INTRODUCTION

The dioecious moss *Dichelyma capillaceum* (With.) Myr. (*Fontinalaceae*) has a very scattered amphitropical range (Fig. 1), mainly including the northeastern part of North America and Scandinavia (Crum and Anderson, 1981; Ireland, 1989; Nyholm, 1960; Toivonen, 1972). In Europe, most of the populations (19) are situated in Southern Sweden, but they are on the decline there (ECCB, 1995; Hylander, 1998). Elsewhere, a very small number of populations are known from Finland, Poland, and Karelia in Russia (Kotiranta et al., 1998). This moss cannot be found in Estonia, and populations known from Denmark, Italy, and France (all but one) are assumed to be extinct (Allorge and Jovet-Ast, 1948; ECCB, 1995). No data on recent populations from Poland and Karelia are available. Each population from Germany and the one from France seem to be still present. The records from the Netherlands, Greece, and Sicily date from 1760, 1851, and 1888, respectively (Touw, 1989; Preston, 1984; Dia et al., 1987), but none of these has been verified. Thus, the actual centers of distribution of this rare and endangered species are located in northeastern North America and Northern Europe.

There have been no reports of European fossil or subfossil finds of *D. capillaceum* (e.g., Jovet-Ast, 1967; Dickson, 1973), and at least no Quaternary subfossils of this species have been found in North America either (Miller, 1980; Janssens, 1983).

*Dichelyma capillaceum* is a yellowish-brown to green medium-sized pleurocarpous aquatic moss growing in small tufts up to 10 cm long. Leaves are erect-spreading, slightly falcate, second, and lanceolate. The nerve is excurrent in a long aristate point about ½ the length of the leaf. Capsules are extremely rare and immersed. The species is almost without exception sterile in Europe, and it is suspected to have lost its ability to produce capsules due to declining habitat quality and frequencies of male plants (Hedenäs et al., 1996). Current spreading occurred asexually with fragments of young branches. No specialized vegetative dispersal units are known in *D. capillaceum*, but plants can be dispersed vegetatively, either at a limited locality through elongation of stoloniferous shoots that are sometimes seen firmly attached to the substrate or within the watercourse by fragmentation (Toivonen, 1972). There is no evidence of long-distance dispersal, and the main dispersal vector seems to be stream water.
The moss inhabits riparian habitats, growing on tree bark and rocks.

In Europe, this species is treated as vulnerable and is included in Appendix I of the Bern Convention and in Annex 2 of the EC Habitats and Species Directive.

*Dichelyma capillaceum* was found in Germany for the first time in a small village near the town of Brühl at the beginning of the 20th century (Brasch, 1923). The locality is ca. 1500 km as the crow flies away from the main European range in Scandinavia (Sweden). Feld (1958) cited the locality again without confirming the existence of the populations and mentioned two other records for Eastern Germany (Sagan and Westprußen), taken from Mönkemeyer (1927). Searches to find German populations failed over the years, and Düll (1980) stated that the species is impossible to find due to landscape change and finally considered it extinct (Düll, 1994). The species was re-found in the sterile stage in 1997, more than 70 years after previous records (Frahm and Stapper, 1998). Even though the species is cited as exclusively sterile over almost all of its present range in Europe, one herbarium specimen from 1923 bears sporophytes.

A population was recently discovered again in Germany. However, it is not clear if it settled the present habitat as a result of long-distance dispersal from North American or Scandinavian relatives or was present there for a long time already. Considering that the species has not been seen for decades with sporophytes across its range and no propagules for long-range spreading are known, we here examine the question of relationships with representatives in the two present centers of its distribution.

Phylogeographical methods have made it possible to elucidate the effects of large-scale historical events (such as Pleistocene climate change) on the distribution and subdivision of biota and put into context the role of reproductive biology in the genetic structuring of species.
MATERIAL AND METHODS

Chloroplast markers are often successfully applied in plant phylogeography research not only because they are better conserved, but also because genes of chloroplast RNAs have spacers and introns that are useful for studying differences among both populations and genera. Widely used is the highly variable spacer positioned between lysine-tRNA-Exon trnL (UAA-3') and the tRNA gene for phenylalanine trnF (GAA) (e.g., Taberlet et al., 1991; Kelchner, 2000). A previous survey demonstrated that this fragment is likely to exhibit an informative amount of diversity (Vogel et al., 1996). It has also been shown that the chloroplast is maternally (i.e., uniparentally) inherited in most plants (Vogel et al., 1998).

We extracted DNA from herbarium specimens using the 2x CTAB (hexadecyltrimethylammonium bromide) method as described by Sabovlević et al. (2005).

Amplification of the trnL-F region (Taberlet et al., 1991) was carried out using the forward primer C and the reverse primer F slightly modified for bryophytes according to Meiśner et al. (1998) and Sugiyama et al. (2003).

The alignment of sequences was created manually with the alignment editor Align 32 (Happerle, 2003), and PAUP4.0b10 (Swofford, 2002) was used for the calculation of molecular trees.

Maximum parsimony, likelihood, and neighbor-joining analyses were performed with trnL-F data sets of selected specimens and a few specimens borrowed from the GenBank. Heuristic search were carried out with the following options: all characters unweighted and unordered, multiseriate characters interpreted as uncertain, gaps coded as missing data, performing TBR branch swapping, collapse zero length branches, and MulTrees option in effect. Heuristic bootstrap searches were performed with 1000 replicates, 100 random additional replicates per bootstrap, and the same option in effect.

The sequences obtained for selected specimens are deposited in the GenBank (Table 1), and three additional ones were used from the database (AF191505, AF191506, and AF191504).

<table>
<thead>
<tr>
<th>Species</th>
<th>Origin</th>
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<tbody>
<tr>
<td>Dichelyma capillaceum</td>
<td>Sweden, Võxjö, leg. K. Hylander</td>
</tr>
<tr>
<td>Dichelyma capillaceum</td>
<td>Sweden, Sandviken, leg. K. Hylander</td>
</tr>
<tr>
<td>Dichelyma capillaceum</td>
<td>Sweden, Närke, leg. L. Hedenäs</td>
</tr>
<tr>
<td>Dichelyma capillaceum</td>
<td>USA, Missouri, leg. B. Summers and C. D. Scott</td>
</tr>
<tr>
<td>Dichelyma capillaceum</td>
<td>USA, New Hampshire, leg. B. Allen</td>
</tr>
<tr>
<td>Dichelyma capillaceum</td>
<td>USA, Maine, leg. B. Allen</td>
</tr>
<tr>
<td>Dichelyma capillaceum</td>
<td>Germany, Brühl, leg. Sabovlević and Frahm</td>
</tr>
<tr>
<td>Dichelyma falcata</td>
<td>USA, from GenBank AF191505</td>
</tr>
<tr>
<td>Dichelyma falcata</td>
<td>USA, from GenBank AF191506</td>
</tr>
<tr>
<td>Dichelyma uncinatum</td>
<td>USA, from GenBank AF191504</td>
</tr>
</tbody>
</table>

RESULTS

The results obtained in PAUP analyses clearly show that the German population is distant from all the other tested populations. Since the species is quite rare and in danger of extinction, three North European populations and three from the northeastern part of North America were chosen for comparison with the German one. Additionally, the trnL-F sequences of two specimens of Dichelyma falcata and one of D. uncinatum were added.

The alignment was 424 bp long, consisting of 385 constant characters, 23 variable but parsimony-uninformative characters, and 16 parsimony-informative characters. The trnL intron contained only one informative site, whereas high levels of informative variation occurred in the trnL/trnF spacer. Two informative variations are present in the trnF gene. A similar pattern of sequence variability was reported for the family Fontinalaceae (Show and Allen, 2000). When only the sequences of Dichelyma capillaceum are taken into account, no variation has been recognized within trnF sequence genes. Uncorrected sequence divergence among D. capillaceum specimens varied from 0.09612 to 0.07441 (variation was somewhat lower within European specimens: 0.08103-0.07441), while the divergence values between D. falcata and D. capillaceum var-
Fig. 2. The most parsimonious tree of selected *Dichelyma* populations with bootstrap values above branches (USA – United States of America, SE – Sweden, DE – Germany).
ied from 0.00321 to 0.00773 and between *D. uncinatum* and *D. capillaceum* from 0.00101 to 0.00211. The distance values between *D. uncinatum* and *D. falcatum* were 0.01256 and 0.01318. The highest distance value within *D. capillaceum* specimens falls between the German specimen and the American Missouri specimen.

**DISCUSSION**

On the basis of the results obtained, inferred from the plastid *trnL*-F sequence region, the German population can be considered to be more closely related to the North European ones than to those from the American continent. The same tree patterns are obtained by maximum parsimony (Fig. 2) and neighbor-joining analyses. The German populations are clustered with the Swedish Göknäset population, but to judge from branch length have been separated from it for a long time. This clade makes a common branch with the two other tested Swedish populations, which then clustered with the separated branch of the American populations. All are rooted with the branch bearing the other two species of *Dichelyma* used in this study.

In light of the obtained results, it can be assumed that the German population has for a long time been genetically separated from all the others tested. This can be explained in terms of long geographical isolation and non-sexual reproduction. The German population survived *in situ* or was asexually spread from some other site within the sometimes wider but disjoint range of this species.

Several Pleistocene glacial refugia have been proposed for European biota (Taberlet et al., 1998; Hewitt, 1999). The consensus from previous molecular studies is that taxa emerged from one or more of three South European mainlands (the Iberian, Apennine, and/or Balkan) following the last glacial and presumably also during interglacials. However, congruence in phylogeographical patterns is observed only on a broad scale, and most taxa show distinctive patterns of genetic diversity throughout Europe. Molecular and fossil evidence also indicates that some tree species such as common beech (Demesure et al., 1996), black alder (King and Ferris, 1998), and Scots pines (Sinclair et al., 1999) survived the Pleistocene in relatively northern locations (i.e., close to the periglacial zone).

Schneller (1996), Vogel et al. (1999), Suter et al. (2000), and Trewick et al. (2002) demonstrated that low genetic variability is suggestive of recent expansion.

Inasmuch as the German population shows a different genotype from the American ones and is slightly different but genetically distant from the Scandinavian populations, it can be assumed that it originated from the Scandinavian one and that its expansion has occurred relatively recently, bearing in mind sterility of the German population, its size, and the absence of propagules for short- or long-distance dispersal. Since the species inhabits riparian habitats over its range and in North America spreads disjointly from boreal to subtropical climate zones (always decreasing in more southern regions) and in view of the molecular data obtained, it can be assumed that Scandinavian representatives probably have ancestors in relatives in America, where the center of the range of the genus *Dichelyma* is.

The German population spread south by long-distance dispersal after having originated from some Scandinavian ancestor. Since the species is known to have small spores, it cannot be excluded that long-distance dispersal occurred with spores once upon a time when sporophyte production was still common. Such an unexpected appearance of new populations with different genetic structure in geographically distant areas has already been shown for *Hilpertia velenovskyi* (Saboljčević et al., 2006).

The obtained results strongly emphasize the high value of the German population for conservation of this riparian pleurocarp moss and stress the need for urgent action to protect its habitat.

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**REFERENCES**


ГЕНАТИЧКИ ДИВЕРЗИТЕТ И ФИЛОГЕОГРАФИЈА РЕТКЕ РИПАРИЈСКЕ МАХОВИНЕ *DICHELYMA CAPILLACEUM* (WITH.) MYR. НА ОСНОВУ TRNL-F ПЛАСТИДНИХ СЕКВЕНЦИ ДНК

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Изучавани генетски односи маховине *Dichelyma capillaceum* (With.) Мур. базирани на основу секвенци trnL-F хлоропластног генома. На основу ових података евидентно је да немачка популација ове маховине води порекло из Скандинавије (Шведске) пре него из Северне Америке. На основу генетичких дистанци између шведских и немачке популације, одвајање немачке популације се десило пре много времена.