# Phylogeny and species delimitation within the moss genus *Dicranum* Hedw.

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2014

To my family

Annick S. Lang
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ISBN: 978909028491

NUR: 941

Cover: *Dicranum scoparium* Hedw.
Design and layout by Annick S. Lang & Mieke de Roo
All photos and drawing by Annick S. Lang unless noted
Printing: CPI – KONINKLIJKE WÖHRMANN

# Phylogeny and species delimitation within the moss genus *Dicranum* Hedw.

Proefschrift

ter verkrijging van
de graad van Doctor aan de Universiteit Leiden,
op gezag van Rector Magnificus prof.mr. C.J.J.M. Stolker,
volgens besluit van het College voor Promoties
te verdedigen op woensdag 8 oktober 2014
klokke 11.15 uur

door

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geboren te Meyrin (Zwitzerland)
in 1982

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# Contents

# Chapter 1



## General Introduction

*Dicranum* Hedw. (Dicranaceae, Bryophyta) is a large genus essentially found in the Holarctic. It mainly grows on soil in forest and mountain habitats forming dense, tomentose tufts or cushions (Crosby *et al*. 1999; Crum & Anderson 1981). Because of their morphological plasticity and broad distribution range, members of the genus *Dicranum* Hedw. (Dicranaceae, Bryophyta) are often difficult to identify, especially at the species level, hence many morphological species boundaries remain poorly understood. Furthermore, no comprehensive and complete study of this genus is available This study focuses on the phylogeny and species delimitation within *Dicranum*, a Holarctic genus. Morphological and molecular species circumscription of species complexes are first studied, forming the basis for a phylogenetic reconstruction of the genus.

### Meeting Bryophyta

Bryophyta (mosses) is a highly diverse group of non-vascular land plants. With c. 12,500 species, bryophyta represents the second most diverse phylum of land plants and is found in every terrestrial ecosystem (Frey & Stech 2009; Crosby *et al*. 1999). Together with Marchantiophyta and Anthocerotophyta, Bryophyta have a haplodiplobiontic life cycle, with a haploid vegetative and dominant gametophyte, while the ephemeral diploid sporophyte remains attached to the maternal plant. Bryophyta display great morphological diversity, although they are characterized by few morphological synapomorphies such as multicellular rhizoids, leafy gametophytes and sporophytes with a capsule possessing a columella and stomata but lacking elaters (Frey & Stech 2009). They are generally small but their size can range from few millimetres (e.g. Ephemeropsis) up to 70 centimetres (e.g. Dawsonia superba). The more complex structures and positions of the sporophyte has been particularly important for the classifications of mosses, leading to a division between nematodontous and arthrodontous mosses based on the origin of peristome teeth, with the latter being further divided into acro- and pleurocarpous according to the position of the perichaetia on the stem, and into haplo- and diplolepideous mosses based on the tissue forming the teeth of the peristome (Goffinet & Shaw 2009).

Haplolepids (Dicranidae) represent the second largest subclass of mosses, after the diplolepideous-alternate mosses (Bryidae) (Stech *et al*. 2012). They are usually characterized by a peristome with a single row of arthrodontous teeth around the opening of the capsule (La Farge 2002; Hernández- Maqueda *et al*. 2008; Stech *et al*. 2012). Currently, Dicranidae are divided into six recognized subclasses (Pottiales, Dicranales, Archidiales, Grimmiales, Bryoxiphiales, Scouleriales; Goffinet & Shaw 2009; Frey & Stech 2009) with two additional subclasses included in Frey & Stech (2009) (Mitteniales, Catoscopiales). Together with Grimmiales and Pottiales, Dicranales is one of

the largest order of the subclass and is characterized by usually smooth lamina cells, differentiated alar cells, a strong costa and traberculate and striate peristome teeth (Goffinet *et al*. 2009; Frey & Stech 2009). The family Dicranaceae is complex family of the order Dicranales, which morphological concept has been redefined many times, counting up to 55 genera (e.g. Vitt 1984; Goffinet & Shaw 2009). The advances in molecular phylogenies allowed a clearer circumscription of Dicranaceae and reduced the number of genera to 24 (Stech & Frey 2008; Frey & Stech 2009).

### Molecular and barcode markers

During the past twenty years, DNA sequences of bryophytes have been increasingly used in systematic studies and taxonomical revisions giving new insights in bryology (Stech & Quandt 2010). While the first studies including DNA data utilized only one or two markers, the number of available markers, especially mitochondrial ones, has increased rapidly in the last ten years. Nevertheless, most of the studies still rely on four principal markers, namely *trnL-F*, *rps4*, *rbcL* and ITS (Stech & Quandt 2010). Recently, much effort has been placed in finding universal barcode markers. In addition to these traditional markers, *atpF-atpH*, *matK*, *psbK-psbI*, *rpoB*, *rpoC1* and *trnH-psbA* have been suggested as potential bryophyta barcode markers (CBOL Plant Working Group 2009). Of these ten potential loci, only six showed features that are suitable to delimit moss species (*trnL-F*, *rps4*, *trnH-psbA*, *rbcL*, *matK* and *ITS*; Liu *et al*. 2010, 2011). However, no consensus has been reached yet in finding the optimal combination of barcoding markers that are suitable for delimiting closely related species (Liu *et al*. 2010; Stech & Quandt 2010).

### Molecular Systematics of Dicranaceae with emphasis on *Dicranum*.

Although traditional morphological classification is often incongruent with modern systematics, which is based on molecular data, the monophyly of Dicranidae (arthrodonthous-haplolepideous mosses) is supported by all available phylogenetic reconstructions (e.g. Goffinet *et al*. 2001; Hedderson *et al*. 2004; La Farge *et al*. 2002; Stech *et al*. 2012). However, molecular studies have revealed that neither Dicranales nor Dicranaceae, as defined by Vitt

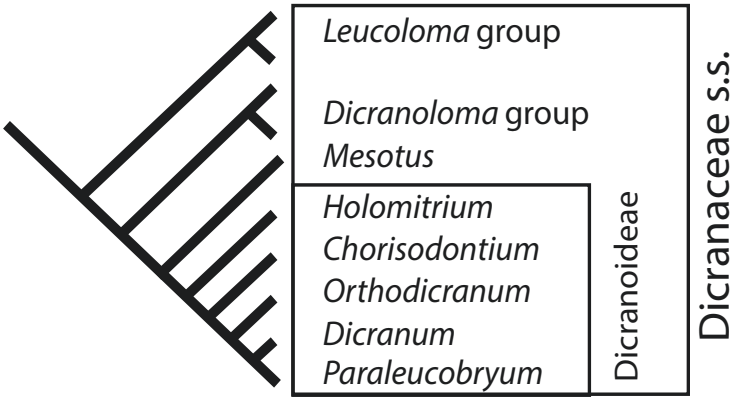


Fig. 1. Simplified cladogram of the Dicranaceae s.s. and relationships of its genera acoording to La Farge 2002 and Stech 1999.

(1984) and Goffinet & Shaw (2009), are a monophyletic group. While many taxa were clearly separated and thus placed in new lineages (Cox *et al*. 2010; Hedderson et al. 2004; La Farge *et al*. 2002; Stech 2008, 2012), the core of the Dicranaceae [Dicranaceae s.s. sensu La Farge (2002)] comprised four subfamilies: Dicranoideae, Mesotoideae, Dicranoloma group, and Leucoloma group, with Dicranoideae encompassing *Dicranum*, *Orthodicranum*, *Paraleucobryum*, *Chorisodontium*, *Eucamptodontopsis*, and *Holomitrium* (Fig. 1). Although molecular data gave new insights in the circumscription of the Dicranaceae, the relationships among genera remained ambiguous. Furthermore, the molecular species circumscription within the genera remained largely underexplored. The available molecular studies on *Dicranum* species complexes reveal ambiguous relationships among species due to their limited genetic variation (Ignatova & Fedosov 2008; Tubanova *et al*. 2010; Tubanova & Ignatova 2011) and is in need of further molecular studies.



### Taxonomical history of *Dicranum*

*Dicranum* is one of the largest moss genera, with more than 880 binomials originally given (van der Wijk *et al*. 1962; Tropicos.org). However, the genus as currently recognized, has ca. 90 accepted species (Frey & Stech 2009; Tropicos.org). Since the first nomenclatural description of the family, several revisions have been made, narrowing considerably the concept of its genera, and particularly the one concerning *Dicranum*.

Hedwig has described the genus in 1801 and considered *D. scoparium* as the type of the genus. A total of 34 other *Dicranum* species were simultaneously described in his Species Muscorum Frondosorum (1801). Of these 34 species, only three remained in the modern concept of the genus: *D. condensatum*, *D. scoparium* and *D. spurium* (Peterson 1979). Bruch, Schimper and Gümbel in 1847 (Bryologia Europea), worked on this family and described seven new genera within Dicranaceae and recognised 11 sections under *Dicranum*. Nearly simultaneously, Müller published his Synopsis Muscorum Frondosorum (1849), in which the treatment of the family encompassed six genera and five sections under *Dicranum*. Most of the genera and *Dicranum* species described by Bruch, Schimper & Gümel and Müller are nowadays placed in other families, respectively other genera of the Dicranales (Goffinet & Shaw 2009; Peterson 1979). Until the beginning of last century, several other revisions of the Dicranaceae and *Dicranum* have been done on the country- or continental level (Japan, North America, Europe) leading to multiple rearrangements of the genus and subdividing it into different subgenera or sections (Table 1; Brotherus 1906, 1924; Mönkemeyer 1927; Nyholm 1953, 1954, 1987; Peterson 1979 Sakurai 1951, 1952; Takaki 1964). However, in the most recent treatments available (Hedenäs & Bisang 2004; Ireland 2007; Gao & He 1999), neither subgenera nor sections were taken into account.

It is evident that the conceptual disharmony and multiple re-classifications at the genus level is the expression of the difficulties to understand this taxon and to find unique morphological characters capable of providing clear species circumscriptions.

### Morphology and characteristics of *Dicranum*

*Dicranum* is a dioicious genus that is characterised by densly tomentose acrocarpus stems. The leaves are generally falcate-second and lanceolate with a distal subula that varies from keeled to tubulose. They possess a strong costa that is percurrent to slightly excurrent. In cross-section, the costa has one row (sometimes two) of guide cells that are surrounded by stereid bands. The

TABLE 1. Taxonomic treatments of Dicranum according to the main European authors. The names of subgenera and sections within *Dicranum* are given.

| Author | Brotherus (1906) | Brotherus (1924) | Mönkemeyer (1927) | Sakurai (1951) | Nyholm (1954) | Takaki (1964) | Nyholm (1986) |
|---|---|---|---|---|---|---|---|
| subgenera | Arctoa | Crassidicranum | Crassicostata | Falcati- | | Crassidicranum | |
| | Chorisodontium | Eudicranum | Crispifolia | Fragili- | | Dicranum | |
| | Crassidicranum | Pseudo- chorisodontium | Fragilifolia | Elongati- | | | |
| | Dicranum | | Fulvella | Nippono- | | | |
| | Holodontium | | Scoparia | Pseudo-chorisodontium | | | |
| | Leiodicranum | | Strumifera | Scopario- | | | |
| | Orthodicranum | | Undulata | Undulati- | | | |
| | Paraleucobryum | | | | | | |
| sections | | | | | Crassidicranum | | Crassinervia |
| | | | | | Fuscescentia | | Dicranum |
| | | | | | Scoparia | | Elongata |
| | | | | | Spuria | | Fuscescentiforma |
| | | | | | | | Montana |
| | | | | | | | Muehlenbeckia |
| | | | | | | | Spuria |

dorsal side of the costa can be smooth or ornamented with lamellae, furrows or mamillae (Fig. 2). The leaves have a well differentiated double-layered alar region. The lower lamina cells are elongated and generally porose, while the upper lamina cells are either prosenchymatous (elongated and porose) or parenchymatous (short and smooth) (Fig. 2). The seta is mostly solitary, erect and twisted. It possesses an inclined capsule (Fig. 3A) with 16 bifid peristome teeth and a long-rostrate operculum (Goffinet & Shaw 2009; Ireland 2007). Morphological characters of *Dicranum* are very plastic and descriptions are usually based on few stable characters, such as sporophytic or costal characters. Some other discriminant characters are largely overlooked, especially in closely related species, as observed in *D. bardunovii*, *D. septentrionale*.

As in many bryophytes, vegetative reproduction plays an important role in *Dicranum*. While most species can easily propagate from gametophyte fragments, some species produce specialised structures such as flagelliform branchlets (*D. leioneuron* Kindb.), or have easily breakable leaf apices (*D. tauricum* Sapjegin). *Dicranum* can also reproduce sexually. Males can be either as large as female (Fig. 3B-C) or dwarf and growing on female stems (Fig. 3D), something called pseudomonoicy (Crawford et al. 2009). Sexual dimorphism in *Dicranum* is rather frequent. It was reported to occur in 20% of the species (Pichonet & Gradstein, 2012). Only few studies on dwarfism in *Dicranum* are available (Bisang & Ehrlén 2002; Briggs 1965; Ehrlén *et al*. 2000; Hedenäs & Bisang 2011; Sagmo Solli *et al*. 1998, 2000) and little is known about the mechanisms that triggers dwarfism, its consequences on populations structure and the possible hybridisation between species.

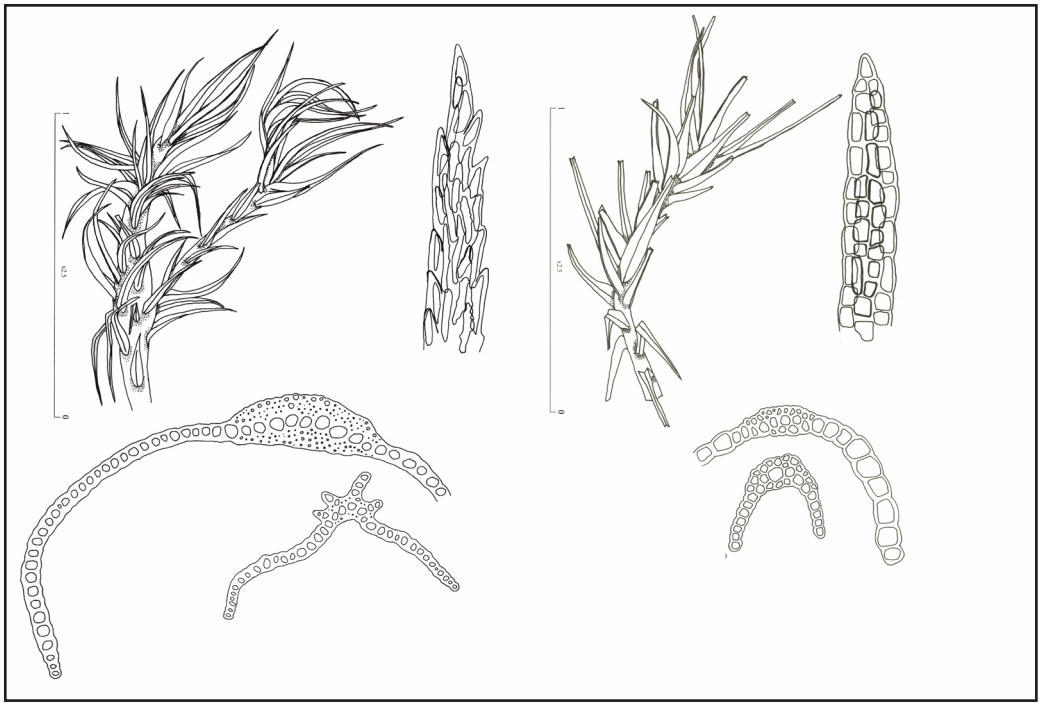

FIG. 2. Plant habit, leaf apex and leaf cross- section at base and upper part of A) *Dicranum scoparium* Hedw. and B) *D. tauricum* Sapjegin.

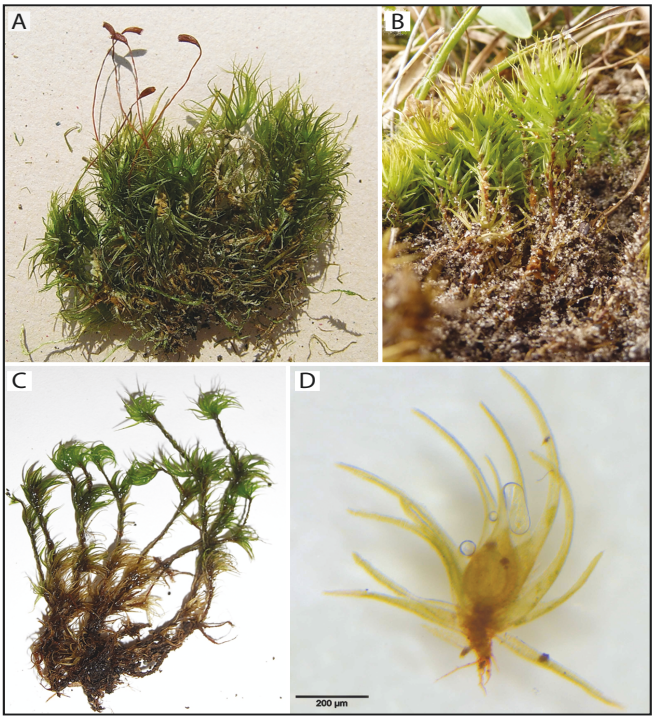


FIG. 3. Habitus of *Dicranum scoparium*. Female cushion with mature **A** sporophytes and **B** without sporophyte. **C** Normal sized males and **D** dwarfed male. Photo D by L. E. van Dijk

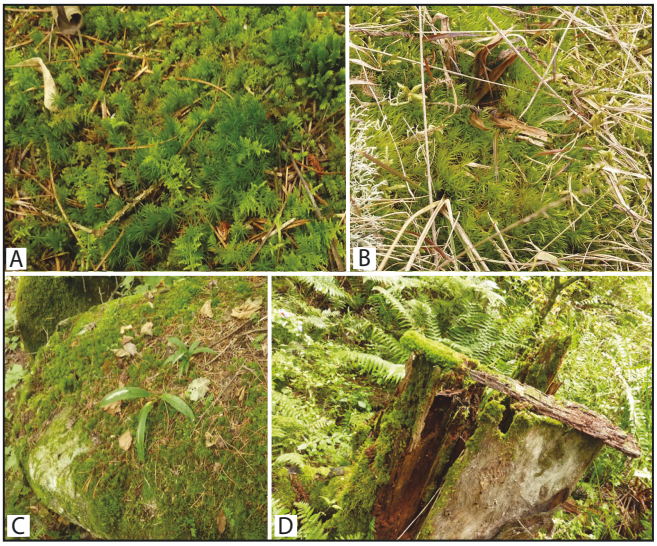


FIG. 4. Habitat type of Dicranum. **A** forest soil, **B** open sandy soil, **C** humus on boulder, **D** rotten tree bark.

### Distribution/ ecology in the Holarctic

The majority of *Dicranum* species is found in the Northern hemisphere. About 30 species are counted for Europe and Asia (Gao & He 1999; Hedenäs & Bisang 2004) and 26 in Northern America (Ireland 2007; Lawton 1971). Generally, *Dicranum* species have large distributions covering more than one continent. One species with a particularly wide distribution is *D. scoparium*. It is typically found in the Holarctic, as defined by Schofield (1992). However, recently, this species has been reported in Australia and New-Zealand (Klazenga 2012). Nonetheless, few endemics exist in the Himalaya (*D. himalayanum*, *D. assamicum*, *D. kashmirense* and *D. orthophyloides*; Chopra 1998; Dandotiya *et al*. 2011), in Japan (*D. leiodontum* and *D. setifolium*; Takaki 1972) and in the west coast of North America (*D. howellii*; Ireland 2007; Lawton 1971), or Hawaii (*D. speirophyllum*; Staples *et al*. 2004).

The distribution of species in the Holarctic is strongly associated with the vegetation zones, the degree of continentality and the altitudinal belts [e.g. *D. scottianum* and *D. canariense* occurring both in oceanic areas (Dierssen 2001; Hedenäs & Bisang 2004)]. Most of the species show a preference for acidic environments. They are found on all kinds of substrate: rocks, decomposed wood, sand, humus, bark, fen and bogs (Fig. 4 A-D). However, complete information about the distribution of many species, especially in species complexes, is still incompletely known, due in part to a poor morphological understanding and confusions between closely related species. Many species are considered as morphologically plastic and consequently as occurring in a broad spectrum of habitats. Recent molecular studies on the *D. acutifolium* species complex, however, revealed that this species complex contained multiple lineages that can be identified by few but distinct characters and occurred in different habitats (e.g. Otnyukova 2007; Tubanova *et al*. 2010; Tubanova & Ignatova 2011). This suggests that other *Dicranum* species with morphological plasticity might encompass several taxa with more restricted habitat preferences.

### Aims and outline of the thesis

This thesis aims at disentangling species circumscriptions of *Dicranum* species based on phylogenetic inferences. Phylogenetic reconstructions have been done sequencing five chloroplast (*rps4-trnT*, *trnL-F, psbA-trnH*, *rps19-rpl2*, *rpoB*) and one nuclear (nrITS) region. Besides molecular approaches, morphological studies were carried out in order to redefine the most suitable combination of characters for identifying species within complexes. A barcoding approach was also used on selected taxa, in order to evaluate the identification power of the markers on closely related species and we further tested the validity of automated species delimitation methods (GMYC and PTP) by comparing the estimated species with morphological and phylogenetic species circumscriptions.

In **chapter 2**, *Dicranum scoparium* species complex has been studied. The molecular analysis shows that *D. majus* is clearly separated from the *D. scoparium* species complex. However, the circumscription of *D. bonjeanii*, *D. nipponense* and *D. howellii* are less clearly distinct. Additionally, in contrary to its numerous phenotypes, *D. scoparium* is proven to be genetically very homogeneous. Nevertheless, a subclade including specimens from both northern America and Asia is revealed.

**Chapter 3** explores the species boundaries of another species complex, the *D. acutifolium* complex. Recent molecular studies have shown that *D. acutifolium* and *D. brevifolium* are poorly circumscribed. Moreover, they revealed two new species, *D. bardunovii* and *D. septentrionale*, which

were further supported by morphological characters. In this chapter, additional molecular studies provide stronger support for the four above-mentioned species. Furthermore, it is shown that the current concept of *D. brevifolium* includes characters attributed to the new species *D. septentrionale*, known from Russia. Additionally, the distribution area of this latter species is extended to Scandinavia.

**Chapter 4** investigates the identification capacity of molecular markers using arctic *Dicranum* species. Phylogenetic studies usually employ several barcoding markers. However, few studies have investigated the circumscription capacity of these barcoding markers in bryophytes. It is shown that none of the markers, taken independently, is sufficiently discriminative for species level identification. However, increasing the number of variable characters by combining several markers provides supported species delineation.

In **chapter 5**, 28 out of 30 European *Dicranum* species are included in a phylogenetic analysis and two methods of species delimitation are compared, namely the general mixed Yule coalescent approach (GMYC) and Poisson tree processes (PTP). In this chapter, we investigate the congruence between morphological and molecular species circumscriptions. In line with the results obtained in chapters 2, 3 and 4, supported species delineation was obtained using five chloroplast and one nuclear markers, but species relationships remained unresolved. The phylogenetic reconstruction reveals that six species are molecularly indistinguishable from closely related allies, reducing the number of species to 24. The GMYC and PTP methods tended to overestimate the number of phylogenetic entities, estimating between 34 and 58 species, and exposed several incongruences between morphological species concept and molecular phylogenetic species delineations. These differences might ensue from evolutionary processes that were so far undiscovered, but might also be linked to methodological issues.

# Chapter 2

## What's in a Name? Disentangling the *Dicranum scoparium* species complex (Dicranaceae, Bryophyta)

A. S. Lang and M. Stech



Published in Systematic Botany, 2014, 39(2): 369-379

ABSTRACT

*Dicranum* is a large (ca. 90 species) and taxonomically complex moss genus. Circumscriptions and relationships of many *Dicranum* species remain ambiguous due to the absence of a worldwide revision and comprehensive phylogenetic analyses. In this study, we address species circumscriptions and relationships of presumed close allies within *Dicranum* sect. *Dicranum*. Molecular phylogenetic reconstructions based on five chloroplast regions and nrITS suggest a close relationship between *D. bonjeanii*, *D. howellii*, *D. nipponense*, *D. japonicum*, *D.* cf. *lorifolium*, and *D. scoparium*, which can be regarded as the *D. scoparium* species complex. In contrast, *D. majus* and *D. polysetum*, as well as *D. fuscescens* and *D. spadiceum* (former varieties of *D. scoparium*), are separated from the complex. Molecular data are generally congruent with the morphological species concept, but the circumscriptions of *D. bonjeanii*, *D. japonicum*, *D.* cf. *lorifolium*, and *D. scoparium* need further study. Most analysed *D. scoparium* specimens from across its Holarctic distribution are contained in one clade (*D. scoparium* S.S.), but a number of North American specimens are resolved as closely related to *D. japonicum* and *D.* cf. *lorifolium*. Costa cross sections and characters of the leaf apex (shape, serrulation of margins) are most relevant for identifying the studied *Dicranum* species morphologically.

INTRODUCTION

In bryophytes, polyphyly of (morpho)species may be the rule rather than the exception, due to a limited number of available morphological characters, the focus on a few key characters with recurrent homoplastic transitions of character states, and the influence of the environment on character variability (Vanderpoorten & Goffinet 2006). Insufficient knowledge of the spatial distribution of morphological variation has furthermore led to the description of high numbers of species and intraspecific taxa, especially in morphologically highly variable genera. In fact, incongruence between morphological species circumscriptions and molecular phylogenetic

reconstructions is increasingly being reported (for review see Heinrichs *et al*. 2009; Vanderpoorten and Shaw 2010). On the other hand, molecular inferences in bryophytes are often based on a single or few molecular markers (Stech & Quandt 2010), and genetic processes such as rapid diversification and incomplete lineage sorting, in particularly in recently diverged species (cf. Rittmeyer and Austin 2012) as well as cryptic speciation (e.g. Bickford *et al*. 2007), have not yet been well studied. Consequently, further comparative analyses of molecular versus morphological characters are necessary to better understand species circumscriptions in bryophytes.

*Dicranum* is a genus of haplolepideous mosses (Dicranidae) with a predominantly Northern Hemisphere, cool-temperate distribution (Frey & Stech 2009). More than 880 binomials were originally described in *Dicranum* (van der Wijk *et al*. 1962; Tropicos.org). To cope with this diversity, several regional taxonomic revisions and systematic treatments have been carried out (e.g. Sakurai 1951; Nyholm 1954, 1987; Takaki 1964, 1972; Peterson 1979; Crum & Anderson 1981; Noguchi 1987; Bellolio-Trucco & Ireland 1990; Otnyukova 2001; Hedenäs & Bisang 2004; Ireland 2007). However, with more than 90 currently accepted species, *Dicranum* (including *Orthodicranum*) is still one of the largest and taxonomically most complex genera of Dicranaceae and the Dicranidae in general (Frey & Stech 2009; Tropicos.org). Morphological species circumscriptions and relationships remain difficult to assess in *Dicranum* as long as neither a worldwide revision nor comprehensive molecular phylogenetic analyses are available.

*Dicranum* species are characterized by falcate-secund, narrowly lanceolate to ovate-lanceolate, usually unistratose leaves; entire to serrate leaf margins; a subpercurrent to shortly excurrent narrow costa that is smooth or with serrate ridges at back; subquadrate to elongate, thick-walled, often porose laminal cells; well-developed alar cells and a haplolepideous, *Dicranum*-type peristome with a single row of teeth around the capsule mouth (e.g. Hedenäs & Bisang 2004; Ireland 2007; Frey & Stech 2009). The main difficulty is to find stable morphological characters at the species level. Gametophytic characters, such as serrulation of leaf margins, number of costal ridges, shape of upper laminal cells, and leaf length and shape, vary considerably depending on environmental conditions (e.g. Hagen 1915; Briggs 1965; Bellolio-Trucco & Ireland 1990; Ireland 2007). Perichaetial leaves and sporophytic characters, which have been considered more significant for species identification (e.g. Hagen 1915; Peterson 1979), are often not available.

The problem of morphological species delimitation is well exemplified in a number of species of section *Dicranum* (Hedw.) Sull. (sensu Nyholm 1987; Bellolio-Trucco & Ireland 1990), whose circumscriptions are unclear due to morphological variability and intergrading forms. These species (including the Holarctic *D. scoparium* Hedw., *D. bonjeanii* De Not. and *D. majus* Turner as well as more narrowly distributed species such as *D. lorifolium* Mitt., *D. japonicum* Mitt. and *D. nipponense* Besch. in Asia, and *D. howellii* Renauld & Cardot in North America), may form a complex of closely related species, or represent intraspecific taxa within one broadly circumscribed species, *D. scoparium* s.l. (e.g. Nyholm 1954, 1987; Lawton 1971; Peterson 1979; Crum & Anderson 1981; Noguchi 1987; Gao & He 1999; Hedenäs & Bisang 2004; Ireland 2007).

In this paper, we address species circumscriptions and relationships of *D. scoparium* and six presumed close allies (*D. bonjeanii*, *D. howellii*, *D. japonicum, D.* cf. *lorifolium*, *D. majus*, and *D. nipponense*), which together may form a species complex. Inferences are based on molecular phylogenetic reconstructions using chloroplast (*rpoB*, *trnH-psbA*, *trnL-trnF*, *rps4-trnT*, *rps19-rpl2*) and nuclear ribosomal ITS sequences. Implications of the molecular data for the suitability of gametophytic characters (e.g. number of costal ridges, serrulation of leaf margins) for species

identification are discussed.

## Materials and Methods

***Sampling—*** A total of 111 *Dicranum* specimens were sampled. The sampling included 17 specimens of which all or part of the sequences had been generated for earlier studies (Stech 1999; Stech *et al*. 2006; Stech & Frey 2008; Lang & Naciri 2010) and 94 newly analysed specimens. As initial molecular analyses showed that several specimens were probably misidentified, morphological re-identifications were carried out by the authors using identification keys for *Dicranum* in Japan (Noguchi & Iwatsuki 1987), China (Gao & He 1999), Europe (Hedenäs & Bisang 2004), and North America (Ireland 2007). These resulted in the following specimen counts and species names used in the final analyses (cf. Figs. 1, 2; Appendix 1): 63 specimens of *D. scoparium*, 40 of other species of section *Dicranum* (five *D. bonjeanii,* five *D. howellii*, six *D. japonicum*, 14 *D.* cf. *lorifolium*, seven *D. majus*, two *D. nipponense*, and one *D. polysetum*), and eight of species of other sections of *Dicranum* (one *D. fragilifolium*, two *D. fuscescens*, one *D. montanum,* and four *D. spadiceum*). Of the latter species, *D. fuscescens* (sect. *Fuscescentiformia*) and *D. spadiceum* (sect. *Muehlenbeckia*) (Chopra 1975; Nyholm 1987; Bellolio-Trucco & Ireland 1990) were former varieties of *D. scoparium*. *Dicranum fragilifolium* (sect. *Elongata*) and *D. montanum* (sect. *Montana*) were included for comparison as representatives of species that have never been associated with sect. *Dicranum*. The sampling of *D. scoparium* covered both the intraspecific morphological variation and distant parts of the species' Holarctic distribution range, i.e. North America (U.S.A., Canada), different parts of Europe (from Iceland to the Caucasus) and Macaronesia, and East Asia (Taiwan, South Korea). However, the sampling was biased towards Continental Europe due to limited availability of collections from other regions and misidentified collections. Besides, *D. scoparium* was recently included in the bryoflora of Australia (Klazenga 2012), but no material from Australia was available. Four samples of *Holomitrium*, one *H. crispulum* Mart. and three *H. arboreum* Mitt., were chosen as outgroup representatives based on the sister-group relationship of *Holomitrium* and *Dicranum* in earlier phylogenetic reconstructions (La Farge *et al*. 2002; Stech *et al*. 2006).

***Molecular Marker Selection—***Five chloroplast DNA regions described in Lang & Naciri (2010), i.e., partial *rpo*B gene, *trn*$H_{GUG}$-*psbA* and *rps*19-*rpl*2 intergenic spacers, and two parts of the *trn*S-F region, namely *rps*4-*trn*$T_{UGU}$ spacer and *trn*L-F (*trn*$L_{UAA}$ intron and *trn*$L_{UAA}$-*trn*$F_{GAA}$ spacer), as well as one nuclear region (nrITS1-5.8S-ITS2) were amplified and sequenced. Except for *rpo*B and *rps*19-*rpl*2, these regions are among the most frequently used phylogenetic markers in bryophytes (Stech & Quandt 2010). The regions rps19-rpl2 and rpoB presented, together with rps4-trnT, the highest sequence variation at the intraspecific level in *Dicranum scoparium* (Lang & Naciri 2010), and were included to overcome the problem of low sequence divergence in *Dicranum* as indicated in earlier phylogenetic studies (Stech 1999; La Farge *et al*. 2002; Stech *et al*. 2006). The rps19-rpl2 region has also been found in the mitochondrial and nuclear genomes of some species of green algae, bryophytes, and angiosperms (e.g. Turmel *et al*. 2002; Raubeson *et al*. 2007; Terasawa *et al*. 2007; Wang *et al*. 2008). Although the primers developed by Lang & Naciri (2010) were based on the chloroplast genome of the moss species *Physcomitrella patens* (Hedw.) Bruch & Schimp. (Sugiura *et al*. 2003), we performed a BLAST search (Altschul *et al*. 1990) and compared our sequences with chloroplast and mitochondrial *rps*19-*rpl*2 sequences of several

other land plants in GenBank, to assure that only orthologous copies from the chloroplast genome were used.

***DNA Extraction, Amplification and Sequencing*—**Several leaves of a single stem apex taken from fresh or herbarium collections were carefully cleaned in demineralised water. DNA was extracted from the dried leaves using the DNeasy plant mini kit (Qiagen, Hilden, Germany) and eluted in 100 µl AE buffer. The PCR reactions were carried out in a final volume of 20 µl. The reaction mixture contained 1× buffer, 2.5 mM $MgCl_2$, 0.2 mM dNTPs, 0.5 µM of both forward and reverse primers, 0.0375 U (5 U/µl) Biotaq polymerase (Gentaur, Brussels, Belgium) and 1 µl DNA. The amplification reactions for the chloroplast markers were performed following Lang & Naciri (2010). Amplification of ITS followed Stech (2004) except for an annealing temperature of 45 °C. The PCR products were purified and sequenced at Macrogen Inc. (www.macrogen.com). GenBank accession numbers of all sequences are listed in Appendix 1.

***Alignment and Phylogenetic Reconstruction*—**Sequences were aligned in Geneious v5.3.6 (Biomatters 2010) using 65% similarity matrix costs, and manually adjusted. Short hairpin-associated inversions in the *trnH-psbA* and *trnL-F* spacers, which can flip at the population level and may significantly reduce phylogenetic structure if undetected (Quandt and Stech 2004; Borsch and Quandt 2009; Whitlock *et al*. 2010), were positionally separated in the alignment and not coded as indels. The dataset used for phylogenetic analyses has been submitted to TreeBASE (study number 13703).

Phylogenetic inferences were based on maximum parsimony (MP), maximum likelihood (ML) and Bayesian inference (BI) analyses. Best-fit models of nucleotide sequence evolution were selected according to the Akaike information criterion in MrModeltest (Posada and Crandall 1998) executed through PAUP* 4.0b10 (Swofford 2002), namely HKY + Γ for the non-coding chloroplast markers (*rps4-trnT*, *trnL-trnF*, *trnH-psbA*, *rps19-rpl2*), and HKY + I for *rpoB* and nrITS. Gaps were coded as informative by a simple indel coding strategy (SIC) (Simmons and Ochoterena 2000) implemented in SeqState (Müller 2004). Sequence and indel data were treated as separate and unlinked partitions, employing the restriction site model ('F81') for the indel matrix.

To check for incongruence, phylogenetic reconstructions based on chloroplast and nuclear sequences (93 and 90 ingroup samples, respectively) were visually compared. In addition, an incongruence length difference test (ILD, Farris *et al*. 1994) as implemented in PAUP* was performed with 100 replicates.

Heuristic searches under parsimony were performed in PAUP* using simple sequence addition and tree bisection-reconnection (TBR) branch swapping. Bootstrap searches under parsimony were performed with 10,000 replicates using the fast bootstrap option. Maximum likelihood analyses were carried out with RAxML v.7.2.6 (Stamatakis 2006) employing the graphical user interface raxmlGUI v.0.93 (Silvestro and Michalak 2012). Bootstrap analyses under ML were done using the thorough bootstrap heuristics algorithm with 20 runs and 200 replicates. Bayesian analyses were run on the Bioportal server (www.bioportal.uio.no). Bayesian posterior probabilities were calculated based on the Markov chain Monte Carlo (MCMC) method, using MrBayes v3.0b4 (Huelsenbeck and Ronquist 2001, Ronquist and Huelsenbeck 2003). The a priori probabilities supplied were those specified in the default settings of the program. Two runs with four chains were run simultaneously ($30 \times 10^6$ generations for chloroplast and combined data and 11 ×

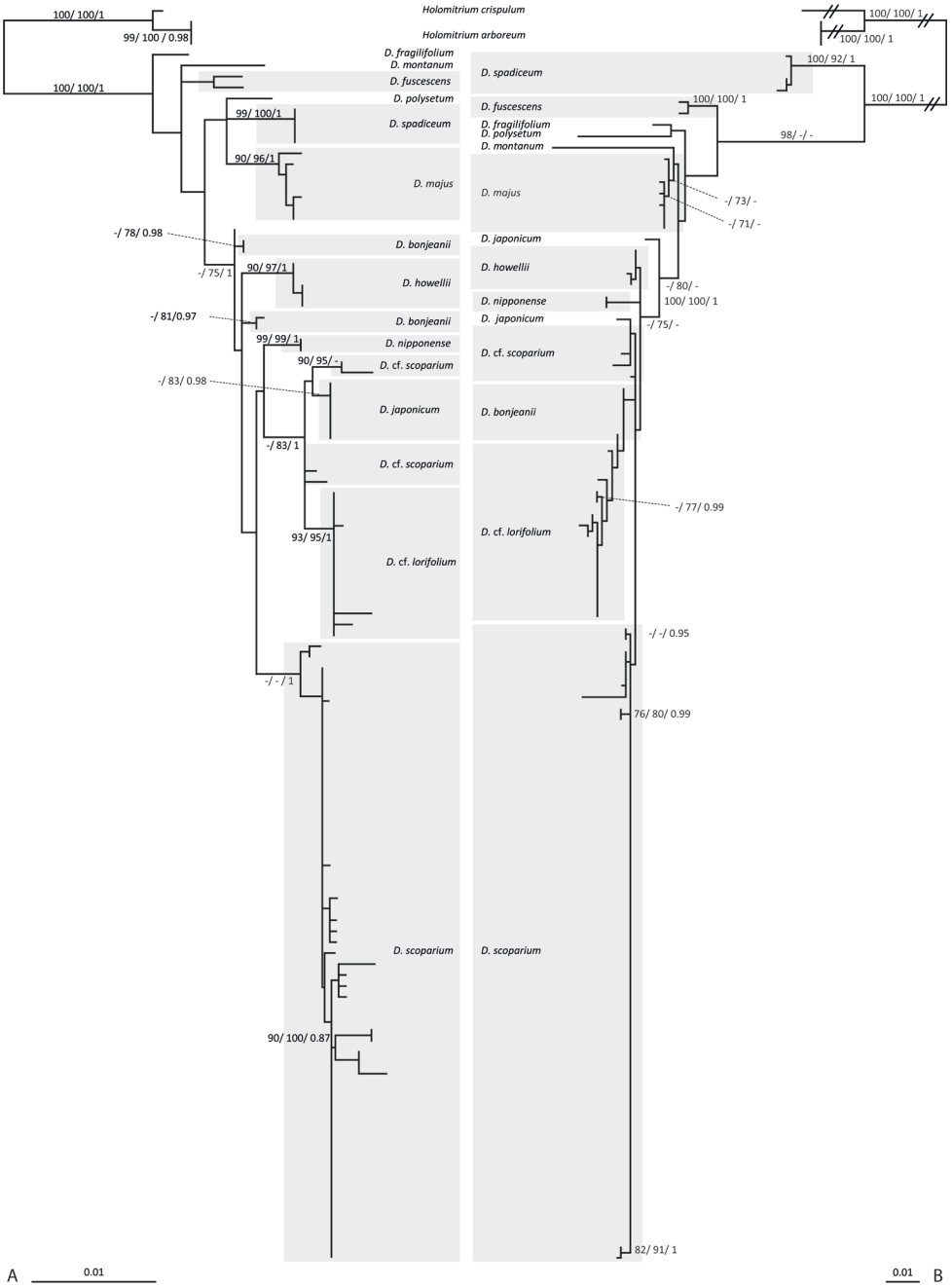


FIG. 1. Single optimal maximum likelihood phylogenetic reconstructions inferred from chloroplast (A) and nrITS (B) sequence data of *Dicranum* species and four specimens of *Holomitrium* as outgroup representatives, including indels coded by simple indel coding (SIC). Support values (maximum parsimony bootstrap, maximum likelihood bootstrap, Bayesian posterior probabilities) are indicated at the branches. Branch lengths are to scale except those indicated by the symbol "//" (shortened 8.5 times).

$10^6$ generations for ITS), with the temperature of the single heated chain set to 0.5. Chains were sampled every 1,000 generations and the respective trees written to a tree file. Fifty percent majority rule consensus trees and posterior probabilities of clades were calculated by combining the four runs and using the trees sampled after the chains converged. Trace plots generated in Tracer v1.5 (Rambaut and Drummond 2007) were used to check for convergence of the runs (plateaus of all runs at comparable likelihoods) and to infer the 'burnin', which was set to 25%.

## Results

Sequence lengths of nrITS ranged from 753–845 nucleotides (nt) in the ingroup (754–990 nt including *Holomitrium*). Corresponding length ranges of the chloroplast markers were 509–521 nt (509–521) for *rps4-trnT*, 449–462 (449–462) for *trnL-trnF*, and 139–140 (139–140) for *trnH-psbA*. No length variation was observed in *rps19-rpl2* (310 nt) or in the sequenced part of *rpoB* (457 nt). The combined chloroplast and ITS alignment comprised 2993 positions including *Holomitrium* (*rps4-trnT* positions 1–521, *trnL-trnF* 522–986, *trnH-psbA* 987–1135, *rps19-rpl2* 1136–1445, *rpoB* 1446–1902, ITS 1903–2993). Of the 2993 positions, 149 ambiguous positions in ITS were removed from the further calculations. Of the remaining 2843 included positions, 309 were variable, and 204 of the variable positions were parsimony-informative (*rps4-trnT* 40/27, *trnL-trnF* 26/23, *trnH-psbA* 17/10, *rps19-rpl2* 21/11, *rpoB* 20/13, ITS 185/120 variable/parsimony-informative positions). Coding gaps by simple indel coding (SIC) yielded a total of 145 indel characters (including *Holomitrium*), of which four corresponding to an inversion in *trnH-psbA* were excluded from phylogenetic analysis. Of the remaining 141 indels characters, 93 (*rps4-trnT* 1, *trnL-trnF* 3, *trnH-psbA* 1, ITS 88) were parsimony-informative.

Maximum parsimony analyses with or without indels included resulted in differently resolved most parsimonious phylogenetic reconstructions, but did not show incongruence with respect to significantly supported clades. Consistency indices of the reconstructions with or without indels included were similar (chloroplast: CI 0.6816 versus 0.6755, ITS: CI 0.9342 versus 0.8731), indicating only a slight increase in homoplasy due to the inclusion of the indel characters in either case.

The single optimal ML trees calculated from the combined chloroplast markers versus ITS including indels (lnL = -3,808.37 and lnL= -3,216.40, respectively), are shown in Fig. 1, with bootstrap support values (BS) from maximum parsimony and maximum likelihood analyses as well as Bayesian posterior probabilities (PP) indicated at the branches. Since both visual inspections of chloroplast versus ITS tree topologies and the ILD test ($p = 0.23$) indicated that the two datasets were congruent, combined analyses of all markers were performed as well, of which the optimal ML tree is shown in Fig. 2 (lnL = -7,367.38). Clade support in the respective analyses without indels was similar to the analyses with indels and therefore not indicated on the trees.

All trees displayed short branches within the ingroup (Figs. 1, 2). A clade comprising six species of sect. *Dicranum* (*D. bonjeanii*, *D. howellii*, *D. japonicum*, *D.* cf. *lorifolium*, *D. nipponense*, and *D. scoparium*) was recovered in both separate analyses (Figs. 1A, B) and with maximal support (100% BS, PP 1) in the combined tree (Fig. 2). *Dicranum majus* and *D. polysetum* were resolved outside this clade. Relationships between them and the other included *Dicranum* species remained largely unsupported. All species with more than one accession sequenced were resolved as monophyletic, except *D. scoparium* in all trees (Figs. 1, 2), *D. bonjeanii* in both separate trees (Figs.

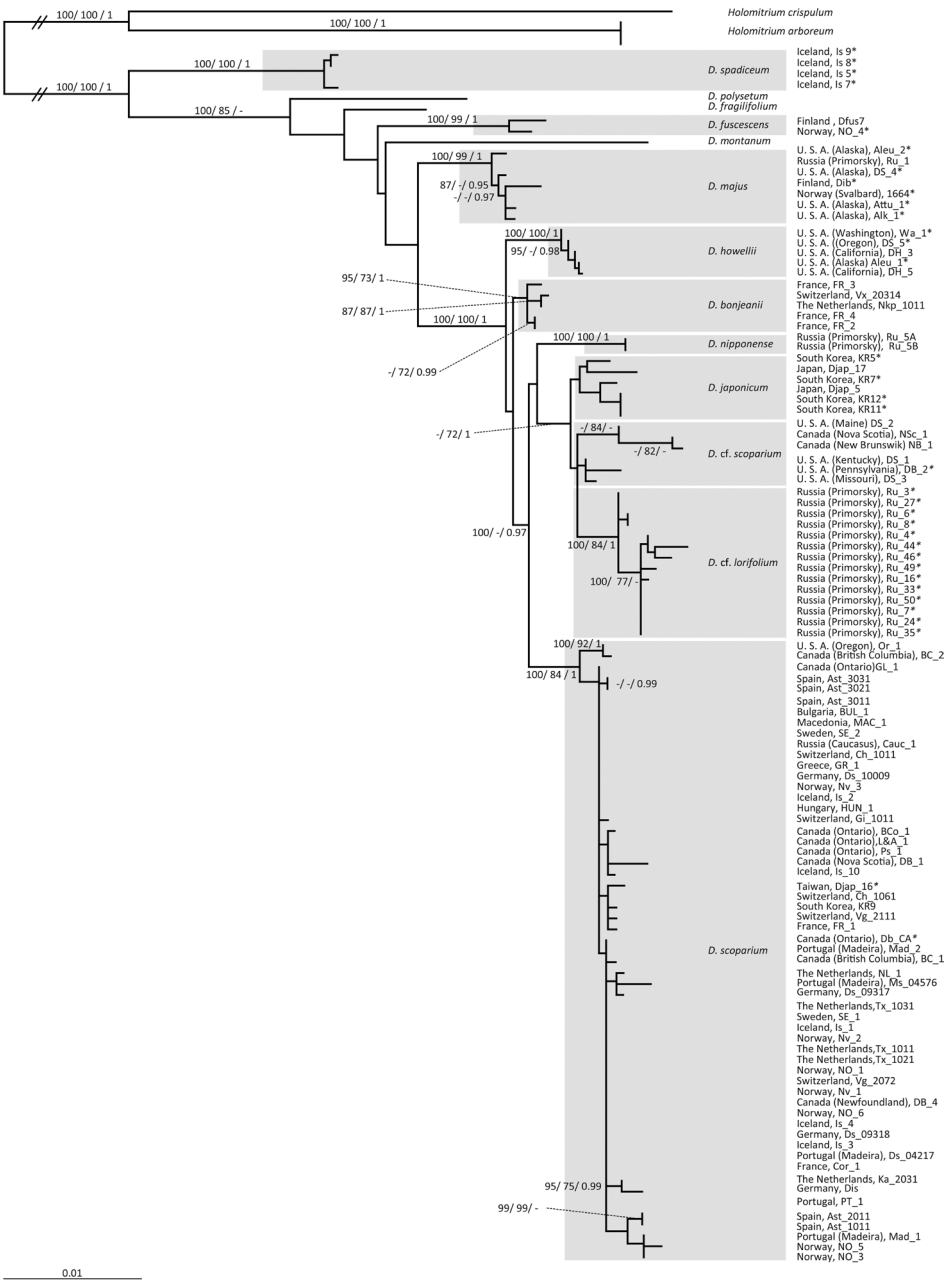


FIG. 2. Single optimal maximum likelihood phylogenetic reconstruction inferred from combined chloroplast and nrITS sequence data of *Dicranum* species and four specimens of *Holomitrium* as outgroup representatives, including indels coded by simple indel coding (SIC). Support values (maximum parsimony bootstrap, maximum likelihood bootstrap, Bayesian posterior probabilities) are indicated at the branches. Samples with an asterisk were re-identified morphologically (original identifications are indicated in Appendix 1). Branch lengths are to scale except those indicated by the symbol "//" (shortend 5.8 times).

1A, B), and *D. japonicum* in the ITS tree (Fig. 1B). The clades of *D. nipponense* and *D. spadiceum* received ≥ 90% BS and PP ≥ 0.99 in all analyses. The clades of *D. howellii*, *D.* cf. *lorifolium*, and *D. majus* were significantly supported in the chloroplast and combined trees (≥90% BS except 84% for *D.* cf. *lorifolium* in Fig. 2, PP 1) but received lower (*D. majus*: -/73% BS) or no support (*D. howellii, D.* cf. *lorifolium*), respectively, in the ITS tree. The clade of *D. fuscescens* received significant support in the ITS and combined trees but no support in the chloroplast tree. The specimens of *Dicranum bonjeanii* and *D. japonicum* were split into two clades in the chloroplast and ITS trees, respectively, and resolved as monophyletic with moderate ( *D. bonjeanii*: 95/73% BS, PP 1) or no support (*D. japonicum*), respectively, in the combined analysis. The majority of the sequenced *D. scoparium* specimens, including all European ones as well as ten from North America and two from East Asia, clustered in one clade in all analyses, with 100/84% BS and PP 1 in the combined analysis. Further putative *D. scoparium* specimens from North America and East Asia were part of a clade together with *D. japonicum* and *D.* cf. *lorifolium* (72/83% BS, PP 1 in the chloroplast and combined trees), but relationships within this clade remained unresolved.



## Discussion

***Tackling Species Circumscriptions and Relationships in Dicranum*—**Earlier molecular phylogenetic analyses indicated low sequence divergence within a clade comprising species of *Dicranum* (including *Orthodicranum*) and the closely related genera *Chorisodontium* and *Paraleucobryum*, contrary to higher sequence divergences in other species-rich genera of Dicranaceae such as *Dicranoloma* and *Leucoloma* (Stech 1999; La Farge *et al.* 2002; Stech *et al.* 2006). However, the respective analyses were based on limited taxon sampling and use of only one or two markers, rendering inferences about the suitability of DNA sequence data to resolve species circumscriptions and relationships in *Dicranum* difficult. Recently Tubanova and Ignatova (2011) analysed Russian *Dicranum* species based on nrITS sequences from a larger taxon sampling. Their study revealed some well-supported clades consisting of a single species or a few closely related species each, but the overall resolution and support of the phylogenetic reconstruction remained low. In cases of little molecular variation and phylogenetic structure, the best strategy would probably be to combine the information of several suboptimal markers to collect a small number of synapomorphic sites from each of them, until well-resolved phylogenetic trees can be produced (e.g. Edwards *et al.* 2007; Leaché and Rannala 2010; Stech & Quandt 2010; Dong *et al.* 2012), provided that issues such as evolutionary model selection per marker, possible incongruence between markers, or incomplete lineage sorting are taken into account (e.g. Holland *et al.* 2004; Kubatko and Degnan 2007; Liu and Pearl 2007). In the present study, trees generated separately from five chloroplast markers and ITS (Fig. 1) displayed an overall similar topology despite less resolution in ITS. Combined analyses of all six markers generally increased statistical support for clades at the species level in *Dicranum* (Fig. 2). Furthermore, all species with more than one accession sequenced (except possibly for *D. scoparium*, see discussion below) were resolved as monophyletic in the combined analysis, indicating that molecular lineages were generally congruent with the morphological species concept in *Dicranum*. However, the respective clades representing species were partly unsupported and their relationships remained largely unresolved. Probably an even higher number of molecular markers, including variable mitochondrial and single-copy nuclear markers, needs to be combined to infer supraspecific relationships in *Dicranum*.

***Molecular Characterisation of the D. scoparium Species Complex*—**The present molecular phylogenetic reconstructions (Figs. 1, 2) suggest a close relationship between *D. bonjeanii*, *D. howellii*, *D. nipponense*, *D. japonicum*, *D.* cf. *lorifolium*, and *D. scoparium*, which can be regarded as the *D. scoparium* species complex. These species were all considered to belong to section (or subgenus) *Dicranum* (e.g. Sakurai (1951) [as subgenus *Scopario-Dicranum*]; Takaki 1964; Nyholm 1987, Bellolio-Trucco & Ireland 1990), except *D. nipponense*, which was placed into a new subgenus *Nippono-Dicranum* by Sakurai (1951) based on peristome characters. As these characters turned out to be insignificant, however, and no other characters supported this segregation, *D. nipponense* was placed close to *D. scoparium* and allies again (Takaki 1964), which is supported by the molecular data. In contrast, other species of section *Dicranum* sensu Nyholm (1987), namely *D. majus* and *D. polysetum*, are separated from the *D. scoparium* complex based on the molecular inferences (Figs. 1, 2). The same holds for the species that were formerly treated as varieties of *D. scoparium* but placed in other sections than sect. *Dicranum* in the more recent literature, i.e., *D. fuscescens* (sect. *Fuscescentiformia*) and *D. spadiceum* (sect. *Muehlenbeckia*) (e.g. Nyholm 1987; Bellolio-Trucco & Ireland 1990). Whether additional *Dicranum* species that are putatively closely related to *D. scoparium* (e.g. *D. crassifolium* Sérgio, Ochyra and Seneca and *D. leioneuron* Kindb.) fall into the *D. scoparium* complex as defined here remains to be tested. Besides, at present no morphological synapomorphies are known that delimit the *D. scoparium* complex from *D. majus* and other *Dicranum* species.

***Morpho-molecular Species Circumscriptions*—**Distinction of the species here comprised as the *D. scoparium* complex and *D. majus* has been considered difficult for a long time due to their phenotypic plasticity. The gametophyte of *D. majus* is typically characterized by large plants with longer and more strongly falcate-secund leaves than in *D. scoparium*, leaf margins serrate in distal half, strongly porose lamina cells, and in particular a costa with a double row of guide cells (single row in *D. scoparium*) and an irregularly furrowed abaxial surface instead of with four continuous longitudinal ridges. However, large plants of *D. scoparium* may resemble *D. majus*, whereas small plants of *D. majus*, especially plants from arctic-alpine regions, which often show only one layer of costa guide cells, are easily confused with *D. scoparium* (e.g. Nyholm 1954; Takaki 1964; Hedenäs & Bisang 2004; Hedenäs *et al*. 2006). The morphological variation of *D. majus* led to the description of multiple varieties, of which two relate to the arctic morphotype: var. *orthophyllum* A. Braun ex Milde was commonly used in North America (Grout 1937; Steere 1978), while var. *condensatum* I. Hagen was recognized in the European and Russian Arctic (Hagen 1915; Brotherus 1923; Abramova *et al*. 1961). Wahlenberg (1814), in contrast, did not consider *D. majus* a separate species with intraspecific variation, but rather a form of *D. scoparium*. The present molecular data support the species status of *D. majus* and its separation from *D. scoparium*. Morphologically, the presence of furrows on the costa and the double row of guide cells, or at least few double guide cells that are always present at the leaf base, can be regarded as diagnostic characters for *D. majus* (Hedenäs *et al*. 2006; Table 1). Whether the distinction of two geographical morphotypes in Europe (Hedenäs *et al*. 2006) holds true at the molecular level remains to be tested.

*Dicranum bonjeanii* can usually be recognized by its transversely undulate upper leaf portions, erect-patent leaves when moist, a narrow costa with two weak dorsal ridges that are serrate to nearly entire, and a rather broad leaf acumen. However, leaf shape and undulation have also been reported to be the most variable characters (Briggs 1965), and the taxonomic

TABLE 1. Recapitulation of gametophytic characters, ecological and geographical ranges for the species included in the *Dicranum scoparium* complex and *D. majus* (Gao & He 1999; Hedenäs & Bisang 2004; Ireland 2007; Klazenga 2012; present study).

| | Gametophytic characters from the literature | Suggested diagnostic characters | Ecological and geographical range |
|---|---|---|---|
| *D. bonjeanii* | Leaves erect-patent, upper portions transversely undulate, margins denticulate, acumen blunt or obtuse, costa narrow, 2 low dorsal ridges, weakly serrate, leaf lamina cells prosenchymatous, porose. | Leaf apex blunt or obtuse, costa narrow, with (usually 2) weakly developed ridges. | Moist soil or temporarily wet depressions in rich fens or mires. Holarctic. |
| *D. howellii* | Leaves falcate-secund to straight and erect, margins strongly serrate in the distal half, acumen acute, keeled or tubulose, costa percurrent to shortly excurrent, with 2 (- 4) toothed ridges, lamina cells prosenchymatous, porose. | Leaf apex acute, costa narrow, with two toothed ridges. | Soil, humus, humus over rock, rotten logs, tree trunks and tree bases. Western North America. |
| *D. japonicum* | Leaves erect-patent, sometimes undulate, margins coarsely serrate, costa slender, percurrent to shortly excurrent, ending in a short hairpoint, apex long subulate or keeled, costa with 2 serrate ridges, lamina cells oblong-rhomboidal in distal part, porose. | Leaf about 10 times as long as wide, leaf margins coarsely serrate, apex subulate or keeled. | Humic soil, rock, or rotten logs. China, Korea, Japan, Russian Far East. |
| *D. lorifolium* | Leaves homomallous, falcate-secund to erect-patent, narrowly lanceolate, margins sharply serrate near the apex, apex long, canaliculate, costa thin, percurrent, serrate distally, lamina cells rhomboidal to short-rectangular in distal part, porose. | Leaf about 10 times as long as wide, leaf margins sharply serrate distally, apex subulate. | Rotten wood or tree bases. China, Bhutan, Nepal, India. |
| *D. majus* | Leaves long, strongly falcate-secund, margins serrate in distal half, costa with 2 rows of guide cells, irregularly furrowed on abaxial surface, lamina cells prosenchymatous, porose. | Costa furrowed with 2 rows of guide cells at the base (or at least few double guide cells). | Humid to wet soil and rock, in forests and more open habitats. Holarctic. |
| *D. nipponense* | Stems unevenly foliate, leaves erect-spreading or falcate-secund, margins coarsely serrate, apex shortly acute to acuminate, costa subpercurrent, slender, 2 ridges, keeled, laminal cells elongate- rhomboidal distally, porose. | Stem apex crowded, leaves shorter at the base of the stem, falcate-lanceolate, margins dentate, apex keeled, obtuse. | Soil, rock, or rotten wood. China, Korea, Japan. |
| *D. scoparium* | Leaves straight to falcate-second, margins serrate, apex keeled, costa percurrent, 4 serrate ridges with several stereid bands proximally, lamina cells prosenchymatous, porose. | Leaf apex keeled, costa with 4 serrate ridges in upper part leaf. | Soil, humus, humus over rock, rotten wood, tree bases, dry and humid environments. Holarctic, Australia. |

rank of *D. bonjeanii* has often been questioned due to the presence of intergrading forms with *D. scoparium*. While in Europe and Asia *D. bonjeanii* is generally accepted as a separate species, North American bryologists preferred to consider it as one of the multiple forms of *D. scoparium,* probably induced by the environment (Grout 1937; Jennings 1951; Lawton 1971; Peterson 1979; Crum & Anderson 1981; but see Ireland 2007). *Dicranum bonjeanii* is mostly found in eutrophic fens, whereas *D. scoparium* s.str. is found on different substrates mainly in dry to mesic woodlands. Although the *D. bonjeanii* clade is only resolved in the combined chloroplast and ITS tree (Fig. 2), the present molecular data indicate that *D. bonjeanii* can, at least in Europe, be separated from *D. scoparium* s.str., which supports the ecological differentiation and morphological differences of *D. bonjeanii*, in particular the narrow costa ending into a broad apex as well as the (usually two) weakly developed costal laminae (Table 1). Whether this taxon occurs in North America as well remains to be tested, which seems to be complicated since most of the specimens collected under this name were wrongly identified (Ireland 2007).

Other species within the *D. scoparium* complex cause identification problems in more restricted geographic areas. The western North American endemic *D. howellii* is characterized, when fertile, by the gradually acuminate inner perichaetial leaves (abruptly long-acuminate, convolute-sheathing in *D. scoparium*), whereas due to the plasticity of *D. scoparium*, the gametophytic characters of both species largely overlap. Besides, both *D. howellii* and *D. scoparium* occur on similar substrates (Table 1). Consequently, some authors considered *D. howellii* as a synonym (Grout 1937) or as a variety of *D. scoparium* (Peterson 1979). At the molecular level, however, *D. howellii* can clearly be distinguished from *D. scoparium* (Figs. 1A, 2). Gametophytically, the most reliable character to recognize *D. howellii* seems to be its narrow costa with two low ridges.

The East Asian *D. nipponense* resembles both *D. japonicum* in its larger forms (Noguchi 1987) and *D. bonjeanii* in its weaker forms (Otnyukova 2001). It is, however, distinguished from closely related *Dicranum* species by a combination of characters including unevenly foliate stems (lower leaves smaller than upper ones) with leaves crowded at the stem apex, falcate-lanceolate leaves that are broadest just above the base and keeled above with a broad and dentate point, and linear-rectangular and strongly porose laminal cells (Otnyukova 2001). Further differences with *D. bonjeanii* are the strongly dentate margins and the costa having usually two to three serrate ridges at the back (Table 1). Besides, *D. nipponense* grows mainly on rotten wood. Together with the degree of molecular divergence (cf. the significant support and comparatively long branches in Figs. 1 and 2), these characters indicate that *D. nipponense* is well characterized.

The circumscription of *D. scoparium* itself and its delimitation from *D. japonicum* and *D.* cf. *lorifolium* seems to be most difficult according to the present results. As the large clade of mainly European specimens (Figs. 1, 2) corresponds to the typical morphology of *D. scoparium* (cf. Hedenäs & Bisang 2004), we consider it to represent *D. scoparium* s. s. The fact that some specimens from North America and East Asia are included in this clade coincides with the wide distribution of *D. scoparium*. A larger sampling from outside Europe is, however, necessary for more solid conclusions on the total distribution and frequency of *D. scoparium* s. s. in the different continental areas.

The other specimens from North America and East Asia originally identified as *D. scoparium* (cf. Appendix 1; Fig. 2) differ from the plants in the *D. scoparium* s. s. clade in having generally narrower, linear leaves ending in an acuminate to setaceous apex. The distal abaxial side of the costa has at least four strongly serrulate lamellae, the leaf margins are strongly serrulate, and the lamina cells are finely porose. A BLAST search of the respective sequences did not correspond to

any North American or Asian accession available on GenBank, including *D. bardunovii* Tubanova & Ignatova, which was recently described from Buryatia, south-central Siberia, based on morphological characters and nrITS sequences (Tubanova and Ignatova 2011). The sequenced specimens from Japan, South Korea, and Russia morphologically resemble two closely related East Asian species, *D. japonicum* and *D. lorifolium*; however, morphological differences between these species and *D. scoparium* are rather subtle. The latter differs from the two Asian species mainly by a broader leaf base and a percurrent instead of excurrent costa (Gao & He 1999). In addition, Noguchi (1987) mentions that the main difference between *D. japonicum* and *D. scoparium* is found in their habitat preference, with the former growing on humus in shady habitats and the latter in sunnier and drier places. The morphological characters distinguishing the two Asian species from each other are mainly based on the sporophyte, namely inclination of the capsule as well as size and papillosity of the spores (Gao & He 1999). The only gametophytic difference is the canaliculate (*D. lorifolium*) versus keeled (*D. japonicum*) leaf apex (Table 1). Considering these slight differences and the fact that two further specimens from Japan were originally identified as *D. japonicum*, we provisionally conclude that the specimens from Japan and South Korea belong to *D. japonicum*. The Russian specimens probably represent *D.* cf. *lorifolium* as judged from the presence of suberect capsules in the two fertile specimens sequenced (Ru_6 and Ru_7). The sequenced North American specimens, in contrast, fall within the range of morphological variation of *D. scoparium*. Their taxonomic status and delimitation from *D. japonicum* and *D.* cf. *lorifolium* needs to be assessed based on further molecular and morphological analyses of a larger sampling of North American and Asian specimens, which may reveal further "cryptic" molecular lineages as well.

Because bryophytes have limited numbers of morphological characters, which are in addition under strong environmental constraints (e.g. Briggs 1965; Vanderpoorten & Goffinet 2006), it is difficult to identify key characters defining species. This is especially true in a genus with polymorphic species such as *Dicranum*. Molecular phylogenetic analyses may allow species to be circumscribed even when morphological characters are ambiguous (e.g. Vanderpoorten & Goffinet 2006; Stech *et al*. 2011; Stech *et al*. 2013). However, it is necessary to re-address the morphology in light of the molecular phylogenetic reconstructions to infer whether morphologically variable but molecularly well-supported species can be identified with certainty by morphological characters (e.g. Sukkharak *et al*. 2011). In the present study, molecular analyses provided useful support for defining part of the species of the *D. scoparium* complex as well as *D. majus*, which allowed reconsideration of the most reliable gametophytic characters to identify them (Table 1). On the other hand, the molecular data raised questions concerning the circumscription of *D. bonjeanii*, *D. japonicum*, and *D. lorifolium*, species for which the morphological definition was also limited. Further data are necessary to reach taxonomic conclusions for these species and to finally decide how many taxa should be distinguished in the *D. scoparium* complex. Nevertheless, the present approach is promising for the study of other taxonomically difficult complexes of closely related species in bryophytes.

# Chapter 3

## Species delimitations in the *Dicranum acutifolium* complex (Dicranaceae, Bryophyta) using molecular markers

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Journal of Bryology, in press



ABSTRACT

Because of their morphological plasticity and broad geographic distribution, the taxonomy of *Dicranum* is difficult. The circumscription of the different species included in the *Dicranum acutifolium* complex is poorly understood and taxonomic confusions are frequent. The present study extends earlier ITS-based phylogenetic reconstructions of the *D. acutifolium* complex by analysing five additional chloroplast markers (*trnT-rps4, trnL-F, psbA-trnH, rps19-rpl2, rpoB*) together with ITS of a larger taxon sampling. The phylogenetic analyses delimit *Dicranum acutifolium* (Lindb. & Arnell) C.E.O Jensen, *D. bardunovii* Tubanova & Ignatova, *D. brevifolium* (Lindb.) Lindb., and *D. septentrionale* Tubanova & Ignatova, which together form the *D. acutifolium* complex, and confirm that *D. pseudoacutifolium* is synonymous with *D. flexicaule*. *Dicranum septentrionale* was known so far from across Russia but also occurs in Scandinavia, where it was probably overlooked due to morphological resemblance with *D. brevifolium*. The problem of mixed collections for identification is exemplified by the holotype of *D. bardunovii*, which contains also individuals of the morphologically most similar *D. acutifolium* according to the molecular data. Morphometric analyses support the differentiation of the *D. acutifolium* complex. Furthermore, ordination analyses point to a continuous range of variation among species within the *D. acutifolium* complex, especially due to the larger variation of *D. septentrionale*.

### INTRODUCTION

*Dicranum* Hedw. (including *Orthodicranum* (Bruch & Schimp.) Loeske) is a large moss genus of more than 90 accepted species (Frey & Stech 2009; Tropicos.org) with a predominantly Holarctic distribution. Several *Dicranum* species are known to be morphologically plastic (Hedenäs & Bisang 2004; Ireland 2007), and their circumscriptions remain ambiguous as neither a thorough worldwide revision nor a complete phylogenetic analysis of *Dicranum* is available yet. Nevertheless, a number of recent studies tackled certain groups of *Dicranum* species based on molecular data, namely

the *D. scoparium* Hedw. complex (Lang & Stech 2014), arctic *Dicranum* species (Lang *et al*. 2014), *Dicranum* species with fragile leaves (Ignatova & Fedosov, 2008) or the *D. acutifolium* (Lindb. & Arnell) C.E.O. Jensen and *D. fuscescens* Turner complexes (Tubanova *et al*. 2010; Tubanova & Ignatova 2011). The former two studies concluded that (closely related) *Dicranum* species can be best delimited based on combined analysis of five chloroplast markers and the nuclear ribosomal ITS region. Inferences on the *D. acutifolium* complex, in contrast, were based on ITS only and need to be re-evaluated based on a larger marker sampling.

The *Dicranum acutifolium* complex is part of *Dicranum* sect. *Spuria* Bruch & Schimp. (sensu Nyholm, 1987) and centred around two circumarctic – alpine species, *D. acutifolium* and *D. brevifolium* (Lindb.) Lindb. They were first described as varieties of *D. bergeri* Blandow (Lindberg & Arnell 1890) or *D. muehlenbeckii* Bruch & Schimp. (Lindberg 1865), respectively, the former being an erroneous name for *D. undulatum* (cf. Hedenäs & Bisang 2004). Otnyukova (2007) described a new species, *D. pseudoacutifolium* Otnyukova, which differed from *D. acutifolium* and *D. brevifolium* in morphological characters such as the absence of bulgings above cell walls, non-porose lower leaf cells, and inner perichaetial leaves abruptly contracted into a subula. However, molecular data revealed that the type specimen of this species corresponded to a weak form of *D. flexicaule* Brid., with whom it has been consequently synonymized (Tubanova *et al*. 2010), while other *D. pseudoacutifolium* specimens had identical ITS sequences with *D. acutifolium* (Tubanova *et al*. 2010). On the other hand, two newly identified molecular lineages were described as species, i.e. *D. septentrionale* Tubanova & Ignatova and *D. bardunovii* Tubanova & Ignatova (Tubanova *et al*. 2010; Tubanova & Ignatova 2011). These two species are morphologically very close to *D. brevifolium*, but also share several features with *D. acutifolium*. However, neither the newly described species nor *D. brevifolium* or *D. acutifolium* formed strongly supported clades based on ITS sequences only. The *Dicranum acutifolium* complex, in line with Tubanova *et al*. (2010), thus comprises four species, *D. acutifolium*, *D. brevifolium*, *D. bardunovii* and *D. septentrionale*. It is characterised by a combination of morphological characters including leaves that are keeled distally with the blade shaped like a pair of tongs in cross-section as well as thick-walled lamina cells that are subquadrate to short rectangular, sometimes irregularly shaped above and elongated and porose below.

This study extends the phylogenetic reconstructions of Tubanova *et al*. (2010) and Tubanova & Ignatova (2011) by analysing five chloroplast markers (*trnT-rps4, trnL-F, psbA-trnH, rps19-rpl2, rpoB*) in combination with ITS of a larger taxon sampling. Based on the molecular data and a re-evaluation of morphological characters, we aim to (i) add further molecular support to the circumscription of the *D. acutifolium* complex, (ii) clarify species circumscriptions in the complex, and in particular evaluate the taxonomic status of *D. bardunovii* and *D. septentrionale*, and (iii) investigate the value of morphological characters used to distinguish the species of the complex.

## Materials and Methods

***Sampling—*** A total of 67 *Dicranum* specimens were sampled (Appendix 1). The sampling included 15 specimens of which ITS sequences had already been generated by Tubanova *et al*. (2010) and Tubanova & Ignatova (2011), 12 specimens newly sequenced for all six markers employed here (see below), and 40 specimens of which chloroplast and ITS sequences were generated for previous studies (Lang & Stech 2014; Lang *et al*. 2014; Stech, 1999; Stech *et al*. 2006). We studied 29 specimens of sect. *Spuria*: six originally identified as *D. acutifolium,* 13 *D. brevifolium,* two *D. septentrionale*, three *D. bardunovii* (including the holotype, from which two

plants were sequenced separately), one *D. drummondii,* and four *D. undulatum*. Eight *Dicranum* species were included as representatives of other sections than sect. *Spuria*: four specimens of *D. spadiceum* J.E. Zetterst. (sect. *Muehlenbeckia* Peterson), three *D. elongatum* Schleich. ex Schwägr. (sect. *Elongata* Hag.), five *D. fuscescens* and 11 D. *flexicaule* (sect. *Fuscescentiformia* Kindb.), seven *D. majus* Turner, two *D. nipponense* Besch., two *D. scoparium*, and three *D. bonjeanii* De Not. (all sect. *Dicranum* Hedw.). *Dicranum muehlenbeckii* (sect. *Muehlenbeckia*) could not be included in the molecular analyses because of unsuccessful DNA amplification. However, two specimens were included in the morphological analyses. Previous studies resolved *Holomitrium* Brid. as sister group of *Dicranum* (La Farge *et al*. 2002; Stech *et al*. 2006). Therefore, four samples, one *H. crispulum* Mart. and three *H. arboreum* Mitt., were chosen as outgroup representatives (Appendix 1).

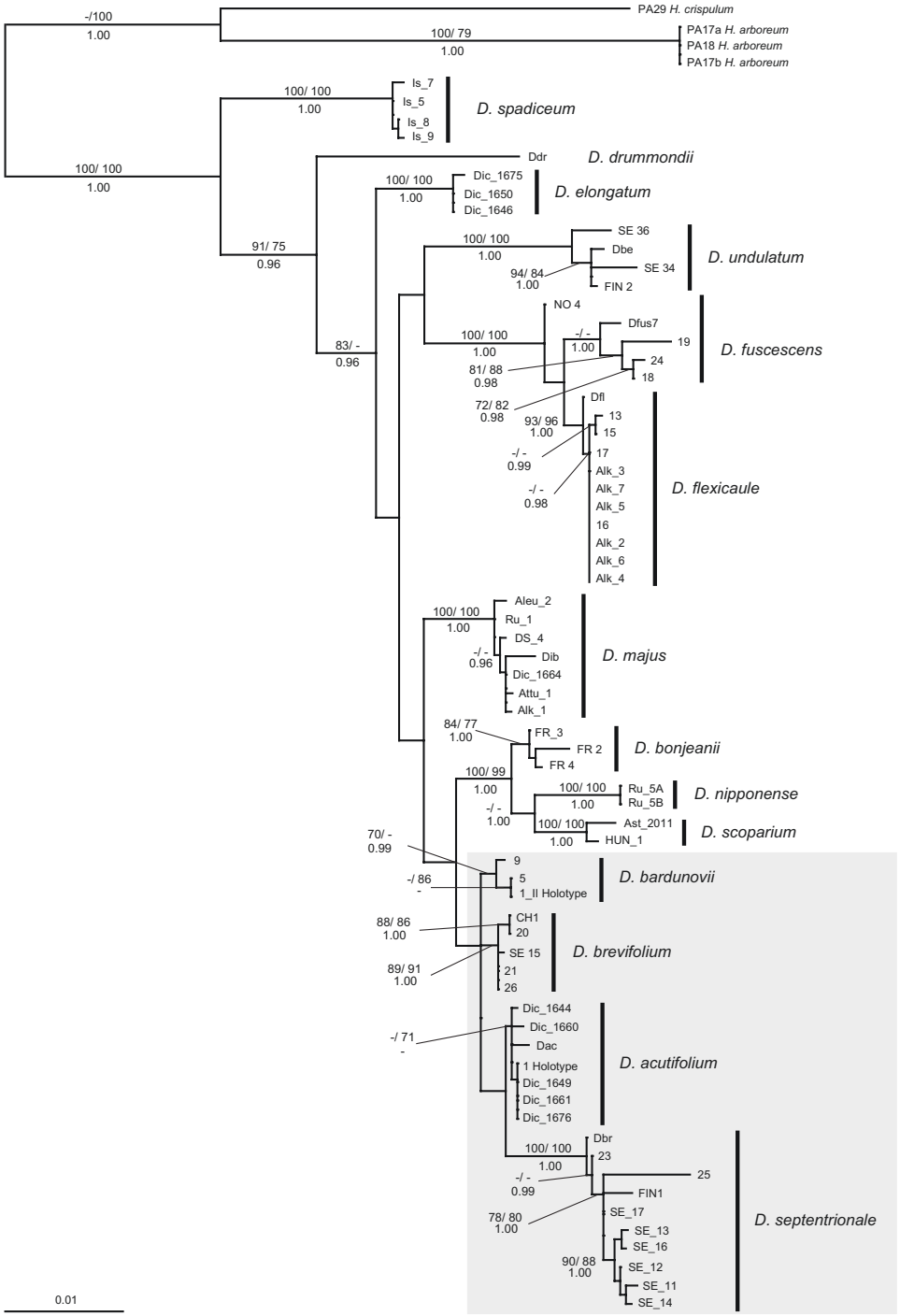
***DNA extraction, amplification and sequencing—*** The greenest part of single gametophyte shoots was selected for DNA extraction. After cleaning the shoot under a binocular, total DNA was extracted using the NucleoSpin® Extract II Kit (Macherey-Nagel, Düren, Germany). Six markers employed to delimit closely related *Dicranum* species in Lang & Stech (2014) and Lang *et al*. (2014) were amplified and sequenced: five chloroplast regions (partial *rpoB* gene, *trnH*$_{GUG}$*-psbA*, *rps19-rpl2*, and *rps4-trnT*$_{UGU}$ intergenic spacers, and *trnL*$_{UAA}$ intron / *trnL*$_{UAA}$*-trnF*$_{GAA}$ intergenic spacer) and the nuclear ribosomal nrITS1-5.8S-ITS2 region. PCR amplifications were performed as described in Lang & Stech (2014). All PCR products were purified and sequenced at Macrogen Inc. (www.macrogen.com). GenBank accession numbers of all sequences are listed in Appendix 1.

***Alignment and phylogenetic reconstruction—*** Alignment and phylogenetic reconstruction

Sequences were aligned in Geneious v6.1.6 (Biomatters, available from www.geneious.com) using 65% similarity matrix costs, and manually adjusted. One short hairpin-associated inversion in the *trnH-psbA* spacer, which can flip at the population level and may significantly reduce phylogenetic structure if undetected (Quandt & Stech 2004; Borsch & Quandt 2009; Whitlock *et al*. 2010), was positionally separated in the alignment and not coded as indels.

The best substitution model was selected for each locus according to the Akaike information criterion (AIC) using MrModeltest (Posada & Crandall, 1998) executed through PAUP* 4.0b10 (Swofford 2002). Gaps were coded as informative by simple indel coding (SIC) (Simmons & Ochoterena 2000) as implemented in SeqState (Müller 2004). To check for incongruence, phylogenetic reconstructions based on chloroplast and nuclear sequences were visually compared. In addition, an incongruence length difference test (ILD, Farris *et al*. 1994) as implemented in PAUP* was performed with 100 replicates. As both visual inspections and the ILD test indicated that the plastid and nuclear tree topologies were congruent ($p$=0.03), the two datasets were combined for analysis in a total evidence approach.

Phylogenetic inferences were based on maximum parsimony (MP), maximum likelihood (ML) and Bayesian inference (BI) analyses, with and without indels coded by SIC included. Heuristic searches under parsimony were performed in PAUP* using simple sequence addition with 1000 replicates and tree bisection-reconnection (TBR) branch swapping. The nucleotide matrix was divided into three partitions for ML and BI, namely the non-coding chloroplast markers (*rps4-trnT*, *trnL-trnF*, *trnH-psbA*, *rps19-rpl2*), the chloroplast gene *rpoB*, and the nrITS region. Maximum likelihood analyses were carried out with RAxML v.7.2.6 (Stamatakis 2006) employing the graphical user interface raxmlGUI v.0.93 (Silvestro & Michalak 2012). As implemented in RAxML, the GTR model


PA29 H. crispulum
PA17a H. arboreum
PA18 H. arboreum
PA17b H. arboreum
Is_7
Is_5
Is_8
Is_9
D. spadiceum
Ddr
D. drummondii
Dic_1675
Dic_1650
Dic_1646
D. elongatum
SE 36
Dbe
SE 34
FIN 2
D. undulatum
NO 4
Dfus7
19
24
18
D. fuscescens
Dfl
13
15
17
Alk_3
Alk_7
Alk_5
16
Alk_2
Alk_6
Alk_4
D. flexicaule
Aleu_2
Ru_1
DS_4
Dib
Dic_1664
Attu_1
Alk_1
D. majus
FR_3
FR 2
FR 4
D. bonjeanii
Ru_5A
Ru_5B
D. nipponense
Ast_2011
HUN_1
D. scoparium
9
5
1_II Holotype
D. bardunovii
CH1
20
SE 15
21
26
D. brevifolium
Dic_1644
Dic_1660
Dac
1 Holotype
Dic_1649
Dic_1661
Dic_1676
D. acutifolium
Dbr
23
25
FIN1
SE_17
SE_13
SE_16
SE_12
SE_11
SE_14
D. septentrionale
0.01

of nucleotide substitution with Γ model of rate heterogeneity was used for all partitions. Bootstrap searches under ML were done using the thorough bootstrap heuristics algorithm with 20 runs and 1000 replicates. Bayesian analyses were run on the CIPRES science gateway (Miller *et al*. 2010). Bayesian posterior probabilities were calculated based on the Markov chain Monte Carlo (MCMC) method, using MrBayes v3.2.1 x64 (Huelsenbeck & Ronquist 2001; Ronquist & Huelsenbeck 2003), with MrModeltest best fit models HKY + Γ for the non-coding chloroplast markers and HKY + I for *rpoB* and nrITS. Nucleotide and indel data were treated as separate and unlinked partitions, employing the restriction site model ('F81') for the indel matrix as recommended by Ronquist *et al*. (2005). The a priori probabilities supplied were those specified in the default settings of the program. Two runs with four chains were run simultaneously ($11 \times 10^6$ generations), with the temperature of the single heated chain set to 0.5. Chains were sampled every 1000 generations and the respective trees written to a tree file. Fifty percent majority rule consensus trees and posterior probabilities of clades were calculated by combining the four runs and using the trees sampled after the chains converged. Trace plots generated in Tracer v1.5 (Rambaut & Drummond 2007) were used to check for convergence of the runs (plateaus of all runs at comparable likelihoods) and to infer the 'burnin', which was set to 25%.

***Morphological analysis—*** A total of 47 specimens were included in the morphological analyses: all six *D. acutifolium*, five *D. brevifolium*, three *D. bardunovii* and ten *D. septentrionale* as well as two *D. scoparium*, two *D. nipponense*, three *D. bonjeanii*, three *D. majus*, four *D. undulatum* and the four *D. spadiceum*, plus three additional *D. drummondii* and two *D. muehlenbeckii* specimens that could not be sequenced. Thirty-four gametophytic characters were scored according to their relevance for species identification (Nyholm, 1987; Hedenäs & Bisang 2004; Tubanova *et al*. 2010). As none of the examined samples carried sporophytes, sporophytic characters were not included in the statistical analyses. Presence or absence of character states was scored for each sample (Appendix 2). Morphological scoring was made under a light microscope on three branch leaves removed from the upper part of the stem, excluding the uppermost part. Three additional leaves were removed and used for scoring characters of costa cross-section. Multistate characters were artificially separated into binary characters for analytical reasons.

A multivariate approach was used to investigate the phenotypic affinities between the taxa of the *D. acutifolium* complex and other putatively closely related species. Morphological discontinuities were first explored through a hierarchical cluster analysis based on Jaccard distances and the complete-linkage method as clustering strategy using the vegan package (Oksanen *et al*. 2013) in R 2.15 (R Development Core Team 2013). Pearson's correlation coefficient was calculated to evaluate the optimal number of clusters. To further explore the morphological similarity of species, we performed an ordination with nonmetric multidimensional scaling (NMDS; Kruskal 1964) applying the metaMDS function of vegan with its default arguments. We used the Jaccard matrix to produce a five dimensional ordination (i.e., $k=5$) and plotted against species

◄— Fig. 1. Single optimal maximum likelihood phylogenetic reconstruction inferred from the partitioned matrix for the non-coding chloroplast loci( trnT-rps4- trnL-F- trnH-psbA- rps19- rpl2), the coding region *rpo*B and nrITS, including indels coded by simple indel coding (SIC). The default GTR+ Γ model was applied for all DNA partitions and F81 was employed for the indel matrix. Bootstrap analyses under ML were done using the thorough bootstrap heuristics algorithm with 20 runs and 1000 replicates. BI was obtained with the best fit models HKY + Γ for the first partition, and HKY + I for rpoB and nrITS and F81 for the indel matrix, after 11,000,000 generations with two runs and four chains and the temperature of the single heated chain set to 0.5. Trees were sampled every 1000 generations and a burnin was set to 25%. Four specimens of Holomitrium were used as outgroup representatives. Support values (MP and ML BS ≥70%, BI PP ≥0.95) are indicated at the branches. Grey boxes delimit species of the D. acutifolium complex.

groups. Differences between and among groups of the ordinations were tested using an analysis of similarities (ANOSIM; Clarke 1993; Chapman & Underwood 1999).

## Results

The total chloroplast alignment comprised 1901 positions, of which 122 were variable, and 90 of the variable characters were parsimony-informative. Of the 1077 positions in the ITS alignment, 124 ambiguous positions were removed from the subsequent calculations. The remaining 953 positions comprised 160 variable characters, of which 113 were parsimony-informative. Simple indel coding of the combined dataset yielded 155 additional characters (excluding three corresponding to an inversion in *psbA-trnH*), of which 99 were parsimony-informative.

Similar consistency indices resulted from the most parsimonious phylogenetic reconstructions with and without indels included (CI 0.7206 versus 0.7386), indicating a slightly lower amount of homoplasy in the indel characters. The single optimal ML tree of the combined markers is shown in Fig. 1, with bootstrap support values (≥70% BS) from the parsimony and likelihood analyses as well as posterior probabilities (PP ≥95) from Bayesian inference indicated on the branches.

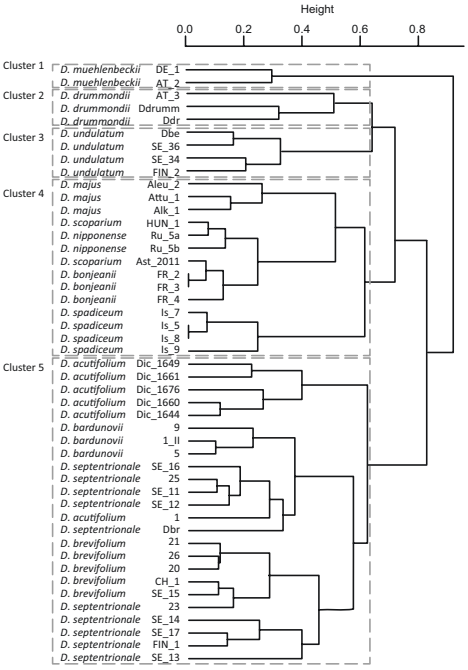



Fig. 2. Cluster dendrogram of 34 morphological characters and 47 specimens of *D. acutifolium*, *D. brevifolium*, *D. bardunovii*, *D. septentrionale*, *D. scoparium*, *D. bonjeanii*, *D. nipponense*, *D. majus*, *D. spadiceum*, *D. drummondii*, *D. muehlenbeckii* and *D. undulatum*, based on a Jaccard distance matrix and complete-linkage clustering strategy. The optimal number of clusters (k=5), delimited by the dashed rectangles, was calculated based on Pearson's correlation coefficient (r=0.769). Species names are based on the clades resolved in the molecular phylogenetic reconstructions.

Regardless of the phylogenetic inference, *D. brevifolium* and *D. septentrionale* were resolved in well-supported clades (≥89% BS, PP 1; Fig. 1). *Dicranum bardunovii* was less strongly supported (70% ML BS, PP 0.99), whereas *D. acutifolium* only received 71% BS in the ML analysis. Relationships among these four lineages remained ambiguous. *Dicranum drummondii*, which shares common morphological characters with species of the *D. acutifolium* complex, was clearly separated from the latter as well as from *D. undulatum* (100% BS, PP 1). One of the two plants of the holotype of *D. bardunovii* corresponded to *D. acutifolium*. Furthermore, eight of the 13 samples identified as *D. brevifolium* corresponded to *D. septentrionale* and only five were attributed to *D. brevifolium*. These confusions were due to mis-identifications of the samples, as the species are readily confused morphologically.

The *D. acutifolium* complex was resolved as sister group to the *D. scoparium* complex, but relationships between these two complexes as well as *D. majus*, *D. elongatum* and the *D. fuscescens* complex (*D. flexicaule* and *D. fuscescens*) remained unsupported.

The cluster analysis of morphological characters (Fig. 2) divided the analysed specimens

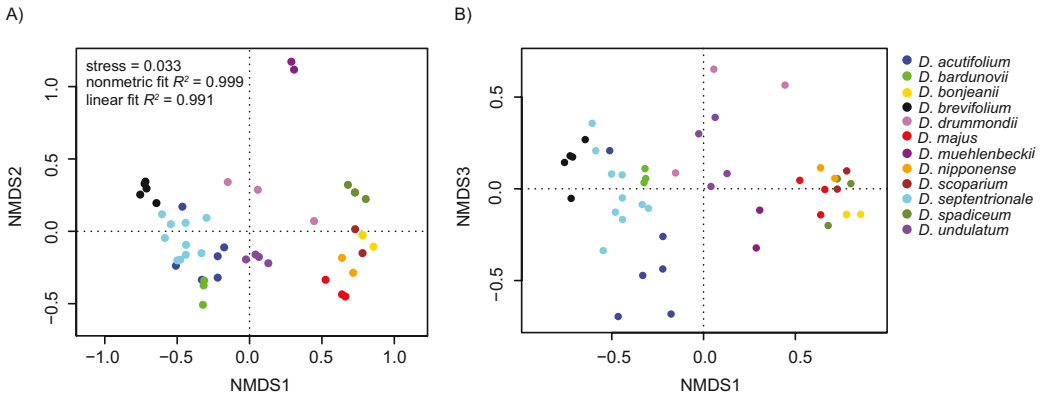



FIG. 3. Nonmetric Multidimensional Scaling (NMDS) ordinations of 47 *Dicranum* specimens using a Jaccard distance matrix and applying the metaMDS fonction of vegan with its default arguments (k=5). Scatterplots are showing the first and second (A), and first and third (B) dimensions. Species names are based on the clades resolved in the molecular phylogenetic reconstructions

into five optimal clusters (Pearson' s correlation coefficient $r$=0.769). *Dicranum muehlenbeckii*, *D. drummondii* and *D. undulatum* were grouped in clusters 1, 2 and 3, respectively. Cluster 4 included all the specimens of the *D. scoparium* complex plus *D. majus* and *D. spadiceum,* while all four species from the *D. acutifolium* complex grouped in cluster 5. Within the cluster of the *D. acutifolium* complex, three subclusters corresponded to *D. acutifolium*, *D. bardunovii* and *D. brevifolium*, respectively, whereas *D. septentrionale* did not form a homogeneous group, in contrast to the molecular tree. SE_11, SE_12, SE_16 and 25 formed a group that was most similar to *D. bardunovii*, SE_13, SE_14, SE_17, and FIN_1 formed a group that was most similar to *D. brevifolium* and one sample (23) clustered with *D. brevifolium*. Furthermore, both samples from the holotype of *D. bardunovii* (1 and 1_II) were situated in different subclusters, but sample 1 was not part of the *D. acutifolium* cluster, in contrast to the molecular tree.

The five clusters identified in the cluster analysis were best distinguishable by the first three axes of the NMDS scatterplot (Fig. 3; stress value= 0.033, nonmetric fit $R^2$=0.999, linear fit $R^2$=0.991). While the specimens included in the *D. acutifolium* complex formed a first group with negative values on axis one, the specimens of the *D. scoparium* complex, plus *D. spadiceum* and *D. majus* formed a second group with positive values. *Dicranum undulatum,* as well as *D. muehlenbeckii* and *D. drummondii,* was plotted between group one and two, with the former in the vicinity of group one. While axis two further allowed a clear distinction of *D. muehlenbeckii* (Fig. 3A), axis 3 confirmed the separation of *D. undulatum* from the *D. acutifolium* complex and the distinction of *D. brevifolium*, *D. acutifolium*, *D. bardunovii* (Fig. 3B). Although *D. septentrionale* was differentiated by axis 1 and 2, its similarity with *D. brevifolium* and *D. bardunovii* was displayed by axis 3. The differentiation among species was supported by the ANOSIM (R= 0.8867; $p$=0.001, 999 permutations).

## DISCUSSION

Both the present molecular phylogenetic reconstructions (Fig. 1) as well as the morphological analyses (Fig. 2) support the current circumscription of the *D. acutifolium* species complex, which comprises *D. acutifolium*, *D. bardunovii*, *D. brevifolium*, and *D. septentrionale*. Morphologically

TABLE 1. Table of characters for D. acutifolium, D. brevifolium, D. septentrionale and D. bardunovii based on the present study and the literature (Tubanova et al. 2010; Tubanova& Ignatova, 2011).

| | Leaf orientation | Leaf margin | Leaf apex | Alar cells | Lamina | Basal lamina cells | Median lamina cells | Upper lamina cells | Costa |
|---|---|---|---|---|---|---|---|---|---|
| *D. acutifolium* | Generally straight or slightly secund, slightly flexuose when dry. Not undulate. | Unistratose, entire. | Acuminate, serrulate, shaped like a pair of tongs in the middle and keeled to circular in apex. | Golden, bistratose. | Unistratose, no projecting cells, incrassate walls. | Rectangular to linear, not sharply different from median cells, porose,. | Quadrate to elongated, generally porose. | Irregularly arranged, irregualrely quadrate or elongated, generally eporose. | One row of guide cells, differentiated dorsal epidermis, smooth. |
| *D. brevifolium* | Generally secund, curled or twisted when dry. Not or slightly undulate in distal half. | Unistratose, occasionally bistratose in apex with geminate teeth | Acuminate, serrulate, shaped like a pair of tongs. | Golden, bistratose | Occasionally projecting cells in upper part, bulging cell walls, incrassate. | Rectangular, different from median cells, generally eporose. | Quadrate, eporose. | Regularly arranged, quadrate, eporose. | One row of guide cells, differentiated dorsal epidermis, with rugosity on the abaxial side. |
| *D. septentrionale* | Falcate secund, sometimes undulate, strongly curled when dry. | Unistratose, occasionally bistratose in apex with geminate teeth, serrulate. | Acuminate, serrulate, shaped like a pair of tongs, generally keeled in apex. | Generally golden, sometimes hyaline, bistratose. | Unistratose, occasionally with strongly projecting cells, especially in apex, bulging cell walls, incrassate. | Rectangular to linear, not sharply different form median cells, porose. | Quadrate to elongated, generally eporose. | Regularly arranged, irregularely quadrate, eporose. | One row of guide cells, sometimes with duplicated cells, differentiated dorsal epidermis, generally smooth. |
| *D. bardunovii* | Generally straight or slightly secund, slightly undulate, slightly flexuose when dry. | Unistratose, serrulate. occasionally bistratose in apex with geminate teeth | Acuminate, serrate, mostly shaped like a pair of tongs, generally keeled in apex. | Hyaline, bistratose. | Unistratose, with projecting cells, bulging cell walls, incrassate. | Generally rectangular, porose. | Quadrate to elongated, porose or not. | Regularly arranged, irregularely quadrate, projecting cells in upper half, eporose. | One row of guide cells, differentiated dorsal epidermis, projecting cells in upper half. |

all four species together are characterised by incrassate lamina cells that are parenchymatous in the upper half of the leaf, acuminate and serrulate to serrate leaf apices, and a keeled upper leaf with incurved margins, resulting in a tong-shaped transverse section. The molecular data furthermore supports the conclusion of Tubanova *et al*. (2010) that *D. pseudoacutifolium* is synonymous with *D. flexicaule* (samples 13 and 15 in Fig. 1). Although supraspecific relationships in *Dicranum* remain largely unsupported based on the present molecular data (cf. also Lang & Stech 2014), and molecular relationships of *D. muehlenbeckii* await further study, no close relationship of the *D. acutifolium* complex with the other included species of sect. *Spuria*, *D. drummondii* and *D. undulatum*, nor with *D. fuscescens* (Fig. 1) and *D. muehlenbeckii* (Figs. 2, 3) are indicated. The latter two species share certain morphological characters with *D. acutifolium* and *D. brevifolium*, such as bulging cell walls, quadrate apical cells and slightly porose basal cells (e.g. Nyholm 1954, 1987; Ireland 2002), but are easily differentiated from the species of the *D. acutifolium* complex by their leaves not tong–shaped in cross section, thin lamina cell walls, and leaf margins serrate in the distal half. Despite its larger size, keeled leaf and the absence of bulging cell walls, *D. drummondii* is sometimes confused with *D. acutifolium* because of its flexuose leaves in dry state and irregularly shaped upper lamina cells (Ireland 2007; Nyholm 1987). *Dicranum undulatum* and *D. acutifolium* have both straight leaves that have projecting upper cells at back. However, the former has transversely undulate leaves that narrow into an obtuse apex, whereas the leaves of *D. acutifolium* end in a subulate point.

The existence of numerous intergrading forms occurring among the species of the *D. acutifolium* complex has caused much taxonomic confusion and led to frequent misidentifications. Their distinction is based on few subtle gametophytic characters (Table 1), and deviating forms render morphological identification difficult (Ireland 2002, 2007) as exemplified by the sample 1 of *D. acutifolium* or 23 of *D. septentrionale* (Figures 2, 3). Furthermore, herbarium collections of *D. brevifolium* were frequently found under different names, such as *D. drummondii*, *D. flexicaule*, or *D. undulatum* (Ireland 2002, Tubanova *et al*. 2010; Tubanova & Ignatova 2011). At the molecular level *D. acutifolium*, *D. bardunovii, D. brevifolium* and *D. septentrionale* seem more clearly distinguishable (Fig. 1), although the respective clades receive different statistical support, with *D. brevifolium* and *D. septentrionale* being well supported in all analyses, whereas *D. acutifolium* and *D. bardunovii* receive (lower) support only in part of the analyses. The molecular data also helped renaming misidentified specimens within the *D. acutifolium* complex, since a number of specimens identified as *D. brevifolium* were resolved in the clade of the *D. septentrionale* (Fig. 1). *Dicranum septentrionale* is a recently described species defined by few characters that were previously attributed to the morphological variation of *D. brevifolium* (Table 1). Nonetheless, the morphological characters frequently intergrade with *D. bardunovii* or *D. brevifolium* (Fig. 2, 3). According to the present results, *D. brevifolium* is characterised morphologically by tong-like leaf apices, elongated basal cells that are well differenciated from the median one and generally not porose, quadrate upper cells and rugose dorsal surface of the costa. In contrast, *D. septentrionale* is best differentiated by projecting lamina cells, especially at the leaf apex, elongated basal cell that gradually become quadrate, and irregularly shaped in the upper part of the leaf and a generally smooth dorsal epidermis. Furthermore, the distributions and ecological preferences of both *D. brevifolium* and *D. septentrionale* are incompletely known or misunderstood, considering that further *D. brevifolium* collections may appear to belong to *D. septentrionale*. While *D. septentrionale* was known so far from across Russia (Fig. 4), our study shows that its distribution range extends to

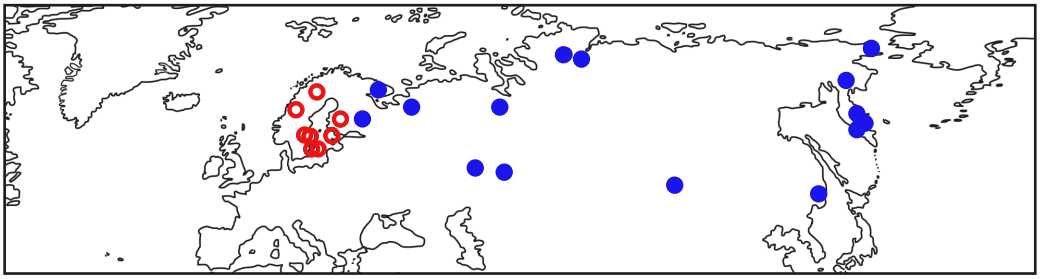


FIG. 4. Geographical distribution of *Dicranum septentrionale* according to collections studied in Tubanova et al. (2010) (filled blue dots) and the present study (empty red dots).

Scandinavia. This indicates that *D. septentrionale* has a Eurasian or possibly Holarctic distribution. In addition, the herbarium material we examined suggests that *D. septentrionale* could be a boreo-montane species, whereas *D. brevifolium* would be a (sub)arctic-alpine species, as suggested by previous studies (Amann *et al.* 1918; Dierssen 2001). A morpho-molecular re-identification of further specimens, including *D. brevifolium* from North America, is needed to delimit the distribution patterns and ecological preferences of *D. brevifolium* and *D. septentrionale* with more confidence.

Another part of the confusion between the species of the *D. acutifolium* complex may stem from the presence of mixed collections. A striking example is the holotype of *D. bardunovii*, which contains also individuals of the morphologically most similar *D. acutifolium* according to the molecular data (samples 1 and 1_II, Fig. 1). Not only are the morphological differences between *D. acutifolium* and *D. bardunovii* small (Table 1, Figs. 2-3), *D. bardunovii* also shows morphological variation departing from the holotype description. This is the case in the sequenced specimen 1, which resembles *D. septentrionale* by the presence of projecting cells in the lamina, the coloured alar cells and non porose, quadrate to elongated median lamina cells. Morphologically similar species growing in mixed cushions is not uncommon in *Dicranum*. For example, collections containing *D. scoparium* and *D. bonjeanii* have been found in locations where both species occurred also separately (own observations). Environmental conditions have a strong influence on morphological characters, especially in extreme conditions (Hedenäs *et al.* 2006), altering also typical characters. The distinction of closely related species is then even more difficult. Additionally, dwarf males growing on female stems (pseudomonoicy) are found both in *D. brevifolium* and *D. acutifolium* and have been seen in one *D. bardunovii* specimens (Tubanova & Ignatova 2011) a number of morphologically distinct specimens were revealed. They are similar to D. acutifolium (Lindb. & Arnell. Whether hybridisation, a process that affects also the morphology, occurs in mixed patches is still unknown. The present molecular data do not indicate any hybridisation for *D. acutifolium* and *D. bardunovii*, however, the absence of support for the *D. acutifolium* complex suggest that such a process might have occurred. The use of other molecular methods and more variable markers could be useful to understand the species dynamics at population level.

In line with a number of recent studies (e.g. Sukkharak *et al.* 2011; Carter 2012; Medina *et al.* 2012; Stech *et al.* 2013; Lang & Stech 2014) the present study displays the importance of molecular data for clarifying species circumscriptions, resolving taxonomical issues and for the re-evaluation of morphological characters in bryophytes and *Dicranum* in particular.

# Chapter 4

## DNA barcoding of Arctic bryophytes – an example from the moss genus *Dicranum* (Dicranaceae, Bryophyta)

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Published in Polar Biology, 2014, 37 (8): 1157-1169

ABSTRACT

The identification of bryophytes from the Arctic is often difficult due to deviating morphologies under the extreme environmental conditions. This is especially true for species-rich and taxonomically complex genera, such as the moss genus *Dicranum*. DNA barcoding is expected to improve the identification of Arctic bryophyte species, but the optimal combination of barcoding markers for mosses in general, especially for delimiting closely related species, is still under discussion. In this paper, we test the discrimination capacity of six potential barcode markers (*rps4-trnT*$_{UGU}$, *trnL*$_{UAA}$-*trnF*$_{GAA}$, *trnH*$_{GUG}$-*psbA*, *rps19-rpl2*, *rpoB,* nrITS1-5.8S-ITS2) based on phylogenetic reconstructions of 30 *Dicranum* samples from Spitsbergen (Svalbard, Norway) and reference samples from all ten *Dicranum* species confirmed for the Svalbard archipelago and six additional Arctic *Dicranum* species. All 16 species (possibly except *D. fuscescens*), were distinguishable with bootstrap support >70% based on the combined sequence data, but none of the individual markers could delimit all included species. All Svalbard collections could be readily assigned to five species, *D. acutifolium*, *D. elongatum*, *D. laevidens*, *D. majus*, and *D. spadiceum*, respectively. It is concluded that DNA barcoding improves species identification of Arctic *Dicranum* plants, but that a combination of several markers is necessary in order to obtain reliable identification results, with the single loci ITS1, *trnL-F* and *rps4-trnT* being the most promising regions.

INTRODUCTION

Bryophytes comprise three different phylogenetic lineages of land plants, namely liverworts, hornworts, and mosses (e.g. Qiu *et al*. 2006). Of these, liverworts and mosses play an essential role in Arctic terrestrial ecosystems and constitute a major component of different types of tundra vegetation (e.g., Callaghan *et al*. 2004; Lang *et al*. 2012; Longton 1997). The Arctic bryoflora comprises a considerable diversity of approximately 700 species (Longton 1988; Frisvoll & Elvebakk 1996; Afonina & Czernyadjeva 1995; Konstantinova & Potemkin 1997).

Bryophytes, especially mosses, have been widely employed in studies of ecosystem processes and organismal interactions in (sub-)Arctic environments (e.g., Alsos *et al*. 1998; Gordon *et al*. 2001; Gornall *et al*. 2007; Jasmin *et al*. 2008; Krab *et al*. 2008; van der Wal & Brooker 2004). The Arctic has been divided into bioclimatic regions, where the High Arctic is characterized by an open herbaceous vegetation with some dwarf-shrubs on mineral soils, while the Low Arctic generally consists of a closer herbaceous vegetation, composed of dwarf and low shrubs on peat-rich soils (Walker *et al*. 2005). Biodiversity-based investigations of Arctic ecosystem processes, however, are still severely hampered by insufficient knowledge of bryophyte taxonomy and by the ability of species recognition based on morphological characters. In response to the extreme environmental conditions bryophytes display unusual growth forms and deviant gametophytic characters in the (High) Arctic. This plasticity makes morphological identification to species level difficult or even impossible (e.g., Buryová & Shaw 2005; Frisvoll & Elvebakk 1996; Hesse *et al*. 2012). This is especially true for species-rich and taxonomically complex genera such as *Bryum* Hedw., *Dicranum* Hedw. and *Schistidium* Brid. (e.g., Steere 1978; Hesse *et al*. 2012). Consequently, ecological studies have largely been limited to a few easily distinguishable species or genus-level identifications (e.g., Okitsu *et al*. 1998), or treated bryophytes as a single category without distinction of species (e.g., van der Wal *et al*. 2001). Sometimes bryophytes are even grouped with lichens and fungi as the outdated group of 'cryptogams' (e.g., Hudson & Henry 2010; Wahren *et al*. 2005; Epstein *et al*. 2004). The development of new identification tools to treat bryophytes in a more comprehensive way would surely increase the significance of ecological studies in the (High) Arctic, especially with respect to the potential of bryophytes for investigating the impact of global climate change (Tuba *et al*. 2011).

DNA barcoding is a molecular tool for species identification based on species-specific sequence differences in a short, standardized DNA region. In contrast to this original idea, however, barcoding in land plants (including bryophytes) is supposed to be based on one or two core markers plus additional information from other DNA regions where necessary (e.g., Hollingsworth *et al*. 2009, 2011). In bryophytes, especially mosses, the plastid markers recently proposed for barcoding of land plants (CBOL Plant Working Group 2009; Kress *et al*. 2005) either tend to be short (*psbA-trnH* spacer; Stech & Frey 2008; Stech & Quandt 2010), have different discrimination capacity at the species level (*rbcL*; Liu *et al*. 2010; Stech & Quandt 2010), or need more study concerning primer design and amplification strategy (*trnK*/*matK*, e.g., Bell *et al*. 2012). Although the optimal combination of barcoding markers for bryophytes is still under discussion (e.g., Liu *et al*. 2010; 2011; Bell *et al*. 2012; Hassel *et al*. 2013; Stech *et al*. 2013), several other molecular markers have already shown to be useful for inferring species delimitations and identifying species in bryophytes (e.g., Bell *et* al. 2012; von Cräutlein *et al*. 2011; Draper & Hedenäs 2009; Stech *et al*. 2011, 2013).

*Dicranum* (Dicranaceae) is a large genus belonging to the second largest subclass of mosses, Dicranidae. It comprises ca. 90 species essentially found in the Holarctic (Crosby *et al*. 1999; Ireland 2007), including about 30 species in the boreo-arctic region, of which ten species were accepted for Svalbard (Frisvoll & Elvebakk 1996). Several *Dicranum* species show a high morphological variability (Hedenäs & Bisang 2004; Hedenäs *et al*. 2006; Smith 2004; Lang & Stech 2014), which renders their identification challenging, in particular in plants (Hedenäs *et al*. 2006; pers. obs.). Previous phylogenetic studies revealed low sequence divergence in commonly employed plastid markers (La Farge *et al*. 2002; Stech *et al*. 2006). The nuclear ribosomal ITS region allowed

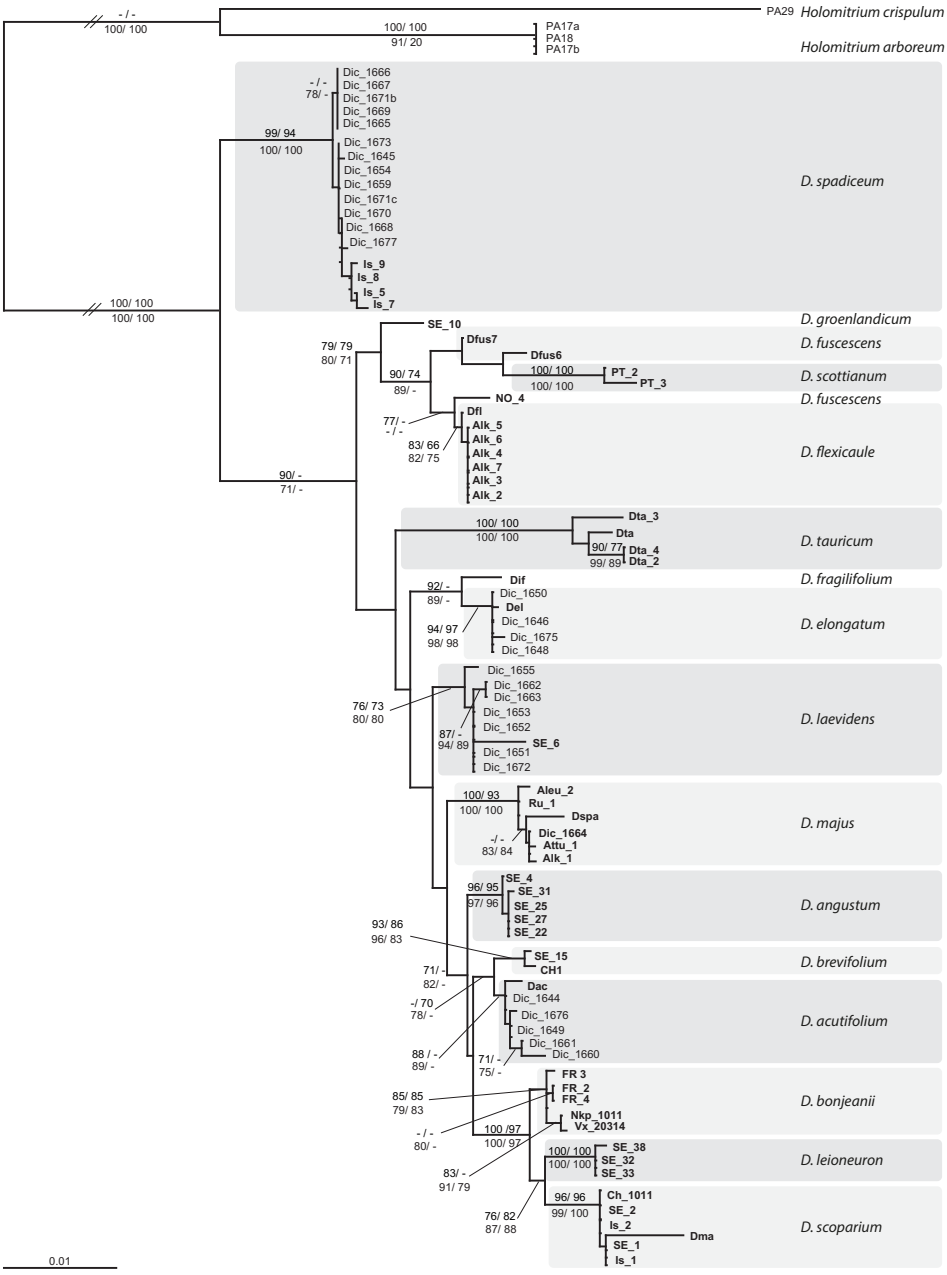


FIG. 1. Single optimal maximum likelihood phylogenetic reconstruction inferred from combined chloroplast and nrITS sequence data of *Dicranum* species, including indels coded by simple indel coding (SIC). Four specimens of *Holomitrium* were used as outgroup representatives and names in bold represent the reference samples. Numbers above branches indicate MP bootstrap support and numbers below the branch indicate ML bootstrap support, with and without indels. Branch lengths are to scale except those indicated by the symbol "//" (shortened 2 times).

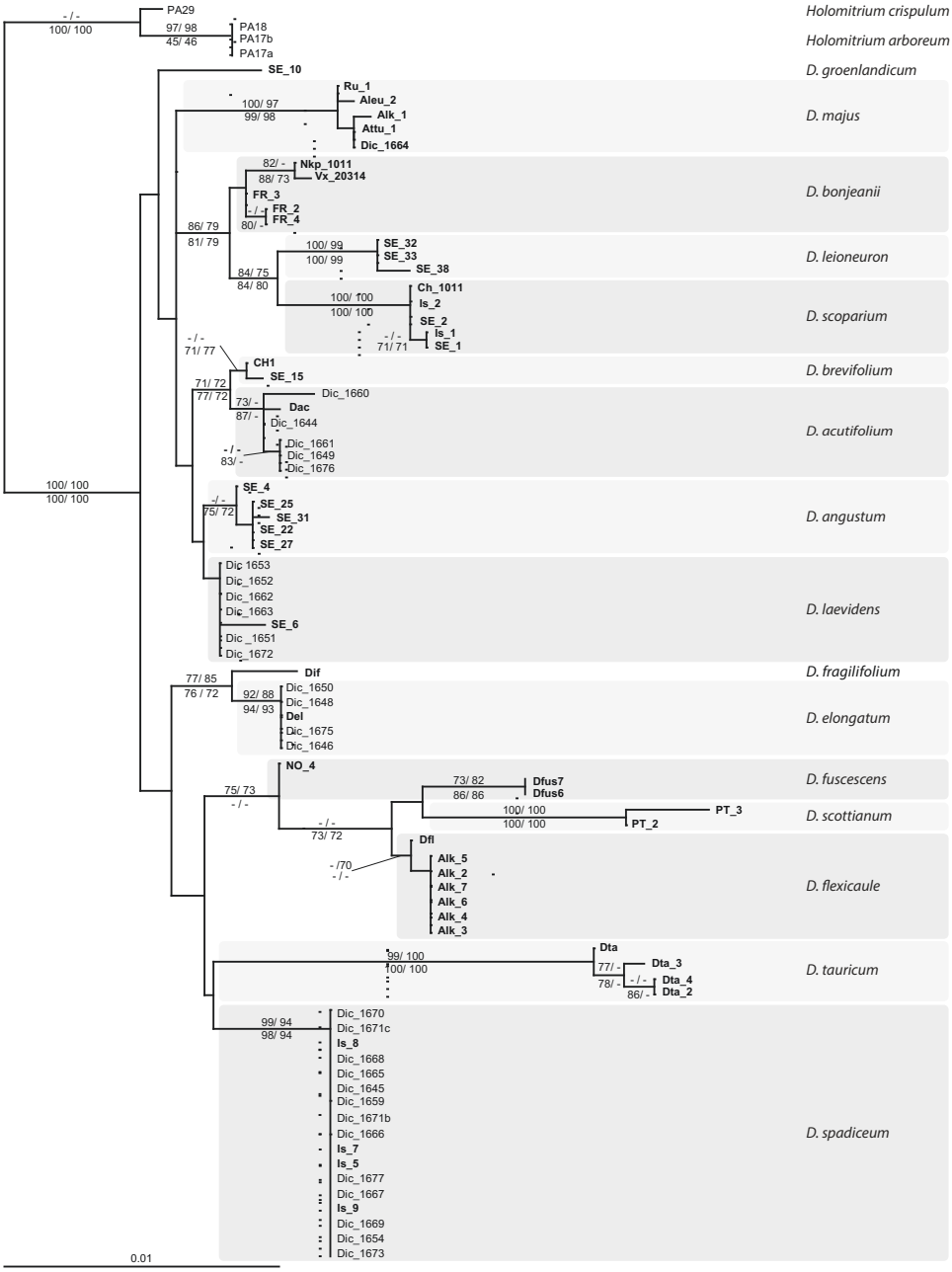


FIG. 2. Single optimal maximum likelihood phylogenetic reconstruction inferred from chloroplast sequence data of *Dicranum* species, including indels coded by simple indel coding (SIC). Four specimens of *Holomitrium* were used as outgroup representatives and names in bold represent the reference samples. Numbers above branches indicate MP bootstrap support and numbers below the branch indicate ML bootstrap support, with and without indels.

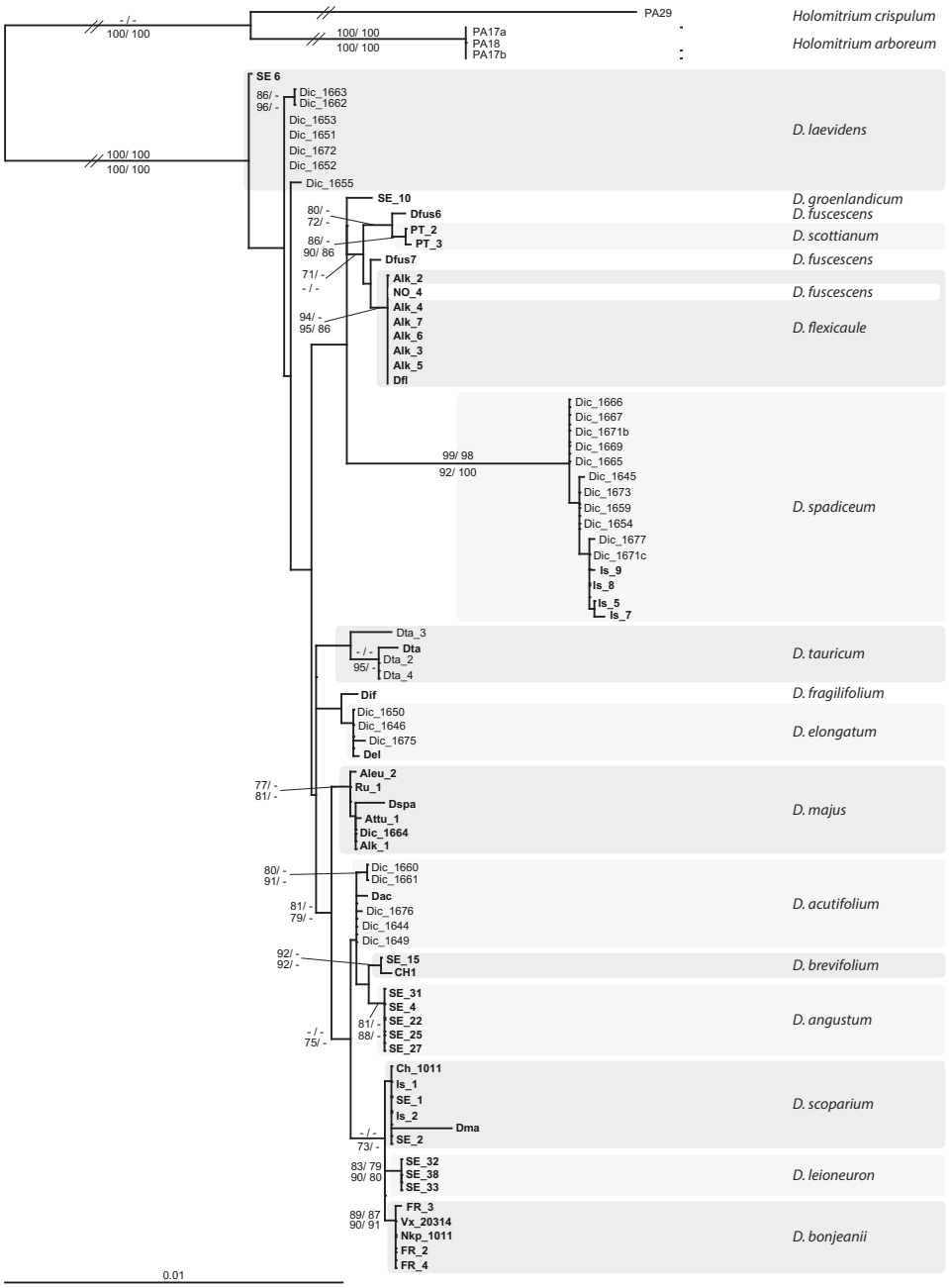


FIG. 3. Single optimal maximum likelihood phylogenetic reconstruction inferred from ITS sequence data of *Dicranum* species, including indels coded by simple indel coding (SIC). Four specimens of *Holomitrium* were used as outgroup representatives and names in bold represent the reference samples. Numbers above branches indicate MP bootstrap support and numbers below the branch indicate ML bootstrap support, with and without indels. Branch lengths are to scale except those indicated by the symbol "//" (shortened 2 times).

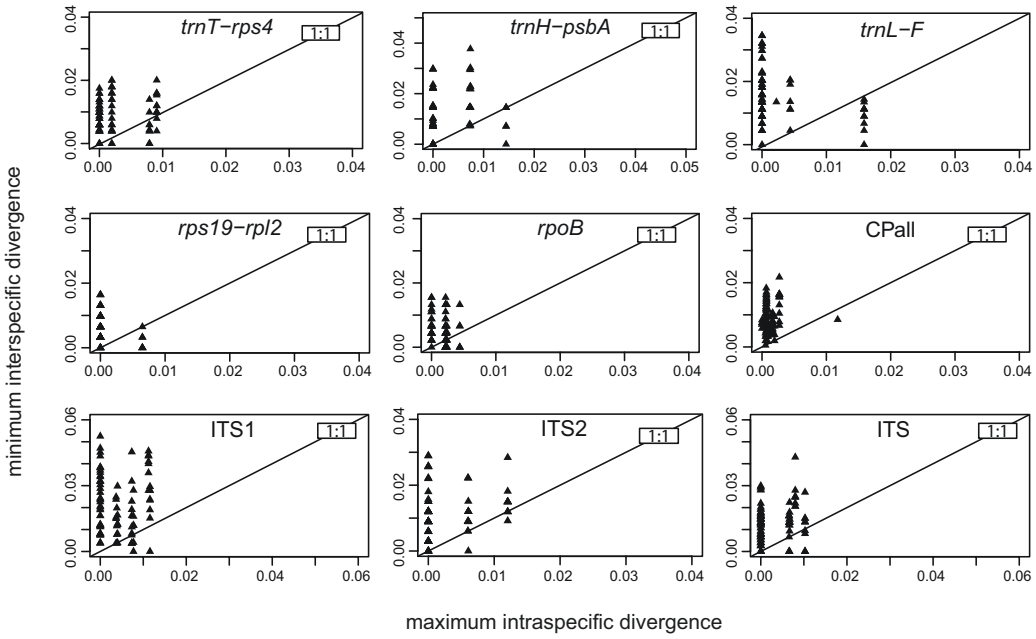


FIG. 4. Comparison of maximum intraspecific versus minimum interspecific divergence distances for *Dicranum* species pairs with more than one specimen sequenced. Genetic distances have been calculated using a K2P model of sequence evolution for *trnT-rps4*, *trnL-F*, *trnH-psbA*, *rps19-rpl2*, *rpoB*, all chloroplast markers combined (CPall), ITS and its partitions ITS1 and ITS2.

species identification in a study of Russian *Dicranum* species albeit with low support (Tubanova & Ignatova 2011; Tubanova *et al*. 2010). Most recently however, Lang & Stech (2014) showed that closely related species of the *Dicranum scoparium* Hedw. species complex were best resolved by combining five plastid regions and the nuclear ribosomal ITS region.

In the present study we aim to test how well *Dicranum* collections from the High Arctic archipelago of Svalbard can be identified to species level based on a DNA barcoding approach. Inferences are based on molecular phylogenetic reconstructions using chloroplast (*rpoB*, *trnH-psbA*, *trnL-trnF*, *rps4-trnT*, *rps19-rpl2*) and nuclear ribosomal ITS sequences from Svalbard and reference collections. The reference sequences were generated from well-identified, morphologically typical specimens from temperate to boreal regions. Furthermore, we examine the species discrimination efficacy of the six markers individually based on phylogenetic inference and comparison of maximum intraspecific versus minimum interspecific genetic distances.

## MATERIALS AND METHODS

***Sampling—*** According to the most recent checklist of the bryophytes of Svalbard (Frisvoll & Elvebakk 1996), 17 *Dicranum* species have been reported for the archipelago, of which ten were accepted in the checklist (*Dicranum acutifolium* (Lindb. & Arn.) C.Jens., *D. angustum* Lindb., *D. elongatum* Schleich. ex Schwägr., *D. flexicaule* Brid., *D. fuscescens* Sm., *D. laevidens* R.S. Williams, *D. majus* Sm., *D. scoparium* Hedw., *D. spadiceum* J.E. Zetterst., and *D. tauricum* Sapjegin), and seven were excluded due to erroneous identification (*D. bonjeanii* De Not., *D. brevifolium* (Lindb.) Lindb.,

*D. fragilifolium* Lindb., *D. groenlandicum* Brid., *D. leioneuron* Kindb., *D. muehlenbeckii* Bruch & Schimp., and *D. scottianum* Turner ex Scott). DNA sequences of 52 reference specimens comprising 16 out of the 17 species were compiled from earlier studies (Lang & Naciri 2010; Stech 1999; Lang & Stech 2014) or newly generated for this study (Appendix 1): *Dicranum acutifolium* (1 sample), *D. angustum* (5), *D.brevifolium* (2), *D. bonjeanii* (5), *D. elongatum* (1), *D. flexicaule* (7), *D. fragilifolium* (1), *D. fuscescens* (3), *D. groenlandicum* (1), *D. laevidens* (1), *D. leioneuron* (3), *D. majus* (6), *D. scoparium* (6), *D. spadiceum* (4), *D. scottianum* (2) and *D. tauricum* (4). The reference specimens originated from temperate to boreal regions, generally displayed morphologies typical for the respective species, and were mostly identified, or their identifications checked, by the authors or L. Hedenäs (Stockholm). *Dicranum muehlenbeckii,* however, could not be included in the analysis because of unsuccessful DNA amplification. Thirty *Dicranum* specimens from Svalbard (including one specimen already included in Lang & Stech, 2014) collected by the second and third author in Adventdalen (Longyearbyen area), Colesbukta, and Kongsfjorden (Ny-Ålesund area) in 2008-2010 were analysed (DNA numbers Dic_1644-Dic_1646, Dic_1648-Dic_1655, Dic_1659-Dic_1673, Dic_1675-Dic_1677). The collection strategy was to collect as many morphotypes as possible from the sampled areas. Four samples of *Holomitrium* Brid., one of *H. crispulum* Mart. and three of *H. arboreum* Mitt., were chosen as outgroup representatives according to earlier molecular phylogenetic reconstructions (La Farge *et al.* 2002; Stech *et al.* 2006). Voucher information and GenBank accession numbers of the DNA sequences generated from the 30 specimens are listed in Appendix 1. The nomenclature of *Dicranum* used in this study follows Hedenäs & Bisang (2004), which corresponds to the accepted species in Tropicos (Tropicos.org) and The Plant List (2013), except three species considered as synonyms in the latter two databases (*D. angustum* and *D. laevidens* as synonyms of *D. spadiceum* and *D. flexicaule* as synonym of *D. fuscescens*).

***Molecular marker selection—*** For this study, we sequenced five chloroplast regions, i.e., partial *rpoB* gene, *trnH*$_{GUG}$*-psbA* and *rps19-rpl2* intergenic spacers, and two parts of the *trnS-F* region, namely *rps4-trnT*$_{UGU}$ spacer and *trnL-F* (*trnL*$_{UAA}$ intron and *trnL*$_{UAA}$*-trnF*$_{GAA}$ spacer), and the nuclear ribosomal nrITS1-5.8S-ITS2 region. Amplification and sequencing success as well as haplotype diversity of the chloroplast markers were inferred for *54 Dicranum scoparium* specimens by Lang & Naciri (2010). Subsequently these markers provided, together with nrITS sequences, valuable results in delimiting species of the *Dicranum scoparium* complex, based on a sampling of 111 *Dicranum* specimens (Lang & Stech 2014).

***DNA extraction, amplification and sequencing—*** DNA was extracted from the dried leaves of a single plant using the NucleoSpin® Extract II Kit (Macherey-Nagel, Düren, Germany). Polymerase chain reaction (PCR) was performed following Lang & Stech (2014). PCR products were purified and sequenced at Macrogen Inc. (www.macrogen.com). GenBank accession numbers of all sequences are listed in Appendix 1.

***Alignment and phylogenetic reconstruction—*** Sequences were aligned in Geneious v6.1.6 (Biomatters 2010) using 65% similarity matrix costs, and manually adjusted. Gaps were treated as missing data or coded as informative by a simple indel coding strategy (SIC) (Simmons & Ochoterena 2000) as implemented in SeqState (Müller 2004). Short hairpin-associated inversions in the *trnH-psbA* spacer were positionally separated in the alignment and not coded as indels.

TABLE 1. Alignment length (Length), number of constant characters (Constant), variable characters (Variable), parsimony-informative characters (Parsi-info) and percentage of parsimony-informative characters (% parsi-info) for nucleotide and indel matrices. Values were calculated from alignments of each marker with outgroup and ingroup only (ingr).

| | *trnT-rps4* | | *trnL-trnF* | | *psbA-trnH* | | *rps19-rpl2* | | *rpoB* | | ITS 1 | | ITS 2 | | ITS | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | ingroup | | ingroup | | ingroup | | ingroup | | ingroup | | ingroup | | ingroup | | ingroup | |
| Species | | 15 | | 16 | | 16 | | 14 | | 16 | | 16 | | 16 | | 16 |
| Length | 522 | 522 | 471 | 471 | 149 | 149 | 310 | 310 | 457 | 457 | 360 | 304 | 427 | 371 | 966 | 854 |
| Constant | 485 | 498 | 432 | 436 | 136 | 138 | 292 | 299 | 441 | 443 | 287 | 274 | 369 | 348 | 821 | 798 |
| Variable char. | 37 | 24 | 39 | 35 | 13 | 11 | 18 | 11 | 16 | 14 | 73 | 30 | 67 | 23 | 145 | 56 |
| Parsi-info | 33 | 20 | 35 | 29 | 9 | 7 | 11 | 9 | 12 | 1 | 49 | 20 | 42 | 19 | 96 | 42 |
| % parsi- info | 6 | 4 | 7 | 6 | 6 | 5 | 4 | 3 | 3 | 2 | 14 | 7 | 10 | 5 | 10 | 5 |
| #indels | 3 | 3 | 6 | 6 | 5 | 5 | 1 | 1 | 0 | 0 | 64 | 34 | 59 | 32 | 123 | 66 |
| Parsi-info | 3 | 3 | 4 | 5 | 3 | 3 | 1 | 1 | 0 | 0 | 49 | 28 | 37 | 20 | 87 | 53 |
| Total parsi-info | 36 | 23 | 39 | 34 | 12 | 10 | 12 | 10 | 12 | 10 | 98 | 48 | 79 | 39 | 183 | 95 |

Numbers of constant, variable and parsimony-informative sites were calculated for each locus using PAUP* v4.0b10 (Swofford 2002).

Phylogenetic analyses using Maximum Parsimony (MP) optimality criterion and maximum likelihood (ML) were performed on every marker separately (including separate analyses of ITS1 and ITS2) and the combined markers (total evidence trees *sensu* Kluge 1989), both with and without indels included. Before combining markers, we tested for incongruence by visual inspection of the separate trees and by applying an incongruence length difference test (ILD, Farris *et al*. 1994) as implemented in PAUP* with 100 replicates.

MP analyses were performed using PAUP*. Heuristic searches were performed with 100 replicates using random sequence addition, one tree held at each step and tree bisection-reconnection (TBR) branch swapping, saving up to 10,000 trees. ML analyses were carried out with RAxML v. 7.3.0 (Stamatakis *et al*. 2006) employing the raxmlGUI v.0.93 interface (Silvestro & Michalak 2012). The default GTR+Γ model was chosen for all markers. Bootstrap analyses under ML were done using the thorough bootstrap heuristics algorithm with 1,000 replicates.

Pairwise nucleotide distances between all sequences were calculated in PAUP* under the Kimura 2-parameter (K2P) model for the combined chloroplast dataset, ITS and all partitions. Maximum intraspecific distances were plotted against minimum interspecific distances for all possible species pairs with more than one specimen sequenced to infer the presence of a barcoding gap (cf. Stech *et al*. 2013).

## RESULTS

Lengths of the sequenced chloroplast markers within *Dicranum* and *Holomitrium* ranged from 509-521 nucleotides for *rps4-trnT*, 449-471 nt for *trnL-F*, 136-140 nt for *trnH-*

*psbA*, 309-310 nt for *rps19-rpl2* and 457 nt for *rpoB*. The total plastid alignment comprised 1909 positions, of which 123 were variable, and 100 of the variable positions were parsimony-informative. Simple indel coding yielded a total of 15 additional characters, of which 11 were parsimony-informative. Hence, a total of 111 parsimony-informative characters resulted from the plastid markers. Sequences length of nrITS1-5.8S-ITS2 ranged from 747-900 nt (747-839 within *Dicranum*). The alignment comprised 1086 positions, of which 120 were removed from further calculations due to ambiguous alignment. Of the 966 remaining positions, 145 were variable and 96 of the variable positions were parsimony-informative. Simple indel coding yielded 123 characters of which 87 were parsimony informative. In total, 183 parsimony-informative characters resulted from nrITS. Respective numbers of parsimony-informative characters per plastid marker and for ITS1 and ITS2 separately are summarized in Table 1. The partitions with the most parsimony-informative characters were ITS1 (13.61%) and ITS2 (9.84%), followed by the chloroplast markers *trnL-F* (7.43%), *rps4-trnT* (6.32%), *trnH-psbA* (6.04%), *rps*19-*rpl*2 (3.55%), and partial *rpoB* gene (2.63%).

Maximum parsimony analyses with or without indels included resulted in most parsimonious phylogenetic reconstructions with similar consistency indices (combined chloroplast: CI 0.7306 versus 0.7159, ITS: CI 0.7939 versus 0.8394), indicating only a slightly higher amount of homoplasy in the indel characters in ITS. Both visual inspections of plastid versus ITS tree topologies and the ILD test ($p = 0.29$) indicated that the two datasets were congruent and could be combined.

The single optimal ML tree of the combined analysis of all markers including indels is shown in Fig. 1 (lnL = -7188.5806). The optimal ML trees calculated from the combined chloroplast markers versus ITS (lnL = -3728.5203 and lnL= -3159.2803, respectively) are shown in Figs. 2 and 3, with bootstrap support values (BS) from maximum parsimony and maximum likelihood analyses. Separate clades of all 16 included *Dicranum* species were resolved in the combined tree (Fig. 1) and the plastid marker tree (Fig. 2) according to the positions of the reference specimens, except *Dicranum fuscescens* that was resolved as paraphyletic. The ITS tree (Fig. 3) was less resolved and most clades were weakly supported. The *D. leioneuron, D. majus, D. scottianum and D. spadiceum* clades received bootstrap support of ≥70% in all three phylogenetic inferences. The *D. acutifolium, D. elongatum*, *D. scoparium*, and *D. tauricum* clades yielded high support in the chloroplast and combined analyses, whereas the *D. angustum, D. bonjeanii and D. brevifolium* clades were supported (≥80% BS) in ITS and the combined tree. The *D. flexicaule* and *D. laevidens* clades received high boostrap support (≥78%) only in the combined analysis (Table 2). High support in the combined trees was furthermore obtained for the sister group relationships of *D. elongatum* + *D. fragilifolium*, *D. flexicaule* + *D. fuscescens* + *D. scottianum*, as well as *D. bonjeanii* + *D. scoparium* + *D. leioneuron* of the *D. scoparium* complex. The Svalbard specimens clustered in five clades, namely the *D. acutifolium* (5 specimens), *D. elongatum* (4), *D. laevidens* (7), *D. majus* (1) and *D. spadiceum* (13) clades (Figs. 1-3).

Clades with more than one sequence were generally weakly supported in each of the seven single partitions (Table 2). Moreover, only six clades were recovered in at least four partitions, namely the *D. leioneuron*, *D. majus*, *D. scoparium, D. scottianum*, *D. spadiceum* and *D. tauricum* clades. ITS1 recovered the most clades with statistical support ≥ 70% BS, whereas *trnH-psbA* recovered only the *D. elongatum* clade with strong bootstrap support (84/ 86%).

Genetic distances were generally small in all markers. The ranges of intraspecific versus interspecific pairwise genetic distances overlapped for all markers, except in the combined chloroplast dataset

TABLE 2. Bootstrap values of MP and ML analysis including indels, for clades with more than one sequence. Values for each single markers as well as for the concatenated chloroplast markers (CPall), nrITS1-5.8S-ITS2 (ITS) and the combined sequence data (combined) are shown. Values ≥70% BS are in bold. Dashes denote clades that were absent in the respective phylogenetic reconstruction.

| species | *trnT-rps4* | *trnL-trnF* | *psbA-trnH* | *rps19-rpl2* | *rpoB* | ITS 1 | ITS 2 | CPall | ITSI | combined |
|---|---|---|---|---|---|---|---|---|---|---|
| *Dicranum acutifolium* | ≤ 50 | **85/91** | | | | | | **73/87** | ≤ 50 | **88/89** |
| *Dicranum angustum* | | **83/85** | | | | **72/82** | | 67/**75** | **81/88** | **96/97** |
| *Dicranum bonjeanii* | 66/66 | ≤ 50 | ≤ 50 | | | **87/94** | | 52/53 | **89/90** | **85/79** |
| *Dicranum brevifolium* | | ≤ 50 | | ≤ 50 | | **80/72** | **76**/64 | 55/**71** | **92/92** | **93/96** |
| *Dicranum elongatum* | ≤ 50 | | **84/86** | | | 59/61 | | **92/94** | 62/64 | **94/98** |
| *Dicranum fragilifolium* + | | | | | | | | | | |
| *D. elongatum* | 52/49 | | | | 62/**71** | **71**/65 | ≤ 50 | **77/76** | 60/62 | **92/89** |
| *Dicranum fuscescens* | **81/86** | | | | | | | | | |
| *Dicranum flexicaule* | ≤ 50 | 56/53 | | | | **75/93** | ≤ 50 | 64/63 | | **83/81** |
| *Dicranum scottianum* | **86/85** | **100/100** | -/ 66 | | 60/62 | **84/92** | 52/56 | **100/100** | **86/90** | **100/100** |
| *Dicranum fuscescens* + | | | | | | | | | | |
| *Dicranum flexicaule* | ≤ 50 | | -/ 54 | | 61/64 | | 56/60 | | | |
| *Dicranum fuscescens* + | | | | | | | | | | |
| *Dicranum flexicaule* + | | | | | | | | | | |
| *Dicranum scottianum* | **91/95** | **85/86** | | | | | **79/77** | **75**/41 | **71**/66 | **90/89** |
| *Dicranum laevidens* | | ≤ 50 | | **70**/63 | 61/**71** | 68/- | | ≤ 50 | | **78/80** |
| *Dicranum leioneuron* | 57/**77** | 53/**74** | -/53 | ≤ 50 | | 55/**73** | 62/62 | **100/100** | **83/90** | **100/100** |
| *Dicranum majus* | 59/**87** | **89/94** | 51/65 | ≤ 50 | ≤ 50 | **77**/60 | 72/67 | **100/99** | **77/81** | **100/100** |
| *Dicranum scoparium* | 62/**70** | **91/88** | ≤ 50 | **82/85** | 65/63 | 60/**70** | | **100/100** | ≤ 50 | **96/99** |
| *Dicranum spadiceum* | -/64 | **64/87** | 56/65 | ≤ 50 | 52/58 | **100/81** | **100/97** | **99/98** | **99/92** | **99/100** |
| *Dicranum tauricum* | **100/99** | **99/99** | ≤ 50 | **92/100** | | **82/78** | | **99/100** | 69/61 | **100/100** |

(Table 3). Tables of all nucleotide distances measured are available on request. Furthermore, the comparison of maximum intraspecific versus minimum interspecific genetic distances (Fig. 4) showed greater intraspecific than interspecific distances for a number of pairwise comparisons in every partition (data points below the 1:1 line). Therefore, no clear barcode gap was obtained for all pairwise comparisons, i.e. none of the markers was powerful enough to discriminate all studied species.

## Discussion

***DNA barcoding in Dicranum and implications for mosses in general—*** All *Dicranum* species included in this study except *D. fuscescens* were distinguishable based on the combined sequence data of five chloroplast markers and nrITS, with bootstrap support >70% for all clades of species represented by more than one sample (Fig. 1, Table 2). However, an increased sampling of the species represented in this study by one or few specimens would be necessary to confirm their monophyly and infer intra- versus interspecific sequence variation with more confidence. The present results support our earlier study focusing on closely related species within the *D. scoparium* complex (Lang & Stech 2014), namely the close relationship between *D. bonjeanii* and *D. scoparium* and the separation of *D. majus* from the *D. scoparium* complex. In addition, *D. leioneuron*, which was not included in Lang & Stech (2014), is resolved as a member of the *D. scoparium* complex here. As the molecular clades of these species coincide with the morphological species circumscriptions, we conclude that the sequenced entities are in fact separate species. *Dicranum flexicaule* is morphologically very similar to *D. fuscescens* and frequently regarded as a variety or a form of the latter (Ireland 2007; Mönkemeyer 1927; Podpěra 1954; Savicz-Lyubitskaya & Smirnova 1970; The Plant List 2013). However, several other authors accept *D. flexicaule* as a separate yet doubtful species (Bellolio-Trucco & Ireland 1990; Hedenäs & Bisang 2004). The present molecular data support a close relationship of *D. flexicaule* with *D. fuscescens*, and in addition *D. scottianum*, but analyses of a larger number of specimens or additional markers are necessary to resolve their relationships with confidence and conclude about the taxonomic status of *D. flexicaule*.

Each individual locus provided insufficient variability to distinguish all sequenced species (Table 2, Fig. 4). The ITS region as well as the chloroplast markers *trnL-F* and *rps4-trnT* showed the highest species discrimination capacity in terms of statistical support, in accordance with previous studies (e.g., La Farge *et al*. 2002; Hernández-Maqueda *et al*. 2008; Hollingsworth *et al*. 2009). However, the *D. elongatum* clade was only supported by *psbA-trnH* and none of the markers delimited *D. laevidens* (Table 2). The *psbA-trnH* spacer possessed a relatively high proportion of parsimony-informative characters in *Dicranum*, as in other moss species (Liu *et al*. 2010; Hassel *et al*. 2013), although the resulting tree was still poorly resolved (cf. Table 2). Neither the combined chloroplast loci nor the generally variable ITS region could discriminate all included *Dicranum* species with confidence. A recent study of the *Racomitrium canescens* (Hedw.) Brid. complex (Stech *et al*. 2013), another representative of subclass Dicranidae, showed that nrITS performed better in terms of species discrimination capacity than chloroplast data. In *Dicranum*, the concatenated chloroplast markers provided clades with generally better support, which may be due to the larger number of chloroplast markers included here than in the study by Stech *et al*. (2013), which employed solely the *rps4-trnT-trnL* region. Few studies have so far compared the performance of ITS1 vs ITS2 alone as barcoding markers for bryophytes. ITS2 was considered as universal barcode marker for plants and animals because of conserved regions in the adjacent genes, suitable for

TABLE 3 Pairwise Kimura 2-parameter (K2P) distances for ITS, the combined chloroplast markers and different partitions. The upper two rows indicate the ranges of intraspecific and interspecific distances for all *Dicranum* species. The last row indicates the overlap between the maximum intraspecific and minimum interspecific distances.

| | trnT-rps4 | trnL-trnF | pbA-trnH | rps19-rpl2 | rpoB | ITS 1 | ITS 2 | ITS | CPall | Combi |
|---|---|---|---|---|---|---|---|---|---|---|
| intra- specific | 0-0.0091 | 0-0.0159 | 0-0.0144 | 0-0.0065 | 0-0.0045 | 0-0.0116 | 0-0.0121 | 0-0.0103 | 0-0.0118 | 0-0.0089 |
| inter- specific | 0-0.0279 | 0-0.0391 | 0-0.0467 | 0-0.0165 | 0-0.0177 | 0-0.0638 | 0-0.0316 | 0-0.0627 | 0.0005-0.0296 | 0.0015-0.0346 |
| overlap | 0.0091 | 0.0159 | 0.0144 | 0.0065 | 0.0044 | 0.0116 | 0.0121 | 0.0103 | 0.0112 | 0.0073 |

primer design. Additionally, ITS2 had sufficient variability for identification of closely related species (Yao *et al*. 2010) and has proven to be conclusive for some bryophyte taxa, among them also Arctic species (Hassel et al. 2013). In contrast, other studies reported higher variation and species discrimination capacity of ITS1 in bryophytes (Liu *et al*. 2010; Stech *et al*. 2013), which was also the case in *Dicranum* (Table 2). As suggested by Stech *et al*. (2013), further analyses would be necessary in order to infer which part of the ITS region performs best as DNA barcode in bryophytes.

The present study is another example that a combination of several markers may be necessary to identify moss species with confidence based on molecular data. While one marker was sufficient to discriminate two closely related *Orthodontium* species (Rowntree *et al*. 2010), most complexes of closely related species needed several markers to be discriminated at species level (e.g., Carter 2012; Draper & Hedenäs 2009; Medina *et al*. 2012). However, finding the optimal combination of barcoding markers capable of delimiting closely related species is still a major concern in bryophytes and no consensus has been reached yet (Hollingsworth *et al*. 2011; Liu *et al*. 2010; Stech & Quandt 2010). The combination of ITS1 and/or ITS2, *rps4-trnT-trnL*, and *psbA-trnH* seems to be suitable for moss genera with generally low sequence divergence such as *Dicranum* (compared, for example, with its southern Hemisphere sister genus *Dicranoloma* (Renauld) Renauld; Stech *et al*. 2006). Additional markers such as *rps19-rpl2* may be required for certain species. The *rpoB* gene, in contrast, does not provide any additional resolution.

***Molecular versus morphological identification of Dicranum specimens from Svalbard—*** According to the molecular data, the 30 sequenced *Dicranum* specimens from Svalbard belong to five species, *D. acutifolium*, *D. elongatum*, *D. laevidens*, *D. majus*, and *D. spadiceum*. All of them are among the ten species accepted for the archipelago in the checklist by Frisvoll and Elvebakk (1996). The sequenced specimens represent most of the morphological variation of *Dicranum* in the respective sampled habitats and areas on Svalbard, and consequently, most, if not all, *Dicranum* species occurring there. Nonetheless, molecular analysis of an extended sampling across Svalbard would be necessary to assess whether the five other species accepted in the checklist are actually occurring on the archipelago or not, and to confirm the absence of the seven rejected species. Such an extended sampling would require extensive additional fieldwork or PCR amplification and sequencing of older herbarium material. The latter, however, seems to be difficult according to preliminary analyses (unpublished results) of collections from Edgeøya, eastern Svalbard, dating from the 1980s (cf. Hesse *et al*. 2012).

*Dicranum* species occurring in the Arctic are difficult to identify morphologically, especially in the field, but also microscopically. In few species the diagnostic characters seem to be stable, such as the strong costa and strongly incrassate, short and smooth upper lamina cells of *D. elongatum*, which could be recognized relatively easily. Most species present more variability in their diagnostic characters and are thus more often misidentified. For example, in temperate habitats, *Dicranum majus* is characterized by strongly falcate leaves, prosenchymatous and porose upper lamina cells, furrows on the costa and a double row of guide cells in the lower leaf. These typical characters are much less distinct in High Arctic specimens (Hedenäs *et al*. 2006). High Arctic specimens can therefore readily be mistaken for other species such as *D. scoparium* or *D. spadiceum* (Hedenäs & Bisang 2004; pers. obs.). The most difficult group of species to identify is comprised of *D. angustum*, *D. groenlandicum*, *D. laevidens,* and *D. spadiceum*. *Dicranum spadiceum* has long and narrow leaves ending in a tubular apex. Its leaf margins are slightly denticulate near the apex and the lamina cells are thin-walled and slightly porose. The basal cells are elongate, gradually becoming shorter and irregular, and lack pores, while the typical parenchymatous cells are sometimes restricted to the tip of the lamina. While *D. angustum* and *D. laevidens* are considered synonyms of *D. spadiceum* in The Plant List (2013), the former is distinguished by long, narrow, tubular and acuminate leaves as well as thin-walled and non-porose lamina cells. *Dicranum laevidens* is distinguished by an entire leaf margin as well as incrassate and porose, prosenchymatous lamina cells (Hedenäs & Bisang 2004). According to the molecular data, *D. angustum* and *D. laevidens* are clearly separated from *D. spadiceum* (Figs. 1-3), supporting their status as separate species. All respective Svalbard specimens belong to either *D. laevidens* or *D. spadiceum*, which corresponds well with the conclusions of Frisvoll and Elvebakk (1996) based on morphology that true *D. angustum* may be rare on Svalbard and further study is necessary to delimit *D. angustum* from *D. laevidens* or *D. spadiceum*. Again, additional fieldwork or molecular analysis of (old) herbarium specimens possibly representing *D. angustum* would be necessary. Species boundaries of *D. laevidens* and *D. groenlandicum* remained unclear because of their strong morphological similarities (Bellolio-Trucco & Ireland 1990; Hedenäs & Bisang 2004; Nyholm 1987; Steere 1978; Tuomikoski *et al*. 1973). In the absence of sporophytes, the distinction between the two species is essentially based on the different growth form, as *D. groenlandicum* grows in very dense and *D. laevidens* in looser tufts. Both the reference specimen of *D. groenlandicum* and one Svalbard specimen (Dic_1651) formed dense cushions, but the latter belonged to *D. laevidens* according to the molecular data, whereas the reference specimen was clearly separated molecularly (Figs. 1-3). This is a first indication that the habit may not always be reliable for identifying *D. groenlandicum*, and that in case no other gametophytic diagnostic characters can be found, sterile plants of *D. groenlandicum* can best be identified by DNA barcoding. Examination of supplementary material would be necessary to confirm this result.

Correct species identification is important in various fields of biodiversity assessments, ecology and conservation (Cornelissen *et al*. 2007; Dinnage *et al*. 2012; Steele & Pires 2011; Winter *et al*. 2012). Morphological identification of Arctic mosses requires taxonomic expertise and a combination of several stable characters. However, gametophytic characters often show deviating morphologies (Bellolio-Trucco & Ireland 1990; Hedenäs & Bisang 2004; Hesse *et al*. 2012). In *Dicranum* and many other genera, sporophytic characters are useful to distinguish gametophytically similar species, e.g., *D. groenlandicum* vs. *D. laevidens* and *D. fuscescens* vs. *D.*

*flexicaule*. Yet, sporophytes are rarely present in Arctic material. Therefore, species identification of Arctic plants of *Dicranum*, as well as of other complex moss species and genera, could greatly benefit from DNA barcoding. In *Dicranum*, identification can be best achieved using a combination of nuclear and plastid DNA sequences. Additional taxon sampling would, however, be necessary to better understand the relationships between morphological variability and genetic variation, solve taxonomic issues and build up a reference sequence database for molecular identification of unknown specimens by local BLAST searches in addition to molecular phylogenetic approaches.

# Chapter 5

## Phylogeny and species delimitations in European *Dicranum* (Dicranaceae, Bryophyta) inferred from nuclear and plastid DNA.

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Submitted to Molecular Phylogenetics and Evolution

ABSTRACT

DNA sequences are increasingly used for taxonomy, inferring phylogenetic relationships and identifying species boundaries. Many specific methods to define species delimitation have appeared recently, with the generalized mixed Yule coalescent (GMYC) method being the most popular. However, only few studies on land plants have been published so far and GMYC analyses of bryophytes are largely missing. *Dicranum* is a large genus of mosses whose (morpho-) species are partly ill-defined and frequently confused. To infer molecular species delimitations, we reconstructed phylogenetic trees based on five chloroplast markers and nuclear ribosomal ITS sequences from 28 out of 30 species occurring in Europe. We further applied GMYC and PTP species delimitation methods in order to compare their discriminatory power with species boundaries inferred from the molecular phylogenetic reconstructions and with the morphological species concept. Phylogenetic circumscriptions were congruent with the morphological concept for 24 species, while three taxa were molecularly indistinguishable from other closely related species. Phylogenetic relationships between *Dicranum* species remained largely unsupported. Automated species delimitation achieved similar results but tended to overestimate the number of potential species and exposed several incongruences between the morphological concept and inference from molecular phylogenetic reconstructions. It is concluded that GMYC and PTP methods potentially provide a useful and objective way of delimiting bryophyte species, but studies on further bryophyte data sets are necessary to infer whether incongruences might ensue from evolutionary processes and to test the suitability of these approaches.

INTRODUCTION

DNA sequence data are widely used for inferring species delimitations and phylogenetic

relationships. Specific methods to analyze species boundaries based on molecular phylogenetic reconstructions, however, have appeared only recently (cf. Carstens et al. 2013 for review), with the generalized mixed Yule coalescent (GMYC) method (Fontaneto et al. 2007; Pons et al. 2006) being most popular. This method estimates the point of transition from the level of species to population evolutionary processes, i.e. it detects species boundaries based on differences in branching rates at both species and population levels. Automated species delimitation methods are therefore considered especially useful in organisms with unclear species boundaries, due to poor taxonomy knowledge and because processes such as lineage sorting and introgression can obscure the species tree signal (O'Meara 2010 and references therein). Most GMYC studies so far focused on different animal groups (e.g. Poulakakis *et al*. 2012; Zaldívar-Riverón *et al*. 2010) and very few examples of analyses of other organisms such as algae (e.g. Leliaert *et al*. 2009), fungi (e.g. Parnmen *et al*. 2012) and land plants (e.g. Hernández-León *et al*. 2013) have been published. GMYC analyses of plant, and especially bryophyte, species are hence still largely missing.

Bryophytes are an important component of terrestrial ecosystems and count up to 18,000 known species (*Goffinet & Shaw 2009*). Nevertheless, because of the limited number of morphological characters available, the morphological plasticity of species and the generally broad geographical distribution, the taxonomy of many bryophyte lineages is still ambiguous. Molecular data can facilitate the circumscription of species, especially in taxa with extreme morphological similarities (e.g. Dong *et al*. 2012; *Hedenäs & Eldenäs 2007*; Heinrichs *et al*. 2009; Stech *et al*. 2013).

Species circumscription and identification in the Holarctic moss genus *Dicranum* (Dicranaceae, Bryophyta) has been notoriously difficult. The genus counts more than 90 species (www.Tropicos.org; Frey & Stech 2009), many of which are broadly distributed and display a great range of morphological plasticity, and only few species are habitat-specific (Hedenäs & Bisang 2004). Moreover, *Dicranum* and related genera display little molecular variation, as shown in previous studies (Cox *et al*. 2010; La Farge *et al*. 2002; Stech 1999; Stech *et al*. 2012). Assessing species delimitations in *Dicranum* is thus challenging both at the morphological and molecular level. Our recent studies on the *Dicranum scoparium* and *D. acutifolium* species complexes (*Lang & Stech 2014*; Lang *et al*. *in press*) as well as on boreal-arctic *Dicranum* species (Lang *et al*. 2014) showed that in several cases conclusive species delimitations could only be obtained from combined analyses of several chloroplast markers and nuclear ribosomal ITS sequences.

The present study aims to elucidate species boundaries within *Dicranum* on a broader geographic scale, including 27 of the 29 *Dicranum* species occurring in Europe (Hedenäs & Bisang 2004) plus *D. septentrionale* Tubanova & Ignatova, a newly recorded species in Scandinavia (Lang *et al*. *in press*). Molecular phylogenetic reconstructions based on five chloroplast markers (*trnH*$_{GUG}$*-psbA*, *rps4-trnT*$_{UGU}$ and *trnL*$_{UAA}$*-trnF*$_{GAA}$ intergenic spacers, *rps19-rpl2, rpoB*) plus the nrITS1-5.8S-ITS2 region will be used to test, for the first time in bryophytes, the congruence of two automated species delineation approaches, the general mixed Yule-coalescent (GMYC) models and Poisson tree processes (PTP). Sequence-based species delimitations will furthermore be compared with morphologically recognized species.

## Material and methods

***Sampling—*** A total of 202 *Dicranum* specimens were sampled (Appendix 1), representing 27 species of the 29 European species recognized by Hedenäs and Bisang (2004) and including the new European species record of *D. septentrionale*: six *Dicranum acutifolium* (Lindb. & Arnell)

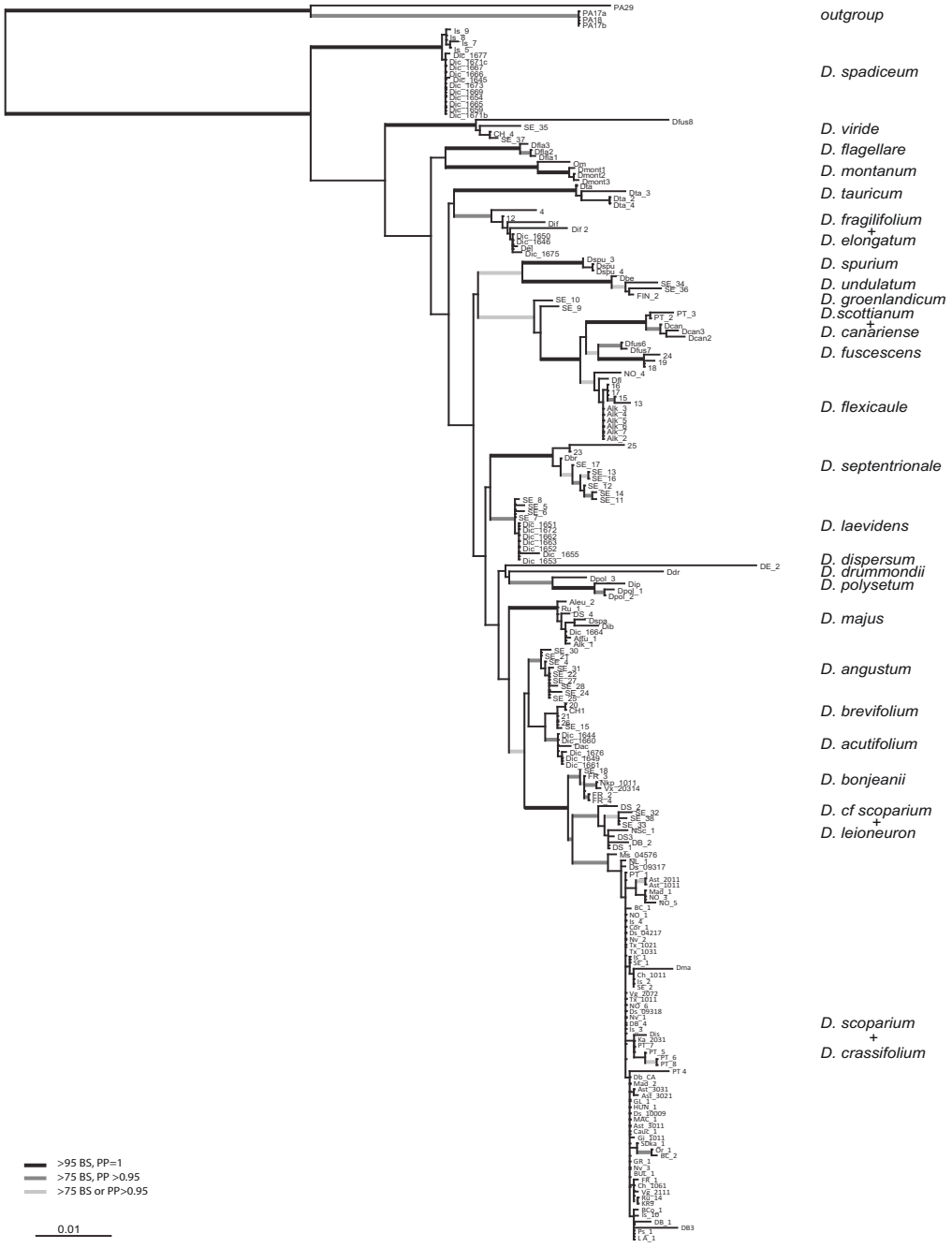



FIG. 1. Single optimal maximum likelihood phylogenetic reconstruction of 28 European *Dicranum* species inferred from the partitioned (non-coding chloroplast, chloroplast and ITS) nucleotide matrix including indels coded by simple indel coding (SIC). Branch thickness and colours indicate bootstrap support and posterior probabilities from ML and respectively Bayesian analyses.

C.E.O. Jensen, nine *D. angustum* Lindb., six *D. bonjeanii* De Not., five *D. brevifolium* (Lindb.) Lindb., three *D. canariense* Hampe ex Müll. Hal., five *D. crassifolium* Sérgio, Ochyra & Séneca, one *D. dispersum* Engelmark, one *D. drummondii* Müll. Hal., four *D. elongatum* Schleich. ex Schwägr., three *D. flagellare* Hedw., 11 *D. flexicaule* Brid., four *D. fragilifolium* Lindb.,six *D. fuscescens* Turner, two *D. groenlandicum* Brid., 11 *D. laevidens* R.S. Williams, three *D. leioneuron* Kindb., eight *D. majus* Turner, four *D. montanum* Hedw., four *D. polysetum* Sw., 65 *D. scoparium* Hedw., two *D. scottianum* Turner ex Robt. Scott, nine *D. septentrionale*, 15 *D. spadiceum* J.E. Zetterst., three *D. spurium* Hedw., four *D. tauricum* Sapjegin, four *D. undulatum* Schrad. ex Brid. and four *D. viride* (Sull. & Lesq.) Lindb. specimens. The sampling included 40 specimens newly sequenced for all six markers employed here, four specimens of which ITS sequences had already been generated by Tubanova *et al*. (2010) and Ignatova and Fedosov (2008) and 162 specimens of which chloroplast and ITS sequences were generated for previous studies (Lang & Stech 2014; Lang *et al*. 2014, *in press*; Stech 1999; Stech *et* al. 2006). As previous studies showed that *Holomitrium* is sister to *Dicranum* (La Farge *et al*. 2002; Stech *et* al. 2006), four samples, one *H. crispulum* Mart. and three *H. arboreum* Mitt, were chosen as outgroup representatives.

***DNA extraction, amplification and sequencing—*** The greenest parts of single gametophyte stems were selected for DNA extraction and cleaned manually with demineralised water under a binocular. Total DNA extraction was carried out using the NucleoSpin® Extract II Kit (Macherey-Nagel, Düren, Germany). Six markers employed to delimit closely related *Dicranum* species in Lang and Stech (2014) and Lang *et al*. (2014 *in press*) were amplified and sequenced, i.e. five chloroplast regions (partial *rpoB* gene, $trnH_{GUG}$-*psbA*, *rps19-rpl2*, *rps4-*$trnT_{UGU}$ and $trnL_{UAA}$-$trnF_{GAA}$ intergenic spacer) and the nuclear ribosomal nrITS1-5.8S-ITS2 region. PCR amplifications were performed as described in Lang and Stech (2014). All PCR products were purified and sequenced at Macrogen Inc. (www.macrogen.com). GenBank accession numbers of all sequences are listed in Appendix 1.

***Alignment and phylogenetic reconstruction—*** Sequences were aligned in Geneious v5.3.6 (Biomatters 2010) using 65% similarity matrix costs, and manually adjusted. Short hairpin-associated inversions in the *trnH-psbA* spacer, which can flip at the population level and may significantly reduce phylogenetic structure if undetected (Borsch & Quandt 2009; Quandt & Stech 2004; Whitlock *et al*. 2010), were positionally separated in the alignment and the corresponding indels were excluded.

Phylogenetic inferences were based on maximum likelihood (ML) and Bayesian inference (BI) analyses. Gaps were coded as informative by a simple indel coding strategy (SIC) (Simmons and Ochoterena 2000) implemented in SeqState (Müller 2004). To check for incongruence, phylogenetic reconstructions based on chloroplast and nuclear sequences were visually compared. In addition, an incongruence length difference test (ILD, Farris *et al*. 1994) as implemented in PAUP* 4.0b10 (Swofford 2002) was performed with 100 replicates. As both visual inspections and the ILD test indicated that the plastid and nuclear tree topologies were congruent ($p$=0.06), the two datasets were combined.

Three nucleotide partitions were used in ML and BI, namely the non-coding chloroplast markers (*rps4-trnT*, *trnL-trnF*, *trnH-psbA*, *rps19-rpl2*), the coding chloroplast region *rpoB* and the nuclear spacer nrITS. ML analyses were carried out with RAxML v.7.2.6 (Stamatakis 2006) employing the graphical user interface raxmlGUI v.0.93 (Silvestro & Michalak 2012) with the default GTR model

of nucleotide substitution and +Γ rate heterogeneity for all partitions. Bootstrap analyses under ML were done using the thorough bootstrap heuristics algorithm with 20 runs and 1000 replicates. BI analyses were run on the CIPRES science gateway (Miller *et al*. 2010). Bayesian posterior probabilities were calculated based on the Markov chain Monte Carlo (MCMC) method, using MrBayes v3.2.1 x64 (Huelsenbeck & Ronquist 2001; Ronquist & Huelsenbeck 2003). The a priori probabilities supplied were those specified in the default settings of the program. Best-fit models of nucleotide sequence evolution were selected according to the Akaike information criterion in MrModeltest (Posada and Crandall 1998) executed through PAUP*, namely HKY + Γ for the non-coding chloroplast partition, and HKY + I for coding and nuclear partitions. Sequence and indel data were treated as separate and unlinked partitions, employing the restriction site model ('F81') for the indel matrix as recommended by Ronquist *et al*. (2005). Two runs with four chains were run simultaneously ($11 \times 10^6$ generations), with the temperature of the single heated chain set to 0.5. Chains were sampled every 1,000 generations and the respective trees written to a tree file. Fifty percent majority rule consensus trees and posterior probabilities of clades were calculated by combining the two runs and using the trees sampled after the chains converged. Trace plots generated in Tracer v1.5 (Rambaut & Drummond 2007) were used to check for convergence of the runs (plateaus of all runs at comparable likelihoods) and to infer the 'burnin', which was set to 25%.

***Sequence-based species delimitation—*** Species boundaries were estimated using the GMYC (Fontaneto *et al*. 2007; Monaghan *et al*. 2009; Pons *et al*. 2006) and the PTP (Zhang *et al*. 2013) approaches. As GMYC requires a fully resolved topology with branch length estimates, we reconstructed an ultrametric tree with a strict molecular clock using parameters specified in BEAUti v. 2 and implemented in BEAST version 2.1.1 (Bouckaert *et al*. 2014). Branch lengths were estimated under a Yule prior with HKY nucleotide substitution model for each data partition. We included a gamma rate heterogeneity and no invariant sites for the chloroplast partition and both *rpoB* and ITS partitions included no gamma rate heterogeneity but estimated invariant sites. In the absence of fossil records, we applied a plastid substitution rate of $5.0 \times 10^{-4}$ SD of 2.0-8.0 $\times 10^{-4}$ subst./site/My following Villarreal and Renner (2014) for chloroplast and *rpoB* partitions and a substitution rate of $1.35 \times 10^{-3}$ subst./site/My for ITS as used in Heinrichs *et al*. (2006). The MCMC chains were run with $20 \times 10^6$ generations, saving the results every 2000th generation. The convergence of the runs was examined in Tracer v1.5. The maximum clade credibility tree was built from the combined runs after eliminating 25% of the trees for burnin in TreeAnnotator v1.7.2. The GMYC approach was carried out in R 2.15 (R Development Core Team 2013) using the splits (Ezard *et al*. 2009) and ape (Paradis *et al*. 2004) packages. The number of clusters and singletons were estimated by running both single and multiple threshold optimisations and using a multimodel Akaike information criterion with a model cutoff of deltaAICc = 7 (Monaghan *et al*. 2009; Pons *et al*. 2006; Powell 2012). On the contrary to the GMYC approach, PTP neither requires an ultrametric tree nor a sequence similarity threshold as input data because speciation rate is modelled by using the number of substitutions between branching and speciation events (Zhang *et al*. 2013). We therefore used the RaxML trees as input data, with 500,000 MCMC generations, thinning set to 100 and burnin at 25%. The calculations were conducted on the bPTP websever (http://species.h-its.org/ptp/).

Unbalanced sampling can affect the estimates of haplotypes and thus might overestimate the number of potential species (Bergsten *et al*. 2012; Zhang *et al*. 2013). Therefore, GMYC and PTP

analyses were additionally conducted on a reduced alignment containing only unique sequences (haplotypes). This reduced alignment, automatically obtained from the raxmlGUI interface, contained 145 sequences, with the strongest reduction in *D. scoparium* sequences retaining 18 out of the initial 65 sequences. The ultrametric and RaxML trees were reconstructed following the above-mentioned methods.

## Results

***Phylogenetic reconstruction—*** The total chloroplast alignment comprised 1914 positions, of which 222 were variable, and 132 of the variable characters were parsimony-informative. Of the 1142 positions in the ITS alignment, 124 ambiguous positions were removed from the subsequent calculations. The remaining 1019 positions comprised 217 variable characters, of which 139 were parsimony-informative. Simple indel coding of the combined dataset yielded 240 additional characters (excluding three corresponding to an inversion in *psbA-trnH*), of which 148 were parsimony-informative.

The single optimal ML tree of the combined markers is shown in Fig. 1, with bootstrap support (≥75% BS) from likelihood analyses and posterior probabilities (PP ≥95) from Bayesian inference indicated on the branches. The phylogenetic reconstruction resolved 23 clades that corresponded to morphological species, including the two species with only one sample (Fig. 1). While the clades of *D. acutifolium, D. angustum, D. bonjeanii, D. brevifolium, D. flagellare, D. fuscescens, D. laevidens, D. majus, D. montanum, D. polysetum*, *D. scoparium* s.l. (including *D. leioneuron*, *D.* cf. *scoparium*, and *D. scoparium* s.s., cf. Lang & Stech 2014), *D. septentrionale, D. spadiceum, D. spurium, D. tauricum, D. undulatum* and *D. viride* were strongly supported (≥81% BS, PP <0.97), *D. flexicaule* was supported only in the Bayesian reconstruction (62% BS, PP 0.99).

*Dicranum groenlandicum* did not form a monophyletic clade. Six species were molecularly indistinguishable from other closely related species: *D. fragilifolium* and *D. elongatum* formed a highly supported clade (95% BS, PP 0.99). While *D. crassifolium* was intermingled with *D. scopariums* s.s., *D leioneuron* clustered with North American specimens of *D.* cf. *scoparium* in a highly supported clade (92% BS, PP 1). Finally, *D. scottianum* and *D. canariense* formed a highly supported clade (100% BS, PP 1). However, the samples of both *D. scottianum* and *D. canariense* clustered in supported subclade (94, 97% BS, PP 1, respectively).

***Sequence- based species delimitation—*** The lineage through-time plot (Fig. 2b, c) indicated an exponential increase in branching rate near the tip of the tree. The single threshold GMYC model using the ultrametric phylogenetic tree created in BEAST resulted in the identification of 24 *Dicranum* clusters with high probabilities (CI= 23-26, lnL of null model= 741.079, ML of GMYC model= 748.162, $p$= 0.00269**) and 10 additional lineages consisting of single sequences, resulting in a total of 34 entities, excluding the outgroup (Fig. 2 a, b). The multiple threshold method gave four threshold times, resulting in a total of 58 entities that consisted of 38 clusters (CI= 30-39, lnL of null model= 741.079, ML of GMYC model= 752.849, $p$=0.000634***) and 20 singletons, excluding the outgroup (Fig. 2 c; Appendix 2). Although the multiple-threshold option was statistically preferred over the single-threshold option (deltaAIC=2.944), neither model was significantly different (Chi-square= 9.375, d.f.= 9, $p$= 0.40339). An inspection of the results obtained from both analyses revealed that the multiple-threshold GMYC model considered a higher number of clusters from samples that belonged to single lineages (Fig. 2a). Therefore, we

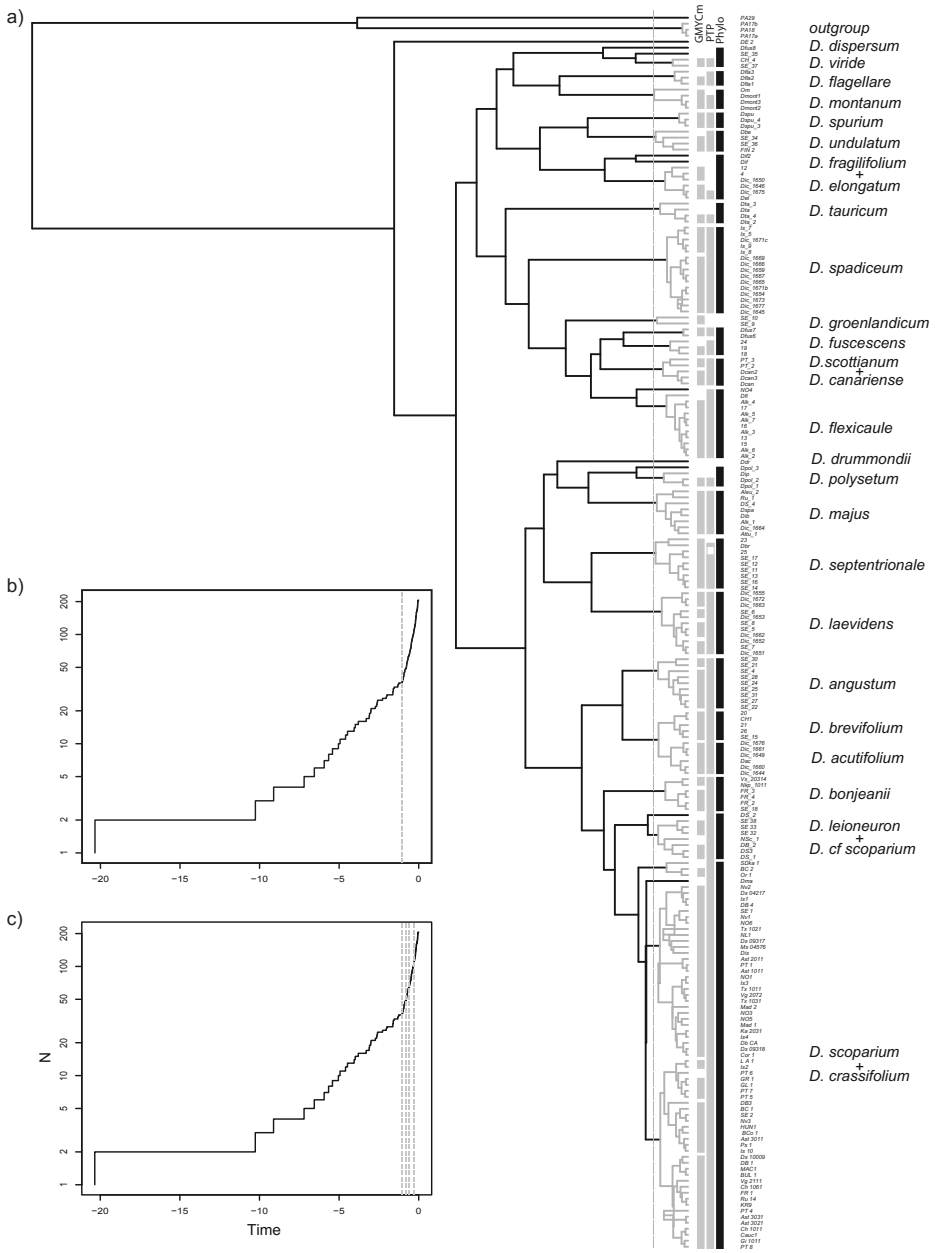



FIG. 2. Ultrametric tree depicting the relationship of *Dicranum* based on Bayesian analysis using a Yule model in BEAST and with fit of the general mixed Yules coalescent (GMYC) single threshold model from plastid and nuclear data. Branch length fitted a strict molecular clock (a). Lineages-through-time plot for single (b) and multiple (c) GMYC thresholds are illustrated. Estimated entities are indicated in grey. The vertical dashed line represent the timing of the earliest coalescent event. The two grey columns indicate the estimated entities from the multiple-threshold model (GMYCm) and the maximum likelihood solution of the Poisson tree processes (PTP), respectively. The black column indicates the phylogenetic boundaries of the *Dicranum* taxa (Phylo).

took a more conservative approach and discussed only the results of the single-threshold method.

The trees resulting from PTP gave similar results to GMYC (Fig. 2a). The number of estimated species varied between 25 and 116, excluding the outgroup (acceptance rate= 0.593), with 37 partitions supported by the ML search, excluding the outgroup (Fig. 2a, Appendix 3).

GMYC results based on the reduced alignment were similar to the results based on the extended alignment. The single-threshold model indicated the presence of 23 clusters and 31 entities while the multiple models resulted in four threshold times and resulted in 30 clusters and 48 entities, excluding the outgroup (CI= 9-40/ 38-68, lnL of null model= 259.1778/ 259.1778, ML of GMYC model= 263.0662/ 266.5076, $p$=0.051/ 0.023*, respectively; Table 1). The number of estimated species obtained from PTP method ranged between 49-102 entities, excluding the outgroup (acceptance rate= 0.716), with 42 partitions supported by the ML search, excluding the outgroup (Appendix 4).

## Discussion

***Phylogenetic reconstruction versus morphological species—*** The present study comprises the largest molecular dataset of *Dicranum* available so far, including all but two *Dicranum* species occurring in Europe following Hedenäs and Bisang (2004), plus *D. septentrionale*, recently described from Russia and newly identified in Scandinavia (Tubanova *et al*. 2010; Lang *et al*. 2014). The majority of the analysed species (23 out of 28, including two singletons), were molecularly recognisable based on the combined analysis of five chloroplast markers and nuclear ribosomal ITS sequences (Fig. 1), albeit not all with significant statistical support. The results support our recent phylogenetic studies on *Dicranum* species complexes and Arctic *Dicranum* species (Lang & Stech 2014; Lang *et al*. 2014; Lang et al. in press) in that a combination of molecular markers data can clarify species circumscriptions in *Dicranum*, and that the low resolution and clade support within *Dicranum* in earlier analyses (e.g. La Farge *et al*. 2002; Stech et al. 2006; Tubanova *et al*. 2010; Tubanova & Ignatova 2011) was a result of too few molecular markers analysed (cf. also Stech & Quandt 2010). Furthermore, the present study shows that, at least for Europe, the molecular data to a large extent support the morphological species concept, despite morphological confusions and subtle diagnostic characters in several species (e.g. Lang *et al*. in press; Tubanova *et al*. 2010).

In contrast to these results, nine species showed discrepancies between their morphological concepts and their molecular circumscription, namely *D. groenlandicum*, *D. elongatum*, *D. fragilifolium*, *D. scottianum, D. canariense*, *D. leioneuron* and *D.* cf *scoparium*. *Dicranum groenlandicum* was resolved as paraphyletic but without significant statistical support (Fig. 1). This arctic species is morphologically very similar to *D. laevidens* and, in absence of sporophytes, both species are essentially differentiated based on the growth form. However, recent molecular studies on arctic *Dicranum* suggested that both species represent two separate entities (Lang *et al*. 2014). The present phylogenetic reconstruction confirms the separation of *D. groenlandicum* from *D. laevidens* and further confirms the delineation of the latter species. Nevertheless, additional sequences of *D. groenladicum* are necessary to infer its delimitation. *Dicranum elongatum* and *D. fragilifolium* are morphologically different and occupy different habitats (Ireland 2007). Moreover, *Dicranum elongatum* is frequently confused with *D. groenlandicum*, while *D. fragilifolium* shares morphological similarities with *D. tauricum* (Hedenäs & Bisang 2004; Ireland 2007). Despite their clear morphological distinctions, the present molecular phylogenetic reconstruction indicates that both *D. elongatum* and *D. fragilifolium* belong to the same taxon (Fig. 1). The two Macaronesian-Atlantic European species *D. canariense* and *D. scottianum* were resolved in one well-supported clade.

Because of their morphological resemblance, *D. canariense* has been considered as a subspecies or variety of *D. scottianum* (Tropicos.org). In the current concept, *D. canariense* differs from the latter by its strongly denticulate margins and thick and denticulate costa (Hedenäs & Bisang 2004). The sampling included in this study confirms their close relationship and indicates that both taxa should be distinguished at subspecies level, however a larger sampling would be necessary to confirm these results. Morphological and ecological characters of *D. leioneuron* have been discussed several times, as it is frequently confused with either *D. bonjeanii* or *D. scoparium* (Ahti & Isoviita 1962; Corley 1991). Consequently, *D. leioneuron* has been sometimes considered as an ecotype of *D. scoparium* or a variety of *D. bonjeanii* (Ahti & Isoviita 1962), a hypothesis that is rejected by the present phylogenetic reconstructions (Fig. 1), which in turn confirm the observations of Corley (1991). Despite being molecularly separated from *D. bonjeanii* and *D. scoparium* s.str., the *D. leioneuron* specimens included in this study clustered in a well-supported lineage together with North American samples, named as *D.* cf. *scoparium* in Lang and Stech (2014). Morphology and habitat of these two groups are, however, clearly different: the North American specimens have falcate-secund leaves that are serrate on the margins and a lamellate costa. The *D. leioneuron* specimens, on the other hand, have all the characteristics of this species, i.e. small and erect-patent leaves; very thin nerve and without dorsal lamellae. Additionally, flagellary shoots are common in this species. Although the present data does not indicate any hybridization processes, the use of other molecular methods or more variable markers could bring new insights in understanding the relationship between *D. leioneuron* and *D. scoparium*. Finally, *D. crassifolium* is a species that has been described recently (Sérgio *et al*. 1995) and that has been found only in few places in Europe. This species resembles *D. scoparium* but is most similar to *D. transylvanicum* (not included here) due to a bi- or even tristratose leaf lamina and denticulate leaf margins. The present molecular phylogenetic inferences, however, show that this species actually corresponds to *D. scoparium*. *Dicranum scoparium* is known to be very plastic morphologically and occurs in a very broad range of habitats (Hedenäs & Bisang 2004; Ireland 2007; Lang & Stech 2014; Smith 2004), including soil or humus, as well as on rocks or tree bases, in open and shady places where *D. crassifolium* grows as well (Sérgio *et al*. 1995). What triggers the deviating leaf lamina morphology of *D. crassifolium*, and how *D. transylvanicum* relates to *D. crassifolium* and *D. scoparium*, remais to be tested.

Various factors such as the environment or polyploidisation may account for the observed morphological variability of *D. crassifolium*, *D. scottianum* and *D. canariense,* for exemple. Deviating morphologies are frequently observed in bryophytes, especially in species growing in stressful environmental conditions (Buryová & Shaw 2005; Hedenäs *et al*. 2006; Pereira *et al*. 2013; Såstad 1998; Såstad *et al*. 1999; Spitale & Petraglia 2010). Most of the *Dicranum* species are widespread and found in a great range of habitats. Hence, local adaptation could partly explain the morphological differences of genetically similar taxa, such as observed in *D. fragilifolium* and *D. elongatum* or *D. leioneurion*. Although the present data does not indicate any hybridisation events, this genetic process is known to influence the morphology (Draper & Hedenäs 2009; Hedenäs 2008; Natcheva & Cronberg 2004; Sotiaux *et al*. 2009). Moreover, the consequences of the special sexual reproduction of *Dicranum*, i.e. dwarf males growing on the branch of a female plant (pseudomonoicy), are largely unknown and would deserve further investigations, in order to explain genetic relationship of closely related species.

TABLE 1. Type of alignment, species delimitation method and number of estimated entities obtained for Dicranum. LR and LR test of the GMYC single- threshold (GMYCs) and multiple-threshold (GMYCm) analyses are also mentioned. Significant values are indicated with an asterisk. The species delimitation results are compared with the number of supported phylogenetic entities (phylo) obtained from maximum likelihood analyses.

| Alignment | method | Number of sequences | Number of estimated clusters | entities | LR | LR test |
|---|---|---|---|---|---|---|
| extended | GMYCs | 206 | 24 | 33 | 9.723 | 0.021* |
| | GMYCm | | 35 | 58 | 19.778 | 0.011* |
| | PTP | | | 37 | | |
| | phylo | | 21 | 23 | | |
| reduced | GMYCs | 145 | 23 | 31 | 7.778 | 0.051 |
| | GMYCm | | 30 | 47 | 14.660 | 0.023* |
| | PTP | | | 42 | | |
| | phylo | | 20 | 22 | | |

***Species delineation using GMYC and PTP—*** The definition of boundaries between species clusters is essential, as it will influence the interpretation of the phylogenetic reconstructions (Powell 2012). However, one of the major drawbacks of molecular taxonomy is putting a non-arbitrary threshold for delineating species. The main advantage of general mixed Yule-coalescent (GMYC) or Poisson tree processes (PTP) methods is the objective estimation of phylogenetic entities and the circumscription of taxa based on branch length dynamics rather than sequences similarities (Monaghan *et al*. 2009; Pons *et al*. 2006). Although GMYC and PTP performances have been proven to be stable under a wide range of conditions, the accuracy of species delimitation methods will principally depend on the singularities of the data set and the initial species concept used (Talavera *et al*. 2013; Zhang *et al*. 2013). In this study, 34 species were recovered by GMYC single threshold methods, which corresponds generally well with the phylogenetic reconstruction.

However, disagreements were observed, such as in *D. scoparium* but also *D. viride*, *D. fragilifolium- D. elongatum*, *D. flexicaule D. fuscescens* and *D. polysetum*, where overestimations in the number of entities occurred compared to the molecular and morphological delimitations (Fig. 2a). Each of these species counted one additional entity when compared to the phylogenetic tree, except for *D. fragilifolium- D. elongatum* and *D. scoparium* which counted a total of three, respectively four entities. Simultaneously, GMYC calculations considered both sample of *D. groenlandicum* as one species and both *D. brevifolium* and *D. acutifolium* were considered as belonging to the same lineage. The number of ML estimates obtained from the PTP of the extended dataset were relatively similar to the results obtained from GMYC methods (Table 1). However, the number of PTP estimates based on the reduced dataset was slightly higher (Table 1). Simulations have shown that an unbalanced sampling are likely to increase the estimates of haplotypes of the oversampled species (Bergsten *et al*. 2012; Zhang *et al*. 2013) and each specimen of an undersampled species might be counted as separate entity (Zhang *et al*. 2013). In our study, the reduced sampling of *D. scoparium* did not decrease the number of potential species. On the contrary, most of the haplotypes or unique sequences, in particular within *D. scoparium*, were considered as single lineages (Appendix 4). The effect of unbalanced sampling in our dataset has probably less impact on the species delimitation due to the generally low variability in *Dicranum*. Indeed, weak signals and high levels of uncertainty can explain the large range of estimated

species in both PTP estimations (J. Zhang, pers. communication).

Overestimations in the GMYC have been observed in previous studies (e.g. Miralles and Vences 2013; Puillandre *et al*. 2012; Talavera *et al*. 2013) and were often related with errors in the GMYC methods or in the construction of the ultrametric, rather than to taxonomical knowledge gaps (Talavera *et al*. 2013; Zhang *et al*. 2013). As our PTP estimates, obtained from a RaxML tree, were relatively close to the phylogenetic clades and not substantially different from the GMYC results, we considered that errors in the ultrametric tree construction had little effects on the species delimitation. As for now, the GMYC and PTP analysis revealed multiple lineages within species in *Dicranum* that lack morphological and ecological support. Simultaneously, these methods showed an absence of DNA divergences between *D. acutifolium* and *D. brevifolium* as well as between *D. scottianum* and *D. canariense*, which indicates that these four morpho-species might belong to two single taxa.

## Conclusions

Biodiversity assessments rely on the correct delimitation of species. The identification of bryophyte species is largely based on morphological characters, which are often subtle and difficult to apply, or prone to plasticity induced by environmental conditions. Phylogenetic species delimitations, on the other hand, also rely on a certain degree of subjectivity. Automated methods such as GMYC and PTP may provide a more objective approach to molecular species delineation based on maximum likelihood inferences, although inferred boundaries are only putative. Our results showed that DNA-based circumscriptions were generally congruent with morphological species delimitations. Nevertheless, GMYC and especially PTP methods exposed several incongruences between morphological concepts and inference from molecular phylogenetic reconstructions. These incongruences might ensue from evolutionary processes, but also display the need for further testing on other bryophyte data sets to infer the suitability of GMYC and PTP methods for species delimitation in bryophytes.

# Chapter 6

## Summary and conclusions

*Dicranum* is a large genus essentially found in the Holarctic (Crosby *et al*. 1999; Frey & Stech 2009). With more than 90 accepted species, *Dicranum* is one of the largest genera of Dicranaceae (Missouri Botanical Garden; Frey & Stech 2009) and about 30 species are recorded for Europe (Hedenäs & Bisang 2004). *Dicranum* species grow in a broad range of habitats, forming dense, tomentose tufts or cushions (Crum & Anderson, 1981), and are easily recognized in the field by their typical « *Dicranum*-look »: acrocarpous stems and leaves that are lanceolate, gradually acuminate and sometimes secund. When fertile, sporophytes have long-rostrate opercula, cucullate calyptrae and 16 peristome teeth that are divided to half-way (Nyholm 1987; Hedenäs & Bisang 2004; Smith 2004; Ireland 2007). However, the taxonomy of this genus is controversial (Allen 1998; Ireland 2007). It has been divided into seven sections (Peterson 1979; Nyholm 1987) whose characteristics are not always distinctive. Moreover, many species are difficult to distinguish due to their morphological plasticity. Intergrading forms are often found, leading to frequent confusions and unclear taxonomy.

In this thesis, species delimitations of temperate and arctic *Dicranum* lineages were investigated using molecular phylogenetic reconstructions and barcoding methods. Four potential barcode markers (*rps4-trnT*, *trnL-F*, *psbA-trnH*, nrITS) and two additional chloroplast markers (*rps19-rpl2* and *rpoB)* were sequenced for 90% of the species known in Europe. Molecular data were analysed with maximum parsimony, maximum likelihood and Bayesian inferences for phylogenetic investigations. Furthermore, Bayesian approaches were used for testing automated species delimitation methods (generalised mixed Yule coalescent (GMYC) and Poisson tree processes (PTP)). Morphological characters were re-addressed in the light of the molecular phylogenetic inferences. Finally, gametophytic characters were re-examined and scored for statistical analyses in order to evaluate their relevance for distinguishing closely related species.

*What is the Dicranum scoparium complex? What are the morphological characters of* Dicranum scoparium *and how is it related to its morphologically close species?*

Bryophytes have a limited number of morphological characters that are strongly influenced by the environment (Briggs 1965; Vanderpoorten & Goffinet 2006). Therefore, it can be difficult to define stable characters that are distinctive for each species. In this thesis, the problem of species delimitations was first investigated in a number of species of section *Dicranum* (Hedw.) Sull. (sensu Nyholm 1987; Bellolio-Trucco & Ireland 1990) whose morphological forms intergrade into one another (Lang & Stech 2014; **chapter 1**), with a focus on the widespread and polymorphic *D.*

*scoparium*. Molecular phylogenetic reconstructions indicated that molecular lineages are generally congruent with morphological species concepts in *Dicranum*. They further suggested a close relationship of the Holarctic *Dicranum scoparium* Hedw. and *D. bonjeanii* De Not. with the more narrowly distributed *D. howellii* Renauld & Cardot (North America), *D. lorifolium* Mitt., *D. japonicum* Mitt. and *D. nipponense* Besch. (Asia), which together could be regarded as the *D. scoparium* species complex. However, other species of section *Dicranum*, namely *D. majus* Turner and *D. polysetum* Sw. were separated from the *D. scoparium* complex, although frequent morphological confusion with *D. scoparium* are reported. The large sampling of *D. scoparium*, including North American, European and Asian specimens and covering the high degree of morphological trait variation, revealed a monophyletic lineage, defined as *D. scoparium* s.s., that corresponds to the morphological concept of this species: the leaves are straight to falcate-secund ending in a keeled apex, margins are serrate, the costa is percurrent with four serrate ridges on its dorsal side and several stereid bands can be seen in cross-sections, lamina cells are prosenchymatous and porose. Nonetheless, *D. crassifolium* Sérgio, Ochyra & Séneca also corresponded to *D. scoparium* s.s. despite its bistratose lamina and dentate margins (**chapter 5**). Furthermore, several *D. scoparium*-looking specimens from North America (*D.* cf. *scoparium*) were separated from *D. scoparium* s.s. and cluster with *D. leioneuron* Kindb. (**chapter 5**).

*What are* D. bardunovii *and* D. septentrionale*? Are they separate species? Are their morphological characters adequate for distinguishing them from the closely related* D. acutifolium and D. brevifolium*?*

Two new species have been recently described from Russia: *D. bardunovii* Tubanova & Ignatova and *D. septentrionale* Tubanova & Ignatova. These two species resemble in many characters to *D. acutifolium* and *D. brevifolium* and are easily overlooked. Thus, the four mentioned species are considered to belong to a complex of species named the *D. acutifolium* species complex. The discovery of *D. bardunovii* and *D. septentrionale* is based on phylogenies using only the nuclear spacer nrITS (Tubanova *et al*. 2010; Tubanova & Ignatova 2011). Although this marker is often considered as sufficient for species delimitation (Chen *et al*. 2010; Liu *et al*. 2011), species circumscription in *Dicranum* lacked support and resolution (Lang & Stech 2014, **chapter 1**) due to the generally low genetic variability in Dicranaceae (Stech 1999; La Farge *et al*. 2002). Hence, several markers were necessary for clearer species circumscriptions as shown in the previous study (Lang & Stech 2014). Therefore, we studied the molecular relationship of the *D. acutifolium* species complex and its close allies by combing additional chloroplast markers to the existing ITS phylogeny and analysed the relevance of morphological characters. The combined molecular analyses corroborated the results obtained by Tubanova *et al*. (2010) and Tubanova & Ignatova (2011). While the delimitation of *D. septentrionale* became strongly supported, the circumscription of *D. bardunovii* remained less clearly defined. Nevertheless, both of them could be recognized as species and were distinct from *D. brevifolium* and *D. acutifolium*. The molecular circumscription of *D. bardunovii*, *D. acutifolium* and *D. brevifolium* and especially *D. septentrionale* was in sharp contrast with their morphological resemblance because the characters differentiating the four species are minute and may be easily overlooked. The recognition of these species was moreover hampered by the occurrence of mixed collections, as exemplified by the holotype of *D. bardunovii*, which contained also individuals of *D. acutifolium*. In line with previous morphological and phylogenetic

analyses (e.g. Sukkharak *et al*. 2011; Carter, 2012; Medina, 2012; Stech *et al*. 2013; Lang & Stech, 2014) the present study highlighted the importance of molecular data for clarifying species circumscriptions.

*Is barcoding a method that can be used for identifying* Dicranum *species? How do barcode markers perform in terms of species identification?*

Correct species identification is important in various fields of biodiversity assessments, ecology and conservation (Cornelissen *et al*. 2007; Dinnage *et al*. 2012; Steele & Pires 2011; Winter *et al*. 2013) but morphological identification of organisms with reduced sizes such as bryophytes can be difficult, especially of arctic bryophytes species, whose gametophytic characters show extreme deviating morphologies and sporophytes are mostly absent (Bellolio-Trucco & Ireland 1990; Hedenäs & Bisang 2004; Hesse *et al*. 2012). DNA barcoding method is an alternative approach to investigate species delimitation. In **chapter 4**, we demonstrated that molecular circumscription of arctic *Dicranum* species using a high number of barcode markers was possible. However, analyses of each individual marker indicated that they tended to possess little interspecific variability. Moreover, the performance of ITS1 and ITS2 was overrated and failed at discriminating all *Dicranum* species. Interspecific genetic distances were generally small and overlapped with intraspecific genetic distances. Used in combinations, the most commonly used markers, *trnT- rps4*, *trnL-F psbA-trnH* and nrITS, did not contain a natural "barcode gap" either, meaning that some species remain difficult to circumscribe (**chapter 5**). However, since the overlap was reduced and in line with other barcode studies (Liu *et al*. 2011; Stech *et al*. 2013; Hassel *et al*. 2013), these four markers could be considered as potential barcode markers in *Dicranum*.

*Is automated species delimitation congruent with the morphological concept of European species?*

Generalized mixed Yule coalescent approach (GMYC) or Poisson tree processes (PTP) are species delimitation methods based on likelihood (ML) phylogenetic inferences (Pons *et al*. 2006; Monaghan *et al*. 2009). While GMYC requires a time-calibrated tree, PTP also works on standard phylogenetic trees. Nevertheless, both methods calculate a number of entities that represent theoretical species. Based on a phylogenetic reconstruction that included 28 of the 30 *Dicranum* species found in Europe, the number of entities recovered by the GMYC methods varied between 34 and 58 and 37 estimated entities were recovered by PTP approaches (**chapter 5**). When considering the single threshold GMYC model and the ML tree obtained from the PTP, both methods were relatively congruent with 34 and 37 species, respectively. These estimations, however, were slightly higher than the number of morphological species. The overestimations concerned *D. scoparium* but also *D. viride*, *D. fragilifolium*, *D. fuscescens* and *D. polysetum*. Simulations have shown that an unbalanced dataset and low intraspecific genetic variability are likely to increase the estimates of haplotypes of the oversampled species (Bergsten et al., 2012; Zhang et al., 2013). In such cases, each specimen of a species with small sampling might be counted as a separate entity (Zhang *et al*. 2013). In our study, analyses on a reduced dataset did not decrease the number of potential species. Therefore, the effect of unbalanced sampling probably had less impact on species delimitations than the generally low genetic variability within the genus *Dicranum*.

Despite the overestimated number of species obtained with automated estimation methods, phylogenetic clades were delimiting species that were generally congruent with the actual morphological circumscriptions. Molecular phylogenetic inferences also brought useful insights in several morpho-species such as *D. leioneuron*, *D. crassifolium*, *D. scottianum* and *D. canariense*, *D. angustum* and *D. laevidens* or species of the *D. acutifolium* complex (**chapter 3, 4, 5**), whose taxonomy was until now unclear. Furthermore, the division of the genus into sections had no biological means, as depicted from the phylogenetic tree in **chapter 5**.

*Future studies*

In this thesis, we show that molecular methods enabled us to clarify the molecular circumscription of a subset of *Dicranum* species and to examine the relationship within species complexes. Although most of the studied taxa were statistically strongly supported, three European species were indistinguishable from other species (*D. crassifolium* from *D. scoparium*, *D. scottianum* from *D. canariense, D. elongatum* from *D. fragilifolium*). Additionally, *D. leioneuron* grouped with North American specimens of *D. scoparium* despite clear morphological differences between the two species. A detailed re-interpretation of morphological differences and possibly re-examination of diagnostic characters should be carried out. Moreover, the delimitation of three European species, *D. fulvum, D. muehlenbeckii* and *D. transylvanicum*, could not be included in the study. Morpho-molecular analyses of these species would allow us to better understand their genetic affiliation.

The sampling of this thesis represent roughly a third of the known *Dicranum* species. The missing taxa are mainly found in Asia and America. A complete revision of *Dicranum*, including all the species, would allow us to complete our knowledge of this species-rich genus.

Furthermore, the inclusion of additional variable markers and studies of microsatellites loci would help to understand the relationship between the different taxa and to explore the influence of male dwarfism on species reproduction as well as to help us answering the question whether hybridization event occurs in *Dicranum*.

# Samenvatting en Conclusies

*Dicranum* (Gaffeltandmos) is een groot geslacht dat hoofdzakelijk voorkomt in het holarctisch gebied (Crosby *et al*. 1999; Frey & Stech 2009). Met meer dan 90 geaccepteerde soorten is *Dicranum* één van de grotere genera in de Dicranaceae (Frey & Stech 2009) en ongeveer 30 soorten zijn geregistreerd voor Europa (Hedenäs & Bisang 2004). *Dicranum* soorten worden in veel verschillende habitats aangetroffen en vormen dichte viltige polletjes of kussentjes (Crum & Anderson, 1981). Ze zijn in het veld aan hun typische « *Dicranum*-habitus » gemakkelijk te herkennen: acrocarpe stengels en eirond-lancetvormige bladeren die geleidelijk zijn toegespitst en soms homotroop gekromd zijn. De sporenkapsels hebben lang-snavelvormige opercula, kapvormige calyptra's en 16 peristoomtanden die halverwege in tweeën gedeeld zijn (Nyholm 1987; Hedenäs & Bisang 2004; Smith 2004; Ireland 2007). Niettemin is de taxonomie van dit genus controversieel (Allen 1998; Ireland 2007). Het genus is verdeeld in zeven secties (Peterson 1979; Nyholm 1987) waarvan de kenmerken niet altijd onderscheidend zijn. Bovendien zijn veel soorten moeilijk van elkaar te onderscheiden vanwege hun morfologische plasticiteit.

In dit proefschrift is de soortomgrenzing van gematigde en Arctische *Dicranum* soorten onderzocht met behulp van moleculaire fylogenetische reconstructies en DNA-barcodering. Vier potentiële barcode merkers (*rps4-trnT*, *trnL-F*, *psbA-trnH*, nrITS) en twee aanvullende chloroplast merkers (*rps19-rpl2* en *rpoB)* zijn gesequenced voor 90% van de soorten bekend uit Europa. Moleculaire data werden geanalyseerd met maximale parsimonie, maximum likelihood en Bayesiaanse methodes voor fylogenetisch onderzoek. Bovendien zijn Bayesiaanse methodes gebruikt om automatische soortsbegrenzing-methodes te testen. De bruikbaarheid van morfologische kenmerken werd geëvalueerd in het licht van de moleculaire fylogenetische resultaten. Om de toepasbaarheid van gametofytkenmerken te evalueren, zijn deze heronderzocht en gescoord om statische analysen uit te voeren.

*Wat is het* Dicranum scoparium *complex? Wat zijn de morfologische kenmerken van* Dicranum scoparium *en hoe is* D. scoparium *gerelateerd aan de morfologisch meest verwante soorten?*

Bryofyten hebben een beperkt aantal morfologische kenmerken die sterk worden beïnvloed door de milieufactoren (Briggs 1965; Vanderpoorten & Goffinet 2006). Daarom kan het moeilijk zijn om stabiele kenmerken te definiëren die onderscheidend zijn voor elke soort. In dit proefschrift zijn de problemen van soortsbegrenzing eerst onderzocht in een aantal soorten van de sectie *Dicranum* (Hedw.) Sull. (sensu Nyholm 1987; Bellolio-Trucco & Ireland 1990) waarvan de morfologische vormen met elkaar overlappen (Lang & Stech 2014; **hoofdstuk 1**) met een focus op de wijdverspreide polymorfe soort *Dicranum* Hedw. *scoparium*. Moleculaire fylogenetische reconstructies wijzen erop dat de moleculaire lijnen meestal congruent zijn met het morfologische soortconcept in *Dicranum*. Zij suggereren verder een nauwe verwantschap tussen de holarctische *D. scoparium*. en *D. bonjeanii* De Not. met de meer beperkt verspreide *D. howellii* Renauld &

Cardot (Noord-Amerika), *D. lorifolium* Mitt., *D. japonicum* Mitt. en *D. nipponense* Besch. (Azië) die gezamenlijk kunnen worden beschouwd als het *D. scoparium* soortencomplex. Echter, andere soorten uit de sectie *Dicranum*, namelijk *D. majus* Turner en *D. polysetum* Sw. zijn gescheiden van het *D. scoparium* complex hoewel morfologische verwarring met *D. scoparium* vaak voorkomt. Onze uitgebreide geografische bemonstering van *D. scoparium,* inclusief Noord-Amerikaanse, Europese en Aziatische exemplaren, en tevens uitgebreide bemonstering van de morfologische kenmerkvariatie onthulde een monofyletische groep, gedefinieerd als *D. scoparium* s.s., die overeenkomt met het morfologische concept van deze soort: de bladeren zijn recht tot homotroop gekromd en eindigen in een gekielde apex, de bladranden zijn gezaagd, de bladnerf is percurrent met vier gezaagde lamellen op de dorsale zijde en verscheidene stereïde banden zijn zichtbaar in dwarsdoorsnede, de bladcellen zijn prosenchymatisch en poraat. Echter, *D. crassifolium* Sérgio, Ochyra & Séneca komt ook overeen met *D. scoparium* s.s. ondanks het tweelagige blad en getande bladranden (**hoofdstuk 5**). Bovendien zijn verscheidene *D. scoparium*-achtige exemplaren uit Noord-Amerika (*D.* cf. *scoparium*) gescheiden van *D. scoparium* s.s. en clusteren ze met *D. leioneuron* Kindb. (**hoofdstuk 5**).

*Wat zijn* D. bardunovii *en* D. septentrionale*? Zijn het aparte soorten? Zijn hun morfologische kenmerken voldoende om ze te onderscheiden van de nauwverwante soorten* D. acutifolium *en* D. brevifolium*?*

Twee nieuwe soorten zijn recentelijk beschreven uit Rusland: *D. bardunovii* Tubanova & Ignatova en *D. septentrionale* Tubanova & Ignatova. Deze twee soorten lijken in veel kenmerken op *D. acutifolium* en *D. brevifolium* en worden gemakkelijk over het hoofd gezien. De vier genoemde soorten worden daarom beschouwd als een complex van soorten, namelijk het *D. acutifolium* soortcomplex. De ontdekking van *D. bardunovii* en *D. septentrionale* is gebaseerd op fylogenieën die alleen gebruik maken van de nucleaire spacer nrITS regio (Tubanova *et al.* 2010; Tubanova & Ignatova 2011). Hoewel deze merker vaak als geschikt wordt beschouwd voor soortsbegrenzing (Chen *et al.* 2010; Liu *et al.* 2011), ontbraken bij de soortomschrijvingen in *Dicranum* ondersteuning en resolutie (Lang & Stech 2014, **hoofdstuk 1**) door de doorgaans lage genetische variabiliteit in de Dicranaceae (Stech 1999; La Farge *et al.* 2002). Verdere merkers zijn bijgevolg noodzakelijk voor het verkrijgen van duidelijkere soortomschrijvingen (Lang & Stech 2014). Daarom onderzochten wij de moleculaire verwantschappen van het *D. acutifolium* soortcomplex en zijn naaste verwanten door aanvullende chloroplast merkers met de bestaande ITS-fylogenie te combineren; ook analyseerden wij de relevante morfologische kenmerken. De gecombineerde moleculaire analyse bevestigt de resultaten van Tubanova *et al.* (2010) en Tubanova & Ignatova (2011). Hoewel de begrenzing van *D. septentrionale* nu significant ondersteund is, blijft de omgrenzing van *D. bardunovii* minder duidelijk. De kenmerken die de soorten *D. bardunovii*, *D. acutifolium* en *D. brevifolium* en vooral *D. septentrionale* onderscheiden zijn onopvallend en worden gemakkelijk over het hoofd gezien. De herkenning van deze soorten wordt bovendien bemoeilijkt door het bestaan van mengcollecties, zoals geïllustreerd bij het holotype van *D. bardunovii*, dat ook individuen van *D. acutifolium* bevat. In overeenkomst met eerdere morfologische en fylogenetische analyses (e.g. Sukkharak *et al.* 2011; Carter, 2012; Medina, 2012; Stech *et al.* 2013; Lang & Stech, 2014) benadrukt dit onderzoek het belang van moleculaire data om soortsomschrijvingen te verduidelijken.

*Is barcodering een methode die gebruikt kan worden om* Dicranum *soorten te identificeren? Hoe presteren barcode merkers in termen van soortsidentificatie?*

Soorten correct op naam te kunnen brengen is belangrijk in biodiversiteitsevaluaties, ecologie en natuurbescherming (Cornelissen *et al*. 2007; Dinnage *et al*. 2012; Steele & Pires 2011; Winter *et al*. 2013), maar morfologische identificatie van organismes met gereduceerde maaten zoals bryofyten kan zeer moeilijk zijn. Dit geldt in het bijzonder voor Arctische mossoorten, waarvan de kenmerken van de gametofyten extreem afwijkende morfologieën laten zien en waar de sporofyten bijna altijd afwezig zijn (Bellolio-Trucco & Ireland 1990; Hedenäs & Bisang 2004; Hesse *et al*. 2012). Het gebruik van DNA-barcodes is een alternatieve methode om soortsomgrenzing te onderzoeken. In **hoofdstuk 4** tonen wij aan dat de moleculaire omschrijving van Arctische *Dicranum* soorten moleculair omschreven kunnen worden door een combinatie van een aantal barcode merkers. Analyses van elke individuele merker tonen echter aan dat zij weinig interspecifieke variabiliteit bezitten. Bovendien faalt de nrITS regio in het onderscheiden van de bestudeerde *Dicranum* soorten. De combinatie van de meest-gebruikte merkers, *trnT- rps4*, *trnL-F, psbA-trnH* en nrITS, heeft duidelijk het grootste potentieel als barcode merker in *Dicranum*.

*Is geautomatiseerde soortsomgrenzing congruent met het morfologische concept van de Europese soorten?*

Gegeneraliseerde 'mixed Yule coalescent' methodes (GMYC) of 'Poisson tree' processen (PTP) soortbegrenzingmethodes die gebaseerd zijn op 'likelihood' (ML) fylogenetische inferenties (Pons *et al*. 2006; Monaghan *et al*. 2009). Terwijl GMYC een tijd-gekalibreerde stamboom nodig heeft, werkt PTP ook met standaard fylogenetische stambomen. Gebaseerd op een fylogenetische reconstructie die 28 van de 30 *Dicranum* soorten van Europa omvat, is het aantal entiteiten teruggevonden met GMYC en PTP methodes tussen de 34 en 58, respectievelijk 37 entiteiten (**hoofdstuk 5**). Als men het GMYC model met enkele drempelwaarde en de ML stamboom verkregen met PTP in beschouwing neemt, dan zijn beide methodes relatief congruent met respectievelijk 34 en 37 species. Deze schattingen zijn echter hoger dan het aantal morfologische soorten. De overschattingen betreffen *D. scoparium* maar ook *D. viride*, *D. fragilifolium*, *D. fuscescens* en *D. polysetum*. Simulaties tonen aan dat een ongebalanceerde dataset en een lage intraspecifieke genetische variabiliteit waarschijnlijk het aantal haplotypen van overbemonsterde soorten zullen laten toenemen (Bergsten et al., 2012; Zhang et al., 2013). In die gevallen zal elk exemplaar van een soort met een kleine steekproef kunnen worden beschouwd als een aparte entiteit (Zhang *et al*. 2013). In ons onderzoek verminderden analyses op een gereduceerde dataset niet het aantal potentiële soorten. Daarom heeft het effect van ongebalanceerde bemonstering waarschijnlijk minder invloed op soortsomgrenzingen dan de normaalgesproken lage genetische variabiliteit binnen het geslacht *Dicranum*.

Ondanks het overschatte aantal soorten verkregen met de geautomatiseerde schattingsmethoden, bakenen de verkregen clades soorten af die meestal congruent zijn met de actuele morfologische omschrijvingen. Moleculaire fylogenetische analyses brengen ook nuttige inzichten in verscheidene morfologische soorten zoals *D. leioneuron*, *D. crassifolium*, *D. scottianum*, *D. canariense*, *D. angustum, D. laevidens* of soorten van het *D. acutifolium* complex (**hoofdstuk 3**, **4**, **5**), waarvan de taxonomie tot nu toe onduidelijk was. Bovendien heeft de verdeling van het genus

in secties geen biologische betekenis, zoals aangetoond wordt in de fylogenetische stamboom in **hoofdstuk 5**.

*Toekomstige studies*

In dit proefschrift hebben wij aangetoond dat moleculaire methodes geschikt zijn om moleculaire omgrenzingen van een aantal *Dicranum* soorten op te helderen en om de verwantschappen binnen soortcomplexen te onderzoeken. Hoewel de moleculaire clades van de meeste van de onderzochte taxa significant werden ondersteund, waren drie Europese soorten niet te onderscheiden van andere soorten (*D. crassifolium* van *D. scoparium*, *D. scottianum* van *D. canariense, D. elongatum* van *D. fragilifolium*). Verder groepeerde *D. leioneuron* met Noord-Amerikaanse exemplaren van *D. scoparium* ondanks duidelijke morfologische verschillen tussen de twee soorten. Een gedetailleerde herinterpretatie van de morfologische verschillen en een heronderzoek van diagnostische kenmerken dient te worden uitgevoerd. Bovendien moet de soortomgrenzing van drie Europese soorten, *D. fulvum, D. muehlenbeckii* en *D. transylvanicum*, verder worden onderzocht waarvan geen materiaal ter beschikking stond voor moleculaire analysen. Morfo-moleculaire analyses van de drie ontbrekende soorten zou ons in staat stellen om hun genetische verwantschap beter te begrijpen.

De bemonstering van dit proefschrift representeert ruwweg een derde van de bekende *Dicranum* soorten. De ontbrekende taxa komen voornamelijk voor in Azië en Amerika. Een complete revisie van alle *Dicranum* soorten omvat, zou ons in staat stellen om onze kennis van dit soortenrijke genus te voltooien.

Bovendien, zou de analyse van meer variabele merkers en van microsatellieten kunnen helpen om de relaties tussen de verschillende taxa te begrijpen, om de invloed van mannelijke dwerggroei op de reproductie van de soorten te onderzoeken, en de vraag te beantwoorden in hoeverre hybridisatie in *Dicranum* voorkomt.

# References


Abramova A. L. A., Savicz-Ljubitskaja L. I. & Z. N. Smirnova. 1961. *Opredelitel Listostebelnich Mchov Arktiki SSSR*. Moskva: Izdatelstvo Akademii Nauk SSSR.

Afonina O. M. &I. V. Czernyadjeva. 1995. *Mosses of the Russian Arctic: check-list and bibliography*. Arctoa 5: 99–142.

Ahti T. & P. Isoviita. 1962. *Dicranum leioneuron Kindb. and the other* Dicranum *mosses inhabiting raised bogs in Finland*. Archivum Societatis Zoologicae Botanicae Fennicae "Vanamo" 17: 68–79.

Allen B. 1998. *The genus Orthodicranum (Musci: Dicranaceae) in Maine*. Evansia 15: 9–20.

Alsos I. G., Elvebakk A & G. W. Gabrielsen. 1998. *Vegetation exploitation by barnacle geese* Branta leucopsis *during incubation on Svalbard*. Polar Research 17: 1–14.

Altschul S. F., Gish W. , Miller W. , Myers E. W. & D. J. Lipman. 1990. *Basic Local Alignment Search Tool*. Journal of Molecular Biology 215: 403–410.

Amann J. J., Meylan C., & P. Culmann. 1918. *Flore des mousses de la Suisse, Deuxième partie, Bryogéographie de la Suisse*. Lausanne: Imprimeries réunies Pp. 49-62.

Bell D., Long D. G., Forrest A. D., Hollingsworth M. L., Blom H. H. & P. M. Hollingsworth. 2012. *DNA barcoding of European* Herbertus *(Marchantiopsida, Herbertaceae) and the discovery and description of a new species*. Molecular Ecology Resources 12: 36–47.

Bellolio-Trucco, G. & R. R. Ireland. 1990. *A taxonomic study of the moss genus* Dicranum *(Dicranaceeae) in Ontario and Quebec*. Canadian Journal of Botany 68: 867–909.

Bergsten J., Bilton D. T., Fujisawa T., Elliott M., & M. T. Monaghan. 2012. *The Effect of Geographical Scale of Sampling on DNA Barcoding*. Systematic Biology 61: 851–869.

Bickford, D., D. J. Lohman, N. S. Sodhi, P. K. L. Ng, R. Meier, K. Winker, K. K. Ingram & I. Das. 2007. *Cryptic species as a window on diversity and conservation*. Trends in Ecology & Evolution 22: 148–155.

Biomatters. 2010. Geneious v5.3.6. Available at: http://www.geneious.com.

Bisang I. & J. Ehrlén. 2002. *Reproductive Effort and Cost of Sexual Reproduction in Female* Dicranum polysetum. Bryologist 105: 384–397.

Borsch, T. & D. Quandt. 2009. *Mutational dynamics and phylogenetic utility of noncoding chloroplast DNA*. Plant Systematics and Evolution 282: 169–199.

Bouckaert R., J. Heled, D. Kühnert, T. Vaughan, C.-H. Wu, D. Xie, M. A. Suchard, Rambaut A. & A. J. Drummond. 2014. *BEAST 2: A software platform for bayesian evolutionary analysis*. PLoS Computational Biology 10: e1003537.

Briggs D. 1965. *Experimental taxonomy of some British species of the genus* Dicranum. New Phytologist 64: 366–366.

Brotherus V. F. 1906. Musci (Laubmoose) III. In Engler A. & K.Prantl [eds] *Die Natürlichen*

*Pflanzenfamilien Teil 3*. Leipzig: Englemann. Pp. 92-197.

Brotherus V. F. 1923. *Die Laubmoose Fennoskandias*. Helsingfors: Akademische Buchhandlung. P. 635.

Bruch P., Schimper W. P. & T. Gümbel. 1847. Dicranaceae. In Schimper W. P. [ed.] *Bryologia Europea seu genera muscorum europaeorum vol I*. Stuttgart: Sumptibus Librariae E. Schweizerbart. P. 44.

Buryová B. & A. J. Shaw. 2005. *Phenotypic plasticity in* Philonotis fontana *(Bryopsida: Bartramiaceae)*. Journal of Bryology 27: 13–22.

Callaghan T. V., Björn L. O., Chernov Y., Chapin T., Christensen T. R., Huntley B., Ims R. A., Johansson M., Jolly D., Jonasson S., Matveyeva N., Panikov N., Oechel W., Shaver G., Elster J., Henttonen H., Laine K., Taulavuori K., Taulavuori E. &C. Zöckler. 2004. *Biodiversity, distributions and adaptations of Arctic species in the context of environmental change*. Ambio 33: 404–417.

Carstens B. C., Pelletier T. A., Reid N. M. & J.D. Satler. 2013. *How to fail at species delimitation*. Molecular Ecology 22: 4369-4383.

Carter B. E. 2012. *Species delimitation and cryptic diversity in the moss genus* Scleropodium *(Brachytheciaceae)*. Molecular Phylogenetics and Evolution 63: 891–903.

CBOL Plant Working Group. 2009. *A DNA barcode for land plants*. Proceedings of the National Academy of Sciences of the United States of America 106: 12794–12797.

Chapman M. G. & A.J. Underwood. 1999. *Ecological patterns in multivariate assemblages: information and interpretation of negative values in ANOSIM tests*. Marine Ecology Progress Series180: 257-265.

Chopra R. S. 1975. *Taxonomy of Indian mosses: An introduction*. New Dehli: CSIR. P. 631.

Chopra, R. N. 1998. *Topics in Bryology*. New Delhi: Allied Publishers Limited. P. 202.

Clarke K. R. 1993. *Non-parametric multivariate analyses of changes in community structure*. Australian Journal of Ecology 18: 117-143.

Corley M. F. V. 1991. *The habitat range of* Dicranum leioneuron *Kindb*. Journal of Bryology 16: 485–486.

Cornelissen J. H. C., Lang S. I., Soudzilovskaia N. A. & H. J. During. 2007. *Comparative cryptogam ecology: a review of bryophyte and lichen traits that drive biogeochemistry*. Annales of Botany 99: 987–1001.

Cox C. J., Goffinet B., Wickett N. J.,Boles S. B. & A.J. Shaw. 2010. *Moss diversity : A molecular phylogenetic analysis of genera*. Phytotaxa 9: 175 – 195.

Crawford M., Jesson L. K. & P. J. Garnock-Jones. 2009. *Correlated evolution of sexual system and life-history traits in mosses*. Evolution 63:1129–1142.

Crosby M. R., Magill R. E., Allen B. & S. He. 1999. *A Checklist of Mosses. Prospectus*. St. Louis: Missouri Botanical Garden. P. 21.

Crum H. A. & L. E. Anderson. 1981. *Mosses of eastern North America volumes 1*. New York: Columbia University Press. Pp. 151-217.

Dandotiya D., Govindapyari H., Suman S. & P. L. Uniya. 2011. *Checklist of the bryophytes of India*. Archives for Bryololgy 88: 1-126.

Dierssen K. 2001. *Distribution, ecological amplitude and phytosociological classification of European bryophytes*. Bryophytorum Bibliotheca 56: 1–289.

Dinnage R., Cadotte M. W., Haddad N. M., Crutsinger G. M. & D. Tilman. 2012. *Diversity of plant evolutionary lineages promotes arthropod diversity*. Ecology Letters 15: 1308–1317.

Dong S., Schäfer-Verwimp A., Meinecke P., Feldberg K., Bombosch A., Pócs T., Schmidt A. R., Reitner J., & H. Schneider. 2012. *Tramps, narrow endemics and morphologically cryptic species in the epiphyllous liverwort Diplasiolejeunea*. Molecular Phylogenetics and Evolution 65: 582–594.

Dong W., Liu J., Yu J., Wang L. & S. Zhou. 2012. *Highly variable chloroplast markers for evaluating plant phylogeny at low taxonomic levels and for DNA barcoding*. PloS one 7: e35071,

Donskov D. G. 2011. *On the leaf fragility in* Dicranum *(Dicranaceae, Bryophyta)*. Arctoa 20: 99–105.

Draper I. & L. Hedenäs. 2009. *Circumscription of European taxa within the* Sciuro-hypnum reflexum *complex (Brachytheciaceae, Bryophyta), based on molecular and morphological data*. Taxon 58: 572–584.

Edwards S. V, Liu L. & D. K. Pearl. 2007. *High-resolution species trees without concatenation*. Proceedings of the National Academy of Sciences of the United States of America 104: 5936–5941.

Ehrlén J. Bisang I. & L. Hedenäs. 2000. *Costs of sporophyte production in the moss,* Dicranum polysetum. Plant Ecology 149:207–217.

Epstein H. E., Calef M. P., Walker M. D., Chapin F. S. III & A. M. Starfield. 2004. *Detecting changes in arctic tundra plant communities in response to warming over decadal time scales*. Global Change Biology 10: 1325–1334.

Ezard, T., Fujisawa T. & T. Barraclough. 2009. *splits: SPecies' LImits by Threshold Statistics*. R package version 1.0-19. Available at: http://r-forge.r-project.org/projects/splits.

Farris J. S., Källersjö M., Kluge A. G. & C. Bult. 1994. *Testing significance of congruence*. Cladistics 10:315–319.

Fontaneto D., Herniou E. A., Boschetti C., Caprioli M., Melone G., Ricci C. & T.G. Barraclough. 2007. *Independently evolving species in asexual bdelloid rotifers*. PLoS biology 5: e87.

Frey W. & M. Stech. 2009. Marchantiophyta, Bryophyta, Anthocerotophyta. *In* Frey W. [ed.] *Syllabus of Plant Families. A. Engler´s Syllabus der Pflanzenfamilien, 13th ed., Part 3 Bryophytes and seedless Vascular Plants*. Stuttgart: Gebr. Borntraeger Verlagsbuchhandlung. P. 419.

Frisvoll A. & Elvebakk A. 1996. Bryophytes. *In* Elvebakk A. & Pestrud P. [eds.] *A catalogue of Svalbard plants, fungi, algae and cyanobacteria*. Oslo: Norsk Polarinstitutt Skifter. Pp. 57–172.

Gao C. & S. He. 1999. Sphagnaceae–Leucobryaceae. 1. *In* Gao C. & Crosby M. R. [eds.] *Moss flora of China*. Beijing, New York and St. Louis: Science Press & Missouri Botanical Garden. P. 273.

Goffinet B. & A. J. Shaw. 2009. *Bryophyte Biology. 2nd Edition*. Cambridge: Cambridge University Press. P. 565.

Goffinet B., Cox C. J., Shaw A. J. & T. A. J. Hedderson. 2001. *The Bryophyta (mosses): systematic and evolutionary inferences from an rps4 gene (cpDNA) phylogeny*. Annales of Botany 87:191–208.

Gordon C., Wynn J. M., S. J. Woodin. 2001. *Impacts of increased nitrogen supply on high Arctic heath: the importance of bryophytes and phosphorus availability*. New Phytologist 149: 461–471.

Gornall J. L., Jónsdóttir I. S., Woodin S. J., van der Wal R. 2007. *Arctic mosses govern below-ground environment and ecosystem processes*. Oecologia 153: 931–41.

Grout A. J. 1937. Dicranum. *In* Grout A. [ed.] *Moss flora of North America north of Mexico*. Newfane, Vermont: published by the author. Pp. 77–89.

Hagen I. 1915. *Forarbejder til en norsk løvmosflora*. Trondheim: Kongel. Norske Vidensk. Selsk. Skr. P. 192.

Hassel K., Segreto R. & T. Ekrem. 2013. *Restricted variation in plant barcoding markers limits identification in closely related bryophyte species*. Molecular Ecology Resources 13:1047-1057.

Hebert P. D. N., Cywinska A., Ball S. L. & J.R. DeWaard. 2003. Biological *identifications through DNA barcodes*. Proceedings of the Royal Society B Biological Sciences 270: 313–321.

Hedderson T. A., Murray D. J., Cox C. J. & T. L.Nowell T. L. 2004. *Phylogenetic relationships of haplolepideous mosses (Dicranidae) Inferred from rps4 gene sequences*. Systematic Botany 29: 29–41.

Hedenäs L. & I. Bisang I. 2011. *The overlooked dwarf males in mosses—Unique among green land plants*. Perspectives in Plant Ecology, Evolution and Systematics 13: 121–135.

Hedenäs L. & I. Bisang. 2004. *Key to European* Dicranum *species*. Herzogia 17: 179–197.

Hedenäs L. & P. Eldenäs. 2007. *Cryptic speciation, habitat differentiation, and geography in* Hamatocaulis vernicosus *(Calliergonaceae, Bryophyta)*. Plant Systematics and Evolution 268: 131–145.

Hedenäs L. 2008. *Molecular variation in* Drepanocladus aduncus *s.l. does not support recognition of more than one species in Europe*. Journal of Bryology 30: 108–120.

Hedenäs L., Bisang I., Lüth M. & N. Schnyder. 2006. *Variation in* Dicranum majus *in central, western and northern Europe*. Journal of Bryology 28: 293–298.

Heinrichs J., Klugmann F., Hentschel J. & H. Schneider. 2009. *DNA taxonomy, cryptic speciation and diversification of the Neotropical-African liverwort,* Marchesinia brachiata *(Lejeuneaceae, Porellales)*. Molecular Phylogenetics and Evolution 53: 113–21.

Heinrichs, J., J. Hentschel, K. Feldberg, A. Bombosch & H. Schneider. 2009. *Phylogenetic biogeography and taxonomy of disjunctly distributed bryophytes*. Journal of Systematics and Evolution 47: 497–508.

Hernández-Maqueda R., Quandt D., Werner O & J. Muñoz. 2008. *Phylogeny and classification of the Grimmiaceae/Ptychomitriaceae complex (Bryophyta) inferred from cpDNA*. Molecular Phylogenetics and Evolution 46: 863–77.

Hesse C., Jalink L. M., Stech M. & J. D. Kruijer. 2012. *Contributions to the moss flora of Edgeøya and Barentsøya, Svalbard (Norway)*. Polish Botanical Journal 57: 167–179.

Holland B. R., Huber K. T., Moulton V. & P. J. Lockhart. 2004. *Using consensus networks to visualize contradictory evidence for species phylogeny*. Molecular Biology and Evolution 21: 1459–1461.

Hollingsworth M. L., Clark A., Forrest L. L., Richardson J., Pennington R. T., Long D. G., Cowan R., Chase M. W., Gaudeul M. & P. M. Hollingsworth. 2009. *Selecting barcoding loci for plants: Evaluation of seven candidate loci with species-level sampling in three divergent groups of land plants*. Molecular Ecology Resources 9: 439–457.

Hollingsworth P. M., Graham S. W., P. D. Little. 2011. *Choosing and using a plant DNA barcode*. PloS ONE 6: e19254.

Hudson J. M. G. & G. H. R. Henry. 2010. *High Arctic plant community resists 15 years of experimental warming*. Journal of Ecology 98: 1035–1041.

Huelsenbeck J. P. & F. Ronquist. 2001. *MRBAYES: Bayesian inference of phylogenetic trees*. Bioinformatics 17: 754–755.

Ignatova E. A. & V. F. Fedosov. 2008. *Species of* Dicranum *(Dicranaceae, Bryophyta) with fragile leaves in Russia*. Arctoa 17: 63–83.

Ireland R. R. 2002. Dicranum brevifolium *new to the moss flora of Michigan and the Eastern United States*. Michigan Botanist 41: 27–30.

Ireland R. R. 2007. Dicranum (Family Dicranaceae). *In* Zander R. H. [ed.] *Flora of North America*. New York and Oxford: Oxford University Press. Pp. 397–420.

Jasmin J.-N., Rochefort L. & G. Gauthier. 2008. *Goose grazing influences the fine-scale structure of a bryophyte community in arctic wetlands*. Polar Biology 31: 1043–1049.

Jennings O. E. 1951. *A manual of the mosses of western Pennsylvania and adjacent regions*. Indiana: University of Notre Dame Press. P. 412.

Klazenga, N. 2012. Australian Mosses Online. 33. Dicranaceae: Dicranum. http://www.anbg.gov.au/abrs/Mosses_online/Dicranaceae_Dicranum.pdf

Kluge A. G. 1989. *A concern for evidence and a phylogenetic hypothesis of relationships among* Epicrates *(Boidae, Serpentes)*. Systematic Zoology 38: 7–25.

Konstantinova N. A. & A. D. Potemkin. 1997. *Liverworts of the Russian Arctic: an annotated check-list and bibliography*. Arctoa 6: 125–150.

Krab J., Cornelissen J. H. C., Lang S. I. & R. S. P. Logtestijn. 2008. *Amino acid uptake among wide-ranging moss species may contribute to their strong position in higher-latitude ecosystems*. Plant and Soil 304: 199–208.

Kress W. J., Wurdack K. J., Zimmer E. A., Weigt L. A. & D. H. Janzen. 2005. *Use of DNA barcodes to identify flowering plants*. Proceedings of the National Academy of Sciences of the United States America 102: 8369–8374.

Kruskal J. B. 1964. *Multidimensional scaling by optimizing goodness of fit to a non-metric hypothesis*. Psychometrika 29: 1-27.

Kubatko L. S. & J. H. Degnan. 2007. *Inconsistency of phylogenetic estimates from concatenated data under coalescence*. Systematic Biology 56: 17–24.

La Farge C., Shaw A. J. & D. H. Vitt. 2002. *The circumscription of the Dicranaceae (Bryopsida) based on the chloroplast regions trnL — trnF and rps4*. Systematic Botany 27: 435–452.

Lang A .S. & M. Stech. 2014. *What's in a name? Disentangling the* Dicranum scoparium *species complex (Dicranaceae, Bryophyta)*. Systematic Botany 39: 369–379.

Lang A. S., Kruijer J. D. & M. Stech. 2014. *DNA barcoding of arctic bryophytes- an example from the moss genus* Dicranum *(Dicranaceae, Bryophyta)*. Polar Biology 37: 1157-1169.

Lang A. S., Tubanova D. & M. Stech. In press. *Species delimitations in the* Dicranum acutifolium *complex (Dicranaceae, Bryophyta)*. Journal of Bryology.

Lang S. I., Cornelissen J. H. C., Shaver G. R., Ahrens M., Callaghan T. V., Molau U., Ter Braak C. J. F., Hölzer A. & R. Aerts. 2012. *Arctic warming on two continents has consistent negative effects on lichen diversity and mixed effects on bryophyte diversity*. Global Change Biology 18: 1096–1107.

Lang, A. S. & Y. Naciri. 2010. *New chloroplast primers for intraspecific variation in* Dicranum scoparium *Hedw. (Dicranaceae) and amplification success in other bryophyte species*. Molecular Ecology Resources 10: 735–737.

Lawton E. 1971. *Moss Flora of Pacific Northwest*. Nichinan: Hattori Botanical Laboratory. Pp. 72-

81.
Leaché A. D. & B. Rannala. 2010. *The accuracy of species tree estimation under simulation: a comparison of methods*. Systematic Biology 60: 126–137.
Leliaert F., Verbruggen H., Wysor B. & O. De Clerck. 2009. *DNA taxonomy in morphologically plastic taxa: algorithmic species delimitation in the* Boodlea complex *(Chlorophyta: Cladophorales)*. Molecular Phylogenetics and Evolution 53: 122–133.
Lindberg O. S. 1865. *Adnotationes bryologicae*. Botaniska Notiser 4: 73- 81.
Lindberg, O. S. & Arnell, H. W. 1890. *Musci asiae borealis. II Laubmose*. Kongliga Svenska Vetenskaps Academiens Handlingar 23: 74-83.
Liu L. & D. K. Pearl. 2007. *Species trees from gene trees: reconstructing Bayesian posterior distributions of a species phylogeny using estimated gene tree distributions*. Systematic Biology 56: 504–514.
Liu Y., Cao T. & X.-J. Ge. 2011. *A case study of DNA barcoding in Chinese Grimmiaceae and a moss recorded in China for the first time*. Taxon 60: 185–193.
Liu Y., Yan H.- F., Cao T. & X.- J. Ge. 2010. *Evaluation of 10 plant barcodes in Bryophyta (Mosses)*. Journal of Systematics and Evolution 48: 36–46.
Longton R. E. 1988. *Adaptations and strategies of polar bryophytes*. Botanical Journal of the Linnean Society 98: 253–268.
Longton R. E. 1997. The role of bryophytes and lichens in polar ecosystems. *In* Woodin S. J. & M. Marquiss [eds.] *Ecology of Arctic Environments*. Oxford:Blackwell Science Ltd. Pp. 69–96.
Medina R., Lara F., Goffinet B., Garilleti R. & V. Mazimpaka. 2012. *Integrative taxonomy successfully resolves the pseudo-cryptic complex of the disjunct epiphytic moss* Orthotrichum consimile *s. l. (Orthotrichaceae )*. Taxon 61: 1180–1198.
Miller M. A., Pfeiffer W. & T. Schwartz. 2010. C*reating the CIPRES Science Gateway for inference of large phylogenetic trees*. Proceedings of the Gateway Computing Environments Workshop (GCE), 14 Nov. 2010, New Orleans, LA. Pp. 1 - 8. http://www.phylo.org
Monaghan M. T., Wild R., Elliot M., Fujisawa T., Balke M., Inward D. J. G., Lees D. C., RanaivosoloR., Eggleton P., Barraclough T. G. & A. P. Vogler. 2009. *Accelerated species inventory on Madagascar using coalescent-based models of species delineation*. Systematic biology 58: 298–311.
Mönkemeyer W. 1927. Die Laubmoose Europas: Andreaeales-Bryales. *In* Rabenhorsts D. L. [ed.] *Kryptogamen-Flora von Deutschland,* Österreich *und der Schweiz*. Leipzig: Akademische Verlagsgesellschaft m. b. H. Pp. 171–231.
Müller C. 1949. *Synopsis Muscorum Frondosorum omnium hucusque cognitorum Vol 1*. Berlin: Alb. Foerstner. Pp.351-415.
Müller K. 2004. *SeqState – primer design and sequence statistics for phylogenetic DNA data sets*. Applied Bioinformatics 4: 65–69.
Natcheva R. & N. Cronberg. 2004. *What do we know about hybridization among bryophytes in nature?* Canadian Journal of Botany 82: 1687–1704.
Noguchi A. & Z. Iwatsuki. 1987. *Illustrated moss flora of Japan Part 1*. Nichinan: Hattori Botanical Laboratory. Pp. 203-229.
Nyholm E. 1953. *Bryologiska notiser*. Botanika Notiser. 3:290–300.
Nyholm E. 1954. Musci. *In* The Botanical Society of Lund [ed.] *Illustrated moss flora of Fennoscandia*. Lund: CWK Gleerups. Pp. 61–71.

Nyholm E. 1987. Fissidentaceae-Seligeriaceae. *In* Nyholm E. [ed] *Illustrated Flora of Nordic Mosses Fasc. 1*. Copenhagen and Lund: Nordic Bryological Society. Pp. 47–58.

Okitsu S., Minami Y., Kanda H. 1998. *Relationship between plant occurences and surface conditions on a recently deglaciated moraine at Ny-Alesund, Svalbard, Arctic Norway*. Proceedings of the NIPR Symposium on Polar Biology 11: 119–127.

Oksanen J., Blanchet F. G., Kind, R., Legendre P., Minchin P. R., O'Hara R. B., Simpson G. L., Solymos P., Stevens M. H. H. & H. Wagner, H. 2013. *Vegan: Community Ecology Package*. R package version 2.0-6. http://CRAN.R-project.org/package=vegan.

Otnyukova T. N. 2001. *Notes on* Dicranum *(Dicranaceae, Musci) in Russia. 1*. Dicranum nipponense *found in Far East*. Arctoa: 157–160.

Otnyukova T. N. 2007. *Notes on Dicranum (Dicranaceae, Musci) in Russia. 2*. Dicranum pseudoacutifolium *sp. nov. from North Siberia*. Arctoa 16: 163–168.

Paradis E.,Claude J. & K. Strimmer. 2004. *APE 3.0-8: analyses of phylogenetics and evolution in R language*. Bioinformatics 20: 289–290.

Pereira M. R., de S. Dambros C. & C.E. Zartman. 2013. *Will the real Syrrhopodon leprieurii please stand up? The influence of topography and distance on phenotypic variation in a widespread Neotropical moss*. The Bryologist 116: 58–64.

Peterson W. 1979. *A revision of the genera* Dicranum *and* Orthodicranum *(Musci) in North America north of Mexico*. Ph. D. thesis. Edmonton, Alberta: University of Alberta.

Pichonet A. & S. R. Gradstein. 2012. *Male dwarfism in the genus Dicranum (Dicranaceae)-a review*. Cryptogamie, Bryologie 33: 299–311.

Podpěra J. 1954. Dicranum. *In* Podpěra J. [ed.] *Conspectus muscorum Europaeorum*. Praha: Ceskoslovenské akademie v d. Pp. 141-153.

Pons J., Barraclough T. G., Gomez-Zurita J., Cardoso A., Duran D. P., Hazell S., Kamoun S., Sumlin W. D. & A. P. Vogler. 2006. *Sequence-Based Species Delimitation for the DNA Taxonomy of Undescribed Insects*. Systematic Biology *55*: 595–609.

Poulakakis N., Russello M., Geist D., & A. Caccone. 2012. *Unravelling the peculiarities of island life: vicariance, dispersal and the diversification of the extinct and extant giant Galápagos tortoises*. Molecular Ecology. 21:160–173.

Posada D. & K. A. Crandall. 1998. *MODELTEST: Testing the model of DNA substitution*. Bioinformatics 14: 817–818.

Powell J. R. 2012. *Accounting for uncertainty in species delineation during the analysis of environmental DNA sequence data*. Methods in Ecology and Evolution 3: 1–11.

Qiu Y.- L., Li L., Wang B., Chen Z., Knoop V., Groth-Malonek M., Dombrovska O., Lee J., Kent L., Rest J., Estabrook G. F., Hendry T. A., Taylor D. W., Testa C. M., Ambros M., Crandall-Stotler B., Duff J., Stech M., Frey W., Quandt D. & C. C. Davis 2006. *The deepest divergences in land plants inferred from phylogenomic evidence*. Proceedings of the National Academy of Sciences of the United States of America 103: 15511–15516.

Quandt D. & M. Stech. 2004. *Molecular evolution of the trnTUGU-trnFGAA region in bryophytes*. Plant Biology 6: *545–554*.

R Development Core Team. 2013. *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna, Austria. Available at: http://www.r-project.org.

Rambaut A. & A. J. Drummond. 2007. *Tracer v1.5*. Available at http://beast.bio.ed.ac.uk/Tracer.

Raubeson L. A., Peery R., Chumley T. W., Dziubek C., Fourcade H. M., Boore J. L. & R. K. Jansen. 2007. *Comparative chloroplast genomics: analyses including new sequences from the angiosperms* Nuphar advena *and* Ranunculus macranthus. BMC Genomics 8: 174.

Rittmeyer, E. N. and C. C. Austin. 2012. *The effects of sampling on delimiting species from multi-locus sequence data*. Molecular Phylogenetics and Evolution 65: 451–463.

Ronquist F. & J. P. Huelsenbeck. 2003. *MRBAYES 3: Bayesian phylogenetic inference under mixed models*. Bioinformatics 19: 1572–1574.

Ronquist F., Huelsenbeck J. P. & P. van Dermark. 2005. *MrBayes3.1 manual*, Draft 5/17/2005. http://mrbayes.csit.fsu.edu/mb3.1_manual.pdf

Rowntree J. K., Cowan R. S., Leggett M., Ramsay M. M. & M. F. Fay. 2010. *Which moss is which? Identification of the threatened moss* Orthodontium gracile *using molecular and morphological techniques*. Conservation Genetics 11: 1033–1042.

Sagmo Solli I. M., Soderstrom L., Bakken S., Flatberg K. I. & B. Pedersen. 1998. *Reproductive phenology of* Dicranum majus *in central Norway*. Journal of Bryology 20: 311–321.

Sagmo Solli I. M., Soderstrom L., Bakken S., Flatberg K. I. & B. Pedersen. 2000. *Studies of fertility of* Dicranum majus *in two populations with contrasted sporophyte production*. Journal of Bryology 22: 3–8.

Sakurai, K. 1951. *A new classification of the genus Dicranum in Japan (1)*. Journal of Japanese Botany 28: 359–364.

Såstad S. M., Pedersen B. & K. Digre. 1999. *Habitat-specific genetic effects on growth rate and morphology across pH and water-level gradients within a population of the moss* Sphagnum angustifolium *(Sphagnaceae)*. American Journal of Botany 86: 1687–1698.

Såstad S. M. 1998. *Genetic and environmental sources of varation in leaf morphology of* Sphagnum fallax *and* Shagnum isoviitae *(bryopsida): comparison of experiments conducted in the field and laboratory*. Canadian Journal of Botany 77: 1–10.

Savicz-Lyubitskaya L. I. & Z. N. Smirnova. 1970. *The handbook of the mosses of the USSR. The Acrocarpous mosses*. Leningrad: Academy of Science of the USSR. P. 824.

Schofield W. B. 1992. Bryophyte distribution patterns. *In* Bates J. W. & A. M. Farmer [eds.] *Bryophytes and Lichens in a Changing World*. Oxford: Clarendon Press. Pp. 103–130.

Sérgio C., Ochyra R. & A. Séneca. 1995. Dicranum crassifolium *(musci, Dicranaceae), a new species from southern Europe*. Fragmenta Floristica et Geobotanica 40: 203–214.

Silvestro D. & I. Michalak. 2012. *raxmlGUI: a graphical front-end for RAxML*. Organisms Diversity and Evolution 12: 335–337.

Simmons M. P. & H. Ochoterena. 2000. *Gaps as characters in sequence-based phylogenetic analyses*. Systematic Biology 49: 369–381.

Smith A. J. E. 2004. *The moss flora of Britain and Ireland. Second Edition*. Cambridge University Press: Cambridge. P. 1026.

Sotiaux A., Enroth J., Olsson S., Quandt D. & A. Vanderpoorten. 2009. *When morphology and molecules tell us different stories: a case-in-point with* Leptodon corsicus, *a new and unique endemic moss species from Corsica*. Journal of Bryology 31: 186–196.

Spitale, D., and A. Petraglia. 2010. Palustriella falcata *(Brid.) Hedenäs (Amblystegiaceae, Bryopsida) with pluristratose lamina: morphological variability of specimens in springs of the Italian Alps*. Plant Systematics and Evolution 286: 59–68.

Stamatakis A. 2006. *RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with*

*thousands of taxa and mixed models*. Bioinformatics 22: 2688–2690.
Staples G. W., Imada C. T., Hoe W. J. & C. W. Smith. 2004. *A revised checklist of Hawaiian mosses*. Tropical Bryology 25:35–69.
Stech M. & D. Quandt. 2010. *20,000 species and five key markers: The status of molecular bryophyte phylogenetics*. Phytotaxa 9: 196–228.
Stech M. & W. Frey. 2008. *A morpho-molecular classification of the mosses (Bryophyta)*. Nova Hedwigia 86: 1–21.
Stech M. 1999. *A reclassification of Dicranaceae (Bryopsida) based on non-coding cpDNA sequence data*. Journal of the Hattori Botanical Laboratory 86: 137–159.
Stech M. 2004. *Supraspecific circumscription and classification of* Campylopus *Brid. (Dicranaceae, Bryopsida) based on molecular data*. Systematic Botany 29: 817–824.
Stech M. & W. Frey. 2008. *A morpho-molecular classification of the mosses (Bryophyta)*. Nov. Hedwigia 86:1–21.
Stech M., Kolvoort E., Loonen M. J. J. E., Vrieling K. & J. D. Kruijer. 2011. *Bryophyte DNA sequences from faeces of an arctic herbivore, barnacle goose (*Branta leucopsis). Molecular Ecology Resources 11: 404–408.
Stech M., Pfeiffer T. & W. Frey. 2006. *Molecular relationships and divergence of palaeoaustral* Dicranoloma *species (Dicranaceae, Bryopsida). Studies in austral temperate rain forest bryophytes 31*. Journal of the Hattori Botanical Laboratory 100: 451–464.
Stech M., Veldman S., Larraín J. , Muñoz J., Quandt D. , Hassel K. & H. Kruijer. 2013. *Molecular species delimitation in the* Racomitrium canescens *complex (Grimmiaceae) and implications for DNA barcoding of species complexes in mosses*. PLoS ONE 8: e53134.
Stech M., Werner O., González-Mancebo J. M., Patiño J., Sim-Sim M., Fontinha S., Hildebrandt I. & R. M. Ros. 2011. *Phylogenetic inference in* Leucodon *Schwägr. subg.* Leucodon *(Leucodontaceae, Bryophyta) in the North Atlantic region*. Taxon 60: 79–88.
Steele P. R., Pires J. C. 2011. *Biodiversity assessment: state-of-the-art techniques in phylogenomics and species identification*. American Journal of Botany 98: 415–425.
Steere W. C. 1978. *The Mosses of Arctic Alaska*. Bryophytorum Bibliotheca 14: 1–508.
Sugiura C., Kobayashi Y., Aoki S., Sugita C. & M. Sugita. 2003. *Complete chloroplast DNA sequence of the moss* Physcomitrella patens*: evidence for the loss and relocation of rpoA from the chloroplast to the nucleus*. Nucleic Acids Research 31: 5324–5331.
Sukkharak, P., S. R. Gradstein & M. Stech. 2011. *Phylogeny, taxon circumscriptions, and character evolution in the core Ptychanthoideae (Lejeuneaceae, Marchantiophyta)*. Taxon 60: 1607–1622.
Swofford D. L. 2002. *PAUP*. Phylogenetic analysis using parsimony (*and other methods). version 4*. Sunderland: Sinauer Associates Inc.
Takaki N. 1964. *A revision of Japanese* Dicranum. Journal of the Hattori Botanical Laboratory 27: 73–123.
Takaki N. 1972. *Geographical distribution of Japanese* Dicranum *species in the Northern Hemisphere*. Journal of the Hattori Botanical Laboratory 35: 31–40.
Talavera G., Dincă V. & R. Vila. 2013. *Factors affecting species delimitations with the GMYC model: insights from a butterfly survey*. Methods in Ecology and Evolution 4: 1101–1110.
Taylor J. W., Jacobson D. J., Kroken S., Kasuga T., Geiser D. M., Hibbett D. S. & M. C. Fisher. 2000. *Phylogenetic species recognition and species concepts in fungi*. Fungal Genetics and Biology

31: 21–32.
Terasawa K., Odahara M., Kabeya Y., Kikugawa T., Sekine Y., Fujiwara M. & N. Sato. 2007. *The mitochondrial genome of the moss Physcomitrella patens sheds new light on mitochondrial evolution in land plants*. Molecular Biology and Evolution 24: 699–709.
The Plant List (2013). Version 1.1. http://www.theplantlist.org
Tropicos.org, Missouri Botanical Garden, 2014, Available at http://www.tropicos.org
Tuba Z., Slack N. G., Stark L. R. 2011. *Bryophyte Ecology and Climate Change*. Cambridge University Press: Cambridge. P. 528.
Tubanova D. Y. & E. A. Ignatova. 2011. *A new species of* Dicranum *(Dicranaceae, Bryophyta) from Asiatic Russia*. Arctoa 20: 183–190.
Tubanova D. Y., Goryunov D. V, Ignatova E. A. & M. S. Ignatov. 2010. *On the taxonomy of Dicranum acutifolium and D. fuscescens complexes (Dicranaceae, Bryophyta) in Russia*. Arctoa 19: 151–164.
Tuomikoski R., Koponen T. & T. Ahti. 1973. *The mosses of the island of Newfoundland*. Annales Botanici Fennici 10: 217–264.
Turmel M., Otis C. & C. Lemieux. 2002. *The chloroplast and mitochondrial genome sequences of the charophyte* Chaetosphaeridium globosum*: insights into the timing of the events that restructured organelle DNAs within the green algal lineage that led to land plants*. Proceedings of the National Academy of Sciences of the United States of America 99: 11275–11280.
van der Wal R., R. W. Brooker. 2004) *Mosses mediate grazer impacts on grass abundance in arctic ecosystems*. Funct Ecol 18: 77–86.
van der Wal R., van Lieshout S. M. J. & M. J. J. E Loonen. 2001. *Herbivore impact on moss depth, soil temperature and arctic plant growth*. Polar Biology 24: 29–32
van der Wijk R., Margadant W. D. & P. A. Florschütz. 1962. *Index Muscorum*. International Bureau for Plant Taxonomy and Nomenclature of the International Association for Plant Taxonomy: Utrecht. P. 535.
Vanderpoorten A. & A. J. Shaw. 2010. *The application of molecular data to the phylogenetic delimitation of species in bryophytes : A note of caution*. Phytotaxa 9: 229–237.
Vanderpoorten A. & B. Goffinet. 2006. *Mapping uncertainty and phylogenetic uncertainty in ancestral character state reconstruction: an example in the moss genus* Brachytheciastrum. Systematic Biology 55: 957–971.
Vitt D. H. 1984. The classification of Bryopsida. *In* Schuster, R. M. [ed.] *New manual of bryology, vol 2*. Nichinan: The Hattori Botanical Laboratory. Pp. 696–759.
von Cräutlein M., Korpelainen H., Pietiläinen M., J. Rikkinen. 2011. *DNA barcoding: a tool for improved taxon identification and detection of species diversity*. Biodiversity and Conservation 20: 373–389.
Wahlenberg G. 1814. *Flora Carpatorum Principalium exhibens plantas in montibus Carpaticis inter flumina Waagum et Dunajetz eorumque ramos Arvam et Popradum crescentes, cui praemittitur Tractatus de altidudine, vegetatione, temperatura et meteoris horum montium in genere*. Gottingae: Impensis Vandenhöck et Ruprecht P. 408.
Wahren C.- H., Walker M. D., M. S. Bret-Harte. 2005. *Vegetation responses in Alaskan arctic tundra after 8 years of a summer warming and winter snow manipulation experiment*. Global Change Biology 11: 537–552.

Walker D. A., Raynolds M. K., Daniëls F. J. A., Einarsson E., Elvebakk A., Gould W. A., Katenin A. E., Kholod S. S., Markon C. J., Melnikov E. S., Moskalenko N. G., Talbot S. S., Yurtsev B. A. & the other members of the CAVM Team. 2005. *The Circumpolar Arctic vegetation map*. Journal of Vegatation Science 16: 267.

Wang R. J., Cheng C. L., Chang C. C., Wu C. L., Su T. M. & S. M. Chaw. 2008. *Dynamics and evolution of the inverted repeat-large single copy junctions in the chloroplast genomes of monocots*. BMC Evolutionary Biology 8: 36.

Whitlock B. A., Hale A. M. & P. A. Groff. 2010. *Intraspecific inversions pose a challenge for the trnH-psbA plant DNA barcode*. PLoS ONE 5: e11533.

Winter M., Devictor V. & O. Schweiger. 2013. *Phylogenetic diversity and nature conservation: where are we?* Trends in Ecolology and Evolution 28: 199–204.

Yao H., Song J., Liu C., Luo K., Han J., Li Y., Pang X., Xu H., Zhu Y., Xiao P. & S. Chen. 2010. *Use of ITS2 region as the universal DNA barcode for plants and animals*. PloS ONE 5: e13102.

Zaldívar-Riverón A., Martínez J. J., Ceccarelli F. S., De Jesús-Bonilla V. S., Rodríguez-Pérez A. C., Reséndiz-Flores A., & M. A. Smith. 2010. *DNA barcoding a highly diverse group of parasitoid wasps (Braconidae: Doryctinae) from a Mexican nature reserve*. Mitochondrial DNA 21:18–23.

Zhang J., Kapli P., Pavlidis P. & A. Stamatakis. 2013. *A general species delimitation method with applications to phylogenetic placements*. Bioinformatics 29: 2869–76.

# Appendices

APPENDIX 1. List of all sample used for molecular analyses and morphological studies analyses. DNA, herbarium location Herb., voucher information, collection date, geographic origin, original label and GenBank accession numbers *rps4-trn*T, *trn*L-*trn*F, *trn*H-*psb*A, *rps*19-*rpl*2, *rpo*B, nrITS of the specimens included in the study.

| Voucher | species | Herb. | collector and number | date of collection | Locality | | original label | *rps4-trn*T | *trn*L-*trn*F | *trn*H-*psb*A | *rps*19-*rpl*2 | *rpo*B | ITS |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | *D. acutifolium* | UUH | Krivobokov 262 | 12.07.2000 | RU | Buryatia | *D. bardunovii* | KJ796621 | KJ796598 | KJ796531 | - | KJ796572 | JN897272 |
| 4 | *D. fragilifolium* | MW | Churakova s.n. | 03.07.2001 | RU | Arkhangels Province | *D. fragilifolium* | - | KM502760 | KM502638 | - | KM502705 | FJ952597 |
| 5 | *D. bardunovii* | MW | Ivanova s.n. | 01.08.1991 | RU | Yakutia | *D. bardunovii* | KJ796622 | KJ796599 | KJ796532 | - | KJ796573 | JN897273 |
| 9 | *D. bardunovii* | MW | Volotovskiy s.n | 20.06.1985 | RU | SouthYakutia | *D. bardunovii* | - | KJ796600 | KJ796533 | - | - | JN897274 |
| 12 | *D. fragilifolium* | MW | Ignatov & Ignatova s.n. | 12.08.2001 | RU | Vologda Province | *D. fragilifolium* | KM502604 | KM502761 | KM502639 | - | KM502706 | FJ952596 |
| 13 | *D. flexicaule* | KRF | Molokova s.n. | 23.07.1995 | RU | Tuva | *Dicranum sp.* | KJ796606 | KJ796581 | KJ796513 | - | KJ796555 | HQ830328 |
| 15 | *D. flexicaule* | KRF | Otnyukova s.n. | 29.07.1991 | RU | Krasnoyars Territory | *Dicranum sp.* | - | KJ796582 | KJ796514 | - | KJ796556 | HQ830330 |
| 16 | *D. flexicaule* | IRK | Dudareva s.n. | 04.08.2006 | RU | Zabaikalsk Territory | *Dicranum sp.* | KJ796607 | KJ796583 | KJ796515 | - | KJ796557 | HQ830331 |
| 17 | *D. flexicaule* | MHA | Ignatov 06-2637 | s.d. | RU | Primorsky Territory | *Dicranum sp.* | - | KJ796584 | - | - | KJ796558 | HQ830332 |
| 18 | *D. fuscescens* | MHA | Bezgodov & Shkaraba s.n. | 31.07.2005 | RU | Perm Province | *D. fuscescens* | - | KJ796578 | KJ796510 | - | KJ796552 | HQ830334 |
| 19 | *D. fuscescens* | MHA | Ignatov 06-2588 | 03.09.2006 | RU | Primorsky Territory | *D. fuscescens* | KJ796605 | KJ796579 | KJ796511 | - | KJ796553 | HQ830337 |
| 20 | *D. brevifolium* | MW | Egorov s.n. | 27.07.1996 | RU | Karachaevo- Cherkessia | *D. brevifolium* | KJ796611 | KJ796587 | KJ796520 | - | KJ796563 | HQ830342 |
| 21 | *D. brevifolium* | KRF | Otnyukova s.n. | 12.06.2007 | RU | Tuva | *D. brevifolium* | KJ796612 | KJ796588 | KJ796521 | KJ796629 | KJ796564 | HQ830341 |
| 23 | *D. septentrionale* | LE | Neshataeva 986 | 04.08.2007 | RU | Kamchatka | *D. septentrionale* | KJ796608 | KJ796585 | KJ796516 | KJ796627 | KJ796559 | HQ830338 |
| 24 | *D. fuscescens* | MHA | Ignatov 06-14 | 20.08.2006 | RU | Sakhalin | *D. fuscescens* | - | KJ796580 | KJ796512 | - | KJ796554 | HQ830335 |
| 25 | *D. septentrionale* | MW | Churakova 864 | 19.07.2000 | RU | Arkhangels Province | *D. septentrionale* | - | KJ796586 | KJ796517 | - | KJ796560 | HQ830339 |
| 26 | *D. brevifolium* | MW | Korotkov s.n. | 06.09.2002 | RU | NorthOssetia | *D. brevifolium* | - | KJ796589 | KJ796522 | KJ796630 | KJ796565 | HQ830343 |
| 1_II | *D. bardunovii* | UUH | Krivobokov 262 | 12.07.2000 | RU | Buryatia | *D. bardunovii holotype* | KJ796620 | KJ796597 | KJ796530 | KJ796638 | - | KJ796547 |
| Aleu_1 | *D. howellii* | UBC | Talbot TAN1C-17 | 16.08.2002 | US | Alaska, Aleutian-Island | *D. scoparium* | - | KF423914 | KF423447 | - | KF423658 | - |
| Aleu_2 | *D. majus* | UBC | Talbot & Schofield ADA42-29 | 24.08.2006 | US | Alaska, Sea Parrot Island | *D. scoparium* | KF423824 | KF423915 | KF423448 | KF423752 | KF423659 | KF423553 |
| Alk_1 | *D. majus* | UBC | Talbot & Solomesch 05-41-18 | 07.07.2005 | US | Alaska, Northwest Arctic borough | *D. scoparium* | KF423825 | KF423916 | KF423449 | KF423753 | KF423660 | KF423554 |

| Voucher | species | Herb. | collector and number | date of collection | Locality | original label | *rps4-trn*T | *trn*L-*trn*F | *trn*H-*psb*A | *rps*19-*rpl*2 | *rpo*B | ITS |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Alk_2 | *D. flexicaule* | L | Morgado & Geml Bry-280712 001 | 28.07.2012 | US Alaska | *Dicranum sp.* | KJ651000 | KJ651051 | KJ650824 | - | KJ650907 | KJ650849 |
| Alk_3 | *D. flexicaule* | L | Morgado & Geml Bry-280712 002 | 28.07.2012 | US Alaska | *Dicranum sp.* | KJ651001 | KJ651052 | KJ650825 | - | KJ650908 | KJ650850 |
| Alk_4 | *D. flexicaule* | L | Morgado & Geml Bry-280712 003 | 28.07.2012 | US Alaska | *Dicranum sp.* | KJ651002 | KJ651053 | KJ650826 | - | KJ650909 | KJ650851 |
| Alk_5 | *D. flexicaule* | L | Morgado & Geml Bry-280712 004 | 28.07.2012 | US Alaska | *Dicranum sp.* | KJ651003 | KJ651054 | KJ650827 | - | KJ650910 | KJ650852 |
| Alk_6 | *D. flexicaule* | L | Morgado & Geml Bry-280712 005 | 28.07.2012 | US Alaska | *Dicranum sp.* | KJ651004 | KJ651055 | KJ650828 | - | KJ650911 | KJ650853 |
| Alk_7 | *D. flexicaule* | L | Morgado & Geml Bry-280712 006 | 28.07.2012 | US Alaska | *Dicranum sp.* | KJ651005 | KJ651056 | KJ650829 | - | KJ650912 | KJ650854 |
| Ast1011 | *D. scoparium* | FCO-Briof | Fdez. Ordóñez 269 | 24.10.2001 | ES Asturias | *D. scoparium* | KF423826 | KF423917 | KF423450 | KF423754 | KF423661 | KF423555 |
| Ast2011 | *D. scoparium* | FCO-Briof | Fdez. Ordóñez 1077 | 22.02.2002 | ES Asturias | *D. scoparium* | KF423827 | KF423918 | KF423451 | KF423755 | KF423662 | KF423556 |
| Ast3011 | *D. scoparium* | FCO-Briof | del Collado 3784-1 | 01.08.2002 | ES Asturias | *D. scoparium* | KF423828 | KF423919 | KF423452 | KF423756 | KF423663 | KF423557 |
| Ast3021 | *D. scoparium* | FCO-Briof | del Collado 2993-1 | 27.07.1999 | ES Asturias | *D. scoparium* | KF423829 | KF423920 | KF423453 | KF423757 | KF423664 | KF423558 |
| Ast3031 | *D. scoparium* | FCO-Briof | del Collado 3744-1 | 18.08.2005 | ES Asturias | *D. scoparium* | KF423830 | KF423921 | KF423454 | KF423758 | KF423665 | KF423559 |
| AT_2 | *D. muehlenbeckii* | L | van Melick 208859 | s.d. | AT Steiermark | *D. muehlenbeckii* | | | | | | |
| AT_3 | *D. drummondii* | L | van Melick 212270 | 06.07.1973 | AT Tirol | *D. muehlenbeckii* | | | | | | |
| Attu_1 | *D. majus* | UBC | Schofield &Talbot 120253 | 15.09.2002 | US Alaska, Attu Island | *D. scoparium* | - | KF423922 | KF423455 | KF423759 | KF423666 | KF423560 |
| BC_1 | *D. scoparium* | UBC | Schofield & Klinkenberg 119252 | 24.03.2002 | CA British Columbia, Lulu Island | *D. scoparium* | KF423898 | KF423997 | KF423536 | - | - | KF423644 |
| BC_2 | *D. scoparium* | UBC | Schofield & Williams 117252A | 18.05.2001 | CA British Columbia, Lac les Jeune Road | *D. scoparium* | KF423899 | KF423998 | KF423537 | - | - | KF423645 |
| BCo1 | *D. scoparium* | NY | Buck 54100 | 19.09.2008 | CA Ontario, Bruce Co. | *D. scoparium* | KF423831 | KF423923 | KF423456 | KF423760 | KF423667 | KF423561 |
| BUL_1 | *D. scoparium* | L | Papp 10/101/1 | 06.08.2010 | BG Sofia Prov. | *D. scoparium* | KF423832 | KF423924 | KF423457 | KF423761 | KF423668 | KF423562 |

| Voucher | species | Herb. | collector and number | date of collection | Locality | | original label | *rps4-trn*T | *trn*L-*trn*F | *trn*H-*psb*A | *rps*19-*rpl*2 | *rpo*B | ITS |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Cauc_1 | *D. scoparium* | S | Ignatov & Ignatova B113001 | 12.09.2005 | RU | Karachaevo- Cherkessian Rep. | *D. scoparium* | KF423833 | KF423925 | KF423458 | KF423762 | KF423669 | KF423563 |
| CH1 | *D. brevifolium* | S | Hedenäs B98890 | 14.08.2004 | CH | Canton Wallis | *D. brevifolium* | KJ651039 | KJ651095 | KJ650837 | KJ650990 | KJ650953 | KJ650895 |
| Ch_1011 | *D. scoparium* | G | Lang 20080907.1 | 07.09.2008 | CH | Canton Geneva | *D. scoparium* | GQ428082 | GQ428036 | GQ427991 | GQ427953 | GQ427914 | KF423564 |
| Ch_1061 | *D. scoparium* | G | Lang 20080907.6 | 07.09.2008 | CH | Canton Geneva | *D. scoparium* | GU068393 | GU068477 | GU068448 | GU068364 | GU068422 | KF423565 |
| CH_4 | | L | Greven s.n. | 28.07.1990 | CH | Canton Wallis | *D. muehlenbeckii* | XXXXXXXX | XXXXXXXX | XXXXXXXX | XXXXXXXX | XXXXXXXX | XXXXXXXX |
| Cor_1 | *D. scoparium* | S | Sotiaux & Sotiaux 462 | 25.05.2004 | FR | Haute- Corse | *D. scoparium* | KF423834 | KF423926 | KF423459 | KF423763 | KF423670 | KF423566 |
| Dac | *D. acutifolium* | L | Stech B970831.1 | 31.08.1997 | FI | Kuusamo Prov. | *D. acutifolium* | KJ651030 | DQ462590 | KJ650818 | KJ650981 | KJ650941 | KJ650881 |
| DB_1 | *D. scoparium* | MO | King &Garvey B657 | 05.10.2001 | CA | Nova Scotia, Richmond Co. | *D. bonjeanii* | KF423835 | KF423927 | KF423460 | KF423764 | KF423671 | - |
| DB_2 | *D. cf. scoparium* | MO | Davis 270 | 12.08.2007 | US | Pennsylvania, Cambria Co. | *D. bonjeanii* | GU068416 | GU068500 | GU068471 | GU068387 | GU068443 | KF423567 |
| DB_3 | *D. scoparium* | MO | Weber, Wittmann, Andrus &Cooper B-111031 | 12.08.1996 | US | Colorado, San Juan Co. | *D. bonjeanii* | - | KM502773 | KM502649 | - | - | KM502686 |
| DB_4 | *D. scoparium* | MO | Allen 28704 | 09.09.2003 | CA | Newfoundland, Avalon Peninsula | *D. bonjeanii* | GU068418 | GU068502 | GU068473 | GU068389 | GU068445 | KF423568 |
| Db_CA | *D. scoparium* | L | Allen 9479 | 13.08.1990 | CA | Ontario, Thunder-bay district | *D. bonjeanii* | - | KF423999 | KF423538 | - | - | KF423646 |
| Dbe | *D. undulatum* | L | Stech B970824.2 | 24.08.1997 | FI | North Karelia Prov. | *D. bergeri* | KJ796626 | KJ796604 | KJ796537 | - | KJ796577 | KJ796551 |
| Dbr | *D. septentrionale* | L | Stech B960801.2 | 01.08.1996 | AT | Tirol | *D. brevifolium* | KJ796610 | DQ462591 | KJ796519 | KJ796628 | KJ796562 | KJ796539 |
| Dcan | *D. canariense* | L | Stech 04-405 | 2004 | SP | Canaries Islands | *D. canariense* | KM502596 | KM502749 | KM502627 | KM502722 | KM502702 | KM502664 |
| Dcan2 | *D. canariense* | L | Stech 04-547 | 2004 | SP | Canaries Islands | *D. canariense* | KM502597 | KM502750 | KM502628 | KM502723 | KM502703 | KM502665 |
| Dcan3 | *D. canariense* | L | 07-113 | 2004 | SP | Canaries Islands | *D. canariense* | KM502598 | KM502751 | KM502629 | KM502724 | KM502704 | KM502666 |
| Ddr | *D. drummondii* | L | Stech B970827.4 | 27.08.1997 | FI | Kuusamo Prov. | *D. drummondii* | KJ796609 | DQ462589 | KJ796518 | - | KJ796561 | KJ796538 |
| Ddrumm | *D. drummondii* | S | Hedenäs, Ohlsson, Odelvik &Myrdal B122041 | s.d. | SE | Härjedalen | | | | | | | |
| DE_1 | *D. muehlenbeckii* | L | van Melick 202606 | s.d. | DE | Baden- Württemberg | *D. muehlenbeckii* | | | | | | |

| Voucher | species | Herb. | collector and number | date of collection | Locality | | original label | *rps4-trn*T | *trn*L-*trn*F | *trn*H-*psb*A | *rps*19-*rpl*2 | *rpo*B | ITS |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| DE_2 | *D. dispersum* | S | Sauer MS95022 | 12.05.1995 | DE | Baden-Württemberg | *D. dispersum* | KJ651041 | KJ651097 | KJ650839 | KJ650992 | KJ650955 | KJ650897 |
| Del | *D. elongatum* | L | Stech B970831.3 | 31.08.1997 | FI | Kuusamo Prov. | *D. elongatum* | KJ651031 | DQ462592 | KJ650819 | KJ650982 | KJ650942 | KJ650882 |
| Dfl | *D. flexicaule* | L | Stech B970827.5 | 27.08.1997 | FI | Kuusamo Prov. | *D. flexicaule* | KJ651032 | - | KJ650820 | - | KJ650943 | KJ650883 |
| Dfla1 | *D. flagellare* | L | Wondergem 1300 | 07.10.2010 | NL | Utrecht | *D. flagellare* | KM502601 | KM502757 | KM502635 | KM502727 | - | KM502672 |
| Dfla2 | *D. flagellare* | L | Bijlsma 12053 | 16.05.2009 | NL | Limburg | *D. flagellare* | KM502602 | KM502758 | KM502636 | KM502728 | - | KM502673 |
| Dfla3 | *D. flagellare* | L | Bijlsma 13104 | 12.02.2011 | NL | Gelderland | *D. flagellare* | KM502603 | KM502759 | KM502637 | KM502729 | - | KM502674 |
| Dfus6 | *D. fuscescens* | L | Wondergem 1134 | 21.05.2009 | NL | Gelderland | *D. fuscescens* | KJ651042 | KJ651098 | KJ650840 | KJ650993 | - | KJ650898 |
| Dfus7 | *D. fuscescens* | L | Stech B970824.3 | 24.08.1997 | FI | Karelia Prov. | *D. fuscescens* | KF423896 | - | KF423534 | KF423819 | KF423742 | KF423642 |
| Dfus8 | | L | Stech B970824.3 | 24.08.1997 | FI | Karelia Prov. | *D. fuscescens* | KM502619 | KM502781 | KM502656 | KM502742 | KM502714 | KM502694 |
| DH_3 | *D. howellii* | MO | Allen 24114 | 24.03.2002 | US | California, Mendocino Co. | *D. howellii* | KF423840 | KF423928 | KF423465 | - | - | KF423569 |
| DH_5 | *D. howellii* | MO | Shevock 19290 | 29.04.2000 | US | California, San Fransisco Co. | *D. howelii* | KF423841 | KF423929 | KF423466 | KF423769 | KF423676 | KF423570 |
| Dib | *D. majus* | L | Stech B970829.4 | 29.08.1997 | FI | Kuusamo Prov. | *D. bonjeanii* | KF423836 | AF135068/ AF136076 | KF423461 | KF423765 | KF423672 | AF144114 |
| Dic_ 1644 | *D. acutifolium* | L | Stech & Kruijer 10-102a | 04.08.2010 | NO | Svalbard | *D. acutifolium* | KJ651006 | KJ651057 | KJ650789 | KJ650961 | KJ650913 | KJ650855 |
| Dic_ 1645 | *D. spadiceum* | L | Stech & Kruijer 08-203 | 14.07.2008 | NO | Svalbard | *D. angustum* | KJ651007 | KJ651058 | KJ650790 | KJ650962 | KJ650914 | KJ650856 |
| Dic_ 1646 | *D. elongatum* | L | Stech & Kruijer 08-250 | 15.07.2008 | NO | Svalbard | *D. elongatum* | KJ651008 | KJ651059 | KJ650791 | KJ650963 | KJ650915 | KJ650857 |
| Dic_ 1648 | *D. elongatum* | L | Stech & Kruijer 09-072 | 28.06.2009 | NO | Svalbard | *D. elongatum* | KJ651022 | KJ651060 | KJ650809 | - | KJ650932 | - |
| Dic_ 1649 | *D. acutifolium* | L | Stech & Kruijer 10-118 | 04.08.2010 | NO | Svalbard | *D. elongatum* | KJ651009 | KJ651061 | KJ650792 | KJ650964 | KJ650916 | KJ650858 |
| Dic_ 1650 | *D. elongatum* | L | Stech & Kruijer 10-202 | 07.08.2010 | NO | Svalbard | *D. elongatum* | - | KJ651062 | KJ650793 | - | KJ650917 | KJ650859 |
| Dic_ 1651 | *D. laevidens* | L | Stech & Kruijer 10-216 | 07.08.2010 | NO | Svalbard | *D. groenlandicum* | KJ651010 | KJ651063 | KJ650794 | KJ650965 | KJ650918 | KJ650860 |
| Dic_ 1652 | *D. laevidens* | L | Stech & Kruijer 10-002 | 01.08.2010 | NO | Svalbard | *D. laevidens* | KJ651011 | KJ651064 | KJ650795 | - | KJ650919 | KJ650861 |

| Voucher | species | Herb. | collector and number | date of collection | Locality | original label | *rps4-trn*T | *trn*L-*trn*F | *trn*H-*psb*A | *rps*19-*rpl*2 | *rpo*B | ITS |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Dic_1653 | *D. laevidens* | L | Stech & Kruijer 10-006a | 01.08.2010 | NO Svalbard | *D. laevidens* | - | KJ651065 | KJ650796 | - | KJ650920 | KJ650862 |
| Dic_1654 | *D. spadiceum* | L | Stech & Kruijer 10-297 | 2010 | NO Svalbard | *D. laevidens* | KJ651012 | KJ651066 | KJ650797 | KJ650966 | KJ650921 | KJ650863 |
| Dic_1655 | *D. laevidens* | L | Stech & Kruijer 09-71 | 2009 | NO Svalbard | *D. laevidens* | - | KJ651067 | KJ650808 | - | - | KJ650874 |
| Dic_1659 | *D. spadiceum* | L | Stech & Kruijer 08-025 | 10.10.2012 | NO Svalbard | *D. laevidens* | - | KJ651068 | KJ650798 | KJ650967 | KJ650922 | KJ650864 |
| Dic_1660 | *D. acutifolium* | L | Stech & Kruijer 08-031 | 11.07.2008 | NO Svalbard | *D. laevidens* | KJ651013 | KJ651069 | KJ650799 | KJ650968 | KJ650923 | KJ650865 |
| Dic_1661 | *D. acutifolium* | L | Stech & Kruijer 08-033a | 11.07.2008 | NO Svalbard | *D. laevidens* | KJ651014 | KJ651070 | KJ650800 | KJ650969 | KJ650924 | KJ650866 |
| Dic_1662 | *D. laevidens* | L | Stech & Kruijer 09-021 | 28.06.2009 | NO Svalbard | *D. laevidens* | KJ651015 | KJ651071 | KJ650801 | - | KJ650925 | KJ650867 |
| Dic_1663 | *D. laevidens* | L | Stech & Kruijer 09-022 | 28.06.2009 | NO Svalbard | *D. laevidens* | KJ651016 | KJ651072 | KJ650802 | - | KJ650926 | KJ650868 |
| Dic_1664 | *D. majus* | L | Stech & Kruijer 10-029 | 01.08.2010 | NO Svalbard | *D. laevidens* | KF423823 | KF423913 | KF423446 | - | KF423657 | KF423552 |
| Dic_1665 | *D. spadiceum* | L | Stech & Kruijer 10-046 | 01.08.2010 | NO Svalbard | *D. laevidens* | KJ651017 | KJ651073 | KJ650803 | KJ650970 | KJ650927 | KJ650869 |
| Dic_1666 | *D. spadiceum* | L | Stech & Kruijer 11-229 | 08.07.2011 | NO Svalbard | *Dicranum sp.* | KJ651018 | KJ651074 | KJ650804 | KJ650971 | KJ650928 | KJ650870 |
| Dic_1667 | *D. spadiceum* | L | Stech & Kruijer 11-237 | 08.07.2012 | NO Svalbard | *D. majus* | KJ651019 | KJ651075 | KJ650805 | KJ650972 | KJ650929 | KJ650871 |
| Dic_1668 | *D. spadiceum* | L | Stech & Kruijer 11-052 | 29.06.2011 | NO Svalbard | *Dicranum sp.* | KJ651023 | KJ651076 | KJ650810 | KJ650975 | KJ650933 | - |
| Dic_1669 | *D. spadiceum* | L | Stech & Kruijer 11-171 | 02.07.2011 | NO Svalbard | *D. spadiceum* | KJ651020 | KJ651077 | KJ650806 | KJ650973 | KJ650930 | KJ650872 |
| Dic_1670 | *D. spadiceum* | L | Stech & Kruijer 11-168 | 02.07.2011 | NO Svalbard | *D. spadiceum* | - | KJ651078 | KJ650811 | - | KJ650934 | - |
| Dic_1671b | *D. spadiceum* | L | Stech & Kruijer 11-167b | 02.07.2011 | NO Svalbard | *D. spadiceum* | KJ651021 | KJ651079 | KJ650807 | KJ650974 | KJ650931 | KJ650873 |
| Dic_1671c | *D. spadiceum* | L | Stech & Kruijer 11-167c | 02.07.2011 | NO Svalbard | *D. laevidens* | KJ651024 | KJ651080 | KJ650812 | - | KJ650935 | KJ650875 |

| Voucher | species | Herb. | collector and number | date of collection | Locality | | original label | *rps4-trn*T | *trn*L-*trn*F | *trn*H-*psb*A | *rps*19-*rpl*2 | *rpo*B | ITS |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Dic_ 1672 | *D. laevidens* | L | Stech & Kruijer 11-0431 | 2011 | NO | Svalbard | *Dicranum sp.* | KJ651025 | KJ651081 | KJ650813 | KJ650976 | KJ650936 | KJ650876 |
| Dic_ 1673 | *D. spadiceum* | L | Stech & Kruijer 11-153 | 01.07.2011 | NO | Svalbard | *D. cf acutifolium* | KJ651026 | KJ651082 | KJ650814 | KJ650977 | KJ650937 | KJ650877 |
| Dic_ 1675 | *D. elongatum* | L | Kruijer & Stech 11-213 | 07.07.2011 | NO | Svalbard | *D. elongatum* | KJ651027 | KJ651083 | KJ650815 | KJ650978 | KJ650938 | KJ650878 |
| Dic_ 1676 | *D. acutifolium* | L | Stech & Kruijer 11-161 | 01.07.2011 | NO | Svalbard | *D. acutifolium* | KJ651028 | KJ651084 | KJ650816 | KJ650979 | KJ650939 | KJ650879 |
| Dic_ 1677 | *D. spadiceum* | L | Stech & Kruijer 11-165 | 02.07.2011 | NO | Svalbard | *D. laevidens* | KJ651029 | KJ651085 | KJ650817 | KJ650980 | KJ650940 | KJ650880 |
| Dif | *D. fragilifolium* | L | Stech B970827.1 | 27.08.1997 | FI | Kuusamo Prov. | *D. fragilifolium* | KF423837 | AF135069/ AF136077 | KF423462 | KF423766 | KF423673 | AF140700 |
| Dif2 | *D. fragilifolium* | L | Stech B970828.8 | 28.08.1997 | FI | Kuusamo Prov. | *D. fragilifolium* | - | KM502762 | - | - | - | KM502675 |
| Dip | *D. polysetum* | L | Stech B9705181.1 | 18.05.1997 | DE | Mecklenburg- Vorpommern | *D. polysetum* | KF423838 | AF129587 | EU163523 | KF423767 | KF423674 | AF144113 |
| Dis | *D. scoparium* | L | Stech B960719.1 | 19.07.1997 | DE | Schleswig- Holstein | *D. scoparium* | KF423839 | AF129588/ AF129561 | KF423464 | KF423768 | KF423675 | AF140699 |
| Djap_16 | *D. scoparium* | MO | He 36007 | 10.08.2006 | TW | Eastern Taiwan | *D. japonicum* | KF423900 | KF424000 | KF423539 | KF423820 | KF423744 | - |
| Djap_17 | *D. scoparium* | S | Mizutani 15102 | 28.10.1995 | JP | Honshu | *D. japonicum* | KF423901 | KF424002 | KF423541 | - | - | KF423648 |
| Djap_5 | *D. scoparium* | MO | He & Song 34527 | 13.07.2004 | KR | Jeju- do | *D. japonicum* | KF423902 | KF424003 | KF423542 | - | KF423745 | - |
| Dma | *D. scoparium* | L | Stech s.n. | 1997 | FI | | *D. majus* | - | KJ651086 | KJ650821 | - | KJ650945 | KJ650884 |
| Dmont_1 | *D. montanum* | L | Wondergem 1302 | 27.10.2010 | NL | North Holland | *D. montanum* | KM502606 | KM502767 | KM502644 | KM502732 | - | KM502680 |
| Dmont_2 | *D. montanum* | L | Zwarts 2033 | 03.12.2008 | NL | Gelderland | *D. montanum* | KM502607 | KM502768 | KM502645 | KM502733 | - | KM502681 |
| Dmont_3 | *D. montanum* | L | Smulders 10139 | 05.11.2010 | NL | North Brabant | *D. montanum* | KM502608 | KM502769 | KM502646 | KM502734 | - | KM502682 |
| Dpol_1 | *D. polysetum* | L | Wondergem 1355 | 25.01.2011 | NL | Utrecht | *D. polysetum* | KM502609 | KM502770 | KM502647 | KM502735 | - | KM502683 |
| Dpol_2 | *D. polysetum* | L | Zwarts 2121 | 28.02.2009 | NL | Gelderland | *D. polysetum* | KM502610 | KM502771 | KM502648 | KM502736 | - | KM502684 |
| Dpol_3 | *D. polysetum* | L | Aptroot 69434 | 31.08.2010 | NL | Overijssel | *D. polysetum* | KM502611 | KM502772 | - | KM502737 | - | KM502685 |
| Ddrumm | *D. drummondii* | S | Hedenäs, Ohlsson, Odelvik & Myrdal B122041 | | SE | Härjedalen | *D. drummondii* | | | | | | |
| DS_ 04217 | *D. scoparium* | L | Stech 04-217 | 28.07.2004 | PT | Madeira | *D. scoparium* | KF423844 | KF423932 | KF423469 | KF423771 | KF423678 | KF423576 |

| Voucher | species | Herb. | collector and number | date of collection | Locality | | original label | *rps4-trn*T | *trn*L-*trn*F | *trn*H-*psb*A | *rps*19-*rpl*2 | *rpo*B | ITS |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| DS_09317 | *D. scoparium* | L | Stech 09-317 | 27.12.2009 | DE | Northrhine-Westphalia | *D. scoparium* | KF423845 | KF423933 | KF423470 | KF423772 | KF423679 | KF423577 |
| DS_09318 | *D. scoparium* | L | Stech 09-318 | 27.12.2009 | DE | Northrhine-Westphalia | *D. scoparium* | KF423846 | KF423934 | KF423471 | KF423773 | KF423680 | KF423578 |
| DS_1 | *D. cf. scoparium* | MO | Risk, Richardson & Newland 14,463 | 20.04.2007 | US | Kentucky, Greenup Co. | *D. scoparium* | GU068413 | GU068497 | GU068468 | GU068384 | GU068440 | KF423571 |
| DS_10009 | *D. scoparium* | L | Stech 10-009 | 02.04.2010 | DE | Rhineland- Palatinate | *D. scoparium* | KF423847 | KF423935 | KF423472 | KF423774 | KF423681 | KF423579 |
| DS_2 | *D. cf. scoparium* | MO | Allen 28074 | 10.06.2007 | US | Maine, Knox Co. | *D.scoparium* | GU068414 | GU068498 | GU068469 | GU068385 | GU068441 | KF423572 |
| DS_3 | *D. cf. scoparium* | MO | Holmberg 1578 | 24.03.2006 | US | Missouri, Jefferson Co. | *D.scoparium* | GU068415 | GU068499 | GU068470 | GU068386 | GU068442 | KF423573 |
| DS_4 | *D. majus* | MO | Talbot AT102-30 | 15.08.1999 | US | Alaska, Attu Island | *D. scoparium* | GU068421 | GU068504 | GU068476 | GU068392 | GU068447 | KF423574 |
| DS_5 | *D. howellii* | MO | Allen 28834 | 26.03.2008 | US | Oregon, Multnomah Co. | *D.scoparium* | KF423843 | KF423931 | KF423468 | KF423770 | KF423677 | KF423575 |
| Dspa | | L | Stech B970828.9 | 28.08.1997 | FI | Kuusamo Prov. | *D. spadiceum* | - | KJ651087 | KJ650822 | - | - | KJ650886 |
| Dspu | *D. spurium* | L | Stech | | | | *D. spurium* | KM502615 | KM502777 | - | KM502738 | KM502712 | KM502690 |
| Dspu_3 | *D. spurium* | L | Bijlsma 13057 | 22.01.2011 | NL | Gelderland | *D. spurium* | KM502616 | KM502778 | KM502653 | KM502739 | - | KM502691 |
| Dspu_4 | *D. spurium* | L | Aptroot 69776 | 11.07.2011 | NL | Drenthe | *D. spurium* | KM502617 | KM502779 | KM502654 | KM502740 | - | KM502692 |
| Dta | *D. tauricum* | L | Stech B911228.3 | 28.12.1991 | DE | Nordrhein-Westfalen | *D. tauricum* | KJ651034 | KJ651088 | KJ650823 | KJ650994 | KJ650944 | KJ650887 |
| Dta_2 | *D. tauricum* | L | Smulders 10140 | 05.11.2010 | NL | NorthBrabant | *D. tauricum* | KJ651043 | KJ651099 | KJ650841 | KJ650995 | - | KJ650899 |
| Dta_3 | *D. tauricum* | L | Pellicaan s.n. | 22.04.2010 | NL | Utrecht | *D. tauricum* | KJ651044 | KJ651100 | KJ650842 | KJ650996 | - | KJ650900 |
| Dta_4 | *D. tauricum* | L | Buter 73747 | 13.07.2010 | NL | NorthBrabant | *D. tauricum* | KJ651045 | KJ651101 | KJ650843 | KJ650997 | - | KJ650901 |
| FIN_1 | *D. septentrionale* | S | Hedenäs & Bisang B194528 | 17.09.2010 | FI | Åland | *D. brevifolium* | KJ796619 | KJ796596 | KJ796529 | KJ796637 | KJ796571 | KJ796546 |
| FIN_2 | *D. undulatum* | S | Hedenäs & Bisang B198289 | 25.05.2013 | FI | Åland | *D. undulatum* | KJ796623 | KJ796601 | KJ796534 | KJ796639 | KJ796574 | KJ796548 |
| FR_1 | *D. scoparium* | L | Martinez s.n. | 10.09.2008 | FR | Alpes-Maritimes | *D. scoparium* | KF423848 | KF423936 | KF423473 | KF423775 | KF423682 | KF423580 |
| FR_2 | *D. bonjeanii* | L | Hovenkamp 10-43 | 01.08.2010 | FR | Savoie | *Dicranum sp.* | KF423849 | KF423937 | KF423474 | - | KF423683 | KF423581 |
| FR_3 | *D. bonjeanii* | L | Hovenkamp 10-47 | 01.08.2010 | FR | Savoie | *Dicranum sp.* | KF423850 | KF423938 | KF423475 | KF423776 | KF423684 | KF423582 |
| FR_4 | *D. bonjeanii* | L | Hovenkamp 10-43 | 01.08.2010 | FR | Savoie | *Dicranum sp.* | KF423851 | KF423939 | KF423476 | - | KF423685 | KF423583 |

| Voucher | species | Herb. | collector and number | date of collection | Locality | | original label | *rps4-trn*T | *trn*L-*trn*F | *trn*H-*psb*A | *rps*19-*rpl*2 | *rpo*B | ITS |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Gi_1011 | *D. scoparium* | G | Lang & Price 20080701.1 | 01.07.2008 | CH | CantonVaud | *D. scoparium* | GQ428088 | GQ428041 | GQ427997 | GQ427960 | GQ427919 | KF423584 |
| GL1 | *D. scoparium* | CANM | Ireland, Dugal & Ley 23775 | 22.06.1989 | CA | Ontario, Gloucester | *D. scoparium* | - | KF423940 | KF423477 | KF423777 | KF423686 | KF423585 |
| GR_1 | *D. scoparium* | L | Papp 10/77/6 | 15.07.2010 | GR | CentralMacedonia | *D. scoparium* | KF423852 | KF423941 | KF423478 | KF423778 | KF423687 | KF423586 |
| HUN_1 | *D. scoparium* | UBC | Schofield 104660 | 31.08.1995 | HU | NorthernHU | *D. scoparium* | KF423853 | KF423942 | KF423479 | KF423779 | KF423688 | KF423587 |
| Is_1 | *D. scoparium* | ICEL | Elmarsdóttir 42630 | 25.07.2001 | IS | Norðurlandeystra | *D. scoparium* | KF423854 | KF423943 | KF423480 | KF423780 | KF423689 | KF423588 |
| Is_10 | *D. scoparium* | ICEL | Egilsson 44119 | 20.08.1997 | IS | Norðurlandvestra | *D. scoparium* | KF423862 | KF423951 | KF423488 | KF423788 | KF423697 | KF423596 |
| Is_2 | *D. scoparium* | ICEL | Egilsson 44218 | 17.07.2003 | IS | Norðurlandeystra | *D. scoparium* | KF423855 | KF423944 | KF423481 | KF423781 | KF423690 | KF423589 |
| Is_3 | *D. scoparium* | ICEL | Elmarsdóttir 44446 | 24.08.2004 | IS | Norðurlandeystra | *D. scoparium* | KF423856 | KF423945 | KF423482 | KF423782 | KF423691 | KF423590 |
| Is_4 | *D. scoparium* | ICEL | Þórisson 43747 | 02.08.2002 | IS | Norðurland eystra | *D. scoparium* | KF423857 | KF423946 | KF423483 | KF423783 | KF423692 | KF423591 |
| Is_5 | *D. spadiceum* | ICEL | Egilsson 44012 | 12.08.2002 | IS | Norðurland vestra | *D. scoparium* | KF423858 | KF423947 | KF423484 | KF423784 | KF423693 | KF423592 |
| Is_7 | *D. spadiceum* | ICEL | Egilsson 43998 | 11.08.2002 | IS | Norðurland eystra | *D. scoparium* | KF423859 | KF423948 | KF423485 | KF423785 | KF423694 | KF423593 |
| Is_8 | *D. spadiceum* | ICEL | Egilsson 41447 | 12.08.2002 | IS | Norðurland eystra | *D. scoparium* | KF423860 | KF423949 | KF423486 | KF423786 | KF423695 | KF423594 |
| Is_9 | *D. spadiceum* | ICEL | Egilsson 43804 | 24.07.2002 | IS | Norðurland vestra | *D. scoparium* | KF423861 | KF423950 | KF423487 | KF423787 | KF423696 | KF423595 |
| Ka2031 | *D. scoparium* | L | Lang 20091203.3 | 03.12.2009 | NL | Norðurland vestra | *D. scoparium* | KF423863 | KF423952 | KF423489 | KF423789 | KF423698 | KF423597 |
| KR9 | *D. cf. japonicum* | JNU | Yoon s.n. | 28.10.2011 | KR | Jeju-doHal lasan | *D. scoparium* | KF423866 | KF423956 | KF423493 | KF423791 | KF423702 | KF423600 |
| KR11 | *D. cf. japonicum* | JNU | Yoon s.n. | 19.05.2011 | KR | Jeollanam- do | *D. scoparium* | KF423867 | KF423957 | KF423494 | - | KF423703 | KF423601 |
| KR12 | *D. cf. japonicum* | JNU | Yoon s.n. | 18.05.2011 | KR | Jeollanam- do | *D. scoparium* | KF423868 | KF423958 | KF423495 | - | KF423704 | KF423602 |
| KR5 | *D. cf. japonicum* | JNU | Yoon s.n. | 08.03.2011 | KR | Jeollanam- do | *D. scoparium* | KF423864 | KF423954 | KF423491 | - | KF423700 | KF423598 |
| KR7 | *D. cf. japonicum* | JNU | Yoon s.n. | 16.08.2010 | KR | Jeju- do | *D. scoparium* | KF423865 | KF423955 | KF423492 | KF423790 | KF423701 | KF423599 |
| L&A1 | *D. scoparium* | CANM | Ley & al. 1222 | 11.07.1990 | CA | Ontario, Lennox & Addinton Co. | *D. scoparium* | - | KF423959 | KF423496 | KF423792 | KF423705 | KF423603 |
| MAC_1 | *D. scoparium* | L | Papp 10/87/2 | 18.07.2010 | MK | Pelagonia region | *D. scoparium* | KF423869 | KF423960 | KF423497 | KF423793 | KF423706 | KF423604 |
| Mad_1 | *D. scoparium* | S | Hedenäs B4566 | 14.06.1998 | PT | Madeira | *D. scoparium* | - | KF423961 | KF423498 | KF423794 | KF423707 | KF423605 |
| Mad_2 | *D. scoparium* | S | Hedenäs & Bisang B22461 | 20.11.1999 | PT | Madeira | *D. scoparium* | - | KF423962 | KF423499 | KF423795 | - | KF423606 |
| MS_ 04576 | *D. scoparium* | L | Stech 04-576 | 08.08.2004 | PT | Madeira | *D. scoparium* | KF423870 | KF423963 | KF423500 | KF423796 | KF423708 | KF423607 |

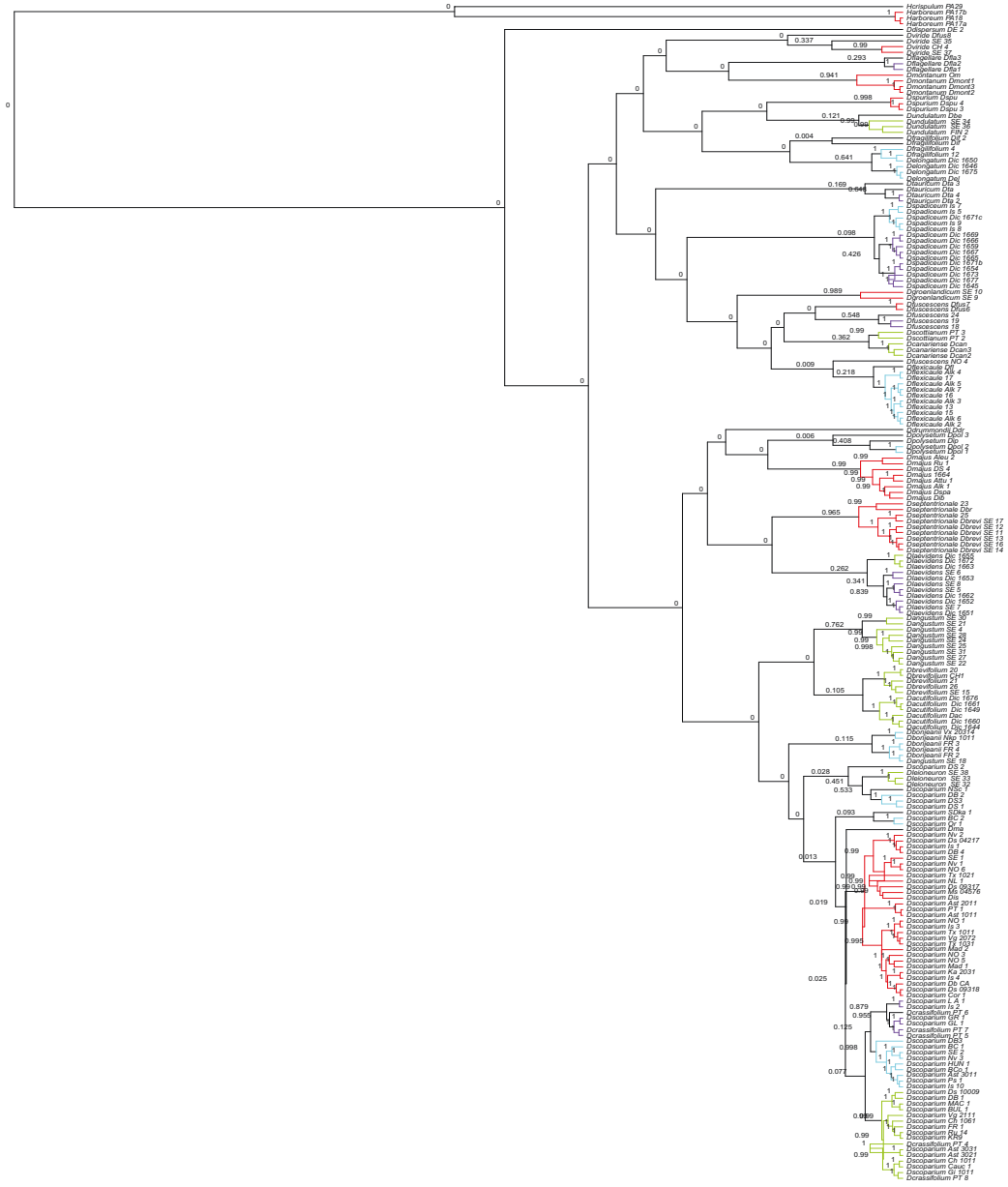
| Voucher | species | Herb. | collector and number | date of collection | Locality | | original label | *rps4-trn*T | *trn*L-*trn*F | *trn*H-*psb*A | *rps*19-*rpl*2 | *rpo*B | ITS |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| NB_1 | *D. scoparium* | UBC | McAlpine s.n. | 21.08.1990 | CA | New Brunswick, Charlotte Co. | *D.scoparium* | - | KF423964 | KF423501 | KF423797 | KF423709 | - |
| Nkp_ 1011 | *D. bonjeanii* | L | Lang 20091126.2 | 26.11.2009 | NL | South Holland | *D. scoparium* | KF423871 | KF423965 | KF423502 | KF423798 | KF423710 | KF423608 |
| NL_1 | *D. scoparium* | L | van den Vaart s.n. | 2012 | NL | South Holland | *D. scoparium* | KF423872 | KF423966 | KF423503 | KF423799 | KF423711 | KF423609 |
| NO_1 | *D. scoparium* | TRH | Prestø B-7239 | 18.04.2003 | NO | Herøy | *D. scoparium* | KF423873 | KF423967 | KF423504 | KF423800 | KF423712 | KF423610 |
| NO_3 | *D. scoparium* | TRH | Hassel B-6584 | 13.04.2005 | NO | Gloppen | *D. scoparium* | - | KF423968 | KF423505 | - | KF423713 | KF423611 |
| NO_4 | *D. fuscescens* | TRH | Hassel s.n. | 11.08.1998 | NO | Troms | *D. scoparium* | - | KF423969 | KF423506 | - | KF423714 | KF423612 |
| NO_5 | *D. scoparium* | TRH | Prestø B-7017 | 31.05.2004 | NO | Gjemnes | *D. scoparium* | - | KF423970 | KF423507 | - | KF423715 | KF423613 |
| NO_6 | *D. scoparium* | TRH | Prestø B-7605 | 30.05.2004 | NO | Frei | *D. scoparium* | KF423874 | KF423971 | KF423508 | KF423801 | KF423716 | KF423614 |
| NSc_1 | *D. cf. scoparium* | UBC | Schofield & Schofield 95348 | 09.05.1990 | CA | Novascotia, Digby Co. | *D. scoparium* | - | KF424001 | KF423540 | - | - | KF423647 |
| NV1 | *D. scoparium* | O | Hanssen 3964/05 | 01.08.1996 | NO | Buskerud | *D. scoparium* | KF423875 | KF423972 | KF423509 | KF423802 | KF423717 | KF423615 |
| NV2 | *D. scoparium* | O | Engan GE-20 | 18.04.2003 | NO | Østfold | *D. scottianum* | KF423876 | KF423973 | KF423510 | KF423803 | KF423718 | KF423616 |
| NV3 | *D. scoparium* | O | Hanssen 5399 | 01.05.1999 | NO | Buskerud | *D. scoparium* | KF423877 | KF423974 | KF423511 | KF423804 | KF423719 | KF423617 |
| Om | *D. montanum* | L | Stech B890721.5 | 21.07.1989 | DE | Nordrhein- Westfalen | *D. montanum* | KF423878 | AF129589/ AF129562 | KF423512 | KF423805 | KF423720 | AF144115 |
| Or_1 | *D. scoparium* | UBC | Schofield, Harpel &Forest Service Personnel 116776 | 10.10.2000 | US | Oregon, Umatilla National Forest | *D. scoparium* | KM502612 | KM502774 | KM502650 | - | KM502710 | KM502687 |
| PA17a | *H. arboreum* | L | Stech PA17a | 11 | BR | Parana, Municipio CampinaGrande do Sul | *Holomitrium* | KF423894 | KF423994 | KF423532 | KF423818 | KF423740 | KF423640 |
| PA17b | *H. arboreum* | L | Stech PA17b | 11 | BR | Parana, Municipio CampinaGrande do Sul | *Holomitrium* | KF423893 | KF423993 | KF423531 | - | KF423739 | KF423639 |
| PA18 | *H. arboreum* | L | Stech PA18 | 11 | BR | Parana, Municipio CampinaGrande do Sul | *Holomitrium* | KF423895 | KF423995 | KF423533 | - | KF423741 | KF423641 |
| PA29 | *H. crispulum* | L | Stech PA29 | 11 | BR | Parana, Municipio CampinaGrande do Sul | *Holomitrium* | KF423892 | KF423992 | KF423530 | - | KF423738 | KF423638 |
| PS1 | *D. scoparium* | CANM | Ireland 23915 | 06.09.1989 | CA | Ontario, Parry Sound | *D. scoparium* | - | KF423975 | KF423513 | KF423806 | KF423721 | KF423618 |

| Voucher | species | Herb. | collector and number | date of collection | Locality | | original label | *rps4-trn*T | *trn*L-*trn*F | *trn*H-*psb*A | *rps*19-*rpl*2 | *rpo*B | ITS |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| PT_1 | *D. scoparium* | S | Hedenäs B44512 | 22.09.2000 | PT | Centro | *D. scoparium* | - | KF423976 | KF423514 | KF423807 | KF423722 | KF423619 |
| PT_2 | *D. scoparium* | Herb. H.Waltje | Waltje AZ-0102 | 09.08.2012 | PT | Azores | *Dicranum sp.* | KJ651046 | KJ651102 | KJ650844 | - | KJ650956 | KJ650902 |
| PT_3 | *D. scoparium* | Herb. H.Waltje | Frahm AZ-0184 | 10.08.2012 | PT | Azores | *Dicranum sp.* | KJ651047 | KJ651103 | KJ650845 | - | KJ650957 | KJ650903 |
| PT_4 | *D. scoparium* | LISU | Sérgio FRID 13g | 25.11.2010 | PT | Trás-os-Montes e Alto Douro | *D. crassifolium* | KM502599 | KM502752 | KM502630 | - | - | KM502667 |
| PT_5 | *D. scoparium* | LISU | Sérgio 13796 | 19.05.2006 | PT | Beira Litora | *D. crassifolium* | - | KM502753 | KM502631 | - | - | KM502668 |
| PT_6 | *D. scoparium* | LISU | Sérgio 14679 | 30.12.2010 | PT | Beira Alta | *D. crassifolium* | KM502600 | KM502754 | KM502632 | KM502725 | - | KM502669 |
| PT_7 | *D. scoparium* | LISU | Garcia 205276 | 26.06.2003 | PT | Douro Litoral | *D. crassifolium* | - | KM502755 | KM502633 | KM502726 | - | KM502670 |
| PT_8 | *D. scoparium* | LISU | Sérgio, Carvalho, Garcia & Louro 212140 | 04.05.2004 | PT | Trás-os-Montes e Alto Douro | *D. crassifolium* | - | KM502756 | KM502634 | - | - | KM502671 |
| Ru_1 | *D. majus* | L | Lang 20100906.2 | 06.09.2010 | RU | Primorsky Prov. | *D. majus* | KF423879 | KF423977 | KF423515 | KF423808 | KF423723 | KF423620 |
| Ru_14 | *D. scoparium* | L | van Melick 214110 | 18.09.2010 | RU | IrkutskProv. | *D. scoparium* | KM502613 | KM502775 | KM502651 | - | KM502711 | KM502688 |
| Ru_16 | *D. cf. lorifolium* | L | Lang 20100910.8 | 11.09.2014 | RU | Primorsky Prov. | *D. scoparium* | KF423909 | KF424010 | KF423549 | - | KF423750 | KF423655 |
| Ru_24 | *D. cf. lorifolium* | L | Lang 20100910.10 | 11.09.2014 | RU | Primorsky Prov. | *D. scoparium* | KF423910 | KF424011 | KF423550 | - | KF423751 | KF423656 |
| Ru_27 | *D. cf. lorifolium* | L | Lang 20100909.9 | 10.09.2014 | RU | Primorsky Prov. | *D. scoparium* | KF423911 | KF424012 | KF423551 | KF423821 | - | - |
| Ru_3 | *D. scoparium* | L | Lang 20100908.17 | 08.09.2010 | RU | Primorsky Prov. | *D. scoparium* | - | KF423979 | KF423517 | - | KF423725 | KF423622 |
| Ru_33 | *D. cf. lorifolium* | L | Lang 20100905.10 | 06.09.2014 | RU | Primorsky Prov. | *D. scoparium* | KF423907 | KF424008 | KF423547 | - | - | KF423653 |
| Ru_35 | *D. cf. lorifolium* | L | Lang & Cherdantseva 20100908.14 | 09.09.2014 | RU | Primorsky Prov. | *D. scoparium* | KF423906 | KF424007 | KF423546 | - | KF423748 | KF423652 |
| Ru_4 | *D. scoparium* | L | Lang 20100908.22 | 08.09.2010 | RU | Primorsky Prov. | *D. scoparium* | KF423881 | KF423980 | KF423518 | KF423809 | KF423726 | KF423623 |
| Ru_44 | *D. cf. lorifolium* | L | Lang 20100906.17 | 07.09.2014 | RU | Primorsky Prov. | *D. scoparium* | KF423904 | KF424005 | KF423544 | - | KF423746 | KF423650 |
| Ru_46 | *D. cf. lorifolium* | L | Lang 20100906.6 | 07.09.2014 | RU | Primorsky Prov. | *D. scoparium* | KF423903 | KF424004 | KF423543 | - | - | KF423649 |
| Ru_49 | *D. cf. lorifolium* | L | Lang 20100906.11 | 07.09.2014 | RU | Primorsky Prov. | *D. scoparium* | KF423908 | KF424009 | KF423548 | - | KF423749 | KF423654 |
| Ru_5_A | *D. nipponense* | L | Lang 20100909.1 | 09.09.2010 | RU | Primorsky Prov. | *D. bonjeanii* | KF423882 | KF423981 | KF423519 | KF423810 | KF423727 | KF423624 |
| Ru_5_B | *D. nipponenese* | L | Lang 20100909.1 | 09.09.2010 | RU | Primorsky Prov. | *D. scoparium* | KF423883 | KF423982 | KF423520 | KF423811 | KF423728 | KF423625 |
| Ru_50 | *D. cf. lorifolium* | L | Lang 20100906.8 | 07.09.2014 | RU | Primorsky Prov. | *D. scoparium* | KF423905 | KF424006 | KF423545 | - | KF423747 | KF423651 |
| Ru_6 | *D. scoparium* | L | Lang 20100910.6 | 10.09.2010 | RU | Primorsky Prov. | *D. scoparium* | - | KF423983 | KF423521 | - | KF423729 | KF423626 |

| Voucher | species | Herb. | collector and number | date of collection | Locality | | original label | *rps4-trn*T | *trn*L-*trn*F | *trn*H-*psb*A | *rps*19-*rpl*2 | *rpo*B | ITS |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Ru_7 | *D. scoparium* | L | Lang 20100911.3 | 11.09.2010 | RU | Primorsky Prov. | *D. scoparium* | KF423884 | KF423984 | KF423522 | - | KF423730 | KF423627 |
| Ru_8 | *D. scoparium* | L | Lang 20100908.6 | 08.09.2010 | RU | Primorsky Prov. | *D. scoparium* | KF423885 | KF423985 | KF423523 | - | KF423731 | KF423628 |
| SDak_1 | *D. scoparium* | UBC | Churchill & Churchill 19597 | 18..8.1998 | US | South Dakota, Pennington Co. | *D. scoparium* | KM502614 | KM502776 | KM502652 | - | - | KM502689 |
| SE_1 | *D. scoparium* | S | Odelvik B163166 | 12.04.2009 | SE | Gästrikland | *D. scoparium* | KF423886 | KF423986 | KF423524 | KF423812 | KF423732 | KF423629 |
| SE_10 | *D. groenlandicum* | S | Hedenäs B74363 | 17.08.2002 | SE | Torne Lappmark | *D. groenlandicum* | - | KJ651089 | KJ650830 | KJ650984 | KJ650946 | KJ650888 |
| SE_11 | *D. septentrionale* | S | Hedenäs B74004 | 07.09.2002 | SE | Uppland | *D. brevifolium* | KJ796613 | KJ796590 | KJ796523 | KJ796631 | KJ796566 | KJ796540 |
| SE_12 | *D. septentrionale* | S | Hedenäs B122921 | 10.07.2007 | SE | Härjedalen | *D. brevifolium* | KJ796614 | KJ796591 | KJ796524 | KJ796632 | KJ796567 | KJ796541 |
| SE_13 | *D. septentrionale* | S | Hedenäs B183369 | 26.05.2011 | SE | Gotland | *D. brevifolium* | KJ796615 | KJ796592 | KJ796525 | KJ796633 | - | KJ796542 |
| SE_14 | *D. septentrionale* | S | Hedenäs B193369 | 26.07.2012 | SE | Södermanland | *D. brevifolium* | KJ796616 | KJ796593 | KJ796526 | KJ796634 | KJ796568 | KJ796543 |
| SE_15 | *D. brevifolium* | S | Hedenäs B175744 | 21.05.2010 | SE | Hälsingland | *D. brevifolium* | KJ651040 | KJ651096 | KJ650838 | KJ650991 | KJ650954 | KJ650896 |
| SE_16 | *D. septentrionale* | S | Hallingbäck 46166 | 07.08.2008 | SE | TorneLappmark | *D. brevifolium* | KJ796617 | KJ796594 | KJ796527 | KJ796635 | KJ796569 | KJ796544 |
| SE_17 | *D. septentrionale* | S | Hedenäs & Bisang B84948 | 11.08.2003 | SE | Gotland | *D. brevifolium* | KJ796618 | KJ796595 | KJ796528 | KJ796636 | KJ796570 | KJ796545 |
| SE_18 | | S | Norin B132878 | 29.06.2002 | SE | Torne Lappmark | *D. angustum* | KM502595 | KM502748 | KM502626 | KM502721 | KM502701 | KM502663 |
| SE_2 | *D. scoparium* | S | Hedenäs B164630 | 03.08.2009 | SE | Jämtland | *D. scoparium* | KF423887 | KF423987 | KF423525 | KF423813 | KF423733 | KF423630 |
| SE_21 | *D. angustum* | S | Hedenäs, Rönblom, Odelvik & Hamnede B139061 | 26.06.2012 | SE | Dalarna | *D. angustum* | KM502592 | KM502744 | KM502622 | KM502717 | KM502697 | KM502659 |
| SE_22 | *D. angustum* | S | Hedenäs B193541 | 29.08.2012 | SE | Jämtland | *D. angustum* | KJ651036 | KJ651092 | KJ650834 | KJ650987 | KJ650950 | KJ650892 |
| SE_24 | *D. angustum* | S | Westerberg B132876 | 25.08.2006 | SE | Norrbotten | *D. angustum* | KM502593 | KM502745 | KM502623 | KM502718 | KM502698 | KM502660 |
| SE_25 | *D. angustum* | S | Hedenäs, Bisang & Persson B105001 | 09.09.2005 | SE | Jämtland | *D. angustum* | - | KJ651090 | KJ650832 | KJ650986 | KJ650948 | KJ650890 |
| SE_27 | *D. angustum* | S | Norin B132922 | 11.08.2002 | SE | TorneLappmark | *D. angustum* | KJ651037 | KJ651093 | KJ650835 | KJ650988 | KJ650951 | KJ650893 |
| SE_28 | *D. angustum* | S | Johansson B132925 | 08.08.2006 | SE | TorneLappmark | *D. angustum* | KM502594 | KM502746 | KM502624 | KM502719 | KM502699 | KM502661 |
| SE_30 | *D. angustum* | S | Hedenäs B107583 | 14.09.2009 | SE | Jämtland | *D. angustum* | - | KM502747 | KM502625 | KM502720 | KM502700 | KM502662 |
| SE_31 | *D. angustum* | S | Johansson B132926 | 08.07.2003 | SE | TorneLappmark | *D. angustum* | KJ651038 | KJ651094 | KJ650836 | KJ650989 | KJ650952 | KJ650894 |
| SE_32 | *D. leioneuron* | S | Hedenäs B116708 | 06.10.2002 | SE | Medelpad | *D. leioneuron* | KJ651048 | KJ651104 | KJ650846 | - | KJ650958 | KJ650904 |
| SE_33 | *D. leioneuron* | S | Laegaard, Gustafsson, Poulsen, Brunbjerg 23200L | 23.05.2010 | SE | Hälsingland | *D. leioneuron* | KJ651049 | KJ651105 | KJ650847 | KJ650998 | KJ650959 | KJ650905 |

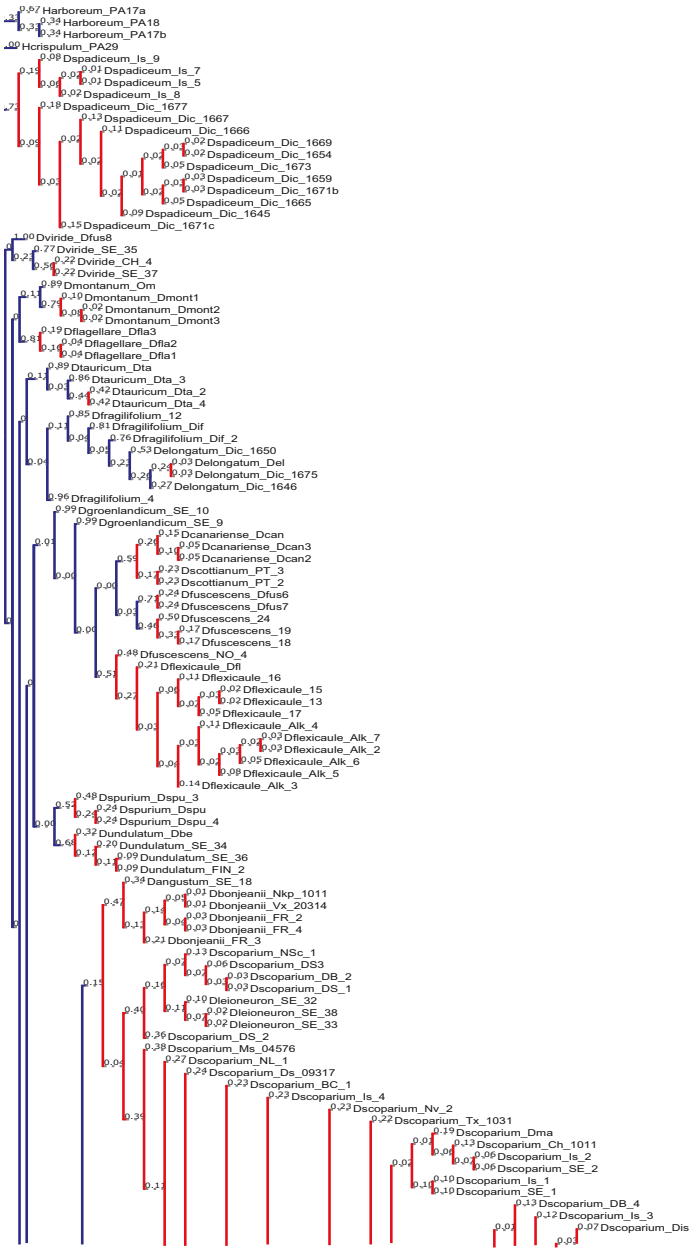
| Voucher | species | Herb. | collector and number | date of collection | Locality | | original label | *rps4-trnT* | *trnL-trnF* | *trnH-psbA* | *rps19-rpl2* | *rpoB* | ITS |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| SE_34 | *D. undulatum* | S | Hedenäs, Odelvik & Rönblom B199960 | 12.09.2013 | SE | Ångermanland | *D. undulatum* | KJ796624 | KJ796602 | KJ796535 | KJ796640 | KJ796575 | KJ796549 |
| SE_35 | *D. viride* | S | Hagström B172407 | 27.05.2009 | SE | Småland | *D. viride* | KM502620 | KM502782 | KM502657 | KM502743 | KM502715 | KM502695 |
| SE_36 | *D. undulatum* | S | Hedenäs, Odelvik & Rönblom B199806 | 09.09.2013 | SE | Ångermanland | *D. undulatum* | KJ796625 | KJ796603 | KJ796536 | KJ796641 | KJ796576 | KJ796550 |
| SE_37 | *D. viride* | S | Lönnell B48734 | 15.06.1999 | SE | Gotland | *D. viride* | KM502621 | KM502783 | KM502658 | - | KM502716 | KM502696 |
| SE_38 | *D. leioneuron* | S | Hedenäs & Persson B135011 | 07.05.2008 | SE | Dalsland | *D. leioneuron KJ651050* | KJ651050 | KJ651106 | KJ650848 | KJ650999 | KJ650960 | KJ650906 |
| SE_4 | *D. angustum* | S | Norin B131031 | 06.08.2002 | SE | Torne Lappmark | *D. laevidens* | - | KJ651091 | KJ650833 | - | KJ650949 | KJ650891 |
| SE_5 | *D. laevidens* | S | Westerberg B131027 | 13.07.2002 | SE | Lule Lappmark | *D. laevidens* | - | KM502764 | KM502641 | KM502730 | KM502708 | KM502677 |
| SE_6 | *D. laevidens* | S | Hedenäs, Bisang & Persson B105000 | 09.09.2005 | SE | Jämtland | *D. laevidens* | KJ651035 | - | KJ650831 | KJ650985 | KJ650947 | KJ650889 |
| SE_7 | *D. laevidens* | S | Hedenäs B85000 | 10.10.1995 | SE | Dalarna | *D. laevidens* | - | KM502765 | KM502642 | - | - | KM502678 |
| SE_8 | *D. laevidens* | S | Hedenäs B74327 | 21.09.2002 | SE | Jämtland | *D. laevidens* | KM502605 | KM502766 | KM502643 | KM502731 | KM502709 | KM502679 |
| SE_9 | *D. groenlandicum* | S | Hedenäs B74365 | 21.09.2002 | SE | Jämtland | *D. groenlandicum* | - | KM502763 | KM502640 | - | KM502707 | KM502676 |
| Tx1011 | *D. scoparium* | L | Lang 20100314.1 | 14.03.2010 | NL | North Holland | *D. scoparium* | KF423888 | KF423988 | KF423526 | KF423814 | KF423734 | KF423631 |
| Tx1021 | *D. scoparium* | L | Lang 20100314.5 | 14.03.2010 | NL | North Holland | *D. scoparium* | KF423889 | KF423989 | KF423527 | KF423815 | KF423735 | KF423632 |
| Tx1031 | *D. scoparium* | L | Lang 20100314.9 | 14.03.2010 | NL | North Holland | *D. scoparium* | KF423890 | KF423990 | KF423528 | KF423816 | KF423736 | KF423633 |
| Vg_2072 | *D. scoparium* | G | Lang & Price 20070719.31 | 19.07.2007 | CH | Canton Geneva | *D. scoparium* | GU068406 | GU068490 | GU068461 | GU068377 | GU068434 | KF423634 |
| Vg_2111 | *D. scoparium* | G | Lang & Price 20070719.35 | 19.07.2007 | CH | Canton Geneva | *D. scoparium* | GQ428101 | GQ428056 | GQ428011 | GQ427973 | GQ427933 | KF423635 |
| Vx_ 20314 | *D. bonjeanii* | G | Lang, Price & Naciri 20070523.21 | 23.05.2007 | CH | Canton Geneva | *D. bonjeanii* | GQ428105 | GQ428059 | GQ428014 | GQ427976 | GQ427936 | KF423636 |
| Wa_1 | *D. howellii* | UBC | Schofield & Harpel 120572 | 23.10.2002 | US | Washington, Snohomish Co. | *D. scoparium* | KF423891 | KF423991 | KF423529 | KF423817 | KF423737 | KF423637 |

Appendix 2.

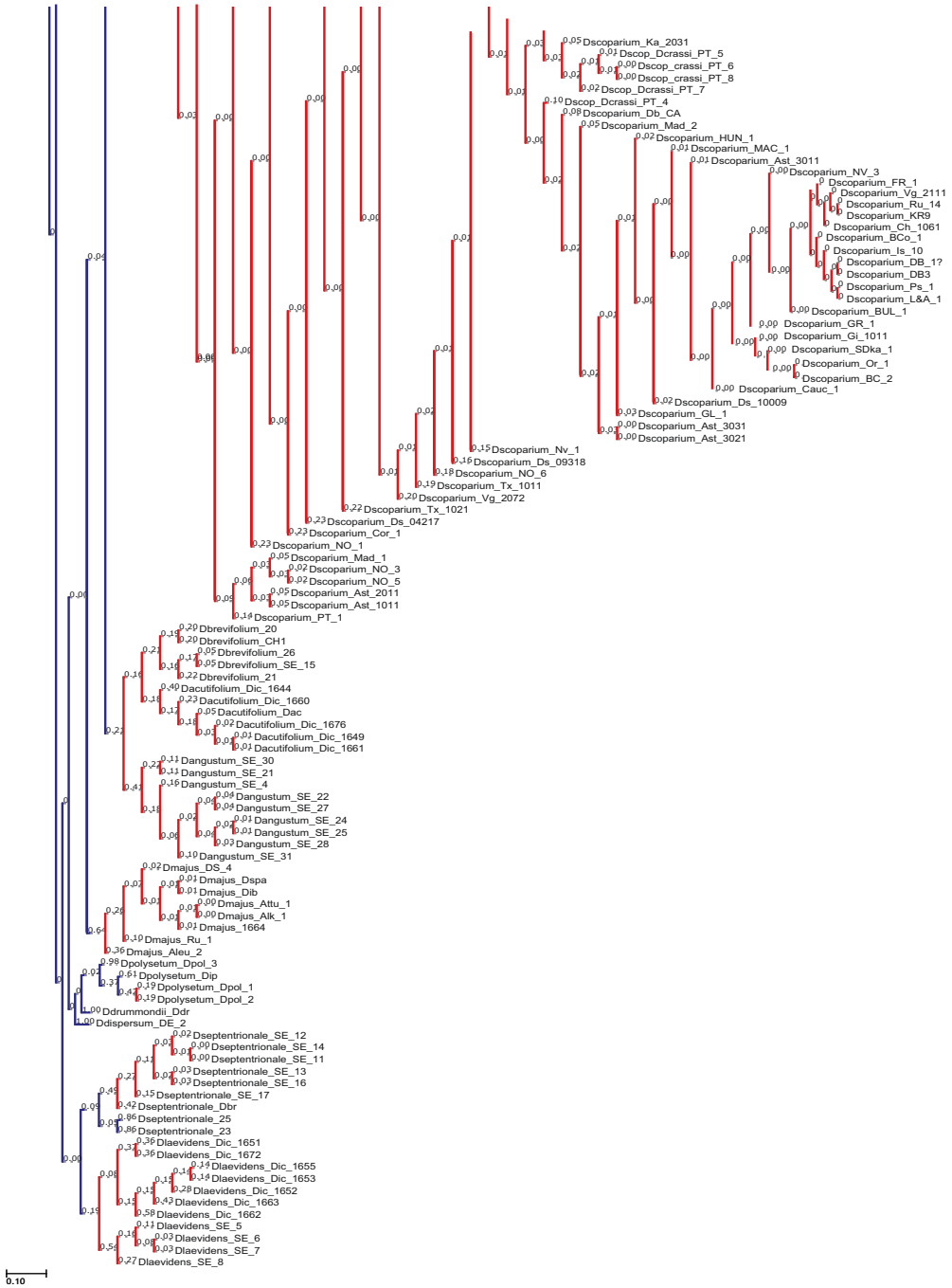


Appendix 2. General mixed Yules coalescent (GMYC) multiple threshold model from plastid and nuclear data. Branch length fitted a strict molecular clock, with estimated entities indicated in colors fitting the lineages-through-time plot for multiple GMYC thresholds.

## Appendix 3.

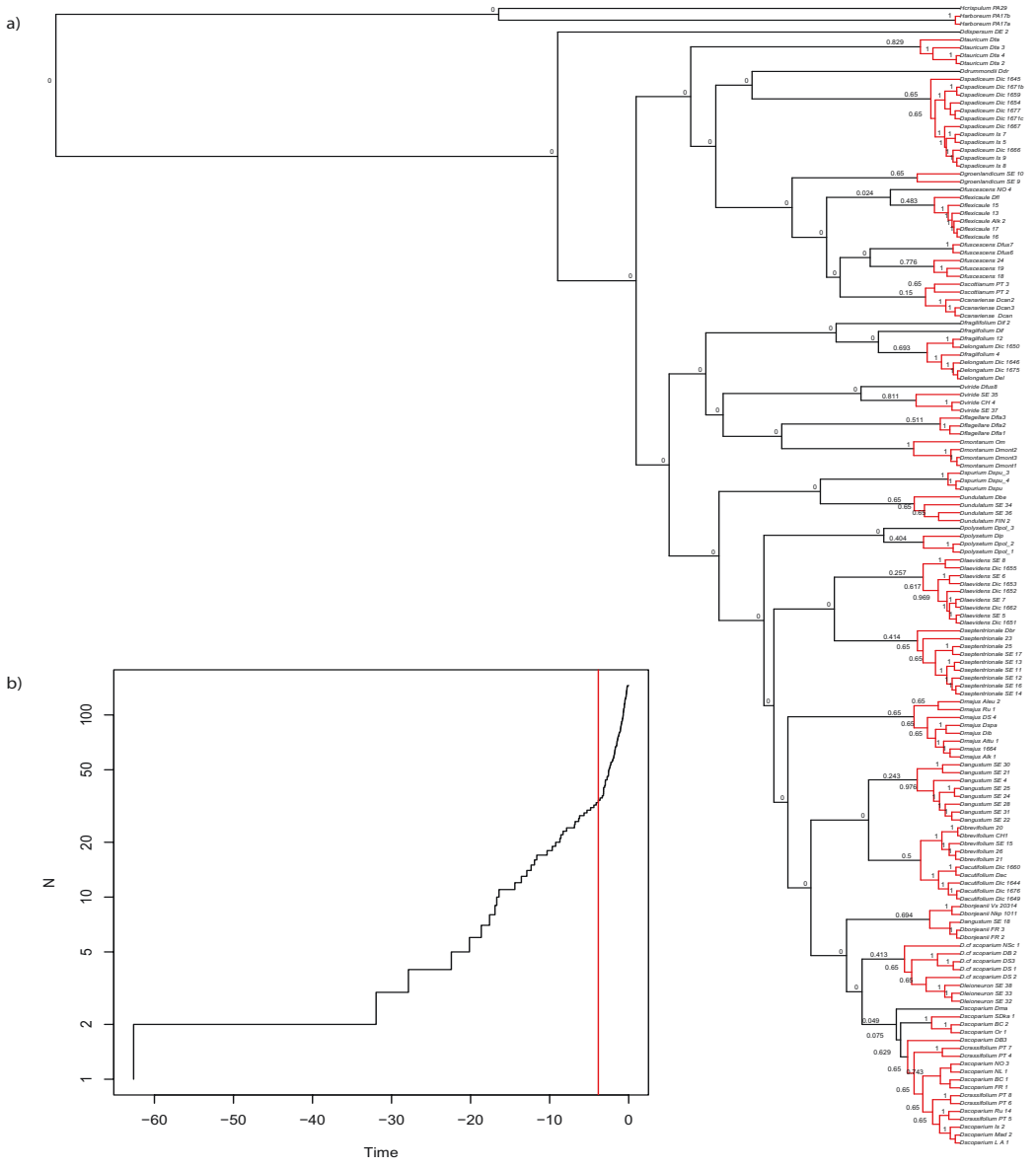



Appendix 3. PTP ML partitions obtained from http://species.h-its.org/. Species boundaries are indicated in red.

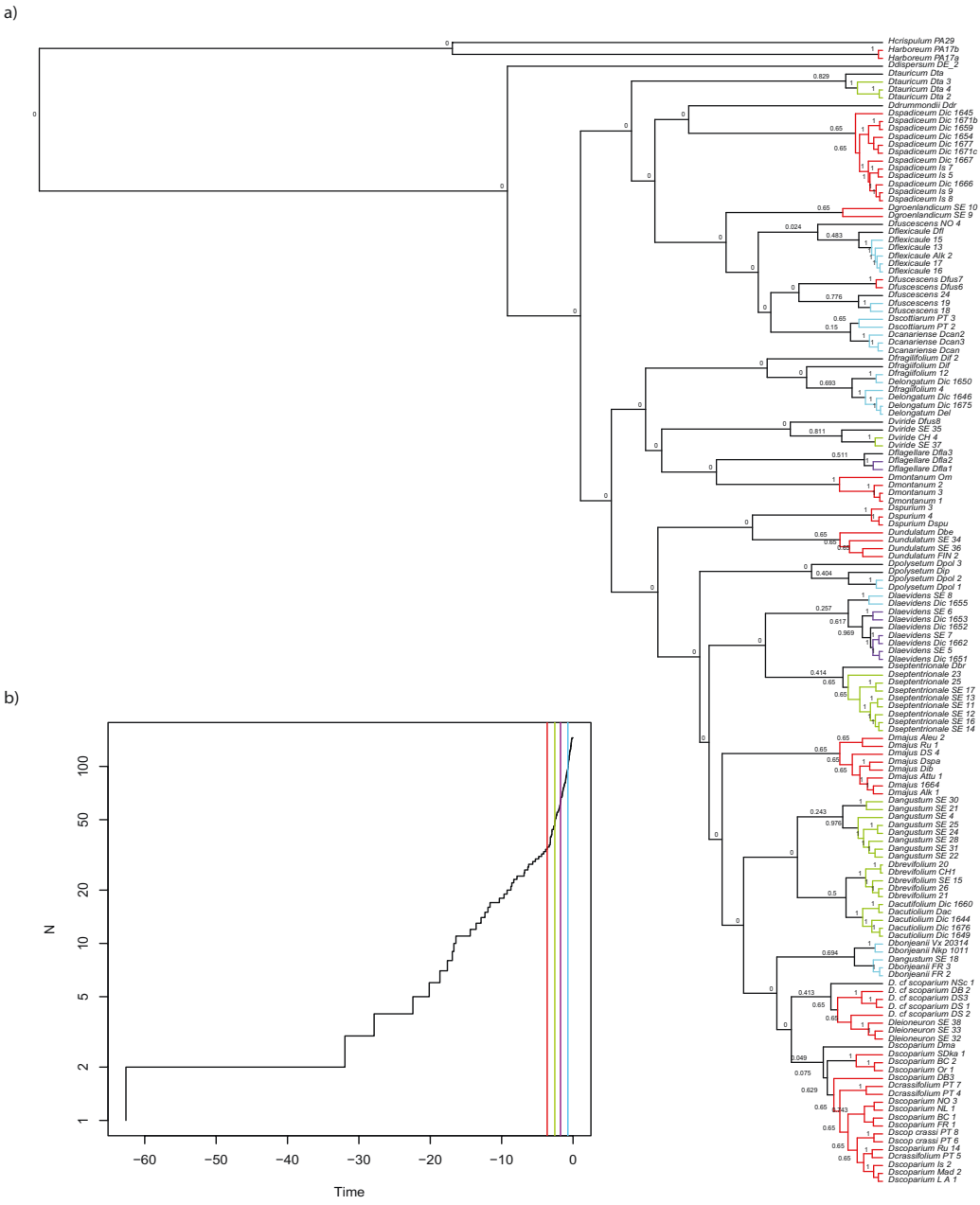



APPENDIX 3. suite

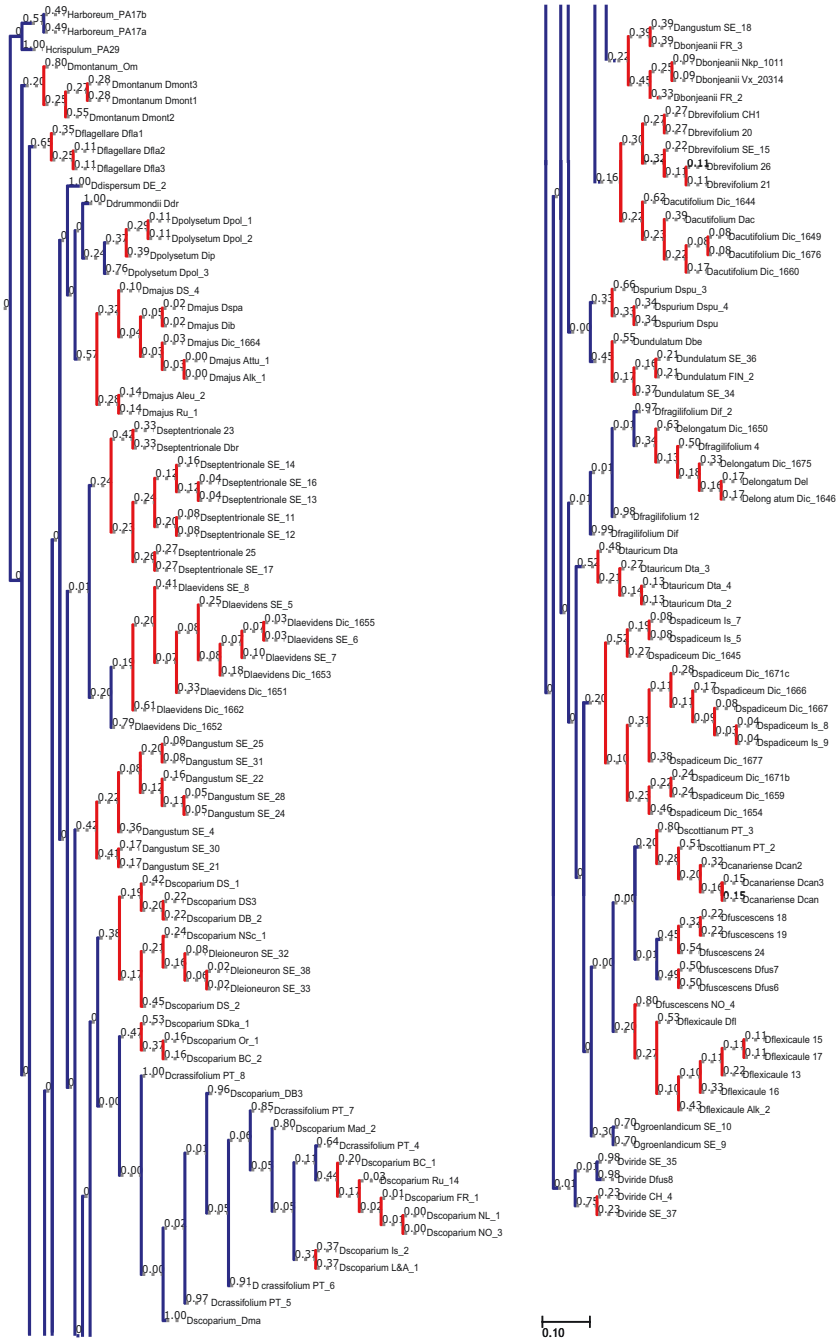
APPENDIX 4 GMYC tree obtained from a reduced dataset




GMYC ultrametric tree depicting species delimitation of 20 morpho-species *Dicranum* based on Bayesian analysis using a Yule model in BEAST and with fit of the general mixed Yules coalescent (GMYC) single threshold model from plastid and nuclear data. Branch length fitted a strict molecular clock, with estimated entities indicated in red. (a). Lineages-through-time plot for single (b) GMYC threshold is illustrated. The vertical red line represent the timing of the earliest coalescent event.




GMYC ultrametric tree depicting species delimitation of 20 morpho-species *Dicranum* with fit of the general mixed Yules coalescent (GMYC) multiple threshold model from plastid and nuclear data. Branch length fitted a strict molecular clock, with estimated entities indicated in colors (a) fittinng tha lineages-through-time plot for multiple GMYC threshold (b). The vertical color lines represent the timing of the four coalescent event.




PTP ML partitions obtained from http://species.h-its.org/. Species boundaries are indicated in red.

# Curriculum Vitae

Annick S. Lang was born on January 19, 1982 in Meyrin, Switzerland. During her secondary education, Annick participated in a language exchange program in Australia. It was in the Austrialian rainforest of Fraser Island that she discovered her interest for botany and ecological studies. She obtained her high school diploma in modern languages at Collège Rousseau in Geneva in 2001. That same year, she started biology studies at the University of Neuchâtel, Switzerland, where she obtained a bachelor's degree three years later. While studying various subjects related to plant biology (plant- insect interactions, phytosociology, plant ecology), her interest for evolutionary botany, and especially bryology, grew stronger. In 2006, Annick obtained a master's degree in Behavioural Ecology and Evolution from the same university, with a major in Botany. Her master's project focused on population genetics of a rare European fen orchid, *Liparis loeselii* (L.) Rich, in Franche-Conté (France) and Switzerland. During her master's, she also participated in the European project 'Intrabiodiv' and worked as a teaching assistant for botanical practicum courses.

After obtaining her master's degree, she went back to Geneva where she worked for three years at the Conservatory and Botanical Garden on a liverworts type collection digitalisation project. This is where Annick was introduced to the moss genus *Dicranum* and undertook preliminary investigations on *D. scoparium* with the bryophyte, fern and gymnosperm curator Dr. M. J. Price.

Annick started her PhD in November 2009 at the National Herbarium of the Netherlands (now Naturalis Biodiversity Center) where she pursued bryological studies under the supervision of Dr. M. Stech. During her PhD, she participated in international conferences in Vladivostok (Russia), Melbourne (Australia), and New York (U. S. A.) and was involved in the organisation of Naturalis's seminars. Furthermore, she had the opportunity to supervise one MSc project on male dwarfism in *D. scoparium*. After her graduation, Annick intends to continue her research on the genus *Dicranum* with the aim to study the effect of pseudomonoicy on population level in more detail.

# List of publications

## Papers

Price M. J. & **A. S. Lang**. 2007. *A new types digitalisation project underway at G. Databasing of the Stephani non-African liverwort types: a GBIF.ch project*. Tropical Bryology Group Newsletter 22: 3-4.

**Lang A. S.** & Y. Naciri. 2010. *New chloroplast primers for intraspecific variation in* Dicranum scoparium *Hedw. (Dicranaceae) and amplification success in other bryophyte species*. Molecular Ecology Resources 10: 735-737.

Price M. & **A. Lang**, 2011, Dicranum tauricum *Sapjegin ( Dicranaceae ) new for the Canton of Geneva, Switzerland*. Meylania 47: 29-33.

**Lang A. S.** & M. Stech. 2014. *What's in a name? Disentangling the* Dicranum scoparium *species complex (Dicranaceae, Bryophyta)*. Systematic Botany 39: 369–379.

**Lang A. S.**, Kruijer J. D. & M. Stech. 2014. *DNA barcoding of arctic bryophytes- an example from the moss genus* Dicranum *(Dicranaceae, Bryophyta)*. Polar Biology 37: 1157-1169.

**Lang A. S.**, Tubanova D. & M. Stech. *Species delimitations in the* Dicranum acutifolium *complex (Dicranaceae, Bryophyta*. Journal of Bryology, in press.

**Lang A. S.**, Bocksberger G. & M. Stech. *Phylogenetic relationship and species delimitation in* Dicranum *(Dicranaceae, Bryophyta) inferred from nuclear and plastid DNA*. Molecular Phylogenetics and Evolution. Submitted to Molecular Phylogenetics and Evolution

## Conferences abstract

Price M. J., **Lang A. S.** & Y. Naciri, *Do Bryophytes Evolve?* Conference series, Conservatory and Botanical Garden, Geneva. 15.11.2007 (talk).

**Lang A. S.** & M. J. Price. *The Franz Stephani collection: digitalisation and hightlights of this Liverwort collection*. Conference series, Conservatory and Botanical Garden, Geneva. 13.12.2007 (talk).

Price M. J., **Lang A. S.**, Gendre M., Lugeon, J., Loizeau P.-A., Palese R., Gautier L., & P. Clerc. *The Bryophyte Types in G: Databasing, Digitalisation and Discovery*. Andrew W. Mellon Foundation Meeting, Buenos Aires, Argentina. November 2008 (poster).

**Lang A. S.** & M. J. Price. *Type catalogues and species concepts: databasing of the Franz Stephani*

*liverwort collection at G*. NCCR Plant Survival International Conference on Plant Species Concepts and Evolution. University of Neuchâtel, Switzerland. 30 January- 01 February 2008 (poster).

**Lang A. S.** & M. Stech. *Morphological variability and biogeography of* Dicranum scoparium *Hedw.*, International conference "Bryoflora of the Russian Far East: taxonomy, genesis, and its phytogeographical relations", Vladivostok, 3-12 September 2010 (talk).

**Lang A. S.** & M. Stech. *Biogeography of mosses with an emphasis in* Dicranum scoparium *Hedw.*, Staff seminar series, Leiden, 2.12.2012 (talk).

**Lang A. S.** & M. Stech. *What does the phylogeography of widespread moss species reveal? New insight with the Holarctic moss* Dicranum scoparium *Hedw*. IBC meeting, Melbourne, 24-30 July 2011 (talk).

**Lang A. S.** & M. Stech. *What does the phylogeography of widespread moss species reveal? New insight with the Holarctic moss* Dicranum scoparium *Hedw*. PhD day, Leiden, 9.12.2012 (talk)

**Lang A. S.** & R. Gama De Nato. *All you've ever wondered about mosses!* Staff seminar series, Leiden, 20.3.2012 (talk).

Stech M., Cornelder B., **Lang A. S.**, Mennes C.B. Veldman S. & J.D. Kruijer. *Molecular identification of arctic moss species for ecosystem and biodiversity studies*. MOSS 2012 and Mol. Sys. Conference, New York, 22.6.2012 (talk).

THESIS

**Lang A. S.** 2006. Liparis loeselii *(L.) Rich.: a study on population viability*. Master Thesis, Laboratoire de botanique évolutive, University of Neuchâtel, Switzerland.

# Acknowledgments

I see earning a PhD like a hike in the mountains: the start is easy and delightful, maybe a little scary, as one cannot fully grasp what is really going to happen. The journey continues with ups and downs; still nice although sometimes difficult. During these harder moments, it is always gratifying to check on a map to see what one has already accomplished. The most difficult moment is probably when one reaches the end: the path gets steeper and the hardest passes remain to be crossed. But the view from the summit is worth the effort, I can tell!

First I would like to thank my supervisor Dr. Michael Stech for his guidance, support and patience throughout my PhD. I also would like to thank the National Herbarium of the Netherlands, now Naturalis Biodiversity Center, for financially supporting my project. I am very grateful to the herbaria FCO, ICEL, MO, O, NY, S, TRH, G and UBC, Huub van Melick, Beata Papp, Yoon-Jun Young, Peter Hovenkamp, Karen Martinez who provided me material and I also would like to thank my colleagues Marcel Eurlings, Regine Niba, Bertie Joan van Heuven, Wim Star for their help in the lab. Furthermore, I would like to share all my gratitude to Dr. Michelle Price, Dr. Yamama Naciri and Eva Meier from Geneva, without whom this project would not have started and for their enthusiasm, encouragement, and fruitful discussions.

I very likely inherited my love for hiking from my family. So, thank you Mam, Pap, Stef, Phil and Adriana for all your encouragement and support during my 'trek' in Leiden. Thank you also to my dear old friends from Switzerland, who stayed there or went hiking their own mountain. It was great to share this experience with you.

Any trip, whether it is a hike or a road trip, is filled with meeting amazing people. Thanks to all of you, my Leiden 'trek' went a bit further away and allowed me to realise that there are as many views on life and truths as there are cultures. Gracias Fatima and tack så mycket Patrik, my first housemates in Leiden, for caring and sharing so many great moments. Grazie Chiara, Paolo, Stefano and Alex for your big hearts and hospitality. Thank you, merci, obrigada, Дякую, gracias, danke my dear housemates, it was a musical and tasteful pleasure to live with you. Finally, I would like to say Danke, gracias, merci, dziękuję, kop khun, Dhanyavaad, Спасибо, Terima kasih, Ευχαριστώ, kamsahamnida and of course hartelijke bedankt to all of you, for all the good moments, laughs and friendship! You made me feel like at home in Leiden and I feel very spoiled by having had the chance to meet you!