Phylogeny and species delimitation within the moss genus Dicranum Hedw.

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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

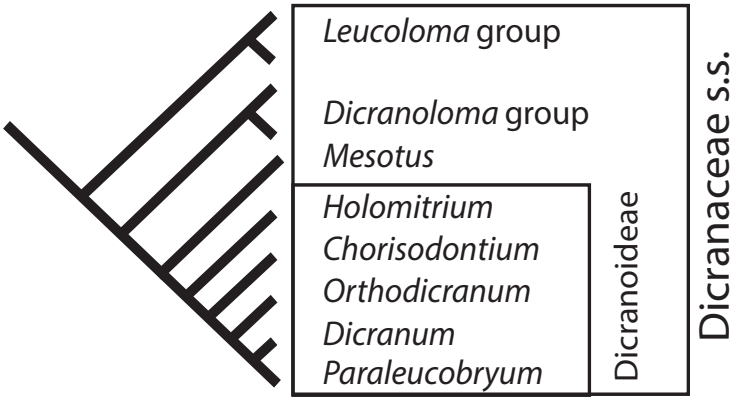

Fig. 1. Simplified cladogram of the Dicranaceae s.s. and relationships of its genera acoording to La Farge 2002 and Stech 1999.

(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.

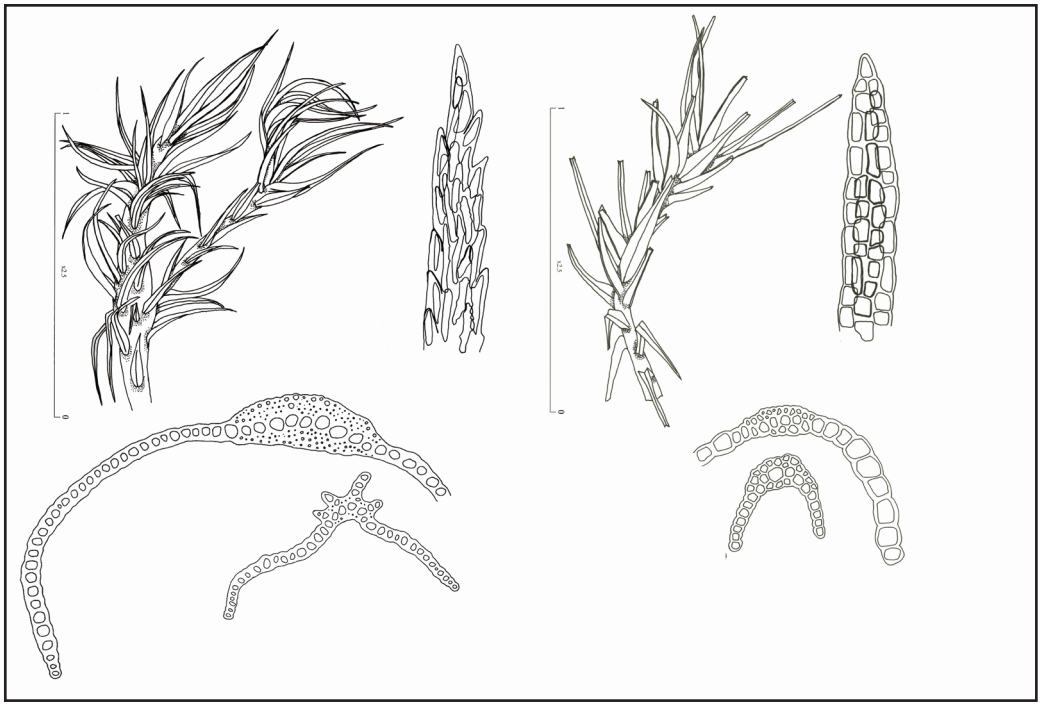

FIG. 2. Plant habit, leaf apex and leaf cross- section at base and upper part of A) *Dicranum scoparium* Hedw. and B) *D. tauricum* Sapjegin.

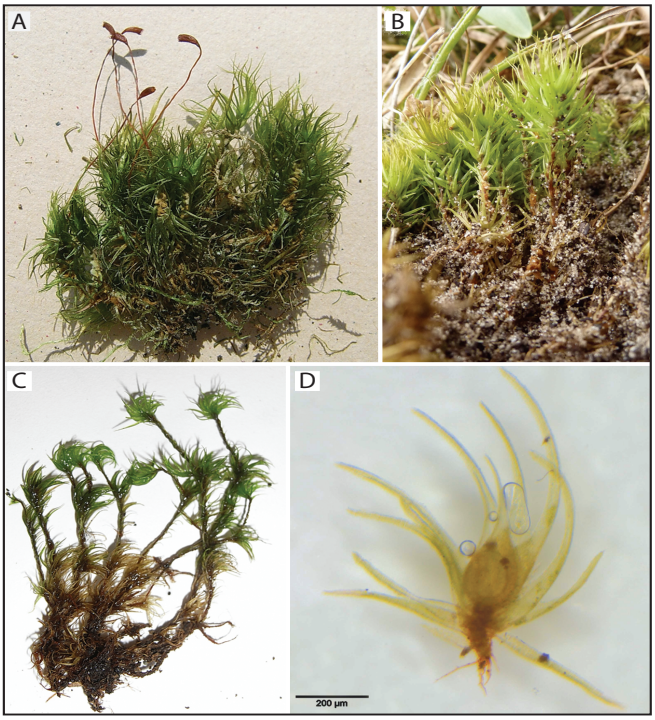

FIG. 3. Habitus of *Dicranum scoparium*. Female cushion with mature **A** sporophytes and **B** without sporophyte. **C** Normal sized males and **D** dwarfed male. Photo D by L. E. van Dijk

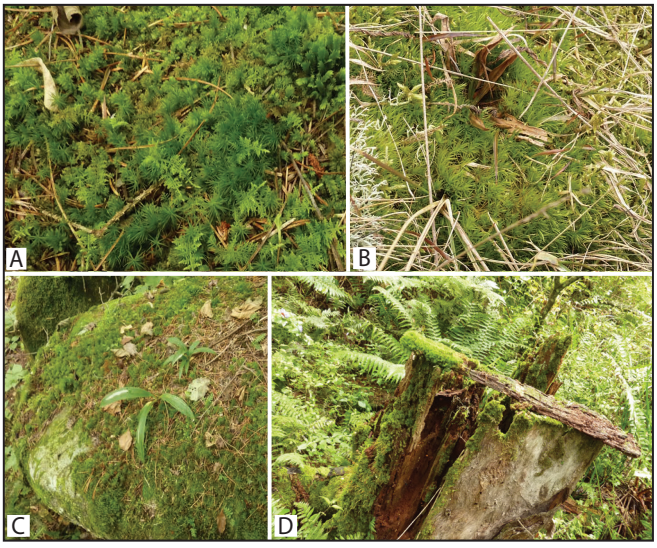

FIG. 4. Habitat type of Dicranum. **A** forest soil, **B** open sandy soil, **C** humus on boulder, **D** rotten tree bark.

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.