

**GENETIC DIVERSITY AND PHYLOGEOGRAPHY OF THE RARE RIPARIAN MOSS *DICHELYMA CAPILLACEUM* (WITH.) MYR. INFERRED FROM *TRNL-F* PLASTID DNA SEQUENCES**M. SABOVLJEVIĆ<sup>1</sup> and J.-P. FRAHM<sup>2</sup><sup>1</sup>*Institute of Botany and Jevremovac Botanical Garden, Faculty of Biology, University of Belgrade, 11000 Belgrade, Serbia*<sup>2</sup>*AG Bryologie, Nees Institut für Biodiversität der Pflanzen, RFW Universität Bonn, D-53115 Bonn, Germany*

**Abstract** — The genetic relationships of *Dichelyma capillaceum* (With.) Myr. are studied from chloroplast sequences of the trnL-F region. On the basis of the molecular data obtained, the German population can be considered to be derived from Scandinavian (Swedish) rather than North American populations. To judge from the genetic distances between the Swedish and German populations, the separation must have occurred a long time ago.

**Key words:** Mosses, *Dichelyma capillaceum*, genetic relationship, phylogeography, relictness

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**INTRODUCTION**

The dioecious moss *Dichelyma capillaceum* (With.) Myr. (Fontinalaceae) has a very scattered amphiatlantic range (Fig. 1), mainly including the north-eastern 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.



Fig. 1. The world distribution of *Dichelyma capillaceum*.

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 20<sup>th</sup> 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 Sabovljević 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 Meißner et al. (1998) and Sugiura 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 1.** Specimens used in this study, with GenBank accession numbers.

Species	Origin
<i>Dichelyma capillaceum</i>	Sweden, Vöxjö, leg. K. Hylander
<i>Dichelyma capillaceum</i>	Sweden, Sandviken, leg. K. Hylander
<i>Dichelyma capillaceum</i>	Sweden, Närke, leg. L. Hedenäs
<i>Dichelyma capillaceum</i>	USA, Missouri, leg. B. Summers and C. D. Scott
<i>Dichelyma capillaceum</i>	USA, New Hampshire, leg. B. Allen
<i>Dichelyma capillaceum</i>	USA, Maine, leg. B. Allen
<i>Dichelyma capillaceum</i>	Germany, Brühl, leg. Sabovljević and Frahm
<i>Dichelyma falcatum</i>	USA, from GenBank AF191505
<i>Dichelyma falcatum</i>	USA, from GenBank AF191506
<i>Dichelyma uncinatum</i>	USA, from GenBank AF191504

## 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 falcatum* 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. falcatum* and *D. capillaceum* var-

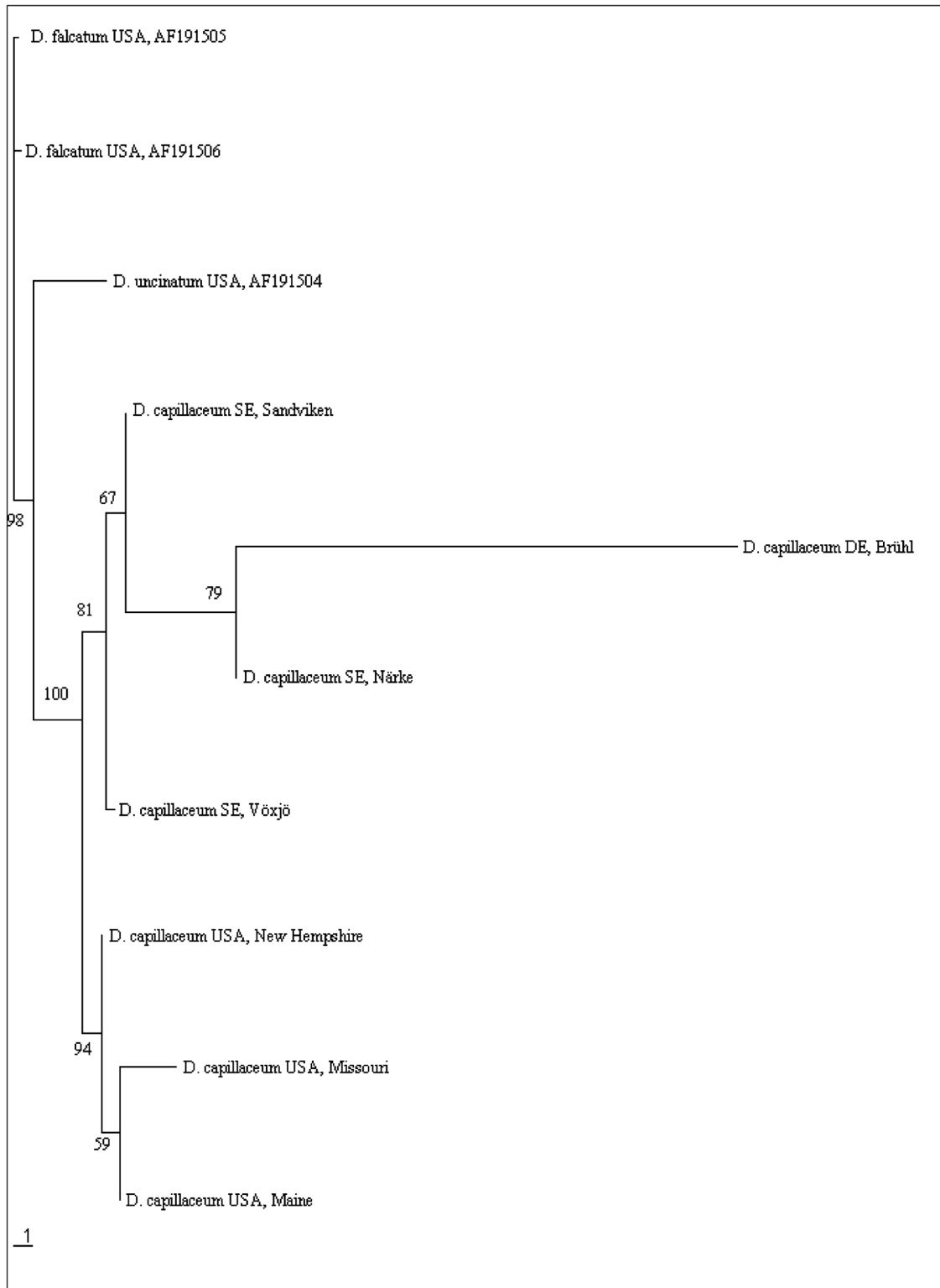


Fig. 2. The most parsimonious tree of selected *Dichelyma* populations with bootstrap values above branches (USA – United States of America, SE – Sweden, DE – Germany).

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 Göknaät 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 pine (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 Treweek 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* (Sabovljević 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

- Allorge, V., and S. Jovet-Ast (1948). *Dichelyma capillacea* dans le sud-ouest de la France. *Compt. Rend. Som. Séa. Soc. Biogéogr.* **25**, 17-18.

- Brasch, H. (1923). Beitrag zur Laubmoosflora. *Sitzungber. Naturhist. Ver. Preuss. Rheinlande Westfalen* **1920-1922**, 18-22.
- Crum, H. A., and L. E. Anderson (1981). *Mosses of Eastern North America*. 1328 pp. Columbia University Press, New York.
- Demeseure, B., Comps, B., and R. Petit (1996). Chloroplast DNA phylogeography of the common beech (*Fagus sylvatica*) in Europe. *Evolution* **50**, 2515-2520.
- Dia, M. G., Miceli, G., and F. M. Raimondo (1987). Check-list dei muschi noti in Sicilia. *Webbia* **41**, 61-123.
- Dickson, J. H. (1973). *Bryophytes of the Pleistocene. The British Record and Its Chorological and Ecological Implications*. Cambridge University Press, London.
- Düll, R. (1980). Die Moose (Bryophyta) des Rheinlandes (Nordrhein-Westfalen, BRD). *Dech. Beih.* **24**, 1-365.
- Düll, R. (1994). *Deutschlands Moose*, 3. Teil. IDH Verlag, Bad Münstereifel.
- ECCB (1995). Red Data Book of European Bryophytes. Trondheim.
- Feld, J. (1958). Moosflora der Rheinprovinz. *Dech. Beih.* **6**, 1-94.
- Frahm, J.-P., and N. Stapper (1998). Das Laubmoos *Dichelyma capillaceum* nach 70 Jahren an seinem einzigen Fundort in Deutschland gefunden. *Decheniana* **151**, 109-113.
- Happerle, D. (2003). *Align v01/2003: Manual Sequence Alignment for PCs*. Heidelberg.
- Hedenäs, L., Godow, S., and K. Hylander (1996). Bryophyte profiles, No. 1: *Dichelyma capillaceum* (Dicks.) Myr. (Bryopsida: Fontinalaceae). *J. Bryol.* **19**, 157-179.
- Hewitt, G. (1999). Post-glacial re-colonization of European biota. *Biol. J. Linn. Soc.* **68**, 87-112.
- Hylander, K. (1998). Härklomossa, *Dichelyma capillaceum* - ekologi och aktuell förekomst i Sverige. *Svensk Bot. Tidskr.* **92**, 95.
- Ireland, R. R. (1989). Distribution of the moss genus *Dichelyma* in Canada. *Lindbergia* **15** (2), 65-69.
- Janssens, J. A. (1983). Quaternary fossil bryophytes in North America: new records. *Lindbergia* **9**, 137-151.
- Jovet-Ast, S. (1967). Bryophyta, In: *Traité de Paléobotanique*, Vol. 2 (Ed. E. Boureau), 17-186. Maisson de Cie, Paris.
- Kelchner, S. A. (2000). The evolution of non-coding chloroplast DNA and its application in plant systematics. *Ann. Missouri Bot. Garden* **87**, 482-498.
- King, R. A., and C. Ferris (1998). Chloroplast DNA phylogeography of *Alnus glutinosa* (L.) Garetn. *Mol. Ecol.* **7**, 1151-1161.
- Kotiranta, H., Uotila, P., Sulkava, S., and S. L. Peltonen (1998). *Red Data Book of East Fennoscandia*. 351 pp. Ministry of the Environment, Helsinki.
- Meißner, K., Frahm, J.-P., Stech, M., and W. Frey (1998). Molecular divergence patterns and infragenetic relationship of *Monoclea* (Monocleales, Hepaticae). Studies in austral temperate rain forest bryophytes 1. *Nova Hedwigia* **67**, 289-302.
- Miller, N. G. (1980). Quaternary fossil bryophytes in North America: catalog and annotated bibliography. *J. Hattori Bot. Lab.* **47**, 1-34.
- Mömkemeyer, W. (1927). *Die Laubmoose Europas*. Rabenhorsts Kryptogamenflora, Bd. IV. Leipzig.
- Nyholm, E. (1960). *Illustrated Moss Flora of Fennoscandia, II. Musci*, 4. 289-408. CWK Gleerup, Lund.
- Preston, C. D. (1984). A check-list of Greek mosses. *J. Bryol.* **13**, 43-95.
- Sabovljević, M., Frahm, J.-P., and U. Herbiniaux (2005). Taxonomic value, systematic position, and the origin of German populations of *Isoetecium holtii* Kindb. based on molecular data. *Lindbergia* **30** (3), 107-112.
- Sabovljević M., Frahm, J.-P., and F. Schaumann (2006). The origin of the German populations of *Hilpertia velenovskyi* (Pottiaceae, Bryopsida): inferences from variation in the nuclear ITS region. *Cryptogam. Bryol.* **27** (3), 357-365.
- Schneller, J. J., and R. Holderegger (1966). Colonization events and genetic variability within populations of *Asplenium ruta-muraria*, In: *Pteridology in Perspective* (Eds. J. M. Camus, M. Gibby, and R. J. Johns), 571-580. Royal Botanical Gardens, London.
- Show, A. J., and B. Allen (2002). Phylogenetic relationship, morphological incongruence, and geographic speciation in the Fontinalaceae (Bryophyta). *Mol. Phylogenet. Evol.* **16** (2), 225-237.
- Sinclair W. T., Mormon, J. D., and R. A. Enos (1999). The post-glacial history of Scots pine (*Pinus sylvestris* L.) in Western Europe: evidence from mitochondrial DNA variation. *Mol. Ecol.* **8**, 83-88.
- Sugiura, C., Kobayashi, Y., Setsuyuki, A., Sugita, C., and S. Mamoru (2003). Complete chloroplast DNA sequence of the moss *Physcomitrella patens*: evidence for the loss and relocation of rpoA from the chloroplast to the nucleus. *Nucl. Acid Res.* **31** (18), 5324-5331.
- Suter, M., Schneller, J. J., and J. C. Vogel (2000). Investigation into genetic variation, population structure, and breeding systems of the fern *Asplenium trichomanes* ssp. *quadri-valens*. *Intern. J. Plant Sci.* **161**, 233-244.
- Swofford, D. (2002). *PAUP\*: Phylogenetic Analyses Using Parsimony (\*and Other Methods), version 4.0b1.0*. Sinauer, Sunderland, MA.
- Taberlet, P., Gielly, L., Pautou, G., and J. Bouvet (1991). Universal primers for amplification of three non-coding regions of chloroplast DNA. *Plant Mol. Biol.* **17**, 1105-1109.
- Taberlet, P., Fumagalli, L., Wust-Saucy, A. G., and J. F. Cossons

- (1998). Comparative phylogeography and postglacial colonization route in Europe. *Mol. Ecol.* **7**, 453-464.
- Touw, A. (1989). Fontinalaceae, In: *De Nederlandse Bladmossen. Flora en Verspreidingsatlas van de Nederlandse Musci (Sphagnum Uitgezonderd)* (Eds. A. Touw and W. V. Rubers), 345-347. Stichting Uitgeverij Koninklijke Nederlands Natuurhistorische Vereniging, Utrecht.
- Trewick, S. A., Morgan-Richards, M., Russell, S. J., Henderson, S., Rumsey, F. J., Pinter, I., Barrett, J. A., Gibby, M., and J. C. Vogel (2002). Polyploidy, phylogeography, and Pleistocene refugia of the rockfern *Asplenium ceterach*: evidence from chloroplast DNA. *Mol. Ecol.* **11**, 2003-2012.
- Toivonen, H. (1972). Distribution of *Dichelyma capillaceum* (With.) Myr. emend. Br. et Scimp. (Bryophyta), especially in northwestern Europe. *Ann. Bot. Fennici* **9**, 102-106.
- Vogel, J. C., Russell, S. J., Barrett, J. A., and M. Gibby (1996). A non-coding region of chloroplast DNA as a tool to investigate reticulate evolution in European *Asplenium*, In: *Pteridology in Perspective* (Eds. J. M. Camus, M. Gibby, and R. J. Johns), 313-327. Royal Botanical Gardens, London.
- Vogel, J. C., Russell, S. J., Rumsey, F. J., Barrett, J. A., and M. Gibby (1998). Evidence for maternal transmission of chloroplast DNA in the genus *Asplenium* (Aspleniaceae, Pteridophyta). *Bot. Acta* **111**, 247-249.
- Vogel, J. C., Rumsey, F. J. and S. J. Schneller (1999). Genetic structure, reproductive biology, and ecology in isolated populations of *Asplenium ciskii* (Aspleniaceae, Pteridophyta). *Heredity* **83**, 604-612.

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

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Изучавани су генетски односи маховине *Dichelyma capillaceum* (With.) Myr. 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.