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Is the sword moss (*Bryoxiphium*) a preglacial Tertiary relict? ☆

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ABSTRACT

The disjunction of floras between East Asia, Southeast North America, West North America, and Southwest Eurasia has been interpreted in terms of the fragmentation of a once continuous mixed mesophytic forest that occurred throughout the Northern Hemisphere due to the climatic and geological changes during the late Tertiary. The sword moss, *Bryoxiphium*, exhibits a distribution that strikingly resembles that of the mesophytic forest elements such as *Liriodendron* and is considered as the only living member of an early Tertiary flora in Iceland. These hypotheses are tested here using molecular dating analyses and ancestral area estimations. The results suggest that the extant range of *Bryoxiphium* results from the fragmentation of a formerly wider range encompassing North America and Southeast Asia about 10 million years ago. The split of continental ancestral populations is too recent to match with a continental drift scenario but is spatially and temporally remarkably congruent with that observed in Tertiary angiosperm relict species. The timing of the colonization of Iceland from Macaronesian ancestors, about two million years ago, is, however, incompatible with the hypothesis that *Bryoxiphium* is the only living member of an early Tertiary flora of the island. Alaska was recurrently colonized from East Asia. The ability of *Bryoxiphium* to overcome large oceanic barriers is further evidenced by its occurrence on remote oceanic archipelagos. In particular, Madeira was colonized twice independently from American and East Asian ancestors, respectively. The striking range disjunction of *Bryoxiphium* is interpreted in terms of its mating system, as the taxon exhibits a very singular pattern of spatial segregation of the sexes.

1. Introduction

Cooling climates in the later part of the Tertiary period (65–2 million years ago; hereafter Ma) forced large assemblages of warm temperate to subtropical biotas to retreat from medium to high latitude circumboreal distributions southwards to large refugial regions in East Asia, Eastern and Western North America, and Southwest Eurasia, which preserved the warm wet climate that they needed (Wen, 1999; Milne and Abbott, 2002). The floras concerned are considered as Tertiary relict and have long captured the interest of botanists (Tiffney, 1985a,b; Wen et al., 2010). In angiosperms, over 100 genera exhibit disjunct distributions between two or more of these regions (Wen, 1999; Milne and Abbott, 2002; Milne, 2006), with disjunctions between Eastern North America and Eastern Asia being by far the most common (Xiang et al., 2000; Donoghue and Smith, 2004). The last Tertiary land connection between Eurasia and North America was the Bering land bridge, which experienced significant climate cooling from 15 Ma onwards, initiating divergence and speciation between North American and Eurasian species of many Tertiary relict genera (Milne and Abbott, 2002).

The tulip tree, *Liriodendron*, offers one of the most striking examples of the fragmentation of a once continuous mixed mesophytic forest that occurred throughout the Northern Hemisphere following climatic and geological changes during the late Tertiary and Quaternary (Wen, 1999; Xiang et al., 2000). *Liriodendron* currently occurs in Eastern North America (*L. tulipifera*) and Southeast Asia (*L. chinense*),

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but unequivocal fossils dating back to the Miocene in Idaho, upper Pliocene in Europe, middle and late Miocene in Japan (Xiang et al., 2000), and upper to middle Miocene in Iceland (Denk et al., 2005) attest of a previously much wider, and more continuous range.

Similar striking disjunctions are commonly observed in mosses, and are hence sought to be interpreted by a common process, namely vicariance (Shaw, 2001). However, molecular dating evidence revealed that patterns that are spatially congruent with an ancient vicariance scenario might in fact be temporally congruent with a more recent long-distance dispersal hypothesis (for review see Villarreal and Renner, 2014). In fact, bryophytes appear as extremely efficient dispersers based on world-scale patterns of floristic turnover (Patiño et al., 2014), correlations between floristic patterns and wind connectivity (Muñoz et al., 2004), experimental evidence (Lönnell et al., 2012, 2014; Sundberg, 2013) and substantial allele sharing among trans-oceanic populations (Szövényi et al., 2008; Shaw et al., 2014, 2015a,b). Furthermore, bryophytes may be prone to biotic dispersal across continents and oceans (Lewis et al., 2014). Altogether, these observations support the hypothesis that, in spore-producing plants (van Zanten and Pócs, 1981; Wolf et al., 2001) and other mobile organisms (Sanmartín and Ronquist, 2004; Yoder and Nowak, 2006; Perrie and Brownsey, 2007; Sanmartín et al., 2007; De Queiroz, 2014), dispersal has obscured any signature of vicariance. In fact, recent re-analysis of the only study inferring geographic vicariance due to continental drift in mosses (McDaniel and Shaw, 2003) yielded much younger ages for the relevant split that are incompatible with a continental drift scenario (Villarreal and Renner, 2014).

The sword moss *Bryoxiphium*, which exhibits a strikingly disjunct distribution spanning East Asia, North and Central America, Iceland, Mauritius, and Macaronesia, appears as one of the best remaining candidates for a vicariant origin in bryophytes. As Steere (1937) suggested, '*B. norvegicum* is an old species. It is, to judge from its present distribution, undoubtedly a member of a once widespread flora, which extended over the Northern Hemisphere in late Cretaceous or nearly Tertiary times. The great uniformity of appearance and lack of variation within each species are also marks of old groups. One is reminded here of some of the conservative and unchanging phanerogams, as *Ginkgo*, *Sequoia*, or *Liriodendron*, which may be contemporary in geological age with this loss. The fossil distribution of the tulip tree resembles the present distribution of *Bryoxiphium*, and the two may be members of the same flora, and a relic of an epoch with a much more equable climate' (Steere, 1937, p. 350). Given the extremely old stem age of 137 [62–219] Ma inferred for *Bryoxiphium* in the context of a dating phylogenetic analysis of the entire moss phylum (Laenen et al., 2014), it is tempting to see in the highly disjunct distribution of *Bryoxiphium*, which, as Steere (1937) noticed, is strikingly reminiscent of the past distribution of *Liriodendron*, the signature of such a relictual origin. In this context, *Bryoxiphium* has been considered to be the only living member of an early Tertiary flora in Iceland (Lawton, 1971). As Schuster (1983) noted indeed, and in contrast to the global equilibrium of higher plant species distributions with present climate (Araujo and Pearson, 2003), bryophytes are able to persist in microhabitats where a suitable microenvironment endures, long after the general climate of the region has changed. As Anderson (1963) suggested, 'the diminutive bryophytes, therefore, potentially offer a better clue to the solution of phytogeographical problems than do many vascular plants'.

Here we employed phylogeographic reconstructions based on phylogenetic analyses, molecular dating analyses, and estimations of ancestral ranges, to infer the historical biogeography of *Bryoxiphium* and test the hypothesis that its disjunct distribution results from the fragmentation of the once continuous mixed mesophytic forest that occurred throughout the Northern Hemisphere until the late Tertiary.

2. Methods

2.1. Taxon and molecular sampling

Bryoxiphium is the sole genus of the Bryoxiphiales (Goffinet et al., 2009). The plants are conspicuous and so readily recognized by their shiny, keeled-conduplicate leaves overlapping in two rows and becoming longer and subulate-awned toward the stem apex (Fig. 1), that misidentifications or oversight are unlikely to account for its hypothesized disjunct distribution. The genus is dioicous and sporophyte production is extremely rare across large parts of its range. *Bryoxiphium* comprises four taxa of very similar morphology and challenged taxonomic status (Hax, 2006) with a strikingly disjunct distribution (Fig. 1): *Bryoxiphium norvegicum* subsp. *norvegicum*, distributed in North America, East Asia, Mauritius and Iceland; *B. norvegicum* subsp. *japonicum*, distributed in East Asia; *B. madeirense* in Madeira; and *B. mexicanum* in Central America.

Thirty-nine specimens of *Bryoxiphium* were sampled from East Asia, Central and North America, Iceland and the Atlantic archipelagos of Madeira, Azores, covering the entire range except for the disjunct occurrence on Mauritius (Table 1 and see also Table S1 in supplementary material). *Drummondia sinensis* (accession numbers, FJ883993, FJ572610, FJ572456), *Trichostomum tenuirostre* (FJ884001, FJ572590, FJ572457), and *Orthodicranum montanum* (FJ572644, FJ883997, FJ572644, FJ572464) were used as outgroups based on large-scale phylogenetic analyses of mosses (Cox et al., 2010).

Plant material was ground in liquid nitrogen and total genomic DNA was isolated from each specimen using a Cetyltrimethylammonium Bromide (CTAB) extraction protocol without RNase treatment, and purified using the Geneclean III Kit (MP Biomedicals, OH, USA). Four chloroplast regions, including *rpoC*1 (Chase et al., 2007), *rps*4 (Nadot et al., 1994; Souza-Chies et al., 1997), *trn*G (Pacak and Szweykowska-Kulinska, 2000), and *trnL*–*trnF* (Taberlet et al., 1991) were selected for exhibiting the appropriate level of polymorphism at the species level in *Bryoxiphium*. These loci were amplified and sequenced following the protocols of Patiño et al. (2015a).

Forward and reverse sequencing of the purified amplicons was conducted using the BigDye Terminator v. 3.1 cycle sequencing kit (Applied Biosystems, Foster City, CA, USA). Forward and reverse sequences were assembled and edited using Sequencher 4.01 (Gene Codes, Ann Arbor, MI, USA). Contigs were aligned using Clustal W (Larkin et al., 2007) and GUIDANCE (Penn et al., 2010), and the alignments obtained were manually refined using PhyDE (Müller et al., 2006). Regions of incomplete data at the 3′ and 5′ ends of the target loci were excluded from subsequent analyses. All GenBank accession numbers are available in Table 1.

2.2. Phylogenetic and molecular dating analyses

The concatenated dataset was analyzed in PartitionFinder (Lanfear et al., 2012) to select the best partitioning scheme, using the greedy algorithm with linked branch lengths and Bayesian information criteria. Two partitions were selected: the genes *rpoC1, rps4, trnL* (HKY+G), and *trnG* (HKY+I).

In order to set the phylogeny within an explicit time-frame, we ran uncorrelated lognormal relaxed clock analyses of our molecular data sets using BEAST version 1.8.0 (Drummond et al., 2012). Because the inclusion of identical sequences results in many zero length branches at the tip of the tree and can cause the model to over-partition the data set (Reid and Carstens, 2012), we performed the BEAST analyses at the level of haplotypes. Twenty-six haplotypes were defined (Table 1) with DnaSP version 5.10.1 (Librado and Rozas, 2009) and the haplotypic identity of each specimen is provided in Table 1 (see also Table S1 in supplementary material).