining patterns of genetic structure in cosmopolitan mosses.

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Appendix 1. Sample identification (referring to elevation of collection), name of the species, voucher information including Herbarium where they are kept, original publication if they have been previously published, origin of the samples, geographic coordinates in decimal degrees, haplotypes and GenBank accession numbers for the samples used in this study.

Sample ID	Species	Herbarium	Original publication	Location	Geographic Coordinates	haplotype	ITS1	ITS2	*rps4*
100 m 1	*Bryum argenteum*	MUB 40250	-	Spain, Granada province	N 36.75117 W 003.19887	hap. 1	KC493840		KC493936
100 m 2	*Bryum argenteum*	MUB 40251	-	Spain, Granada province	N 36.75117 W 003.19887	hap. 2	KC493841		KC493937
100 m 3	*Bryum argenteum*	MUB 40252	-	Spain, Granada province	N 36.75117 W 003.19887	hap. 2	KC493842		KC493938
100 m 4	*Bryum argenteum*	MUB 40253	-	Spain, Granada province	N 36.75117 W 003.19887	hap. 2	KC493843		KC493939
100 m 5	*Bryum argenteum*	MUB 40254	-	Spain, Granada province	N 36.75117 W 003.19887	hap. 2	KC493844		KC493940
100 m 6	*Bryum argenteum*	MUB 40255	-	Spain, Granada province	N 36.75117 W 003.19887	hap. 2	KC493845		KC493941
200 m 1	*Bryum argenteum*	MUB 40220	-	Spain, Granada province	N 36.84465 W003.50905	hap. 3	KC493868	KC493894	KC493911
200 m 2	*Bryum argenteum*	MUB 40221	-	Spain, Granada province	N 36.84465 W003.50905	hap. 3	KC493873	KC493899	KC493912
200 m 3	*Bryum argenteum*	MUB 40222	-	Spain, Granada province	N 36.84465 W003.50905	hap. 3	KC493874	KC493900	KC493914
200 m 4	*Bryum argenteum*	MUB 40223	-	Spain, Granada province	N 36.84465 W003.50905	hap. 3	KC493872	KC493898	KC493913
350 m 1	*Bryum argenteum*	MUB 40256	-	Spain, Granada province	N 36,80025 W 003.21738	hap. 4	KC493867	KC493893	KC493942
550 m 1	*Bryum argenteum*	MUB 40257	-	Spain, Granada province	N 36,80025 W 003.21738	hap. 2	KC493875	KC493901	KC493944
550 m 2	*Bryum argenteum*	MUB 40258	-	Spain, Granada province	N 36,80025 W 003.21738	hap. 2	KC493876	KC493902	KC493945
550 m 3	*Bryum argenteum*	MUB 40259	-	Spain, Granada province	N 36,80025 W 003.21738	-	KC493877	KC493903	-
550 m 4	*Bryum argenteum*	MUB 40260	-	Spain, Granada province	N 36,80025 W 003.21738	hap. 2	KC493878	KC493904	KC493947
550 m 5	*Bryum argenteum*	MUB 40261	-	Spain, Granada province	N 36,80025 W 003.21738	hap. 5	KC493858	KC493884	KC493943
550 m 6	*Bryum argenteum*	MUB 40262	-	Spain, Granada province	N 36,80025 W 003.21738	hap. 2	KC493879	KC493905	KC493946
800 m 1	*Bryum argenteum*	MUB 40263	-	Spain, Granada province	N 36.79475 W 003.24682	hap. 6	KC493865	KC493891	KC493948
800 m 2	*Bryum argenteum*	MUB 40264	-	Spain, Granada province	N 36.79475 W 003.24682	hap. 7	KC493864	KC493890	KC493949
800 m 3	*Bryum argenteum*	MUB 40314	-	Spain, Granada province	N 36.79475 W 003.24682	hap. 8	KC493866	KC493892	KC493950
1000 m 1	*Bryum argenteum*	MUB	-	Spain, Granada	N 37.16320 W	hap. 2	KC49388	KC49390	KC49393

		40248		province	003.46658		2	8	4
1000 m 2	*Bryum argenteum*	MUB 40249	-	Spain, Granada province	N 37.16320 W 003.46658	hap. 2	KC493839		KC493935
1500 m 1	*Bryum argenteum*	MUB 40224	-	Spain, Granada province	N 37.00218 W 003.27027	hap. 2	KC493824		KC493910
1500 m 2	*Bryum argenteum*	MUB 40225	-	Spain, Granada province	N 37.00218 W 003.27027	hap. 2	KC493825		KC493915
1500 m 3	*Bryum argenteum*	MUB 40226	-	Spain, Granada province	N 37.00218 W 003.27027	hap. 2	KC493826		KC493916
1500 m 4	*Bryum argenteum*	MUB 40227	-	Spain, Granada province	N 37.00218 W 003.27027	hap. 2	KC493827		KC493917
1500 m 5	*Bryum argenteum*	MUB 40228	-	Spain, Granada province	N 37.00218 W 003.27027	hap. 2	KC493828		KC493918
1650 m 1	*Bryum argenteum*	MUB 40265	-	Spain, Granada province	N 37.12633W 003.43677	hap. 2	KC493869	KC493895	KC493951
2170 m 1	*Bryum argenteum*	MUB 40236	-	Spain, Granada province	N 37.09725 W 003.39753	hap. 2	KC493881	KC493907	KC493924
2170 m 2	*Bryum argenteum*	MUB 40237	-	Spain, Granada province	N 37.09725 W 003.39753	hap. 2	KC493880	KC493906	KC493925
2170 m 3	*Bryum argenteum*	MUB 40238	-	Spain, Granada province	N 37.09725 W 003.39753	-	KC493862	KC493888	-
2170 m 4	*Bryum argenteum*	MUB 40239	-	Spain, Granada province	N 37.09725 W 003.39753	hap. 10	KC493833		KC493926
2170 m 5	*Bryum argenteum*	MUB 40240	-	Spain, Granada province	N 37.09725 W 003.39753	hap. 2	KC493834		KC493928
2170 m 6	*Bryum argenteum*	MUB 40241	-	Spain, Granada province	N 37.09725 W 003.39753	hap. 2	KC493835		KC493927
2170 m 7	*Bryum argenteum*	MUB 40242	-	Spain, Granada province	N 37.09725 W 003.39753	hap. 10	KC493836		KC493929
2170 m 8	*Bryum argenteum*	MUB 40243	-	Spain, Granada province	N 37.09725 W 003.39753	hap. 9	KC493837		KC493930
2200 m 1	*Bryum argenteum*	MUB 40266	-	Spain, Granada province	N 37.10975 W 003.41837	hap. 2	KC493870	KC493896	KC493952
2250 m 1	*Bryum argenteum*	MUB 40229	-	Spain, Granada province	N 37.09725 W 003.39753	hap. 2	KC493829		KC493919
2250 m 2	*Bryum argenteum*	MUB 40235	-	Spain, Granada province	N 37.09725 W 003.39753	hap. 11	KC493863	KC493889	KC493923
2250 m 3	*Bryum argenteum*	MUB 40231	-	Spain, Granada province	N 37.09725 W 003.39753	hap. 12	KC493830		KC493920
2250 m 4	*Bryum argenteum*	MUB 40232	-	Spain, Granada province	N 37.09725 W 003.39753	hap. 12	KC493831		KC493921
2250 m 5	*Bryum argenteum*	MUB 40233	-	Spain, Granada province	N 37.09725 W 003.39753	hap. 2	KC493832		KC493922
2500 m 1	*Bryum argenteum*	MUB 40244	-	Spain, Granada province	N 37.09512 W 003.38668	hap. 2	KC493838		KC493932
2500 m 2	*Bryum argenteum*	MUB 40245	-	Spain, Granada province	N 37.09512 W 003.38668	-	KC493883	KC493909	-
2500 m 3	*Bryum argenteum*	MUB	-	Spain, Granada	N 37.09512 W	hap. 12	KC49386	KC49388	KC49393

		40246		province	003.38668		1	7	1
2500 m 4	*Bryum argenteum*	MUB 40247	-	Spain, Granada province	N 37.09512 W 003.38668	hap. 12	KC493860	KC493885	KC493933
2680 m 1	*Bryum argenteum*	MUB 40276	-	Spain, Granada province	N 37.07605 W 003.37648	hap. 13	KC493854		KC493961
2680 m 2	*Bryum argenteum*	MUB 40277	-	Spain, Granada province	N 37.07605 W 003.37648	hap. 13	KC493855		KC493962
2680 m 3	*Bryum argenteum*	MUB 40278	-	Spain, Granada province	N 37.07605 W 003.37648	hap. 13	KC493856		KC493963
2680 m 4	*Bryum argenteum*	MUB 40279	-	Spain, Granada province	N 37.07605 W 003.37648	hap. 2	KC493871	KC493897	KC493964
2700 m 1	*Bryum argenteum*	MUB 40267	-	Spain, Granada province	N 37.0,943 W 003.38662	hap. 9	KC493846		KC493953
2700 m 2	*Bryum argenteum*	MUB 40268	-	Spain, Granada province	N 37.0,943 W 003.38662	-	KC493847		-
2700 m 3	*Bryum argenteum*	MUB 40269	-	Spain, Granada province	N 37.0,943 W 003.38662	hap. 9	KC493848		KC493954
2700 m 4	*Bryum argenteum*	MUB 40270	-	Spain, Granada province	N 37.0,943 W 003.38662	hap. 2	KC493849		KC493955
2700 m 5	*Bryum argenteum*	MUB 40271	-	Spain, Granada province	N 37.0,943 W 003.38662	hap. 9	KC493850		KC493956
2700 m 6	*Bryum argenteum*	MUB 40272	-	Spain, Granada province	N 37.0,943 W 003.38662	hap. 15	KC493851		KC493957
2700 m 7	*Bryum argenteum*	MUB 40273	-	Spain, Granada province	N 37.0,943 W 003.38662	hap. 14	KC493859	KC493885	KC493958
2700 m 8	*Bryum argenteum*	MUB 40274	-	Spain, Granada province	N 37.0,943 W 003.38662	hap. 16	KC493852		KC493959
2700 m 9	*Bryum argenteum*	MUB 40275	-	Spain, Granada province	N 37.0,943 W 003.38662	hap. 9	KC493853		KC493960
2870 m 1	*Bryum argenteum*	MUB 40280	-	Spain, Granada province	N 37.07153 W 003.37355	hap. 13	KC493857		KC493965
Bryum apiculatum	*Bryum apiculatum*	HBNU	Wang and Zhao (2009)	China, Yunnan province	-	-	EU878213		FJ593892
Bryum funckii	*Bryum funckii*	HBNU	Wang and Zhao (2009)	China, Hunan province	-	-	EU878209		AY078332
Bryum recurvulum	*Bryum recurvulum*	HBNU	Wang and Zhao (2009)	China, Hebei province	-	-	EU878217		FJ593887
Bryum yuennanense	*Bryum yuennanense*	HBNU	Wang and Zhao (2009)	China, Yunnan province	-	-	EU878211		FJ593890

CHAPTER II

THE COSMOPOLITAN MOSS *BRYUM ARGENTEUM* IN ANTARCTICA: RECENT COLONISATION OR *IN SITU* SURVIVAL?

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Abstract

Since the onset of glaciation following the Oligocene (30-28 Ma), the prevalence of increasingly cold conditions has shaped the evolution of the Antarctic biota. Two hypotheses, post-glacial recruitment from extra-regional locations and *in situ* persistence, have been proposed to explain the biogeography of the contemporary species-poor terrestrial Antarctic biota. Bryophytes, which form a major group of the Antarctic flora, exhibit a strong, inherent ability to survive cold conditions but also have high long-distance dispersal capacities, which are compatible with both hypotheses. Here, we test these hypotheses by means of population genetic and phylogeographic analyses of the cosmopolitan moss *Bryum argenteum*. We find evidence for at least three independent colonisation events of the species in Antarctica. Ancestral area reconstruction coupled with molecular dating suggest colonisation times of the different Antarctic clades ranging from four million years for the oldest lineage to half a million years for the youngest lineage. This suggests multiple colonisation events of Antarctica by this species during several glacial cycles within the Pleistocene, Pliocene and possibly late Miocene. This is the first study to demonstrate *in situ* persistence of bryophytes in Antarctica throughout previous glaciations.

Keywords: glacial refugia, dispersal, bryophytes, molecular dating, phylogeography

Introduction

The prevalence of increasingly cold conditions has shaped the evolution of the Antarctic biota since its separation from the other Southern Hemisphere continents during the final stages of the breakup of the supercontinent Gondwana (Convey & Stevens 2007; Convey *et al*. 2009; Fraser *et al*. 2012). Climate cooling was accompanied by a decrease in diversity of the angiosperm-dominated vegetation that inhabited the Antarctic Peninsula and parts of the continent during the Eocene. A mosaic of southern beech and conifer-dominated woodlands and tundra, comparable to that in parts of southern South America (Patagonia) today, characterised the region throughout the Oligocene (approximately 34-23 Ma). By the middle Miocene (approximately 16-11.6 Ma), localized pockets of tundra persisted until at least 12.8 Ma (Anderson *et al*. 2011). The transition to cold-based, alpine glacial regimes characterized by perennially dry and frozen conditions from 13.85 Ma has not subsequently been reversed (Lewis *et al*. 2008). Since the late Miocene, the Antarctic ice sheets have repeatedly thickened and advanced beyond their current limits onto the continental shelf. Ice sheets are believed to have overrun most currently ice-free areas during glaciations in the Pliocene (5-2.6 Ma) and the Pleistocene (2.6 Ma-10 Ka), including the Last Glacial Maximum (LGM; around 22-17 Ka) (Convey *et al*. 2009, and references therein; Mackintosh *et al*. 2013). The terrestrial biota remaining in Antarctica today is depauperate in terms of species diversity, and is often cryptic. The major groups of organisms represented include micro-invertebrates, cryptogams and microbial groups (Convey 2013).

Two main hypotheses have been proposed to explain how the contemporary terrestrial Antarctic biota endured the glacial events of the Pleistocene (Convey &

Stevens 2007; Convey *et al*. 2008). The first, and long-held but generally untested, view is that the large majority of the Antarctic terrestrial biota was eradicated from both the Antarctic continent and the associated offshore islands and archipelagos of the Scotia Arc (including sub-Antarctic South Georgia). The considerably expanded and thickened ice sheets would have caused a complete loss of terrestrial exposures and habitats during the glacial maxima, as is consistent with current glaciological model reconstructions. As a consequence, most or all current Antarctic terrestrial biota would have had to (re-)colonise the continent after the LGM, either from disjunct populations or from refugia. However, while the long-distance dispersal (LDD) capacity of bryophytes would potentially have facilitated (re)colonisation on such a timescale, the ability to disperse over long distances is apparently more limited for many other groups of Antarctic organisms. An additional weakness of the hypothesis is that many elements of the contemporary Antarctic biota show high levels of endemism (Øvstedal & Lewis Smith 2001; Pugh & Convey 2008), which could not have arisen after the LGM. The persistence of such an endemic biota would require refugia beyond the current confines of Antarctica (for instance in the more distant sub-Antarctic islands or the other Southern Hemisphere continents), which would have been situated beyond the current distributions of many species. Therefore, this hypothesis would imply multiple colonisation events out of Antarctica when refugia were required, and subsequent extinction from them once Antarctica was recolonised. The alternative hypothesis is that species have survived *in situ*, in multiple refugia that must have been present in different regions across Antarctica. This hypothesis has received increasingly strong support in recent years from both molecular and classic biogeographical studies, as well as from geological and geomorphological evidence demonstrating the diachrony of ice-sheet expansions around Antarctica, and refining both the thickness and timing of previous episodes of maximum ice sheet extent (Convey *et al*. 2008, 2009; Pugh & Convey 2008; Vyverman *et al*. 2010; Fraser *et al*. 2012).

Bryophytes (mosses and liverworts) are the dominant land plant flora in Antarctica, reaching their greatest diversity and extent in the Antarctic Peninsula and Scotia Arc (Ochyra *et al*. 2008; Convey 2013). As a group, they are generally regarded as possessing strong LDD capacities, supported by both direct (Lewis *et al*. 2014; Lönnel *et al*. 2012; Sundberg 2013; van Zanten 1978, 1981) and indirect (see Szövényi *et al*. 2012 for review) evidence. These characteristics would, in principle, equip them well for recolonisation of Antarctica following any episode of regional extinction. Elsewhere, recent evidence points to the major role of oceanic islands as glacial refugia for the subsequent (re-)colonisation of continents (Laenen *et al*. 2011, Hutsemékers *et al*. 2011). However, the geographic scale of Antarctic isolation from other land-masses, along with protection from direct north-south transfer by atmospheric and ocean currents, give the continent considerable geographic isolation (Barnes *et al*. 2006). Alternatively, a feature common among most bryophytes is their ability to grow at low (sub-optimal) temperatures. More than half of the 40 temperate species investigated by Furness and Grime (1982) showed a growth reduction of less than 50% at 5°C compared to growth at their optimal temperature, and this feature has also been described in Arctic and Antarctic bryophytes (Longton 1988). Indeed, many species, including some from the tropics, seem to be physiologically pre-adapted to cold and can survive temperatures ranging from -10 to -27°C (Glime 2007). Recently, La Farge *et al*. (2013) have provided evidence for totipotent capacity (the ability of a cell to dedifferentiate into a meristematic state, and subsequently regrow) in Arctic bryophyte tissue buried by ice for 400 years, and Roads *et al.* (2014) have reported regrowth from gametophytes in moss banks preserved in permafrost for over 1.5 Ka. Furthermore, population genetic data for the temperate moss *Homalothecium sericeum* (Hedw.) Schimp. support persistence of the species in micro-refugia within the extensively glaciated northern Europe during the LGM (Désamoré et al. 2012). These examples suggest that bryophytes may be viable candidates to have survived Antarctic glacial cycles *in situ.*

Using the cosmopolitan moss *Bryum argenteum* Hedw. as a model, Hills *et al*. (2010) interpreted the lower genetic diversity observed in Antarctic *vs* non-Antarctic samples as a consequence of a lower rate of DNA substitution and isolation in refugia within Victoria Land since the Pleistocene. However, in the absence of 1) evidence for heterogeneity of DNA substitution rate among lineages, 2) an explicit time frame, 3) representative sampling across the entire range of the species, and 4) explicit analyses of population genetic structure, the hypothesis of survival in extra-Antarctic areas with subsequent (re-) colonisation cannot be excluded.

Here, we present a reconstruction of the phylogeography of *B. argenteum* at the global scale, and use molecular dating techniques to determine whether its presence in Antarctica is the result of recent (re-)colonisation from Pleistocene refugia outside Antarctica (H1), or whether it survived the Quaternary and Tertiary glaciations *in situ* (H2). If H1 holds true, we would expect Antarctic populations to be of recent, post-glacial origin, and therefore to show relatively little genetic differentiation from populations from other regions. Under that hypothesis, colonisation events might occur more frequently than under a scenario of long-term *in situ* persistence. Therefore, we would also expect under H1 a high gene flow from populations outside Antarctica, which could lead to a decrease in the signature of any founder effect. Conversely, if H2 holds true, we would predict that extant Antarctic populations derive from ancestors distributed on this continent before the LGM. We would further expect, provided that gene flow with the sub-Antarctic islands and other Southern Hemisphere areas has been limited, Antarctic populations to be genetically isolated from other regions and exhibit a clear phylogeographic signal (*sensu* Pons and Petit 1996).

Materials and Methods

Specimen sampling and molecular protocols

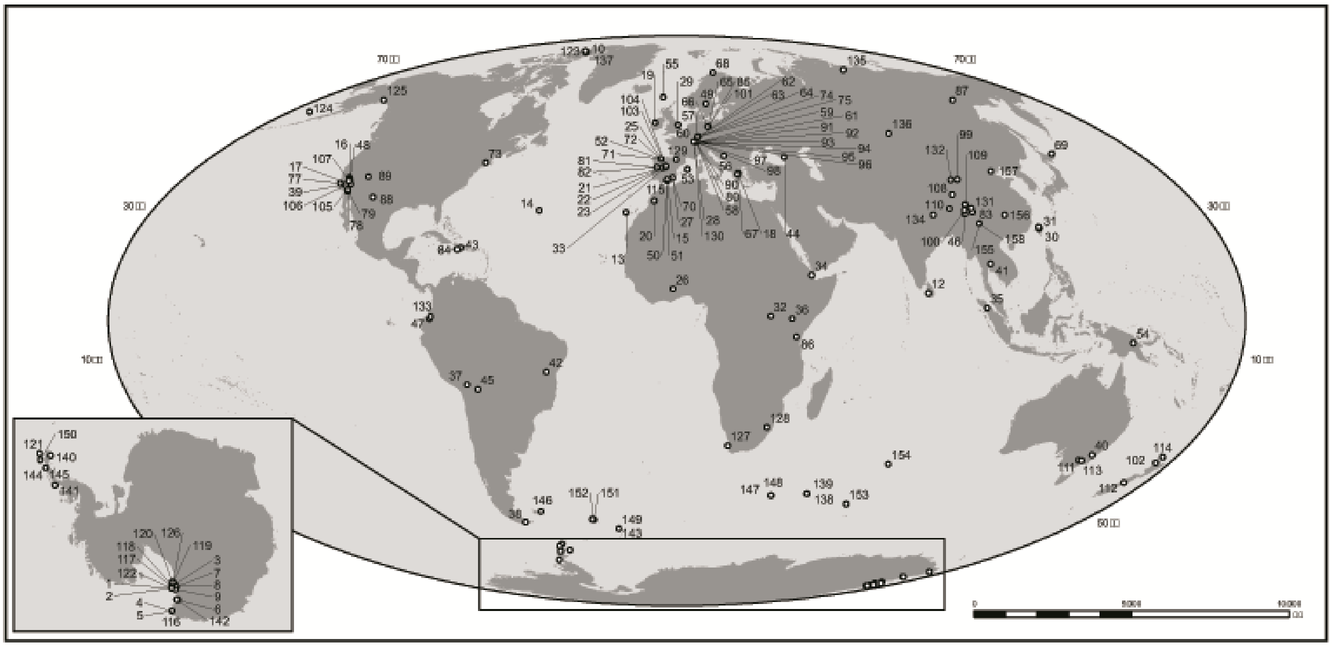
B. argenteum is a cosmopolitan, weedy moss species that thrives in disturbed environments. Its natural occurrence in Antarctica is indicated by its presence in the earliest botanical records for the continent and its widespread distribution within the regions where it occurs (Ochyra *et al*. 2008; Cannone *et al*. 2013). A total of 154 accessions of *B. argenteum* were sampled from Africa, America, Asia, Antarctica, the sub-Antarctic islands, Australasia and Europe (Appendix 1). From these accessions, 28 samples were taken from previously published papers (Hills *et al*. 2010; Pisa *et al*. 2013; Skotnicki *et al*. 2005) available in GenBank. The remaining samples were sequenced for this study, and included 47 specimens collected by the authors and colleagues, all retained at the herbarium of the Universidad de Murcia, Spain (MUB) and 70 specimens held at the institutional herbaria of the British Antarctic Survey, U.K. (AAS); California Academy of Sciences, U.S.A (CAS); Eszterházy Károly College, Hungary (EGR); Institute of Terrestrial Ecology, U.K. (ACHE); Main Botanical Garden of the Russian Academy of Sciences, Russia (MHA); Moscow State University, Russia (MW); New York Botanical Garden, U.S.A. (NY); Royal Botanic Garden Edinburgh, U.K. (E); Swedish Museum of Natural History, Sweden (S), University of Connecticut, U.S.A. (CONN), and the private herbaria of D.T. Holyoak and B.

Goffinet. Four of the closely related species to *B. argenteum* (Wang and Zhao 2009) were selected as outgroup species (Appendix 1). The geographic location of the accessions is detailed in Fig. 1.

Total genomic DNA was extracted following the protocol described in Werner *et al*. (2002), or using the DNeasy Plant Mini Kit (Qiagen GmbH, Hilden, Germany). PCR amplification and sequencing of the nuclear internal transcribed spacer (ITS) region were performed using the protocol described in Pisa *et al*. (2013). Most of the amplifications were carried out using universal primers AB101 and AB102 (Douzery *et al*. 1999), with some nucleotide modifications to adapt these primers to *B. argenteum*. In some cases, sequences were generated using universal primers ITS-A and ITS-B as described in Blattner (1999), employing a similar PCR step as in Pisa *et al*. (2013), with exceptions being the use of the Taq PCR Core Kit (Qiagen GmbH, Hilden, Germany) and an annealing temperature of 50°C. Forward and reverse sequence fragments for both ITS1 and ITS2 were edited and assembled using Bioedit 7.05 (Hall 1999) and every polymorphism was checked from the chromatograms. The sequences were aligned by eye, adding gaps where necessary to conserve homology between sequences (Appendix 2). Gaps were counted with SeqState (Müller 2005) using complex indel-coding. The number of polymorphic sites was calculated with DnaSP (Librado & Rozas 2009).

ITS remains the most widely used source of information on genetic variation at the species level in plants and fungi (reviewed by Nagy *et al*. 2012), although its use in phylogenetics has been questioned due to the potential presence of paralogs and pseudogenes (see Nieto Feliner & Rosselló 2007 for review). In mosses in particular, evidence for ITS paralogy was recently reported (Košnar *et al*. 2012). We consider that the use of ITS in *B. argenteum* was justified in the current study as no conflicting base calls during direct sequencing were observed, suggesting that the presence of intragenomic paralogous copies is unlikely.

Fig. 1. Geographic location of *Bryum argenteum* and outgroup accessions used



Population genetic analyses

The sequences were grouped into haplotypes using DnaSP. For each of the seven geographic regions defined above, we calculated haploid diversity (h), unbiased haploid diversity by population (uh) and frequency of endemic haplotypes (x) using GENALEX 6.5 (Peakall &Smouse 2006) and Tajima's D using Arlequin 3.5 (Excoffier et al. 2005). Genetic differentiation among geographic regions and presence of phylogeographic signal in the data were assessed by means of comparative analyses employing fixation index (F_{ST}) and N_{ST}. N_{ST} is a measure of genetic differentiation among populations; it is analogous to F_{ST} but takes the genetic distances among genotypes (here, the pairwise distance among them) into account (Pons & Petit 1996).

When N_{ST}>F_{ST}, it means that mutation rates are higher than dispersal rates between geographical regions, generating a phylogeographic pattern. The significance of F_{ST} and N_{ST} were tested by constructing the distribution of the null hypothesis by means of 9,999 random permutations of individuals among geographic regions, as implemented by Spagedi 1.3 (Hardy & Vekemans 2002). The existence of a phylogeographic signal was tested by assessing the significance of the observed difference between N_{ST} and F_{ST} values by means of 9,999 random permutations of the allele distance matrix. Global *F* and *N* statistics among the seven geographic regions were computed, as well as pairwise statistics among regions. The correction of Benjamini &Yekutieli (2001) for multiple tests was applied to determine the significance of the pairwise statistics.

Phylogeny, molecular dating, and ancestral area reconstructions

The phylogeny and divergence time among ITS genotypes within *B. argenteum* were investigated using BEAST v1.7.5 (Drummond *et al*. 2012). In the absence of fossil evidence, a prior on the absolute rate of molecular evolution was used, following the procedure described in Huttunen et al. (2008) and Aigoin *et al*. (2009). In the absence of a substitution rate for ITS in bryophytes, we used a normal distribution with a mean and standard deviation of $4.125e^{-3}$ and $1.807e^{-3}$ substitutions per site per million years, respectively, which corresponds to the average absolute substitution rates of ITS across a wide range of annual herbaceous species (Kay *et al*. 2006). However, we consider that this rate is likely to overestimate substitution rates in mosses, which are longer-lived and rely for a large part on asexual reproduction. This is particularly the case in *B. argenteum*, which is thought to be sterile in Antarctica (Ochyra *et al*. 2008). On average, the substitution rate of 18S rDNA, the neighbouring region of ITS, in mosses is suggested to be less than half that of vascular plants (Stenøien 2008). It is likely that the substitution rate used here may therefore be an overestimate of the true rate for this species, and therefore that divergence times derived from this substitution rate may be significantly underestimated.

The Hasegawa, Kishino and Yano (HKY) model with gamma distribution and invariant sites had the best Bayesian information criterion (BIC) score for the ITS dataset using jModeltest 2.1.4 (Darriba *et al*. 2012). A relaxed clock with lognormal distribution was employed for the analysis. Before running the final dating analysis, the performance of five tree models (i.e. coalescent with constant size population, coalescent under an extended Bayesian skyline including the two linear and stepwise models, speciation under a birth-death process and speciation under Yule process) were compared by using a model selection procedure based on Bayes factors calculated in TRACER v1.5 (Rambaut & Drummond 2009). Overall, the model using the coalescent under a stepwise extended Bayesian skyline model (Heled & Drummond 2008) performed best (data not shown). Four independent Markov chain Monte Carlo (MCMC) analyses were each run for 100,000,000 generations for every model. Parameter values were sampled every 10,000 generations and convergence and acceptable mixing of the samples were checked using the program TRACER v1.5. After discarding the burn-in steps (2,000 trees), the runs were combined to obtain an estimate of the posterior probability distributions of the dates of divergence.

To estimate ancestral areas, we used the maximum likelihood dispersal-extinction-cladogenesis (DEC) method (Ree *et al*. 2005; Ree & Smith 2008a) as implemented in the software Lagrange build 20091004 (Ree & Smith 2008b) on the BEAST chronogram. Each genotype was assigned to one or more of the seven geographic regions defined above. We conducted the analysis in Lagrange based on an unconstrained model permitting an equal probability of dispersal between all areas at any time.

Results

The complete alignment had a total length of 928 bp after the exclusion of the 5.8S rRNA gene. No further region was excluded from the alignment. There were 328 sites with gaps corresponding to106 indels and 111 polymorphic sites. The alignment excluding outgroup sequences had a total length of 844 bp. There were 173 sites with gaps corresponding to 78 indels and 81 polymorphic sites allowing for the identification of 77 haplotypes (Appendix 1). Haploid diversity unbiased by population size was lowest in Antarctica (uh=0.62) as compared to other regions (0.79 - 0.94) (Table 1). The frequency of endemic haplotypes exhibited the reverse trend, reaching its highest value (x=0.90) in Antarctica. None of Tajima's D statistics differed significantly from 0.

There was a significant difference in genotype frequency among geographic regions (Global F_{ST}=0.146, $P<0.0001$). The global N_{ST} (0.267, $P<0.0001$) was significantly higher than F_{ST} ($P<0.0001$) providing evidence that, on average, the genotypes from the same region were more closely related than the genotypes from different regions. This geographic structure was largely due to the significant genetic isolation of Antarctica. The phylogeographic signal between Antarctica and any of the other six regions was consistently significant, whereas a significant phylogeographic signal could not be detected among any other pairs of regions (Table 2).

Accessions from Antarctica belonged to three clades (Fig. 2). Clade I was mainly composed of Antarctic genotypes, with the exception of one European genotype and one common genotype shared between Antarctica, Europe, Asia and America. Clade II was composed of Antarctic, Sub-Antarctic, American and Austalasian genotypes. Clade III was composed of Antarctic and American genotypes. The Lagrange analysis indicated that the most recent common ancestor of clade I, which may have been distributed across Asia, Europe, and America, colonised Antarctica 4.36 Ma (Highest Posterior Density, HPD, 1.79-14.72). In clades II and III, the earliest colonisation of Antarctica dates back to 1.43 Ma (HPD 0.42-4.97) and 0.55 Ma (HPD 0.13-1.97), respectively, from an ancestor most closely related to American populations. Potential dispersal events from Antarctica to other regions are not excluded as genotypes in clade II located in Australasia and the sub-Antarctic islands come from ancestors distributed across Antarctica and America.

Table 1. Sample size (N), number of genotypes (Na), haploid diversity (h), haploid diversity unbiased by population (uh), frequency of endemic haplotypes (x) and Tajima's D (D) in seven geographic regions of the worldwide distributed moss *Bryum argenteum* based on the nuclear ITS *locus*

Continental area	**N**	**Na**	**h**	**uh**	**x**	**D**
Asia	20	17	0.89	0.94	0.71	-0.89 (0.19)
America	27	19	0.87	0.91	0.63	-1.27 (0.09)
Europe	56	22	0.77	0.79	0.68	-0.86 (0.21)
Australasia	7	6	0.80	0.93	0.83	0.35 (0.65)
Antarctica	25	10	0.59	0.62	0.90	-0.93 (0.28)
Africa	11	9	0.80	0.88	0.56	0.20 (0.61)
sub-Antarctic islands	8	6	0.75	0.93	0.83	0.08 (0.36)

Table 2. Pairwise F_{ST} values (below diagonal) and N_{ST} values (above diagonal) among seven geographic regions of the worldwide distributed moss *Bryum argenteum* based on the nuclear ITS *locus*. The *P*-values (in brackets) are associated to the null hypotheses that $F_{ST}=0$ and that $F_{ST}=N_{ST}$ respectively

Continental area	**Asia**	**America**	**Europe**	**Australasia**	**Antarctica**	**Africa**	**sub-Antarctic**
Asia	-	0.012 $^{n.s.}$	0.157 $^{n.s.}$	0.124 (0.0358)	0.383 (0.0003)*	0.060 $^{n.s.}$	0.100 $^{n.s.}$
America	0.033 (0.0159)*	-	0.135 $^{n.s.}$	0.049 $^{n.s.}$	0.479 (0.0001)*	0.027 $^{n.s.}$	0.062 $^{n.s.}$
Europe	0.105 (0.0004)*	0.120 (0.0001)*	-	0.180 $^{n.s.}$	0.568 (0.0001)*	0.077 $^{n.s.}$	0.181 $^{n.s.}$
Australasia	0.034 $^{n.s.}$.	0.025 $^{n.s.}$	0.122 (0.0085)*	-	0.565 (0.0002)*	0.114 $^{n.s.}$	0.016 $^{n.s.}$
Antarctica	0.186 (0.0001)*	0.210 (0.0001)*	0.272 (0.0001)*	0.251 (0.0001)*	-	0.549 (0.0002)*	0.498 (0.0047)*
Africa	0.056 (0.0082)*	0.044 (0.0334)*	0.107 (0.0031)*	0.066 (0.0466)	0.256 (0.0001)*	-	0.073 $^{n.s.}$
Sub-Antarctic	0.096 (0.0007)*	0.088(0.0046)*	0.156 (0.0015)*	0.077 (0.0468)	0.298 (0.0001)*	0.127 (0.0025)*	-

$^{n.s.}$ indicates that the test is not significant (*P*>0.05)

* indicates that the test remains significant after the correction of Benjamini and Yekutieli (2001) for multiple tests at the p<0.05 significance level

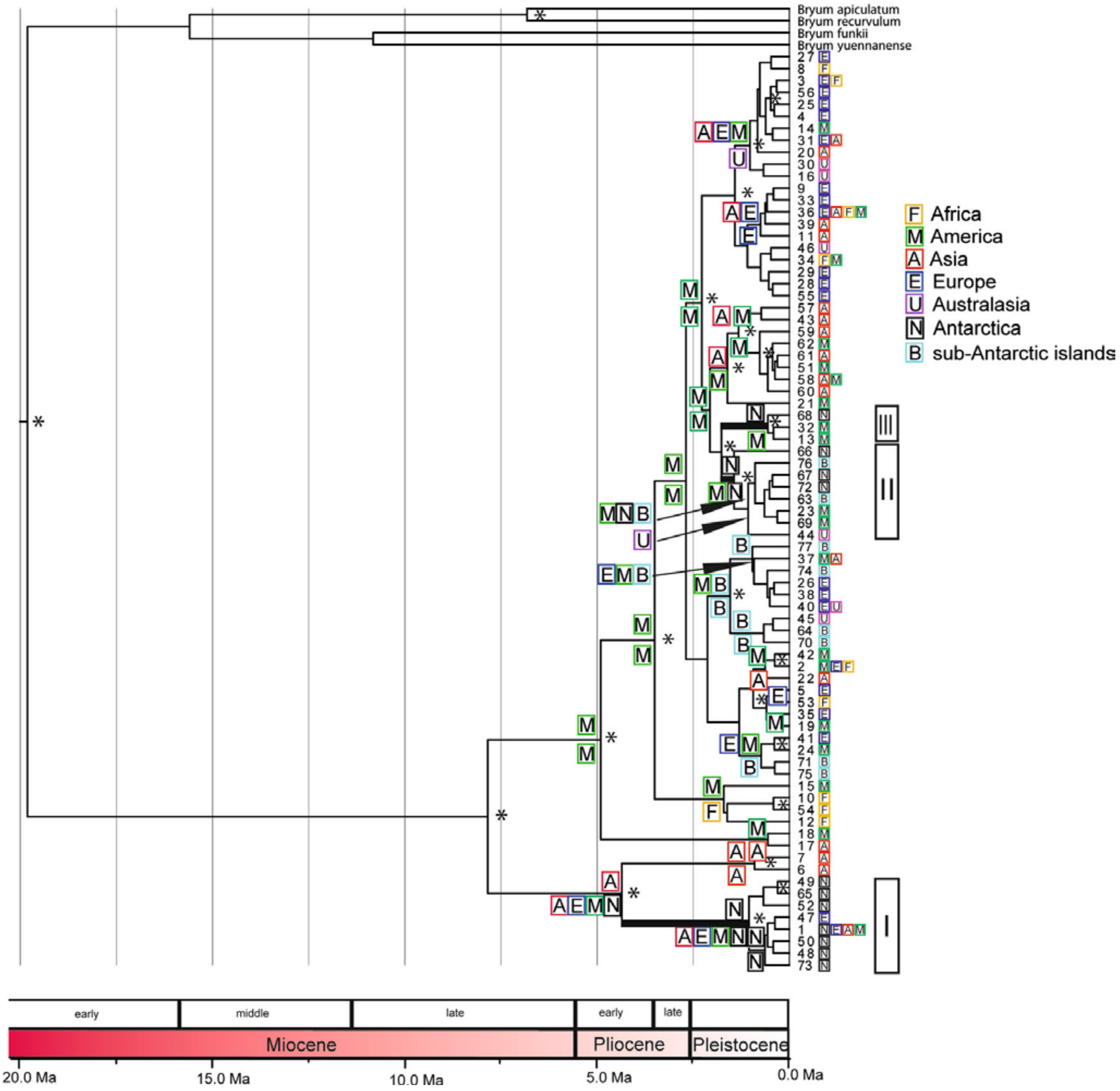
Discussion

Evidence for at least three distinct origins of *B. argenteum* in Antarctica was found, with colonisation times ranging between approximately 4.4 Ma (clade I), 1.4 Ma (clade II), and 0.6 Ma (clade III). However, as mentioned above, the substitution rate of Kay *et al*. (2006) is 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 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* in Antarctica may be considerably more ancient than estimated here. The current study therefore provides the first support for the long-term persistence *in situ* of a bryophyte species in Antarctica, with time-scales in the order of millions of years. No evidence supporting strict post-Pleistocene (i.e. recent) colonisation (H1) was found in any of the lineages. Our results, however, do not exclude potential dispersal events from Antarctica to other regions and future studies with a larger sample size and obtained from more locations may identify evidence for recent colonisation events.In agreement with our finding of long-term persistence of *B. argenteum* in Antarctica, a significant phylogeographical signal was found in all pairwise comparisons between Antarctica and each of the six other global regions, while no such signal was identified amongst the latter. This indicates that extant patterns of genetic diversity of Antarctic *B. argenteum* populations are better explained in terms of *in situ* diversification than recruitment of migrants from other areas, resulting in the highest proportion of endemic haplotypes as compared to other regions of the world. Such an interpretation is consistent with recent developments in biogeographical knowledge of much of the contemporary terrestrial biota in Antarctica.

Evidence for long-term history *in situ* has been reported in all major groups except the bryophytes, with timescales ranging from mid-Pleistocene (e.g. diatoms, rotifers, cladocerans) to Pliocene, Miocene and Gondwana-breakup (e.g. springtails, chironomid midges, mites, copepods, microorganisms) (see Convey *et al*. 2008, 2009, and references therein; Vyverman *et al*. 2010). McGaughran *et al*. (2010), in a comparative phylogeographic study of different springrail (Collembola) species in Victoria Land and along the Scotia Arc and Antarctic Peninsula, identified analogous evidence of intraregional differentiation and hence colonisation patterns on timescales dating back to at least the earliest Pleistocene.

How and where bryophytes and other terrestrial biota could have survived through glaciation events within Antarctica is not yet well understood. The lowest ITS haplotypic diversity within *B. argenteum* worldwide was observed in Antarctica, as in the cosmopolitan moss *Ceratodon purpureus* Hedw. (Brid.) (Clarke *et al*. 2008, 2009). Although the hypothesis of lower mutation rates in Antarctic *B. argenteum* populations cannot be ruled out (Hills *et al*. 2010), such a low regional genetic diversity can also be interpreted in terms of the sterile condition of *B. argenteum* in Antarctica (Ochyra *et al*. 2008), either due to the regional absence of one of the sexes in this dioicous species, and/or inhibition of sex expression due to prevailing cold and dry conditions (Longton 1988). Yet, analyses of patterns of genetic diversity in Antarctic populations of *B. argenteum* failed to evidence a significant bottleneck. This observation does not support the hypothesis of a substantial past decrease in population size and points to the persistence of sufficiently large and numerous populations of the species through time.

Fig. 2. Spatial and temporal dimensions of *Bryum argenteum* evolution. Chronogram of the fifty per cent majority-rule consensus of the trees sampled from the posterior probability distribution generated by the BEAST analysis of ITS sequences of the *B. argenteum* genotypes sampled worldwide. The maximum likelihood reconstruction of geographical range evolution under the unconstrained dispersal-extinction-cladogenesis (DEC) model (ln L=214.9) implemented in Lagrange is given in boxes at each node of interest. The geographical areas defined for this study and each haplotype are provided. The two series of reconstructions at each internal node indicate the ML ancestral range estimate for the upper and lower branch connecting that node, respectively. Boxes at terminal nodes indicate the geographic areas occupied by the accessions belonging to each haplotype. The vertical bars (I, II, and III) indicate the clades with Antarctic genotypes. Thicker branches indicate the support for the Antarctic clades (PP > 0.9). * indicates the support for clades with PP > 0.9).



In Antarctica, areas of heated ground associated with geothermal activity, where *B. argenteum* is known to occur (Convey *et al*. 2000), may be particularly relevant in considering the locations of some potential refuge sites (Convey & Lewis Smith 2006, Fraser *et al*. 2014). Although individually ephemeral, the presence of geothermal habitats may have extended over considerable time periods, as volcanism has been widespread throughout the Tertiary in parts of the northern Antarctic Peninsula and elsewhere in Antarctica (Baker *et al*. 1975, Convey *et al*. 2000; Convey & Lewis Smith 2006; Fraser *et al*. 2014). Therefore, geothermal habitats might have played a key role in the longer-term regional persistence of species with rapid colonisation capacities such as bryophytes, allowing survival through periods of apparently greater environmental extremes than are currently experienced (Convey & Lewis Smith 2006).

Conclusion

This is study demonstrates for the first time *in situ* persistence of bryophytes in Antarctica throughout previous glacial cycles and contradicts the hypothesis of post-glacial recruitment from extraregional locations. .Although *B. argenteum,* like other moss species, exhibits the lowest levels of genetic diversity worldwide in Antarctica, ITS variation is substantial within and among Antarctic populations, making it a highly suitable model for investigating fine-scale patterns of genetic structure and diversity at continental scale in order to reconstruct its biogeographic history. In particular, detailed phylogeographic information at the Antarctic scale would allow for testing Fraser's *et al*. (2014) hypothesis that refugia indeed correspond to areas of high geothermal activity and contribute, together with information from other terrestrial organisms

(Terauds *et al*. 2012), to enhancing the identification of bioregions that are not fully represented in the current Antarctic Specially Protected Area network.

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Appendix 1 Accessions used in this study. For each sample the following information is given: Identification number, *Bryum argenteum* haplotype based on nrITS sequences, or species used as outgroup; geographic origin; herbarium where it is retained or publication source if published previously; collection data for the samples sequenced for this study; geographic coordinates for the ingroup samples; GenBank accession numbers for ITS1 and ITS2 separately, or for the whole ITS region in the case the number is given in the middle of ITS1 and ITS2 columns; and the geographic region from the seven regions considered.

Id no.	Haplotype id or outgroup	Geographic origin	Herbarium or literature reference if	Collection	Coordinates in decimal degrees	GenBank Accession N. ITS		Geographic
						ITS1	ITS2	
1	1	Antarctica, Beaufort Island	Hills *et al.* (2010)	-	S 76.91667 E 166.90000	GU907063		Antarctica
2	1	Antarctica, Beaufort Island	Hills *et al.* (2010)	-	S 76.91667 E 166.90000	GU907064		Antarctica
3	1	Antarctica, Canada Glacier, Taylor Valley, S. Victoria Land	Skotnicki *et al.* (2005)	-	S 77.58333 E 163.25000	AY611432		Antarctica
4	1	Antarctica, Cape Hallet	Hills *et al.* (2010)	-	S 72.30000 E 170.30000	GU907066		Antarctica
5	1	Antarctica, Cape Hallet	Hills *et al.* (2010)	-	S 72.30000 E 170.30000	GU907067		Antarctica
6	1	Antarctica, Edmonson Point, N. Victoria Land	Skotnicki *et al.* (2005)	-	S 74.33333 E 164.50000	AY611430		Antarctica
7	1	Antarctica, Granite Harbour	Hills *et al.* (2010)	-	S 77.00000 E 162.50000	GU907065		Antarctica
8	1	Antarctica, Granite Harbour, S. Victoria Land	Skotnicki *et al.* (2005)	-	S 77.00000 E 162.50000	AY611434		Antarctica
9	1	Antarctica, Marble Point	Hills *et al.* (2010)	-	S 76.43333 E 163.83333	GU907069		Antarctica
10	1	Canada, Northwest Territories, District of Fanklin, Ellesmere	NY 69323	*G.W. Scotter 45657*	N 79.71667 W 85.83333	KF952783	KF952892	America
11	1	Norway, Svalbard archipelago, Spitsbergen	MUB 44625	*M. Stech & J.D. Kruijer 08-088*	N 78,91667 E 11,93333	KF952782	KF952891	Europe
12	1	Sri Lanka (Ceylon), Central province, Nuwara Eliya District	E 00416780	*C.C. Townsend 73/1779*	N 6.95694 E 80.10889	KF952781	KF952890	Asia
13	2	Spain, Canary Islands, La Gomera	MUB 44654	*J.M. González-Mancebo et al. s.n.*	N 28.10737 W 17.23866	KF952785	KF952894	Africa
14	2	Spain, Canary Islands, La Palma	MUB 44653	*J.M. González-Mancebo & J. Leal*	N 28.67562 W 47.11100	KF952784	KF952893	Africa
15	2	Spain, Granada province, Albuñol	Pisa *et al.* (2013)	-	N 36.80025 W 3.21738	KC493867	KC493893	Europe
16	2	USA, California, Mariposa County, Yosemite National Park	CAS 1083159	*J.R. Shevock 29944*	N 37.51889 W 119.60000	KF952787	KF952896	America
17	2	USA, California, Tulare County, Sierra Nevada	CAS 1039823	*J.R. Shevock 20946*	N 36.06111 W 118.59306	KF952786	KF952895	America
18	3	Greece, Prefecture of Ilia, Ráhes	MUB 12158	*M.J. Cano, J. Muñoz, R.M. Ros &*	N 38.90861 E 22.83500	KF952794	KF952903	Europe
19	3	Ireland, E. County Mayo	Herb. D.T. Holyoak	*D.T. Holyoak 03-186*	N 53.61889 W 9.30639	KF952788	KF952897	Europe
20	3	Morocco, High Atlas, Oukaimeden	MUB 13090	*R.M. Ros s.n.*	N 31.21667 W 7.86667	KF952793	KF952902	Africa
21	3	Spain, Ávila province, Sierra de Gredos	MUB 44652	*R.M. Ros & O. Werner s.n.*	N 40.42231 W 5.29667	KF952796	KF952905	Europe
22	3	Spain, Ávila province, Sierra de Gredos	MUB 44653	*R.M. Ros & O. Werner s.n.*	N 40.42231 W 5.29667	KF952789	KF952898	Europe
23	3	Spain, Ávila province, Sierra de Gredos	MUB 44652	*R.M. Ros & O. Werner s.n.*	N 40.42231 W 5.29667	KF952795	KF952904	Europe
24	3	Spain, Canary Islands, Tenerife	MUB 44656	*J. Patiño s.n.*	N 28,35770 W 0,72512	KF952797	KF952906	Africa
25	3	Spain, León province, Abelgas	MUB 44660	*S. Pisa s.n.*	N 42.89139 W 5.97389	KF952790	KF952899	Europe
26	3	Spain, Murcia province, Campus de Espinardo	MUB 44663	*R. M. Ros s.n.*	N 8.02056 W 1.16944	KF952791	KF952900	Europe
27	3	Spain, Murcia province, Sierra de las Herrerías	MUB 44664	*R.M. Ros & O. Werner s.n.*	N 37.58278 W 1.42667	KF952792	KF952901	Europe
28	4	France, Vosges Department, Vosges Mountains	MUB 44641	*R.M. Ros & O. Werner s.n.*	N 47.90478 E 7.10286	KF952798	KF952907	Europe
29	5	UK, Great Britain, N. Northumberland	Herb. D. T. Holyoak	*D.T. Holyoak 08-670*	N 52.96667 E 0.55000	KF952799	KF952908	Europe
30	6	Taiwan, Hwalien County, Ta-yu-ling	E 00416777	*C.-C. Chuang 5781*	N 23.81667 E 121.23333	KF952800	KF952909	Asia
31	7	China, Taiwan, Nantou County, Central Mountain Range	CAS 995161	*J.R. Shevock 17888*	N 24.12500 E 121.21667	KF952801	KF952910	Asia
32	8	Uganda, Western Region,	Herb. D. T.	*M.J. Wigginton*	N 1.08333	KF952802	KF952911	Africa

		Kabale	Holyoak	*U5011a*	E 29.80000			
33	9	Spain, Madrid province, Sierra de Guadarrama	MUB 44668	*S. Pisa s.n.*	N 40.59030 W 3.98505	KF952803	KF952912	Europe
34	10	Union of the Comoros, Grande Comore (Ngazidja)	EGR	*R.E. Magill & T. Pócs 10946*	N 11.61222 E 43.33222	KF952804	KF952913	Africa
35	11	Indonesia, Sumatra, Gunung Sinabung	NY 1229461	*L. Hoffmann 89-196*	N 3.18139, E 98.44111	KF952805	KF952914	Asia
36	12	Kenya, Nyandarua County, Aberdare Mts.	EGR	*J. Spence s.n.*	N 0.41083 E 36.61667	KF952806	KF952915	Africa
37	13	Bolivia, La Paz, Murillo	NY 1229477	*S. Churchill 22828*	S 16.61667 W 68.07528	KF952807	KF952916	America
38	14	Chile, Antarctica Chilena province, Isla Navarino,	Herb. B. Goffinet	*B. Goffinet 6765*	S 54.95000 W 67.63333	KF952808	KF952917	America
39	15	USA, California, Tulare County, Sierra Nevada	CAS 989901	*J.R. Shevock 17562*	N 35.88889 W 118.34583	KF952809	KF952918	America
40	16	Australia, Canberra	Hills *et al.* (2010)	-	S 35.30000 E 149.13333	GU907059		Australasia
41	17	Thailand, Khao Yai National Park	NY 1229462	*C. Charoenphol 4395 with Larsen &*	N 14.53333 E 101.36667	KF952810	KF952919	Asia
42	18	Brazil, Bahia state, Municipality of Abaíra, Campo de Ouro Fino	NY 1229480	*D.J.N. Hind H50910 with R.F. Queiroz*	S 13.25000 W 41.90000	KF952811	KF952920	America
43	19	Dominican Republic, La Vega province, Alto de la Bandera	NY 635898	*W.C. Steere 23098*	N 18.81667 W 70.61667	KF952812	KF952921	America
44	20	Russia, NW Caucasus	MW	*V. Onipchenko 99/95*	N 43.45000 E 41.68333	KF952813	KF952922	Asia
45	21	Boliva, Cochabamba, Carrasco	NY 1229478	*S. Churchill 22558*	S 17.75000 W 64.80000	KF952814	KF952923	America
46	22	China, Yunnan province, Gonshan County	E 00477214	*D. G. Long 36064*	N 27.68539 E 98.30422	KF952815	KF952924	Asia
47	23	Ecuador, Pichinga province	NY 1229481	*S.P. Churchill 13518 with I.*	N 0.38333 W 78.21667	KF952816	KF952925	America
48	24	USA, California, Fresno County, Sierra Nevada	CAS 989203	*J.R. Shevock 13927*	N37.16250 W 119.09583	KF952817	KF952926	America
49	25	Sweden, Värmland	S B178200	*L. Hedenäs & G. Odelvik s.n.*	N 59.78333 E 14.36667	KF952818	KF952927	Europe
50	26	Spain, Granada province, Sierra Nevada	Pisa *et al.* (2013)	-	N 37.09725 W 3.39753	KC493863	KC493889	Europe
51	27	Spain, Granada province, Vélez de Benaudalla	Pisa *et al.* (2013)	-	N 36.84465 W 3.50905	KC493873	KC493899	Europe
52	28	Spain, León province, Abelgas	MUB 44661	*S. Pisa s.n.*	N 42.89139 W 5.97389	KF952819	KF952928	Europe
53	29	Spain, Balearic Islands, Menorca	MUB 44665	*R.M. Ros & O. Werner s.n.*	N 39.89944 E 4.10389	KF952820	KF952929	Europe
54	30	Papua New Guinea, Chimbu province, Wilhelm Mountain	NY 1229460	*J.L. De Sloover 42892*	S 5.75667 E 145.03556	KF952821	KF952930	Australasia
55	31	Denmark, Faroe Islands, Streymoy, Thorshavn	S B185204	*R. Fargerstén & M. Haapasaari s.n.*	N 62.01139 W 6.75361	KF952834	KF952943	Europe
56	31	France, Vaucluse Department, commune de Bonnieux,	MUB 1097	*R.M. Ros s.n.*	N 43.81389 E 18.43333	KF952835	KF952944	Europe
57	31	Germany, Baden-Württemberg, Black Forest	MUB 44630	*S. Pisa s.n.*	N 47.84417 E 8.01917	KF952832	KF952941	Europe
58	31	Germany, Baden-Württemberg, Black Forest	MUB 44637	*S. Pisa s.n.*	N 47.86444 E 8.02194	KF952824	KF952933	Europe
59	31	Germany, Baden-Württemberg, Black Forest	MUB 44638	*S. Pisa s.n.*	N 47.85972 E 8.03639	KF952825	KF952934	Europe
60	31	Germany, Baden-Württemberg, Black Forest	MUB 44639	*S. Pisa s.n.*	N 47.85972 E 8.03639	KF952826	KF952935	Europe
61	31	Germany, Baden-Württemberg, Black Forest	MUB 44640	*S. Pisa s.n.*	N 47.85972 E 8.03639	KF952827	KF952936	Europe
62	31	Germany, Baden-Württemberg, Eberbach	MUB 44649	*R.M. Ros & O. Werner s.n.*	N 49.46111 E 8.98778	KF952830	KF952939	Europe
63	31	Germany, Baden-Württemberg, Eberbach	MUB 44650	*R.M. Ros & O. Werner s.n.*	N 49.46111 E 8.98778	KF952837	KF952946	Europe
64	31	Germany, Baden-Württemberg, Sankt Leon-Rot	MUB 44651	*R.M. Ros & O. Werner s.n.*	N 49.24333 E 8.65222	KF952831	KF952940	Europe
65	31	Germany, Berlin	MUB 44645	*R.M. Ros & O. Werner s.n.*	N 52.44028 E 13.58250	KF952828	KF952937	Europe
66	31	Germany, Berlin	MUB 44647	*R.M. Ros & O. Werner s.n.*	N 52.51861 E 13.39694	KF952829	KF952938	Europe
67	31	Greece, Prefecture of Fokida, Delphi	MUB 12010	*M.J. Cano, J. Muñoz, R.M. Ros &*	N 38.48278 E 22.50444	KF952836	KF952945	Europe
68	31	Norway, Finnmark	S B176550	*L. Hedenäs s.n.*	N 70.78333 E 23.33333	KF952822	KF952931	Europe
69	31	Russia, Kuril Islands, Kunashir Island	MHA	*M.S. Ignatov 06-1810*	N 44.35000 E 146.26667	KF952833	KF952942	Asia
70	31	Spain, Granada province, Sierra Nevada	Pisa *et al.* (2013)	-	N 37.09725 W 3.39753	KC493862	KC493888	Europe
71	31	Spain, León province, Abelgas	MUB 44659	*S. Pisa s.n.*	N 42.89139 W 5.97389	KF952823	KF952932	Europe
72	32	Spain, León province, Abelgas	MUB 44662	*S. Pisa s.n.*	N 42.89139	KF952838	KF952947	Europe

					W 5.97389			
73	32	USA, Connecticut, Hartford County	CONN	*D. Les s.n.*	N 41.85278 W 72.51611	KF952839	KF952948	America
74	33	Germany, Baden-Württemberg, Heidelberg	MUB 44658	*R.M. Ros & O. Werner s.n.*	N 49.41111 E 8.70639	KF952841	KF952950	Europe
75	33	Germany, Baden-Württemberg, Heidelberg	MUB 44648	*R.M. Ros & O. Werner s.n.*	N 49.41111 E 8.70639	KF952840	KF952949	Europe
76	34	Spain, Canary Islands, Tenerife	MUB 44655	*J.M. González-Mancebo s.n.*	N 28,14167 W 16,65361	KF952844	KF952953	Africa
77	34	USA, California, Monterey County, Los Padres National	CAS 1083051	*J.R. Shevock 29347*	N 36.09639 W 121.44167	KF952845	KF952954	America
78	34	USA, California, Riverside Co, San Bernardino National Forest	CAS 1047424	*J.R. Shevock 24084*	N 33.77672 W 116.67814	KF952843	KF952952	America
79	34	USA, California, San Bernardino Co, San Bernardino National	CAS 1047515	*J.R. Shevock 24099*	N 34.22497 W 117.06186	KF952842	KF952951	America
80	35	Germany, Baden-Württemberg, Black Forest	MUB 44628	*S. Pisa s.n.*	N 47.84056 E 8.01917	KF952847	KF952956	Europe
81	35	Portugal, Beira Interior, Guarda	MUB 44643	*R.M. Ros s.n.*	N 40.53250 W 7.26667	KF952848	KF952957	Europe
82	35	Portugal, Beira Interior, Guarda	MUB 44657	*R.M. Ros s.n.*	N 40.53250 W 7.26667	KF952846	KF952955	Europe
83	36	China, Schuan Province, Muli Co	CAS 1140938	*J.R. Shevock 36226*	N 28.16667 E 101.22233	KF952849	KF952958	Asia
84	36	Dominican Republic, Independencia province, Sierra	NY 635904	*S.A. Thompson 9237*	N 18.30000 W 71.70000	KF952851	KF952960	America
85	36	Germany, Berlin	MUB 44646	*R.M. Ros & O. Werner s.n.*	N 52.51861 E 13.39694	KF952852	KF952961	Europe
86	36	Tanzania, Kilimanjaro	EGR	*J. Elia 144*	S 4.25722 E 37.99028	KF952850	KF952959	Africa
87	37	Russia, Yakutia	MHA	*M.S. Ignatov 00-29*	N 61.13333 E 138.05000	KF952855	KF952964	Asia
88	37	USA, New Mexico, Doña Ana County, Las Cruces	NY 710573	*R.M. King & R.G. Garvey B315*	N 32.31667 W 106.75000	KF952853	KF952962	America
89	37	USA, Utah, Dixie National Forest	NY 53227	*J.L. Pawek s.n.*	N 37.79667 W 112.80722	KF952854	KF952963	America
90	38	Germany, Baden-Württemberg, Black Forest	MUB 44626	*S. Pisa s.n.*	N 47.83333 E 8.01667	KF952856	KF952965	Europe
91	38	Germany, Baden-Württemberg, Black Forest	MUB 44627	*S. Pisa s.n.*	N 47.83333 E 8.01667	KF952857	KF952966	Europe
92	38	Germany, Baden-Württemberg, Black Forest	MUB 44629	*S. Pisa s.n.*	N 47.84417 E 8.01917	KF952864	KF952973	Europe
93	38	Germany, Baden-Württemberg, Black Forest	MUB 44631	*S. Pisa s.n.*	N 47.86444 E 8.02194	KF952862	KF952971	Europe
94	38	Germany, Baden-Württemberg, Black Forest	MUB 44632	*S. Pisa s.n.*	N 47.86444 E 8.02194	KF952863	KF952972	Europe
95	38	Germany, Baden-Württemberg, Black Forest	MUB 44633	*S. Pisa s.n.*	N 47.86444 E 8.02194	KF952858	KF952967	Europe
96	38	Germany, Baden-Württemberg, Black Forest	MUB 44634	*S. Pisa s.n.*	N 47.86444 E 8.02194	KF952859	KF952968	Europe
97	38	Germany, Baden-Württemberg, Black Forest	MUB 44635	*S. Pisa s.n.*	N 47.86444 E 8.02194	KF952860	KF952969	Europe
98	38	Germany, Baden-Württemberg, Black Forest	MUB 44636	*S. Pisa s.n.*	N 47.86444 E 8.02194	KF952861	KF952970	Europe
99	39	China, Qinghai province, Menyuan County	E 00477222	*D.G. Long 27295*	N 37.11889 E 102.31639	KF952865	KF952974	Asia
100	39	China, Yunnan province, Degin County	E 00477223	*D.G. Long 23999*	N 28.63333 E 98.73278	KF952866	KF952975	Asia
101	40	Germany, Berlin	MUB 44644	*R.M. Ros & O. Werner s.n.*	N 52.50833 E 13.33444	KF952867	KF952976	Europe
102	40	New Zealand, Huntley	Hills *et al.* (2010)	-	S 37.56667 E 175.15000	GU907062		Australasia
103	41	Spain, Madrid province, Sierra de Guadarrama	MUB 44666	*S. Pisa s.n.*	N 40.83139 W 3.95278	KF952868	KF952977	Europe
104	41	Spain, Madrid province, Sierra de Guadarrama	MUB 44667	*S. Pisa s.n.*	N 40.83139 W 3.95278	KF952869	KF952978	Europe
105	42	USA, California, Inyo County, Mojave Desert	CAS 1113251	*J.R. Shevock 24458*	N 35.85364 W 117.38125	KF952870	KF952979	America
106	42	USA, California, Kern County, Sierra Nevada	CAS 957141	*J.R. Shevock 14859*	N 35.56250 W 118.95417	KF952872	KF952981	America
107	42	USA, California, Tulare County, Sierra Nevada	CAS 993294	*J.R. Shevock 13658*	N 36.73750 W 118.85833	KF952871	KF952980	America
108	43	China, Quinghai Province, Yushu Tibetan Autonomous	NY 1229472	*B.C. Tan 95-1733*	N 33.00250 E 97.27611	KF952873	KF952982	Asia
109	43	China, Sichuan Province, Litang County, Hengduan Mountains	CAS 1140922	*J.R. Shevock 35853*	N 30.17792 E 100.00311	KF952875	KF952984	Asia
110	43	China, Xizang province, SE Tibet	Herb. D.T. Holyoak	*G. Miehe & U. Wündisch 94-164-*	N 29.05000 E 93.98333	KF952874	KF952983	Asia
111	44	Australia, Mount Buffalo	Hills *et al.* (2010)	-	S 36.78333 E 146.05000	GU907057		Australasia
112	44	New Zealand, Christchurch	Hills *et al.* (2010)	-	S 43.08333	GU907056		Australasi

					E 172.11667			a
113	45	Australia, Mount McKay	Hills *et al.* (2010)	-	S 36.86667 E 147.25000	GU907061		Australasia
114	46	New Zealand, Hamilton	Hills *et al.* (2010)	-	S 35.88333 E 175.46667	GU907060		Australasia
115	47	Spain, Granada province, Sierra Nevada	Pisa *et al.* (2013)	-	N 37.06943 W 3.38662	KC493852		Europe
116	48	Antarctica, Cape Hallet	Hills *et al.* (2010)	-	S 72.30000 E 170.30000	GU907068		Antarctica
117	49	Antarctica, Cape Bird	Hills *et al.* (2010)	-	S 77.21667 E 166.43333	GU907070		Antarctica
118	49	Antarctica, Cape Royds, Ross Island	Skotnicki *et al.* (2005)	-	S 77.58333 E 166.16667	AY611433		Antarctica
119	49	Antarctica, Garwood Valley	Hills *et al.* (2010)	-	S 78.05000 E 164.16667	GU907072		Antarctica
120	49	Antarctica, Miers Valley	Hills *et al.* (2010)	-	S 78.08333 E 164.75000	GU907071		Antarctica
121	49	South Shetland Islands, King George Island	AAS 1750	*R. Ochyra s.n.*	S 62.18000 W 58.58000	KJ409559	KJ409572	Antarctica
122	50	Beaufort Island, Ross Sea	Skotnicki *et al.* (2005)	-	S 76.91667 E 166.90000	AY611431		Antarctica
123	51	Canada, Northwest Territories, District of Fanklin, Ellesmere	NY 69322	*G.W. Scotter 45680*	N 79.48333 W 85.26667	KF952878	KF952987	America
124	51	USA, Alaska, Pribilof Islands, St. Paul Island	NY 321062	*W.B. Schofield 108239*	N 57.15000 W 170.25000	KF952876	KF952985	America
125	51	USA, Alaska, Valdez-Cordova Census Area	MUB 44624	*F. Lara, R. Garilleti & B. Albertos s.n.*	N 61.12583 W 146.35111	KF952877	KF952986	America
126	52	Antarctica, Cape Chocolate, S. Victoria Land	Skotnicki *et al.* (2005)	-	S 77.95000 E 164.50000	AY611429		Antarctica
127	53	South Africa, Cape province, Vredenburg	MUB 5343	*J.M. Egea s.n.*	S 32.76667 E 18.00000	KF952879	KF952988	Africa
128	54	South Africa, KwaZulu-Natal province, Vryheid	EGR	*J. van Rooy 55*	S 27.76667 E 30.78333	KF952880	KF952989	Africa
129	55	France, Department Hautes Pyrénées	Herb. D. T. Holyoak	*D.T. Holyoak 01-553*	N 42.73333 W 0.05000	KF952881	KF952990	Europe
130	56	France, Vosges Department, Vosges Mountains	MUB 44642	*R.M. Ros & O. Werner s.n.*	N 47.90478 E 7.10286	KF952882	KF952991	Europe
131	57	China, Sichuan Province, Jiulong County, Hengduan Mountains	CAS 1141190	*J.R. Shevock 36078*	N 29.22078 E 101.45050	KF952883	KF952992	Asia
132	58	China, Qinghai province, Gonghe County	E 00477221	*D.G. Long 26787*	N 36.97722 E 99.90056	KF952885	KF952994	Asia
133	58	Colombia, Nariño Department , Municipality of Cumbal	NY 1229483	*B.R. Ramirez P. 6489*	N 0.95250 W 77.81944	KF952884	KF952993	America
134	59	Nepal, Taplejung District	E 00477231	*D.G. Long 21500*	N 27.43333 E 87.46667	KF952886	KF952995	Asia
135	60	Russia, Siberia, Taimyr	MHA	*V.E. Fedosov 08-162*	N 71.88083 E 110.78806	KF952887	KF952996	Asia
136	61	Altai Republic	MHA	*M.S. Ignatov 0/111*	N 50.50000 E 89.16667	KF952888	KF952997	Asia
137	62	Canada, Northwest Territories, District of Fanklin, Axel Heiberg	NY 69321	*G.W. Scotter 45482*	N 80.03333 W 88.75000	KF952889	KF952998	America
138	63	French Southern and Antarctic Lands, Crozet Islands	Hills *et al.* (2010)	-	S 46.45000 E 52.000	GU907058		sub-Antarctic
139	64	French Southern and Antarctic Lands, Crozet Islands,	AAS	*B.G. Bell* 1687	S 46.42000 E 51.83000	KJ409558	KJ409571	sub-Antarctic
140	65	Antarctica, Antarctic Peninsula, Cockburn Island	AAS	*R.I. Lewis Smith 7922*	S 64.20000 W 56.85000	KJ409560	KJ409573	Antarctica
141	65	Antarctica, Antarctic Peninsula, Jenny Island	AAS	*R.I. Lewis Smith 4713*	S 67.73000 W 68.38000	KJ409561	KJ409574	Antarctica
142	66	Antarctica, Ross Sector, Victoria Land	AAS	*R.I. Lewis Smith 11794*	S 74.33000 E 165.13000	KJ409562	KJ409575	Antarctica
143	67	British Overseas Territories, South Sandwich Islands,	AAS	*P. Convey* 202B	S 57.07000 W 26.70000	KJ409563	KJ409576	Antarctica
144	68	Antarctica, Antarctic Peninsula, Danco Coast	AAS	*R.I. Lewis Smith 4176*	S 64.68000 W 62.63000	KJ409564	KJ409577	Antarctica
145	68	Antarctica, Antarctic Peninsula, Danco Coast, Cuverville Island	AAS	*R. Weinstein* 8812	S 64.68000 W 62.63000	KJ409567	KJ409580	Antarctica
146	69	British Overseas Territories, Falkland Islands	AAS	*R.I. Lewis Smith 5437*	S 51.70000 W 57.85000	KJ409565	KJ409578	America
147	70	South Africa, Prince Edward Islands, Marion Island	AAS 235	*B.J. Huntley s.n.*	S 46.87000 E 37.85000	KJ409566	KJ409579	sub-Antarctic
148	71	South Africa, Prince Edward Islands	ACHE 296	*N.J.M. Gremmen s.n.*	S 46.92000 E 37.75000	KJ409569	KJ409582	sub-Antarctic
149	72	British Overseas Territories, South Sandwich Islands,	AAS	*P. Convey* 207A	S 57.07000 W 26.70000	KJ409568	KJ409581	Antarctica
150	73	South Shetland Islands, Deception Island	AAS	*D. Mason* 40	S 62.9500 W 60.55000	KJ409570	-	Antarctica
151	74	British Overseas Territories, South Georgia Island	AAS	*R.I. Lewis Smith 8397*	S 54.10000 W 36.72000	-	KJ409583	sub-Antarctic
152	75	British Overseas Territories,	AAS	*R.I. Lewis Smith*	S 54.00000	-	KJ409584	sub-

		South Georgia Island		3102	W 37.13000			Antarctic
153	76	French Southern and Antarctic Lands, Kerguelen island	AAS	*B.G. Bell* 3192B	S 49.35000 E 70.20000	-	KJ409585	sub-Antarctic
154	77	French Southern and Antarctic Lands, Amsterdam Island	AAS	*B.G. Bell* 3217	S 37.92000 E 77.67000	-	KJ409586	sub-Antarctic
155	*Bryum apiculatum*	China, Yunnan province	Wang and Zhao (2009)	-	-	EU878213		-
156	*Bryum funkii* Mitt.	China, Hunan province	Wang and Zhao (2009)	-	-	EU878209		-
157	*Bryum recurvulum*	China, Hebei province	Wang and Zhao (2009)	-	-	EU878217		-
158	*Bryum yuennanense* Broth.	China, Yunnan province	Wang and Zhao (2009)	-	-	EU878211		-

CHAPTER III

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, typical 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 colonization events, erasing any signature of a bottleneck that would be expected in the case of a recent colonization event. The molecular dating analyses indicated that the first colonization of the island took place more than one hundred thousand 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. Altogether, 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 distinguishing native from alien populations.

Keywords: Bryophytes; Dispersal; Oceanic islands; Island colonization; Phylogeography.

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 biotas 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 geographical 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 of their local abundance and distribution range due to anthropogenic activities (Schuster 1983). This is reminiscent of invasive species' behavior (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 geographical 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 & Söderström 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 south-west 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 characterized by a natural disturbance regime. All British populations are male, which suggests a single or very low number of successful introductions of this dioicous 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 characterized by a fast recolonization 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 for 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 characterizing 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 ones

(H2); and (iii) the species is of recent, alien origin and colonized natural habitats secondarily (H3). If H1 applies, we expect the timing of the colonization of the island by *B. argenteum* to predate human colonization 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 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 colonization, 500 years BP. Given this recent time-frame, 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 colonization events, we expect that any signal of isolation-by-distance would be erased by recurrent long-distance dispersal events.

Materials 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 2,151 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). 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 been also 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 AIC and BIC criteria, as implemented by 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 5,000 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 standard deviation 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 by 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 by 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 the 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 by BEAST v1.8.0 (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 standard deviation 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 stepwise 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 v1.5 (Rambaut & Drummond 2009). Overall, the model using the coalescent under a stepwise 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 v1.5. 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 by 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 geographical regions defined above and splitting Tenerife into anthropogenic and natural habitats. Genetic divergence between Tenerife and each of the geographical regions was assessed through F_{ST} and N_{ST} as implemented by 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 pairwise distance among genotypes obtained with Mega 5. A significantly higher value of N_{ST} compared to F_{ST} provides evidences for a phylogeographical 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 9,999 random permutations of individuals among regions. Significance of the phylogeographical signal ($N_{ST} > F_{ST}$) was tested by 9,999 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 habitats 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, geographical distance, and altitudinal difference (used as a measure of local environmental variation) to seek for 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 9,999 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 geographical 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 9,999 randomizations.

Results

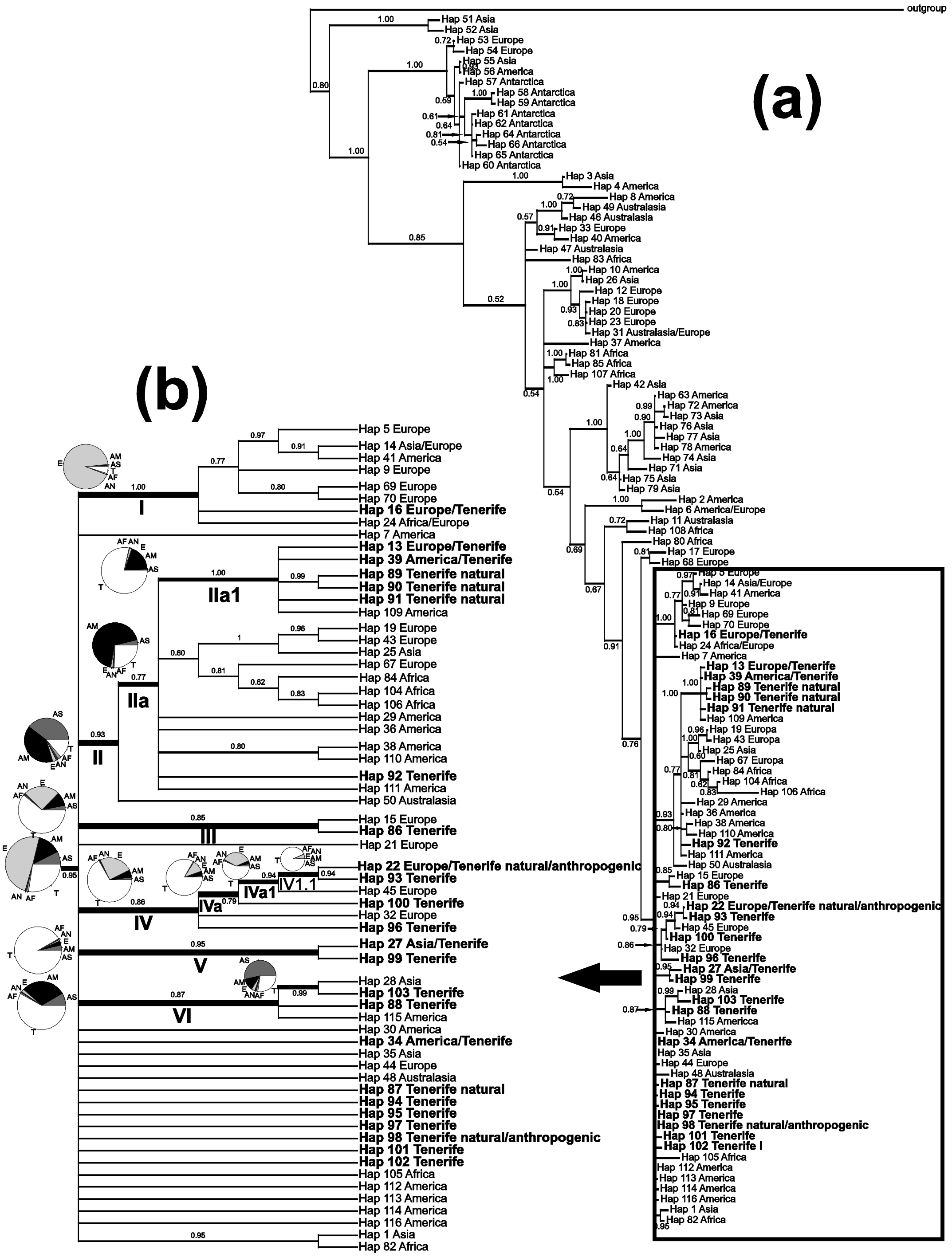
There were 131 polymorphic positions in the matrix resolving a total of 24 genotypes sampled in Tenerife, 18 of which were endemic to it (Table 1). Sample size, number of genotypes, genetic diversity and frequency of endemic genotypes for each geographical region are shown in Table 1. The Tenerife accessions 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.

Table 1. Sample size, number of genotypes, haploid diversity, haploid diversity unbiased by population and frequency of endemic genotypes for each defined geographical 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 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 1,641 Ka (Fig. 2; 942 - 2,227 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 lnL = -5673.14 for the backbone constrained topology (informed prior) and lnL = -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.

Fig. 1. Phylogenetic relationships among *Bryum argenteum* ITS genotypes estimated by Bayesian inference: (a) Fifty per cent 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) which is a cladogram view of the subclade of Fig. 1 (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).



The molecular dating analyses indicated that multiple colonization of Tenerife took place during a period of time ranging between the recent past for the six genotypes shared with continental areas to more than 100 Ka BP for the others (Table 2). Pairwise F_{ST} values among Tenerife and each continental region of the world were consistently significant ($F_{ST} > 0$) (Table 3). Pairwise 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.

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^{b}	0.92	500 (216 - 901)
VI/ VI-bis^{b}	0.58	830 (595 - 1,095)

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

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 geographical component of the matrix of altitudinal distance (partial Mantel test, $P = 0.0001$).

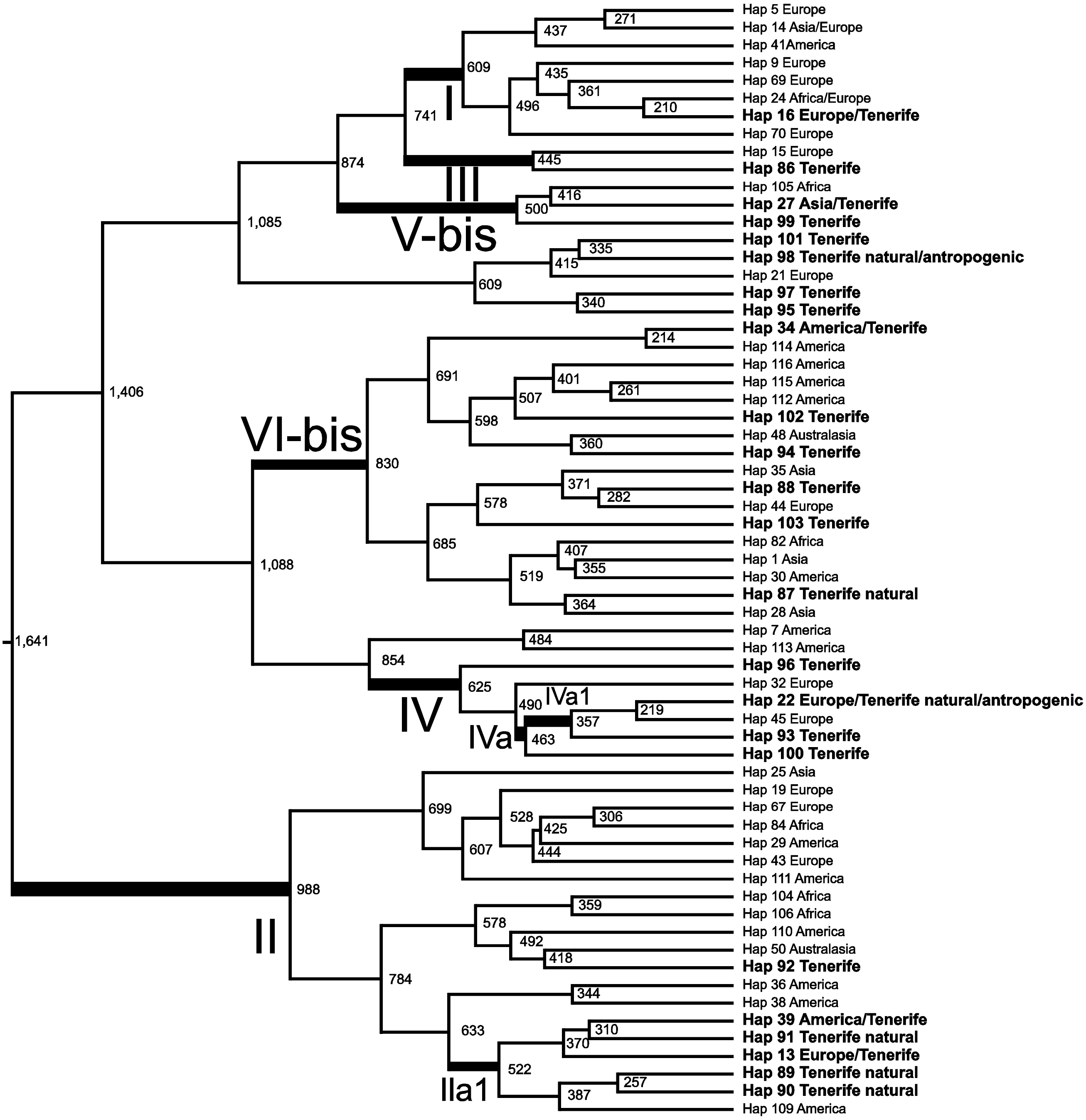
Neutral tests (Tajima's *D* and Fu's *Fs*) for the Tenerife accessions as a whole and for each habitat type (natural versus disturbed) showed that the Tenerife populations in anthropogenic and natural habitats do not significantly depart from 0 (Table 4), failing to provide evidence for recent demographic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

Table 3. Pairwise 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 to the null hypotheses that $F_{ST} = 0$ and that $F_{ST} = N_{ST}$ respectively.

Population indexes	**Geographical 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, $P > 0.05$; $^*P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$.

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 Fig 1(b) for clade labels.



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 colonized 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 colonization 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 colonization events erasing the signal of a recent founding event (Dlugosch & Parker 2008).

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)

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 colonized multiple times independently. This observation parallels recurrent colonization patterns reported in island bryophytes (Grundmann *et al.* 2007; Vanderpoorten *et al.* 2008; Hutsemékers *et al.* 2011; 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, point to these two continents as main sources for the island. Nevertheless, even in a weed like *B. argenteum*, which is characterized by a dual mating system involving both sexual (spores) and asexual (production of specialized 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. 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 colonization of Tenerife dates back to more than one hundred thousand years BP, i.e. well before the first human settlements, about 2500 years BP (Rando *et al*. 1997). 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 (Korpelainen *et al*. 2013; Hutsemékers *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 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, erase any spatial patterns of genetic variation (St. Quinton *et al*. 2011; Fussi *et al*. 2012), and therefore, also point 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 geographical origin and assess the timing of colonization 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 upon 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 an 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 like bryophytes (Essl *et al*. 2013, 2014).

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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 in the case the number is given in the middle of ITS1 and ITS2 columns. for each Tenerife accession, the altitude (in metres above sea level) and the habitat (anthropogenic vs natural) are also provided.

Haplotype name	Habitat type in Tenerife accessions	Altitude (m a.s.l.) in Tenerife accessions	Geographic origin	Herbarium or literature reference if previously published	Collection	Geographical coordinates in decimal degrees				GenBank accession numbers		Geographic region
										ITS1	ITS2	
										ITS		
1	-		Indonesia, Sumatra, Gunung Sinabung	Pisa et al. (2014)	-	N	3,18139	E	98,44111	KF952805	KF952914	Asia
2	-		Bolivia, La Paz, Murillo	Pisa et al. (2014)	-	S	16,61667	W	68,07528	KF952807	KF952916	America
3	-		Thailand, Khao Yai National Park	Pisa et al. (2014)	-	N	14,53333	E	101,36667	KF952810	KF952919	Asia
4	-		Brazil, Bahia state, Municipality of Abaíra, Campo de Ouro Fino	Pisa et al. (2014)	-	S	13,25000	W	41,90000	KF952811	KF952920	America
5	-		Greece, Prefecture of Fokida, Delphi	Pisa et al. (2014)	-	N	38,48278	E	22,50444	KF952836	KF952945	Europe
5	-		Norway, Finnamark	Pisa et al. (2014)	-	N	70,78333	E	23,33333	KF952822	KF952931	Europe
6	-		Spain, León province, Abelgas	Pisa et al. (2014)	-	N	42,89139	W	5,97389	KF952838	KF952947	Europe
6	-		USA, Connecticut, Hartford County	Pisa et al. (2014)	-	N	41,85278	W	72,51611	KF952839	KF952948	America
7	-		Boliva, Cochabamba, Carrasco	Pisa et al. (2014)	-	S	17,75000	W	64,80000	KF952814	KF952923	America
8	-		Ecuador, Pichinga province	Pisa et al. (2014)	-	N	0,38333	W	78,21667	KF952816	KF952925	America
9	-		Sweden, Värmland	Pisa et al. (2014)	-	N	59,78333	E	14,36667	KF952818	KF952927	Europe
10	-		USA, New Mexico, Doña Ana Co, Las Cruces	Pisa et al. (2014)	-	N	32,31667	W	106,75000	KF952853	KF952962	America
10	-		USA, Utah, Dixie National Forest	Pisa et al. (2014)	-	N	37,79667	W	112,80722	KF952854	KF952963	America
11	-		Papua New Guinea, Chimbu Province, Wilhelm Mountain	Pisa et al. (2014)	-	S	5,75667	E	145,03556	KF952821	KF952930	Australasia
12	-		Spain, Granada province, Sierra Nevada	Pisa et al. (2013)	-	N	37,09725	W	3,39753	KC493863	KC493889	Europe
13	-		Spain, Granada province, Albuñol	Pisa et al. (2013)	-	N	36,80025	W	3,21738	KC493867	KC493893	Europe
13	Anthropogenic	1487	Spain, Canary Islands, Tenerife	MUB 44727	N. Rancel & J. Patiño s.n.	N	28,33780	W	16,52177	KF953033	KF953119	Tenerife

13	Anthropogenic	1487	Spain, Canary Islands, Tenerife	MUB 44728	N. Rancel & J. Patiño s.n.	N	28,33780	W	16,52177	KF953034	KF953120	Tenerife
13	Anthropogenic	1487	Spain, Canary Islands, Tenerife	MUB 44729	N. Rancel & J. Patiño s.n.	N	28,33780	W	16,52177	KF953035	KF953121	Tenerife
13	Anthropogenic	1487	Spain, Canary Islands, Tenerife	MUB 44730	N. Rancel & J. Patiño s.n.	N	28,33780	W	16,52177	KF953036	KF953122	Tenerife
14	-		Denmark, Feroe Islands, Streymoy, Thorshavn	Pisa et al. (2014)	-	N	62,01139	W	6,75361	KF952834	KF952943	Europe
14	-		France, The Vaucluse, commune de Bonnieux, domaine de la Chambarelle	Pisa et al. (2014)	-	N	43,81389	E	18,43333	KF952835	KF952944	Europe
14	-		Germany, Berlin	Pisa et al. (2014)	-	N	52,44028	E	13,58250	KF952828	KF952937	Europe
14	-		Germany, Berlin	Pisa et al. (2014)	-	N	52,51861	E	13,39694	KF952829	KF952938	Europe
14	-		Germany, Baden-Württemberg, Black Forest	Pisa et al. (2014)	-	N	47,84417	E	8,01917	KF952832	KF952941	Europe
14	-		Germany, Baden-Württemberg, Black Forest	Pisa et al. (2014)	-	N	47,86444	E	8,02194	KF952824	KF952933	Europe
14	-		Germany, Baden-Württemberg, Black Forest	Pisa et al. (2014)	-	N	47,85972	E	8,03639	KF952825	KF952934	Europe
14	-		Germany, Baden-Württemberg, Black Forest	Pisa et al. (2014)	-	N	47,85972	E	8,03639	KF952826	KF952935	Europe
14	-		Germany, Baden-Württemberg, Black Forest	Pisa et al. (2014)	-	N	47,85972	E	8,03639	KF952827	KF952936	Europe
14	-		Germany, Baden-Württemberg, Eberbach	Pisa et al. (2014)	-	N	49,46111	E	8,98778	KF952830	KF952939	Europe
14	-		Germany, Baden-Württemberg, Eberbach	Pisa et al. (2014)	-	N	49,46111	E	8,98778	KF952837	KF952946	Europe
14	-		Germany, Baden-Württemberg, Sankt Leon-Rot	Pisa et al. (2014)	-	N	49,24333	E	8,65222	KF952831	KF952940	Europe
14	-		Russia, Kuril Islands, Kunashir Island	Pisa et al. (2014)	-	N	44,35000	E	146,26667	KF952833	KF952942	Asia
14	-		Spain, Granada province, Sierra Nevada	Pisa et al. (2013)	-	N	37,09725	W	3,39753	KC493862	KC493888	Europe
14	-		Spain, León province, Abelgas	Pisa et al. (2014)	-	N	42,89139	W	5,97389	KF952823	KF952932	Europe
15	-		Spain, Granada province, Vélez de Benaudalla	Pisa et al. (2013)	-	N	36,84465	W	3,50905	KC493873	KC493899	Europe
16	-		Greece, Prefecture of Ilia, Ráhes	Pisa et al. (2014)	-	N	38,90861	E	22,83500	KF952794	KF952903	Europe
16	-		Ireland, E. County Mayo	Pisa et al. (2014)	-	N	53,61889	W	9,30639	KF952788	KF952897	Europe
16	-		Spain, León province, Abelgas	Pisa et al. (2014)	-	N	42,89139	W	5,97389	KF952790	KF952899	Europe
16	-		Spain, Ávila province, Sierra de Gredos	Pisa et al. (2014)	-	N	40,42231	W	5,29667	KF952796	KF952905	Europe

16	-		Spain, Ávila province, Sierra de Gredos	Pisa et al. (2014)	-	N	40,42231	W	5,29667	KF952789	KF952898	Europe
16	-		Spain, Ávila province, Sierra de Gredos	Pisa et al. (2014)	-	N	40,42231	W	5,29667	KF952795	KF952904	Europe
16	Anthropogenic	322	Spain, Canary Islands, Tenerife	MUB 44670	J. Patiño s.n.	N	28,40470	W	16,50429	KF953014	KF953100	Tenerife
16	Anthropogenic	322	Spain, Canary Islands, Tenerife	MUB 44671	J. Patiño s.n.	N	28,40470	W	16,50429	KF953015	KF953101	Tenerife
16	Anthropogenic	322	Spain, Canary Islands, Tenerife	MUB 44672	J. Patiño s.n.	N	28,40470	W	16,50429	KF953016	KF953102	Tenerife
16	Anthropogenic	322	Spain, Canary Islands, Tenerife	MUB 44673	J. Patiño s.n.	N	28,40470	W	16,50429	KF953017	KF953103	Tenerife
16	Anthropogenic	322	Spain, Canary Islands, Tenerife	MUB 44674	J. Patiño s.n.	N	28,40470	W	16,50429	KF953018	KF953104	Tenerife
16	Anthropogenic	322	Spain, Canary Islands, Tenerife	MUB 44675	J. Patiño s.n.	N	28,40470	W	16,50429	KF953019	KF953105	Tenerife
16	Anthropogenic	1046	Spain, Canary Islands, Tenerife	Pisa et al. (2014)	-	N	28,35770	W	16,49263	KF952797	KF952906	Tenerife
16	Anthropogenic	1046	Spain, Canary Islands, Tenerife	MUB 44707	J. Patiño s.n.	N	28,35770	W	16,49263	KF953023	KF953109	Tenerife
16	Anthropogenic	1046	Spain, Canary Islands, Tenerife	MUB 44704	J. Patiño s.n.	N	28,35770	W	16,49263	KF953020	KF953106	Tenerife
16	Anthropogenic	1046	Spain, Canary Islands, Tenerife	MUB 44705	J. Patiño s.n.	N	28,35770	W	16,49263	KF953021	KF953107	Tenerife
16	Anthropogenic	1046	Spain, Canary Islands, Tenerife	MUB 44706	J. Patiño s.n.	N	28,35770	W	16,49263	KF953022	KF953108	Tenerife
16	Anthropogenic	1275	Spain, Canary Islands, Tenerife	MUB 44719	N. Rancel & J. Patiño s.n.	N	28,34320	W	16,52180	KF953024	KF953110	Tenerife
16	Anthropogenic	1275	Spain, Canary Islands, Tenerife	MUB 44723	N. Rancel & J. Patiño s.n.	N	28,34320	W	16,52180	KF953028	KF953114	Tenerife
16	Anthropogenic	1275	Spain, Canary Islands, Tenerife	MUB 44720	N. Rancel & J. Patiño s.n.	N	28,34320	W	16,52180	KF953025	KF953111	Tenerife
16	Anthropogenic	1275	Spain, Canary Islands, Tenerife	MUB 44721	N. Rancel & J. Patiño s.n.	N	28,34320	W	16,52180	KF953026	KF953112	Tenerife
16	Anthropogenic	1275	Spain, Canary Islands, Tenerife	MUB 44722	N. Rancel & J. Patiño s.n.	N	28,34320	W	16,52180	KF953027	KF953113	Tenerife
16	Anthropogenic	1387	Spain, Canary Islands, Tenerife	MUB 44724	N. Rancel & J. Patiño s.n.	N	28,34250	W	16,50836	KF953029	KF953115	Tenerife
16	Anthropogenic	1387	Spain, Canary Islands, Tenerife	MUB 44725	N. Rancel & J. Patiño s.n.	N	28,34250	W	16,50836	KF953030	KF953116	Tenerife
17	-		Spain, León province, Abelgas	Pisa et al. (2014)	-	N	42,89139	W	5,97389	KF952819	KF952928	Europe
18	-		Germany, Baden-Württemberg, Black Forest	Pisa et al. (2014)	-	N	47,83333	E	8,01667	KF952856	KF952965	Europe
18	-		Germany, Baden-Württemberg, Black Forest	Pisa et al. (2014)	-	N	47,83333	E	8,01667	KF952857	KF952966	Europe
19	-		Germany, Baden-Württemberg, Black Forest	Pisa et al. (2014)	-	N	47,84056	E	8,01917	KF952847	KF952956	Europe
19	-		Portugal, Beira Interior, Guarda	Pisa et al. (2014)	-	N	40,53250	W	7,26667	KF952848	KF952957	Europe
20	-		Germany, Baden-Württemberg, Black Forest	Pisa et al. (2014)	-	N	47,84417	E	8,01917	KF952864	KF952973	Europe
20	-		Germany, Baden-Württemberg, Black Forest	Pisa et al. (2014)	-	N	47,86444	E	8,02194	KF952862	KF952971	Europe

20	-		Germany, Baden-Württemberg, Black Forest	Pisa et al. (2014)	-	N	47,86444	E	8,02194	KF952858	KF952967	Europe
20	-		Germany, Baden-Württemberg, Black Forest	Pisa et al. (2014)	-	N	47,86444	E	8,02194	KF952859	KF952968	Europe
20	-		Germany, Baden-Württemberg, Black Forest	Pisa et al. (2014)	-	N	47,86444	E	8,02194	KF952860	KF952969	Europe
20	-		Germany, Baden-Württemberg, Black Forest	Pisa et al. (2014)	-	N	47,86444	E	8,02194	KF952861	KF952970	Europe
21	-		Spain, Balearic Islands, Menorca	Pisa et al. (2014)	-	N	39,89944	E	4,10389	KF952820	KF952929	Europe
22	-		Germany, Berlin	Pisa et al. (2014)	-	N	49,41111	E	8,70639	KF952840	KF952949	Europe
22	Anthropogenic	322	Spain, Canary Islands, Tenerife	MUB 44677	J. Patiño s.n.	N	28,40470	W	16,50429	KF953068	KF953154	Tenerife
22	Anthropogenic	1387	Spain, Canary Islands, Tenerife	MUB 44726	N. Rancel & J. Patiño s.n.	N	28,34250	W	16,50836	KF953069	KF953155	Tenerife
22	Natural	2005	Spain, Canary Islands, Tenerife	MUB 44739, TFC-Bryo 18992	J.M. González-Mancebo et al. s.n.	N	28,21870	W	16,64903	KF953070	KF953156	Tenerife
23	-		Germany, Baden-Württemberg, Black Forest	Pisa et al. (2014)	-	N	47,86444	E	8,02194	KF952863	KF952972	Europe
24	-		Morocco, High Atlas, Oukaimeden	Pisa et al. (2014)	-	N	31,21667	W	7,86667	KF952793	KF952902	Africa
24	-		Spain, Murcia province, Campus de Espinardo	Pisa et al. (2014)	-	N	38,02056	W	1,16944	KF952791	KF952900	Europe
24	-		Spain, Murcia province, Sierra de las Herrerías	Pisa et al. (2014)	-	N	37,58278	W	1,42667	KF952792	KF952901	Europe
25	-		China, Yunnan province, Gonshan County	Pisa et al. (2014)	-	N	27,68539	E	98,30422	KF952815	KF952924	Asia
26	-		Russia, Yakutia	Pisa et al. (2014)	-	N	61,13333	E	138,05000	KF952855	KF952964	Asia
27	-		Russia, NW Caucasus	Pisa et al. (2014)	-	N	43,45000	E	41,68333	KF952813	KF952922	Asia
27	Anthropogenic	932	Spain, Canary Islands, Tenerife	MUB 44703	J. Patiño s.n.	N	30,35890	W	16,50874	KF953063	KF953149	Tenerife
28	-		China, Qinghai province, Menyuan County	Pisa et al. (2014)	-	N	37,11889	E	102,31639	KF952865	KF952974	Asia
28	-		China, Yunnan province, Degin County	Pisa et al. (2014)	-	N	28,63333	E	98,73278	KF952866	KF952975	Asia
29	-		Dominican Republic, La Vega province, Alto de la Bandera Mountain	Pisa et al. (2014)	-	N	18,81667	W	70,61667	KF952812	KF952921	America
30	-		Dominican Republic, Independencia province, Sierra de Baoruco	Pisa et al. (2014)	-	N	18,30000	W	71,70000	KF952851	KF952960	America
31	-		Germany, Berlin	Pisa et al. (2014)	-	N	52,50833	E	13,33444	KF952867	KF952976	Europe

31	-		New Zealand, Huntley	Hills et al. (2010)	-	S	37,56667	E	175,15000	GU907062		Australasia
32	-		Spain, Madrid province, Sierra de Guadarrama	Pisa et al. (2014)	-	N	40,59030	W	3,98505	KF952803	KF952912	Europe
33	-		Spain, Madrid province, Sierra de Guadarrama	Pisa et al. (2014)	-	N	40,83139	W	3,95278	KF952868	KF952977	Europe
33	-		Spain, Madrid province, Sierra de Guadarrama	Pisa et al. (2014)	-	N	40,83139	W	3,95278	KF952869	KF952978	Europe
34	Anthropogenic	596	Spain, Canary Islands, Tenerife	MUB 44688	A. Rodríguez & J. Leal s.n.	N	28,37730	W	16,51842	KF953010	KF953096	Tenerife
34	Anthropogenic	1258	Spain, Canary Islands, Tenerife	Pisa et al. (2014)	-	N	28,14400	W	16,65361	KF952844	KF952953	Tenerife
34	Anthropogenic	1258	Spain, Canary Islands, Tenerife	MUB 44715	J.M. González-Mancebo s.n.	N	28,14400	W	16,65361	KF953011	KF953097	Tenerife
34	Anthropogenic	1258	Spain, Canary Islands, Tenerife	MUB 44716	J.M. González-Mancebo s.n.	N	28,14400	W	16,65361	KF953012	KF953098	Tenerife
34	Anthropogenic	1258	Spain, Canary Islands, Tenerife	MUB 44717	J.M. González-Mancebo s.n.	N	28,14400	W	16,65361	KF953013	KF953099	Tenerife
34	-		USA, California, Riverside Co, San Bernardino National Forest	Pisa et al. (2014)	-	N	33,77672	W	116,67814	KF952843	KF952952	America
34	-		USA, California, San Bernardino Co, San Bernardino National Forest	Pisa et al. (2014)	-	N	34,22497	W	117,06186	KF952842	KF952951	America
34	-		USA, California, Monterey County, Los Padres National forest	Pisa et al. (2014)	-	N	36,09639	W	121,44167	KF952845	KF952954	America
34	-		USA, Nevada, Carson City Co, Carson	CAS 1040044	J.R. Shevock 21942	N	39,1642	W	119,7231	KF953082	KF953168	America
35	-		China, Schuan Province, Muli Co, Hengduan Mountains	Pisa et al. (2014)	-	N	28,16667	E	101,22233	KF952849	KF952958	Asia
36	-		USA, California, Inyo Co, Mojave Desert	Pisa et al. (2014)	-	N	35,85364	W	117,38125	KF952870	KF952979	America
36	-		USA, California, Kern Co, Sierra Nevada	Pisa et al. (2014)	-	N	35,56250	W	118,95417	KF952872	KF952981	America
37	-		USA, California Tulare Co, Sierra Nevada	Pisa et al. (2014)	-	N	35,88889	W	118,34583	KF952809	KF952918	America
38	-		USA, California, Tulare Co,Sierra Nevada	Pisa et al. (2014)	-	N	36,73750	W	118,85833	KF952871	KF952980	America
39	Anthropogenic	1070	Spain, Canary Islands, Tenerife	MUB 44709, TFC-Bryo 19158	J. Patiño s.n.	N	28,10534	W	16,61250	KF953037	KF953123	Tenerife
39	-		USA, California, Mariposa Co, Yosemite National Park	Pisa et al. (2014)	-	N	37,51889	W	119,60000	KF952787	KF952896	America

39	-		USA, California, Tulare Co, Sierra Nevada	Pisa et al. (2014)	-	N	36,06111	W	118,59306	KF952786	KF952895	America
40	-		USA, California, Fresno Co, Sierra Nevada	Pisa et al. (2014)	-	N	37,16250	W	119,09583	KF952817	KF952926	America
41	-		Chile, Prov. of Antarctica Chilena, Isla Navarino, Magallanes	Pisa et al. (2014)	-	S	54,95000	W	67,63333	KF952808	KF952917	America
42	-		China, Sichuan Province, Litang Co, Hengduan Mountains	Pisa et al. (2014)	-	N	30,17792	E	100,00311	KF952875	KF952984	Asia
43	-		Portugal, Beira Interior, Guarda	Pisa et al. (2014)	-	N	40,53250	W	7,26667	KF952846	KF952955	Europe
44	-		Germany, Berlin	Pisa et al. (2014)	-	N	52,51861	E	13,39694	KF952852	KF952961	Europe
45	-		Germany, Baden-Württemberg, Heidelberg	Pisa et al. (2014)	-	N	49,41111	E	8,70639	KF952841	KF952950	Europe
46	-		Australia, Mount Buffalo	Hills et al. (2010)	-	S	36,78333	E	146,05000	GU907057		Australasia
47	-		Australia, Mount McKay	Hills et al. (2010)	-	S	36,86667	E	147,25000	GU907061		Australasia
48	-		Australia, Canberra	Hills et al. (2010)	-	S	35,30000	E	149,13333	GU907059		Australasia
49	-		New Zealand, Christchurch	Hills et al. (2010)	-	S	43,08333	E	172,11667	GU907056		Australasia
50	-		New Zealand, Hamilton	Hills et al. (2010)	-	S	35,88333	E	175,46667	GU907060		Australasia
51	-		Taiwan, Hwalien County, Ta-yu-ling	Pisa et al. (2014)	-	N	23,81667	E	121,23333	KF952800	KF952909	Asia
52	-		China, Taiwan, Nantou Co, Central Mountain Range	Pisa et al. (2014)	-	N	24,12500	E	121,21667	KF952801	KF952910	Asia
53	-		Norway, Svalbard archipelago, Spitsbergen	Pisa et al. (2014)	-	N	78,91667	E	11,93333	KF952782	KF952891	Europe
54	-		Spain, Granada province, Sierra Nevada	Pisa et al. (2013)	-	N	37,06943	W	3,38662	KC493852		Europe
55	-		Sri Lanka (Ceylon), Central province, Nuwara Eliya District	Pisa et al. (2014)	-	N	6,95694	E	80,10889	KF952781	KF952890	Asia
56	-		Canada, Northwest Territories, District of Fanklin, Ellesmere Island	Pisa et al. (2014)	-	N	79,71667	W	85,83333	KF952783	KF952892	America
57	-		Antarctica, Cape Hallet,	Hills et al. (2010)	-	S	72,30000	E	170,30000	GU907068		Antarctica
58	-		Antarctica, Garwood Valley	Hills et al. (2010)	-	S	78,05000	E	164,16667	GU907072		Antarctica

58	-		Antarctica, Miers Valley	Hills et al. (2010)	-	S	78,08333	E	164,75000	GU907071		Antarctica
59	-		Antarctica, Cape Bird	Hills et al. (2010)	-	S	77,21667	E	166,43333	GU907070		Antarctica
59	-		Antarctica, Cape Royds, Ross Island	Skotnicki et al. (2005)	-	S	77,58333	E	166,16667	AY611433		Antarctica
60	-		Antarctica, Canada Glacier, Taylor Valley, S. Victoria Land	Skotnicki et al. (2005)	-	S	77,58333	E	163,25000	AY611432		Antarctica
60	-		Antarctica, Cape Hallet	Hills et al. (2010)	-	S	72,30000	E	170,30000	GU907066		Antarctica
60	-		Antarctica, Cape Hallet	Hills et al. (2010)	-	S	72,30000	E	170,30000	GU907067		Antarctica
60	-		Antarctica, Marble Point	Hills et al. (2010)	-	S	76,43333	E	163,83333	GU907069		Antarctica
61	-		Antarctica, Beaufort Island, Ross Sea	Skotnicki et al. (2005)	-	S	76,91667	E	166,90000	AY611431		Antarctica
62	-		Antarctica, Beaufort Island	Hills et al. (2010)	-	S	76,91667	E	166,90000	GU907063		Antarctica
62	-		Antarctica, Beaufort Island	Hills et al. (2010)	-	S	76,91667	E	166,90000	GU907064		Antarctica
62	-		Antarctica, Granite Harbour	Hills et al. (2010)	-	S	77,00000	E	162,50000	GU907065		Antarctica
63	-		Canada, Northwest Territories, District of Fanklin, Ellesmere Island	Pisa et al. (2014)	-	N	79,48333	W	85,26667	KF952878	KF952987	America
63	-		USA, Alaska, Pribilof Islands, St. Palul Island.	Pisa et al. (2014)	-	N	57,15000	W	170,25000	KF952876	KF952985	America
63	-		USA, Alaska, Valdez-Cordova Census Area	Pisa et al. (2014)	-	N	61,12583	W	146,35111	KF952877	KF952986	America
66	-		Antarctica, Cape Chocolate, S. Victoria Land	Skotnicki et al. (2005)	-	S	77,95000	E	164,50000	AY611429		Antarctica
64	-		Antarctica, Edmonson Point, N. Victoria Land	Skotnicki et al. (2005)	-	S	74,33333	E	164,50000	AY611430		Antarctica
65	-		Antarctica, Granite Harbour, S. Victoria Land	Skotnicki et al. (2005)	-	S	77,00000	E	162,50000	AY611434		Antarctica
67	-		U.K., Great Britain, N. Northumberland	Pisa et al. (2014)	-	N	52,96667	E	0,55000	KF952799	KF952908	Europe
68	-		France, Department Hautes Pyrénées	Pisa et al. (2014)	-	N	42,73333	W	0,05000	KF952881	KF952990	Europe
69	-		France, Vosges Department, Vosges Mountains	Pisa et al. (2014)	-	N	47,90478	E	7,10286	KF952882	KF952991	Europe

70	-		France, Vosges Department, Vosges Mountains	Pisa et al. (2014)	-	N	47,90478	E	7,10286	KF952798	KF952907	Europe
71	-		China. Sichuan Province, Jiulong Co, Hengduan Mountains	Pisa et al. (2014)	-	N	29,22078	E	101,45050	KF952883	KF952992	Asia
72	-		Colombia, Departamento de Nariño, Municipio de Cumbal	Pisa et al. (2014)	-	N	0,95250	W	77,81944	KF952884	KF952993	America
73	-		China, Qinghai province, Gonghe County	Pisa et al. (2014)	-	N	36,97722	E	99,90056	KF952885	KF952994	Asia
74	-		Nepal, Taplejung District	Pisa et al. (2014)	-	N	27,43333	E	87,46667	KF952886	KF952995	Asia
75	-		China, Quinghai Province, Yushu Tibetan Autonomous Prefecture	Pisa et al. (2014)	-	N	33,00250	E	97,27611	KF952873	KF952982	Asia
76	-		Russia, Siberia, Taimyr	Pisa et al. (2014)	-	N	71,88083	E	110,78806	KF952887	KF952996	Asia
77	-		Altai Republic	Pisa et al. (2014)	-	N	50,50000	E	89,16667	KF952888	KF952997	Asia
78	-		Canada, Northwest Territories, District of Fanklin, Axel Heiberg Island	Pisa et al. (2014)	-	N	80,03333	W	88,75000	KF952889	KF952998	America
79	-		China, Xizang province, SE Tibet	Pisa et al. (2014)	-	N	29,05000	E	93,98333	KF952874	KF952983	Asia
80	-		Uganda, Western Region, Kabale	Pisa et al. (2014)	-	N	1,08333	E	29,80000	KF952802	KF952911	Africa
81	-		Union of the Comoros, Grande Comore (Ngazidja)	Pisa et al. (2014)	-	N	11,61222	E	43,33222	KF952804	KF952913	Africa
82	-		Tanzania, Kilimanjaro	Pisa et al. (2014)	-	S	4,25722	E	37,99028	KF952850	KF952959	Africa
83	-		Kenya, Nyandarua County, Aberdare Mts.	Pisa et al. (2014)	-	N	0,41083	E	36,61667	KF952806	KF952915	Africa
84	-		South Africa, Cape province, Vredenburg	Pisa et al. (2014)	-	S	32,76667	E	18,00000	KF952879	KF952988	Africa
85	-		South Africa, KwaZulu-Natal province, Vryheid	Pisa et al. (2014)	-	S	27,76667	E	30,78333	KF952880	KF952989	Africa
86	Anthropogenic	322	Spain, Canary Islands, Tenerife	MUB 44669	J. Patiño s.n.	N	28,40470	W	16,50429	KF953008	KF953094	Tenerife
86	Anthropogenic	596	Spain, Canary Islands, Tenerife	MUB 44678	A. Rodríguez & J. Leal s.n.	N	28,37730	W	16,51842	KF952999	KF953085	Tenerife
86	Anthropogenic	596	Spain, Canary Islands, Tenerife	MUB 44685	A. Rodríguez & J. Leal s.n.	N	28,37730	W	16,51842	KF953006	KF953092	Tenerife
86	Anthropogenic	596	Spain, Canary Islands, Tenerife	MUB 44686	A. Rodríguez & J. Leal s.n.	N	28,37730	W	16,51842	KF953007	KF953093	Tenerife
86	Anthropogenic	596	Spain, Canary Islands, Tenerife	MUB 44679	A. Rodríguez & J. Leal s.n.	N	28,37730	W	16,51842	KF953000	KF953086	Tenerife

86	Anthropogenic	596	Spain, Canary Islands, Tenerife	MUB 44687	A. Rodríguez & J. Leal s.n.	N	28,37730	W	16,51842	KF953009	KF953095	Tenerife
86	Anthropogenic	596	Spain, Canary Islands, Tenerife	MUB 44681	A. Rodríguez & J. Leal s.n.	N	28,37730	W	16,51842	KF953002	KF953088	Tenerife
86	Anthropogenic	596	Spain, Canary Islands, Tenerife	MUB 44682	A. Rodríguez & J. Leal s.n.	N	28,37730	W	16,51842	KF953003	KF953089	Tenerife
86	Anthropogenic	596	Spain, Canary Islands, Tenerife	MUB 44683	A. Rodríguez & J. Leal s.n.	N	28,37730	W	16,51842	KF953004	KF953090	Tenerife
86	Anthropogenic	596	Spain, Canary Islands, Tenerife	MUB 44684	A. Rodríguez & J. Leal s.n.	N	28,37730	W	16,51842	KF953005	KF953091	Tenerife
86	Anthropogenic	596	Spain, Canary Islands, Tenerife	MUB 44680	A. Rodríguez & J. Leal s.n.	N	28,37730	W	16,51842	KF953001	KF953087	Tenerife
87	Natural	2151	Spain, Canary Islands, Tenerife	MUB 44740,TFC-Bryo 16124	J.M. González-Mancebo & J. Leal s.n.	N	28,22480	W	16,61852	KF953031	KF953117	Tenerife
88	Anthropogenic	1195	Spain, Canary Islands, Tenerife	MUB 44712	N. Rancel & J. Patiño s.n.	N	28,35480	W	16,50134	KF953032	KF953118	Tenerife
89	Natural	2005	Spain, Canary Islands, Tenerife	MUB 44732,TFC-Bryo 18990	J.M. González-Mancebo et al. s.n.	N	28,21870	W	16,64903	KF953038	KF953124	Tenerife
89	Natural	2005	Spain, Canary Islands, Tenerife	MUB 44733, TFC-Bryo 18991	J.M. González-Mancebo et al. s.n.	N	28,21870	W	16,64903	KF953039	KF953125	Tenerife
89	Natural	2005	Spain, Canary Islands, Tenerife	MUB 44734, TFC-Bryo 18994	J.M. González-Mancebo et al. s.n.	N	28,21870	W	16,64903	KF953040	KF953126	Tenerife
89	Natural	2005	Spain, Canary Islands, Tenerife	MUB 44735, TFC-Bryo 18996	J.M. González-Mancebo et al. s.n.	N	28,21870	W	16,64903	KF953041	KF953127	Tenerife
89	Natural	2005	Spain, Canary Islands, Tenerife	MUB 44736, TFC-Bryo 18997	J.M. González-Mancebo et al. s.n.	N	28,21870	W	16,64903	KF953042	KF953128	Tenerife
90	Natural	2005	Spain, Canary Islands, Tenerife	MUB 44737, TFC-Bryo 18998	J.M. González-Mancebo et al. s.n.	N	28,21870	W	16,64903	KF953043	KF953129	Tenerife
91	Natural	1882	Spain, Canary Islands, Tenerife	MUB 44731, TFC-Bryo 19163	J.M. González-Mancebo & J. Leal s.n.	N	28,31300	W	16,64362	KF953044	KF953130	Tenerife
92	Anthropogenic	322	Spain, Canary Islands, Tenerife	MUB 44676	J. Patiño s.n.	N	28,40470	W	16,50429	KF953047	KF953133	Tenerife
92	Anthropogenic	1070	Spain, Canary Islands, Tenerife	MUB 44710, TFC-Bryo 19152	J. Patiño s.n.	N	28,10534	W	16,61250	KF953045	KF953131	Tenerife
92	Anthropogenic	1070	Spain, Canary Islands, Tenerife	MUB 44711, TFC-Bryo 19155	J. Patiño s.n.	N	28,10534	W	16,61250	KF953046	KF953132	Tenerife
93	Anthropogenic	932	Spain, Canary Islands, Tenerife	MUB 44700	J. Patiño s.n.	N	28,35890	W	16,50874	KF953048	KF953134	Tenerife
94	Anthropogenic	810	Spain, Canary Islands, Tenerife	MUB 44697	J.M. González-Mancebo s.n.	N	28,16000	W	16,62028	KF953049	KF953135	Tenerife
95	Anthropogenic	1195	Spain, Canary Islands, Tenerife	MUB 44713	N. Rancel & J. Patiño s.n.	N	28,35480	W	16,50134	KF953050	KF953136	Tenerife

96	Anthropogenic	777	Spain, Canary Islands, Tenerife	MUB 44689	J. Patiño s.n.	N	28,36890	W	16,52347	KF953051	KF953137	Tenerife
97	Anthropogenic	777	Spain, Canary Islands, Tenerife	MUB 44690	J. Patiño s.n.	N	28,36890	W	16,52347	KF953052	KF953138	Tenerife
98	Anthropogenic	777	Spain, Canary Islands, Tenerife	MUB 44691	J. Patiño s.n.	N	28,36890	W	16,52347	KF953053	KF953139	Tenerife
98	Anthropogenic	777	Spain, Canary Islands, Tenerife	MUB 44692	J. Patiño s.n.	N	28,36890	W	16,52347	KF953054	KF953140	Tenerife
98	Anthropogenic	777	Spain, Canary Islands, Tenerife	MUB 44693	J. Patiño s.n.	N	28,36890	W	16,52347	KF953055	KF953141	Tenerife
98	Anthropogenic	777	Spain, Canary Islands, Tenerife	MUB 44694	J. Patiño s.n.	N	28,36890	W	16,52347	KF953056	KF953142	Tenerife
98	Anthropogenic	1061	Spain, Canary Islands, Tenerife	MUB 44708	J.M. González-Mancebo s.n.	N	28,11670	W	16,65000	KF953059	KF953145	Tenerife
98	Anthropogenic	1195	Spain, Canary Islands, Tenerife	MUB 44714	N. Rancel & J. Patiño s.n.	N	28,35480	W	16,50134	KF953057	KF953143	Tenerife
98	Natural	2005	Spain, Canary Islands, Tenerife	MUB 44738, TFC-Bryo 18995	J.M. González-Mancebo et al. s.n.	N	28,21870	W	16,64903	KF953058	KF953144	Tenerife
99	Anthropogenic	777	Spain, Canary Islands, Tenerife	MUB 44695	J. Patiño s.n.	N	28,36890	W	16,52347	KF953060	KF953146	Tenerife
100	Anthropogenic	932	Spain, Canary Islands, Tenerife	MUB 44701	J. Patiño s.n.	N	28,35890	W	16,50874	KF953061	KF953147	Tenerife
100	Anthropogenic	932	Spain, Canary Islands, Tenerife	MUB 44702	J. Patiño s.n.	N	29,35890	W	16,50874	KF953062	KF953148	Tenerife
101	Anthropogenic	1258	Spain, Canary Islands, Tenerife	MUB 44718	J.M. González-Mancebo s.n.	N	28,14400	W	16,65361	KF953064	KF953150	Tenerife
102	Anthropogenic	810	Spain, Canary Islands, Tenerife	MUB 44698	J.M. González-Mancebo s.n.	N	28,16000	W	16,62028	KF953065	KF953151	Tenerife
103	Anthropogenic	777	Spain, Canary Islands, Tenerife	MUB 44696	J. Patiño s.n.	N	28,36890	W	16,52347	KF953067	KF953153	Tenerife
103	Anthropogenic	835	Spain, Canary Islands, Tenerife	MUB 44699	J. Patiño s.n.	N	28,35980	W	16,52361	KF953066	KF953152	Tenerife
104	-		South Africa, West Cape province, Pienaarskloof	BOLUS 46296	T.A.J. Hedderson 17693	S	33,23216667	E	20,1576	KF953071	KF953157	Africa
105	-		South Africa, West Cape province, Loxton	BOLUS 46293	T.A.J. Hedderson16975	S	31,42500	E	22,32778	KF953072	KF953158	Africa
106	-		South Africa, West Cape province, Beaufort West	BOLUS 46294	T.A.J. Hedderson16922	S	32,20833	E	22,55778	KF953073	KF953159	Africa
107	-		Union of the Comoros, Grande Comore, Kartala Forest	BOLUS 46301	T.A.J. Hedderson16795a	S	11,75641	E	43,33548	KF953074	KF953160	Africa
108	-		France, La Réunion, Piton des Neiges	MUB 44753	C. Ah-Peng s.n.	S	21,08000	E	55,44000	KF953075	KF953161	Africa
109	-		USA, California, San Francisco Co, San Francisco Bay	CAS 993259	J.R. Shevock 18994	N	37,7653	W	122,3931	KF953076	KF953162	America
110	-		USA, California, Stanislaus Co, Modesto	CAS 993318	J.R. Shevock 17847	N	37,6417	W	121,0250	KF953077	KF953163	America
111	-		USA, Arizona, Huachuca Mountains	CAS 1095635	J.R. Shevock 29540	N	31,4597	W	110,3655	KF953078	KF953164	America
112	-		USA, California, Riverside Co, Murrieta	CAS 1080846	E. Laeger 3214	N	33,6006	W	117,1722	KF953079	KF953165	America
113	-		USA, California, Alameda Co, San Leandro	CAS 1095222	J.R. Shevock 29404	N	37,7010	W	122,1892	KF953080	KF953166	America

114	-		USA, California, Riverside Co, Temecula Canyon	CAS 1019492	J.R. Shevock 20489	N	33,4528	W	117,1744	KF953081	KF953167	America
115	-		USA, California, Los Angeles Co, Santa Susana Mountains	CAS1137829	J.R. Shevock 34041	N	34,3933	W	118,6842	KF953083	KF953169	America
116	-		USA, Tulare Co, Moorehouse	CAS 989805	J.R. Shevock 15646	N	36,1542	W	118,6583	KF953084	KF953170	America

GENERAL CONCLUSIONS

1. There were patterns of genetic structure and diversity in *B. argenteum* along an altitudinal gradient in Sierra Nevada Mountains of Spain. Those patterns completely support the EiE tenet. The lack of evidence for isolation by distance suggests that the first half of the tenet, "Everything is everywhere", fully applies (at a local level) in Sierra Nevada. The last part of the tenet, "but the environment selects", is supported by evidence for an environmentally-driven pattern of genetic differentiation that suggests adaptive divergence.
2. Genetic diversity on the mountains of Sierra Nevada peaks above 1,900 m a.s.l. and suggest that *B. argenteum* persisted in a range that was extensively glaciated during the late Pleistocene.
3. The worldwide reconstruction of the phylogeography of *B. argenteum* suggests that the extant patterns of genetic distribution are best explained by intercontinental dispersal rather than vicariance.
4. The strong phylogeographic signal between Antarctica and any other continental mass and the absence of a significant phylogeographic signal among any other pair of continents suggest that Antarctica is by far the most isolated continent of all in terms of bryophyte dispersal.
5. The hypothesis of ubiquitous dispersal, and therefore the EiE tenet at a global scale, is rejected by the significant genetic isolation of Antarctica and by the significant geographic partitioning of allele frequencies between pairs of continents.
6. Population genetic analyses, phylogeny, ancestral area reconstructions and molecular dating analyses indicate altogether that *B. argenteum* colonized Antarctica on at least three occasions and those events took place in time scales in the order of millions to hundred thousands of years. Thus, *B. argenteum*

successfully persisted *in situ* during several glacial cycles within the Pleistocene, Pliocene and perhaps late Miocene. Therefore, the hypothesis that the species (re-)colonize Antarctica after the last glacial maximum is rejected by the data.

7. Population genetic analyses, phylogeny, ancestral area reconstructions and molecular dating analyses indicate altogether that *B. argenteum* colonized the island of Tenerife on multiple occasions. Earlier events of colonization on the island took place in time-scales of hundred thousand years, well before the first human settlements and mainly came from Africa and Europe. Therefore, the hypothesis that humans introduced for the first time the species onto the island is rejected.
8. The patterns of genetic differentiation along altitudinal gradients in Tenerife suggest that environmental variation plays a key role in shaping the genetic structure of *B. argenteum*, as already suggested in the study from the mountains of Sierra Nevada. Nevertheless, genetic drift also plays a role in establishing the observed patterns of genetic variation in the island.
9. No signature of demographic shifts was detected in the populations of *B. argenteum* on Sierra Nevada, Antarctica or Tenerife.

The results and conclusions derived from the scientific research of this thesis contribute to a better understanding of the present and past biogeographic structure of cosmopolitan bryophyte species, which will be useful to an array of disciplines such as biogeography, bryophyte biology, evolutionary biology, ecology, and conservation management.