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INTERCONTINENTAL MEDITERRANEAN DISJUNCT MOSSES: MORPHOLOGICAL AND MOLECULAR PATTERNS[1]

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This study focused on three species that occur disjunctly between western North America and the Mediterranean region of southern Europe, northern Africa, and western Asia, forming the so-called Madrean-Tethyan distribution pattern. Quantitative morphological characters were measured in New and Old World plants to find any subtle phenotypic differentiation between the disjunct populations. Sequences from the nuclear ribosomal internal transcribed spacer region were obtained from the same populations to assess differentiation at the molecular level and to compare molecular diversity with patterns of morphological similarity among plants. Little or no morphological differentiation existed between New and Old World plants in any of the species, but internal transcribed spacer (ITS) sequences revealed some phylogeographic structure. Patterns of morphological similarity in all three species were incongruent with phylogeographic structure revealed by sequence data. New World populations were more variable than Old World populations at the molecular level in the three species. Despite some evidence for differentiation between disjunct plants, no plausible mutation rate would date the divergence at ≥20 million years ago (MYA), as implied by the Madrean-Tethyan hypothesis. Recent long-distance dispersal is a more likely explanation for intercontinental disjunctions in these species.

Key words: bryophyte phylogeography; *Claopodium*; cryptic speciation; *Dicranoweisia*; disjunctions; internal transcribed spacer; Madrean-Tethyan; *Scleropodium*.

Moss species tend to have much broader geographic ranges than species of seed plants. Few mosses are cosmopolitan and weedy, i.e., common in anthropogenic habitats and likely dispersed by human activity (e.g., *Bryum argenteum* Hedw., *Funaria hygrometrica* Hedw.). Many species, in contrast, have well-defined bi- or tricentric distributions that parallel disjunct patterns long known in seed plants, for example, eastern North America-eastern Asia (Iwatsuki, 1958), western North America-Europe (Schofield and Crum, 1972; Schofield, 1988). Such seed plant disjunctions as these have been interpreted generally in the context of previously continuous ranges disrupted by climate change during the Tertiary (see, however, Wen, 1999). Because many mosses exhibit similar patterns, it has been argued that disjunctions within moss species share a common historical explanation with seed plant genera. Two corollaries of this view are that many extant moss species are exceedingly old and that they have been in a state of morphological stasis for tens of millions of years (Crum, 1972).

This paper deals with mosses that are disjunct between the Pacific coast of North America and the Mediterranean region of southern Europe (and adjacent parts of Africa and western Asia). Patterns of disjunction between southern Europe and western North America have gained the attention of paleobotanists (Axelrod, 1973, 1975; Wolfe, 1975), systematists (Raven, 1973; Raven and Axelrod, 1978; Fritsch, 1996, 2001), and ecologists (Cody and Mooney, 1978). Axelrod (1973, 1975) proposed a vicariance explanation for Mediterranean seed plant disjunctions embodied in the Madrean-Tethyan hypothesis. Accordingly, Mediterranean disjuncts reflect fragmentation of an ancestral, widespread, sclerophyllous flora that existed across western North America and Eurasia under subhumid conditions until the end of the Oligocene, about 25–20 million years ago (MYA). This theory has been challenged on paleobotanical grounds because of limited evidence that sclerophyllous vegetation ever formed a continuous band between presently disjunct regions (Wolfe, 1975). Inferences from molecular biogeographic analyses of extant taxa have shed new light on this question. Divergence dates estimated from DNA are consistent with Tertiary vicariance in *Styrax* (Fritsch, 2001), *Arbutus* (Hileman et al., 2001), and *Datisca* (Liston et al., 1989a, 1992; Liston, 1997), although inferences from isozymes are sometimes in conflict (Fritsch, 1996).

This paper focuses on three species of moss, *Claopodium whippleanum* (Sull.) Ren. & Card., *Dicranoweisia cirrata* (Hedw.) Lindb., and *Scleropodium touretii* (Brid.) L.F. Koch, that are disjunct between the west coast of North America from California to British Columbia or Alaska and the Mediterranean region of southern Europe, northern Africa, and southwestern Asia. Species disjunct between Europe and the Pacific coast of North America account for approximately 5% of the mosses and 7% of the liverworts of Pacific North America (Schofield, 1984). While liverworts with this pattern tend to be widespread in Europe, the mosses are more typically Mediterranean (i.e., regions with hot dry summers and wet winters). Although infraspecific disjunctions of this magnitude are known in angiosperms (e.g., *Styrax*—Fritsch, 1996, 2001; *Senecio*—Liston et al., 1989b), more often the disjunction is reflected in the distribution of congeneric species or groups of species (e.g., *Arbutus*—Hileman et al., 2001). The mosses considered in this paper, in contrast, appear to be morphologically undifferentiated between continents, and the North American and European populations have never been proposed to represent different species. If their disjunct patterns reflect ancient vicariance dating to the Oligocene, morphological evolution has remained at a virtual standstill for some 20 or more million years.

The three species considered in this paper are fairly com-

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mon in live oak or dry Douglas-fir forests in coastal areas of California northward to southern Canada. In southern Europe and northern Africa, they occur in ecologically similar zones and, although not rare, may be less common than in western North America. (*Claopodium whippleanum* is in fact rather rare in southern Europe.) They do not occur in the eastern United States, but isolated records exist for *Dicranoweisia cirrata* eastward in North America to Idaho and also in Mexico. They are not, in any event, widespread species in the Northern Hemisphere but rather have highly disjunctive distributions that are essentially bicentric. The purposes of this study were (1) to determine if subtle morphological differentiation exists between North American and European populations and (2) to assess cryptic differentiation between disjunct population systems using DNA sequence data from the nuclear ribosomal internal transcribed spacer (ITS) region. Although divergence times could not be reliably estimated in the absence of a fossil record for calibration, we aimed to test the Madrean-Tethyan prediction that intercontinentally disjunct populations are clearly divergent at the molecular level.

MATERIAL AND METHODS

Study organisms—*Claopodium whippleanum* (Leskeaceae) occurs from California to Alaska (Lawton, 1971) and grows on mesic to xeric soils at the bases of trees, on humus or rotting wood, and on exposed soil banks. Gametophytes are unisexual and sporophytes occur occasionally in western North American populations. In the Mediterranean area, it is a rare species known from some countries and islands of southwest Europe (Düll, 1985, 1992) and northern Africa (Jiménez et al., 2002). It grows on humid screes in *Quercus rotundifolia* and *Pinus* forests with acidic soils. It is rarely found with sporophytes. Eighteen populations were sampled for morphological and molecular analyses (listed in the Appendix, http://ajbsupp.botany.org/v90/). These include five populations from the Old World and 13 from North America.

Dicranoweisia cirrata (Dicranaceae) generally grows as small round colonies on rocks, in open live oak forests, or sclerophyllous chaparral in the United States and in comparable microsites within coniferous forests northward to Alaska. It is also known from Arizona and northern Mexico (Sharp et al., 1994). In Europe *D. cirrata* is widespread northward to southern Scandinavia. It is also known from the Macaronesian Islands, southwest Asia, and northern Africa (Hill et al., 1992), where it usually forms cushions on tree trunks, especially at the base of conifers. The species has bisexual gametophytes (autoicous; separate male and female buds), and plants commonly produce sporophytes. Thirty-two populations were included in the analysis, 10 from Old World and 22 from North America (see Appendix at http://ajbsupp.botany.org/v90/).

Scleropodium touretii (Brachytheciaceae) grows in the same sorts of habitats as *Claopodium whippleanum* in western North America, but is more common. It typically occurs on soil banks and sometimes rocks on a thin layer of soil. It has unisexual gametophytes, and sporophytes are produced occasionally. In Europe it is present in the Mediterranean, central and western parts of the continent, north to Scotland and Denmark, also in Macronesia, northern Africa, and southwestern Asia (Hill et al., 1994). Its ecology is very similar to that in North America. Our sampling included six populations from the Old World and seven from western North America.

Morphological measurements—Analyses of specimens we collected were supplemented with specimens in DUKE and MUB. Additional North American collections were provided by J. Shevock (National Forest Service and UC), W. B. Schofield (UBC), and F. Lara (Universidad Autónoma de Madrid). Sampling for morphological and molecular analyses utilized different plants from the same collection. Except where noted, vouchers for the analyses are preserved in DUKE and MUB; in the case of loans from UC and UBC, the original collections are preserved in those herbaria with a single stem used for the analyses preserved at DUKE.

Claopodium whippleanum and Scleropodium touretii—Morphological measurements included both continuous and discrete characters. Quantitative characters measured for each species are listed in Table 1. For growth form, type of branching, position of leaves on the stem, and a general impression of the voucher was considered. Two microscopic slides were prepared with about 15–20 leaves each, one with leaves of the primary or secondary stem and the other with branch leaves. For each quantitative leaf character, 10 measurements were made from these preparations always taking length and width from the same leaf. Leaf apex length was estimated by measuring the distance from the base to the apex (defined by where the angle of the leaf margin changed fairly abruptly, to the tip of the leaf). For leaf cell dimensions, 10 cells were measured, always taking length and width from the same cell. Upper cells were measured in the upper third of leaves, basal cells in the one or two most basal rows of cells in the leaf, and alar cells in the outer angles of leaves. Because branch and stem leaves are different in size and shape in *Claopodium* and *Scleropodium* (as in many other pleurocarpous mosses), leaf and cell measurements were made on both types. Sporophyte features were not taken into account for these species because of their scarcity in the collections studied.

Dicranoweissia cirrata—For gametophyte size, the lengths of 10 plants were measured, and one of the plants was selected for additional measurements of leaf and cell dimensions. A microscopic slide was prepared with about 15–20 leaves from the upper third of the stem. For leaf length and width, and costa width, 10 leaves were measured. Cell dimensions were taken from all the leaves in the slide preparation. When present, sporophytes were described, but because of their scarcity a maximum of five were measured.

Morphological analyses—Analyses to detect geographic patterns in morphological variation included both uni- and multivariate approaches. Means from multiple measurements per population (previous section) were estimated for use in the statistical analyses comparing Old and New World samples. Within each species, populations were classified as Old or New World, and significant differentiation in each morphological trait was tested separately using univariate analyses of variance (ANOVAs). The ANOVA models specified region (Old vs. New World) as the main effect with error variances derived from populations within these regions.

Multivariate patterns of variation were assessed using principal components analyses (PCA) and UPGMA cluster analyses. For the PCA, plots of populations within each species in relation to the first two principal components were constructed with populations designated as New or Old World (N and o in Fig. 1). Only characters with no missing data for any of the populations are shown in the plots. (For example, gemmae characters in *Dicranoweisia* are not included in the PCA because some populations lacked them.) All characters were included in UPGMA cluster analyses for each species (with missing data coded as appropriate). Statistical analyses were conducted in SAS (SAS Institute Inc., Cary, North Carolina, USA).

DNA extraction, PCR amplification, and sequencing—DNA extractions followed a modification of Doyle and Doyle's (1990) protocol, and nuclear ribosomal DNA (nrDNA) was amplified as in Shaw (2000). Our nrDNA sequences include the last 5–25 bases of the 18s ribosomal RNA gene, *ITS*-1, 5.8s rRNA gene, *ITS*-2, and the first few bases of the 26s rRNA gene. Boundaries of the subregions were identified by comparison to available GenBank sequences. Multiple ITS haplotypes were detected within plants from two populations of *Scleropodium* (MED3, SP7), and two haplotypes were identified from each based on cloned sequences. Initial polymerase chain reaction (PCR) products were cloned using the TOPO-TA cloning kit. A 0.8-μL sample of fresh PCR product was mixed with 0.2 μL of salt solution and 0.2 μL of vector and incubated at room temperature for 30 min. The ligation/cloning reaction was added to 10–15 μL of competent cells and incubated on ice for 30 min. The cells were heat shocked for 30 s at 42°C, transferred to ice, and 150 μL SOC medium (at room temperature) was added to the cells. The

TABLE 1. Morphological characters in New and Old World populations of *Claopodium whippleanum*, *Dicranowiesia cirrata*, and *Scleropodium touretii* (means ± 1 SE). *$P \leq 0.05$; **$P \leq 0.01$; ns = not significant. All units in micrometers.

Character	Old World	New World	Significance level
Claopodium whippleanum			
Stem leaf length	654.6 ± 54.3	661.4 ± 29.5	ns
Stem leaf width	309.5 ± 27.4	298.0 ± 16.5	ns
Stem leaf costa width	28.0 ± 2.1	30.2 ± 1.3	ns
Stem leaf apical cell length	20.0 ± 0.6	21.8 ± 0.9	ns
Stem leaf cell length	13.6 ± 0.6	14.5 ± 0.6	ns
Stem leaf cell width	5.6 ± 0.3	5.5 ± 0.1	ns
Branch leaf length	581.9 ± 44.0	625.5 ± 29.6	ns
Branch leaf width	236.0 ± 26.6	243.3 ± 11.0	ns
Branch leaf costa width	25.1 ± 1.8	28.5 ± 1.3	ns
Branch leaf apical cell length	20.0 ± 0.7	20.0 ± 0.6	ns
Branch leaf cell length	12.7 ± 0.4	12.3 ± 0.3	ns
Branch leaf cell width	5.9 ± 0.1	5.7 ± 0.2	ns
Branch leaf basal cell length	16.5 ± 0.6	15.4 ± 0.7	ns
Branch leaf basal cell width	6.3 ± 0.2	6.5 ± 0.2	ns
Dicranoweisia cirrata			
Plant height	8.9 ± 1.1	12.1 ± 0.6	**
Leaf length	1934.9 ± 71.9	2134.2 ± 56.6	*
Leaf width	373.6 ± 24.9	379.5 ± 9.2	ns
Costa width	49.1 ± 1.1	48.6 ± 1.4	ns
Upper cell diameter	9.3 ± 0.4	10.0 ± 0.2	ns
Basal cell length	31.3 ± 0.9	37.0 ± 1.6	*
Basal cell width	15.6 ± 0.5	14.1 ± 0.3	*
Seta length	5.6 ± 0.6	5.1 ± 0.3	ns
Capsule length	1387.1 ± 80.0	1486.1 ± 54.4	ns
Capsule diameter	588.8 ± 32.4	559.1 ± 17.1	ns
Peristome length	112.5 ± 3.5	120.5 ± 4.4	ns
Spore diameter	17.9 ± 0.6	17.3 ± 0.3	ns
Gemma length	117.1 ± 11.7	127.6 ± 14.2	ns
Gemma diameter	40.5 ± 1.2	46.9 ± 1.9	*
Scleropodium touretii			
Stem leaf length	1306.2 ± 59.8	1356.3 ± 86.0	ns
Stem leaf width	760.9 ± 70.8	767.9 ± 52.9	ns
Stem leaf apical cell length	78.1 ± 17.6	99.6 ± 33.5	ns
Stem leaf costa length (% leaf)	65.4 ± 2.1	60.0 ± 3.7	ns
Stem leaf costa width	61.7 ± 8.6	53.9 ± 6.6	ns
Stem leaf upper cell length	57.5 ± 5.1	60.2 ± 4.1	ns
Stem leaf upper cell width	4.6 ± 0.2	5.0 ± 0.3	ns
Stem leaf basal cell length	28.4 ± 2.0	29.3 ± 2.4	ns
Stem leaf basal cell width	10.9 ± 0.4	11.0 ± 0.6	ns
Stem leaf alar cell length	22.9 ± 2.5	23.4 ± 2.6	ns
Stem leaf alar cell width	13.3 ± 0.7	13.1 ± 0.7	ns
Branch leaf length	1212.8 ± 54.8	1269.6 ± 88.6	ns
Branch leaf width	655.2 ± 31.1	665.4 ± 72.9	ns
Branch leaf apical cell length	97.8 ± 21.4	53.8 ± 13.5	ns
Branch leaf costa length (% leaf)	72.4 ± 3.1	73.8 ± 2.5	ns
Branch leaf costa width	53.6 ± 3.5	50.9 ± 6.4	ns
Branch leaf upper cell length	54.5 ± 1.9	54.8 ± 4.3	ns
Branch leaf upper cell width	4.7 ± 0.3	4.8 ± 0.3	ns
Branch leaf basal cell length	27.0 ± 2.1	28.1 ± 0.4	ns
Branch leaf basal cell width	10.5 ± 0.4	10.6 ± 0.4	ns
Branch leaf alar cell length	24.6 ± 2.4	20.1 ± 1.9	ns
Branch leaf alar cell width	11.3 ± 0.7	11.3 ± 0.6	ns

mixture was shaken horizontally at 200 rpm for 1 h. The broth culture was plated on agar (IPTG 0.5 mmol/L, X-gal 80 μg/mL, tetracycline 12.5 μg/mL, ampicillin 100 μg/mL) and incubated overnight at 37°C. Colonies containing the vector with a PCR insert were used as template for a second PCR amplification.

Phylogenetic analyses—Sequence chromatograms were compiled using Sequencher software (version 2.0, Gene Codes, Ann Arbor, Michigan, USA) to produce contigs based on nucleotide identifications from both DNA strands. All sequences were aligned by eye, with gaps inserted where needed to preserve nucleotide homology. Separate alignments were made for each species. A total of five indels and one duplication were scored (0,1) across the three species as follows; *Claopodium*, 2; *Dicranoweisia*, 1; *Scleropodium*, 3. *Claopodium* had a 17-base pair (bp) insertion/deletion (indel) in the ITS-1 and a three-nucleotide (apparent) duplication (GTT) in ITS-2. The remaining indels in other species were single-nucleotide differences in ITS-1.

The ITS region was sequenced for outgroup taxa in order to root phylogenetic trees from each study species: *Claopodium crispifolium* (Hook.) Ren & Card. for *C. whippleanum*, *Dicranoweisia crispula* (Hedw.) Lindb. *ex* Milde for *D. cirrata*, and *Scleropodium obtusifolium* (Jaeg.) Kindb. *in* Macoun &

Kindb. for *S. touretii*. For *Claopodium* and *Dicranoweisia*, however, outgroup sequences, although readily alignable, were sufficiently divergent relative to ingroup sequences that the root could attach almost anywhere, yielding completely unresolved topologies in the strict consensus trees for rooted phylogenies. Consequently, a rooted tree is presented only for *Scleropodium*. Although historical inferences for *Claopodium* and *Dicranoweisia* are limited by having only unrooted networks, patterns of genetic diversity and differentiation could still be investigated.

Because of the small sizes of the three data sets, exhaustive searches for the most parsimonious trees were conducted in PAUP (Swofford, 2001). Trees were produced in McClade (Maddison and Maddison, 1992) to reflect relationships implied by unweighted pair group method with arithmetic means (UPGMA) clustering of morphological similarities in each species. These trees were enforced as topological constraints in parsimony analyses of ITS data. Most parsimonious (MP) trees under the constraints were compared to unconstrained trees using the parametric Kishino-Hasegawa and nonparametric Templeton tests as implemented in PAUP. Additional topological constraints in which monophylly of North American vs. European populations was forced were also compared to unconstrained ITS trees. The MP trees for each species produced under such constraints were compared to the unconstrained trees.

Population genetic analyses—Estimates of nucleotide diversity and differentiation between North American and European populations of each species were conducted using SITES (Hey and Wakeley, 1997) and ARLEQUIN version 2.00 (Schneider et al., 2000). Two estimates of theta (θ) were calculated separately for disjunct populations of each species to evaluate levels of molecular diversity in North American and Old World plants. The numbers of pairwise differences between pairs of haplotypes yields an estimate of $\theta\pi$ (Tajima, 1983), and θs is based on the numbers of segregating sites (Watterson, 1975). Patterns of differentiation were assessed by the number of fixed differences between plants disjunct on different continents, the number of shared polymorphic nucleotide sites, and genetic diversity among populations (F_{ST}). The F_{ST} and tests of its significance were computed according to Excoffier et al. (1992), using ARLEQUIN (Schneider et al., 2000).

RESULTS

Morphological analyses—All specimens of *Claopodium whippleanum* examined from both Old and New World populations were very similar morphologically, with the exception of population BC18 from Canada (Vancouver Island). Plants in this population were erect rather than prostrate, as is typical of this species, and the leaves were not conduplicate as in the rest of specimens studied.

American specimens of *Scleropodium touretii* appeared to show greater qualitative variability than those from Europe, where they seemed homogeneous. However, a difference in level of variation was not detected in statistical analyses of the quantitative data.

The morphology of *Dicranoweisia cirrata* has not been accurately described in floristic treatments. According to descriptions in moss floras for Britain and Ireland (Smith, 1978), Fenncoscandia (Nyholm, 1986), Mexico (Sharp et al., 1994), and the Pacific Northwest of North America (Lawton, 1971), *D. cirrata* has smooth upper laminal cells, 9–14 μm wide, alar cells not forming auricles or only slightly differentiated, and lamina unistratose or bistratose at apex and margins. We observed that this species is more variable than has been described. For instance the upper cells range from (6–)8–13 μm wide, smooth in the Old World specimens but sometimes papillose in the American samples. The alar cells are sometimes clearly differentiated in the American specimens but never in the Old World. In both New and Old World plants, the upper part of the lamina of the leaf can be uniformly bistratose, bistratose in patches, or unistratose. The margins are consistently bistratose or sometimes tristratose at least in the upper and median part of leaf; they are only unistratose in the basal part of leaf. Some of these characters have been described in the related species, *D. crispula* (Hedw.) Milde, but not in *D. cirrata*.

There was no evidence of strong morphological differentiation in quantitative traits between Old World and North American populations of any species. For *Claopodium* and *Scleropodium*, no character even approached significance, whereas limited differentiation was suggested for *Dicranoweisia* (Table 1). North American plants of *Dicranoweisia* tended to be taller, with larger leaves, larger basal leaf cells, and larger asexual gemmae. Despite these subtle differences in gametophyte sizes between North American and European plants of *Dicranoweisia*, comparable differences in sporophyte dimensions were not detected (capsule and peristome traits in Table 1).

Additional subtle patterns of morphological variation were noted in the gametophytes of *Dicranoweisia*. Five samples from North America (BC37, OR40, CA103, CA1276, and CA1277) are here referred to as NA2 to distinguish them from the rest of the North American plants (NA1) and from the Old World plants (OW). Plants from the NA2 populations were taller (OW = 8.95 mm, NA1 = 11.75 mm, and NA2 = 13.18 mm) with longer (OW = 31.3 μm, NA1 = 33.7 μm, and NA2 = 48.4 μm) but narrower (OW = 15.6 μm, NA1 = 14.6 μm, and NA2 = 12.4 μm) basal cells. The last two measurements are related to the presence of auricles in these five samples; such auricles are absent in all Old World samples and are only slightly developed in one sample from NA1. Papillae are absent from the leaf cells in all samples from the Old World, present in seven of 17 populations from NA1, and present in all five populations of NA2. On the other hand, gemmae were present in six of 10 populations from the Old World, in seven of 17 populations from NA1, and none of the five populations from NA2. These morphological variations do not correspond to any geographic pattern in North America, nor are they reflected in the ITS data. (In fact, plants belonging to the NA2 group of populations can be found on the widely divergent branches of the ITS tree.) These five populations do not belong to *Dicranoweisia crispula*, a related species that has auriculate leaves and papillose leaf cells, but is differentiated from *D. cirrata* by such other characters as plane leaf margin, smaller midleaf cells, fine longitudinal cuticular striations on the leaf cells, and ovate to ellipsoid capsules.

An absence of general morphological differentiation between Old World and North American populations of the three species was evident in the multivariate analyses. Neither the PCA nor UPGMA cluster analyses yielded any hint of generalized differentiation (Figs. 1–4). In many instances, European and North American populations were most similar to one another, and the phenograms (Figs. 2–4) suggest a random pattern with regard to New and Old World populations. Within these broader geographic regions, proximate populations did not tend to be more similar than those separated by greater distances. It is clear that morphological variation is not structured geographically to any significant extent in any of the three species we investigated.

Molecular analyses—The 5.8 rRNA gene was 157 bp in all three species. In *Claopodium*, ITS-1 was 236–254 bp with 29 variable nucleotide sites and one indel, and ITS-2 was 277–

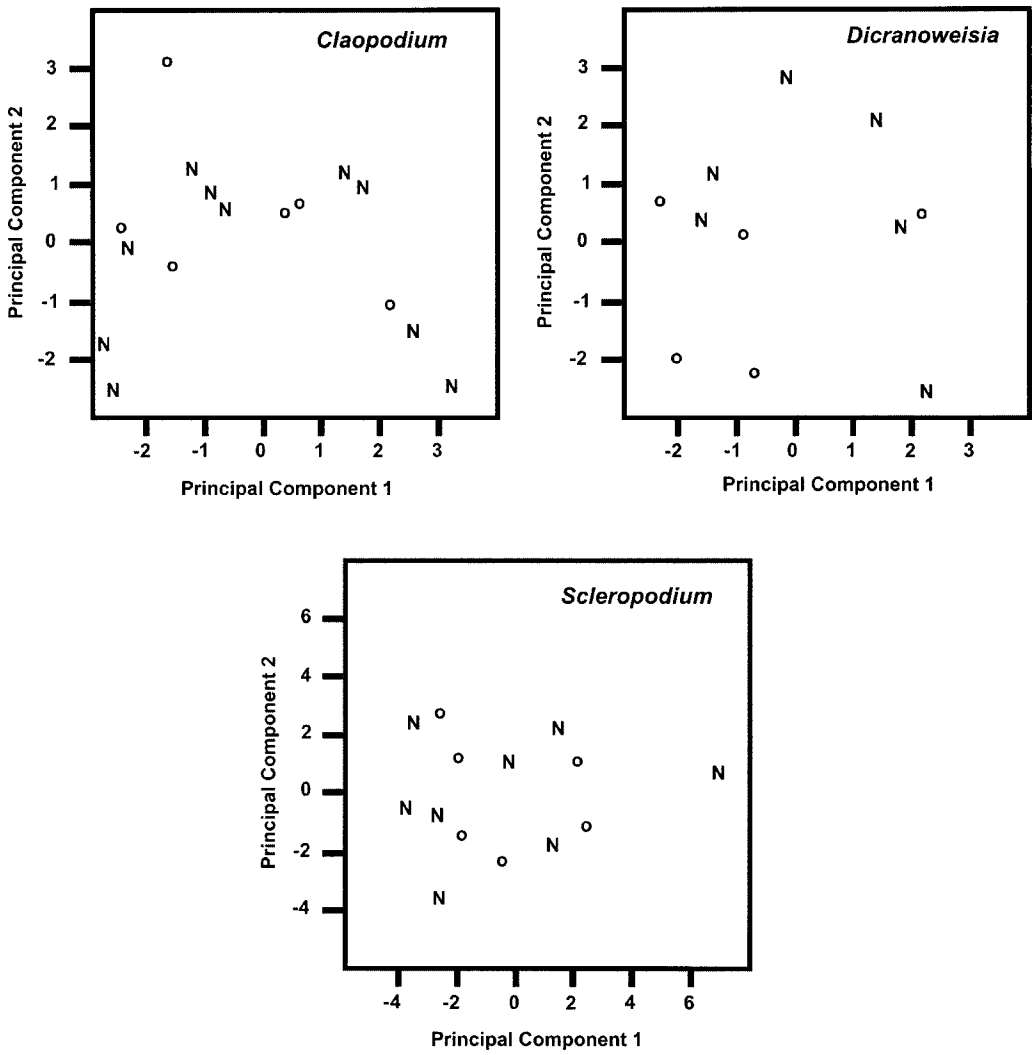

Fig. 1. Plots of New (N) and Old (o) World populations of *Claopodium whippleanum*, *Dicranoweisia cirrata*, and *Scleropodium touretii* in relation to the first two principal components in a multivariate analysis of morphological characters. See Table 1 for lists of characters measured in each species.

279 bp with 30 variable sites and two indels. The 5.8s rRNA gene was invariant. Two equally most parsimonious trees were found in an exhaustive search of the ITS data (Fig. 2). The five European populations were monomorphic in terms of ITS sequences and were differentiated from North American populations by a single mutation (Fig. 2). North American populations, in contrast, were more variable, with 37 mutations differentiating populations. Among the North American plants, three nucleotide sites (two in ITS-1 and one in ITS-2) were polymorphic for more than two nucleotides. Both $\theta\pi$ and θs were high for North American populations of *Claopodium* relative to the two other species (Table 2). Theta (θ) was of course zero for the monomorphic European populations. Only about 20% of the nucleotide variation in *Claopodium* was attributable to differentiation between Old and New World plants, but F_{ST} was significantly greater than zero.

Two populations from California (CA1265, CA1268) shared an ITS haplotype with a population from British Columbia (BC17), and two other California populations (CA1266, CA105) likewise shared a haplotype. Otherwise, all of the American populations were unique, although no fine-scale geographic patterns were evident among the populations. When the phylogenetic analysis of ITS sequences was constrained to the branching topology suggested by UPGMA clustering of morphological variation, tree length increased from 39 steps (unconstrained) to 65 steps, an increase of 67%, and homoplasy indices increased substantially (Table 3). The constrained and unconstrained trees were significantly different using both the Kishino-Hasegawa (parametric) and Templeton (nonparametric) tests. Constraining the analyses to enforce monophylly of North American vs. European populations did not increase tree length.

In *Dicranoweisia*, ITS-1 was 291–292 bp with nine variable nucleotide sites and two indels, and ITS-2 was 288 bp with seven variable sites. One site was polymorphic in the 5.8s rRNA gene. An exhaustive search of the ITS data yielded a single most parsimonious tree with no homoplasy (Fig. 3, Table 3). A group of 17 populations, including plants from both North America and Europe, were monomorphic and identical in terms of ITS sequences. This group is distinguished by at least three mutations from five other populations, all Old World. This latter group of Old World plants is distinguished from all remaining populations, all American, by a single mutation. Within the latter group of American plants, nine pop-

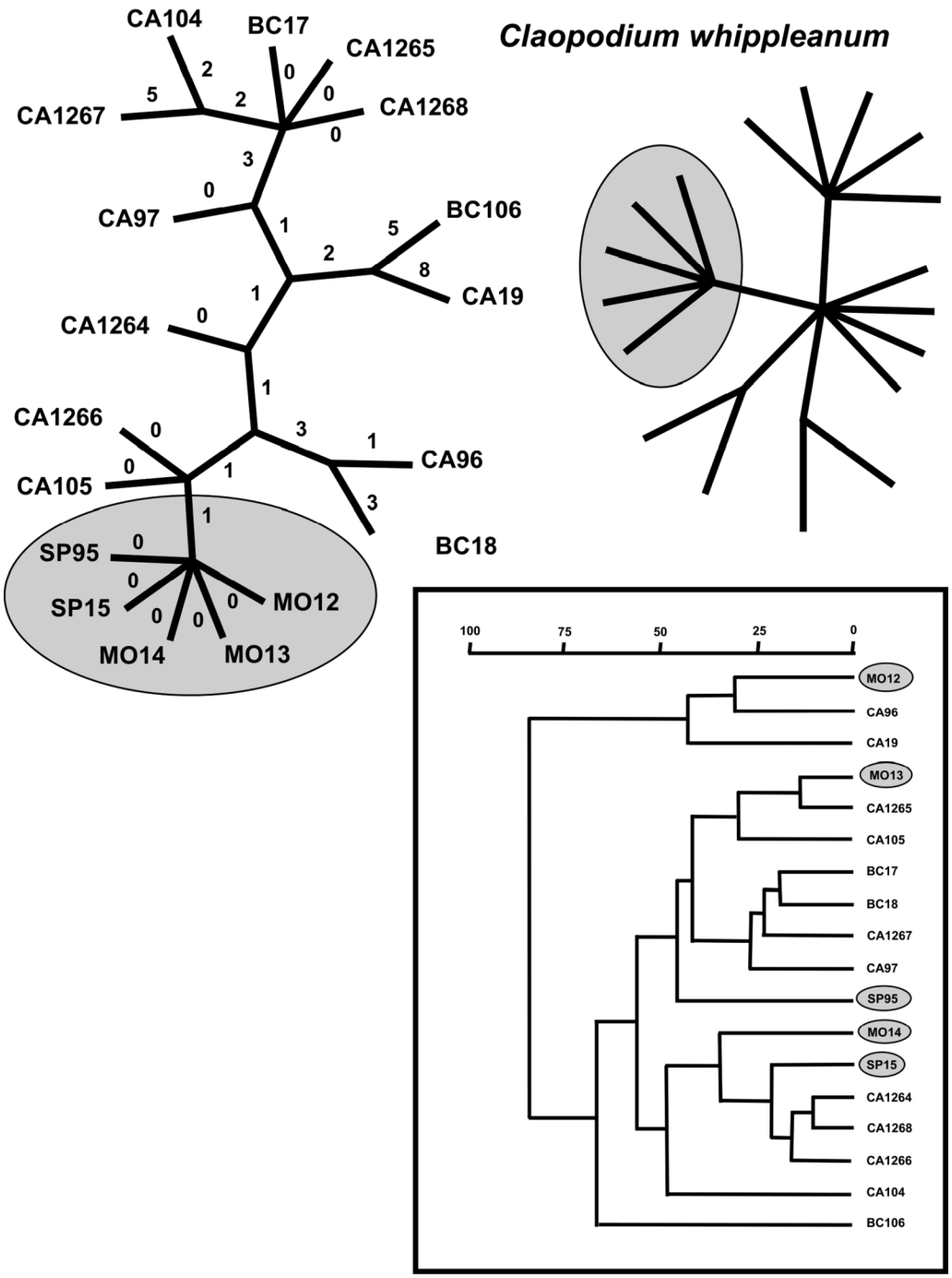

Fig. 2. *Claopodium whippleanum*. One of two most parsimonious networks (top, left) and the consensus network (top, right) from ITS sequence data and a UPGMA phenogram based on morphological characters listed in Table 1.

ulations from the United States are differentiated by five mutations from the Mexican population, which was most similar to the European plants (Fig. 3). Constraining North American and European populations as monophyletic groups increased branch length by four steps over the unconstrained analyses. The two topologies were significantly incongruent at $P < 0.05$ (Kishino-Hasegawa and Templeton tests).

Constraining the phylogenetic analysis of the ITS data to the branching topology revealed in the UPGMA phenogram from morphological data increased branch length from 16 steps to 103 steps (500%) and, not surprisingly, estimates of homoplasy increased equivalently (Table 3). Neither molecular nor morphological data resolved European and North American populations as distinct groups, and grouping of populations was highly incongruent between data sets. California populations CA1271 and CA1274, for example, were different in terms of ITS sequences but were very similar morphologically (Fig. 3). In contrast, the Spanish populations SP30 and

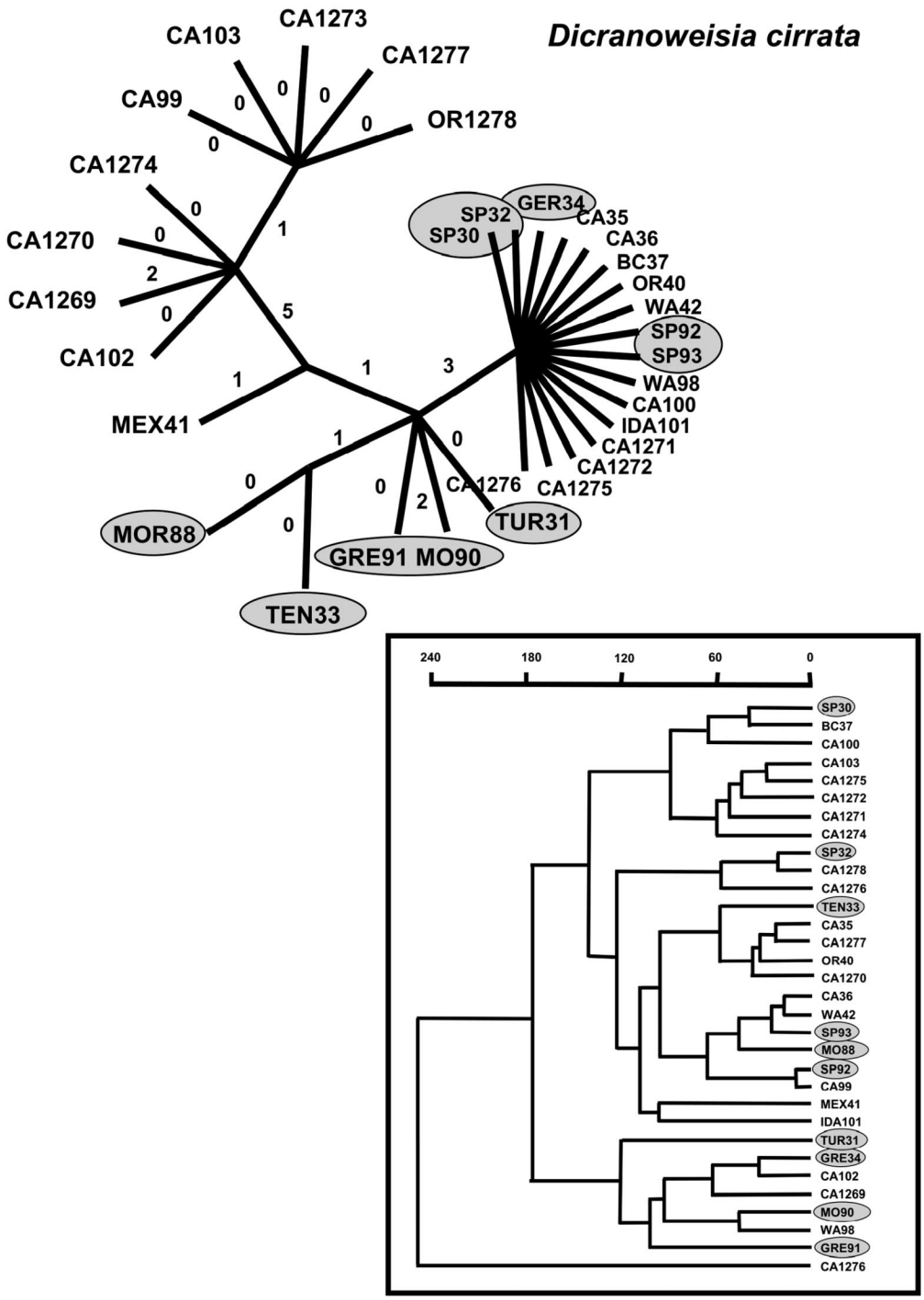

Fig. 3. *Dicranoweisia cirrata*. Single most parsimonious network (top) and a UPGMA phenogram based on morphological characters listed in Table 1.

SP32 had identical ITS sequences but were relatively different morphologically.

Within the group of North American plants distinguished by five mutations, two subgroups differed by a single mutation, and one population (CA1269) was characterized by two autapomorphies. Otherwise, populations were monomorphic. Taking into account all of the North American plants, however, $\theta\pi$ was more than twice as high in North American than European plants, and θs was also higher (Table 2). However, standard deviations of these estimates were high and overlapping. The F_{ST} for North American vs. European plants was lower than in either of the other two species, but was significantly greater than zero.

In *Scleropodium*, ITS-1 was 286–289 bp with eight variable nucleotide sites and four indels, and ITS-2 was 292 bp with seven of them variable. Phylogenetic analyses of ITS sequences from *Scleropodium* yielded one most parsimonious tree (Fig. 4). Two samples from the Old World, populations MO3

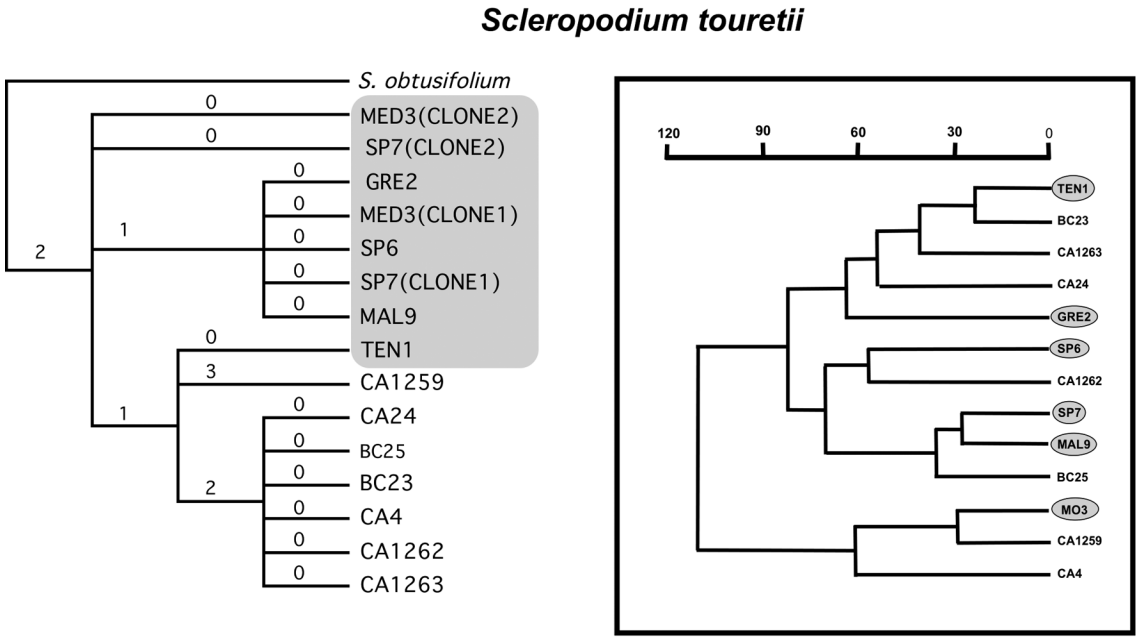

Fig. 4. *Scleropodium touretii*. Single most parsimonious tree, rooted with a sequence from *S. obtusifolium* (shaded) and a UPGMA phenogram based on morphological characters listed in Table 1.

and SP7, yielded sequences that suggested intragenomic polymorphism. Subsequently, two ITS sequences differing in a single nucleotide substitution were cloned from each of these plants. One of the two cloned sequences from each plant (clone 1 in Fig. 4) was identical to ITS sequences obtained from other European plants of *Scleropodium*, whereas the other (clone 2) was uniquely shared by these two populations. Clone 2 differed from the outgroup by only two mutations and may represent an early diverging ITS sequence within *S. touretii* (Fig. 4).

Inclusion of an outgroup sequence from a North American population of *S. obtusifolium* in the phylogenetic analysis indicates that the North American populations are nested within a paraphyletic basal grade of European populations of *S. touretii*. Clone 2 of the two polymorphic plants is most similar to the outgroup sequence, and the widespread European ITS haplotype differs by only one additional substitution. A population from Tenerife (TEN1) shares a synapomorphic mutation with the seven North American populations. The remaining North American populations, with the exception of CA1259, are united by 2 mutations (Fig. 4). The F_{ST} between North American and European populations was higher than for *Claopodium* and *Dicranoweisia* and was significantly greater than zero. However, constraining the ITS analysis to enforce monophylly of the North American and European populations increased tree length by only one step, and the two topologies were not significantly incongruent. Constraining the ITS phylogeny to the topology obtained from the UPGMA analysis of morphological data increased branch length from eight to 23 steps (190%), with an expected increase in homoplasy (Table 2). As in the previous two species, relationships implied by morphological vs. molecular data were significantly incongruent in terms of molecular evolution, as assessed by the Kishino-Hasegawa test. Neither American nor Old World popula-

TABLE 2. Estimates of nucleotide diversity and differentiation within and between North American and European populations of disjunct species.

Population	$\theta\pi$	θs	F_{ST}	Fixed differences	Shared polymorphic sites
Claopodium					
Europe	0.000	0.000			
North America	7.269 ± 4.093	8.3784 ± 3.484			
Europe × North America			0.1987 ($P \leq 0.007$)	1	0
Dicranoweisia					
Europe	2.422 ± 1.620	2.121 ± 1.164			
North America	5.169 ± 2.903	3.566 ± 2.903			
Europe × North America			0.1665 ($P \leq 0.04$)	0	3
Scleropodium					
Europe	1.036 ± 0.874	0.385 ± 0.385			
North America	1.714 ± 1.296	2.041 ± 1.223			
Europe × North America			0.6533 ($P \leq 0.0001$)	0	0

TABLE 3. Tree length and tree statistics for constrained and unconstrained analyses of ITS variation in *Claopodium whippleanum*, *Dicranowiesia cirrata*, and *Scleropodium touretii*. HK and T = significance of Kishino-Hasegawa and Templeton tests, respectively, of differences in tree length. CI = consistency index; RI = retention index; RC = rescaled consistency index. $*P < 0.05$; $**P < 0.01$; $***P < 0.001$; ns = not significant.

Statistic	Unconstrained	Constrained	HK/T
Claopodium whippleanum			
Length	39	65	**/***
CI	0.769	0.462	
RI	0.750	0.028	
RC	0.577	0.013	
Dicranoweisia cirrata			
Length	16	103	**/***
CI	1.000	0.155	
RI	1.000	0.094	
RC	1.000	0.015	
Scleropodium touretii			
Length	8	23	*/ns
CI	1.000	0.348	
RI	1.000	0.250	
RC	1.000	0.087	

tions of *Scleropodium* are variable in the ITS region, and this is reflected in low estimates for θ from nucleotide substitutions alone (Table 3).

DISCUSSION

Despite observations of some subtle patterns of morphological variation in qualitative characters that appear to have a geographic component, quantitative measurements of morphological characters did not reveal significant differentiation between Old and New World populations in any of the three species we studied. Some limited amount of differentiation, mainly in gametophyte size, was revealed within *Dicranoweisia*, but the differences accounted for a minimal proportion of the overall variation and were barely significant.

Molecular data, in contrast, did reveal some geographic patterns in both cladistic structure and levels of molecular diversity. Relationships among populations implied by morphological patterns are significantly incongruent with genealogical relationships inferred from molecular data. For *Claopodium* and *Dicranoweisia*, ITS phylogenies were unrooted so it was not possible to assess monophyly of infraspecific clades. Nevertheless, the five Old World populations of *Claopodium* shared a mutation that set them apart from all New World populations. It is possible that rooting the network would resolve both Old and New World populations as monophyletic, or, alternatively, populations of one of the two regions may represent a basal grade that gave rise to a disjunct monophyletic group of populations.

In *Dicranoweisia*, neither the Old nor New World population forms a monophyletic group. Plants belonging to a clade of North American plants share five mutations, but other North American populations are identical to Old World plants. Again, in the absence of a rooted phylogeny, it is difficult to interpret this pattern in a historical context. Intercontinental dispersal might be the basis for the disjunct distribution of ITS haplotypes, or shared haplotypes between New and Old World plants may have resulted from retention of ancestral polymorphism. The well-marked clade of North American plants could be derived from plants with such ancestral haplotypes. This interpretation would imply that the group of 17 New and Old World populations that share a common ITS haplotype are basal and that other Old World plants (i.e., populations TUR31, GRE 91, MO90, TEN33, and MOR88) are derived from them. The haplotype network, polarized in this way, further implies that the species spread to Mexico, and then northward into the United States and Canada.

The molecular pattern in *Scleropodium* is more like that observed in *Claopodium*. New and Old World populations are differentiated, but one population from California lacks the mutations that otherwise characterize New World plants, and a population from Tenerife shares that same mutation that is otherwise limited to New World plants. The rooted phylogeny for *Scleropodium touretii* suggests that the Old World plants form a paraphyletic basal grade from which the New World plants were derived. Indeed, plants from two Old World populations (one from Spain, one from Morocco) had two different ITS haplotypes, and one of them differed from that found in the outgroup (*S. obtusifolium*) by only two mutations. Most of the infraspecific ITS sequence variation in *Scleropodium* was attributable to differentiation between North American and European plants (i.e., $F_{ST} = 0.6533$), although the overall level of variation was lower than in the other two species.

It may be significant that in all three species, North American populations contained higher levels of nucleotide diversity than did their Old World conspecific counterparts. In *Claopodium*, Old World populations were monomorphic and fixed for a single ITS haplotype, whereas New World populations were characterized by numerous nucleotide substitutions. Similarly, in both *Dicranoweisia* and *Scleropodium*, New World populations were characterized by higher levels of θ, although high standard deviations associated with estimates made these differences not statistically significant. The highest θ value was from North American populations of *Claopodium*, followed by American populations of *Dicranoweisia*, then *Scleropodium*. The values of θ from Old World populations were uniformly low or, as in *Claopodium*, zero.

Nucleotide diversity estimated by θ can reflect a number of biological attributes. Population size (at present as well as in the recent past), sex ratios, generation time, and age of the species are among those factors that could underlie differences in standing levels of molecular diversity. There are no obvious differences in population structure (large vs. small breeding populations) or generation time between Old and New World plants in any of the three mosses. Sex ratios were not investigated. It could be argued that higher levels of molecular diversity in North American plants might result from longer occupation of the American parts of their ranges, but the rooted phylogeny of *Scleropodium touretii* contradicts that interpretation. It should be noted, however, that broader sampling within *Scleropodium* might change interpretations of evolutionary polarity within *S. touretii*. The pattern of differing levels of nucleotide diversity is most obvious in *Claopodium*. This species is more common in western North America than in southern Europe, where it is quite rare. This pattern of abundance, as well as the difference in molecular diversity, is consistent with a more recent colonization of Europe from North America, but in the absence of a rooted phylogeny, this hypothesis is speculative.

Cryptic speciation, the evolution of phylogenetically distinct taxa that are not marked morphologically, has been documented in both mosses and liverworts (Shaw, 2001). Mosses

and liverworts are morphologically simple plants, and it is perhaps not surprising that evolutionary differentiation is not always evident from morphological characters. In this study, subtle differentiation revealed by ITS sequences are not reflected in morphological variation among populations. Nevertheless, none of the three species investigated here appear to consist of cryptic sister species disjunct between New and Old Worlds. We observed morphological variation within *Dicranoweisia* that is at odds with published descriptions of this species in regional floras, and some of the features we observed are more characteristic of the related species, *D. crispula*. This latter species is distinguished by other characters and our observations cannot be explained by misidentifications, nor by the occurrence of cryptic speciation within the morphologically defined *D. cirrata*. Morphological variation did not correspond to molecular differentiation in any way that suggests cladogenesis.

Our results are not consistent with a Madrean-Tethyan explanation for the disjunct distributions of *Claopodium whippleanum*, *Dicranoweisia cirrata*, or *Scleropodium touretii*. We were not able to assess absolute ages by calibrating rates of molecular evolution in these species, but no plausible rate of nucleotide substitution could yield a divergence time ≥20–25 MYA. Even in *Claopodium*, where North American and Old World plants are distinguishable by their ITS sequences, and in *Scleropodium*, where, with two exceptions, a similar pattern of differentiation was found, the limited degree of differentiation almost certainly reflects more recent divergence. In *Dicranoweisia*, Old and New World plants neither form distinct clades, nor differ to an extent that makes Tertiary vicariance plausible. More recent long-distance dispersal is the most likely explanation for the intercontinental disjunctions characterizing these species.

There has been much discussion about infraspecific intercontinental disjunctions in bryophytes. Because bryophyte species exhibit many of the same patterns found in seed plants at the generic level (i.e., vicariant species rather than populations) and because many such patterns are thought to have historical bases dating as far back as the early Tertiary, these patterns have been used to argue that many bryophyte species are extremely old (e.g., Crum, 1972). A corollary of this interpretation is that such species have remained morphologically unchanged for tens of millions of years. The western North American–southern Europe disjunction as exemplified by the three species included in this study is one such pattern of disjunction in mosses. Our results, however, do not support the interpretation that this disjunction is ancient and therefore obviate any need to hypothesize morphological stasis over vast amounts of time.

Bryophytes represent an underutilized group for biogeographic studies. In addition to widespread species with more or less continuous distributions across the three northern continents, many patterns of infraspecific disjunction are ripe for analyses based on newly available molecular methods. Recent work on mosses disjunct between North America and Europe (Shaw and Allen, 2000) demonstrate the efficacy of molecular tools for studying bryophyte distributions. A substantial number of species are disjunct between eastern Asia and eastern North America, but none has been subjected to molecular phylogeographic analyses. Our results suggest that when the utility of morphological variation for biogeographic inferences is limited by homogeneity across continents, molecular data may reveal patterns that permit additional insights into population level processes such as dispersal and mating patterns.

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